Discussion

Epizootic ulcerative syndrome (EUS) broke out for the first time in India in 1988 in some regions of the north-east India. Subsequently, it affected West Bengal. Gradually the disease spread to almost all the states of India except, Jammu Kashmir, Himachal Pradesh, Punjab and Gujarat by 1993. At present the disease is under control in most of the states of India but in some areas of North Bengal, the disease has been occurring every year especially during winter months (Roy, 2003; Routh, 2006).

Immune system of animals is the defense machinery to fight against the diseases and the disease state occurs through interaction of the host defense and pathogen. However, the host parasite relationship in fishes is highly infiuenced by the environment. As the fishes are ectothermic in nature and they live in aquatic habitats, they are more susceptible to environmental stress than the homeothermic animals (Trust, 1986).Therefore, studies on the immune system of fishes have been imperative for better management of diseases. Head kidney, spleen and thymus are important in immunologic defense mechanism and haemopoiesis in teleost fish (Ellis et al., 1976; Ellis, 1980; Secombes and Manning 1980; Turner et al., 1984).

Morphological studies of kidney of healthy *Cirrhinus mrigala* showed that it was divided into an anterior part, the head kidney, and a posterior part, the trunk kidney. Routine histological studies of head kidney under light microscope showed the presence of erythropoietic cells as well as lymphoid cells in the cortex and medulla (Fig.8). Ultra structure of head kidney confirmed the presence of lymphomyeloid and erythroid cells. Erythrocytes, immature and mature, lymphocytes, monocytes, neutrophilic myelocytes, neutrophils with type I and type II granules, thrombocytes and macrophages were detected in ultrastructural studies (Figs. 9 and 10).

Morphological studies of spleen in healthy *C. mrigala* showed that it was a dispersed structure found in between the loops of intestine. Histological studies showed presence of red pulp and white pulp regions where white pulp region is surrounded by the red pulp (Fig. 12). Ultra structural studies of spleen showed the presence of almost all types cells found in head kidney. But here the cells are loosely bound in comparison to head kidney.

Morphological studies of thymus showed that it was a paired small organ present dorsolaterally and triangular in shape (Fig. 20). Histological study showed the presence of erythroid cells arranged in cords and presence of trabeculae which divided the organ into incompletely separated lobules. Along with the presence of erythroid cells epithelial cells and some other cell types were also observed. The thymus was not divided into cortex and medulla and Hassall's corpuscles like structure were detected (Fig. 21). Ultra structural studies showed the presence of cell types already found in head kidney and spleen. But cells like hypertrophied epithelial cells and a particular type of cells with a huge number of granules were also found. A number of cells with secretory granules were also found (Figs. 22, 23 and 24).

Sailendri and Muthukarruppan (1975) reported two distinct zones, a deeply stained lymphoid zone and non – lymphoid zone within head kidney of *Tilapia mossumbica.* They detected lymphocytes of all sizes, monocytes, plasma cells, granulocytes, and erythrocytes in the head kidney. Tatner and Manning (1983) reported that head kidney of Rainbow trout was packed with many lymphocytes along with erythrocytes and suggested that head kidney was a lympho-haemopoietic organ.

Sailendri and Muthukarruppan (1975) reported that spleen in *Tilapia mossmbica* was an elongated and flattered structure and was situated along the left side of the stomach. They mentioned that histologically spleen was divided into white pulp and red pulp regions. The red pulp region was mainly erythroid with a few lymphocytes while white pulp region contained reticular centers. Various cells types like lymphocytes, plasma cells, granulocytes, monocytes and erythrocytes were also found by them. Tatner and Manning (1983) observed that the spleen contained both erythrocytes and lymphocytes. They could not detect red pulp and white pulp regions within spleen. Ultra structural studies by Bodammer et al. (1990) in striped Bass showed that the spleen was composed of tightly packed lymphomyeloid cells and erythroid cells.

Sailendri and Muthukarruppan (1975) showed that the thymus in *Tilapia mossumbica* was encapsulated by a thin strand of collagen fibers and consisted of outer, middle and inner zones. Outer zone consisted of predominantly of thymocytes while the

inner zone consisted of predominantly of lymphocytes. The gland was found highly vascularized. Zapata (1981) studied the histological structure of the thymus of a teleost fish, *Rutilus rutilus* and found that the thymus contained a huge number of lymphoblasts and thymocytes within a reticular network, formed by epithelial cells. He also reported presence of macrophages, secretory like cells and epithelial cells with cysts but no Hassall's corpuscles like structure. De and Pal (1998) examined the histological and ultramicroscopic structure of thymus of a Gobiid fish, *Pseudopocryptes lanceolatus*. They showed that histologically the gland lacked cortex, medulla and Hassall's corpuscles. But they found the existence of a huge number of lymphoidal, epithelial and erythroidal cells. Besides those cells, macrophages and cystic cells were also detected. Romano et al. (1999) also studied the histological and cytological structures of thymus of sharpsnout seabream, *Diplodus putazzo* and showed that the thymus was divided into cortex and medulla. The cortical region mainly contained lymphocytes while blast-like lymphoid cell were localized in medulla. Cytological studies by Romano et al. (1999) identified four types of epithelial cells like i) limiting, adjacent to the connective tissue ii) medullary and cortical reticular cells iii) nurse cells iv) Hassall's corpuscle and huge number of erythroid cells. Xie et al. (2006) also found thymic epithelial cells (TEC) nurse – like cells and different types of leucocytes like lymphocytes, granulocytes and macrophages but no Hassall's corpuscles were detected. Along with different leucocytes, erythrocytes of different developmental stages were also detected by them.

From above discussion it can be concluded that head kidney, spleen and thymus are lympho–haemopoietic organs in *C. mrigala*. Consistent reports by different authors regarding the presence of different leucocytes and different developmental stages of erythroid in histological section and ultra microscopic structure of the three organs have strongly established the lympho haemopoietic role of these three organs.

Concentrations of different types of leucocytes and different developmental stages of erythrocytes in these three organs indicated that the head kidney played the most active haemopoietic role among these three organs in the teleost, *C. mrigala*.

Histological studies and ultra microscopic studies of head kidney in different teleost by different researchers have showed almost similar observations. Observations of most of the researchers are in conformity with our observations. Like head kidney, results of histological studies and ultramicroscopic studies of spleen of different researchers showed more or less uniform result.

Histological studies of thymus in healthy C. *mrigala* and thymus in other teleost by different workers like Tatner and Manning (1983), Zapata (1981),.De and Pal (1998) and Romano et al. (1999) showed that it was covered by epithelial membrane containing mucous cells. Epithelial membrane covering thymus provides a biological interface between thymus gland and aquatic environment. The mucous cells secrete mucous which can prevent microbial colonization and infection in the thymus (Ottesen and Olafsen, 1977). Macrophages and granulocytes present in thymus may have roles in the deletion of self reactive thymocytes.

Different types of epithelial cells, especially epithelial cells with secretory granules may be related with hormones secreted from thymus, other epithelial cells and myoid cells are not only unknown in fishes but also are still unknown in higher vertebrates (Zapata, 1996). They probably play an important role in creating the milieu, within T lymphocytes as occurs in birds (Kendall, 1991) and mammals (Ritter and Crispe, 1992).

In case of EUS affected C. *mrigala* morphological structures of head kidney and spleen did not show any significant change. But the thymus showed an increase in size ($p < 0.001$) in EUS affected fish compared to healthy fish.

There were evidences that thymus played a vital role in the ontogeny of immunological competence (Miller, 1961; Good et al., 1962; Cooper 1973). Beard (1894) suggested that in elasmobranches the thymus was the source of lymphoid cells. Naturally in EUS affected C. *mrigala*, the thymus was more active than in healthy fishes.

Though morphology of head kidney of EUS affected C. *mrigala* did not show any significant change, but histological structure of head kidney in EUS affected fishes

showed changes like haemorrhages, presence of haemosiderin laden macrophages and necrosis (Figs. 28 and 29).

Ultra Structural studies of head kidney of EUS affected fishes showed some changes. Presence of bacteria throughout the tissue was found (Fig. 30). A considerable degree of necrosis in some regions was also detected. Fibrin clumps were noticed along with the presence of melanin granules (Fig. 31). Apart from above mentioned changes significant decrease in the number of erythrocytes was the feature of head kidney of EUS affected fishes (Fig. 32).

The nature of histopathological changes has some similarities with the observations reported by various authors on histopathological changes caused by bacteria in infected fishes. In Japanese eel *(Anguilla japonica)* affected with red spot disease atrophy of haemopoietic tissue in the head kidney was detected by Miyazaki and Egusa (1977). Miyazaki (1980) observed the degeneration of renal tubules, and atrophy of renal haemopoietic tissue in *Anguilla japonica* affected by *Aeromonas hydrophila.* Later Miyazaki and Kaige (1985) observed the deposition of haemosiderin in the renal tissue of fishes in which experimentally bacteria were introduced. Saha (1998) observed the almost similar histopathological changes in head kidney of EUS affected *Channa punctatus.*

Roy (2003) reported histopathological changes in the haemopoietic organs of different EUS affected fishes.

Flano et al., (1996) studied the histopathological changes in the renal and splenic tissues of Coho salmon (*Oncorhynchus kisutch*) experientially infected with *Renibacterium solmoninarum* and observed following changes. In both the organs presence of bacteria was detected but in spleen bacteria was detected at much earlier stage of infection compared to thymus.

Histological studies of spleen of EUS affected C. *mrigala* showed necrotic changes along with vacuolation in the white pulp regions (Fig. 33). Hemorrhages were also noticed. Ultra microscopic studies of spleen also showed more or less similar changes found in head kidney of affected fish i;e presence of bacteria in the tissue with a considerable degree of necrosis. Fibrin clumps and melanin depositions were also noticed (Fig. 34). Like head kidney in spleen also considerable decrease in number of erythrocytes were also detected (Fig. 35). Bacteria are quite common in the spleen tissue. Melano macrophages with melanin depositions were also found. Fibrin clumps were also found in splenic tissue (Fig. 36).

Miyazaki (1980, 1985) reported similar changes in the splenic tissue of Japanese eel (*Anguilla japonica)* infected artificially with pathogenic bacteria.

Histological and ultramicroscopic studies of splenic tissue of Coho salmon (*Oncorhynchus Kistuch*) infected experimentally with *salmoninarum* showed fibrin clumps and melanin deposition along with presence of bacteria (Flano et al., 1996).

Roy (2003) studied the histopathological changes of spleen of different EUS affected fishes like *Channa gachua, Puntius* sp. and *Catla catla.* In all species of EUS affected fishes, he observed the vacuolation, haemorrhages and necrotic changes.

In the present study histological structure of thymus of EUS affected C. *mrigala* showed some highly eosinophillic areas, containing erythroblastic islets, cords of mature erythrocytes and reticular epithelial cells (Figs. 38 and 39). Sinuses filled with blood vessels were also found. Hassall's corpuscle like structures was also detected. Ultra microscopic studies of thymus of EUS affected fish showed the presence of bacteria in the outer part of thymus (Fig. 41) but fibroblasts cells were also found invaded with bacteria. The necrosis of the connective tissue was also noticed. Macrophages containing bacteria in their cytoplasm were also detected in thymic parenchyma.

Flano et al. (1996) studied histological and ultra microscopic changes in the thymus of Coho salmon experimentally infected with bacteria and found presence of bacteria in

thymic parenchyma, macrophages containing bacteria and necrotic changes in some areas.

Many workers have opined the need for establishment of normal haematological values in fish with a view to the diagnosis of disease (Hesser, 1960; Snieszko, 1960; Larsen, 1961; Summerfelt, 1967).

By applying different staining procedures and techniques, three types of blood cells such as erythrocytes, leucocytes and thrombocytes were identified. The erythrocytes appeared either elliptical or oval in form with clearly visible nucleus (Fig. 42). The erythrocytes were found PAS negative. All the leucocyte subpopulations analogous to the mammalian leucocyte subpopulations were also identified in peripheral blood of healthy *C. mrigala.*

Seasonal variations of the total erythrocyte count in the peripheral blood of healthy *C. mrigala* were noticed (Fig. 60). The total erythrocyte count in peripheral blood of healthy *C. mrigala* dropped significantly during July to September (3.5 to 3.8 $\times 10^6/mm^3$) and then slowly increased being the highest during December (5.1 $\times 10^6/mm^3$). From January onwards the TEC or total erythrocyte count started decreasing slowly till the end of spawning season. In EUS affected fishes total erythrocyte count dropped drastically ($1.44 \pm 0.16 \times 10^6/mm^3$) compared to that of healthy fishes ($4.5 \pm 0.32 \times 10^6/mm^3$) (Fig. 57).

Mott (1957) mentioned that total erythrocyte counts in fishes were low compared with those of mammals. Knoll (1957) noticed that total erythrocyte count in fish varied considerably with species. Mahajan and Dheer (1979) showed that total erythrocyte count in peripheral blood of healthy *Channa panctatus* varied from 2.90 to 3.18 $\times 10^6/m.m^3$. They also showed that TEC varied with seasons. Das and Mukherjee (2000) reported that the total erythrocyte count in the fingerlings of healthy *Labeo rohita* varied from 2.1 to 2.15 $\times$ $10^6/mm^3$. Martins et al. (2004) observed that total erythrocyte count in the peripheral blood of healthy *Leoporinus macrocephalus* was $1.772 \times 10^6/\mu l$.

Kori–Siakpere et al. (2005) showed that mean total erythrocyte count in peripheral blood of African snake head, *Parachanna obscura* was $1.67 \times 10^{12}/L$.

Das et al. (2006) reported that in *Catla catla, Labeo rohita* and *Cirrhinus mrigala* total erythrocyte count varied from 4.0 to 6.0 $\times 10^6/mm^3$, and the total erythrocyte count in healthy *C. mrigala* was the highest 6.0 $\times$ $10^6/mm^3$.

Drastic fall in total erythrocyte count of EUS affected *C. mrigala* may be due to continuous loss of blood through ulcers developed on the body and the degeneration of red blood cells. Seasonal variations of total erythrocyte count may be the function of more than one factor such as temperature, food and metabolic state etc.

In peripheral blood the lymphocytes are the most common. The number varies from 41 to 60% in peripheral blood of healthy *C. mrigala.* According to the size two types of lymphocytes were identified, small and large.

The average diameter of small lymphocyte was 3.72±0.39µm and nuclei diameter was 2.5±0.64µm. The nucleus was round shaped and was surrounded by a thin film of cytoplasm (Figs.42, 43 and 44).

The average diameter of large lymphocyte was 11.2±0.42 and diameter of the nucleus was 9.4±0.12. The nucleus was almost round in shape and was encircled by a thin film of cytoplasm. These lymphocytes were slightly PAS positive. The number of lymphocytes also varied with seasons.

It was noticed that in EUS affected fishes total leucocyte count or TLC was much higher (90.93±13.86) $\times 10^3/mm^3$ in comparison to that of healthy fishes (26.93± 10.13) $\times 10^3/mm^3$ (Fig. 58).

Ellis (1977) noticed two types of lymphocytes large and small in fish and mentioned that the lymphocytes were slightly PAS positive. Andrew (1965) mentioned that the total leucocytes count (TLC) in fish was higher than that of other vertebrates. The total leucocytes count in fish varied from species, 23,000/mm^3 in roach (Catton, 1951), more than 100,000/mm^3 in some bony fishes (Pukhkov, 1964). Weinreb and Weinreb (1969) found variations in differential counts of lymphocytes in teleosts.

Mahajan and Dheer (1979) during their studies on cell types present in peripheral blood of *Channa punctatus* found two types of lymphocytes, small and large. Total number of leucocytes varied from 60 to 62 × 10^3/mm^3.

Das and Mukherjee (2000) during their studies on sublethal effect of Quinolphos on selected blood parameters of *Labeo rohita* found that total leucocyte count in healthy fish was 20 × 10^3/mm^3.

Kori-Siakpere (2005) reported that the TLC in peripheral blood of African snake head, *Parachanna obscura* was 19.07 × 10^3/mm^3.

Das et al. (2006) also observed that the total leucocyte count in peripheral blood of healthy *Cirrhinus mrigala* at normal pH varied from 20 to 22× 10^3/mm^3.

The total leucocyte count decreased during winter (January and February) with a sharp increase during March and April. This increase is followed by a decrease during May and June and finally increased during breeding season i; e July, August. After the end of breeding season the total leucocyte cocent of showed a tendency of decrease till the March of next year.

Mahajan and Dheer (1979) also noticed the similar nature of change in total leucocyte number in peripheral blood of healthy *C. punctatus.*

Among the leucocytes, monocytes are the biggest in size. The shapes of nuclei varied from oval-round to kidney shaped and occupied a peripheral position in the cell (Fig. 45). The monocytes were found PAS positive (Fig. 46). The average n-c ratio of monocytes is 1: 1.67

The differential counts of monocytes in blood smear of healthy *C. mrigala* showed that it constituted 5% to 25% of the total leucocyte population of peripheral blood.

Ellis (1977) described that morphologically fish monocytes were not only similar to mammalian monocytes, even they were also PAS positive like mammalian monocytes. The morphology of monocytes described by Mahajan and Dheer (1979) is almost similar to the morphology found in healthy *C. mrigala.*

Hammers et al. (1995) described the structure of monocytes in common carp *Cyprinus carpio* and mentioned that the monocytes were the largest leucocytes with basophilic cytoplasm and were PAS positive.

Pavlidis (2007) described the morphology of monocytes in six Mediterranean species of fish. The monocytes are the largest in size and cell diameter varied from 16.65± 0.14µm to 20.33± 0.19µm.

Neutrophils are the most numerous among different granulocytes. The nuclei of neutrophils appeared oval to round in shape and seldom had the nuclei appeared bilobed in structure (Fig. 47). The neutrophils showed the strongest PAS reaction (Fig. 48). Neutrophils stained with Sudan Black- B showed presence of granules in the cytoplasm of the cells (Fig. 44).

The probable cause of the change in number of monocytes in peripheral blood of *C. mrigala* is to compensate the opposite change in number of neutrophil in peripheral blood, so that cell mediated immunological status of fish remains intacstaint.

The average cell diameter of neutrophils of healthy *C. mrigala* is 15.53±0.27 µm while the average nuclei diameter is 9.6±1.97 µm making the n-c ratio 1: 1.62. Neutrophils constituted the 20% to 38% of total leucocyte population in healthy *C. mrigala.*

Ellis (1977) suggested that unlike mammalian neutrophils, the nuclei of fish neutrophils either oval or round in appearance. Ellis (1977) also suggested that neutrophils were PAS positive and granules of cytoplasm appeared dark grey in colour when stained with Sudan Black- B. Mahajan and Dheer (1979) also suggested that neutrophils in peripheral blood of *C. punctatus* were large cells with average cell diameter 10.5 µm (±0.14) and that of nucleus was 6.0 µm (±0.31). The nuclei of neutrophils were either round or oval in shape. Hammers et al. (1995) mentioned that neutrophils in common carp, *C. carpio* were strongly PAS positive and round in shape with eccentrically placed nucleus of different shapes ranging from round or oval to bi-lobed and tri- lobed in structure. Palvidis et al. (2007) during their studies on different

blood cells in six species of Mediterranean fish showed that neutrophils were round in shape with an eccentric nucleus. The shape of the nuclei varied from round to oval, to the shape of human kidney. In some rare occasions, it appeared bi- lobed or tri- lobed in structure. The cell diameter varied from 12.24 ±0.3 µm to 15.12 ±0.13 µm.

Literatures containing reports of neutrophils in the peripheral blood of fish especially in teleosts are very few in number.

Mahajan and Deer (1978) during their studies on seasonal variation of cell types in peripheral blood showed that like monocytes the number of neutrophils also changed throughout the year but just in opposite in manner to the monocytes.

The reciprocal change in number of neutrophils and monocytes in peripheral blood of healthy *C. mrigala* during this study may be due to a kind of compensating function so that immune status of fish remains unaltered.

Eosinophils were very rare in peripheral blood of healthy *C. mrigala.* The shape of nuclei appeared irregular and the cytoplasm was found full of acidophilic granules (Fig. 45). Cells were PAS negative. It constituted only 1.5% - 2% of total leucocyte population.

Presence of eosinophil was reported in the blood of many species of fish and in cyclostomes (Jordan, 1938) and in elasmobranches (Fange, 1968). Ellis (1977) reported that the cytoplasm of eosinophils in fish was loaded with acidophilic granules which appeared bright red with Romnowsky's stain. Mahajan and Dheer (1979) also noticed the presence of eosinophils in the peripheral blood of *C. punctatus.* The number varied from 3% - 6% of total leucocyte population. Hammers (1995) showed that eosinophils of peripheral blood in common carp, *C. carpio* were PAS negative.

Palvidis et al. (2007) also found the presence of eosinophils in the peripheral blood of some Mediterranean species.

Basophils were found very rare in peripheral blood of healthy *C. mrigala.* The size of the cells was almost equal to the size of neutrophils but the nuclei were either centrally located or peripherally located (Fig. 42). They were PAS positive.

Haider (1968) reported presence of basophils in carp, but Hines and Yashouv (1970) failed to find basophils in the Israeli strain of this species.

Duthie (1939) and Catton (1951) found basophil in *Triglida* sp. but more in *Ctenolabrus* sp. Basophils have been reported also from blood of brown trout (Blaxhall and Daisley, 1973). The description of the basophil in gold fish stained with Romnowsky's dyes by Watson et al. (1963) and Weinreb (1963) was typical. According to them the nucleus of a basophil was large and eccentric and the cytoplasm contained basophilic large granules. Pitombeira and Martins (1970) claimed that basophils in Spanish mackerel were PAS positive.

Mahajan and Dheer (1979) mentioned that basophils in peripheral blood of *C. punctatus* could be easily identified by the presence of bluish cytoplasm. Nuclei were either rounded or showed considerable variations. Hammers (1995) showed that basophils of *C. carpio* were PAS negative.

Thrombocytes were seldom found in the peripheral blood of healthy *C. mrigala.* Shapes of thrombocytes vary greatly from spindle shaped to irregular structures. Thrombocytes were PAS negative.

Mahajan and Dheer (1979) described thrombocytes as oval cells with an ellipsoid nucleus in peripheral blood of *C. punctatus.* They also reported the presence of a considerable number of thrombocytes of irregular shapes. Palvidis et al. (2007) described thrombocytes as round, oval, elongate and spindle shaped in peripheral blood of six Mediterranean species.

Six distinct developmental stages of erythrocytes were identified, namely small lymphoid haemoblast or slh, basophilic erythroblasts or be, polychromatophilic erythroblasts or pe, acidophilic erythroblasts or ae, young reticulocytes and mature erythrocytes or mr in the tissue imprints of head kidney, spleen and thymus (Figs. 50 and

51). Tissue imprints of three lympho-haemopoietic organs showed that presence of developmental stages of erythrocytes was the highest in the imprints of head kidney and the lowest in thymus.

Sailendri and Muthukkaruppan (1975) identified different developmental stages of erythrocytes in the tissue imprints of three lympho-haemopoietic organs of *T. mossambica*. Mahajan and Dheer (1980) studied the process of erythropoiesis in the head kidney and spleen of healthy *C. punctatus* by preparing tissue imprints as well as using autoradiography techniques and identified six distinct developmental stages of erythrocytes.

There are two views regarding the development of blood cells in fish. One is known as diphyletic view propounded by Catton (1951) and the other is monophyletic view advocated by Jordan and Speidel (1924) and Duthie (1939).

The major changes noticed during present investigation on erythropoiesis were as follows

i. Cytoplasm was primarily basophilic and slowly became acidophilic passing through an intermediate stage.

ii. Finally the acidophilic cytoplasm was slowly replaced by haemoglobin resulting the change in shape and size of erythrocytes.

iii. Mature erythrocytes appeared smaller in size compared to the immature erythrocytes.

iv. Immature erythrocytes possess mitochondria, golgibodies and other cell organelles, but mature erythrocytes do not possess them. May be for this loss of cell organelles, shrinkage has taken place resulting the decrease in size.

Differential count of different subpopulations of leucocytes in peripheral blood of EUS affected *C. mrigala* showed a considerable increase in number of lymphocytes (65.0 ±9.47) compared to healthy fishes (52.49±2.03).

The number of monocytes in EUS affected *C. mrigala* also showed a considerable increase compared to healthy fishes.

Unlike lymphocytes and monocytes, neutrophils in EUS affected *C. mrigala* showed a significant decrease in number in comparison to that of healthy fishes. The number of neutrophils in peripheral blood of EUS affected fishes was 20.43±6.37 while average per cent of neutrophil in peripheral blood of healthy *C. mrigala* was 32.39±8.08.

Eosinophils and Basophils did not show any significant change in number.

Comparison of erythropoietic efficiency of head kidney and thymus of healthy fish was performed. The results showed that head kidney was more erythropoietically efficient than thymus which was consistent with the results of tissue imprints where developmental stages of erythrocytes were more in head kidney than in thymus (Figs. 52 and 53).

The results on erythropoietic efficiency of EUS affected fishes revealed that erythropoietic efficiencies of head kidney, spleen and thymus in EUS affected fishes were significantly less compared to that of healthy fishes.

Literatures dealing with erythropoietic efficiency of lympho-haemopoietic organs of fishes and infected fishes or diseased fishes are extremely rare. The significant decline in erythropoietic efficiency of lympho-haemopoietic organs in EUS affected fish may be explained by the presence of bacteria in those organs and the necrotic changes done by the bacteria.

The loss of blood from ulcers of EUS affected fishes along with decline of erythropoietic efficiency of lympho-haemopoietic organs might have led the acute anaemic condition in EUS affected fish as reported by various authors.

In peripheral blood of both healthy and EUS affected fishes per cent of round shaped erythrocytes representing immature stages and elliptical shaped erythrocytes representing mature erythrocytes differed significantly. (Table no.) In healthy fish per

cent of elliptical erythrocytes was 79.6±3.647 while in EUS affected fish it was 62.2±5.404.

The per cent of oval or immature erythrocytes in healthy fish was 20.4±3.647 and in EUS affected fish it increased to 37.8±5.404 (Figs. 54, 55 and 56).

Das et al. (2006) also showed that the shape of erythrocytes was changed when the fish was exposed to stressful conditions. In the peripheral blood of fishes exposed to either 5.5 pH or 9.0 pH of water, number of oval shaped or round shaped erythrocytes increased significantly.

As EUS affected fishes face an acute anaemic condition, resulting low oxygen carrying capacity which was reflected by the abnormal behaviour shown by EUS affected fishes, production of huge number of immature erythrocytes may be compensating mechanism against anaemic conditions.

Results of haemoglobin measurement of healthy fish year round showed a similar mode of decrease and increase with that of total number of erythrocytes which contain haemoglobin in blood (Fig. 60).

Mahajan and Dheer (1979) also found the similar results during their studies on *Channa punctatus*.

Above discussion and result clearly state that change of total erythrocytes count is the cause and change of haemoglobin in blood.

As every year during winter months out break of EUS takes place in the districts of North Bengal, the kinetics of the primary immune response of fish (*C. mrigala)* to particulate antigen, sheep red blood cells (SRBC), was investigated in terms of antibody-secreting cells by a plaque forming cell (PFC) assay and haemagglutination (HA) tests.

Plaque Forming Cell (PFC) assay was performed from two lymphoid organs head kidney and thymus of healthy *C. mrigala* during three seasons such as summer, rainy season and winter. Haemagglutination titre test was performed throughout the year against the particulate antigen sheep red blood cells (SRBC). Plaque Forming Cell (PFC)

assay was performed on 5th day after injections collecting cells from head kidney and thymus. But haemagglutination titre test was performed on 5th and 10th day after injection.

The results showed that during winter months specially from December to January when water temperature dropped significantly, the number of plaques i; e antibody secreting 'B' cells and amount of antibody production also dropped significantly compared to the number of plaques produced and amount of antibody produced during summer and the rainy season (Figs. 71, 72 and 73) suggesting a partial suppression to immune response of *C. mrigala*. Along with the seasonal suppression of immune response of *C. mrigala*, it was also identified that number of plaques produced from thymus is always significantly less than the number of plaques produced from head kidney. The antibody produced against sheep red blood cells was found completely 2-merceptoethanol sensitive.

Seasonal variations, particularly environmental temperature, greatly influence the immune system of fish (Zapata et al. 1992). Higher environmental temperatures enhance immune responses, while lower temperature adversely affects their expression (Daggfeldt et al. 1993; Lobb et al. 1984). Many fish species show lower blood lymphocyte count and suppressed immune response to an antigen in winter (Avtalion, 1969; Rijkers et al. 1980; Wishkovosky and Avtalion, 1987). In gold fish *Carassices auratus*, plasma IgM, level show annual changes, high in summer and low in winter (Suzuki et al. 1996). Also in rainbow trout *Oncorhyncleus myeniss*, the IgM levels declined in winter (Sanchez et al. 1993). In *Clarions batrachus*, an air-breathing teleost, amount of antibody production and number of plaques decreased significantly during winter (Sinha and Chakravarty, 1997). On the other hand, no correlations between water temperature and IgM levels were found in channel catfish, *Ictalurus punctatus* (Klesius, 1990). Bly and Clem (1991) reported that functions of B and T cell were affected by temperature.

Though, head kidney is the major lympho-haemopoietic organs in *C. mrigala* as in may other fish, yet a notable feature was the presence of Plaque Forming Cells in the

thymus of immunized fish, although this was low in comparison to the PFC level in the head kidney.

Similar results were obtained by Sinha and Chakravarty (1997) during their studies on immunological response in an air-breathing teleost, *C. batrachus*. Using MAb (monoclonal antibody), several investigators have reported the presence of small number of SIg^+ cells in thymus of fish (Egberts et al., 1983; Miller et al., 1987; Ellsaesser et al., 1988).

These findings indicated that thymus of fish might have contributed antibody production to some extent. 2-ME sensitive antibody in *C. mrigala* suggested that the antibody produced in *C. mrigala* was equivalent to mammalian IgM class. The works of Rijkers (1981) and Sinha and Chakravarty (1997) also support this view.

The production of low number of PFC and less amount of antibody during winter months clearly indicated that during winter months immune response in *C. mrigala* was partially suppressed making them more susceptible to disease during this period in every year.