EUROPEAN ACADEMIC RESEARCH
Vol. II, Issue 2/ May 2014

ISSN 2286-4822
www.euacademic.org

Impact Factor: 3.1 (UIF)
DRJI Value: 5.9 (B+)

Ultrastructure of Spleen in the Freshwater Fish, *Tilapia mossambica* (*Peters*)

MEENAKSHI SUNDARESAN[1]
Department of Zoology
D. G. Ruparel College, Senapati Bapat Marg, Mahim
Mumbai, Maharashtra
India

Abstract:

Spleen is an important centre of blood cell production and destruction. Fishes, being the first vertebrates to have spleen, a well organized structure as is evident in higher forms is not expected. In fish spleen is externally covered by a capsule which is lined by a layer of cells. Spleen shows presence of reticulocytes cells below the capsule but no distinct trabeculae within. Interior of spleen is represented by red and white pulp, veins, sinusoids and arteries. The red and while pulp regions appear intermixed. Melanomacrophage centres (MMC) are physiological features found in fish spleen and may contain four types of pigments namely, melanin, lipofuscin, ceroid and haemosiderin. Not much work has been done to study the cellular details of the spleen at the electron microscopic level. The present work has been carried out to identify the different cells (endothelial cells, reticulocytes, megakaryocytes, macrophages and cells belonging to the erythrocytic and leucocytic series) found in the ultrastructure of spleen of Tilapia mossambica (Peters).

Key words: Histology, ultrastructure, spleen, *Tilapia mossambica,*

[1] Dr. Meenakshi Sundaresan is Associate Professor in Zoology in D.G. Ruparel College, Mumbai, India. She has been in academics for the past 30 years and her research interests are cell biology and toxicology. She has done extensive research on the effect of cadmium on fish and has published five research papers.

haemopoietic tissue.

Introduction:

Spleen of vertebrates is concerned with the formation, storage and destruction of blood corpuscles. In lower vertebrates, spleen is the only place where haemopoietic tissues assume the condition of discrete organs (Romer, 1963; Aguius and Roberts 2003). It is also concerned with the defense mechanism against diseases. So the gland is expected to counter the ill effects of the pollutants too. The structure of spleen of fish has been described by Bozidar Kurtovic *et al.* (2008) in the European sea bass. There are descriptions of normal histology for some select species (Yasutake and Wales 1983; Rocha and Monteiro, 1999). But however there has been no systematic review of the spleen histology at the cellular level. As fish have no lymph nodes, the spleen alone plays an essential role in antigen trapping (Press, 1998). Melanomacrophage centres (MMC) are physiological features in fish spleen and kidney (Aguis and Roberts, 2003). They are believed to be functional equivalents of the germinal centers of the spleen and lymph nodes in mammals (Ellis, 1980). MMC may contain four types of pigments- melanin, lipofuscin ceroid and haemosiderin (Aguis and Agbede, 1984; Couillard *et al* 1999). Wolke *et al* (1985) first suggested MMC as potential monitors of fish health. Sundaresan (2014) and Montero *et al* (1999) found that stressful situations can result in increased numbers of spleenic and kidney MMC. This study throws light on the histological structure of spleen through light and electron microscopy.

Material and Methods:

Live *Tilapia* fish were obtained from Masunda Lake in Thane district in Maharashtra, India and were kept for a fortnight for

laboratory acclimatization. The spleen of ten fish samples were fixed in 3% glutaraldehyde for 30 minutes at 4^0 C and processed for electron microscopy. Ultra thin sections were cut on the LKB ultramichrotome and picked up on G-200 copper grids. They were stained for 1 hour with uranyl acetate and counter stained with lead citrate. Grids were scanned under a Ziess EM 109 electron microscope and JEM Joel 100 'S' Japan make electron microscope. The haematology of fish reveals variations in the blood cell types. Hence, determination of cell types was carried out through blood smears by Giemsa staining (Gurr, 1956) and the blood cells were observed within blood capillaries and sinuses. Stained sections of gill filaments and their electron micrographs were also used for the same.

Observations and Discussion:

Histology of spleen - Light Microscopy:

Spleen is covered externally by a thin capsule which consists of a single layer of cells (occasionally double layered). The capsular cells are elongated and appear similar to reticulocytes. The cells are however larger and the cytoplasm is full of eosinophilic granules. The capsule therefore appears as a pink coloured lining over the outer extremity in sections stained with H/E. Nuclei are elongated and stained blue. There are no distinct trabeculae extending interiorly as are seen in spleen of mammals; however several of reticulocytes are seen attached to the inner surface of the capsule.

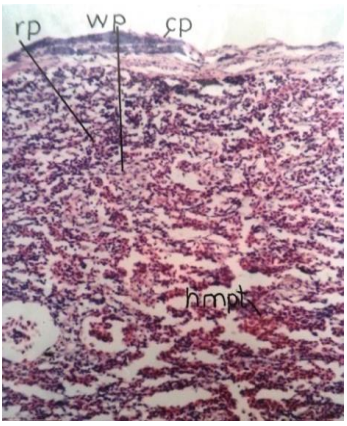

Fig. 1. LS of Spleen – Stain H/E

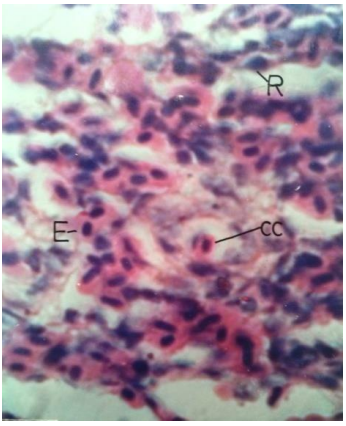

Fig. 2. LS of Spleen - Stain H/E

Key

E- erythrocytes, cp – capsule, R – reticulocyte, hmpt – haemopoietic tissue, cc – central capillary, rp – red pulp, wp – white pulp

The peripheral portion of spleen which is situated immediately below the capsule is represented exclusively by reticulocytes while the interior of the spleen is represented by red and white pulp, veins, sinusoids and arteries (Fig. 1, 2).

The arrangement of red and white pulp is not in an orderly manner as is observed in the spleen of higher vertebrates. The two types of tissues therefore are intermixed; however the presence of the two tissues can be realized by the differential colour when the sections are stained. In H/E stained sections white pulp appears light pink while red pulp appears brownish red. Spleens being concerned with the destruction of blood cells, haemosiderin deposits are seen at various places. Such sites are seen as brown pigmented regions.

Arteries:

Splenic arteries wherever present are cut in sections. Such arteries can be easily differentiated by the presence of endothelial linings. The endothelial cells are stained deeply.

Sinusoids:

These are the blood filled spaces containing blood and blood

cells. They are of varying sizes and do not have any distinct walls but are lined by reticulocytes.

Spleen being concerned with the destruction of blood cells, several of deformed erythrocytic cells can be seen. Besides red cells several of macrophages are also seen. These cells can be distinguished easily form the presence of phagocytic vesicles most of which are filled with phagocytosed blood cells. The vesicles appear dark owing to the presence of haemosiderin within (Fig. 3).

Splenic corpuscles (white pulp):

Spleen of fishes is not as well organized as those of higher vertebrates, hence the differentiation of red and white pulp was not distinctly observed as in higher forms. In section of spleen, most of the area is occupied by reticulocytes and is comparable to the red pulp of higher vertebrate spleen. White pulp region represented by several of small spherical corpuscles have been designated different terms by different authors and the terms such as 'ellipsoids' (Roberts, 1978; Gaikwad, 1981; Awari, 1985; Gaikwad and Rege, 1990; Awari, 1991), 'white pulp' (Guzdar, 1966; Daterao, 1989) and 'splenic corpuscles' (Mariano and Fiore, 1982) are used. In the present work, the term 'splenic corpuscles' has been used.

Each splenic corpuscle consists of a centrally lodged splenic artery termed as 'sheathed artery'. The vessel is lined by endothelial cells. Various stages of leucocytic cells are seen around the central artery arranged in a concentric manner. Splenic corpuscles are short and have uniform size with a diameter of about 25-30 microns. In H/E stained sections splenic corpuscles are somewhat difficult to locate; however, in Giemsa stained sections they are easily noticeable.

Reticulocytes: These cells are represented throughout the splenic tissue. The cells usually have two, at times three or four cytoplasmic processes extending from the central body. The

central regions is spherical or oval and appear dark, situated within this is a roughly spherical nucleus. The cytoplasm is densely filled with coarse granules which impart dark colouration to the cells in stained H/E sections. Owing to the densely filled cytoplasmic granules, it becomes difficult to distinguish the nucleus from the rest of the cytoplasm. The ratio between the nucleus to cytoplasm is 1:1.2 to 1.5. The cytoplasmic processes which extend outward are also full of cytoplasmic granules that impart dark colouration to the region.

The cytoplasmic processes of these cells form a network. Haemopoietic regions are located at various sites within the network of reticulocytes. Haemopoietic tissue regions are however much more at the central region of the spleen. The central region therefore appears dark in stained sections. The whole of the interior of the spleen being represented by the closely packed reticulocytes, in light microscopic studies it becomes very difficult to identify the various types of white blood cells and their structural details. The erythrocytes, wherever present can be detected to a certain extent owing to their oval shape and large size.

Red Pulp:

In the red pulp regions, cells that are observed are mostly the erythrocytic series, granulocytes and megakaryocytes.

Electron microscopy:

Observation of electron micrographs of spleen reveals the presence of several sinusoids, red pulp and the white pulp. The details of various cells that are observed within the spleen are as under:

(1) Endothelial Cells:

These are highly irregular in shape and they line the blood vessels. The cytoplasm is drawn out into two or three processes.

The cytoplasm is full of densely filled granules. Several fibrils are also seen within the cytoplasm. The endoplasmic reticulum is of rough type. The other cytoplasmic constituents are the vesicles, ribosomes and certain tubules of uniform size (Fig. 3, 4).

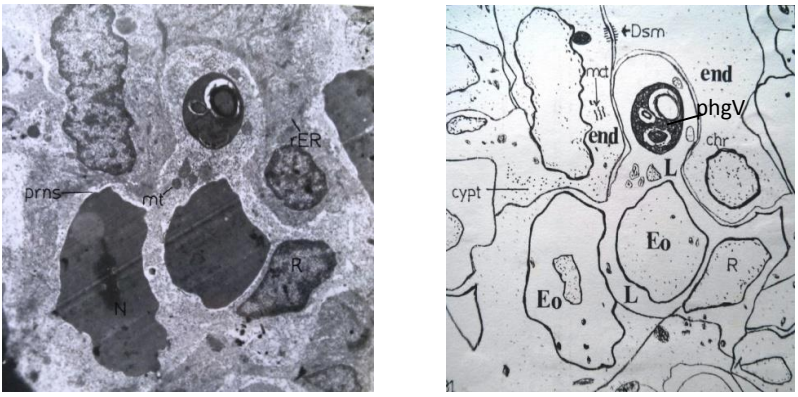

Fig. 3 E.M. and diagrammatic sketch of Spleen showing blood vessel and the surrounding tissue.

Key

Cytp – cytoplasmic process, Dsm – Desmosomes, End – endothelial cell, Eo – older erythrocyte, L – Lumen, mt – mitochondria, N – Nucleus prns – perinuclear space, rER- rough endoplasmic reticulum, R – reticulocyte, mct – microtubules, phgV – phagocytic vesicle

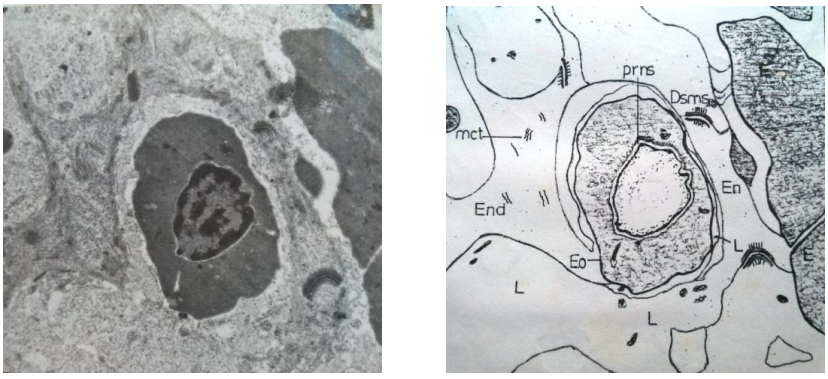

Fig. 4 E.M. and diagrammatic sketch of spleen passing through a capillary.

Key

Dsm – desmosome, End – endothelium, Eo – older erythrocyte, L – Lumen, mct – microtubules, ER – Endoplasmic reticulum, prns – perinuclear space

The cell nucleus is spherical or irregularly oval and has more or less uniformly distributed chromatin material. The cell nuclei with the surrounding cytoplasm are often extended into the lumen of the capillary (Fig 3).

The endothelial cells are little difficult to locate for the reason that the cytoplasmic processes are too long and irregular. In any electron micrograph, therefore only a part of cytoplasmic process may be represented. These cytoplasmic processes can be detected by the presence of characteristic desmosomes between the two neighbouring cells.

(2) Reticulocytes:

These are the elongated cells that taper at the two extremities of the cells. The cells are characterized by the presence of a large prominent nucleus. The cytoplasm is highly granular and has clusters of fibrous material. Such clusters are held parallel to the plasma membrane. Mitochondria are few and are lodged around the nucleus.

Each cell has a large nucleus that tapers at the two ends. The chromatin is of heterochromatin type (Fig 5, 6).

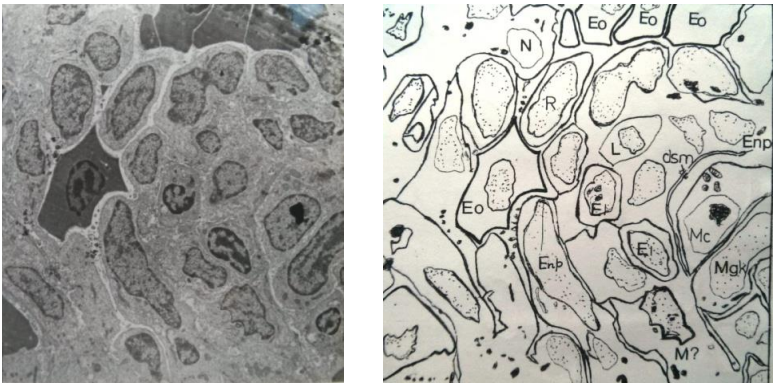

Fig 5 EM and diagrammatic sketch of spleen showing various types of cells.

Key

E1 – erthrocytic stage, Eo – older erythrocyte, Enp – endothelial cell process, L – lymphocyte, N – neutrophil, Mgk – Megakaryocyte, M – Monocyte, R –

Reticulocyte, Mc - Macrophage

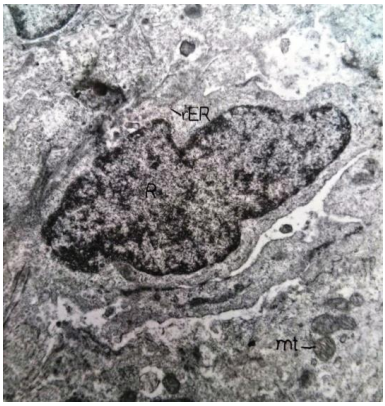

Fig 6 EM of spleen showing single Reticulocyte enlarged.

Key

Mt – mitochondria, rER – rough Endoplasmic Reticulum, R – reticulocyte

(3) Megakaryocytes:

These are the large cells and each is characterized by the presence of a large nucleus. Young cells are oval while fully formed ones may assume spherical or irregular shape. The cytoplasm is densely filled with fine granules. Mitochondria are small and spherical in shape.

Nucleus of the cells is oval or round depending upon the shape of the cell. The chromatin is spread over more or less evenly Fig. 5).

(4) Macrophages:

These cells have irregular boundaries. The cell nucleus is comparatively small and is usually eccentric in position. The cytoplasm is full of granules and a network of endoplasmic reticulum is seen. The mitochondria are oval in shape. The cells have a large number of lysosomes and phagosomes because of which, they can be easily be recognized. The cells are often seen with devoured RBCs or other cell particles. The cells have several other vesicles and vacuoles too (Fig 7).

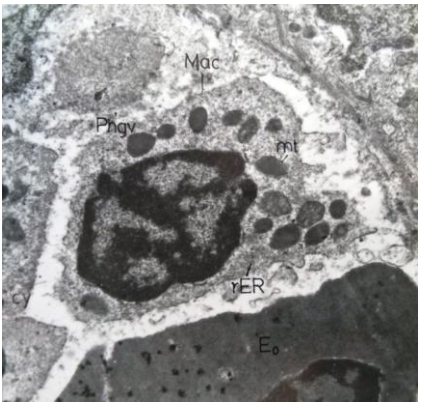

Fig 7 EM of spleen showing a macrophage within a blood vessel.

Key

Mac – macrophage, mt – mitochondria, Phgv – phagocytic vesicle, rER – rough endoplasmic reticulum, Eo – older erythrocytes

(5) Erythrocytes and erythrocytic series:

The erythrocytes of *Tilapia mossambica* (*Peters*) are oval and nucleated. They range in size from 10-13 microns in length and about 6-8 microns in breadth. The matured cells have their cytoplasm densely filled with haemoglobin. The cytoplasm therefore appears dense and no other cytoplasmic organelles other than the nucleus are seen. The cell nucleus is oval with an irregular outer margin. In electron micrographs the region around the nucleus appears as a clear space (perinuclear space). The nuclear material is dense and is of pycnotic type. The immature erythrocytes are more spherical and has a proportionately large circular nucleus with less dense cytoplasm (Fig. 5, 8).

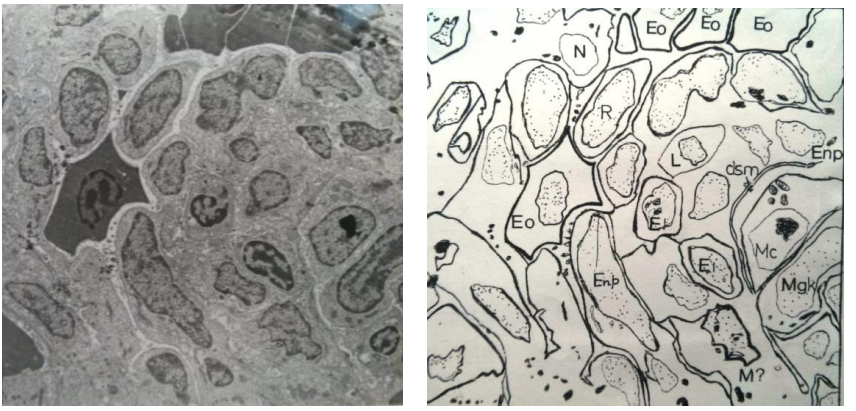

Fig. 5 EM and diagrammatic sketch of spleen showing various types of cells.

Key

E1 – erthrocytic stage, Eo – older erythrocyte, Enp – endothelial cell process, L – lymphocyte, N – neutrophil, Mgk – Megakaryocyte, M – Monocyte, R – Reticulocyte, Mc – Macrophage

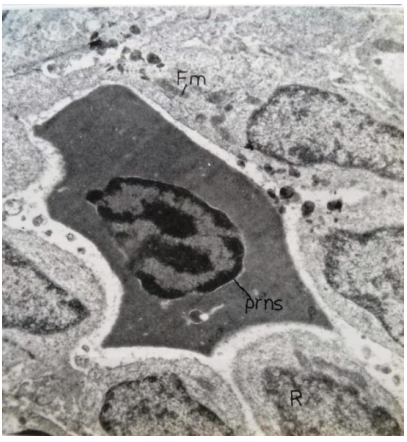

Fig. 8 EM of spleen with a blood sinus

Key

Fm – Fibrous material, prns – perinuclear space, R – Reticulocyte

The young developing erythrocytic stages can be distinguished from their round or oval shape and pycnotic nuclei. The cytoplasm of these cells is full of cytoplasmic granules of varying sizes. Spleen is the region where old RBCs are destroyed. Such cells are seen in large numbers. These cells can

easily be distinguished from other cells of erythrocytic series by their highly irregular shape.

Leucocytic series: Leucocytes are represented by neutrophils and lymphocytes.

Neutrophils: These are roughly irregular cells with clear cytoplasm. The presence of clear cytoplasm helps to recognize cells. The cytoplasmic granules are fine and are uniformly distributed. The cell nucleus is large and spherical. The chromatin material is uniformly distributed (Fig. 5).

Lymphocytes: These cells are comparatively small and have an irregular shape. The nucleus is large and spherical. Cytoplasm is restricted to peripheral extremity. (Fig. 5).

There is no unananimity pertaining to the types of fish leucocytes. While many have reported the presence of all leucocytic cell types in fishes, Yokote (1982) and Pai Vinaya (1993) puts forth the leucocytic cells of fishes to be of only two types viz. the neutrophils and the lymphocytes. In *Tilapia mossambica,* the blood smears show presence of erythrocytes (mature, immature and abnormal), smudged cells, lymphocytes, neutrophils and thrombocytes. These cells were also clearly seen in the electron micrographs of gill lamellae. (Sundaresan et al 2009).

Acknowledgements

Author is grateful to Dr. S. V. Shanbhag for providing valuable guidance and encouragement for this research. I am also grateful to Dr. Gayathri N. for critical review of the paper. Laboratory facility provided by the Principal of Ramnarain Ruia College, Matunga is also appreciated.

REFERENCES

Aguis, C. and S. A. Agbede. 1984. "An electron microscopical study on the genesis of lipofuscin, melanin and haemosiderin in the haemopoietic tissues of fish." *Journal of fish biology* 24: 471-488.

Aqius, C. and R. J. Roberts. 2003. "Melanomacrophage centers and their role in fish pathology." *J. Fish Dis.* 26: 499-509.

Awari Subhash B. 1985. *Toxic effects of cadmium on edible fish Ambassis ranga (Hamilton buchanan)* M.Sc. Thesis University of Bombay.

Awari Subhash B. 1991. *Toxicity and Depuration studies of cadmium on Ambassis ranga (Cuvier valenciennes) with reference to variations in environmental and some biochemical parameters*. Ph.D. Thesis. University of Mumbai.

Basim, M. Jasim. 2008. "Effects of prolonged exposure to cadmium on the haematopoietic organs in grass carp (*Ctenopharyngodon idella*, Cyprinidae)." *Bas. J. Vet., Res.* 7(2): 108-120.

Bozidar, Kurtovic, Emin Teskeredzic and Zlatica Teskeredzic. 2008. "Histological comparison of spleen and kidney tissues from farmed and wild European Sea bass (*Dicentrarchus labrax L.*)" *Acta Adrait*. 49(20): 147-154.

Couillard, C. M., P. J. Williams, S. C. Courtenay and G. P. Rawn. 1999. "Histopathological evaluation of Atlantic tomcod (*Microgadus tomcod*) collected at estuarine sites receiving pulp and paper mill effluent." *Aquatic toxicology* 44: 263-278.

Daterao, M. S. 1989. *Effect of certain heavy metal salts on the physiology of the fish Tilapia mossambica (Peters) alias Saratherodon mossambicus (Trevawas)* Ph.D. Thesis, University of Mumbai.

Ellis, A. E. 1980. "Antigen trapping in the spleen and kidney of

the plaice (*Pleuronectes platessa L.*)" *J. Fish Dis.* 3: 413-426.

Gaikwad, S. A. and Rege, M. S. 1990. "Effects of chronic exposure to pesticides, Thiodon 35 EC and Phenyl mercuric acetate on the various tissues of *Tilapia mossambica* (*Peters*)." *Environ. Concern and Tissue injury*. Vol. IV: 179-192.

Gaikwad, S. A. 1981. *Toxicity studies with Thiodon35 EC and Phenyl mercuric acetate on Tilapia mossambica (Peters)* Ph.D. Thesis, University of Mumbai.

Gurr, E. 1956. *A practical manual of medical and biological staining techniques*. London: Leonard Hill (Books) Ltd.

Guzdar, G. S. 1966. *Haemopoesis in certain fishes*. M.Sc. Thesis, University of Mumbai.

Mariano, S. H. di Fiore. 1982. *Atlas of Human histology*. 5th Edition. Philadelphia: Lea and Febiger.

Montero, D., V. S. Blazer, J. Socorro, M. S. Izquierdo, and L. Tort. 1999. "Dietary and culture influences on macrophage aggregate parameters in gilthead seabream (*Sparus aurata*) juveniles." *Aquaculture* 179: 523-534.

Oguri, M. 1985. "Pigment granules in the renal interstitial tissue of marine teleosts." *Bulletin of the Japanese Society of Scientific Fisheries* 51(9): 1447-1449.

Pai, Vinaya. 1988. *On haematological aspects of two puffer fishes Tetradon oblongus (Bl,), Tetradon lunaris (Bl. Schn.).* M. Phil Thesis submitted to University of Bombay.

Press, C. M. 1998. "Immunology of fishes." In *Handbook of vertebrate immunology,* edited by P. P. Pastoret, P. Griegel, H. Bazin and A. Govaerts, 3-62. San Diego: Academic Press.

Roberts, Ronald. 1978. *Fish Pathology*. Cassel Ltd. 35 Red Lion Square, London WCIR, ISG.

Roberts, R. J., 2001. *Fish Pathology.* 3rd edition. Philadelphia: W. B. Saunders, 367 pp.

Rocha, E. and A. F. Monteiro. 1999. "Histology and Cytology of Fish Liver." A review In *Ichthyology; Research Advances*, edited by D. N. Saksena, 321-344. Science Publishers.

Romer, Alfred Shrewood. 1955. *The Vertebrate Body*. Philadelphia and London: W. B. Saunders Company.

Ruparelia, S. G., Verma Yogendra, Kashyap S. K. and Chatterjee B. B. 1986. "A new approach for the use of standard fish in the toxicological study." *Environmental Biology, Coastal ecosystem*. 89-92: (c) The academy of Environmental Biology, India.

Sundaresan, Meenakshi and Shanbhag, S.V. 2009. "Ultrastructural details of the inter lamaller region in the gill of *Tilapia mossambica (Peters)*." *J. Aqua. Biol.*, 24(2): 160 – 168

Sundaresan, Meenakshi. 2014. "Cadmium induced histological & histochemical changes in the spleen of freshwater fish, *Tilapia mossambica (Peters)*." *Indian Streams Research Journal* 4(3).

Wolke, R. E. G., C. J. George and V. S. Blazer. 1985. "Pigmented macrophage accumulation (MMC, PMB): possible monitors of fish health." In *NOAA Technical Report NMFS. 25*, edited by W. J. Hargis, 93-97. Washington D. C.

Yasutake, W. T. and J. H. Wales. 1983. "Microscopic anatomy of Salmonids: an atlas." In *U. S. Fish and Wildlife Resource Publication*, edited by W.T. Yasutake and J.H. Wales, 150.

Yokote, M. 1982. *An Atlas of fish histology*; Chapter IV – Blood, pp. 64-72. Tokyo: Kodansha Ltd.