CHAPTER VI

HAEMATOLOGICAL STUDIES IN FISHES OF RETTING AND NON-RETTING AREAS

6.1 Introduction

Fishes are poikilothermic animals that are subjected to changes in the environment in which they live. Fishes may be confronted with stress factors such as varied water qualities, pollution, malnutrition and disease. Fishes can adapt themselves to bad environmental conditions by changing their physiological activities. Physiological parameters in fish provide foundation for conclusions as to the physiological status of the organism. The status is directly related to inner and outer factors, biotic and abiotic influences which act on the organism. Due to the extensive biological and chemical variability of water ecosystems, investigation and definition of the physiological status of the inhabiting fish becomes increasingly important for appropriate evaluation of changes which may take place in most individual fish. Hematological studies in fishes have assumed greater significance because these parameters were to be used as an effective and sensitive index to monitor physiological and pathological changes induced by natural or anthropometric factors such as bacterial or fungal infections or pollution of water resources (Blaxhall, 1972). Haematological parameters are a sign of these changes. Haematological values of fishes can be affected by environmental and biological factors such as age, weight, sex, food, bacteria, parasites, and water quality parameters.

Sulphide is an important environmental factor and toxicant for aquatic plants, invertebrates and fishes (Levitt and Arp, 1991; Bagarinao, 1992). In the aquatic environments sulphide is produced mostly from bacterial sulphate reduction in sediments (Hawke *et al.*, 1985). Salt marshes enclosed bays and estuaries, and areas of pollution such as sewage outfalls are characterized by micro molar to milli molar sulphide concentrations in sediments (Bagarinao, 1992).

Studies conducted by Beerman (1994) shows that cell membranes are highly permeable to sulphide and it is therefore, very likely that sulphide quickly penetrates the gill epithelium of fish. Sulphide entering the blood may interact with haemoglobin (Hb) by forming sulphaemoglobin (SHb). The major biochemical lesion caused by sulphide appears to be the inhibition of cytochrome 'c' oxidase, the terminal enzyme in the electron transport chain of mitochondria. Mass fish kills in dredged or impounded salt marshes, or in earthen culture ponds are often attributed routinely to hypoxia/anoxia, salinity and temperature fluctuations, or low pH (Inland Fisheries Project, 1974), but it is quite likely that sulphide is responsible for some fish kills (Torrans and Clemens, 1982).

In this chapter the details of the studies on the haematological parameters of fishes in coir retting and non-coir retting areas of Kadinamkulam estuary were described.

6.2 Review of Literature

Fishes live in very intimate contact with their environment, and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components (Wilson and Taylor, 1993). In fishes, exposure to pollutants can induce either increases or decreases in haematological levels. Blood tissue truly reflects physical and chemical changes occurring in organisms. Early diagnosis is also possible when evaluating haematological data, particularly blood parameters (Luskova, 1997). Blood parameters have been important tools to diagnosis and prognosis of fish diseases. Toxicity testing on multiple species is often required because it is widely recognized that species have differential tolerances to toxicants, i.e. there is no one species that is universally sensitive to all toxicants. The stress caused by environmental pollution changes the structure of red and white blood cells (Larsson *et al.,* 1985). Haematological techniques are the most common method to determine the sub-lethal effects of the pollutants (Larsson *et al.,* 1985). As an indicator of pollution, blood parameters are used in order to diagnose and describe the general health condition of some fishes. Besides, this

type of index reflects certain ecological changes in the environment (Roche and Boge, 1996).

Hematological parameters have been recognized as valuable tools for the monitoring of fish health and in helping fishery biologists to interpret physiological responses to environmental stress, information which is especially relevant when comparing studies of different fish species living in contrasted habitats (Ivanc *et al.,* 1996; Zhiteneva *et al.,* 1997; Leonard and McCormick, 1999; Zhiteneva, 1999). Studies also showed that blood parameters such as haematocrit, haemoglobin concentration and RBC count are related to environmental factors such as water temperature and salinity (Graham, 1997).

When the water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the haematological parameters (van Vuren, 1986). In fishes, exposure to chemical pollutants can induce either increases or decreases in haematological levels. Their changes depend on fish species, age, the cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997). The relationship between haemoglobin and oxygen differs between loading and unloading sites and shows adaptations not only to environmental conditions but also to metabolic requirements, both of which govern oxygen availability and transport to tissues (Weber and Wells, 1989). Such adaptations may involve quantitative changes in total Hb content, or qualitative changes in Hb–oxygen–binding properties, and may appear both at the inter and intra – specific level (Weber and Wells, 1989).

Sulphaemoglobin formation in fish blood was investigated by Bagarino (1992). Leji *et al.* (2007) studied about the thyroidal and osmoregulatory responses in *Tilapia mossambica* the effluents of coconut husk retting and revealed that tilapia has an efficient osmoregulatory mechanism to tolerate the sublethal toxicity of coconut husk retting effluents. Madhukumar and Anirudhan (1996) observed that the effluent of coconut husk retting concentrate released during retting indicates high levels of toxic chemicals including sulphide and ammonia. Studies

also showed that fishes also occur in the sulphidic water column of the Cariaco Trench (Baird *et al.,* 1973). Stosik *et al.* (2002) studied about the selected immunological and haematological indices in Breams *(Abramis brama)* inhabiting various aquatic ecosystems and states that the immunological and haematological parameters are inter dependent to maintain the survival of the fish in response to the water quality parameters. Jawad *et al.* (2004) studied about the relationship between haematocrit and some biological parameters of the Indian shad, *Tenualosa ilisha,* and stated that haematocrit value showed a quadratic relationship to fish size (body length). Blood reactions, anaerobic metabolism, sulfide-insensitive cytochrome 'c' oxidase, and methemoglobinemia are some of the potential mechanisms that may help fish to survive sulfide toxicity (Torrans and Clemens, 1982; Bagarinao and Vetter, 1994).

6.3 Materials and Methods

Animal *Oreochromis mossambicus* (Peters) belonging to the family 'Cichlidae'. The fishes were collected form the four selected stations of Kadinamkulam estuary in pre monsoon, monsoon and post monsoon seasons during the period 2004 to 2005. The details of the study area and sampling stations were described in Chapter III.

Healthy female fishes (n=10) of body weight (30 ± 5 gm) and body length (12 ± 14 cm) were captured and blood samples of the fishes were collected at the sampling site directly from heart in a vial containing 2% ethylene diamine tetra acetic acid (EDTA) for the analysis of hematological parameters. The blood samples were analyzed within few hours after collection.

Chemicals: All chemicals and biochemicals used for analysis were of analytical grade, purchased from Sisco Research Laboratories (India) and E-Merck (India).

6.3.1 Red Blood Cell (RBC) Count

RBC count was done with a Neubauer crystalline counting chamber as described by Sohn and Henry (1969). The blood was drawn upto 0.5 mark in RBC pipette and immediately the diluting fluid was drawn up to the mark 101 (1:200 dilution). The solution was mixed well by shaking gently. It was allowed to stand for 2 or 3 minutes. On the counting chamber the cover slip was placed and a drop of fluid was allowed to flow under the cover slip, holding the pipette at an angle 40°. Afterwards the number of RBCs was counted under the microscope in five small squares and later the number of RBCs per cubic mm was calculated using the following formula:

$$\frac{\text{No. of cells x Dilution factor x Depth factor}}{\text{Area counted (x } 10^6/\text{mm}^3)}$$

6.3.2 White Blood Cell (WBC) Count

WBC count was determined following the procedure described by Hunter and Bomford (1963). The blood was drawn up to 0.5 mark of the WBC pipette and immediately the diluting fluid is drawn up to the '11' mark above the bulb (The dilution fluid consists of 1.5 ml of glacial acetic and 1 ml of aqueous Gentian violet solution made upto 100 ml with distilled water). The solution was mixed thoroughly by shaking gently. It was allowed to stand for 2-3 minutes. The Neubauer counting chamber and cover glass were cleaned and the cover glass was placed over the ruled area. A drop of fluid was allowed to flow under the cover slip holding the pipette at an angle of 40°. It was allowed to stand for 2 to 3 minutes and the WBCs were counted. The number of WBCs per cubic millimetre was calculated using the formula:

$$\frac{\text{No. of cells x Dilution factor x Depth factor}}{\text{Area counted (x } 10^4/\text{mm}^3)}$$

6.3.3 Estimation of Haemoglobin Content

The haemoglobin content was estimated by acid haematin method (Sahli, 1982). N/10 hydrochloric acid (HCl) was taken up to the 10.0 mark in the graduated tube. Blood was collected directly from heart to 20 cm mark in Hb pipette and the outer side was wiped out. This was then transferred into a graduated tube containing N/10 HCl. The pipette was rinsed two or three times with dilute HCl. It was allowed to stand for 10 to 20 minutes after thorough mixing. Then N/10 HCl was added drop by drop by shaking till the blood colour matched with the standard colour. Readings were taken from the scale on the graduated tube and the Hb concentration was expressed in gram percent.

6.3.4 Haematocrit

The haematocrit (Ht) value was calculated by the micro haematocrit method as described by Blaxhall and Daisley (1973). The haematocrit is usually used for measuring the ratio of RBC to plasma in the blood, and in effect measures the packed cell volume (PCV) of the red blood cells contained in the blood. The well mixed anticoagulated blood was drawn into a microhaematocrit tube (75 mm long, 1.1 to 1.2 mm internal diameter and one end was sealed with 'Critaseal'). The tube was then centrifuged in a micro haematocrit centrifuge for 5 min at 10, 500 rev/min. The readings were made with the aid of a micro haematocrit reader. The RBC column in millimetres gave the percentage haematocrit value.

6.3.5 Statistical analysis

All values were expressed as average ± SD. One way ANOVA is used for statistical analysis. The significant difference among means was determined by Duncan's Multiple range tests at the level of $p<0.05$ and $p<0.001$.

6.4 Results and Discussion

6.4.1 Red Blood Cell Count

The major function of red blood cells (RBC) is to transport haemoglobin, which in turn carries oxygen from the gills to the tissues. The RBC also contains a large quantity of carbonic anhydrase, which catalyzes the reaction between carbondioxide and water. The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation (van Vuren, 1986).

The results of the RBC count of fishes in coir retting and non-coir retting areas are given in Figure 8a. The RBC count of fishes collected from the non coir retting area showed almost same values in all three seasons. It is observed that the RBC count in fishes of retting showed a decreasing trend in values with respect to the increasing pollution due to coir retting. The RBC count decreased from 2.92 ± 1.45 x 10^6/mm^3 (SIV) to 2.57 ± 0.01 x 10^6/mm^3 (SII) in pre monsoon season, and showed a decrement of 11.99% in RBC count compared to that of control fishes. In comparison with pre monsoon and post monsoon seasons, RBC count of fishes in monsoon exhibits relatively slight decrement. It ranges from 2.98 ± 0.24 x 10^6/mm^3 (SIV) to 2.77 ± 0.14 x 10^6/mm^3 (SIII) and with respect to the control value the percentage in decrement was only 7.05%. The post monsoon season exhibit the highest decline in RBC count in fishes of retting areas and it ranges from 2.94 ± 0.13 x 10^6/mm^3 (SIV) to 2.56 ± 1.34 x 10^6/mm^3 (SII); the percentage in decline was 12.92%. Here the percentage of decrement in RBC counts in fishes in all three seasons were statistically significant ($P<0.05$, $P<0.001$).

The present investigation showed a diminution in RBC count suggesting decrease in erythropoietin activity due to the interference of pollutants (hydrogen sulphide) in the normal functioning of kidney (Verma *et al.,* 1982). In most vertebrates, including fish, erythropoietic activity is regulated by erythropoietin produced in the kidney (Gordon *et al.,* 1967). Here the stations with hydrogen

sulphide pollution (retting zones) exhibit maximum decrement of red blood cells in *Oreochromis mossambicus*. Studies by Sharan *et al.* (1989) recorded a dose dependent decrease in RBC level of *Channa punctatus* on exposure to the herbicide stamp-30 E.C (Organonitrochlorine group). The present study also states that long term exposure of fishes in coir retting effluents containing different pollutants like hydrogen sulphide, phenol, etc. reduced the RBC count in *Oreochromis mossambicus*. The decrease in RBC count in fishes during hydrogen sulphide exposure (pollution) in coir retting areas might have resulted from the reverse anemic state or haemolysing power of toxicant particularly on the red cell membrane (Ramesh and Saravanan, 2008).

6.4.2 White Blood Cell (WBC) Count

The white blood cells (WBC) are the mobile units of the body's protective system. They are specifically transported to areas of serious inflammation, providing a rapid and potent defence against any infectious agent/toxic substance that might be present.

The results of the WBC count are given in Figure 8b. WBC count of fish exposed to pollutants in coir retting areas registered a slight diminution in WBC count in three seasons. It varies from 5.84 ± 0.04 x 10^4/mm^3 (SIV) to 5.22 ± 2.28 x 10^4/mm^3 (SII) in pre monsoon and from 5.72 ± 0.02 x 10^4/mm^3 (SIV) to 5.33 ± 2.35 x 10^4/mm^3 (SII) in monsoon. In post monsoon season it ranges from 5.68 ± 0.43 x 10^4/mm^3 (SIV) to 5.06 ± 4.01 x 10^4/mm^3 (SII). The WBC count shows relatively slight decline in all seasons with respect to the control values. The pre and post monsoon seasons exhibits the maximum percentage in decrease i.e. 10.62 % and 10.91 % respectively, but in monsoon the WBC count in fishes of polluted stations showed marginal increase (6.82%) with respect to that of the values obtained from the non polluted station. In the present investigation the decrease in the number of WBC might be due to the failure of fishes to meet the pathological conditions arising from the toxicant stress. The reduction in WBC count observed in this study is in agreement with the report by Olanike (2007), he sates that the

release of epinephrine during stress causes a decrease of leucocyte count, which shows the weakening of the immune system. Seasonal influence was observed in the WBC count measured in the fishes of retting areas.

6.4.3 Haemoglobin Content

Haemoglobin (Hb) is the oxygen carrying pigment of the erythrocytes. The most important feature of the Hb is its ability to combine loosely and reversibly with oxygen and thereby involve in oxygen transport. Any quantitative reduction in the pigment is thus likely to affect the oxygen related metabolism of the organism.

The data relating to the Hb content of fishes collected from retting stations of Kadinamkulam estuary shows slight variation in Hb content with respect to that of non retting areas (Figure 8c). In pre monsoon season the Hb content decreases from 9.44 ± 0.31 g% to 9.23 ± 0.02 g% and has a decrease in percentage of 2.22 % over control. In monsoon season it ranges from 9.42 ± 0.14 g% to 9.23 ± 2.08 gram percent showed a percentage decrease of 2.02, in post monsoon season the Hb content varies between 9.49 ± 0.31 g% to 9.22 ± 0.08 g% and has a decrease of 2.84% over control values. In the present investigation the blood samples from retting areas shows perceptible reduction in Hb content in all seasons. But in monsoon season there is slight elevations of Hb content, because of fresh water intrusion will helps to attenuate the pollution from retting areas. This might be the reason for the slight elevation of Hb content in monsoon season.

The results of the present study showed that the haemoglobin content decreases in the fishes of retting areas. Comparatively low levels of Hb indicated anaemic condition in fish which might be due to the stress caused haemolysis as opined by Panigrahi and Mishra (1978). Hydrogen sulphide pollution restrains the Hb content in *Oreocromis mossambicus*. Koundinya and Ramamurthy (1979) suggested the possibility of inhibition of aerobic glycolysis curtailing de novo synthesis of Hb during toxic stress. Also, the lower Hb levels in the H_2S

intoxicated fish might be due to the disruption of the iron synthesizing machinery as supported by Beena and Viswarajan (1987). The decrease in the haemoglobin content in the present study results from the rapid oxidation of haemoglobin or release of O_2 radical brought about by the toxic stress of H_2S (Ramesh and Sarvanan, 2008).

6.4.4 Haematocrit Value

The haematocrit values are valuable in determining the effect of stressors on the health of fish and are also used to determine the oxygen carrying capacity of blood (Larsson *et al.,* 1985).

In the present investigation the Ht content ranges between 31.35 ± 1.22% (SIV) to 28.87 ± 1.22% (SIII) in pre monsoon, from 32.23 ± 0.37% to 30.08 ± 0.14% (SII) in monsoon and in post monsoon the values ranges between 31.24 ± 2.12% (SIV) to 28.31 ± 1.3% (SIII) respectively. The Ht values in fishes of retting areas shows a maximum decrement of 7.91 % in pre monsoon, 6.67 % decrement in monsoon and in post monsoon it decreases to 9.37% compared to that in fishes of non-retting areas (Figure 8d).

The present investigation shows that there is low level of Ht values were observed in fishes of retting zones of Kadinamkulam estuary. This decrease is attributable to shrinking of the red blood cells in fishes due to anoxic conditions in retting areas. The low Ht value would indicate anaemia or oligohaemia (Wepener *et al.,* 1992) or an alteration in the fish metabolism as suggested by Srivastava and Mishra (1979).

Therefore in the present study there is a decrease in concentration of RBC, haemoglobin and haematocrit content in *Oreochromis mossambicus* collected from the Kadinamkulam estuary. The decrease in the values of RBC, Hb results in anaemia, loss of immunity etc.

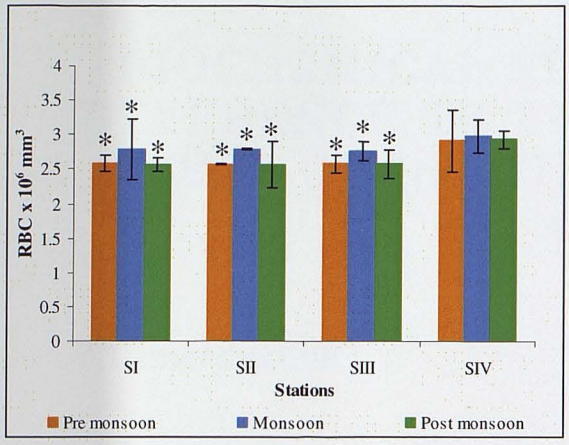

Figure 8a. RBC Count
(n = 10; * P < 0.05)

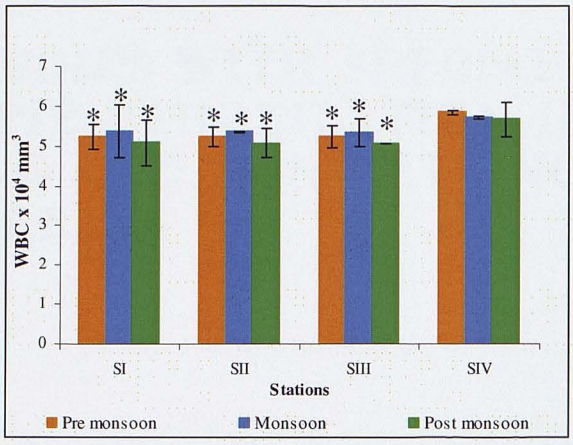

Figure 8b. RBC Count
(n = 10; * P < 0.05)

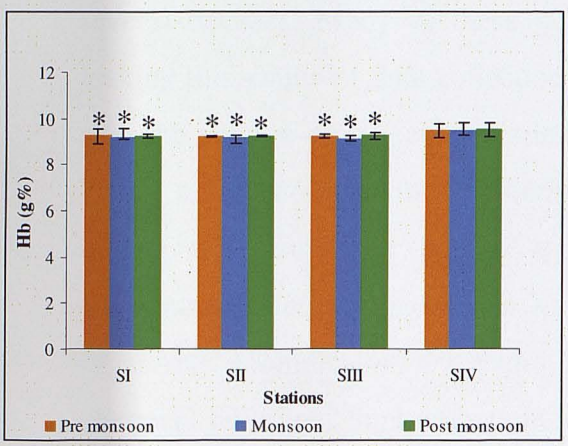

Figure 8c. Haemoglobin content
(n = 10; * P < 0.05)

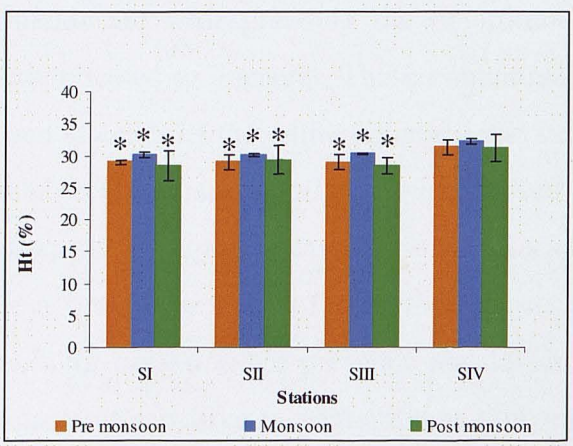

Figure 8d. Haematocrit
(n = 10; * P < 0.05)