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PHYSIOLOGY OF THE BLOOD OF CAT FISHES

THESIS SUBMITTED TO
THE COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY
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1990

To My Late Father

who taught me to search for knowledge

and

To My Mother

whose selfless love contributed a lot

CERTIFICATE

This is to certify that this thesis is an authentic record of research work carried out by Mrs. Valsala Kumari, C.S., under my supervision and guidance in the Department of Industrial Fisheries, Cochin University of Science and Technology in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology and no part thereof has been presented for the award of any other degree, diploma, or associateship in any University.

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DECLARATION

I, Valsala Kumari C.S., do hereby declare that this thesis entitled **"PHYSIOLOGY OF THE BLOOD OF CAT FISHES"** is a genuine record of the research work done by me under the supervision and guidance of Dr. C.T. Samuel, Retired Professor and Head of the Department of Industrial Fisheries, Cochin University of Science and Technology, and has not previously formed the basis for the award of any degree, diploma or associateship in any University.

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ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to my supervising teacher Dr. C.T. Samuel, Retired Head, Department of Industrial Fisheries, Cochin University of Science and Technology for his guidance, encouragement, for scrutinizing the manuscript and for suggesting improvements.

I am greatly indebted to Dr. M. Shahul Hameed, Head, Department of Industrial Fisheries, Cochin University of Science and Technology for his encouragement and for providing laboratory facilities during the final stages of this work.

I thank the authorities of Cochin University of Science and Technology for providing me all the necessary facilities for the successful completion of my work.

I acknowledge my thanks to the Director of Collegiate Education for sending me on deputation for one year for the completion of Ph.D. under the Faculty Improvement Programme (F.I.P) of U.G.C.

I express my deep sense of gratitude to Dr.K.P. Balakrishnan, Head, School of Environmental Studies, for his encouragement and for providing me necessary laboratory facilities during a part of this research work.

I wish to acknowledge my sincere thanks to Dr. N.R. Menon, Head, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences for allowing me to utilize the laboratory facilities.

I am thankful to Dr. George Philip, Professor, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences for allowing me to use the spectrophotometer.

I sincerely thank Dr. K.T. Damodaran, Head, Division of Geology, School of Marine Sciences for allowing me to utilize the microphotographic facilities.

I am indebted to Dr. A. Mohan Das, Reader, School of Environmental Studies for his sincere helps and encouragement.

I express my sincere gratitude to Dr.H. Krishna Ayyar, Scientist, CIFT, Cochin for helping me with the statistical part and Dr.K. Alagar Raja, Head, Division of Statistics, CMFRI, Cochin for his suggestions.

I wish to acknowledge my thanks to Dr. Bright Sing, Assistant Professor, Fisheries College, Panangadu for providing me with the bacteria culture.

The direct and indirect helps of all staff members, Department of Industrial Fisheries are sincerely acknowledged.

I am greatly indebted to Dr. Shaju Thomas, Dr. Mukundan, Dr.K. Suresh, and M/s. K.S. Gopalakrishnan, P.M. Mohan, P.G. Suresh, K.V. Pauly, S. Asok Kumar, and K.C.Bellarmin, Mrs.P. Rajalekshmi Amma, and all my other friends not mentioned here who helped me in one way or other at various stages of my research.

I am grateful to Mr. K. Raju, Technical Assistant, and other non-teaching staff of the Department of Industrial Fisheries, who helped me sincerely in many ways for the successful completion of my research work.

The financial assistance received from the Council of Scientific and Industrial Research in the form of Junior Research Fellowship, The Junior Research Fellowship of the Cochin University of Science and Technology

and the financial help from U.G.C. under Faculty Improvement Programme (F.I.P) are gratefully acknowledged.

Last but not the least, I should express my deep sense of gratitude to my husband for his sincere encouragement and for bearing with all the inconveniences caused to him and our son during the last part of this research.

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CHAPTER - I

GENERAL INTRODUCTION

1.1 RELEVANCE OF AIR BREATHING FISHES IN LAND USE IN INDIA

The vast areas of derelict swamps covered by macrophyton and swarmed by insects scattered in different parts of India are at present either under total negligence or utilized as waste disposal dumps. Eventhough Indian sub-continent is ranked among the first ten fish producing countries in the world, the fish production is not at par with the increasing need of protein in the average Indian diet. So the water areas which become unusable for conventional human activities like the swamps could be used for fish culture which would increase the availability of protein in the form of fish flesh, thus providing new opportunities to the fishermen. But the conversion of swamps for fish culture would entail considerable expenditure. Hence the significance of a group of fresh water fishes which have made their favourable abode the muddy swamps of tropics depending partly on accessory respiration to survive in the inimical environment. The homeostasis achieved in such a hostile, hypoxic medium make them excellent choices for culture in the derelict freshwater bodies of India.

These air breathing fishes form an economically important group which are highly esteemed as food fishes in many parts of South Asia and Africa. Though their natural habitat seems to be the marshes, they have also conquered other freshwater bodies like ponds, tanks, rivers and flooded paddy fields. They can also tolerate slightly brackish waters. They are known for their nutritive, invigorating and therapeutic qualities and are recommended by physicians as diet during convalescence (Jhingran, 1982).

1.2 AIR BREATHING CAT FISHES AND THEIR FEEDING HABITS

Among the unique group of air breathing fishes are present two species of fresh water catfishes which are selected for the present investigation, Heteropneustes fossilis (Bloch) known as Singhi in North India, Kari or Kadu in Kerala and Clarias batrachus (Linn) known as Magur in North India and Muzhi or Musi in Kerala. H. fossilis depends on two lateral sacs on either side of the body for accessary respiration and in Clarias highly vascularised branched structures called dendritic organs are present within the branchial chamber for air breathing. Both these fish species have almost similar feeding habits. The staple food of the fry includes very small crustaceans, but the fingerlings and adults feed on shrimps, ostracods, worms, insect larvae, insects, higher plants and organic debris. They can also subsist on mud from the swamp bottom which contains organic matter in the form of decaying animals and plants. Contrary to general belief, these fishes are thus at the very base of the food chain where they utilize the raw material in the form of organic matter (Dehadrai and Tripathi, 1976).

1.3 CULTURE OF CAT FISHES

For efficient utilization of natural food resources, the fish should be a part of a shorter food chain. So a fish which can convert organic debris into tasty fish flesh is superior to others. From this view point, C. batracbus and H. fossilis are ideal for culture. They are hardy, resistant to diseases and their flesh has high nutritive value.

1.3.1 Cat fish culture in Thailand and Cambodia

In Thailand and Cambodia, C. batrachus is cultured on a large scale. In Thailand, according to Sidhimunka et al. (1968), culture of two species

of Clarias is practised on a large scale. Fifty million fry are collected every year from tanks, paddy fields, irrigation canals and ditches. The production of C. batrachus accounts for about 90% of total production of Clarias (Kloke and Potaros, 1975). The culture of Clarias in Thailand began in the late 1950's which gives a higher annual income than that of other forms of agriculture and the production ranges depending on the source of water, location of the ponds and disease problems (Areerat, 1987).

1.3.2 Culture of cat fishes in India

In India, the ICAR has initiated during the Fourth Five Year Plan, an All India Co-ordinated Research Project on the culture and propagation of air breathing fishes in swamps which is being continued so as to evolve a package of practices in utilizing the swamps for fish culture purposes. This has considerable potential in rural areas because small village swamps are highly productive as a result of domestic sewage. During the Fifth Plan, fish culture operations were proposed to be developed in an intensive manner by bringing over 3 lakh hectares of tanks and ponds under this programme to raise the annual inland fish production to 11.25 lakh tonnes by the end of the plan.

Induced spawning has been successful for the production of seeds for both C. batrachus and H. fossilis in India. On an experimental basis as well as commercially, the culture of cat fishes yielded very high returns. At Kalyani in West Bengal, magur fingerlings (10g average weight), when stocked in a 0.1 hectare pond and supplementary fed, yielded a production of 5.2 tons/hectare/6 months with about 500% profit over the material inputs of feed and fingerlings (Banerjee, 1976 as cited by Jhingran, 1982).

Clarias and Heteropneustes are excellent materials also for intensive culture operations in urban areas as they permit high stocking density and respond to supplementary feeding. Under CIFRI/IDRC Rural Aquaculture Project, 4.8 tons/hectare of singhi in 6 months was obtained when stocked at a rate of 2,50,000/ha (Sengupta et al., 1979 as cited by Jhingran, 1982).

As a result of these recent innovations, inland fish culture has achieved a new dimension in India together with an increase in the status of air breathing fishes, eventhough most of the fresh water culturable bodies like tanks, ponds and swamps are still virgin.

1.4 RELEVANCE OF BLOOD STUDIES IN AN AQUACULTURE SYSTEM

For the successful management of any type of hatchery and fish farm, knowledge of the species concerned is a prerequisite. Unfortunately in most facilities, the stocking density is so high that stress syndromes and diseases are quite frequent. The sudden outbreaks of unknown etiology have caused not a little amount of set back in the aquaculture contingent. So along with the information about the food habits and habitat, the culturists should have the expertise to recognize the unhealthy from the healthy. "Since a change or lack of change in the blood picture is a fundamental characteristic of practically every physiologic or pathologic state, haematologic findings are among the most valuable and most generally useful of all laboratory diagnostic aids" (Wells, 1956). Pickering (1986) suggested that the simplicity of most blood sampling techniques probably accounts for the wide spread use of blood studies as a means of assessing the state of health of teleost fish.

The aquatic environment which is an alien world to humans poses a considerable problem to the adequate investigation of the fish blood.

In spite of this, on the perusal of literature, one can see that within the last two decades, blood physiology has been one of the most sought after branches of life sciences by the scientists all over the world. There are over 25,000 species of teleosts living in fresh, brackish and salt waters. But the literature on blood covers only limited number of them.

1.5 ASPECTS OF BLOOD INVESTIGATED IN THE PRESENT STUDY

The blood of H. fossilis and C. batrachus were studied extensively because of their dual respiratory nature. However, much remains to be understood about various physiological adaptations as reflected in the blood picture which enable them to live in the adverse changing and hypoxic environment. So the normal blood values and the effect of weight on various blood parameters, leucocytes, the effect of environmental factors and reproduction on haematology and the effect of reproduction on blood biochemistry were studied in H. fossilis and C. batrachus.

1.5.1 Normal variation in haematological parameters

The establishment of normal variation in haematological parameters is a prerequisite for the identification of stressful conditions. The normal values may vary throughout the year according to the varying eco-physiological conditions. So the range in normal haematological values were found out for a given weight in H. fossilis and C. batrachus.

Growth is another important factor to be taken into account when considering normal values. In proportion to the physical phenomenon of increasing weight, many of the clinical parameters also change which can be measured using advanced methods. The assessment of normal standard norms for clinically healthy fishes at various stages of life is necessary to identify and assess whether there is any stress or starvation existing

among fishes in a pond. So the important haematological parameters like RBC number, haemoglobin content, haematocrit and erythrocyte constants were estimated in the blood of normal healthy H. fossilis and C. batrachus for a given variation in weight.

1.5.2 Leucocytes and related cells in H fossilis and C. batrachus

To fight against pathological conditions existing in fishes or to defend against new pathogens, a thorough understanding of the defence mechanisms in fishes is warranted. An immune system is one component of the defence system of each and every vertebrate which enables the individual to maintain its homeostasis and thus survive in an environment which is innately hostile. The phagocytic cells which play an important role in vertebrate defence mechanisms are represented in cat fish by monocytes in blood, tissue macrophages and granulocytes. There are a number of substances in blood which are said to have potent defence functions. The complement system in serum with antimicrobial activity exhibited by its lytic properties and interferon which is an important antiviral agent are believed to be produced mainly by macrophages. Lysozyme, a hydrolytic enzyme which is present in the mucus, serum and phagocytic cells of many fishes provides an important defence against many microbial pathogens (Ellis, 1978, Avtalion, 1981, Hine et al., 1986a). According to Ellis (1978), the origin of lysozyme in the serum is probably from the neutrophils and monocytes. The cell mediated immunity, which is basically manifested by two phenomena, delayed hypersensitivity (DHS) and allograft rejection is effected through the action of lymphocytes. Thrombocytes are the most common type of leucocyte and comprise approximately half the leucocytes in all of the 121 species of fish examined by Saunders (1966). They are the biochemical equivalent

of platelet in mammals. Srivastava (1969) provides evidence that clotting rate in teleosts is an apparent function of the number of thrombocytes present. Thus the blood coagulation system has potential as a responsive system capable of serving as an indicator of environmental stress (Casillas and Smith, 1977). A limited amount of phagocytic activity is also attributed to thrombocytes. So it seemed relevant to study the basic morphologic features, some of the cytochemical properties and phagocytic activity of the leucocytes of C. batrachus and H. fossilis.

1.5.3 Haematology in relation to environmental parameters and reproduction

Successful reproduction and maintenance of viable populations are the ultimate determinants of the success of any fish species. Just like mammals, reproduction causes stress for the lower vertebrates like fishes too. In addition many of the phenomena described in relation to reproduction are not the direct result of maturation at all, but can be duplicated by straight forward depletion (Love, 1970) because many a fish abstain from food during exogenous vitellogenesis and spawning.

In a successful culture system, it is a prerequisite to know the various stages of maturation in fish. It is not always feasible to follow tedious histological methods to trace various stages of gonadial maturation. It is known that gonadial cycle induces haematological variations in fishes. Apparently little information is available on haematological changes in relation to the environmental factors affecting the onset and progress of gonadial maturation in culturable fresh water air breathing fishes. So the present investigation also includes the study of variations in patterns of haematological parameters in connection with varying environmental conditions and reproductive stages in H. fossilis and C. batrachus.

1.5.4 Biochemistry of blood in relation to gonadial cycle

The biochemical changes occuring in the gonadial tissues are also reflected in the biochemical composition of the blood. Sex steroid levels in the ovarian tissues and peripheral circulation are well documented in various fish species in relation to gonadial maturation. There are several biochemical factors like blood vitellogenin levels, plasma protein, plasma calcium, mRNA activity and radio active amino acid uptake in the liver which can be used to monitor the progress of exogenous vitellogenesis in fish (Tinsley, 1985). But relatively simple factors like sugar, cholesterol, and blood urea have been very rarely used as indirect indices of maturation, spawning and spent conditions. So the biochemical factors like plasma sugar, plasma protein plasma cholesterol and blood urea were also estimated in relation to gonadial cycle to study whether any changes are involved.

1.6 AVAILABILITY OF FRESH WATER CAT FISHES

Because of the increased industrialisation and urbanisation of Cochin, Kerala, India, most of the swamps and fresh water ponds are reclaimed in the suburban areas. As a result, the availability of H. fossilis and C. batrachus has been considerably decreased. Even in rural areas, these fishes have become very rare in Kerala, may be due to the increased use of insecticides and weedicides in paddy fields which may spread from there to associated ponds and rivers. In addition, the fishes were not available in sufficient numbers from the river through out the year and every year, one of the reasons being the very small number of fishermen engaged in fresh water fishing especially cat fish fishing. So the blood studies in relation to environmental condition and reproduction were to be restricted to a single year and to a few number of fishes. But the pilot studies during the preceding years prove the study to be feasible.

CHAPTER - II

REVIEW OF LITERATURE

2.1 INTRODUCTION

References to the haematology and blood chemistry of fishes are included in the bibliography of Hawkins and Mawdesley-Thomas (1972). Blaxhall (1972) made a review of selected literature regarding the use of haematological techniques in fresh water fish pathology. Hille (1982) reviewed the literature on the blood chemistry of rainbow trout, Salmo gairdneri (Rich.) based on experimental methods.

Blood studies in fishes are relatively recent in India. From the available literature, it seems that Dhar (1948) was the pioneer in this field in India. His investigations were centred on a preliminary study of the total count, morphology and micrometry of RBC and estimation of haemoglobin content in an air breathing fish, Ophiocephalus punctatus. He was followed by Menon (1952). Pillai (1958) studied some aspects of the blood morphology in Hilsa ilisha. Chandrasekhar (1959) investigated the serum proteins of Indian major carps using agar gel electrophoresis. The blood studies in fishes published from India are mainly based on various aspects of the haematology of fresh water fishes.

In this chapter, selected studies on normal blood values in fishes, ontogeny of blood cells, general studies on the relation of blood to growth and size, sex, season, diseases and pollution are reviewed.

2.2 NORMAL BLOOD VALUES

Fish blood like that of other vertebrates is a suspension of formed elements in the fluid plasma. The cells are of two basic types, erythrocytes

and leucocytes (Moyle and Cech., Jr, 1982). In addition, thrombocytes the equivalent of mammalian platelets are also present.

Fish erythrocytes are nucleated and show a wide range of sizes among different species. Among fresh water fishes in India, the smallest sized RBC was found in Amblypharyngodon mola (7.15x5.82 μm) and the largest sized in Amphipnous cuchia (12.56x10.31 μm) as reported by Joshi (1982a). The RBC number in teleost fish may vary from species to species. It may be totally absent as in Chaenocephalus aeratus (Ruud, 1954) or as low as 0.66 - $0.80 \times 10^6/mm^3$ as in Trematomus borchgrevinki (Tyler, 1960) or as high as $6.48 \times 10^6/mm^3$ as in Acanthurus bahianus (Saunders, 1966).

Haemoglobin is a respiratory pigment that vastly increases the binding power of the blood for oxygen. But Antarctic crocodile ice fishes of the family Channichthyidae carry no haemoglobin in their blood (Holeton, 1970). Haemoglobin content in teleosts can be as low as 3.5 to 4 g/dl as in Trematomus borchgrevinki (Grigg, 1967) and as high as 35.4 g/dl as in Amphipnous cuchia (Bhagat and Banerjee, 1986).

Multiple haemoglobins are found in many of the teleost fishes. Four kinds of haemoglobins are found in rainbow trout blood (Binotti et al., 1971), two in American eel blood (Poluhowich, 1972) and three in gold fish blood (Houston and Cyr, 1974). Different combinations of haemoglobin types are observed as adaptations to different environments or ways of life. Gold fish haemoglobin shows functional differences by their responses to temperature (Houston and Cyr, 1974; Houston and Rupert, 1976). Haemoglobin polymorphism for activity levels has also been hypothesized for species of suckers (Powers, 1972 as cited by Moyle and Cech., Jr, 1982).

The haematocrit reading or the percentage of packed cells in the peripheral blood is one of the most important of all clinical constants. Because of its simplicity and high degrees of reproducibility, this procedure is most useful as a routine indication for detection of anemia (Wells, 1956). It was Wintrobe (1934) who introduced haematocrit to haematology. The microhaematocrit method was described by Guest and Siler in 1934. It requires one drop of blood or 20 to 40 µl. In the ultra-microhaematocrit method of Strumia et al. (1954) only 5 to 10 µl of blood is used.

The packed cell volume in marine fishes vary from 20-51% (Kisch, 1949). In Micropterus salmoides, it was reported as 35.05 ± 9.46% (Clark et al., 1979), in Rita rita, a fresh water cat fish, 31% (Pandey and Pandey, 1977) and in Nemacheilus rupicola a hill stream fish, 45.50 ± 3.20% (Sharma and Joshi, 1984).

Glucose concentration varies widely in fishes. Gray and Hall (1930) found that active fishes have a higher blood sugar level as compared to the less active bottom dwelling ones. Khanna and Mehrotra (1968) found a high blood sugar level (80 mg %) in Clarias batrachus. The normal range of glucose in Channa punctatus is comparatively low (Khanna and Singh, 1971). Cornillon et al. (1979) determined different hexose derivatives in rainbow trout serum.

The concentration of total protein in plasma is well documented. It may change from species to species. It is ranging from 1.68 g% in Cynoscion arenarius to 6.19 g% in Sciaenops ocellata (Sulya et al., 1960). Nearly all observations in rainbow trout fall within the range of 2-6 g/100 ml (Wedemeyer and Chatterton, 1970). For the flounder Platichthys flesus, it was reported as 3.50 ± 0.30 g.100 cm^{-3} (Emmerson and Emmerson (1976). The plasma

phosphoprotein levels in Heteropneustes fossilis male is 10.8 ± 1.9 mg P/l Nath and Sundararaj, 1981). It is 15.1 ± 2.8 mg P/l in Carassius auratus (Tinsley, 1985).

There is only sparse data on normal lipid levels in teleosts. The total cholesterol level in Salmo gairdneri fluctuates distinctly and ranged between 161 to 365 mg/100 ml (Wedemeyer and Chatterton, 1970). Chandra (1986) reported normal cholesterol levels of 22 species of fresh water fishes in India. Serum cholesterol levels in fresh water fishes are found to be higher than marine fishes.

Reports on blood urea levels in fishes are few and far between for teleosts. Field et al. (1943) reported blood urea levels in trout and carp. Blood urea levels in Salmo gairdneri falls within a normal range of 1.9 to 9.6 mg 100 ml^{-} (Wedemeyer and Chatterton, 1970; Giorgetti and Ceschia, 1977).

2.3 ONTOGENY OF BLOOD CELLS

The haemocytoblast is considered to be the totipotential free stem cell that gives rise to all other blood cells (Jakowska, 1956; Boomker, 1980). It is derived from the reticulo-endothelial cells. Jordan and Speidle (1924) states that from reticulo-endothilial cells large lymphocytes are formed which in turn give rise to small lymphocytes. The latter are the precursors of haemoblasts and thrombocytoblasts. Yoffey (1929) suggested that the small round cells found in the spleen of fish give rise to the erythrocytic series. According to Duthie (1939), the small lymphocytes (the small lymphoid haemoblasts) found in haemopoietic organs give rise to the blood lymphocytes,

thrombocytes and erythrocytes. But Catton (1951) considers the large lymphocytes or lymphoid haemoblasts are the precursors of all blood cells. Ellis (1976) didn't refer to a stem cell. According to him erythropoiesis mainly occurs in the kidney of plaice, though the spleen is also active. He has described five types of precursor cells to mature erythrocytes; the proerythroblast, erythroblast, late erythroblast, proerythrocyte and young erythrocyte. Boomker (1980) described five types of cells in the erythrocytic series; the haemocytoblast, erythroblast, polychromatophilic erythrocytes, erythrocytes and erythroplastids.

Eventhough early workers have considered the lymphocyte as the stem cell, its role as a precursor cell is doubtful as it is proved to be an immunocompetent cell. However it remains possible that a sub-population of lymphocytes exist which could be considered analogous to that mammalian subpopulation which is speculated to give rise to macrophages (Ellis, 1977).

The production of lymphocytes in the thymus of fish was first described by Murer (1886) as cited by Ellis (1977). His findings were supported by those of Beard (1894). They believed that the epithelial cells of the thymic rudiment gave rise to the thymocytes and the latter was the precursor to all the body's lymphocytes. Hafter (1952) proposed that thymic lymphocytes formed only part of circulating lymphocyte population. Van Hagen (1932) as quoted by Hafter (1952) and Hill (1935) were of the opinion that stem cells entered the thymic bud in fishes and later developed to form thymic lymphocytes. But the hypothesis of Beard was supported by the findings of Turpen et al. (1973).

The development of thrombocyte is still not clear. Jordan and Speidle (1924), Duthie (1939) and Gardner and Yevich (1969) proposed that the small lymphocytes give rise to thrombocytes. Boomker (1980) suggests small lymphoid haemoblast as the precursor cells to thrombocytes.

Monocytes are considered to be developed from lymphocytes in mammals (Bloom and Fawcett, 1968). But later in 1975 they stated that there was enough proof of monocytes developing from a group of proliferating cells in bone marrow which produce monoblasts. Ellis (1977) opined that monocytes are formed from precursor cells in kidney. Boomker (1980) believes that monocytes develop from lymphocytes. Macrophages are considered to be developed from monocytes (Ellis, 1977; Boomker, 1981a).

The kidney appears to be the major granulopoietic organ in the plaice with spleen of secondary importance. The recognition of the developmental stages of the neutrophil are greatly aided by the use of the PAS technique and the acid phosphatase test (Ellis, 1976). He presented the progranuloblast and granuloblast as the precursor cells for granulocyte in plaice. According to Boomker (1981a), haemocytoblast is the stem cell which gives rise to granulocytes. He postulates four types of precursor cells for granulocytes from granuloblast to mature granulocyte.

The plasma cells are believed to be developed from small lymphoid haemoblasts (Boomker, 1981a).

2.4 BLOOD CONSTITUENTS IN RELATION TO GROWTH AND SIZE

Many of the haematological factors are reported to change in relation to length and weight. The total plasma volume in H. fossilis increases from

lower to higher weight groups (Pandey et al., 1975). Juvenile and adult stages of fish can be differentiated by distinct forms of haemoglobin (Hashimoto and Matsuura 1960; Yamanaka et al., 1967). Koch et al. (1964) in Salmo salar and Wilkins and Iles (1966) in Clupea harengus described a series of patterns of Hb as the fish grow. There is evidence that the electrophoretic patterns of the serum proteins also change to some extent with the growth of the fish (Booke, 1964; Haider 1970b; Schlotfeldt, 1975). In Catla catla, constituents of plasma like amylase, glucose, protein, creatinine and chloride were found to increase and plasma calcium and phosphatase were found to decrease with size (Das, 1965). The plasma cholesterol, triglyceride and glucose content increased with the weight of trout (Haider, 1970a; Shimma and Ikeda, 1978; Leger et al., 1979). In Clarias batrachus, blood urea content was found to increase with an increase in weight (Kumari, 1979).

2.5 INFLUENCE OF SEX AND SEASON

Evidences are present to enumerate the role of sex and season in modifying blood values.

Ezzat et al. (1973) showed a definite seasonal variation in leucocyte counts in Tilapia zilli. Red blood cell fragilities showed seasonal variation in Cyprinus carpio (Fourie and Hattingh, 1976).

Umminger and Mahoney (1972) found a haemoglobin maximum during summer in Salmo gairdneri, but Denton and Yousef (1975) indicated high haemoglobin concentration during winter in rainbow trout. Higher blood volume was found in gravid female H. fossilis during the spawning period (Pandey, 1977).

In immature trout almost all plasma constituents as well as electrophoretic pattern were identical (Haider, 1978; Matsuk and Novikov, 1978; Osborn et al., 1978). Biochemical parameters in blood are observed to change with sex and season. Mature females have higher protein, total lipid and cholesterol levels than males (Snieszko et al., 1966; Haider, 1970b).

Changes in the electrophoretic protein pattern in the course of vitellogenesis and maturity are well documented (Borchard, 1978; Kirsipuu, 1979). Transferrin is a female specific protein with iron-binding activity which participates in egg yolk synthesis (Hille, 1982). In male trout, some globulin fractions altered in course of spermatogenesis (Borchard, 1978).

Sano (1960) observed monthly variations of urea, creatinine and glucose. Schlotfeldt (1975) described variations of total protein content which correlated to environmental temperatures with maximal levels at the end of summer. Lipoglobulin fractions fluctuated with season (Matsuk and Novikov, 1978) along with transferrin, ceruloplasmin and iodurophorine (Perrier et al., 1978). The maximal levels of thyroxine, triiodothyronine, androgen and oestrogen occured in winter with low concentrations in summer (Osborn et al., 1978; Scott et al., 1980a,b).

2.6 EFFECT OF DISEASES ON BLOOD CONSTITUENTS

The effect of diseases on blood parameters is relatively a new field and still in a developing state. Bacterial diseases cause extensive damage to fish culture. These infections generally cause changes in the peripheral blood picture of fishes. Reduction of red cell numbers, haemoglobin and

haematocrit has been found in vibriosis of marine fish (Anderson and Conroy, 1970) and juvenile chinook salmon (Cardwell and Smith, 1971). The same changes in haematology was found in furunculosis (Klontz et al., 1966) and in Ulcerative Dermal Necrosis (UDN) in trout (Carbery, 1970). The appearance of macrophages in the blood is considered to be a feature of bacterial infection of rainbow trout (Weinreb, 1958). Aeromonas salmonicida causing ulcerative disease bring about leucopenia in carp (Everberg et al., 1986). Reduction in plasma or serum protein are recorded in UDN in trout (Carbery, 1970), vibrio disease of chinook salmon (Cardwell and Smith, 1971), infectious dropsy in carp (Fleming, 1958), bacterial kidney disease of brook trout (Hunn, 1964) and fungus infection in sea herring (Sindermann and Mairs, 1958). Amend and Smith (1975) observed haematological and blood chemical changes associated with infectious hematopoietic necrosis virus disease in rainbow trout. In rainbow trout infected with bacteria, significant decrease in RBC count, packed cell volume, total plasma protein, blood glucose and electrolytes (were found (Barham et al., 1980).

Trypanosomes are known to affect the haematological and biochemical parameters in fish blood during the course of its infection (Tandon and Joshi, 1974; Joshi and Dabral, 1981). Blood urea and serum acid phosphatase levels in infected fresh water fishes were found to be lowered (Tandon and Chandra, 1978a,b). In Wallago attu, the parasitemia caused lowering of RBC count, haemoglobin concentration, acetyl cholinesterase, acid phosphatase, alkaline phosphatase, ascorbic acid and blood urea. But it elevated the levels of LDH, 5'-Nucleotidase and aldolase (Tandon, 1986). Haemopoietic organs were

found to be affected by trypanosoma and trypanoplasma infections in gold fish (Dykova and Lom, 1979). The experimental infection of rainbow trout with the protozoan, Cryptobia salmositica Katz 1951 caused progressive anaemia and depressed plasma T3, T4, protein and glucose concentrations (Laidley et al., 1988). Thomas and Woo (1988) conducted in vitro and in vivo study on the mechanism of anaemia in Cryptobia infected rainbow trout.

Helminth infections affect the normal blood picture of fishes. They are known to affect the blood cell picture of teleosts (Hoole and Arme, 1982; Elarifi, 1982). Cestode infections caused anaemia in Trichiurus lepturus and Diodon hystrix (Radhakrishnan et al., 1984a,b). Heteropneustes fossilis infested with metacercarie of Diplostomulum species showed reductions in all haematological parameters except leucocyte count and ESR which increased (Murad and Mustafa, 1988).

2.7 EFFECTS OF POLLUTION

Fish toxicity studies in recent years have acquired momentum in relation to increased fish cultural practices. Copper is known to affect the blood picture of brook trout after long term and short term exposure to copper (McKim et al., 1970). Heavy metal cadmium affect the blood oxygen carrying capacity (Johansson - Sjobeck and Larsson, 1978), cause lessions in haemato-poietic sites (Stromberg et al., 1983), and impairment of erythropoietic (McCarthy et al., 1978) and leucopoietic (Murad and Houston, 1988) capacity. Williams and Wootten (1981) reported some effects of therapeutic levels of formalin and copper on blood parameters in rainbow trout.

Investigations have proved that chlorinated hydrocarbons are highly toxic to fish. Changes in serum proteins and free aminoacids were reported in Channa punctatus after exposure to malathion, endrin and dieldrin (Shakoori et al., 1976). In the same fish, DDT and dieldrin were found to cause variations in the normal blood picture (Lone and Javaid, 1976). Effect of aldrin on serum constituents in C. batrachus was reported by Bano (1982). RBC, Hb and PCV in Sarotherodon mossambicus increased when the fish was exposed to 0.1, 0.2 and 0.3 ppm of aldrin for 30 days (Ghosh and Chatterjee, 1986).

Sub lethal concentrations of formalin induced lowering of erythrocyte count and increase in haemoglobin and haematocrit. The sum total of the effect was macrocytosis and hyperchromia (Beevi and Radhakrishnan, 1987). Mahua oil cake and tamarind seed husks, the organic manure-cum-fish toxicants are proved to be haemotoxicants. These were found to cause haemolysis in fresh water fishes (Misra et al., 1986; Chaudhuri et al., 1986a,b).

From the foregoing account, it is evident that fish blood is affected by physical, chemical and biological factors. Normal values in fishes may vary from species to species. It may change according to growth and size, sex and season. Diseases and pollutants affect the haemostatic mechanisms to a large extent. Ontogeny of blood cells is interesting from the academic as well as immunological point of view. So it is of importance to establish normal blood values for all available culturable fishes and it will be interesting to study the deviation from normal blood values caused by various eco-physiological conditions.

CHAPTER - III

MATERIALS AND METHODS

Materials and methods that were used in more than one chapter are given here. All others are given in individual chapters.

3.1 COLLECTION OF FISHES AND TRANSPORTATION

Heteropneustes fossilis (Bloch) and Clarias batrachus (Linn) are fresh water cat fishes. In Kerala, India, they are found in ponds and rivers. During the rainy season, they are captured from paddy fields too. In the present investigation for studying normal variation in haematological components, fishes were collected from streams and ponds in Mavelikara, Alleppey District, Kerala. For all other studies, the collection sites were various ponds and rivers in Panangadu, Edappally and Irimpanam in Ernakulam District, Kerala. Mortality resulting from transportation stress was found to be very high in H. fossilis, if these fishes were transported to the laboratory on the day of collection itself. Sometime the mortality was as high as 98%. The fishes started to die from the third day of transportation up to the seventh day. After one week of acclimatization they rarely died. So the fishes were acclimatized in 500 litre fibre glass tanks near the collection site itself for one week before transportation. As a result, the mortality rate was reduced nearly to zero even after transportation. C. batrachus was found to be sturdier than H. fossilis. They rarely died due to transportation stress.

Fishes were transported in 30 litre plastic buckets. For every 100gm of fish, 4 litres of water space was provided. During the time of transportation, the buckets were covered with provision for air circulation. As

H. fossilis and C. batrachus are air breathers, no aeration was provided during transportation.

3.2 MAINTENANCE OF FISH IN THE LABORATORY

3.2.1 Acclimatization

Fishes were maintained in the laboratory in large fibre glass tanks containing 250 litres to 750 litres of aged tap water. For every 100gm weight of fish, a minimum of 40 litres of water was provided. As these cat fishes are benthic in nature, overcrowding was avoided by keeping small numbers of fishes in each tank. Water was changed on alternate days. Tanks were covered with fish netting to prevent the escape of fishes. The fishes for reproduction studies were kept in tanks placed in open space. They were provided with natural photoperiod.

Fishes for studies on reproduction were kept in the laboratory for seven to ten days only. For all other studies, they were acclimatized for 3 to 4 weeks.

3.2.2 Food and feeding regime

H. fossilis and C. batrachus were provided with a formulated diet and natural food. The artificial food contained wheat powder, dried fish, prawn powder, artemia egg, Vitamin B complex and Vitamin A. To rectify the deficiencies caused by the artificial diet, natural foods like earthworm and beef liver were provided thrice a week. The fishes were fed ad libitum once a day. After feeding, the remaining food particles were immediately removed from the tank bottom.

3.2.3 Diseases and treatment

Common diseases found among these cat fishes were fungal attack, ulceration and black spot disease. Methylene blue was found to be very effective for curing all these diseases.

3.3 IMMOBILIZATION OF FISH AND BLOOD WITHDRAWAL

Feeding was stopped 24 hours prior to the collection of blood. Fish was collected from the tank using a small dip net causing the least amount of disturbance as it is proved that handling stress will alter several of the blood parameters (Chavin and Young, 1970; Wedemeyer, 1972; Robertson et al., 1987). The fishes were immobilized with a sharp blow on the head. No anaesthelizing agent was used because they might affect the results (Houston et al., 1971; Soivio et al., 1977; Smit et al., 1979a,b; Ferreira et al., 1981). For the same reason, sampling time was kept to the minimum. The fish was dried using a towel and the caudal vein was exposed by severing the candal peduncle. Blood was collected in dry vials containing heparin, the anticoagulant (50 IU/ml of blood). The vials were rotated well to ensure the even distribution of the anticoagulant.

3.4 DETERMINATION OF MATURITY

The maturity stages were classified based on the International Council for the Exploration of the Sea ICES Scale (Lovern and Wood, 1937) with modifications. The whole reproductive period was divided into six arbitrary stages based on the morphological appearance of the gonads. The colour and size of the gonads were taken into account for classification.

3.4.1 Gonads in H. fossilis

3.4.1.1 Testis

Stage I Immature	-	Testes minute, tube like, colourless, translucent. Less than 1/3rd of the body cavity.
Stage II Developing	-	Testes elongated, slightly lobed, translucent; a creamy white colour starts to appear at the outer ends. Reaches 2/3rd of the body cavity.
Stage III Maturing	-	Testes highly coiled and lobed, white, opaque. Reach 3/4th of the body cavity.
Stage IV Mature	-	Tightly coiled and convoluted lobules, creamy white in colour.
Stage V Ripe or spawning	-	Highly coiled and convoluted tubules, creamy white colour; if a cut is made in testes, milt freely oozes out.
Stage VI Spent	-	Testes shrunken and blood shot, lobules yellowish.

3.4.1.2 Ovary

Stage I Immature	-	Ovary very small, flesh coloured, translucent.
Stage II Developing	-	Ovaries about 1/3rd length of body cavity, reddish brown in colour.

Stage III Maturing	-	1/2 or 2/3 length of body cavity, greenish brown in colour; eggs visible to the naked eye through the thin tunica.
Stage IV Mature	-	Ovaries very swollen; thin tunica bursts at slight pressure.
Stage V Ripe or Spawning	-	Eggs extruded by slight pressure on the flanks of fish, eggs greenish brown in colour.
Stage VI Spent	-	Ovaries wrinkled and flaccid, shrunken to 1/2 length of body cavity, reddish in colour.

3.4.2 Gonads in C. batrachus

3.4.2.1 Testis

Stage I Immature	-	Testes appear as two thin thread like structures, united at the posterior end. They are semitransparent, colourless and reaches 1/3rd of the length of the body cavity.
Stage II Developing	-	Each testis at this stage gets flattened; appears opaque with smooth surface; light flesh coloured. Occupies 1/3rd of the body cavity.
Stage III Maturing	-	Creamy white, turgid, opaque testis with smooth surface. Occupies 2/3rd of the body cavity.

Stage IV Mature	-	Turgid, reaches 3/4th of the body cavity.
Stage V Ripe or Spawning	-	Size and shape similar to previous stage, but more turgid; if a small part is cut and pressed, milt oozes out freely.
Stage VI Spent	-	Testes shrunken, hollow and flaccid.

3.4.2.2 Ovary

Stage I Immature	-	Paired, lobes small and of equal length; flesh coloured; translucent; reaches less than 1/3rd of the body cavity.
Stage II Developing	-	Reddish in colour; reaches 1/3rd of the body cavity.
Stage III Maturing	-	1/2 or 2/3rd length of the body cavity; reddish brown in colour; eggs visible to the naked eye; ovaries swollen.
Stage IV Mature	-	Ovaries very swollen; fills the body cavity; ovary yellowsih brown in colour.
Stage V Ripe or Spawning	-	Eggs extruded by slight pressure on the flanks of fish; eggs yellowish brown in colour.
Stage VI Spent	-	Ovary appears as flaccid bags with only a few unspawned and immature eggs.

In both species, seminal vesicles were present.

3.5 DETERMINATION OF GONADOSOMATIC INDEX (GSI)

The gonad was removed from the body cavity of fish and weighed to the nearest milligram in a single pan balance (ANAMED). From the total weight of fish and the gonad weight, the GSI was calculated according to the method of June (1953) and Yuen (1955).

$$\text{Gonadosomatic Index (GSI)} = \frac{\text{Gonad weight}}{\text{Total fish weight}} \times 100$$

It is measured as the gram weight of fish per 100gm body weight.

3.6 HAEMATOLOGICAL METHODS

Standard techniques of haematology (Hesser, 1960; Blaxhall and Daisley, 1973) were employed for the haematological determinations. Unless otherwise mentioned, all measurements were done in duplicate.

3.6.1 Preparation of blood smear and staining

A small drop of blood was placed on a slide about 1 cm from one end. Another slide was placed at an angle of about 45° to the first slide and moved back to make contact with the drop of blood. When the blood had spread evenly along the line of contact, the spreader was pushed rapidly along the slide. The smear was allowed to dry in the air.

For the morphological and morphometric examination of blood cells, the slides were stained using Giemsa stain (Merk). For this, the slides should be first fixed in absolute methanol for 3 to 5 minutes and allowed to dry. From each fish, a minimum of 3 slides were made. For staining, to 96 ml

of distilled water, 2 ml of Sorensen's buffer having a pH of 6.8 and 2 ml of Giemsa stain were added. It was well mixed and the fixed smears were stained with it for about 30 minutes.

3.6.2 Counting of Red Blood Corpuscles (RBC)

The haemocytometer with improved Neubauer ruling was used for counting RBC. Hayem's solution (Glaxo) was the diluent. The pipette with red glass bead was used for charging the counting chamber. All countings were done in triplicate.

3.6.3 Estimation of Haemoglobin (Hb) content

Haemoglobin was determined by the Cyanmethemoglobin method (Dacie and Lewis, 1968). In this method, all types of Hb will be converted first to methemoglobin and then to cyanmethemoglobin which can be measured colorimetrically.

0.02 ml of blood was pipetted into 5 ml of Drabkin's reagent (commercial name Aculute by Glaxo). It was shaken well and allowed to stand for 10 minutes. Sometimes a jelly like substance was seen in the solution formed by the ruptured cell walls of RBCs. It can be removed by centrifugation. Optical density was measured at 540 μ in a Bosch and Lomb spectrophotometer against a reagent blank. Using a commercial cyanmethemoglobin standard a standard graph was prepared from which, the values of Hb can be read directly.

3.6.4 Estimation of Packed Cell Volume (PCV)

PCV was determined using the microhaematocrit method (Snieszko, 1960). Heparinized blood was collected in unheparinized even bored capillaries

and sealed with modelling clay. They were centrifuged in a microhaematocrit centrifuge at 11,500 rpm for 5 minutes. PCV was measured directly on a microhaematrocrit reader associated with the centrifuge.

3.6.5 Calculation of RBC constants

Based on the results of the tests which measure total RBC, Hb and PCV, several calculations have been derived which give quantitative information about the RBC. These values are called RBC Constants. Meticulous care was taken to get accurate values for the three basic tests, otherwise these constants would become meaningless. The following constants were computed using respective formula (Lamberg and Rothstein, 1978).

3.6.5.1 Mean Corpuscular Volume (MCV)

MCV, the Mean Corpuscular Volume is the volume of the average cell or the average cell volume of all the red cells.

$$\text{MCV} \qquad \frac{\text{(PCV (\%)}}{\text{RBC in million}} \times 10 \text{ expressed in } \mu^3$$

3.6.5.2 Mean Corpuscular Haemoglobin (MCH)

MCH, the Mean Corpuscular Haemoglobin is the amount of haemoglobin in the average red cell or the average amount of Hb per cell in all the red cells.

$$\text{MCH} = \frac{\text{Hb (g/dl)}}{\text{RBC in million}} \times 10 \text{ expressed in pg}$$

3.6.5.3 Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC, the Mean Corpuscular Haemoglobin Concentration is that portion

of the average RBC containing haemoglobin or the concentration in the average cell.

$$\text{MCHC} = \frac{\text{Hb (g/dl)}}{\text{PCV (\%)}} \times 100 \text{ expressed in \%}$$

3.6.6 Morphometry of blood cells

The erythrocytes and leucocytes were measured by the calibration of an ocular meter with stage micrometer. The length and width of the cells were measured and the Nucleus/Cytoplasmic (N/C) ratio) was calculated.

$$\text{N/C ratio} = \frac{\text{Length x width of nucleus}}{\text{Length x width of cytoplasm}}$$

CHAPTER - IV

NORMAL VARIATION IN HAEMATOLOGICAL PARAMETERS IN HEALTHY *HETEROPNEUSTES FOSSILIS* (BLOCH) AND *CLARIAS BATRACHUS* (LINN)

4.1 INTRODUCTION

The close contact of fish with its medium makes it vulnerable and prey to many agents causing diseases. Pollution is another threat faced by fish. Scientists have stressed the need for the establishment of normal haematological values in fish for the diagnosis of diseases (Snieszko, 1960; Hesser, 1960; Larsen and Snieszko, 1961; Summerfelt, 1967; Blaxhall, 1972) and for studying the effects of pollution (Mawdesley-Thomas, 1971).

'Normal values' is a term which is to be understood clearly. The haematological values of a healthy fish at any time of the year should be normal values. Such values are known to be influenced by age, sex, temperature, breeding period, season and varying eco-physiological conditions. So when defining normal values, all these factors also should be taken into account.

H. fossilis and *C. batrachus* selected for the present investigation were collected from the same location to avoid population differences. They were reared in the laboratory for more than two weeks to avoid stress and for weight related studies, the fishes were collected and utilized in the same season. Diseased fishes were not used for haematological determinations. Sexes and gonadial maturation were always taken into account.

4.2 REVIEW OF LITERATURE

Many investigations have been done in fish blood to establish 'normal'

values (Hattingh, 1973; Pandey et al., 1976; Siddiqui and Naseem, 1979; Clark et al., 1979; Sharma and Joshi, 1984).

Blood values are reported to vary from species to species according to the environmental conditions in which the fish live (Bhatt and Singh, 1981; Sharma and Joshi, 1984) and the activity of fish (Joshi, 1980). Haws and Goodnight (1961) postulated that the activity of the animal and the size of the RBC are closely related, i.e. the more active species have smaller erythrocytes. But according to Srivastava (1968a), it is futile to attempt to trace relationship between the size of RBCs and the activity of fishes. Cameron (1970) has reported that salinity changes have no significant effect on the size of erythrocytes of striped mullet or pin fish. Srivastava and Griffith (1974) observed that species of fundulus occuring in brackish or sea water have relatively small cell areas where as fresh water fishes have larger cells. So further studies are needed before a generalization can be made on the size of the RBC in relation to its environment.

Dube and Datta Munshi (1973) observed an increase in erythrocyte measurements from small to larger fish. The findings of Srivastava (1968a) are in accordance with this. He found the largest sized erythrocytes in Amphipnous which measured 45.0 - 68.5 μm and the smallest erythrocytes in Ophiocephalus which measured 14.0 - 17.0 μm. On the contrary, Pandey et al. (1976) observed a decreasing trend in cell surface area from lower to higher weight groups in H. fossilis.

Younger stages of RBCs in blood was reported by various authors. Boomker (1980) identified 3 stages of polychromatophilic erythrocytes and

erythroblast in the blood of Clarias gariepinus. Joshi (1987) identified three types of erythroblasts in the blood of various fresh water teleosts. As the nomenclature of developing series of erythrocytes are still not clearly explained, it is not known whether Boomker and Joshi are describing the same types of cells. Joshi (1987) also reported the presence of microcytes, macrocytes, crenated red cells and enucleated erythrocytes in the blood of fresh water teleosts.

A size and weight related correlation to haematological factors has been observed in teleosts. Erythrocyte number and haemoglobin concentration in female H. fossilis increased with the body weight (Pandey et al., 1976). Preston (1960), Haws and Goodnight (1961), Dube and Datta Munshi (1973) got similar results in plaice, channel cat fish and Anabas respectively. In Rita rita, Pandey and Pandey (1977) observed an increase in RBC number, Hb concentration and ESR with an increase in weight. But PCV was found to decrease in this fish with an increase in weight. Smith (1977) suggested that small fish have low blood oxygen solubility in spite of a high weight specific oxygen consumption.

Sex is also reported to affect haematological values (Radzinskaya, 1966; Mulcahy, 1970). Male fishes are found to have higher number of RBC and Hb concentration than females in Rita rita (Pandey and Pandey, 1977). Chaudhuri et al. (1986c) made similar observations in Sarotherodon mossambicus. Pickering (1986) found a consistent elevation in the number of circulating erythrocytes in sexually mature male fish. But Clark et al. (1979) found no significant difference between any haematological parameters in male and female Micropterus salmoides.

Seasonal variations affect various blood parameters in many a teleost. It was reported to occur in hill stream fishes like Schizothorax richardsonii, S. plagiostomus and Pseudecheneis sulcatus (Bhatt and Singh, 1986). Seasonal variations in haematological parameters were apparent in Labeo rohita. It was generally high during summer and monsoon months, but low during winter (Siddiqui and Naseem, 1979). Bhagat and Banerjee (1986) found seasonal variation in RBC count, Hb content, PCV and ESR in Amphipnous cuchia.

Asphyxiation causes drastic changes in haematological parameters (Sharma and Joshi, 1985). Asphyxiation increases the haematocrit value of fish blood with varying modes of fluctuations as has been reported by Soivio et al. (1974 a, b). It is suggested that the change in blood parameters might be either due to the release of more erythrocytes into the blood stream or due to a fall in plasma volume of the blood.

Bouck and Ball (1966) have reported on the influence of capture methods on normal blood characteristics in fish and showed that Hb concentrations, erythrocyte sizes and plasma protein varied according to the method of capture.

4.3 MATERIALS AND METHODS

4.3.1 Collection, transportation and maintenance of fishes

Sixty five H. fossilis in the weight group 40 to 60 grams (33 males and 32 females) and C. batrachus in the weight group 100 to 120 grams (20 males and 20 females) were collected whenever available during the year. 40 H. fossilis ranging in weight from 23 to 140 grams (20 males and 20 females) and 40 C. batrachus (20 males of weight 55 to 242 grams and 20

females of weight 55 to 246 grams) were collected during April when the fishes were available in large numbers unlike other periods of the year. The method of collection, transportation and maintenance were as described in 3.1 and 3.2.

4.3.2 Sampling and determination of haematological parameters

The sampling of blood was done as given in 3.3. The blood smears were prepared, fixed and stained as in 3.6.1. The whole blood was analysed for RBC number as in 3.6.2. Hb concentration and PCV were estimated as in 3.6.3 and 3.6.4. The erythrocyte constants were calculated as reported in 3.6.5.

4.3.3 Computation and presentation of data

All values are presented as mean $\pm$ standard deviation. The determination of linear relationships were done on the actual values eventhough in table the values are given for different weight groups for easy presentation. The correlation analysis were performed between weight and RBC, weight and Hb and weight and PCV by the Pearson's formula

$$r = \frac{\Sigma(x-\bar{x})\ (y-\bar{y})}{n\ \sigma x\ \sigma y}$$

where

$$\bar{x} = \frac{\Sigma x}{n}$$

$$\bar{y} = \frac{\Sigma y}{n}$$

$$\sigma x = \sqrt{\frac{\Sigma(x-\bar{x})^2}{n}}$$

$$\sigma y = \sqrt{\frac{\Sigma(y-\bar{y})^2}{n}}$$

n = number of pairs of observations

Data are presented in the form of tables, histograms and line graphs. Linear relationships were calculated by simple regression analysis with RBC number, Hb concentration and PCV as a function of weight.

4.4 RESULTS

4.4.1 Morphology of RBC

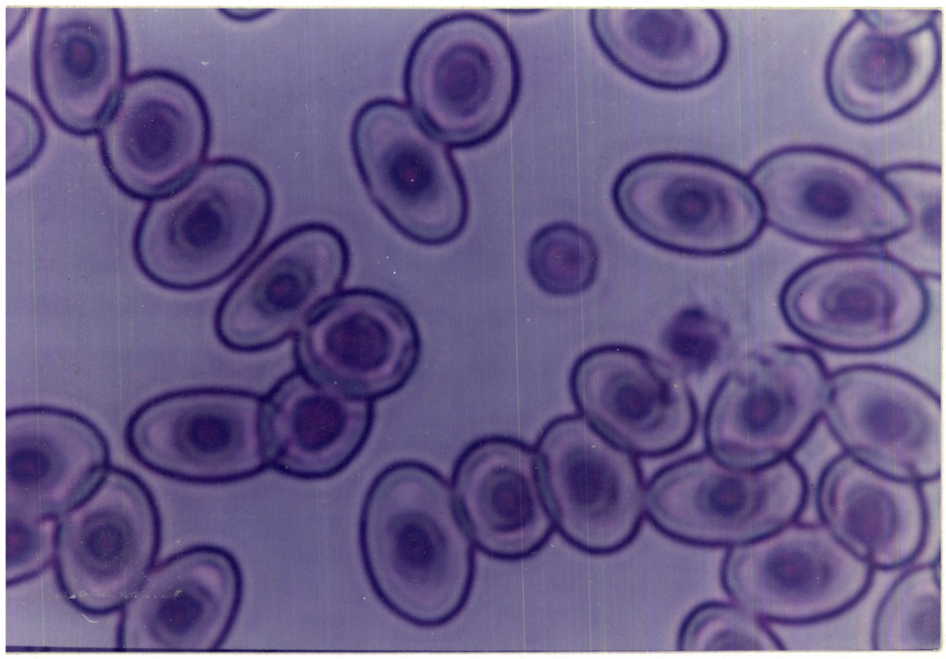
4.4.1.1 Heteropneustes fossilis (Table 1 ; Figure 1a)

Fish RBCs are nucleated. In H. fossilis most of the RBC's were elliptical in shap. Oblong cells were not uncommon. In this fish, circular mature RBC's were found in the stressed condition. But immature erythrocytes were circular in shape and they sometimes appeared in the blood smear. They were usually polychromatophilic cells smaller in size than mature erythrocytes (length 8.82 μm, width 8.82 μm). Enucleated RBCs known as erythroplastids occured at times especially during the breeding season. They resembled mature erythrocytes except for their enucleated condition and smaller size (length 8.24 μm; width 7.84 μm). The mature RBC varied in length from 9.80 to 12.74 μm and in width from 4.90 to 9.80 μm.

In Giemsa stained blood smears, the cytoplasm of mature erythrocytes took light pink and the nucleus a dark purplish violet to purplish blue colour. Cytoplasm was homogeneous in appearance. Small chromatin clumps were uniformly distributed in the nucleus. No nucleolus was observed. The position of the nucleus was central.

4.4.1.2 Clarias batrachus (Table 2 ; Figure 1b)

In C. batrachus most of the RBCs were circular in appearance. Oval cells were also found. Immature cells were sometimes seen in the blood



ig. 1a. RBC in H. fossilis. Note the polychromatophilic erythrocytes with large nucleus and spherical outline.

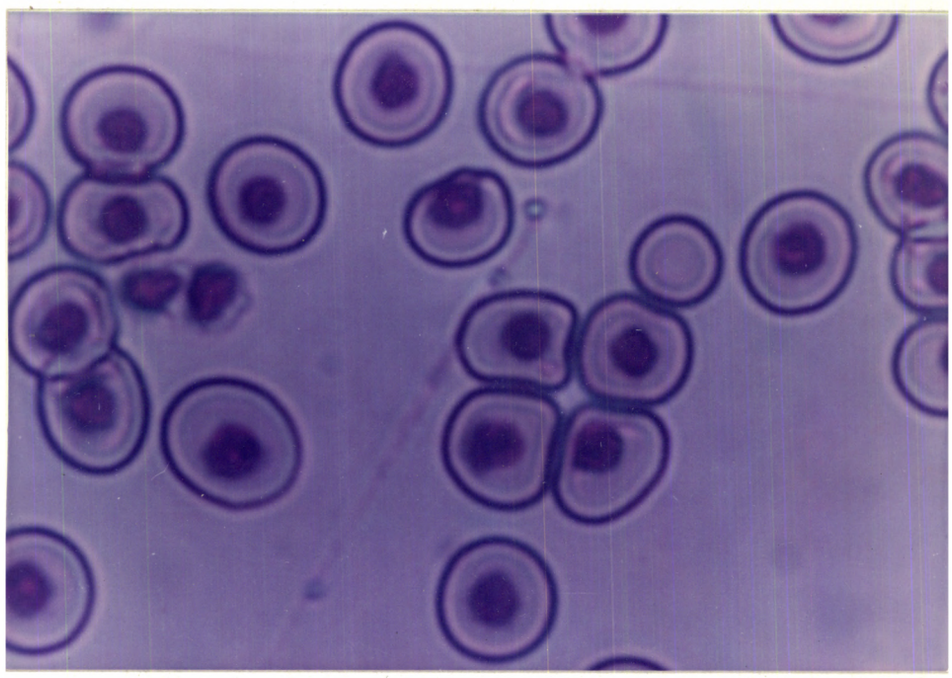


Fig. 1b. RBC in C. batrachus. Note the eccentric nucle in erythrocytes. The cell without a nucleus is erythroplastid.

Table 1. Morphometry of the erythrocytes of Heteropneustes fossilis

Erythrocyte measurements	Mature cell (200 nos.)	Polychromatophilic cell (100 nos.)	Erythroplastid (20 nos.)
Cytoplasm			
Length (L) μm			
Mean ± SD	11.66 ± 1.08	8.82 ± 0.31	8.24 ± 0.45
Range	9.80 - 12.74	8.53 - 8.9	7.92 - 8.50
Width (W) μm			
Mean ± SD	7.84 ± 1.53	8.82 ± 0.19	7.84 ± 0.32
Range	4.90 - 9.80	8.50 - 8.81	7.47 - 7.90
L x W	91.41	77.79	64.80
Nucleus			
Length (L) μm			
Mean ± SD	3.70 ± 0.43	4.45 ± 0.25	-
Range	2.94 - 3.92	4.25 - 4.62	-
Width (W) μm			
Mean ± SD	2.26 ± 0.42	4.40 ± 0.28	-
Range	1.96 - 2.94	4.19 - 4.51	-
L x W	8.36	19.58	-
N/C Ratio (L x W of nucleus/ L x W of cytoplasm)	0.09	0.25	-

Table 2. Morphometry of the erythrocytes of Clarias batrachus

Erythrocyte measurements	Mature cell (200 nos.)	Polychromatophilic cell (100 nos.)	Erythroplastid (20 nos.)
Cytoplasm			
Length (L) µm			
Mean ± SD	10.17 ± 0.92	9.14 ± 0.51	8.33 ± 0.56
Range	8.65 - 11.54	8.65 - 9.63	7.68 - 8.66
Width (W) µm			
Mean ± SD	9.15 ± 0.67	9.14 ± 0.40	7.05 ± 0.56
Range	8.65 - 10.58	8.65 - 9.52	6.73 - 7.69
L x W	93.06	83.55	58.73
Nucleus			
Length (L) µm			
Mean ± SD	3.85 ± 0.15	4.95 ± 0.74	-
Range	3.76 - 3.95	4.82 - 5.77	-
Width (W) µm			
Mean ± SD	3.56 ± 0.46	4.65 ± 0.81	-
Range	2.88 - 3.85	3.85 - 5.52	-
L x W	13.71	22.41	-
N/C Ratio (L x W of nucleus/ L x W of cytoplasm)	0.15	0.27	-

smear. They were identified by their N/C ratio and polychromatophilia. Erythroplastids occured in C. batrachus too (length 8.33 µm; width 7.05 µm). They resembled the erythroplastids of H. fossilis. The size of the mature RBC varied in length from 8.65 to 11.54 µm and in width from 8.65 to 10.58 µm.

In Giemsa stained smears of the blood, the cytoplasm of the RBC appeared light pink to light pinkish blue in colour with a homogeneous appearance. The nucleus was purplish blue. In most of the cells, the nucleus was slightly eccentric in position.

In addition to the above described cells, at times, especially during the breeding period microcytes and macrocytes were found in the blood films of both species. Microcytes are smaller than normal RBC and macrocytes are larger than the latter.

4.4.2 RBC Count

4.4.2.1 Variation in Normal values

4.4.2.1.1 Heteropneustes fossilis (Table 3)

In H. fossilis erythrocyte counts were determined for 65 fishes ranging in weight from 40 to 60 grams (33 males and 32 females) all over the year for determining the normal value for a particular size group. RBC count varied from 228.00 to $450.00 \times 10^4/mm^3$ in the male and 222.00 to $493.00 \times 10^4/mm^3$ in the female. The mean values were found to be $325.82 \pm 62.8 \times 10^4/mm^3$ for the male and $324.06 \pm 73.86 \times 10^4/mm^3$ for the female. In both sexes the lowest number of erythrocytes were found when the sexes were immature

Table 3. Normal haematological values in Heteropneustes fossilis

	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Male (N.33)						
Mean	325.82	10.60	39.66	125.97	32.54	26.35
± SD	62.80	2.27	4.17	11.80	2.63	3.54
Range	228.00-450.00	7.20-14.50	32.00-47.51	106.33-146.33	27.60-37.27	21.53-33.41
Female (N.32)						
Mean	324.06	10.80	39.89	125.26	33.17	26.50
± SD	73.86	2.69	3.52	14.44	2.56	3.83
Range	222.00-493.00	7.70-16.50	32.50-53.33	99.18-146.40	28.21-37.40	20.70-33.84

Table 4. Normal haematological values in Clarias batrachus

	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Male (N.20)						
Mean	326.25	11.07	42.47	131.89	34.10	26.19
± SD	52.65	2.20	5.92	6.15	2.33	2.40
Range	235.00-402.00	7.00-14.35	32.00-50.00	122.78-141.67	29.79-36.67	21.81-29.20
Female (N.20)						
Mean	329.50	11.06	43.12	132.08	33.32	25.55
± SD	66.18	2.58	7.40	9.81	2.40	2.89
Range	217.00-432.00	6.20-15.10	30.00-54.79	120.93-154.80	28.57-36.67	21.06-30.16

and the temperature was low. The highest values were found while the gonads were maturing when the temperature was the highest in summer months. In the immature fish, sexual differences in erythrocyte number was not found. But during the breeding season at some maturity stages RBC count was observed to vary between male and female.

4.4.2.1.2 Clarias batrachus (Table 4)

In C. batrachus ranging in weight from 100 to 120 grams (20 males and 20 females RBC) number varied from 235.00 to 402.00x10^4/mm^3 in the male and 217.00 to 432.00x10^4/mm^3 in the females. The mean was 326.25 $\pm$ 52.65x10^4/mm^3 in the male and 329.50 $\pm$ 66.18x10^4/mm^3 in the female. Here, too the sexual differences were observed only during the breeding season. The highest number of erythrocytes was found during the maturing period and the lowest during the post spawning period.

4.4.2.2 RBC count in relation to weight

4.4.2.2.1 Heteropneustes fossilis (Table 5 and 6 ; Figures 2a and 2b)

For this, erythrocyte numbers were determined for 40 H. fossilis ranging in weight from 23 to 140 grams (20 males and 20 females). Generally with an increase in size group, an increase in RBC count was found irrespective of sex. In the male, RBC number varied from 269.00 to 381.00x10^4/mm^3. Mean was found to be 307.40 $\pm$ 26.54x10^4/mm^3. In the female H. fossilis, the range was 260.00 to 374.00x10^4/mm^3. The mean count was 310.35 $\pm$ 26.15x 10^4/mm^3. Only small differences were found between the mean values of males and females.

Table 5. Body weight groups and corresponding haematological values in male Heteropneustes fossilis (Number of values 5 for each mean)

	Body weight gms	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Mean	32.9	279.20	8.64	34.86	124.86	29.72	24.79
± SD	8.7	7.16	0.77	1.44	4.42	2.86	1.70
Range	23.0-43.5	269.00-286.00	8.10-9.70	33.30-36.59	23.79-127.94	27.37-34.63	23.28-27.14
Mean	63.6	304.00	10.56	36.86	121.25	34.75	28.50
± SD	6.1	4.24	0.44	0.60	1.23	1.34	1.07
Range	55.0-72.0	298.00-308.00	10.09-11.24	35.90-37.50	119.48-122.34	33.72-36.49	27.34-29.97
Mean	92.7	312.20	11.65	38.88	122.03	37.33	30.58
± SD	8.7	18.59	0.97	3.02	3.21	2.25	1.68
Range	83.5-103.5	305.00-342.00	11.07-12.80	36.42-42.75	118.63-125.50	33.68-39.87	28.34-32.69
Mean	120.5	335.00	13.41	39.08	117.07	40.32	34.40
± SD	11.8	32.40	1.11	2.20	6.08	4.29	3.28
Range	111.0-140.0	325.00-381.00	11.80-14.68	35.50-40.90	107.35-123.08	34.93-44.64	29.50-36.86

Table 6. Body weight groups and corresponding haematological values in female Heteropneustes fossilis (Number of values 5 for each mean)

	Body weight gms	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Mean	30.4	276.60	8.48	35.36	127.86	30.71	24.00
± SD	6.4	10.09	0.83	1.39	3.73	3.22	2.14
Range	23.0-38.8	260.00-286.00	7.80-9.70	33.50-36.78	121.82-131.92	28.25-34.54	22.31-26.37
Mean	66.0	310.80	10.82	38.52	123.99	34.82	28.10
± SD	8.4	5.59	0.64	1.57	5.80	1.80	1.41
Range	84.0-107.0	306.00-320.00	10.09-11.49	36.71-41.00	119.19-133.99	32.76-36.08	26.93-30.00
Mean	95.7	313.00	11.84	38.00	121.44	37.82	31.22
± SD	8.2	7.42	1.68	0.89	3.19	3.38	4.90
Range	84.0-107.0	305.00-324.00	10.06-13.59	36.70-39.10	119.94-126.95	32.95-43.01	25.96-36.27
Mean	126.5	341.00	13.74	39.81	116.91	40.40	34.52
± SD	11.1	24.98	0.67	2.03	2.85	2.68	1.70
Range	113.2-140.0	316.00-374.00	12.59-14.22	37.50-42.50	113.97-119.47	37.17-44.58	32.71-37.21

Table 7. Body weight groups and corresponding haematological values in male *Clarias batrachus* (Number of values 5 for each mean)

	Body weight gms	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Mean	69.0	303.40	9.54	39.58	130.11	31.44	24.23
± SD	15.1	17.59	0.95	1.94	1.42	2.38	2.00
Range	51.0-87.0	282.00-331.00	8.42-10.60	37.30-42.60	128.70-132.27	27.97-34.63	21.42-26.92
Mean	126.9	313.20	10.65	39.81	127.13	33.98	26.56
± SD	13.8	5.97	0.56	0.73	1.96	1.20	0.93
Range	115.0-148.3	306.00-320.00	9.81-11.20	38.75-40.70	125.40-129.87	32.05-35.00	25.32-27.59
Mean	175.8	335.60	11.94	41.60	123.45	35.56	28.82
± SD	14.6	15.58	0.59	1.05	2.82	0.52	0.85
Range	156.0-197.0	315.00-349.00	11.20-12.70	40.28-43.00	120.92-127.87	35.05-36.39	27.81-30.09
Mean	227.0	385.60	11.32	43.95	112.91	28.91	25.77
± SD	16.7	16.86	0.68	2.00	2.87	1.16	1.38
Range	204.0-243.0	368.00-405.00	10.41-12.30	42.50-46.67	109.51-116.68	27.88-30.59	23.89-27.46

Table 8. Body weight groups and corresponding haematological values in female Clarias batrachus (Number of values 5 for each mean)

	Body weight gms	RBC x $10^4/mm^3$	Hb g/dl	PCV %	MCV μ^3	MCH pg	MCHC %
Mean	70.4	301.20	8.90	39.62	134.98	29.63	22.41
± SD	17.6	17.30	0.92	1.02	7.44	4.07	1.85
Range	55.0-92.0	283.00-319.00	8.13-10.16	38.50-41.20	130.36-145.07	25.65-35.77	20.43-24.66
Mean	124.8	313.60	10.44	40.25	128.40	33.61	25.95
± SD	15.6	4.4	0.51	0.98	4.36	1.99	1.50
Range	101.9-144.0	309.00-320.00	10.16-11.29	38.95-41.50	124.04-130.00	31.72-36.90	24.48- 26.05
Mean	171.5	337.40	11.77	41.89	124.36	34.87	28.05
± SD	14.7	13.46	0.61	1.12	3.05	0.68	0.81
Range	151.0-190.5	315.00-350.00	10.82-12.20	40.50-43.00	120.71-128.57	34.12-35.74	26.72-28.83
Mean	224.3	388.20	11.34	44.14	113.92	29.31	25.72
± SD	16.6	26.30	0.62	1.79	5.08	2.41	1.75
Range	205.0-246.0	348.00-415.00	10.45-12.05	42.18-45.90	110.76-122.84	26.46-31.88	23.89-28.57

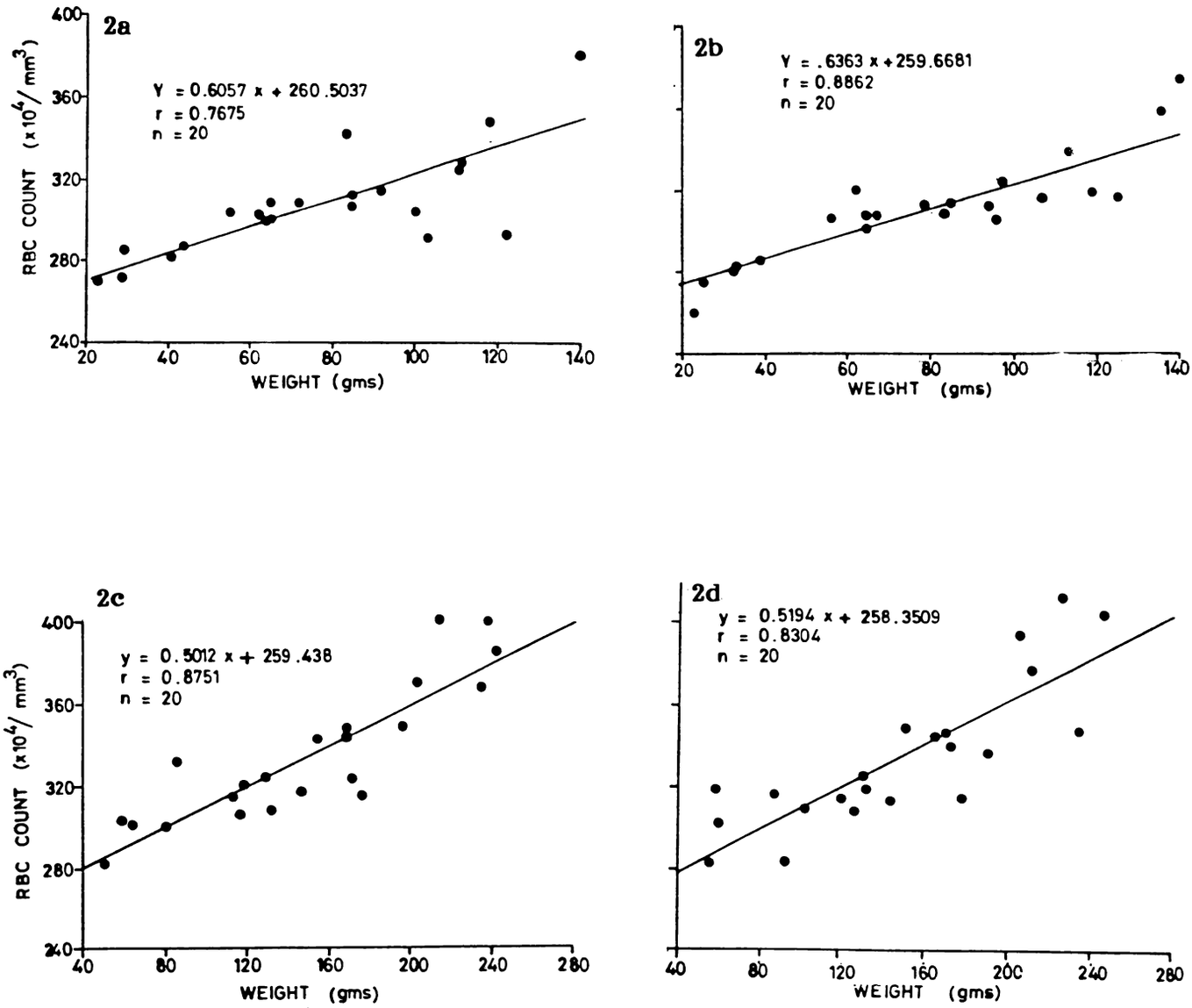


Fig.2. Goodness of fit of regression equation for RBC on weight together with observed data.

2a. Male H. fossilis 2b. Female H. fossilis
2c. Male C. batrachus 2d. Female C. batrachus

Correlation coefficients calculated with RBC count as a function of weight were found to be significant ($P < 0.01$) for both sexes. Linear regression analysis gave the following linear relationships.

RBC count for male = 0.6057 x weight (gms) + 260.5037

RBC count for female = 0.6363 x weight (gms) + 259.6681

The goodness of fit of regression to the observed data are shown in the figures 2a and 2b.

4.4.2.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 2c and 2d)

In C. batrachus RBC counts were determined for 40 fishes (20 males and 20 females). In the male, for a weight variation of 51 to 243 grams, the RBC range was 282.00 to $405.00 \times 10^4/mm^3$. In females, RBC count varied from 283.00 to $415.00 \times 10^4/mm^3$ for a size range of 55 to 246 grams. The mean RBC was $334.45 \times 10^4/mm^3$ for the male and $335.10 \pm 36.71 \times 10^4/mm^3$ for the female. Correlation coefficients with weight as independent variable were found to be significant ($P < 0.01$) for both sexes. Linear regression analysis of RBC count as a function of weight of C. batrachus gave the following linear relationships.

RBC count for male = 0.5012 x weight (gms) + 259.438

RBC count for female = 0.5194 x weight (gms) + 258.3509

The goodness of fit of the regression to the observed data are shown in the figures 2c and 2d.

4.4.3 Hb concentration

4.4.3.1 Variation in normal values

4.4.3.1.1 Heteropneustes fossilis (Table 3)

Hb concentration in H. fossilis (weight, 40 to 60 gms) varied from 7.20 to 14.50g/dl in the male and 7.70g/dl to 16.50g/dl in the female. Mean Hb was 10.60 ± 2.27 g/dl in the male and 10.80 ± 2.69 g/dl in the female. Just like RBC, maximum Hb was found during the breeding season and the minimum when the gonads were immature. No great differences in mean Hb were found between sexes.

4.4.3.1.2 Clarias batrachus (Table 4)

In C. batrachus (weight 100 to 120 gms), Hb varied from 7 to 14.35 g/dl in the male and 6.20to 15.10g/dl in the female. The mean Hb was found to be 11.07 ± 2.20 g/dl in the male and 11.06 ± 2.58 g/dl in the female. Minimum Hb was found during the post spawning period and maximum in summer months. No significant difference between sexes in mean values were found.

4.4.3.2 Hb concentration in relation to weight

4.4.3.2.1 Heteropneustes fossilis (Tables 5 and 6 ; Figures 3a and 3b)

In H. fossilis male, Hb concentration varied from 8.10 to 14.68 g/dl for a size range of 23 to 140 grams. The mean Hb was 11.06 g/dl. In the female, Hb variation was from 7.80 to 14.22 g/dl, the mean Hb being 11.22 g/dl. Only marginal differences were found between sexes.

With an increase in weight of the fish, Hb concentration was found to increase. To test its significance, correlation coefficient was calculated with weight as the independent variable. A highly significant positive corre-

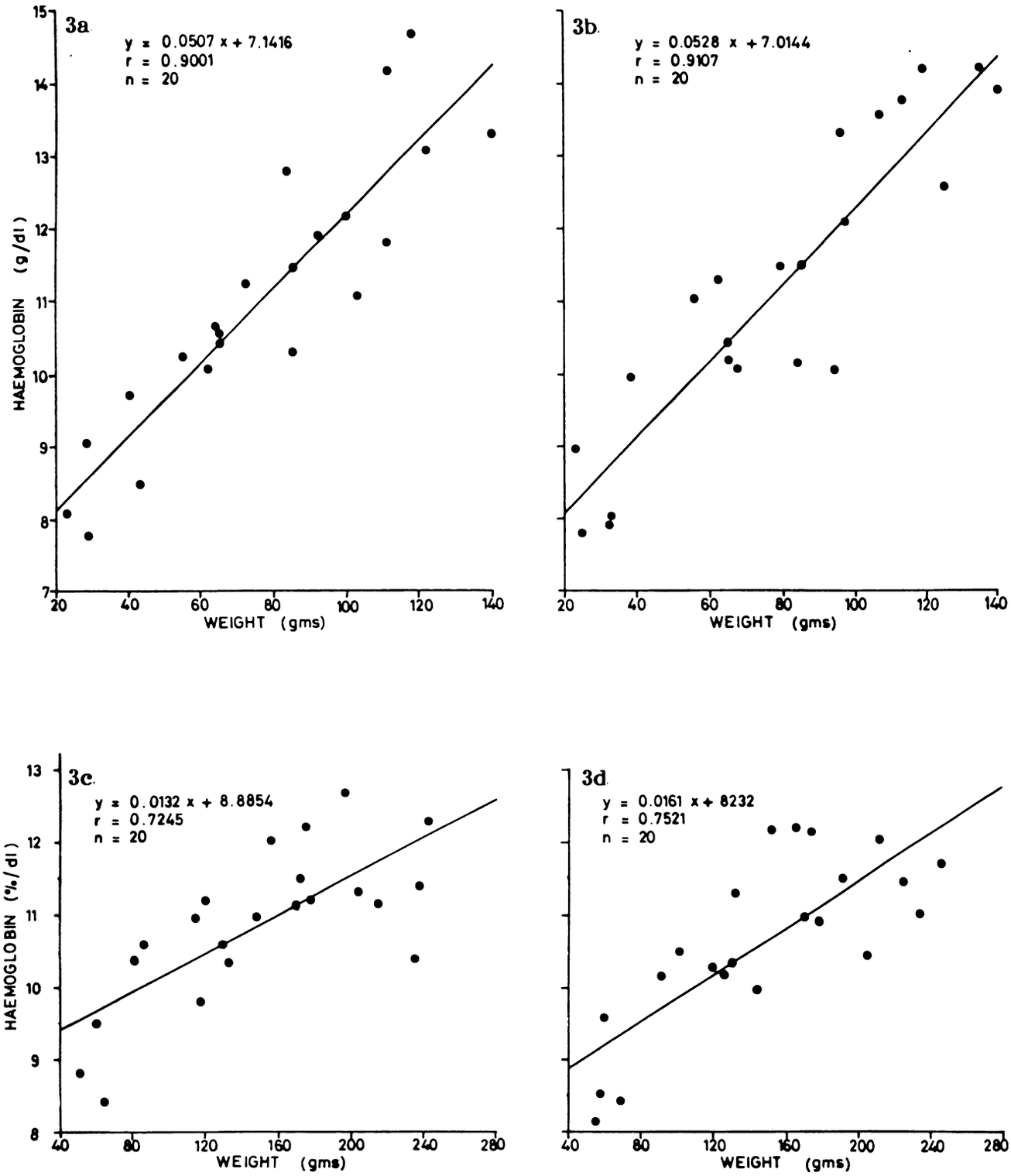


Fig.3. Goodness of fit of regression equation for Haemoglobin on weight together with observed data.

3a. Male H. fossilis
3b. Female H. fossilis
3c. Male C. batrachus
3d. Female C. batrachus

lation ($P < 0.01$) was found for both sexes. Regression analysis was done and the following linear relationships were obtained.

Hb in the male = 0.0507 x weight (gms) + 7.1416

Hb in the female = 0.0528 x weight (gms) + 7.0144

The goodness of fit of regression to the observed data are shown in the figures 3a and 3b.

4.4.3.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 3c and 3d)

In C. batrachus male, Hb concentration varied from 8.42 to 12.70 g/dl for a variation in weight from 51 gms to 243 gms. Mean Hb was 10.86 g/dl. In the female for a body weight range of 55 gms to 246 gms, the range of Hb was 8.13 to 12.20 g/dl. Mean Hb was 10.61 g/dl. Generally in both male and female C. batrachus, Hb concentration was found to be increasing up to the 3rd weight group. Then no increase was found.

To test whether there was any significance in the relationship between weight and Hb in C. batrachus, correlation coefficient was calculated with weight as the independant variable. r was found to be significant ($P < 0.01$). Simple regression analysis was done and the following linear relationships were obtained.

Hb in the male = 0.0132 x weight (gms) + 8.8854

Hb in the female = 0.0161 x weight (gms) + 8.232

The goodness of fit of regression to the observed data are shown in the figures 3c and 3d.

4.4.4 PCV

4.4.4.1 Variation in normal values

4.4.4.1.1 Heteropneustes fossilis (Table 3)

In H. fossilis of 40 to 60 gms in weight, PCV varied from 32% to 47.51% in the male (33 numbers) and 32.50 to 53.33% in the female (32 numbers). The mean PCV was 39.66 ± 4.17% in the male and 39.89 ± 3.52% in the female. Minimum PCV was found during December - January months and maximum from February to April months. No difference was found in mean values between the two sexes.

4.4.4.1.2 Clarias batrachus (Table 4)

In C. batrachus, PCV ranged from 32.00 to 50.00% in male (20 numbers) and 30.00 to 54.79% in the female (20 numbers). The mean PCV was 42.47 ± 5.92% in the male and 43.12 ± 7.40% in the female. Minimum PCV was found during the post-spawning period, and maximum during the summer months. Only a slight difference was found in mean PCV between sexes.

4.4.4.2 PCV in relation to weight

4.4.4.2.1 Heteropneustes fossilis (Tables 5 and 6 ; Figures 4a and 4b)

Packed cell volume in both sexes of H. fossilis changed from lower to higher values with increasing body weights. In the male, PCV increased from 33.30 to 42.75% for a weight increase from 23 to 140 gms. The mean value was 37.23%. In the female, PCV was raised from 33.50 to 42.5% for an increase in weight from 23 to 140 gms. The mean PCV was found to be 37.92%.

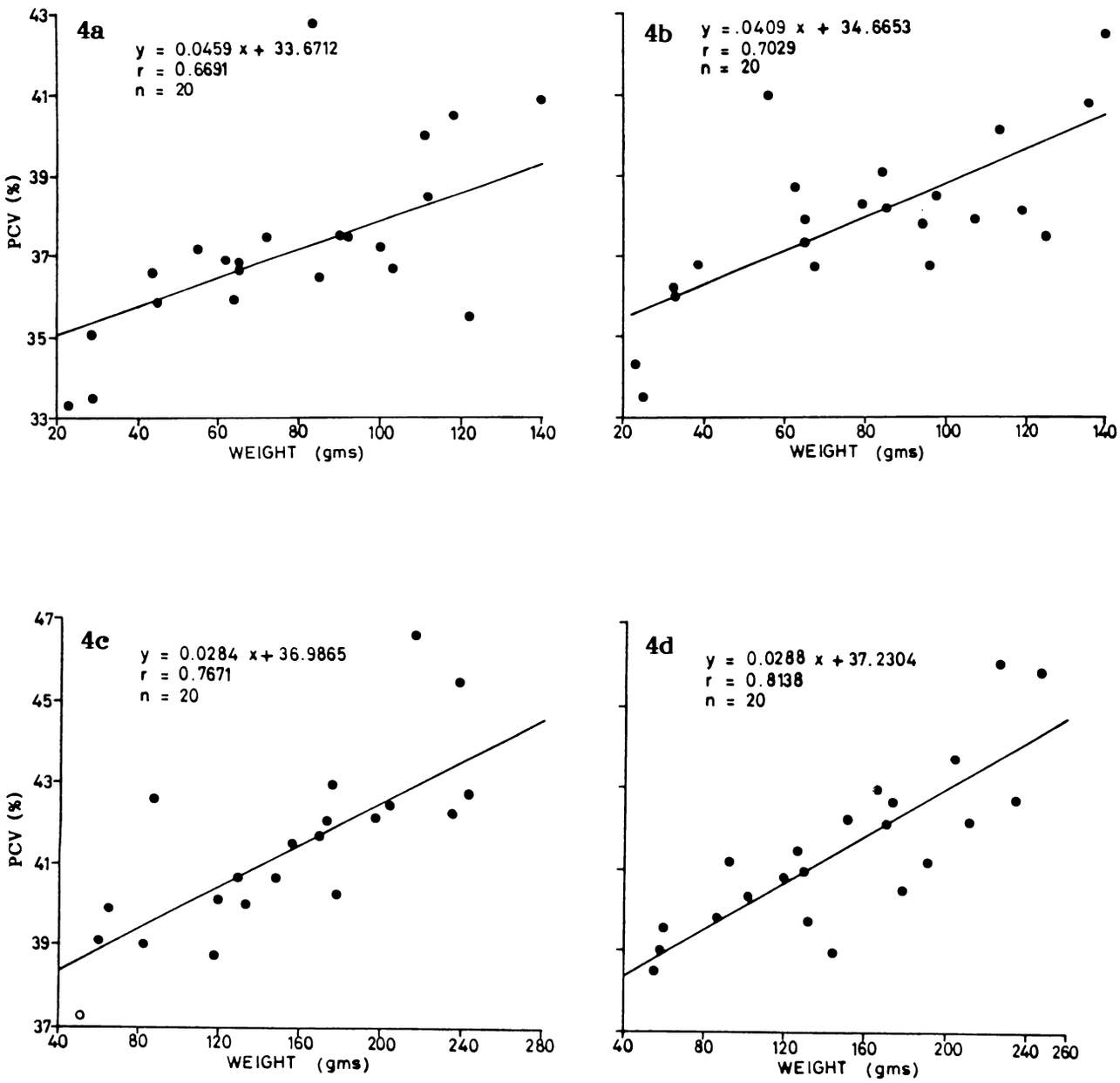


Fig.4. Goodness of fit of regression equation for PCV on weight together with observed data.

4a. Male H. fossilis 4b. Female H. fossilis
4c. Male C. batrachus 4d. Female C. batrachus

Correlation coefficients were calculated with weight as independant variable. To test the significance of the relationship between weight and PCV, correlation coefficient 'r' was calculated and found to be significant. Regression analysis were done and the linear relationship between weight and PCV in both sexes could be presented as follows.

PCV in male = 0.0459 x weight (gms) + 34.6653

PCV in female = 0.0409 x weight (gms) + 34.6653

The goodness of fit of regression to the observed data are shown in the figures 4a and 4b.

4.4.4.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 4c and 4d)

In C. batrachus too, increase in weight brought about an increase in PCV. In the male increase in PCV was 37.30to 46.67% for a weight range of 51 to 243 gms. The mean PCV was 41.23 %. In the female PCV ranged from 38.50 to 46.1%. The mean PCV was 41.48 %.

To test the significance of the relationship between weight and PCV, correlation coefficient was calculated and linear regression analysis was done. The following linear relationships were obtained.

PCV in the male = 0.0284 x weight (gms) + 36.9865

PCV in the female = 0.0288 x weight (gms) + 37.2304

The goodness of fit of regression to the observed data are shown in the figures 4c and 4d.

Table 9. Regression table showing the particulars of haematological parameters as a function of weight in Heteropneustes fossilis

Particulars	Number n	Correlation Coefficient r	Regression Coefficient b	Constant a	Level of Significance P
RBC on weight					
Male	20	0.7675	0.6057	260.5037	< 0.01
Female	20	0.8862	0.6363	259.6681	< 0.01
Hb on weight					
Male	20	0.9001	0.0507	7.1416	< 0.01
Female	20	0.9107	0.0528	7.0144	< 0.01
PCV on weight					
Male	20	0.7245	0.0132	33.6712	< 0.01
Female	20	0.7521	0.0161	34.6653	< 0.01

Table 10. Regression table showing the particulars of haematological parameters as a function of weight in Clarias batrachus

Particulars	Number n	Correlation Coefficient r	Regression Coefficient b	Constant a	Level of Significance P
RBC on weight					
Male	20	0.8751	0.5012	259.4380	< 0.01
Female	20	0.8304	0.5194	258.3509	< 0.01
Hb on weight					
Male	20	0.7245	0.0132	8.8854	< 0.01
Female	20	0.7521	0.0161	8.2320	< 0.01
PCV on weight					
Male	20	0.7671	0.0284	36.9865	< 0.01
Female	20	0.8132	0.0288	37.2304	< 0.01

4.4.5 MCV

4.4.5.1 Variation in normal values

4.4.5.1.1 Heteropneustes fossilis (Table 3)

In H. fossilis MCV ranged from 106.33 to 146.33 μ^3 for 33 males and 99.18 to 146.40 μ^3 for 32 females. The mean MCV for the male was 125.97 $\pm$ 11.80 μ^3 and 125.26 $\pm$ 14.44 μ^3 for the female. The minimum MCV was found in immature fishes and the maximum in fishes in which gonads were maturing.

4.4.5.1.2 Clarias batrachus (Table 4)

In C. batrachus, MCV ranged from 122.78 to 141.67 μ^3 in the male (20 fishes) and 120.93 to 154.80 μ^3 in the female (20 fishes) during the year. The mean MCV for the male was 131.89 $\pm$ 6.15 and for the female 132.08 $\pm$ 9.81. The maximum MCV was found in immature fishes and the minimum in fishes with maturing gonads.

4.4.5.2 MCV in relation to weight

4.4.5.2.1 Heteropneustes fossilis (Tables 5 and 6 ; Figures 5a and 5b)

MCV values, contrary to RBC count, Hb concentration and PCV was found to decrease with an increase in weight of H. fossilis. 20 fishes ranging in weight from 23 to 140 gms coming under each sex of H. fossilis were divided into 4 weight groups with five fishes in each groups in the ascending order. The mean of each weight group and corresponding MCVs were calculated and the mean MCVs were presented in the form of histogram. MCV was found to diminish with higher weights in both sexes of H. fossilis.

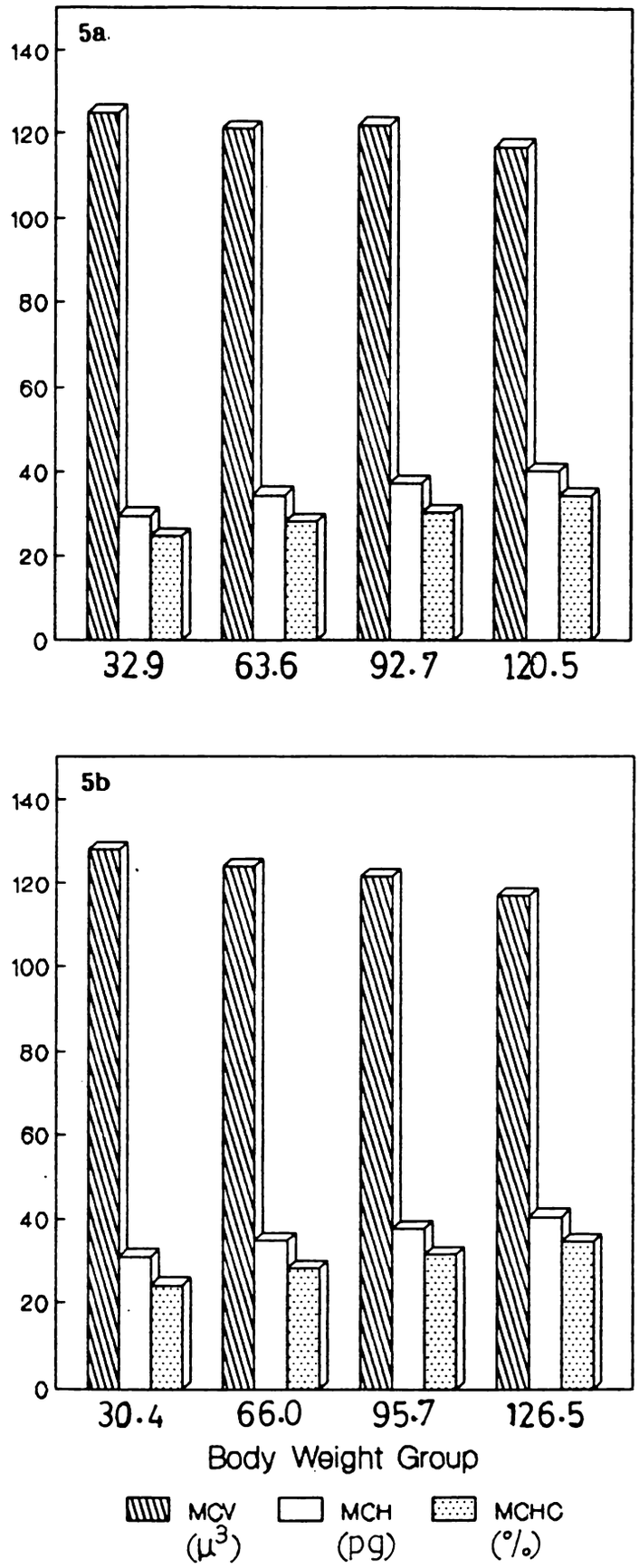


Fig.5. Body weight groups and corresponding MCV, MCH and MCHC.

5a. Male H. fossilis 5b. Female H. fossilis

4.4.5.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 5c and 5d)

In C. batrachus too, 20 fishes of each sex were divided into 4 groups according to their weights and the corresponding mean MCVs were drawn in the form of histograms. It showed that MCV decreased with an increase in weight of C. batrachus.

4.4.6 MCH

4.4.6.1 Variation in normal values

4.4.6.1.1 Heteropneustes fossilis (Table 3)

In H. fossilis, for the weight group 40 - 60 gms, MCH ranged from 27.60 to 37.27 pg in the male and 28.21 to 37.40 pg in the female. The mean was found to be 32.54 $\pm$ 2.63 pg for male and 33.17 $\pm$ 2.56 pg for female. The minimum MCH was found during the post spawning period and the maximum during the summer months.

4.4.6.1.2 Clarias batrachus (Table 4)

In C. batrachus, MCH varied from 29.79 to 36.67 pg in the male and 28.57 to 36.67 pg in the female. The mean was calculated to be 34.10 $\pm$ 2.33 pg in the male and 33.32 $\pm$ 2.40 in the female.

4.4.6.2 MCH in relation to weight

4.4.6.2.1 Heteropneustes fossilis (Tables 5 and 6; Figures 5a and 5b)

Unlike MCV, MCH increased with higher weight groups in H. fossilis. It was linearly related to RBC and Hb. 20 fishes of each sex of H. fossilis were divided into 4 weights groups in the ascending order and their corresponding mean MCHs were drawn as histograms to show the relationship between

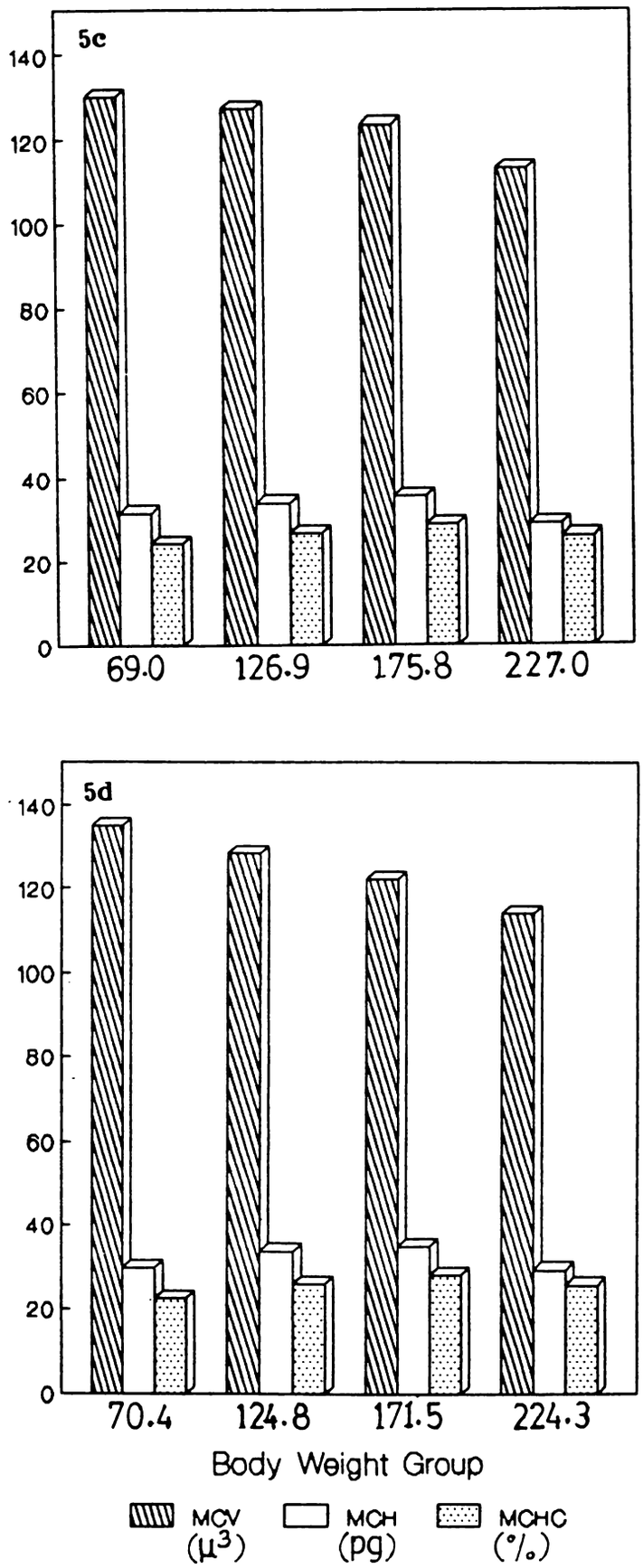


Fig.5. Body weight groups and corresponding MCV, MCH and MCHC.

5c. Male C. batrachus 5d. Female C. batrachus

weight groups and MCH. In H. fossilis, for both sexes, all weight groups showed an increase in MCH.

4.4.6.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 5c and 5d)

In C. batrachus too, 20 fishes of each sex having different weights were divided into 4 weight groups of five fishes in each. The corresponding mean MCH were represented as histograms to establish the weight related increase in MCH. In C. batrachus, MCH increased with an increase in weight only for the first three weight groups. In the last one MCH decreased with an increase in weight.

4.4.7 MCHC

4.4.7.1 Variation in normal values

4.4.7.1.1 Heteropneustes fossilis (Table 3)

In H. fossilis, of 40-60 gms, MCHC varied from 21.53 to 33.41% in the male (33 numbers) and 20.70 to 33.84% in the female (32 numbers). The mean was found to be 26.35 $\pm$ 3.54 for the male and 26.50 $\pm$ 3.83 for the female. The maximum MCHC was found during summer months and the minimum during the post spanning period.

4.4.7.1.2 Clarias batrachus (Table 4)

In C. batrachus, of 100 to 120 gms in the male, MCHC range was from 21.81 to 29.20% (20 fishes) and in the female it ranged from 21.06 to 30.16% (20 fishes). The mean values for male and female were 26.19 $\pm$ 2.40 and 25.55 $\pm$ 2.89 respectively.

4.4.7.2 MCHC in relation to weight

Generally MCHC followed the same trend as that of MCH.

4.4.7.2.1 Heteropneustes fossilis (Tables 5 and 6 ; Figures 5a and 5b)

20 H. fossilis, of each sex were grouped into four based on their weights and the corresponding mean MCHC were plotted as histograms. In both sexes, for all weight groups, MCHC was found to increase with increased weight.

4.4.7.2.2 Clarias batrachus (Tables 7 and 8 ; Figures 5c and 5d)

In C. batrachus too MCHC is directly related to weight. But this positive linear relationship was found only for the first three weight groups. For the last one, no corresponding increase in MCHC was detected. Both sexes showed similar responses.

4.5 DISCUSSION

4.5.1 Morphology of erythrocytes

It is reported that erythrocytes of fishes are usually elliptical in their form (Gulliver, 1875). But in the present study, the erythrocytes of a single species cannot be said to have a single shape. It did vary from elliptical to oblong to circular in H. fossilis. But the most common form was elliptical. In C. batrachus most of the cells were circular. A few oval elliptical and oblong cells were also found. Srivastava (1968a) and Pandey et al. (1976) found elliptical and circular cells in H. fossilis. In this fish, nuclei are elliptical where as in C. batrachus, they are circular. Smith et al. (1952) also observed that the nuclei of 10 species of teleosts take the same shape as the cell in which they are found.

The mean values for the size of the mature erythrocytes were observed to vary greatly between species of fishes (Barron et al., 1956; Haws and Goodnight, 1961; Pradhan, 1961; McKnight, 1966). But, between H. fossilis

and C. batrachus no great variation in size were found. It may be that these two fishes are placed very close phylogenetically and they do belong to the same habitat.

H. fossilis and C. batrachus are relatively sluggish. Milne - Edwards (1857), Dawson (1933), and Haws and Goodnight (1961) proposed that the activity of the animal and the size of the blood cells are closely related, i.e. the more active species have smaller erythrocytes and sluggish ones have larger corpuscles. The size of the RBC of H. fossilis and C. batrachus are medium and it falls within the range of size reported for them by Srivastava(1968a) and Pandey et al. (1976). These two fishes are not very active and they are not extremely sluggish. So the hypothesis stand in good stead in their case.

Polychromatophilic erythrocytes in the blood has been reported by Pandey and Pandey (1977) in Rita rita, Boomker in Clarias gariepinus (1980) and Joshi (1987) in C. batrachus, H. fossilis and some other cat fishes. Here too, they were found in the blood smears of both cat fishes under investigation. So it may be concluded that this is a characteristic feature of cat fish blood.

Erythroplastids, the erythrocytes without nucleus were found in the blood of H. fossilis and C. batrachus. They were earlier reported by Lucas and Jamroz (1961) in birds and Boomker (1980) in C.gariepinus and Sarotherodon mossambicus. In the fishes under investigation they were found during the breeding season though the reason is not known. Boomker (1980) represented them as ending the line of erythrocyte development stages. Microcytes and macrocytes were at times seen in the blood of these two cat fishes. They were also reported by Pandey and Pandey (1977) in Rita rita.

4.5.2 Normal haematological values

4.5.2.1 RBC count

Extreme variation in erythrocyte counts have been reported for teleosts. It may vary from $0.69x10^6/mm^3$ in Opsanus tau to $3.85x10^6/mm^3$ for Pomatomus saltatrix (Eisler, 1965). A very high count of $4.287x10^6/mm^3$ for Ophiocephalus punctatus has been reported by Srivastava (1968a). The mean RBC values for the cat fishes under study are higher when compared to their values reported by Srivastava (1968a), Pandey et al. (1976) and Tandon and Joshi (1976). It might be due to the climatic differences existing between the places where these investigations were done and Kerala where the present work was carried out.

It is noted that seasonal variations do affect RBC number (Cameron, 1970 ; Bridges et al., 1976; Bhatt and Singh, 1986). It might be either due to the effect of changing atmospheric temperature, or by the spawning season or by both. Increases in erythrocyte number during spawning season has been reported by Ezzat et al. (1973) for Tilapia zilli and Fourie and Hattingh (1976) for carp. High numbers of erythrocytes are needed for the high energy demands associated with gonadial maturation. Cameron (1970) has shown that changes in RBC counts in pin fish are of some importance in meeting seasonal increase in respiratory demands. He is of the opinion that other adjustments in erythrocyte size and in the rate of blood circulation would also be required to meet the nearly tenfold change in respiratory metabolism associated with seasonal temperature extremes. Thus the wide variation in the normal erythrocyte counts can be accounted for.

4.5.2.2 Hb concentration

Mean Hb concentration in H. fossilis and C. batrachus are high when compared to other teleosts like Ictalurus punctatus (Haws and Goodnight, 1961), Cyprinus carpio (Houston and De Wilde, 1968) etc. This might be due to the air breathing nature of the cat fishes under present investigation.

A comparative study of the blood of tropical fishes by Willmer (1934) disclosed a definite tendency for higher oxygen carrying capacity in air breathing forms. Dubale (1959) found the loading tension of oxygen to be higher in the blood of air breathers than that of water breathers. The enforced partial unsaturation of blood, resulting from the admixture of oxygenated and venous blood in its passage through the heart may be the cause of the high level of Hb occuring in the blood of many air breathing fishes (Satchell, 1976). The Hb in Clarias is much less sensitive to carbondioxide in the water (Fish, 1956).

For H. fossilis and C. batrachus Srivastava (1968c) got smaller values for Hb (7.6 g/dl for C. batrachus and 9.2 g/dl for H. fossilis) when compared to the present observations. Joshi (1980) reported higher values for the same two species of fishes. But the observations of Pandey et al. (1976) in female H. fossilis agree with the present results. Such wide variations in values of Hb for the same species might be attributed to intra-specific variations existing in fish due to different eco-physiological conditions.

Very high level of Hb during the summer months in both fishes should be due to the high temperature of the seasons. Another reason might be the maturity of gonads. Both these factors increase metabolic activities

needing higher amounts of oxygen resulting in a rise in Hb concentration. The minimum Hb occuring during the immature period should be due to the low metabolic rate of H. fossilis during that period. In C. batrachus, minimum Hb was found just after spawning. This should have been caused by depletion resulting from spawning.

4.5.2.3 PCV

Just like erythrocyte number and haemoglobin concentration, PCV is high in H. fossilis and C. batrachus. High PCV values for these fishes were also reported by Joshi (1980). Just like RBC and Hb, Srivastava (1968a) reported low values for mean PCV too. In these fishes, there is less inter-specific and intra-specific differences between the highest and lowest mean values of PCV than total RBC or Hb values. It may be that according to the number of RBC, the cells become either larger or smaller in size i.e., a reciprocal relationship is said to be existed between the size and number of RBC (Malassez, 1872; Kisch, 1949; Smith et al., 1952; Srivastava, 1968a).

In H. fossilis, the highest values for PCV were obtained during the summer months when gonads were maturing and the lowest during the winter months when the temperature was low and the gonads were immature. The rate of change of metabolic activities causing changes in oxygen requirements can be the reason for this. In C. batrachus the lowest PCV was caused by post spawning depletion of the fish.

4.5.2.4 MCV

MCV values are important in the sense that stress makes erythrocytes to swell causing increased MCV (Holeton and Randall, 1967; Soivio et al.,

1974a,b; Soivio et al., 1977; Soivio and Nikinmaa, 1981). MCV values showed wide variations during different seasons of the year. The smallest MCV was usually found during gonadial maturation as erythropoiesis happened at this time liberating small immature cells into the blood stream.

The variation in MCV during the year was the least in C. batrachus when compared to H. fossilis. The larger body weight of the former might have caused only smaller variation. MCV values derived in the present investigation are smaller than those presented by Srivastava (1968a) for these fishes. Variation in populations or eco-physiological conditions could have caused this disparity in values.

4.5.2.5 MCH and MCHC

No sex difference in MCH and MCHC were found for both species of fishes. Just like other haematological parameters, the highest MCH and MCHC were found during the summer months. These high values are a reflection of very high Hb values during this time, as MCH and MCHC are derivatives from Hb. MCH and MCHC were found to be directly related to Hb in both sexes in both species. The MCH and MCHC values reported by Srivastava (1968c) for H. fossilis and C. batrachus and the MCHC values by Pandey et al. (1976) for H. fossilis are not at par with the results of present investigation. The food availability, feeding regime, the nature of the habitat, the method of Hb and PCV determination all these affects the haematological values. So it is only natural that intra-specific variation may occur from region to region.

4.5.2.6 Effect of sex on normal haematological parameters

Sex doesn't seem to have any effect on the haematological parameters of H. fossilis and C. batrachus except for certain maturity stages during the breeding season. Several studies on salmonid fishes have demonstrated a sexual dimorphism in mature males and females with respect to erythrocyte count or PCV values (Snieszko, 1961; Robertson et al., 1961; Poston, 1966; Lane, 1979). But Conroy (1972) and Korzhuev et al. (1982) failed to observe any sexual differences in PCV or RBC count in mature Salmo salar. Bhagat and Banerjee (1986) suggested that there is no distinct sexual difference in haematological parameters in Amphipnous cuchia and the differences occuring are strictly seasonal.

4.5.3 Haematological factors in relation to weight

The oxygen consumption rate (Vo_2) of fish and many other animals is related to body weight (W) by a relation of the form.

$$Vo_2 = a\ W^r$$

The constant 'a' defines the metabolic rate for a fish of unit weight. The exponent 'r' defines the rate of change of metabolism with 'W' (Smith, 1977). Since oxygen consumption is thus a function of W, it is a probability that changes in W may result in alteration for haematological values. Thus Smith (1977) found that PCV, Hb concentration and RBC numbers are directly correlated with weight in the American plaice, Hippoglossoides platessoides.

In H. fossilis and C. batrachus from the results, it is apparent that an increase in weight is positively correlated to erythrocyte number, Hb con-

centration and PCV for both sexes agreeing with the results of Smith. The higher Hb concentration rate is further supported by the observations on MCH and MCHC that increase with the increasing body weight of fish.

The studies of Chaudhuri et al.(1986c) and Ruparaelia et al. (1986) on Sarotherodon mossambicus showed that total erythrocyte count, haemoglobin content and PCV varied as the fish increased in size with age. Since oxygen consumption is a function of weight (Smith, 1977), with an increase in weight, the oxygen requirements of the body will increase necessitating increased number of erythrocytes. From the present results, it is apparent that under normal conditions, Hb concentration and PCV have a positive linear relationship with RBC number. So elevation in erythrocyte number simultaneously results in increased concentration of Hb and PCV.

But MCV was found to decrease with an increase in body weight in both species of fishes. Pandey et al. (1976) in H. fossilis and Chaudhuri et al. (1986c) in Sarotherodon mossambicus observed the same phenomenon. According to Smith (1977), the relationship of MCV to W is complex and reasonably variable i.e. positive or negative correlations occur at various times of the year. To cope with the increased need of oxygen with higher weights, blood cells might have reduced their volumes. It is established that small cells having greater surface/volume ratios are most efficient in gas exchange (Hartman and Lesler, 1964). Such efficiency would be of advantage in an environment with low dissolved oxygen.

Hb concentration, MCH and MCHC increase with an increase in body weight for all weight groups in H. fossilis. But in C. batrachus, the larger

fish, on reaching the highest weight group, Hb, MCH and MCHC exhibited reduced values. It seems that after attainment of particular weight, blood values don't increase. This finding is consistent with the statement that metabolic activity slows down after certain age (Joshi and Tandon, 1977). But in C. batrachus, the highest weight group displayed the highest erythrocyte number. It may be that to cope with the anaemia as evinced from low Hb, RBC number might have increased.

In addition to their direct relationship to body weight, RBC number is positively related to Hb and PCV and Hb concentration to PCV for the species of fishes under investigation. Such relationships were also reported by Clarke et al. (1979) in Micropterus salmonides. They also detected no significant differences between the blood parameters of male and female. In the present study, females possess slightly higher values of RBC, PCV and MCV for certain weight groups, than the males. The fishes for this study were collected during the summer months and the experiments were carried out at the end of May before spawning when the gonads were fully mature. So reproduction might have affected the haematological factors causing slightly higher blood values in females.

It is apparent from the results that for H. fossilis and C. batrachus normal haematological parameters vary at different times of the year and the variation depends on the body weight, physiological condition of the fish, temperature and season. Sex of the fish doesn't seem to have much effect on the haematological parameters except for some stages during the breeding season. Occurence of immature erthrocytes is a common feature of cat

fish blood. Erythroplastids were usually seen during the breeding period though the reason is not known. When comparing the haematological values of H. fossilis and C. batrachus from Kerala, India, with those obtained from other regions of India, it seems that intra-specific variations in some haematological values do occur. It is only natural when considering the difference in eco-physiological condition existing in geographically distant places.

CHAPTER - V

IDENTIFICATION AND CHARACTERIZATION OF LEUCOCYTES AND RELATED CELLS

5.1 INTRODUCTION

Leucocytes form an important defence mechanism in non-mammalian and mammalian vertebrates. They are indicated in inflammatory reactions in teleosts. An increasing interest in the phylogenetic development of cellular immune mechanisms in fish has underlined the necessity for a clear understanding of the identity of leucocytes of individual species prior to any significant advance in classifying their immunlogical role (Parish et al., 1986).

Identification and characterization of fish blood leucocytes pose problems due to the common practice of comparing them with mammalian leucocytes in stained smears using Romanowsky stains. Ellis (1977) in his review in fish leucocytes highlighted the drawbacks of cellular nomenclature and proposed that for a definitive classification, the basic criteria to be taken into account are functional, ontogenic and morphological characteristics of the leucocytes.

Until recently almost all published works on blood cells identified them on the basis of morphological characteristics in Wright's, Leishman's or Giemsa stained preparations of smears. That practice is now outmoded.

So an attempt has been made to identify the leucocytes of H. fossilis and C. batrachus using ontogenic, cytochemical, functional and morphological characteristics at the light microscopic level.

5.2 REVIEW OF LITERATURE

Studies on fish leucocytes were reported in the beginning of this century

itself (Drzewina, 1906, 1911; Jordan, 1926; Duthie, 1939). Later Boyar (1962) investigated different blood cell types in Clupea harengus harengus. Other important contribution to leucocyte morphology is that of Fey (1963, 1966a,b).

In India, the morphology of leucocytes in fresh water air breathing teleosts was studied by Srivastava (1968b), Pandey et al. (1976) and Mahajan and Dheer (1979). Another major work published in India is that of Joshi (1987) on the cytomorphological classification and key to the identification of normal circulating blood corpuscles based on the blood cell studies in 41 species of fresh water teleosts.

But almost all these works are based on the morphological appearance and morphometry of the cells in blood smears stained with Romanowsky stains. The terminology was applied to the cells by comparing them with mammalian leucocytes on the basis of morphological similarity.

But according to the new trend in cytomorphological studies of blood cells, cellular nomenclatures used in one species can only be applied to the cells of another species if the nature of those cells is in general accord with three basic criteria; functional, ontogenic and morphological (Ellis, 1977).

The functional capacity of the blood cells were tested by applying cytochemical techniques as well as by testing their phagocytosing capacity (Ellis 1976; Page and Rowley, 1983; Parish et al., 1986). Phagocytosing capacity were tested either by in vitro (O'neill, 1985) or in vivo (Ellis et al., 1976) experiments. The ontogeny of the cells were studied from smear

preparations and stained sections of haemopoietic organs like thymus, kidney and spleen (Boomker, 1980; Fange and Pulseford, 1985). Based on these studies, the fish leucocytes are mainly classified into lymphocytes, thrombocytes, monocytes and granulocytes. In addition, macrophages are also recognized.

Melanin containing macrophage like cells are a curious feature of fishes. The cells of the melanin-macrophage centres are a striking feature of all teleosts (Roberts, 1975). Mackmull and Michels (1932) were the first to describe there centres. They demonstrated that circulating macrophages homed specifically on these sites in the cunner by means of intra-peritoneal injection of carbon particles. Roberts (1975) coined the term "melanin-macro--phage". Later, this was modified into melano-macrophage. Agius (1985) published a review on melano-macrophage centres in fish.

Lymphocytes circulate through out the vertebrate body, and congregate in organs which filter body fluids (Ellis, 1977). In fish, lymphocytes are present in neural lymphatic duct lymph (Wardle, 1971). Watson et al. (1963) reported that gold fish lymphocytes comprised about 30% of all blood leucocytes. Gardner and Yevich (1969) reported it as 2-13%.

The number of these cells may be more accurately determined by an immunofluorescent technique which stains only lymphocytes by virtue of the immunoglobulin present in their surface membrane (Ellis and Parkhouse, 1975; Ellis, 1976). This gave the value $12x10^3$ lymphocytes/mm^3. Page and Rowley (1983) found the number of lymphocytes in blood of river lamprey to be 21 $\pm$ 14%. In Clarias gariepinus 12.3 - 20.3% and in Sarotherodon mossambicus 26.7 - 28.6% lymphocytes were reported (Boomker,1981a).

Saunders (1968) described the morphology of thrombocytes in over 225 species of fish and found great variation of cell size and shape and staining characteristics of nucleus and cytoplasm among the various species. Gardner and Yevich (1969) examined the thrombocytes in three species of cyprinodonts and found seasonal variation in members. Srivastava (1969) described thrombocytes in four species of teleosts. Pandey et al. (1976) described thrombocytes as spindle cells in Heteropneustes fossilis. The ratio of lymphocytes to thrombocytes for Salmo gairdneri was given as 25:1 by McCarthy et al. (1973), but Weinreb (1958) claimed it as 2:1.

Monocytes form 1.6 - 8.9% in C. gariepinus and 4.1 - 6.5% in S. mossambicus (Boomker, 1981a). They were found to be 0 - 1.25% in Scyliorhinus canicula (Parish et al., 1986). In plaice, they form about 0.1% of leucocytes.

In the immunological system of teleostean fishes, tissue macrophages have great importance as scavengers of dead cells and foreign material. A definition of the macrophage was put forward at the International Conference on Mononuclear Phagocytes in 1970 and stated that a macrophage is a mononucleated tissue cell derived from circulating monocytes, which adhere to glass and plastic, is characteristically highly phagocytic or pinocytic and has an undulating membrane (Laskin and Lechevalier, 1972 as cited by Ellis, 1977). Three types of granulocytes are reported to occur in fish blood; neutrophil, eosinophil and basophil. Of these, neutrophils were found to be the most abundant.

The number of circulating neutrophils vary over a considerable range. In gold fish it was reported as 1.2% (Watson et al., 1963); 5.12%(Weinreb and

Weinreb, 1969); and in brown trout 0 - 25% of leucocytes.

Axial electron dense crystalloids have been reported in granules of neutrophils (Kelenyi and Nemeth, 1969). Cenini (1984) found crystalloids of fibrillar appearance in carp neutrophil granules.

The reports of the presence of basophils in the blood of fishes vary. Basophils were found in H. fossilis by Pandey et al. (1976), in Channa punctatus by Mahajan and Dheer (1979) and in carp by Loewenthal (1930), Haider (1968), and Cenini (1984). But several authors could not find them in the blood of fish. They were reported to be absent from perch (Yokoyama, 1960), rainbow trout (Klontz, 1972), brown trout (Blaxhall and Daisley, 1973), plaice (Ellis, 1976) and Oreochromis mossambicus (Doggett et al., 1987).

The cells designated mast cells were identified only based on their basophilic staining, cytoplasmic granules and their presence in connective tissue (Ellis, 1977). Roberts et al. (1972) reported the presence of mast cells in plaice dermis. Other scientists who reported the presence of mast cell in fish were Duthie (1939), Catton (1951), Bucke (1971) and Roubal (1986).

Eosinophils were found in the blood of a variety of fishes. They were reported in lampreys (Fey, 1966a; Piavis and Hiatt, 1971; Percy and Potter, 1976), lung fish (Ward, 1969) and in elasmobranchs (Parish et al., 1986). The presence and absence of eosinophils were reported in teleosts. They were observed to be present in Salmo salar (Conroy, 1972), in rainbow trout (Ellis, 1977), in carp (Kelenyi and Nemeth, 1969) and in H. fossilis (Pandey et al., 1976).

Plasma cells are reported to exist in teleosts (Cenini, 1984). Incomplete evidence for the existence of plasma cells in fish comes from the immunofluorescent technique (Ellis, 1976) and the rosette forming technique (Chiller et al., 1969).

Another type of leucocyte found in fish blood is cells with rod shaped granules. They were described for the first time by Bielek (1980). He considered them to be distinct from the rodlet cells found in several fish (Morrison and Odense, 1978). The controversy exists whether they should be considered protozoan parasites (Mayberry et al., 1979; Bannister, 1966) or fish cell (Chaicharn and Bullock, 1967; Paterson and Desser, 1981).

The presence of two sub population of lymphocytes, T lymphocytes of thymus origin which are responsible for cell mediated immunity and also act as helper cells for the second subpopulation B lymphocytes which are responsible for antibody production, have been suggested in fishes. According to Ellis (1986) it is still too early to reach at such a conclusion. But all the general characteristics of cell mediated and humoral immunity attributed to T and B cells are exhibited by fish. A thymus is present and resembles that of higher vertebrates. A hapten carrier effect has been reported in fish. Lymphocytes are known to produce lymphokines which have pharmacological effects. One factor called Macrophage Inhibiting Factor (MIF) inhibits macrophages but stimulates them metabolically (Roitt, 1971). Another factor, Mitogenic factor is MF which produce mixed leucocyte reactions (MLR). The phenomena produced by these two factors have been demonstrated in fish (Ellis, 1986). Allograft rejection by fish is also attributed to the action

of lymphocytes (Hildemann, 1970; Hogarth, 1973). Surface immunoglobulin (sIg) has been recognised in lymphocytes (Warr et al., 1977). Warr and Marchalonis (1980) suggest that sIg acts as an antigen receptor on all fish lymphocytes.

The function of thrombocytes is clotting of the circulating fluids (Srivastava, 1969; Wardle, 1971). Phagocytosis by thrombocytes was reported by Yokoyama (1960) and Fange (1968). Monocytes are associated with phagocytosing capacity (Ellis, 1976; Thuvander et al., 1987). Macrophages also have the capacity for avid phagocytosis (Ellis et al., 1976). Antigen containing macrophages are commonly found in the antibody producing organs of fish (Klontz, 1972). Surface antibody was present in the cell membranes of about 10% of macrophages (Ellis, 1974). They also act as scavenger cells. Neutrophils of fish infiltrate injured tissues (Thorpe and Roberts, 1972; Joy and Jones, 1973). Chemotaxis is found to occur in fish neutrophils (Fletcher and White, 1973). The function of basophils in fishes is not clear. In mammals, they are concerned with histamine production. Mast cells in mammals are mediators of anaphylaxis, but it is not yet clear whether this phenomenon is exhibited by fish (Ellis, 1977).

Phagocytic activity was observed in eosinophils of fish (Jakowska and Nigrelli, 1953; Watson et al., 1963). Jakowska (1956) reported a localised increase in the number of eosinophils in an inflammatory site.

5.3 MATERIALS AND METHODS

5.3.1 Collection and maintenance of fishes

Fishes were collected and maintained as described in 3.2. Blood was

collected as in 3.3 and smears were prepared as specified in 3.6.1.

5.3.2 Preparation of imprints and smears

To study the developing cells and tissue macrophages, the imprints of haemopoietic organs were taken. After the collection of blood from the caudal vein, the fish was killed, abdominal cavity was opened from the ventral side and smear of peritoneal fluid was taken to study the peritoneal macrophages. The haemopoietic organs, the kidney and the spleen were removed. A small piece of each haemopoietic organ was gently macerated in a drop of 0.8% saline (Smith et al., 1967; Boomker, 1980) and the resulting suspension smeared on to cleaned glass slides. Impression smears were made by bringing a freshly cut part of the organ into contact with a glass slide (Ashley and Smith, 1963; Boomker, 1980). Both organ and impression smears were rapidly air dried.

5.3.3 Fixing of smears

Blood smears and organs smears were fixed by acetone free methyl alcohol for 2 to 5 minutes in order to prevent the haemolysing of RBC when it comes in contact with water in which some stains are dissolved and to prevent degenerative autolytic changes in the cells which occur when cells are not fixed.

5.3.4 Staining of smears

The Romanowsky stains i.e., Wright's, Leishman's and Giemsa stain were used for staining blood films and peritoneal, spleen and kidney smears. Giemsa was found to be the best. It gave blood nuclear and cytoplasmic staining. Giemsa staining was done as described in 3.6.1.

5.3.5 Histochemical methods

As the Romanowsky stains were found to be inadequate for identifying the leucocytes, certain histochemical methods were employed for proper identification of different leucocytes. These were applied to all types of smear preparations.

5.3.5.1 Periodic Acid - Schiff (PAS) - Mc Manus and Mowry (1960) as cited by Humason (1966) modified

Fixation - Methyl alcohol

Periodic acid - 1%

Schiff's reagent

Basic fuscin - 1 gm

Distilled water - 85 ml

Sodium metabisulphite - 1.9 gm

N.HCl - 15 ml

Placed in bottle with 50 to 60 ml of free air space. Shaken at intervals for at least 2 hours or overnight. Added 200 mg activated charcoal for 2 minutes. Shaken, filtered and stored in a dark bottle in refrigerator.

Procedure

1. Treated with Periodic acid - 10 minutes
2. Washed in running water - 5 minutes
3. Immersed in Schiff's reagent - 10 minutes
4. Washed in running water - 5 minutes

Counterstained in safranin, washed in distilled water, dried and mounted in glycerine jelly. Control treated to saliva before staining. Majentha colour showed the presence of glycogen.

5.3.5.2 Sudan Black B - Humason (1966) modified

Fixation - 10% formalin

Working solution

Dissolved 7 gms of Sudan Black B in 100 ml of ethylene glycol. Added small amounts at a time and heated to 100 - 110°C, stirred for a few minutes. Filtered hot through Whatman No.2 paper and cooled.

Procedure

1.	Placed in pure ethylene glycol	- 3-5 minutes
2.	Stain	- 2 changes, 5-7 minutes each
3.	Differentiated in glycol and water (85:15)	- 3-5 minutes
4.	Washed in distilled water	- 3-5 minutes
5.	Counterstained in Giemsa	- 10 minutes
6.	Mounted in glycerol jelly Lipids - Blue black to black	

5.3.5.3 Alkaline Phosphatase - Modified Gomori method (Frankel and Peters (1964) as cited by Humason (1966)

Fixation - In acetone, 30 seconds

Incubating solution

0.8% paranitrophenyl phosphate	- 2 ml
2% Sodium barbital	- 2 ml
Distilled Water	- 1 ml
2% $CaCl_2$	- 4 ml

Procedure

1.	Incubated in Substrate	- 45 minutes
2.	Washed well in distilled water	- 1 minute
3.	Treated with 2% Cobalt nitrate	- 3 minutes
4.	Rinsed well in distilled water	- 1 minute
5.	Treated with 2% Yellow Ammonium Sulphide	- 3 minutes

6. Washed well in distilled water - 1 minute
7. Counterstained - In haematoxylin
8. Mounted in glycerine jelly

Sites - Black, sharp and clear non-diffuse granules.

5.3.5.4 Acid Phosphatases - Gomori's method as cited by Humason (1966)

Fixation - In Calcium formalin

Solutions

Sodium acelate buffer - (pH 5.0)
0.6% acetic acid - 300 ml
Sodium acetate (2.7%) - 700 ml

Incubating Solution

Acetate buffer - 125 ml
Distilled water - 500 ml
Lead nitrate - 0.6 gms
3% Sodium glycero-phosphate (Freshly prepared)- 50 ml

Kept at 37°C for 24 hours. Filtered and added about 5-10 ml distilled water to prevent precipation on evaporation. Kept in refrigerator.

Procedure

1. Incubated in Substrate (37°C) - 1-24 hours
2. Rinsed in distilled water - few seconds
3. Washed in 1.2% Acetic Acid - 1 minute
4. Washed in distilled water - few seconds
5. Transferred to 1% yellow Ammonium Sulphide - 2 minutes
6. Washed in distilled water - 5 minutes
7. Counterstained - In haematoxylin
8. Washed in tap water - few seconds
9. Mounted in glycerine jelly

Sites - Black

5.3.5.5 Peroxidase - Humason (1966)

Fixation - In acetone

Solutions

Solution A

Benzidine - 0.2-0.3%

95% ethyl alcohol - 100 ml

Sodium nitroprusside - 5 gms, dissolved in a few ml of water.

Solution B

3% H_2O_2 - 1 ml

Distilled water - 49 ml

Working Solution

Equal volumes of A and B

Procedure

1. Poured working solutions ever slides and allowed to remain - 5-10 minutes
2. Washed in distilled water - 1 minute
3. Counterstained in fresh mixture of Giemsa - 10 minutes
4. Washed in distilled water - 1 minute
5. Smears air dried

   Sites - Grey green to blue black

5.3.6 Phagocytosis

To estimate the functional capacity of leucocytes, their phagocytic ability were tested.

5.3.6.1 Phagocytosis of particulate matter of non-biological origin

0.5 ml of collodial carbon (Rotring water proof ink) diluted with physiological saline (1:1) was injected into the peritoneal cavity of 5 fishes each of H. fossilis and C. batrachus. The fishes were bled after 1 hour,

6 hours and 24 hours. Blood smears and haemopoietic organ smears and peritoneal fluid smears were taken as mentioned earlier and stained with Giemsa.

5.3.6.2 Phagocytosis of bacteria

Heat killed Escherichia coli were injected in physiological saline ($1x10^9$ numbers/cm^3) into the peritoneal cavity of both H. fossilis and C. batrachus. The fishes were bled after 1 hour, 6 hours and 24 hours. Blood and organ smears were taken and stained as mentioned above.

5.3.7 Micrometry

Different leucocytes were measured using an ocular meter calibrated by a stage micrometer. Both the length and width of cytoplasm and nuclei of 50 cells of each type were measured and their mean and standard deviation were calculated. N/c ratio was calculated as in 3.6.6.

5.4 RESULTS

If not otherwise mentioned, the result is common for the blood cells of H. fossilis and C. batrachus. The morphological details of leucocytes are given in Tables 11 and 12 and the results of the histochemical procedures performed on them are given in Tables 13 and 14.

5.4.1 Lymphocytes (Figures: 6a, 6b, 6c and 6d)

Three types of lymphocytes were found in the blood of H. fossilis and C. batrachus; small, medium and large. The ratio of nucleus to cytoplasm is lower in large and medium lymphocytes. In small ones, the nucleus almost fills the cells and the cytoplasm is found as a small rim around the nucleus. In medium and large lymphocytes, the nucleus is concentric which occupies

Table 11. Morphometry of the leucocytes of Heteropneustes fossilis

Cell Particulars	Cytoplasm Length (L) µm	Cytoplasm Width (W) µm	Cytoplasm L x W	Nucleus Length (L) µm	Nucleus Width (W) µm	Nucleus L x w	N/C Ratio
Lymphocytes (N-50 for each)							
Small	5.50 ± 0.41	4.90 ± 0.37	26.95	5.21 ± 0.20	4.62 ± 0.21	24.07	0.89
Medium	6.81 ± 0.45	6.00 ± 0.30	40.86	5.61 ± 0.28	5.49 ± 0.25	30.80	0.75
Large	8.90 ± 0.50	8.10 ± 0.22	72.09	7.50 ± 0.18	6.15 ± 0.42	46.13	0.64
Thrombocytes (N-50 for each)							
Spindle	7.84 ± 1.90	3.92 ± 1.61	30.73	5.36 ± 0.55	1.98 ± 0.16	10.61	0.35
Tear drop	10.29 ± 0.69	3.94 ± 1.39	40.54	4.90 ± 0.25	1.98 ± 0.18	9.70	0.24
Oval	4.50 ± 0.62	4.05 ± 0.42	18.23	3.98 ± 0.27	2.90 ± 0.22	11.54	0.63
Round	3.92 ± 0.35	3.92 ± 0.41	15.37	3.92 ± 0.38	3.25 ± 0.19	12.74	0.83
Monocyte (N-50)	11.84 ± 0.45	10.27 ± 0.66	121.60	9.66 ± 0.35	3.85 ± 0.24	37.19	0.31
Macrophage (N-20 for each)							
Circulating	14.60 ± 1.30	12.71 ± 1.75	185.57	4.95 ± 0.18	3.92 ± 0.20	19.40	0.10
Peritoneal	15.82 ± 1.5	10.95 ± 1.85	173.23	4.20 ± 0.27	4.27 ± 0.19	17.93	0.10
Melano (Coarse type)	16.2 ± 1.5	15.69 ± 0.90	254.18	4.10 ± 0.19	3.95 ± 0.23	16.20	0.06
Neutrophil Single lobed (N-50)	10.29 ± 0.69	9.94 ± 0.35	102.28	5.44 ± 0.50	4.75 ± 0.19	25.84	0.25

Table 12. Morphometry of the leucocytes of Clarias batrachus

Cell Particulars	Cytoplasm Length (L) μm	Cytoplasm Width (W) μm	Cytoplasm L x W	Nucleus Length (L) μm	Nucleus Width (W) μm	Nucleus L x W	N/C Ratio
Lymphocytes (N-50 for each)							
Small	5.20 ± 0.35	4.80 ± 0.30	24.96	4.50 ± 0.40	4.10 ± 0.19	18.45	0.74
Medium	7.00 ± 0.51	5.50 ± 0.45	38.50	6.60 ± 0.28	3.50 ± 0.32	23.10	0.60
Large	9.75 ± 0.42	8.10 ± 0.25	78.98	7.95 ± 0.31	6.08 ± 0.55	48.34	0.61
Thrombocytes (N-50 for each)							
Spindle	8.09 ± 0.95	4.00 ± 1.70	32.36	7.00 ± 0.69	2.20 ± 0.32	15.40	0.48
Tear drop	9.62 ± 0.56	5.77 ± 0.49	55.51	5.07 ± 0.76	3.55 ± 0.87	17.97	0.32
Oval	4.81 ± 0.50	4.25 ± 0.35	20.44	4.17 ± 0.25	2.88 ± 0.42	12.01	0.54
Round	4.10 ± 0.29	4.10 ± 0.25	16.81	3.75 ± 0.29	3.20 ± 0.18	12.00	0.71
Monocyte (N-50)	11.48 ± 0.59	11.09 ± 0.45	127.31	9.85 ± 0.24	4.05 ± 0.19	39.89	0.32
Macrophage (N-50 for each)							
Circulating	13.85 ± 1.56	13.46 ± 1.68	186.42	4.55 ± 0.15	4.47 ± 0.12	20.34	0.11
Peritoneal	15.45 ± 0.80	11.08 ± 0.95	171.86	4.50 ± 0.20	4.45 ± 0.20	20.03	0.12
Melano (Coarse type)	15.98 ± 1.70	15.70 ± 0.81	246.89	4.43 ± 0.29	4.20 ± 0.11	18.61	0.08
Neutrophil Single lobed (N-50)	10.64 ± 0.52	9.61 ± 0.41	102.25	5.77 ± 0.38	4.81 ± 0.25	27.75	0.27

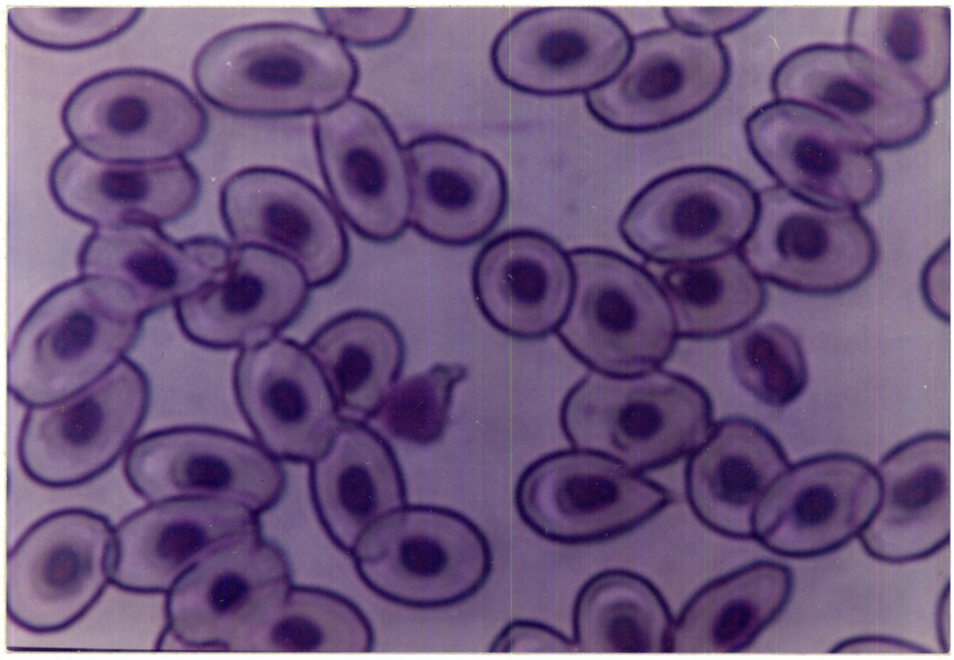


Fig.6a. Small lymphocyte and thrombocyte in H. fossilis. Note the thrombocyte with oval shape and bridge shaped nucleus.

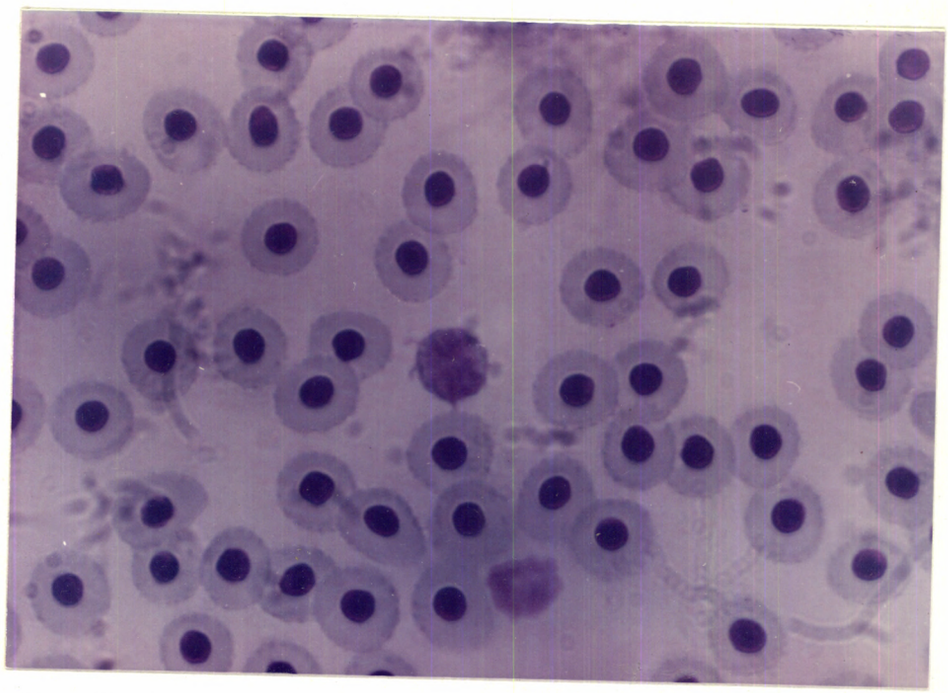


Fig.6b. Medium lymphocyte in C. batrachus. Note the pseudopodia.

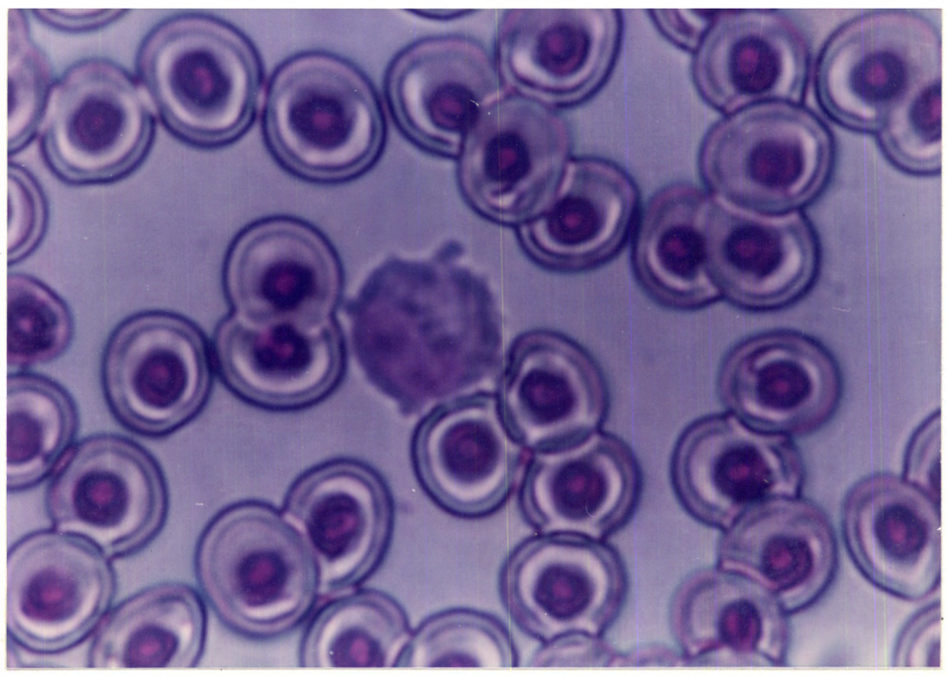


Fig.6c. Large lymphocyte in C. batrachus. Note the characteristic pseudopodia.

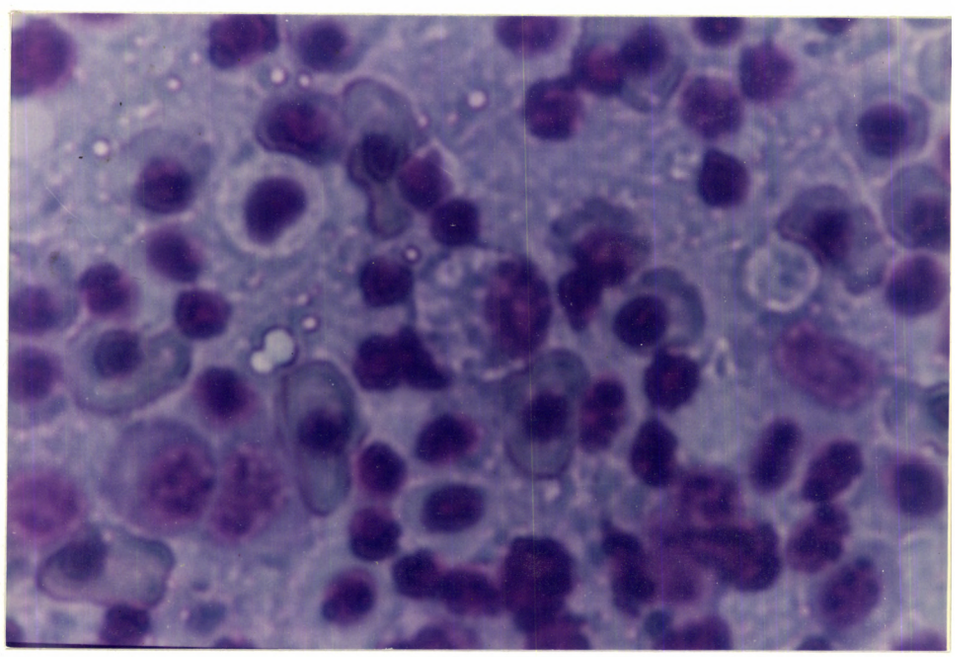


Fig.6d. Lymphoblast in kidney imprint in H. fossilis. Note the large cell with blue black granules presumed to be a precursor of melano-macrophage.

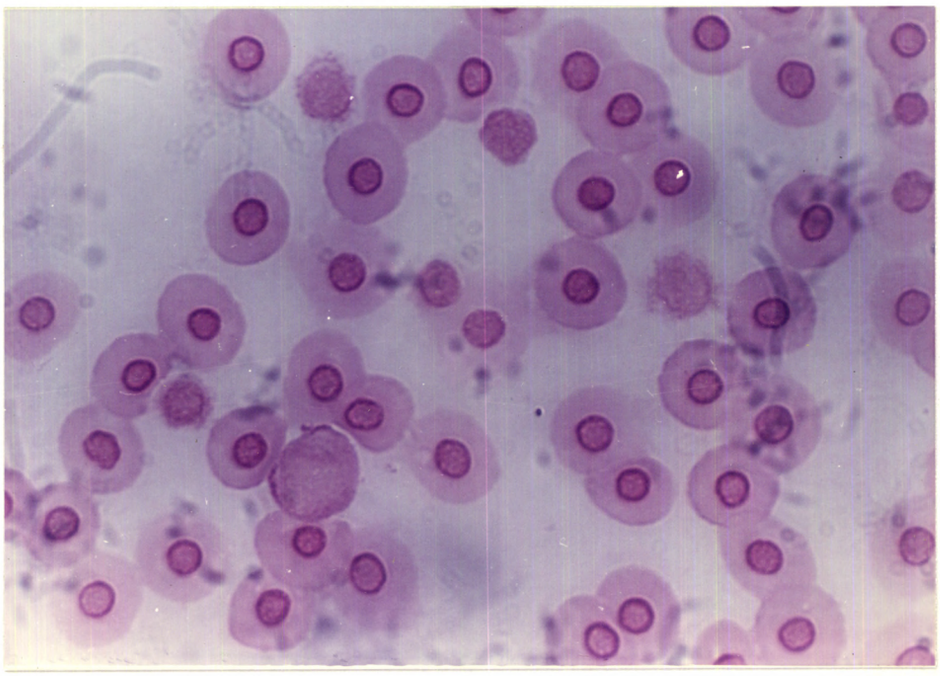


Fig.6e. PAS reaction in lymphocyte and thrombocyte in C. batrachus. Note the light staining of cytoplasm in the lymphocyte and the stained granules in the thrombocyte.

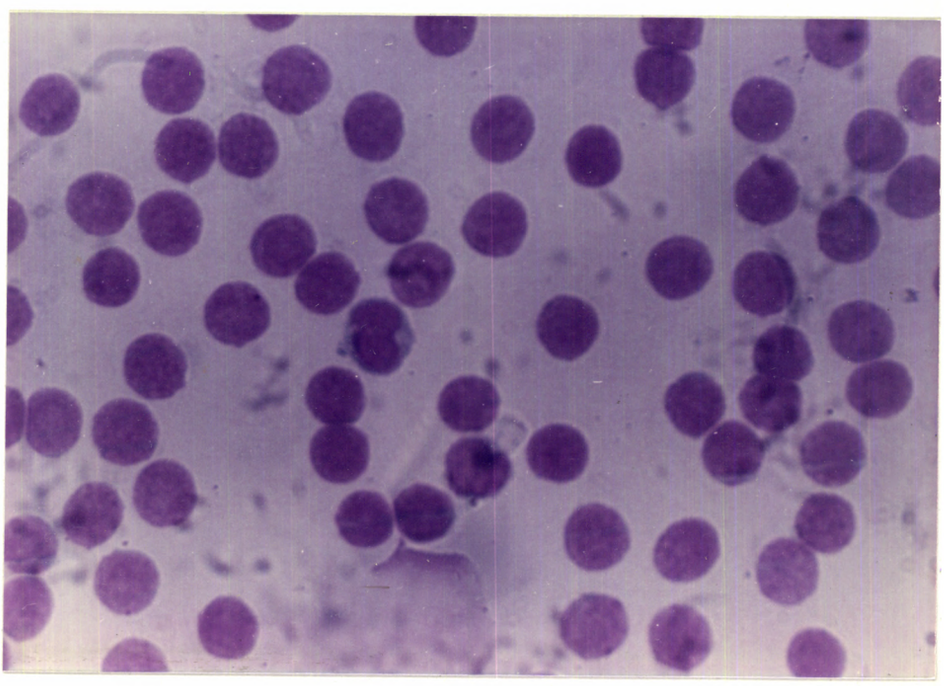


Fig.6f. Acid phosphatase positivity in lymphocyte in C.batrachus.

Table 13. Histochemical procedures performed on the leucocytes of Heteropneustes fossilis

Histochemical Procedures	Lumphocyte	Thrombocyte	Monocyte	Peritoneal/Circulating Macrophages	Melanomacrophage	Neutrophil
PAS	+	++	+++	+++	+++	++++
Sudan B	-	-	++	-	N/P	++++
Acid Phosphatase	+	+	+++	+++	+++	+++
Alkaline Phosphatase	-	-	+++	++	N/P	+++
Peroxidase	-	-	+++	N/P	N/P	++++

N/P - Not Performed

Table 14. Histochemical procedures performed on the leucocytes of Clarias batrachus

Histochemical Procedures	Lymphocyte	Thrombocyte	Monocyte	Peritoneal/Circulating Macrophages	Melanomacrophage	Neutrophil
PAS	+	++	+++	+++	+++	++++
Sudan B	-	-	+	-		+++
Acid Phosphatase	+	+	+++	+++	+++	+++
Alkaline Phosphatase	-	-	+++	++	N/P	++++
Peroxidase	-	-	+++	N/P	N/P	++++

N/P - Not Performed

more than half of the cell. The cytoplasm is usually homogeneous which with Romanowsky stains showed blue colouration. Cytoplasm of both small and larger forms often acquire pseudopodia.

In pronephros smears, large lymphocyte like forms with intense blue cytoplasm and larger nuclei with loose chromatin net work were found. These were absent from the smears of spleen. These may be lymphoblasts. They didn't stain with any of the histochemical methods and they didn't phagocytose any material.

5.4.1.2 Histochemistry (Figures 6e and 6f)

With PAS, lymphocytes were found to take very little staining. With acid phosphatase a slight positive reaction was found. Alkaline phosphatase, peroxidase and Sudan B gave negative results.

5.4.1.3 Phagocytosis

Phagocytosis of any kind of material was found to be absent in cat fish lymphocytes.

5.4.2 Thrombocytes

No inter-specific variations were found between the thrombocytes of the two cat fish species studied. In none of them, the specific azurophilic granule was seen. Thrombocyte nucleus with a wide bridge in between occured in limited numbers. 4 types of thrombocytes were found.

5.4.2.1 Spindle shaped thrombocytes (Figure 7a)

Cells were long, nucleus is also long. Nucleus seen in the middle; cytoplasm usually colourless.

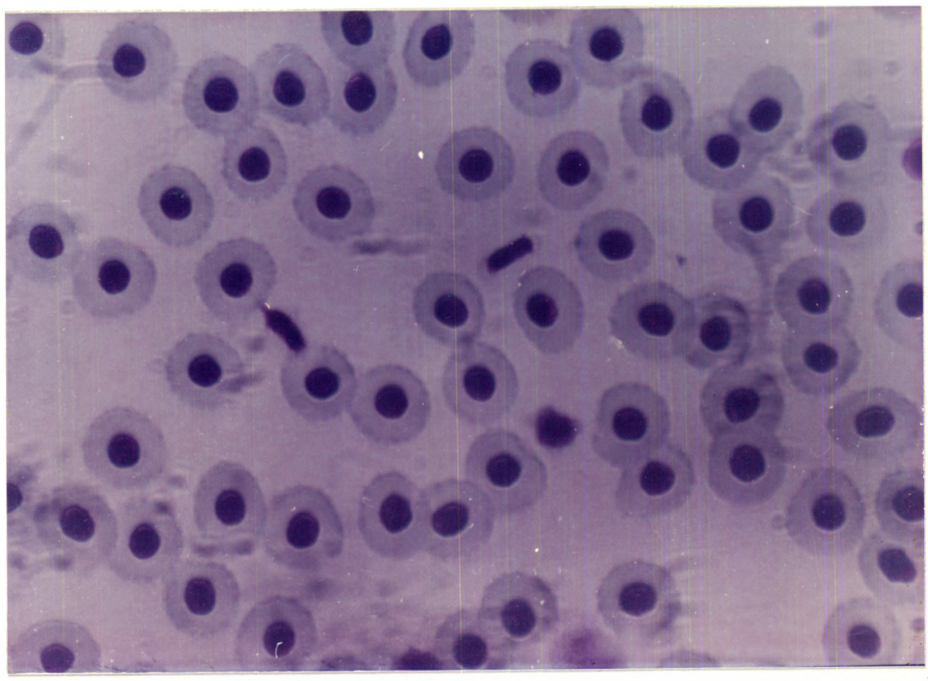


Fig.7a. Spindle shaped thrombocyte in C. batrachus.

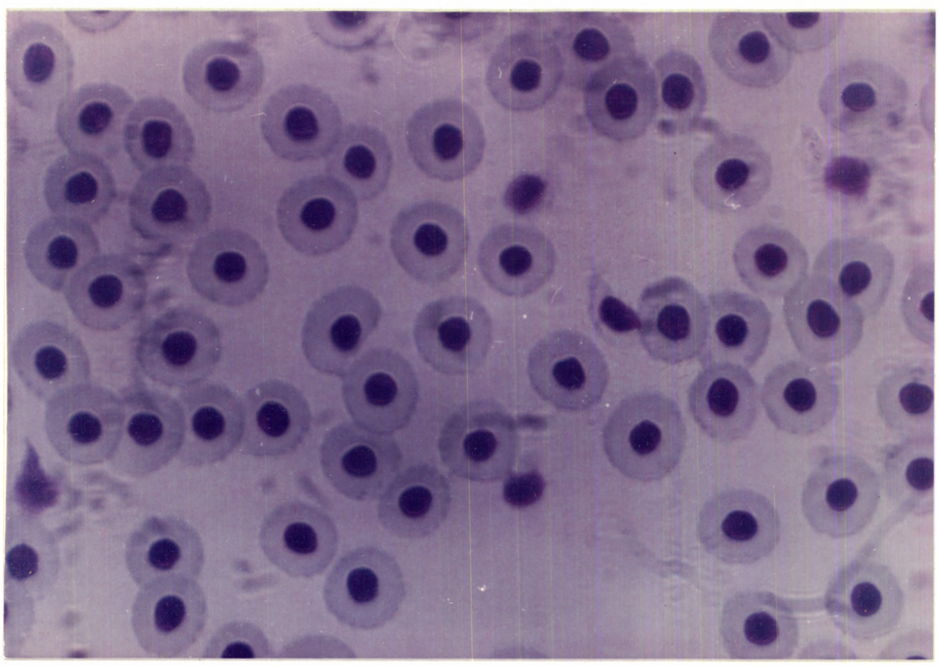


Fig.7b. Tear drop shaped thrombocyte in C. batrachus.

5.4.2.2 Tear drop shaped thrombocytes (Figure 7b)

These were occasionally seen. One end is pointed and the other end round. Nucleus long.

5.4.2.3 Oval thrombocytes (Figure 6a)

Shorter than spindle and tear drop cells. Cytoplasm usually pale blue in colour. Nucleus also oval.

5.4.2.4 Round thrombocytes

Cytoplasm and nucleus round. Around the nucleus a very thin rim of pale blue cytoplasm seen. Sometimes cytoplasm not visible. Smaller than small lymphocyte.

5.4.2.5 Histochemistry (Figure 6e)

Some PAS positive granules were found in the cytoplasm of all thrombocytes. Sudan B was found to be negative. Thrombocytes stained positively with acid phosphatase and negatively with alkaline phosphatase.

5.4.2.6 Phagocytosis

Very small particles of particulate carbon was found within thrombocytes after the fishes were injected with colloidal carbon. No bacteria were found within them.

5.4.3 Monocytes(Figures 8a and 8b)

Monocytes were found in the blood of both H. fossilis and C. batrachus. They were larger cells with mostly vacuolated cytoplasm and large eccentric nucleus. The shape of the nucleus varied from round through bean shaped to horse shoe shaped. The nucleus was stained purple and the cytoplasm

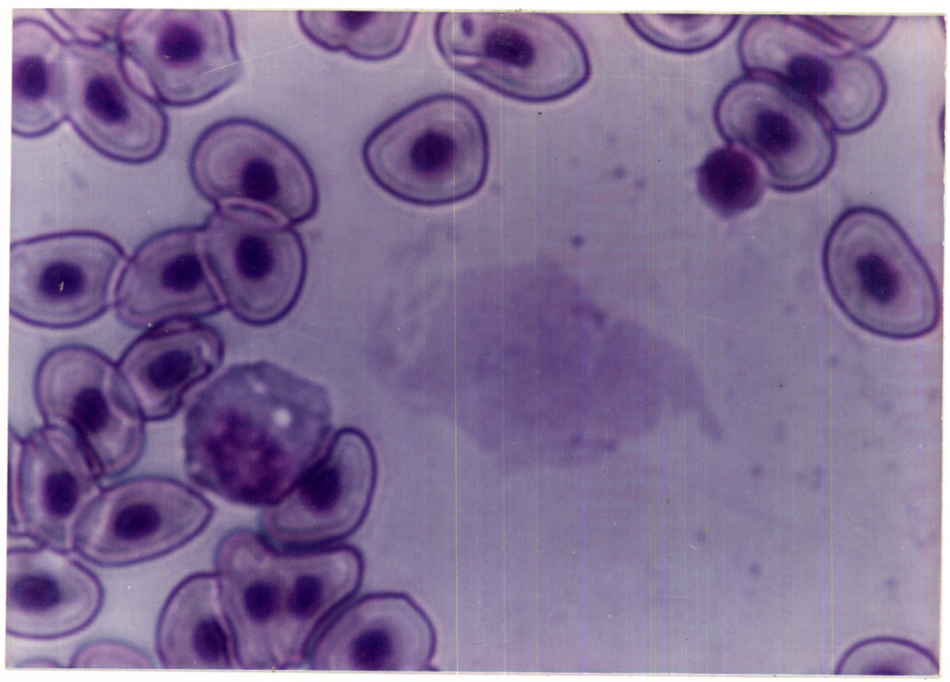


Fig.8a. Monocyte in H. fossilis. Note the cytoplasmic vacuoles and large eccentric nucleus.

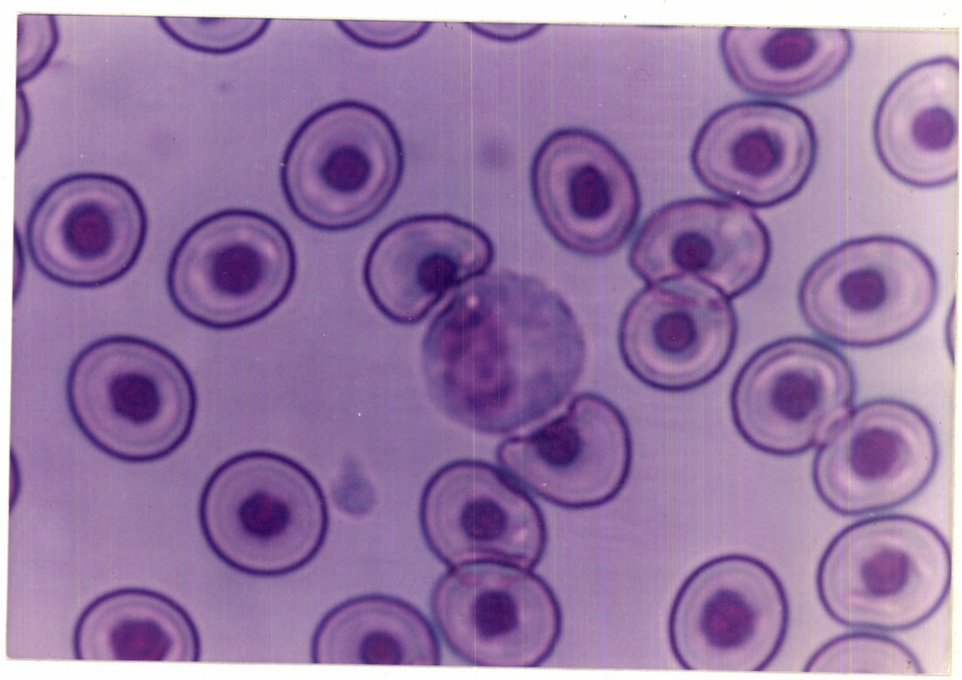


Fig.8b. Monocyte in C. batrachus.

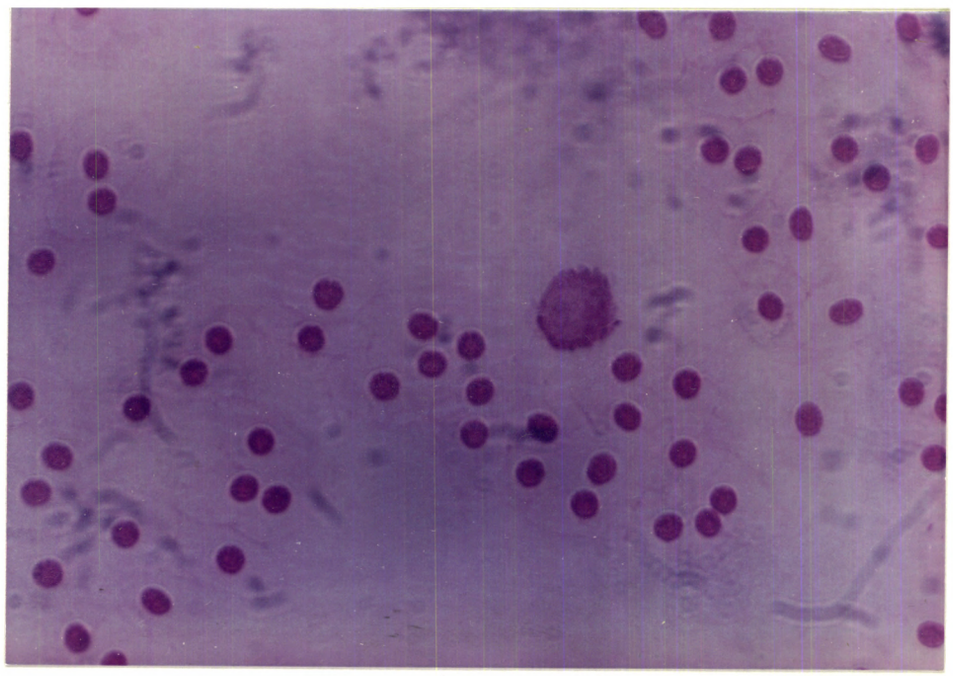


Fig.8c. PAS reaction in monocyte in H. fossilis.

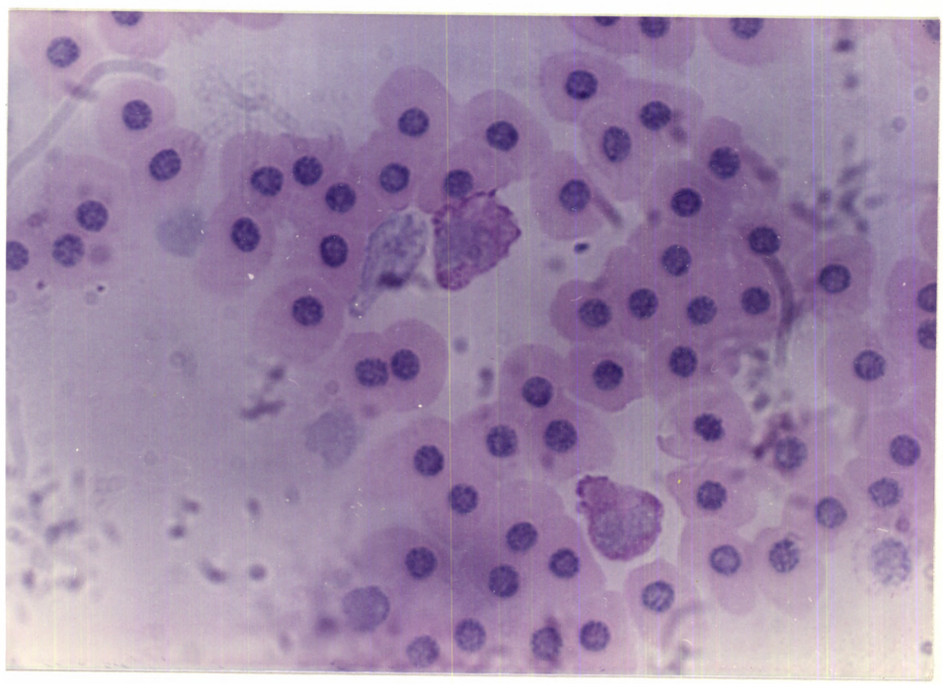


Fig.8d. PAS reaction in monocyte in C. batrachus.

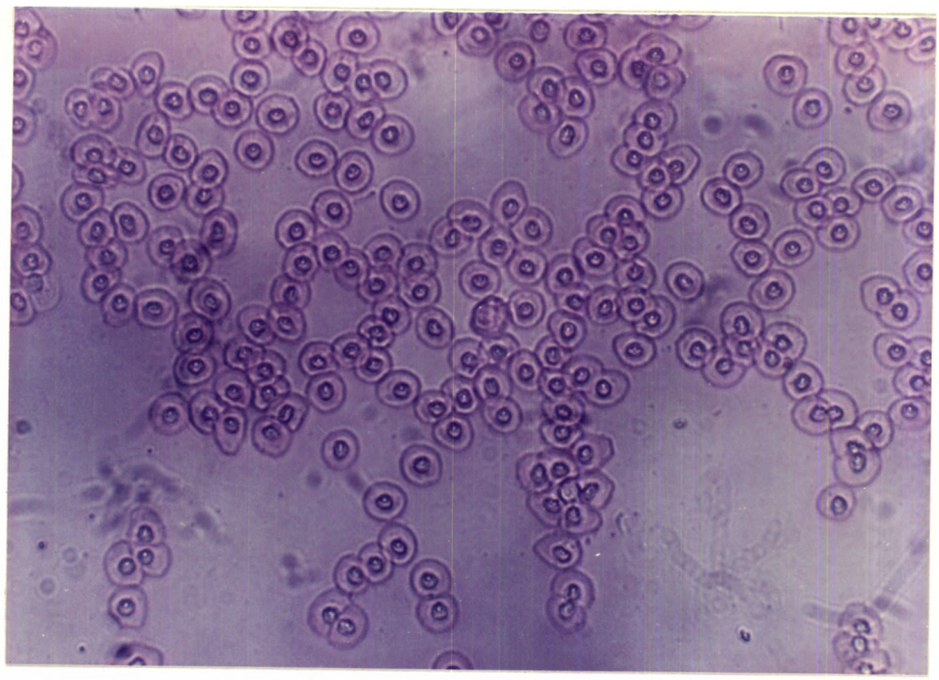


Fig.8e. Alkaline phosphatase in a monocyte. Note the positivity of nucleus and plasma membrane.

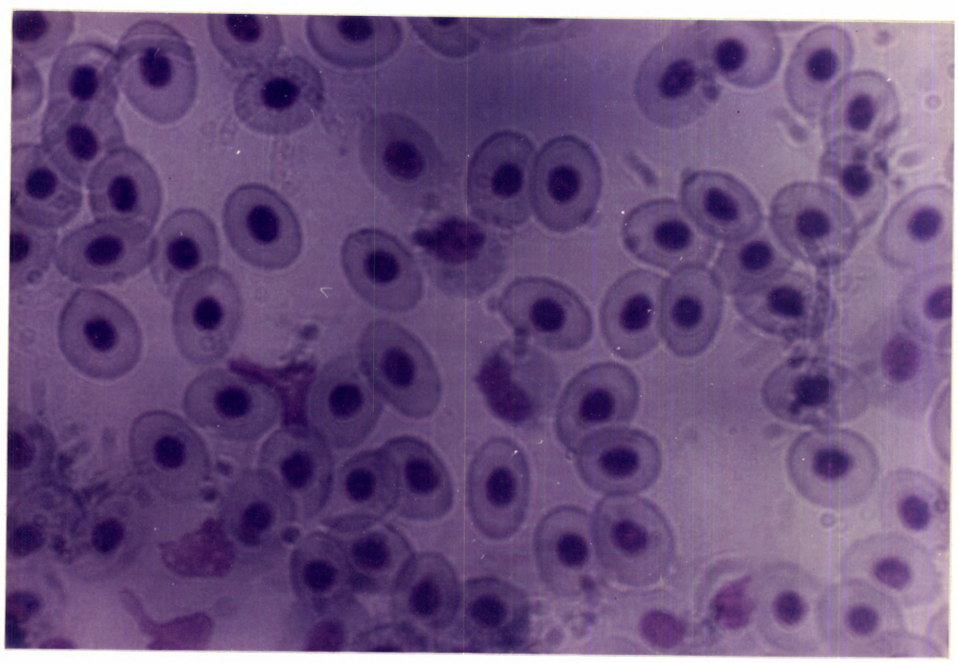


Fig.8f. Phagocytosis : Particulate carbon injested monocyte and neutrophil. Note the very fine carbon particles in the cytoplasm.

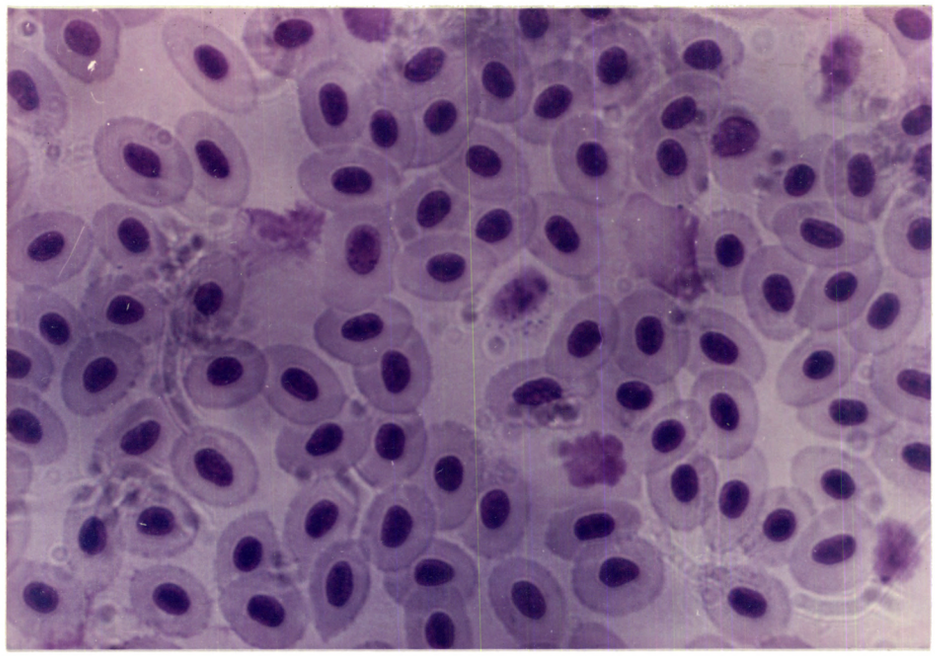


Fig.8g. Phagocytosis : A monocyte with injested bacteria in H. fossilis. Note the bacteria in the cytoplasm.

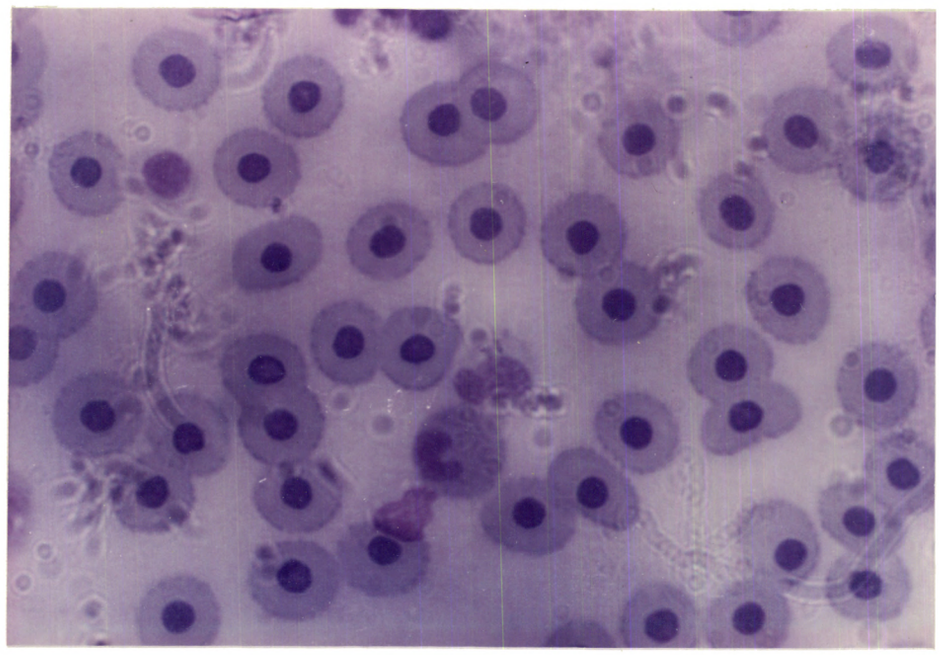


Fig.8h. Phagocytosis : A monocyte with injested bacteria in C. batrachus.

very light grey to bluish grey. In stressed conditions, pseudopodia were found to be produced by the cell. Then very large monocytes with large vacuoles, blue cytoplasm and larger nuclei usually appear in the cytoplasm. These might be monoblasts.

5.4.3.1 Histochemistry (Figures 8c, 8d and 8e)

Stained positive with PAS and Sudan B. Acid phosphatase gave coarse black granules in the cytoplasm. Alkaline phosphatase also gave black granules. With peroxidase, large round bluish black granules were found.

5.4.3.2 Phagocytosis (Figures 8f, 8g and 8h)

After 6 hours of the injection of collodial carbon, they were found in monocytes. The number of monocytes increased in the blood stream after 1 hour. It had taken more than one week for their number to return to the normal level. E. Coli were found within the cytoplasm of monocytes after 24 hours. After the injection of E. Coli, number of monocytes increased in the blood stream.

5.4.4 Macrophages

Two types of macrophages were found in cat fish blood during this investigation. A third type of macrophage was found in peritoneal fluid smears.

5.4.4.1 Circulating macrophages (Figures 9a and 9b)

It is usually a bit difficult to identify the circulating macrophages from monocytes. They usually appear in blood in large numbers under stressed conditions like diseases, presence of parasites and reproduction. Chromatin material is loosely packed in the nucleus. The cytoplasm is vacuolated and sometimes large inclusions are found within. Cytoplasm take a very light

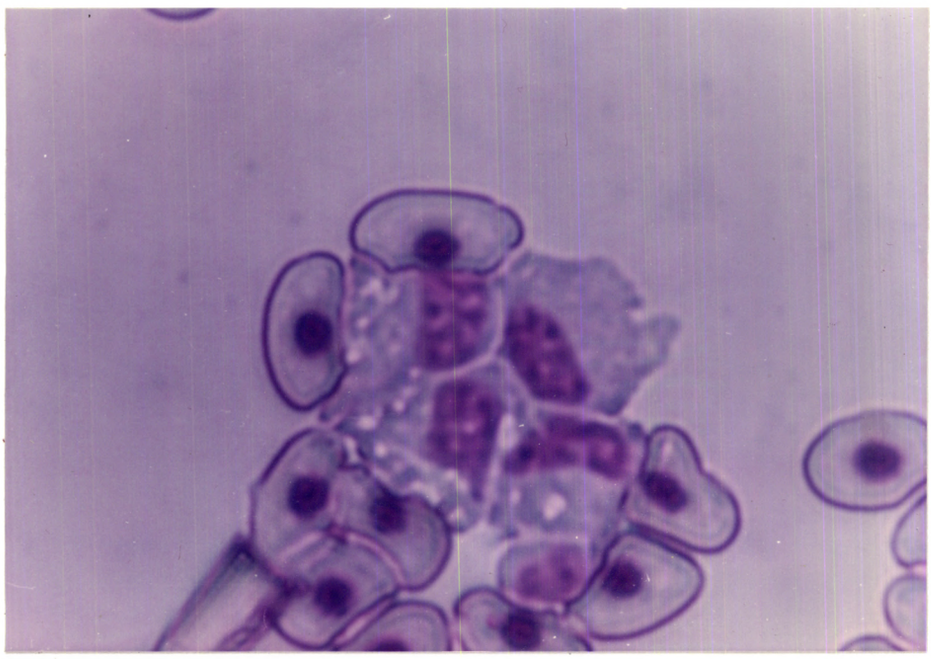


Fig.9a. Circulating macrophages in H. fossilis forming an association. Note the small N/C ratio, characteristic vacuolated cytoplasm and irregular shape.

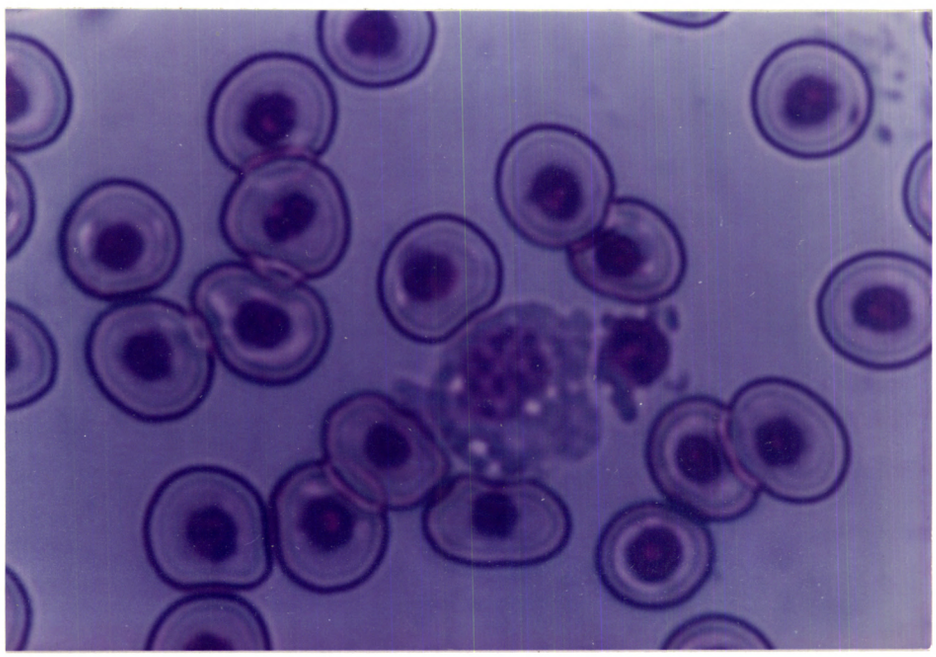


Fig.9b. A circulating macrophage in C. batrachus. Note the small lymphocyte with pseudopodia near it.

stain with Romanowsky stains. Nucleus is relatively small when compared to that of monocytes and it is eccentric. The N/C ratio lower than that of monocyte. In kidney imprints and smears, macrophages occurred in large numbers.

5.4.4.1.1 Histochemistry (Figures 9c and 9d)

Stained positively with PAS and acid phosphatase. With PAS moderate reaction was found as in the case of monocyte. Positive for alkaline phosphatase.

5.4.4.1.2 Phagocytosis (Figure 9e)

Avid phagocytosis was found with carbon and bacteria.

5.4.4.2 Melano-macophages (Figure 6d, 9f and 9g)

These are macrophages containing melanin pigment. They were named melanomacrophages by Roberts in 1974. Only a few description of these cells are available in literature. During this investigation, melanomacrophages were found in the organ smears of kidney and spleen of healthy fishes. But they appeared in blood smears when carbon was injected into the fishes, in spent H. fossilis with trypanosoma infection and in moribund spent C. batrachus male.

Both in smears and imprints, the shape varied from round to irregular. But the nucleus was always eccentric and the cytoplasm pale blue to pale grey with bluish black granules. The nucleus was small, round or indented and stained pale violet or dark violet. Chromatin loosely packed or dense in indented nucleus. N/C ratio was very low. The cytoplasm was usually vacuolated with a few to large number of bluish black coarse granules.

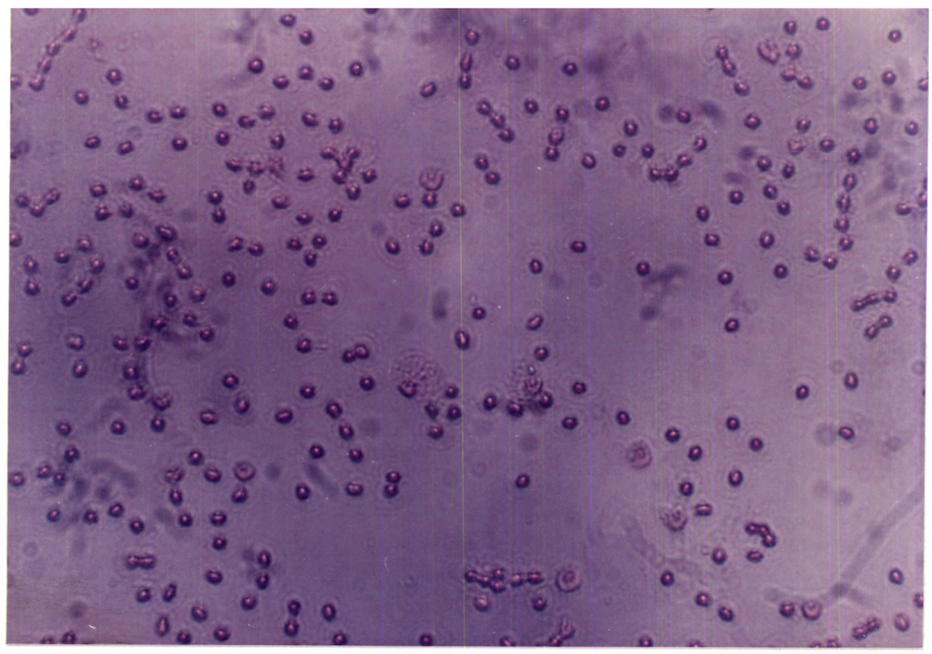


Fig.9c. Circulating macrophages with acid phosphatase positivity in H. fossilis.

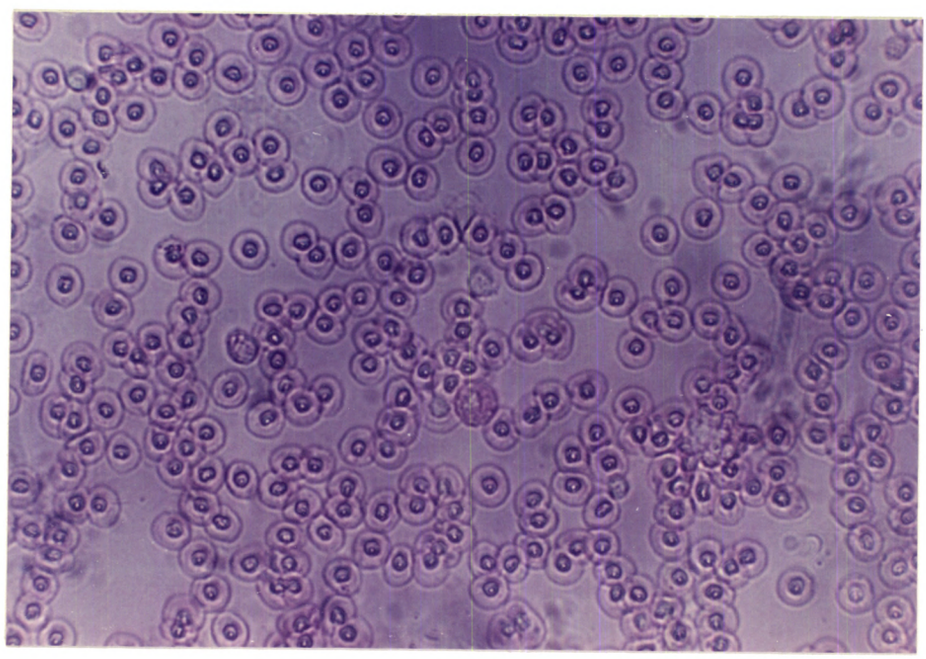


Fig.9d. A circulating macrophage with alkaline phosphatase positivity in C. batrachus.

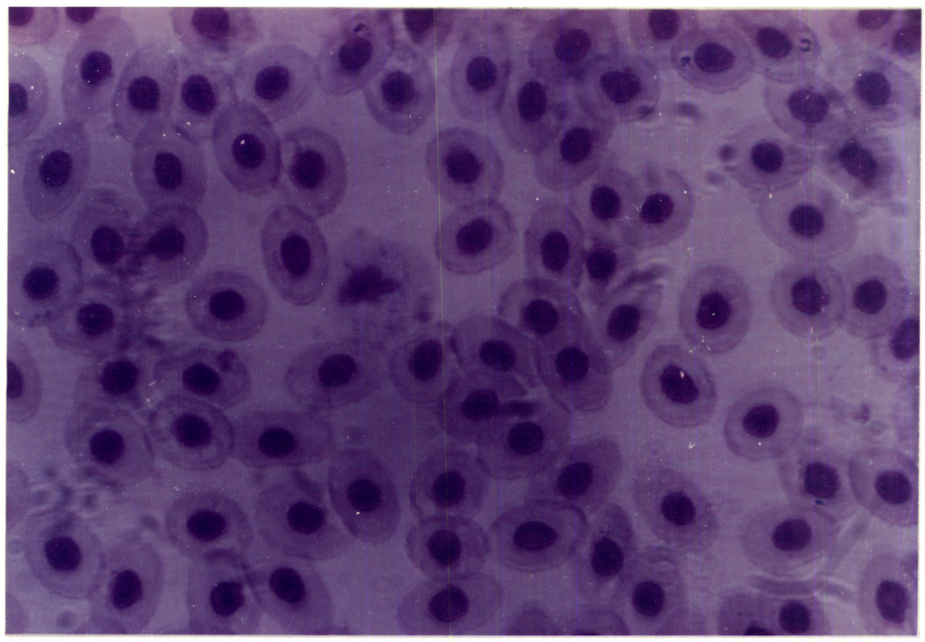


Fig.9e. Phagocytosis : A circulating macrophage with injested bacteria (E. coli) in H. fossilis.

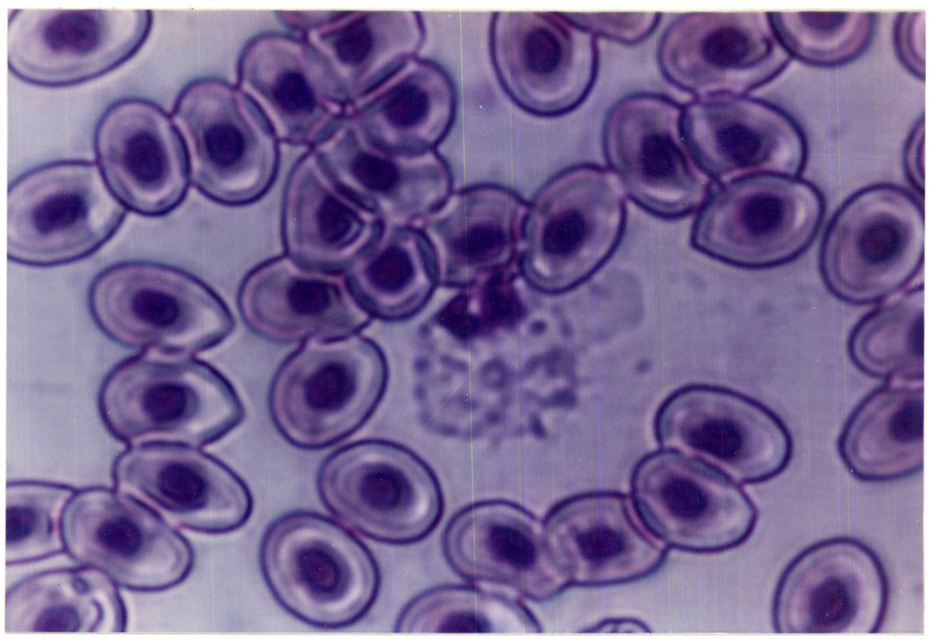


Fig.9f. Circulating melano-macrophage with coarse blue black granules in the blood smear of H. fossilis with trypanosomiasis. Note the indented eccentric small nucleus, large cytoplasmic granules and irregular outline.

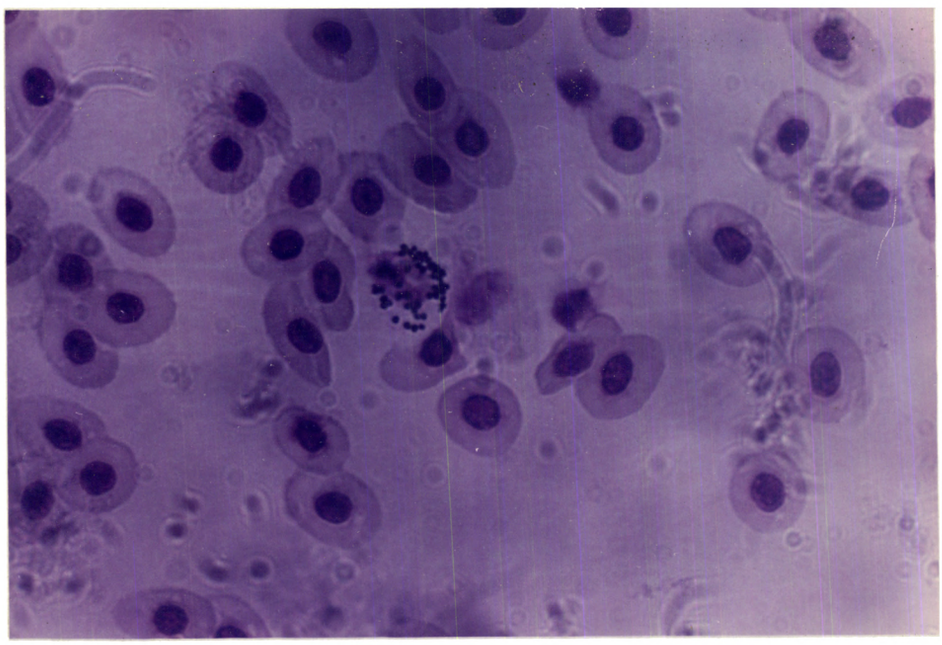


Fig.9g. A circulating melano-macrophage (may be a precursor) in the blood smear of H. fossilis after the injection of E. coli. Note the small round black granules in the cytoplasm and large nucleus.

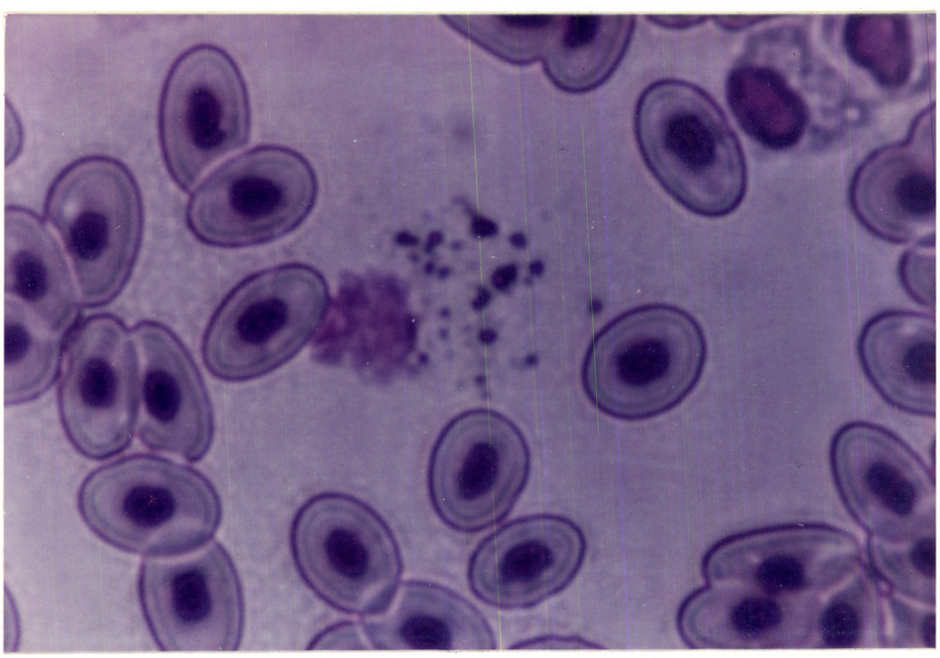


Fig.9h. Phagocytosis : A circulating melano-macrophage in the blood smear of H. fossilis after the injection of carbon. Note carbon in large vacuoles along with blue black granules and the monocyte with carbon.

In the blood when bacteria was injected, another type of melanomacrophage was found. Here the cells were smaller, resembling monocytes, the N/C ratio was larger. The cytoplasm was not much vacuolated; the cytoplasm contained small, round, black granules. In the imprints of kidney, cells resembling monocytes in size and nucleus structure were found to contain coarse blue black granules.

5.4.4.2.1 Histochemistry

Stained moderately with PAS. Showed positive reactions for acid phosphatase. Tests for melanin and haemosiderin (Humason, 1966) sometimes gave positive results showing the presence of them.

5.4.4.2.2 Phagocytosis (Figure 9h)

The melanomacrophages with coarse granules were found to take in carbon particles.

5.4.4.3 Peritoneal macrophages

These were present in peritoneal fluid. They resembled circulating macrophages in their appearance. The N/C ratio was comparatively similar to that of circulating macrophages.

5.4.4.3.1 Histochemistry

Stained positively with PAS and acid phosphatase.

5.4.4.3.2 Phagocytosis (Figure 9i)

They were found to avidly phagocytose foreign materials especially carbon.

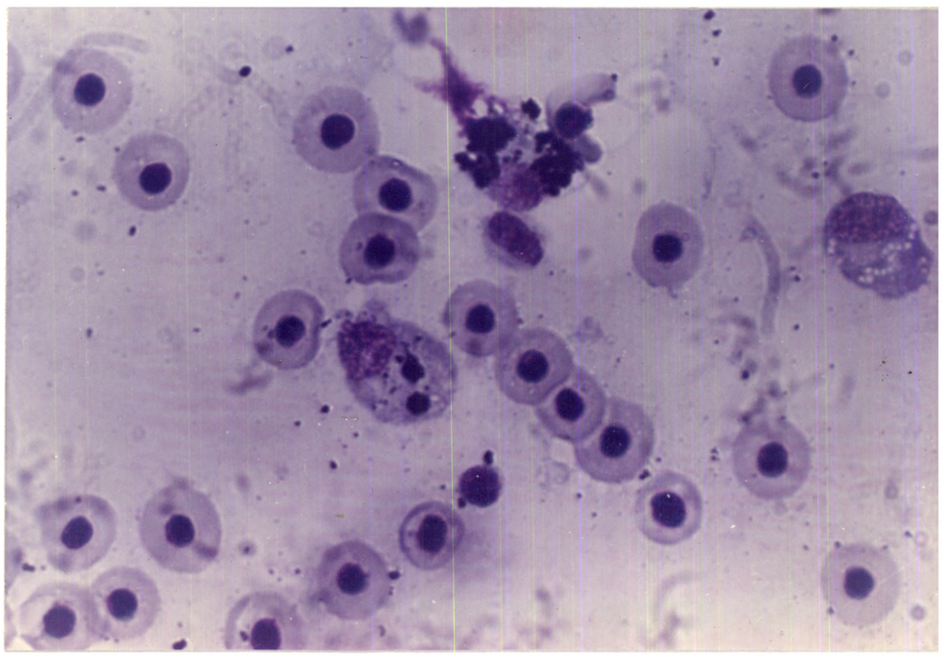


Fig.9i. Phagocytosis : Peritoneal macrophages in C. batrachus with large amount of carbon in the cytoplasm after the injection of carbon.

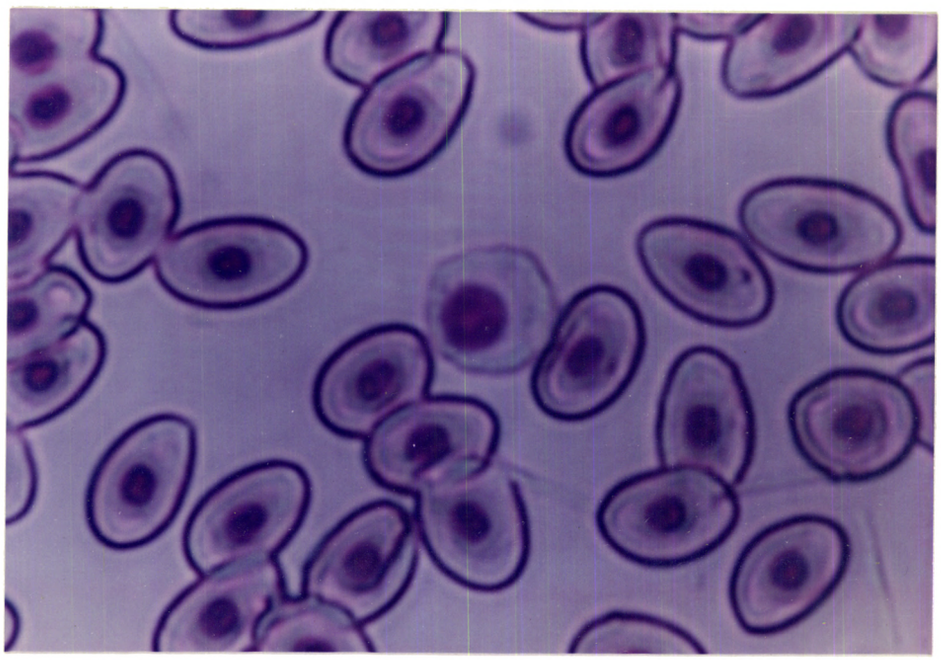


Fig.10a. Neutrophil in H. fossilis. Note the small round nucleus and the blue stained cytoplasm.

5.4.5 Plasma Cell

This is the most rare of all blood cells. It was found in the blood smear of an H. fossilis infected with trypanosome and in some kidney imprints. Cells are either oval or round in shape. Cytoplasm intense blud with a smooth appearance. Nucleus concentric and usually round. Chromatin is arranged in large chunks towards the periphery of the nucleus resembling the spoke of a wheel. Because of its rarity, the cytochemical tests were not done.

5.4.6 Granulocytes (Figures 10a and 10b)

Only one type of granulocyte was observed in the blood of both H. fossilis and C. batrachus. This is a cell almost round in shape with eccentric nucleus. Nucleus stained purple with Giemsa. Nuclear shape varied from round, banded, bilobed, trilobed to tetralobed. But usually it is single lobed i.e. round or oval in shape. The bilobed to tetralobed condition was found during the reproductive stages III to VI of the cat fishes.

Cytoplasm is finely granulated staining from light pink to light blue. Granules are dust like in H. fossilis. In spent fishes, cytoplasm appeared almost colourless and slightly vacuolated. In this condition, most of the neutrophils appeared to be bilobed. In the kidney imprint, two precursor stages of the granulocyte were found, the progranuloblast and granuloblast along with mature granulocyte. The progranuloblast was larger in size with larger nucleus. The granuloblast was intermediate in size and granulocyte the smallest.

5.4.6.1 Histochemistry (Figures 10c, 10d, 10e, 10f,10g,10h and 10i)

With PAS, the cytoplasm stained deep majentha. When the control slide was treated with saliva, no majentha colour developed showing the

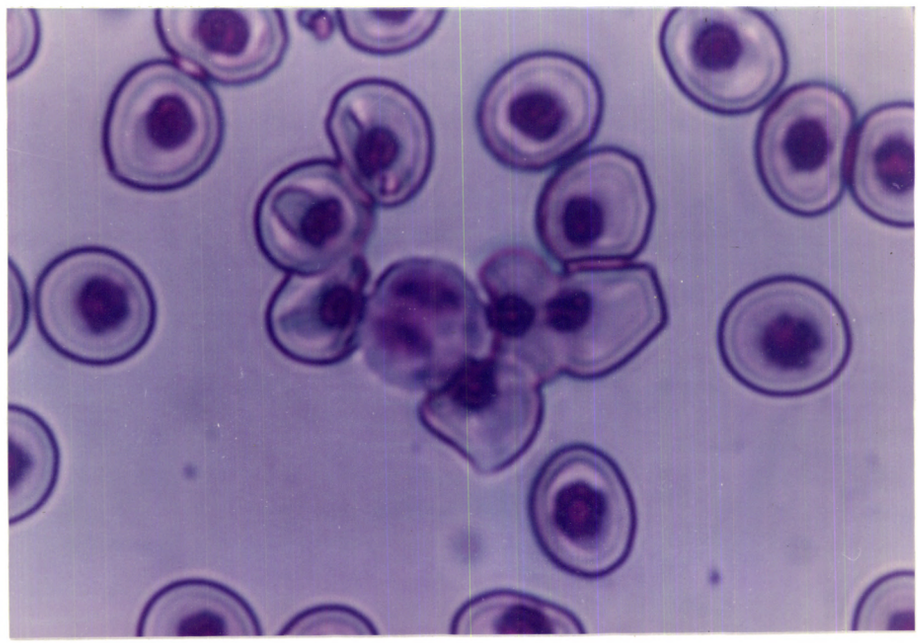


Fig.10b. Neutrophil in C. batrachus. Note the bilobed nucleus.

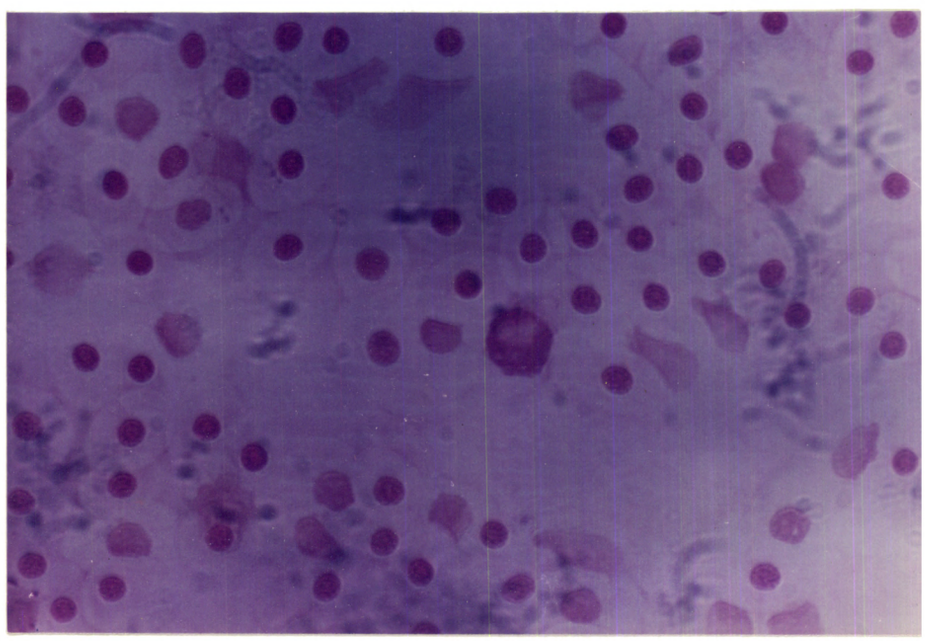


Fig.10c. PAS reaction in the neutrophil in H. fossilis. Note the intense majentha colour of the cytoplasm.

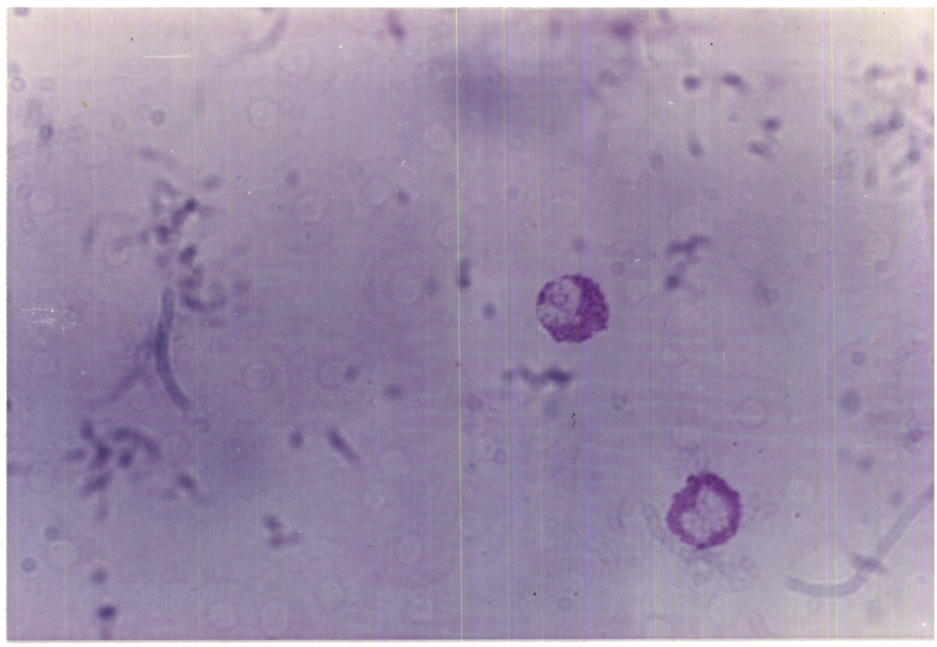


Fig. 10d. PAS reaction in neutrophils in C. batrachus. No counter-staining.

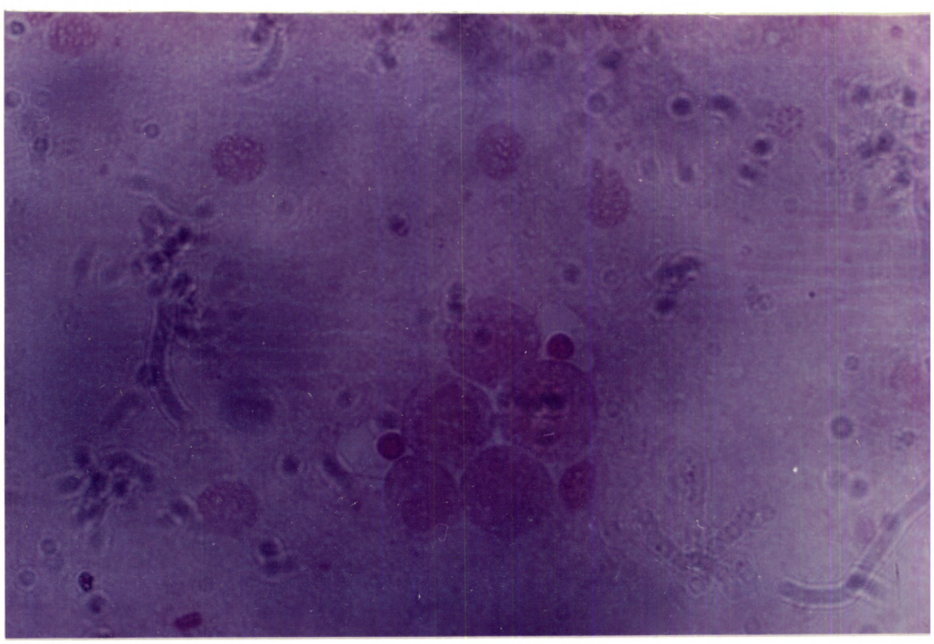


Fig. 10e. PAS positivity in neutrophil series in kidney imprint of C. batrachus. Note granuloblast, progranulocyte and granulocyte.

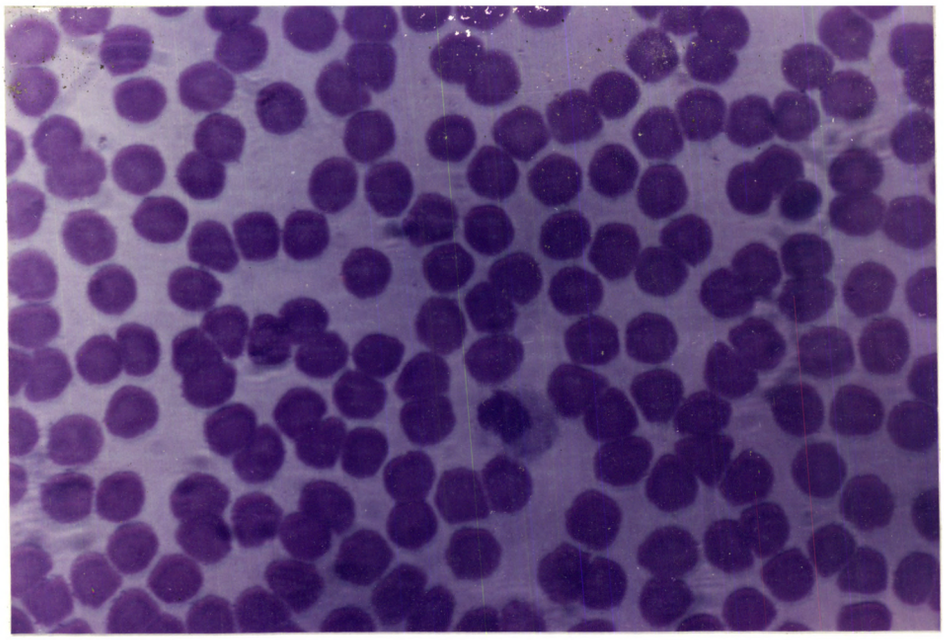


Fig.10f. Acid phosphatase positivity in neutrophil in C. batrachus

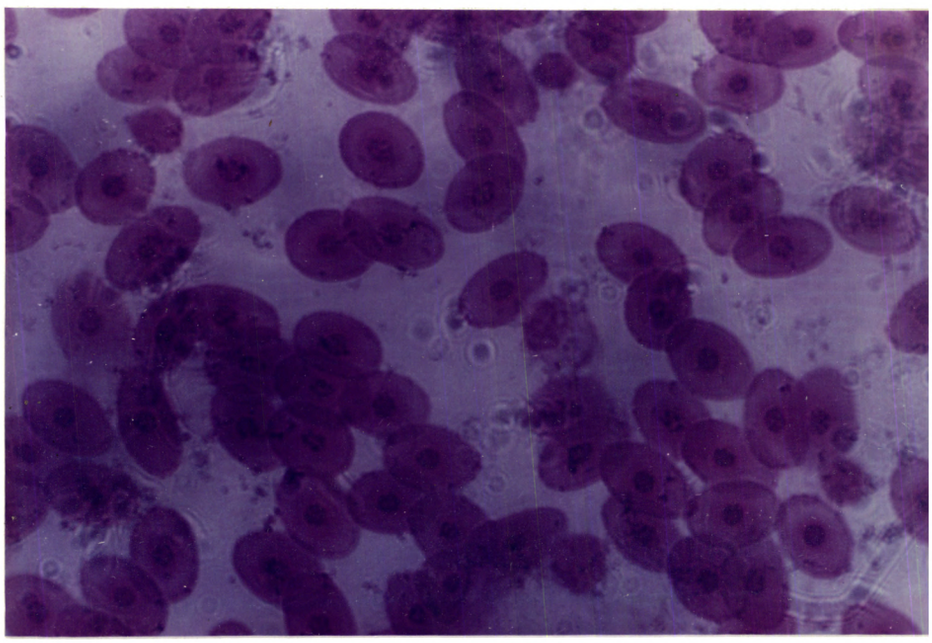


Fig.10g. Alkaline phosphatase positivity in neutrophil in H.fossilis. Note the few large granules in the cytoplasm.

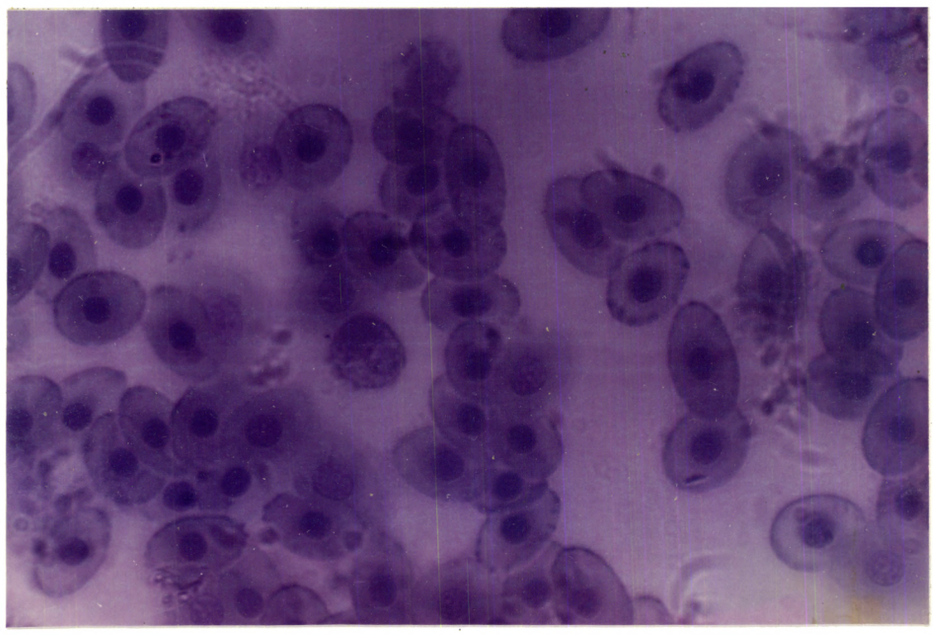


Fig.10h. Sudan B positivity in neutrophil in H. fossilis.

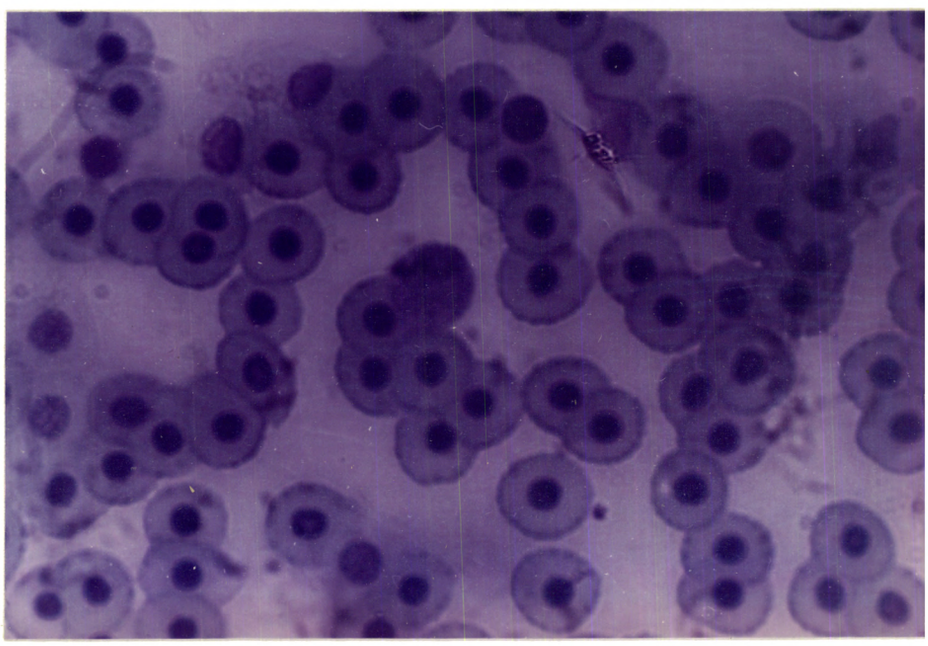


Fig.10i. Sudan B positivity in neutrophil in C. batrachus.

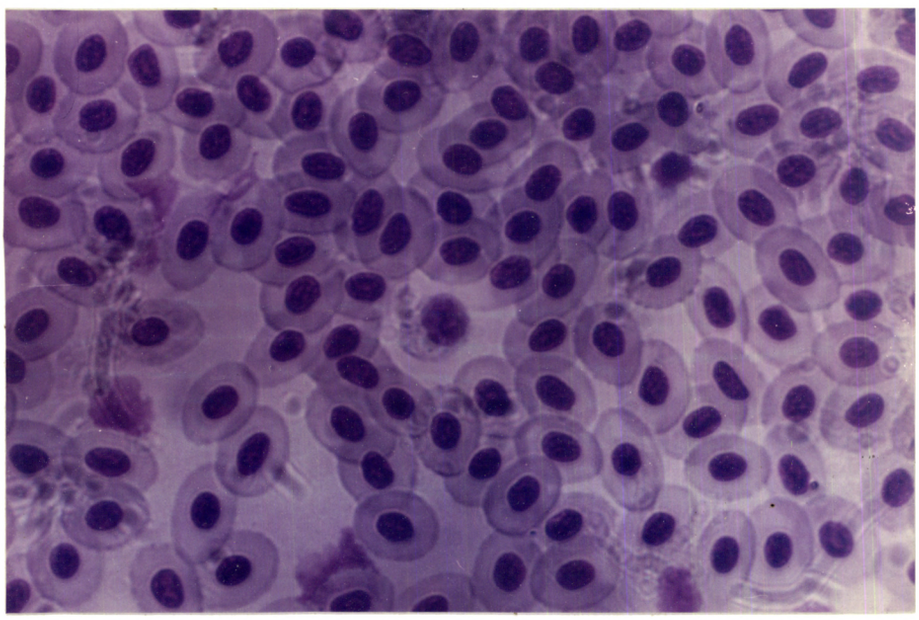


Fig.10j. Phagocytosis : Injestion of bacteria in H. fossilis. Note the bacteria in the cytoplasm.

presence of glycogen in the untreated slide. When kidney imprints were stained for PAS, all stages of the granulocytes were found to be stained. With Sudan Black B, granules of mature neutrophil stained bluish black showing the presence of lipids. With acid phosphatase, small fine black granules were obtained. With alkaline phosphatase too, strong reaction was obtained. With peroxidase, blue black granules were present in the cytoplasm. All these staining properties are characteristics of mammalian neutrophils.

5.4.6.2 Phagocytosis (Figures 8f and 10j)

Very small carbon particles were found to be injested by neutrophils as a result of the injection of particulate carbon into fishes. Neutrophilia was observed in blood and it remained for one week. Small particles of carbon appeared to be scattered in the cytoplasm giving it a blackish tinge. E. Coli cells were found to be injested by neutrophils after 24 hours.

5.5 DISCUSSION

5.5.1 Lymphocytes

The lymphocytes of H. fossilis and C. batrachus are similar in morphological characters to those of other teleosts as well as mammals. The lymphocytes of both species of fishes under investigation gave positive reactions with PAS and acid phosphatase. Pitombeira and Martins (1970), Blaxhall and Daisley (1973) and Ellis (1976) reported the presence of PAS positive lymphocytes in other teleost fishes. Acid phosphatase positive lymphocytes were found by Hine et al. (1986a) in eels and Doggett et al. (1987) in tilapia. Both acid phosphatase and non-specific esterase activity have been observed in the lymphocytes of brown trout (Blaxhall and Hood, 1985) and rainbow trout (Blaxhall and Doggett, 1987).

Three types of lymphocytes were identified, the small, medium and the large. The most commonly occurred type was the small one. There are several similarities in appearance for the small lymphocytes and rount thrombocyte. But the slightly larger size and higher N/C ratio of the small lymphocyte and the denser nuclei of the thrombocyte helps to distinguish them.

The development of lymphocyte is a subject of controversy among scientists and it is still not settled completely. Downey (1909) considered small mononuclear cells found in the kidney of Polyodon spathula to be the precursor of lymphocytes. Lymphocytes were regarded as multi-potential haemopoietic stem cells by early workers like Jordan and Speidle (1924). Jakowska (1956) thought that large lymphoid haemoblast gives rise to erythrocytes and leucocytes. According to Weinreb and Weinreb (1969), the lymphocytes in gold fish can act as stem cells for lymphoid haemoblasts which were considered to be able to produce erythrocytes, granulocytes and lymphocytes. They also suggested that lymphoid haemoblasts can be phagocytic and they were analogous to mammalian macrophages. But it is now proved that lymphocytes are immunocompetent cells which are the cellular basis to adaptive immunity. In his review on teleostean and anuran immune response, Jurd (1985) states that fishes are capable of exhibiting cell mediated immune phenomena including delayed hypersensitivity reactions and mixed lymphocyte reactions. During this investigation lymphocytes were found not to phagocytose any of the injected materials and the histochemical properties characteristic of phagocytic cells were found to be absent in lymphocytes. So these cells can be immunocompetent in cat fishes too.

Thymus is found to be involved in lymphocyte production in addition to kidney. Ellis (1976) found actively dividing lymphoblasts in thymus and kidney mainly and to a lesser extent in spleen. Fange and Pulseford (1985) found large lymphoblasts and three types of lymphocytes in the thymus of angler fish. They also found various types of lymphocyte-macrophage associations. They suggested that certain macrophages might be involved in the maturation of lymphocytes to immunocompetent T cells. However whether distinct populations of T and B lymphocytes exist in fish is open to question and most reports suggest a less specialized function for fish lymphocytes (McKinney et al., 1976; Manning, 1981). But Miller and Tripp (1982) reported that captivity in killifish has a differential effect on a sub-population of lymphocytes analogous to the T helper cells of mammals. According to Ellis (1986), there is much evidence to suggest the existence of distinct sub-populations of lymphocytes in fish homologous to mammalian T and B lymphocytes.

During this investigation too, lymphoblasts were found in organ smears and imprints of pronephros. So it is proposed that lymphoblasts are the precursor cells for large lymphocytes and they gradually condensed to form medium and small lymphocytes.

5.5.2 Thrombocytes

During the course of this study, four types of thrombocytes were found both in H. fossilis and C. batrachus. When blood was used without any anticoagulant for smear preparation, thrombocytes were usually found in groups of two or more numbers. In such cases, some times lone nuclei were also

found like those mentioned by Ellis (1976) in Pleuronectes platessa. The cytoplasmic granules described by Gardner and Yevich (1969) were not present in cat fish blood. Morphological distinction between the round thrombocyte and small lymphocyte is often very difficult. But at the ultrastructural level Cenini (1984) in carp and Thuvander et al. (1987) in rainbow trout showed differences between thrombocyte and small lymphocyte. In thrombocytes, a large number of small vesicles arranged in series or microtubules were found by them.

The main function of the thrombocyte is considered to be the clotting of blood. Srivastava (1969) observed that higher the thrombocyte count, the rapid is the coagulation of blood. Gardner and Yevich (1969) and Wardle (1971) described the part played by thrombocytes during the process of clotting of circulating blood.

Phagocytosis by thrombocytes was described by Yokoyama (1960), Fange (1968) and Thuvander et al. (1987). Ferguson (1976) found carbon particles present in the thrombocytes of plaice after intravenous injection. According to Ellis (1977), the carbon particles found in thrombocytes after intraperitoneal injection of colloidal carbon might be due to mechanical entrapment in the cytoplasmic labyrinthine vesicles which communicate with the environment via stomata in the cell membrane.

In the present investigation too, during phagocytic experiments, carbon particles were found within the cytoplasm of thrombocytes. The presence of acid phosphatase in thrombocytes may be pointing to the phagocytosing capacity of them. The presence of latex bead larger than the labyrinthine vesicle in Thuvander et al.'s experiment in 1987 also tend to show the

phagocytosing capacity of thrombocytes. PAS positive granules described by Ellis (1976) in plaice thrombocytes were found in cat fish thrombocytes too.

The origin of thrombocytes is still an engima. Many workers claim that small lymphocyte is the predecessor of the thrombocyte (Jordan, 1926; Jordan and Speidle, 1924, 1930; Gardner and Yevich, 1969). Ferguson (1976) have shown considerable morphological differences between lymphocytes and thrombocytes in plaice. Considering the lymphocyte position as immuno-competent cell, it seems improbable that it will give rise to another type of cell with entirely different function i.e. clotting of blood.

5.5.3 Monocytes

The reticulo-endothelial system (RES) of teleost fish are considered to be comprised of the premonocyte of the haemopoietic organs, the monocytes of the blood and lymph, the macrophages of loose connective tissue and the free and fixed macrophages of the atrial lining of the heart (Ellis et al., 1978). The criteria to be included in the RES are high phagocytic activity and capacity to concentrate and segregate such phagocytosed material. The monocytes of H. fossilis and C. batrachus showed high phagocytic activity when fishes were injected with carbon and bacteria. Doggett et al. (1987) also found the cell to be phagocytic injesting both carbon and bacteria. They were found to form aggregates with macrophages in smears of stressed fishes. These cells were PAS, acid and alkaline phosphatase positive. Very few peroxidase positive granules were found in some of them. The results of Sudan B staining were erratic. Mostly it was positive.

In the normal cat fishes, the number of circulating monocytes is very small. But when injected with carbon, their number increased in blood circulation within 24 hours. Ellis (1976) also found the same after 18 hours in Pleuronectes platessa.

Many authors deny the presence of monocytes in blood (Weinreb and Weinreb, 1969; Klontz, 1972). They called these cells as macrophages. McCarthy et al. (1973) could not find any monocyte in the blood of rainbow trout. But Thorpe and Roberts (1972) reported the migration of monocytes and neutrophils into an Aeromonas infected tissue from adjacent blood vessels. In Indian fresh water fishes, monocytes were reported by Srivastava (1968b), Pandey et al. (1976), Pandey and Pandey (1977) and Mahajan and Dheer (1979). So by their histochemical and functional properties and their morphological resemblance to mammalian monocytes, it is presumed that monocytes are present in cat fish blood.

Some blood cells which resembled monocytes but with a bluer vacuolated cytoplasm which contained a larger nuclei are considered to be monoblasts. They were also present in kidney imprints. These should be precursor cells to monocytes.

5.5.4 Macrophage

Van Furth et al. in 1972 put forward a new classification of phagocytic cells. They introduced the term Mononuclear Phagocytic System (MPS) which was comprised of phagocytic cells derived from monocytes. Monocytes are regarded as partially differentiated end cells (Gottlieb and Waldman, 1972). They reach their full maturity when they leave circulation and enter the tissues where they develop into macrophages (Ellis, 1977).

Macrophages in fishes are reported to be not normally found among other leucocytes in blood. Phagocytosis of carbon particles in the cells of heart, mesentary, peritoneal cavity, kidney and spleen were observed in teleosts (Mackmull and Michels, 1932; Ellis et al., 1976). In the present investigation, circulating macrophages were found in blood in the spent fishes as well as in fishes stressed physically. In healthy immature fish, they were absent from blood.

In fishes, they might be acting as scavenger cells due to their avid phagocytosis.Chiller et al.(1969) found macrophages from the kidney and spleen of rainbow trout immunised against sheep erythrocytes would form rosettes suggesting the presence of surface antibody in them.

In 1923 Jolly for the first time mentioned melano-macrophage cells as macrophages containing greenish brown pigment. The first detailed study of these structures were published by Roberts (1975). Ellis (1976) demonstrated that intra-peritoneally injected carbon eventually homed to melano-macrophage centres. Roberts (1975) proposed the name melanin-macrophage centres, because of the ultrastructural resemblance of these cells to macrophages, their high pigment content and their tendency to aggregate to form distinct nodules. Oguri (1976) reported the pigment granules present in these cells to be melanins based on its histochemical properties. Three pigment types are normally observed, melanin, haemosiderin and the wear and tear pigment, lipofuscin. The melanin observed in fish melano-macrophages is generally present as discrete granules that resemble integumental melanosomes (Agius, 1985). In H. fossilis too such cells were found, the only difference was that the cells resembled a monocyte more than a macrophage. It may be that

these cells are precursor cells to melano-macrophages. These cells appeared when the fishes were injected with bacteria.

Another type of melano-macrophage found in the cat fishes was with coarse blue black granules. They were usually very large cells with smaller nuclei, either round or indented. These were found in blood of cat fishes injected with carbon as well as in fishes infected with trypanosomes. A precursor cell to this type of melano-macrophage was found in kidney imprint. This round cell was large with blue black coarse granules and larger nuclei. The presence of two types of precursor cells show the possibility of the existence of more than one type of melano-macrophage endowed with different functions, i.e. pigment in the granules of the first mentioned cell could exert a bactericidal effect as suggested by Edelstein (1971) and the second type of cell with coarse granules may have reached in circulation as a scavenger for the collection of carbon particles and the cell debris and haemoglobin break down products resulted from trypanosome infection. In these cells peroxidation of unsaturated lipid cellular components to lipofuscin (Agius, 1985) might take place to be eventually transported to the melano-macrophage centres. Erythrocytes, presumably effete, have been observed to lie both singly and in clumps within melano-macrophages in tilapia Oreochromis niloticus/ O. aureus hybrids (Agius and Agbede, 1984).

The hypothesis of having different types of melano-macrophages with functional difference has been supported by the works of Agius (1979, 1981).

Large macrophages are normally found in small numbers in peritoneal fluids (Ellis et al., 1976). They do resemble circulating macrophages except

that they are more ovoid in shape. When carbon was injected into the peritoneal cavity, these cells were found to be laden with carbon after 24 hours. By then their number was considerably increased. It seems that the major pathway by which peritoneally injected carbon removed was through these macrophages.

5.5.5 Plasma cells

Typically they possess an eccentric nucleus with prominent nucleolus and pyroninophilic cytoplasm packed with rough surfaced endoplasmic reticulum (Ellis, 1977). They were identified in electron microscopic studies by Boomker (1981b) and Cenini (1984). In this investigation, round cells with eccentric nucleus and intense blue cytoplasm and nucleus with chromatin granules arranged in clumps around the nucleoplasm were found. They should be plasma cells. In blood smears they were found only in cat fishes infected with trypanosomes. They are responsible for the production of humoral antibodies in birds and mammals. But their function in fishes is yet to find out.

5.5.6 Granulocyte

In H. fossilis and C. batrachus only a single type of granulocyte was found. Morphologically, the cells are usually rounded with an eccentric nucleus when it is single lobed; sometimes more than one lobe are found. In both cat fishes multilobed condition was observed during the breeding season. The granules are dust like in H. fossilis. The PAS, Sudan B, acid phosphatase, alkaline phosphatase and peroxidase positivity of these cells in both species are characteristic features of mammalian neutrophils.

The staining characteristics of neutrophil seems to vary in different species. The granules are bluish in H. fossilis and pinkish in C. batrachus. But they did'nt stain with Giemsa stain in rainbow trout (Finn and Nielson 1971). Kelenyi and Nemeth (1969) found the neutrophils of Tinca tinca to be peroxidase negative. Hine et al. (1986b) also found peroxidase negative neutrophils in New Zealand eels. He attributed it to their immaturity (Hine et al., 1986a). But peroxidase positivity was found for the neutrophils of brown trout and roach (Catton, 1951), atlantic salmon (Conroy, 1972), brown trout (Blaxhall and Daisley, 1973), Plaice (Ellis, 1976), Scyliorhinus canicula (Parish et al., 1986) and tilapia (Doggett et al., 1987).

Blaxhall and Daisley (1973) also found these cells to be positive for Sudan Black B and PAS and Doggett et al. (1987) found them to be positive for Sudan B, PAS, Acid and alkaline phosphatase and non-specific esterase.

In H. fossilis and C. batrachus the granulocyte was found to phagocytose carbon and bacteria. In plaice, Ellis (1976) couldn't demonstrate phagocytosing activity for neutrophil. But these cells were found to be phagocytosing in tilapia (Doggett et al., 1987) plaice (MacArthur et al., 1984), gold fish (Lester and Budd, 1979), whiting (Elarifi, 1982) and in roach (Hoole and Arme, 1982). So it is only just to call these cat fish granulocytes, neutrophils.

In mammals, the neutrophil is a major phagocytic cell. In fish too Finn and Nielson (1971) reported migration and phagocytosis by neutrophils. Host cells from the brook trout resembling neutrophils, macrophage and pigment cells are reported to phagocytose the bacterial kidney disease organism (Young and Chapman, 1978). Ellis (1977) suggested that chemotaxis could be a feature

of fish neutrophils just like their mammalian counterparts. The phenomenon of neutrophilia under stress condition is another characteristic feature of fish.

In kidney imprints, granuloblasts were present in large numbers. They were bigger than neutrophils with larger nuclei. But their staining affinities were similar so that of adult granulocytes. They stained positive for PAS and acid phosphatase.

So it is concluded that the morphological, functional and ontogenic characters can be utilised in the identification of leucocytes in cat fishes and the leucocytes of H. fossilis and C. batrachus are similar in appearance and function. This may be due to their shared habitat and phylogenetically related position.

CHAPTER - VI

HAEMATOLOGY OF REPRODUCTION IN RELATION TO ENVIRONMENTAL FACTORS

6.1 INTRODUCTION

The annual breeding rhythm exhibited by most of the teleosts is controlled by intrinsic factors like hypothalamo-hypophysial hormones and extrinsic factors like temperature, photoperiod, rainfall etc. In North India, in Heteropneustes fossilis and Clarias batrachus, gonadial recrudescence parallels increase in ambient photoperiod and temperature (Lamba et al., 1983; Singh and Singh, 1987).

Reproduction in itself is a stressful event intended for the propagation of species. Any change in homoeostatic mechanism is known to affect the blood picture of the species concerned. So it is only to be expected that the physiological changes brought about by the maturation and final release of gonadial products in fish should be reflected in its blood picture. Houston (1980) acknowledged that an increase in environmental temperature imposes a variety of stresses upon the teleost, not least among them are those associated with respiratory requirements resulting in responses at the haematological level.

H. fossilis and C. batrachus are two tropical fresh water species which are seasonal spawners whose eggs and ova mature in an extremely inimical environment when the temperature reaches the maximum and the period of day length the longest. Then water bodies in which they live mostly dries up resulting in oxygen deficiency. With the onset of south west monsoon which flood the rivers and ponds they start spawning. Only very little is

known about the haemostatic mechanisms maintained by the fresh water fishes during breeding period. So it seemed worthwhile to investigate whether there was any change in haematological factors like RBC number, haemoglobin content and haematocrit and the derived factors like MCV, MCH and MCHC during various maturity stages in H. fossilis and C. batrachus, which live in such extreme environmental conditions.

6.2 REVIEW OF LITERATURE

In fishes,haematological constituents have been observed to change with season (McKnight, 1966; Gardner and Yevich, 1969; Bridges et al., 1976; van Vuren and Hattin gh, 1978; Mahajan and Dheer, 1979; Srivastava and Agrawal, 1981). Varying ecological conditions and breeding are two of the reasons attributed to it.

The effect of temperature on different haematological parameters in fishes has been reported by many investigators. Acclimation of tilapia to high temperature caused increase in RBC count, WBC count, Hb concentration and PCV while low temperature resulted in a depression in these values (Farghaly et al., 1973). De Wilde and Houston (1967) reported that trout acclimated to low temperature are characterized by relatively low cell counts and a decrease in haematocrit and Hb levels. The MCV tended to be relatively high while MCHC was some what below normal at high temperature. But fishes adopted to 21°C typically had larger numbers of smaller red cells, more Hb and higher MCHC. When Heteropneustes fossilis was transferred from higher to lower temperatures, there was a fall in RBC and Hb values. In Schizothorax plagiostomus, Bhatt and Singh (1985) obtained lower values for RBC, Hb, PCV and MCHC, when fishes were transferred

from 25.5 to 17°C. But in Anguilla anguilla, Kawamoto (1929) reported increase in the number of erythrocytes in winter months. Antony (1961) found an inverse relationship between RBC count and temperature in Carassius auratus. The effect of temperature on haematological parameters in fishes have been reviewed by Houston (1980).

Photoperiod has been demonstrated to be of considerable importance in the timing of functions in animals. Vinberg and Khartove (1953) have shown that illuminated young carp consumed more oxygen than young carp held in darkness. Salmon subjected to reciprocal photoperiod regime from early March has significantly lower standard rate of oxygen in sea in summer than those animals held under a simulated photoperiod regime (Withey and Saunders, 1973). Whitford and Hutchinson (1965) as cited by Choubey et al. (1976) found that at 15°C Amblystoma maculatus has a significantly higher Vo_2 when acclimated to a 16 hour photoperiod than to an 8 hour photoperiod. Increased oxygen consumption may cause increase in haematological parameters. H. fossilis kept under illumination for three months showed significantly higher Vo_2, RBC count and Hb concentration than those subjected to complete darkness (Choubey et al., 1976). But the effect of photoperiod may require or may be modified by interaction with other environmental factors such as temperature (Wolfson, 1964).

Haematological parameters are observed to be fluctuated during the breeding period (Fourie and Hattingh, 1976; Pandey, 1977; Joshi,1982b; Murray, 1984; Pickering, 1986). Thomas et al. (1969) found significantly higher RBC counts in two year old male chinook salmon compared to immature finger lings. Pickering (1986) reported that sexually mature male brown trout had

more erythrocytes in their blood than did immature fish under identical conditions. Naumov (1956) as quoted by Blaxter and Holliday (1963) found that the haemoglobin content of the blood of Clupea harengus is the highest during early maturation but falls sharply at spawning and in the spent stage. But Sindermann and Mairs (1961) reported that haemoglobin in the blood of Alosa pseudoharengus didn't vary between pre and post-spawned fish. The numbers of red and white blood cells do not correlate with the maturity of a number of fresh water fish (Smirnova, 1962). Fourie and Hattingh (1976) suggested that increases in red cell fragility together with other haematological parameters could be used as an indication of breeding season in carp. Circannual variation in haematological and biochemical components in relation to certain eco-physiological conditions were studied in Rita rita by Joshi (1982b). Detailed study of haematology in relation to maturity stages are lacking in literature.

A fluctuation in leucocyte counts were observed in relation to sexual maturity in teleosts (Colgrove, 1966; McCarthy et al., 1975; Pickering, 1986). Pickering (1984) has shown that oral administration of cortisol causes a lymphocytopaenia in immature brown trout.

In mammals, the androgen testosterone stimulates erythropoiesis (Mirand et al., 1965; Alexanian, 1969). It is not proved beyond doubt that such a mechanism exists in fishes. Slicher (1958) showed that hypophysectomy caused a reduction in the number of circulating erythrocytes in Fundulus heteroclitus and that this effect could be reversed by treatment with methyl testosterone. Garavini and Martelli (1978) observed that testosterone stimulates the erythropoietic tissue in the cat fish, Ictalurus melas.

6.3 MATERIALS AND METHODS

6.3.1 Collection, transportation and maintenance of fishes

A pilot study was done during the year 1988 to determine the period of gonadial recrudescence in H. fossilis and C. batrachus. Accordingly, adult specimens of H. fossilis (length 17-20 cm and weight 40 to 65 gms) and C. batrachus (length 20.5 to 26 cm and weight 100 to 130 gms) were collected from fresh water bodies around Cochin every month from January to August, 1989. Transportation and maintenance of fishes were done as described in materials and methods (3.2).

6.3.2 Sampling procedure and determination of haematological parameters

Sampling of blood was done as in 3.3. The whole blood was analysed for RBC, Hb and PCV as described in 3.6.

6.3.3 Determination of maturity and GSI

After collecting the blood in heparinized vials, abdominal cavity was opened and the maturity stages were determined as described in 3.4 and gonadosomatic index was determined as in 3.5.

6.3.4 Collection of environmental data

Environmental temperature, period of day length and rainfall from January to August 1989 were obtained from weather reports published by Meteorological Station, Trivandrum. The terms, period of day length and photoperiod are used in the same sense.

6.3.5 Computation and presentation of data

Data for all parameters were expressed as means $\pm$ standard deviation. All values for individual parameters were subjected to Two Way Analysis

of Variance (ANOVA).

The model assumed being

$x_{ijk} = \mu + \alpha_i + \beta_j + \delta\tau_{ij} + \epsilon_{ijk}$

x_{ijk} = the k^{th} observation in the i^{th} sex and the j^{th} stages;

μ = overall effect

α = effect of i^{th} sex

β_j = effect of the j^{th} stage

$\delta\tau_{ij}$ = the interaction between sex and stages

ϵ_{ijk} = the error

Data are presented in the form of tables and line graphs.

6.4 RESULTS

6.4.1 Environmental factors during 1989 (Table 15)

Mean monthly temperature, period of day length and rainfall from January to August 1989 are given in Table 10. Mean temperature was found to be the minimum in January and maximum in April. Period of day length gradually increased from January to reach the peak in June and then gradually decreased. Rainfall was scanty in January and February, a little in March and April to have the maximum down pour in June, seconded by July. Then it decreased.

6.4.2 Gonadial cycle

6.4.2.1 Heteropneustes fossilis (Table 16 ; Figure 11a)

In male, GSI was the lowest in January to be increased gradually to reach the peak in June and July in the stage V. Then it decreased.

Table 15. Environmental factors at Cochin, Kerala, India from January to August, 1989

Months	January	February	March	April	May	June	July	August
Maximum Temperature °C	31.8	31.4	32.28	33.84	32.29	29.7	29.37	31.71
Minimum	22.3	22.7	24.34	25.45	25.61	23.37	24.03	23.48
Period of day length hrs	11.28	11.41	11.58	12.16	12.29	12.37	12.33	12.21
Rainfall mm	2.13	0.097	40.00	95.00	269.00	680.00	555.00	316.00

Table 16. GSI in Heteropneustes fossilis from I to VI maturity stages (Number of fishes 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Male	Mean	0.117	0.256	0.378	0.523	0.618	0.288
	± SD	0.014	0.022	0.010	0.023	0.010	0.104
	Range	0.105-0.138	0.231-0.278	0.365-0.388	0.495-0.557	0.601-0.625	0.167-0.325
Female	Mean	0.386	0.863	5.223	15.318	17.915	2.716
	± SD	0.011	0.085	0.497	0.293	1.251	0.609
	Range	0.370-0.399	0.778-0.985	4.500-5.759	15.080-15.760	16.196-19.420	2.175-3.580

Table 17. GSI in Clarias batrachus from I to VI maturity stages (Number of values 3 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Male	Mean	0.114	0.279	0.396	0.554	0.633	0.233
	± SD	0.012	0.009	0.020	0.010	0.013	0.017
	Range	0.102-0.125	0.273-0.275	0.375-0.415	0.542-0.565	0.618-0.638	0.215- 0.248
Female	Mean	0.210	0.838	3.770	14.237	15.527	0.922
	± SD	0.045	0.056	0.776	0.497	0.588	0.108
	Range	0.162-0.250	0.790-0.899	3.150-4.642	13.760-14.751	14.981-16.150	0.813-1.098

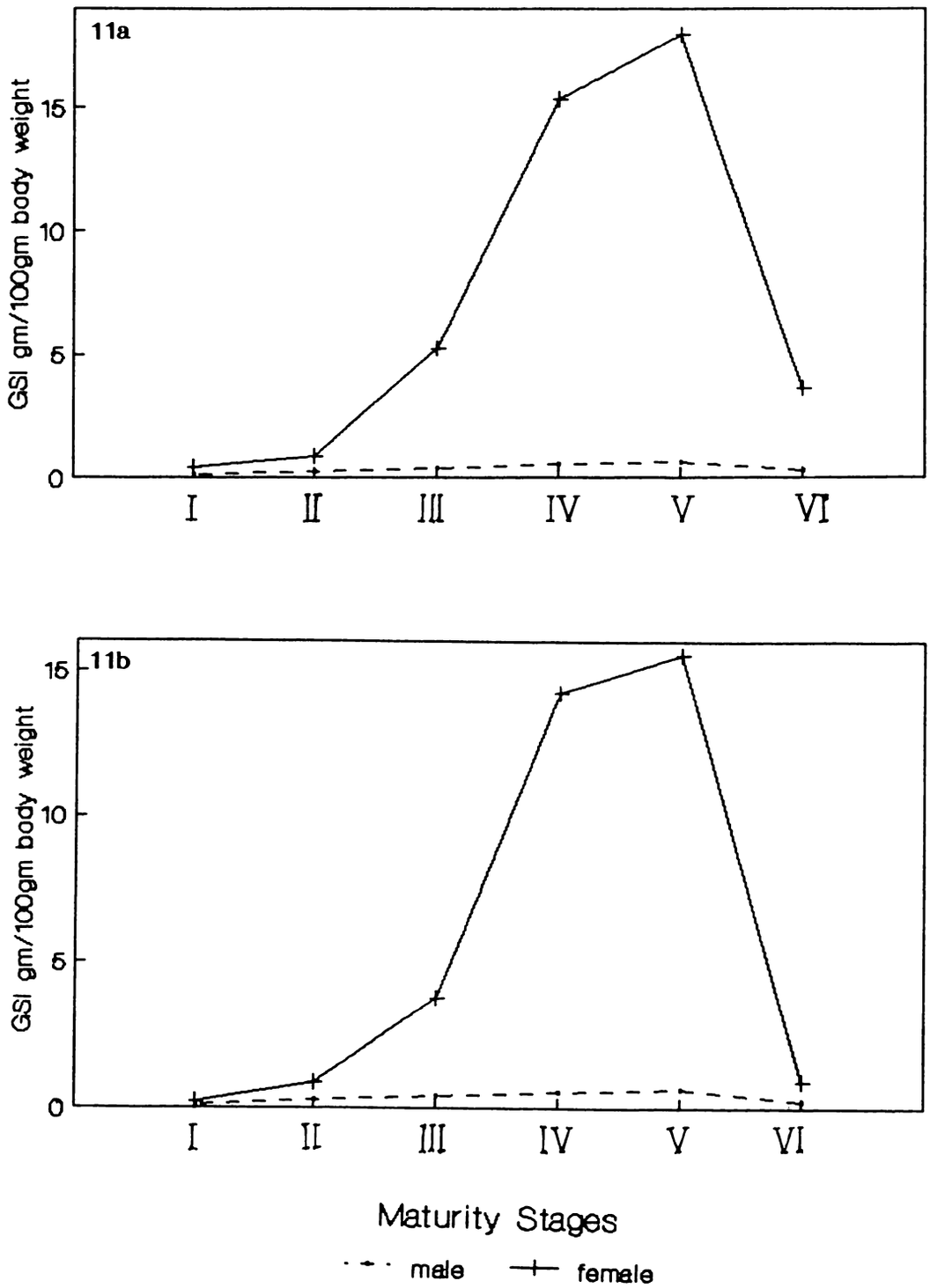


Fig.11 GSI from I to VI maturity stages.

11a. H. fossilis 11b. C. batrachus

Table 22. ANOVA TABLE - Two Way Analysis of Variance of GSI in Heteropneustes fossilis

Source of variations	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
Total	2137.7730	59		
Sex	704.5364	1	704.5364	2991.66**
Stages	744.1168	5	148.8234	631.95**
Sex x Stages	677.8158	5	135.5632	575.64**
Error	11.3040	48	0.2355	

Level of Significance : **P<0.01

Table 23. ANOVA TABLE - Two Way Analysis of Variance of GSI in Clarias batrachus

Source of variations	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
Total	1028.3320	35		
Sex	277.5057	1	277.5057	2725.9892**
Stages	394.0068	5	78.8014	774.0806**
Sex x Stages	354.3753	5	70.8751	696.2191**
Error	2.4442	24	0.1018	

Level of Significance **P< 0.01

In female the ovaries were very small in January (stage I) and the GSI increased gradually during February and March (stage II). In April there was a sudden increase (stage III) to reach its maximum in May (stage IV) and June (stage V). In June and July most of the fishes spawned. In August, the spent fishes (stage VI) occured. The difference between stages, sex and stages and the interaction between sexes and stages were highly significant ($P < 0.01$) when Two Way Analysis of Variance (ANOVA) was done on GSI.

6.4.2.2 Clarias batrachus (Table 17 ; Figure 11b)

The changes in gonadial stages closely followed that of H. fossilis.

In male, the lowest GSI was found in January (stage I). The developing of testes occurred during the stage II in February - March and the maximum GSI was observed during the stage V in June and July. In the spent fish, GSI significantly decreased.

In female too, the pattern is the same as that of male. In January the fishes collected were immature. The increase in GSI occurred from the stage II to V. Spawning happened in the stage V in June and July. Most of the spent fishes were found in August. Spawning was complete in a single spurt, because only very few eggs remained in the ovary of the spent fish which were collected in August. The difference between stages, sex and stages and the interaction between sexes and stages were highly significant ($P < 0.01$; Two Way Analysis of Variance).

6.4.3 Haematology in relation to environmental factors

Temperature is known to affect RBC content in teleosts. But, little is known about the effects of photoperiod and rainfall.

6.4.3.1 RBC count

6.4.3.1.1 Heteropneustes fossilis(Figures 12a and 12b)

In male in general, RBC count increased with an increase in temperature and decreased when the environmental temperature was lowered. Higher counts were obtained from February to April, when high temperature occurred. It was low during January and August when the temperature was low. RBC count generally was high with increased period of day length and the small increase in rainfall until May. Then the count suddenly lowered eventhough photoperiod and rainfall increased.

In the female H. fossilis, RBC count closely followed environmental temperature variations. Count was the highest when the highest temperature occurred in April. It was low when the temperature was low during January and August. The RBC count increased with an increase in photoperiod upto April. Until then, the rainfall only slightly increased. From May onwards the count suddenly decreased eventhough period of day length and rainfall increased.

6.4.3.1.2 Clarias batrachus(Figures 12c and 12d)

In this fish too an environmental temperature related increase and decrease in RBC number was found.

In the male, the highest number of RBC was recorded in April when the temperature was the highest. Then RBC number in blood lowered with a lowering of temperature. Count increased with an increase in photoperiod and the small increase in rainfall upto the stage III in April. Then it decreased

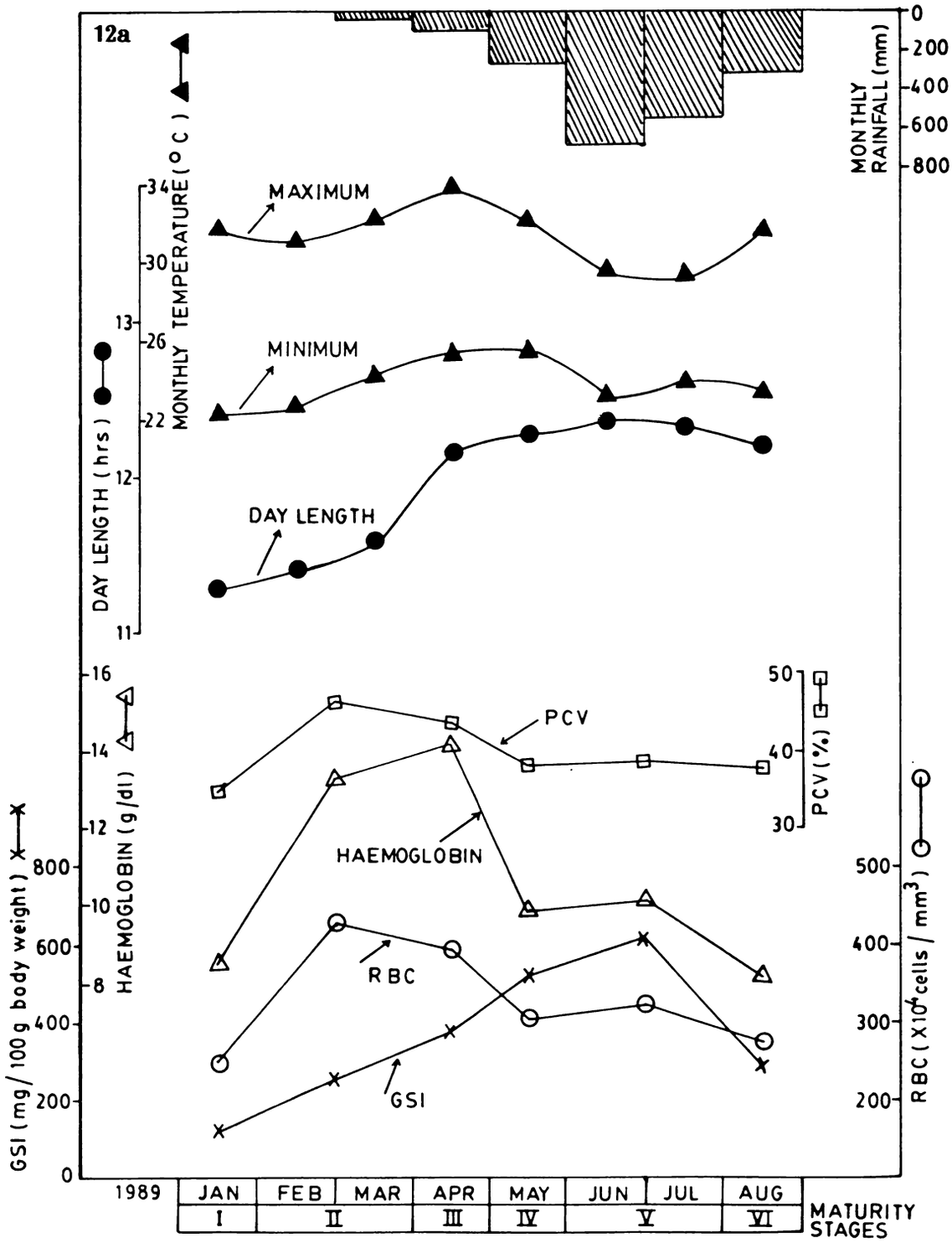


Fig. 12a. Environmental parameters, GSI and haematological values in male H. fossilis from January to August 1989.

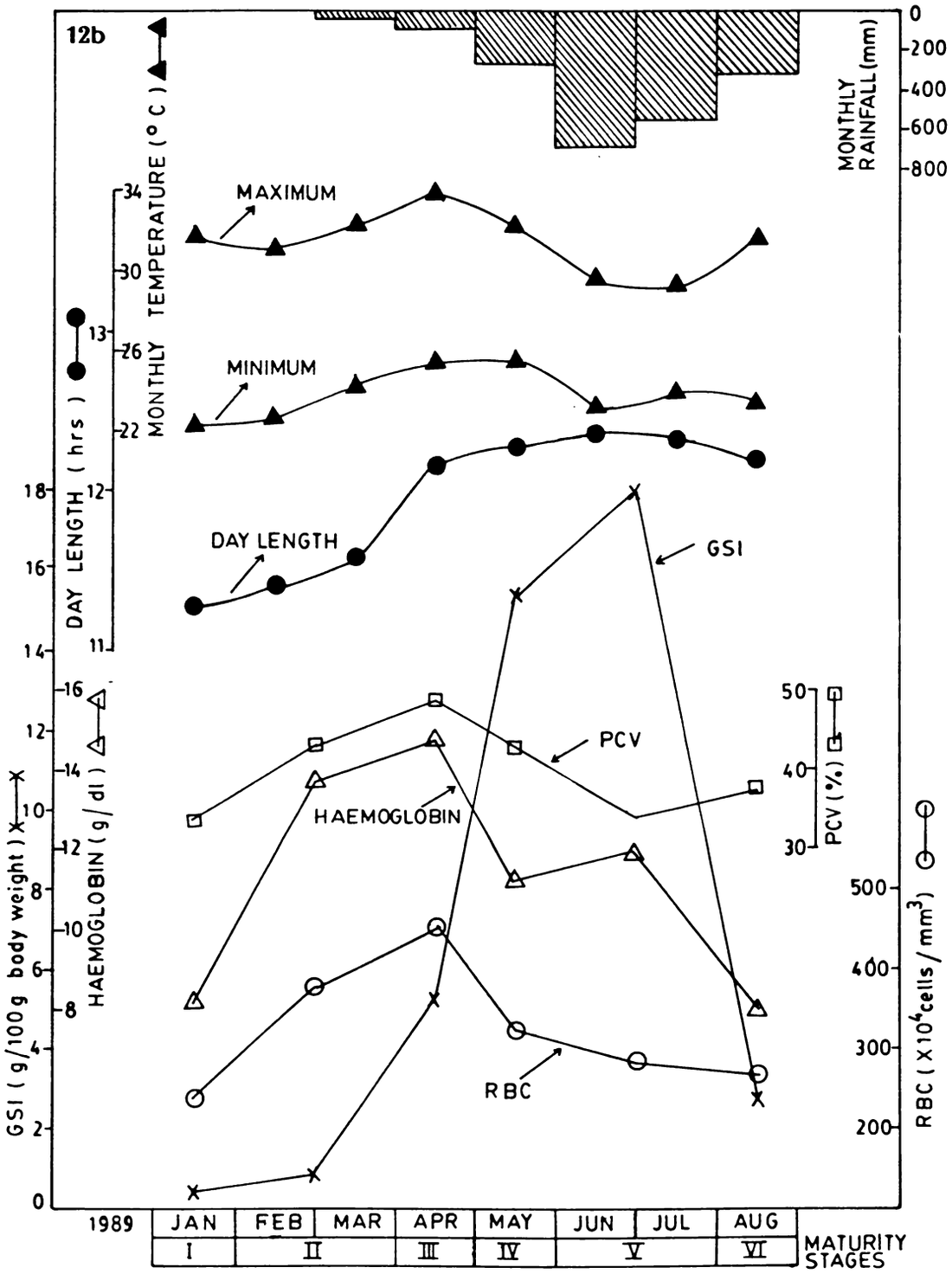


Fig.12b. Environmental parameters, GSI and haematological values in female H. fossilis from January to August, 1989.

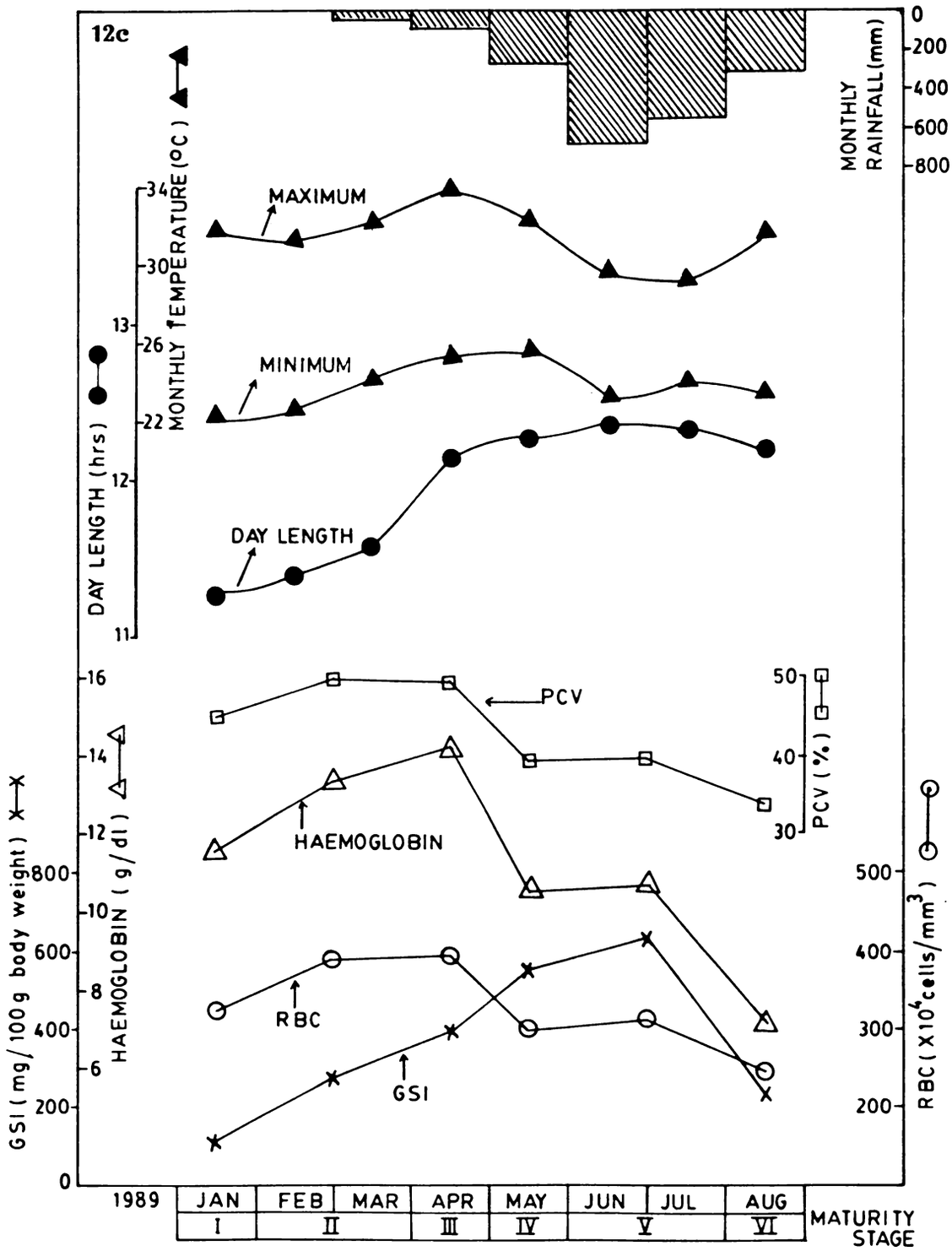


Fig. 12c. Environmental parameters, GSI and haematological values in male C. batrachus from January to August, 1989.

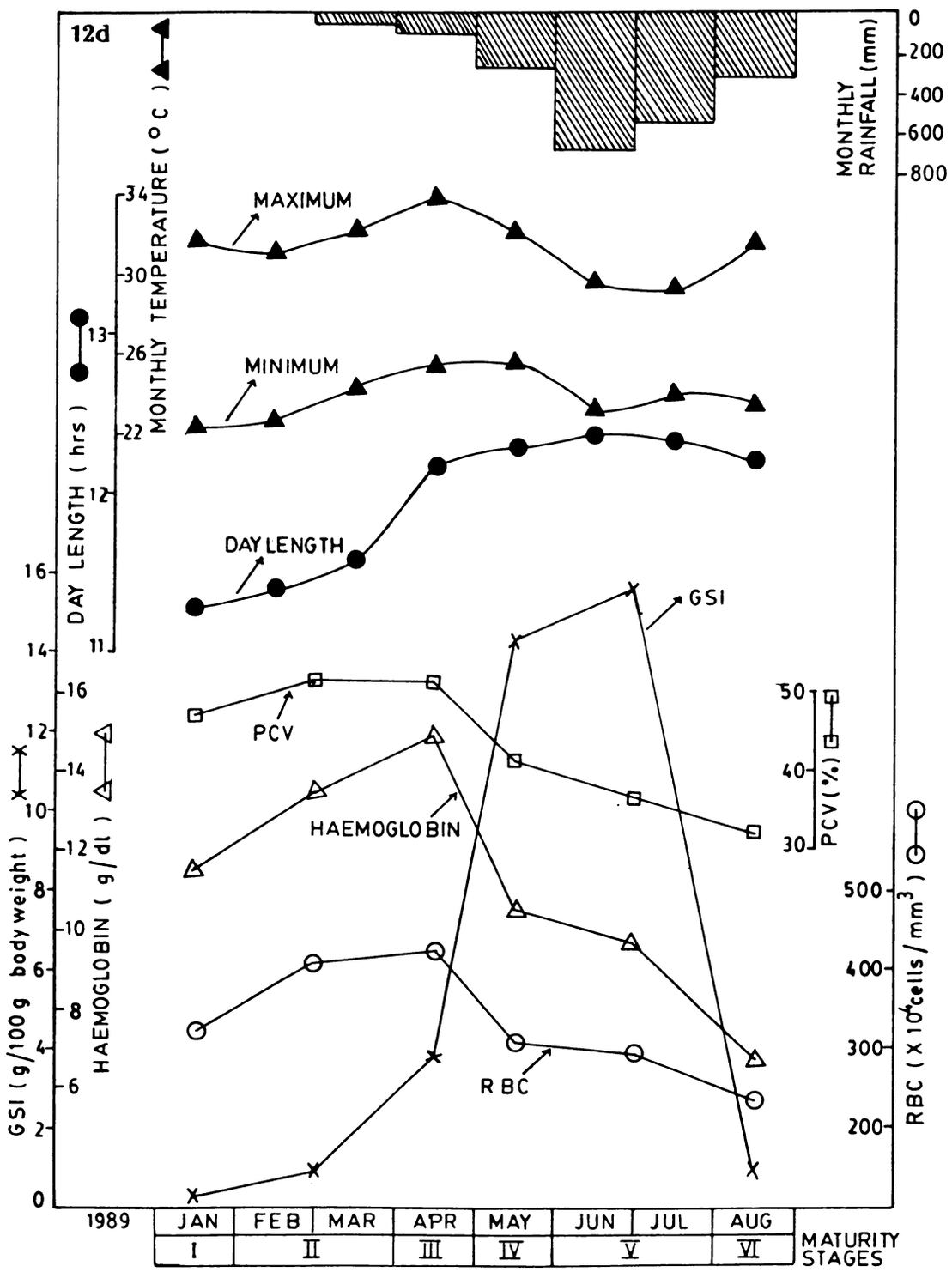


Fig. 12d. Environmental parameters, GSI and haematological values in female C. batrachus from January to August, 1989.

eventhough the period of day length increased along with a rise in the rate of rainfall.

The female followed the same trend as that of the male. Maximum RBC count was found in the stage III when the environmental temperature was the highest in April. Then the count decreased more or less depended on the lowering of temperature. RBC number increased parallel to an increase in the period of day length and the rate of rainfall upto April. Then the count decreased eventhough day length increased along with an increase in rainfall.

6.4.3.2 Hb content

6.4.3.2.1 Heteropneustes fossilis (Figures 12a and 12b)

Haemoglobin concentration in H. fossilis followed the general trend of environmental temperature. Highest Hb was found during the maximum environmental temperature. The decline in Hb was more or less similar to the lowering of temperature. Hb increased with an increase in the day length upto April. Then it decreased regardless of the period of day length and rainfall.

Hb in female closely followed variations in environmental temperature. Maximum Hb during April coincided with the highest environmental temperature. Hb concentration parallelled the period of day length upto April from stage I to III when rainfall increased only slightly. Then Hb decreased, eventhough the photoperiod and rainfall increased.

6.4.3.2.2 Clarias batrachus (Figures 12c and 12d)

In C. batrachus too, maximum environmental temperature coincided

with maximum Hb content in the maturity stage III in April. Then it decreased following the general trend of temperature. Hb increased with the lengthening of day and slight increase in rainfall upto the stage III. Then it lowered inspite of the increase in period of day length and rate of rainfall.

Hb content of Clarias female closely followed the changes in environmental temperature regardless of GSI just like female H. fossilis. The Hb concentration increased with a parallel increase in photoperiod and rainfall only upto April (stage III). Then the rainfall increased along with the lengthening of day, but Hb decreased.

6.4.3.3 PCV

6.4.3.3.1 Heteropneustes fossilis (Figures 12a and 12b)

In the male, the relation between PCV and environmental temperature was found to be less correlated than RBC and Hb. But in general, there was an increase in PCV with raised temperature, and a lowering, as the temperature declined.

The photoperiod and rainfall were found to be not definitely correlated to PCV. Both of them increased upto April alongwith PCV. Then PCV lowered regardless of the increase in both environmental parameters during the next three months.

In the female, PCV closely followed environmental temperature. The maximum level of PCV in the maturing fish coincided with the highest temperature in April. Then it lowered following the general trend in temperature. PCV level elevated along with the lengthening of day and rate of rainfall from stage I to III. Then PCV decreased eventhough period of day length continued to be increased with an increase in the rate of rainfall.

6.4.3.3.2 Clarias batrachus (Figures 12c and 12d)

In Clarias too, the trend of increasing of PCV with an increase in environmental temperature was maintained.

In the male, the changes in PCV were not as pronounced as in the case of H. fossilis, though the general positive correlation was maintained with temperature. PCV increased with an increase in temperature in the stage II in February and March. In the next stage, the high level of PCV was maintained. Then PCV lowered along with the lowering of temperature upto the stage V in June and July. In stage VI temperature increased though PCV decreased. Photoperiod and rainfall followed the same course of temperature upto stage III. Then it increased in the next stage along with an increase in the rainfall even if PCV decreased.

In Clarias female, the relation between PCV and temperature was more pronounced. High levels of PCV were found during the summer when the temperature was high. PCV followed the same trend as that of temperature except for the stage V in May. Photoperiod lengthened along with an elevation in PCV upto stage III in May. Then there was a small increase in rainfall. In the stages IV and V from May to July, the photoperiod and rainfall increased without corresponding rise in PCV value.

6.4.3.4 MCV

6.4.3.4.1 Heteropneustes fossilis

During testicular cycle, MCV produced an inverse relationship with temperature during the months, February, March and April (stage II and III).

Then the changes became less correlated. Upto the stage III, with an increase in photoperiod and rainfall there was a general decrease in MCV. From the stage IV in May onwards, no definite relationship could be found.

In the female, a general negative correlation occurred between temperature and MCV in the stages II and III like that of male. Then the changes became less related. As in temperature, upto the stage III in April MCV showed generally an inverse relationship with photoperiod and rainfall. From stage IV in May onwards, no correlation could be found between period of day length and rainfall and MCV.

6.4.3.4.2 Clarias batrachus

In the male, MCV showed an inverse relationship with temperature at all stages except the stage V in June and July. With photoperiod and rainfall a negative correlation was observed from February to April and then it became erratic.

In the female Clarias, the correlation between MCV and temperature was more like that of H. fossilis. The inverse relationship between both parameters tended to disintegrate and became less correlated from the stage IV in May. The negative relationship with photoperiod and rainfall existed in the stages II and III and then it disappeared.

6.4.3.5 MCH

In general, MCH was found to be closely related to temperature.

6.4.3.5.1 Heteropneustes fossilis

A general positive relationship was found between MCH and temperature. The highest level of MCH coincided with the maximum temperature in April.

The increase and decrease in both parameters were similarly modulated. With the photoperiod, it was difficult to find such a definite relationship. MCH showed a general positive correlation with photoperiod and rainfall until the stage IV in May. Then the correlation was lost.

In H. fossilis female, except for the stage III in April the pattern of variation in environmental temperature and MCH were similar. But for photoperiod and rainfall no such relationship existed.

6.4.3.5.2 Clarias batrachus

In the male, variation in MCH was not as pronounced as in H. fossilis. But a general similarity existed in the pattern of rise and fall of environmental temperature and MCH. Such a relation was lacking in the case of photoperiod and rainfall. C. batrachus female too showed a linearity in changes in the temperature and MCH. Just like the male, here too, the range of variation was narrow. Photoperiod and rainfall showed no direct correlation with MCH.

6.4.3.6 MCHC

6.4.3.6.1 Heteropneustes fossilis

MCHC pattern in male closely paralleled the changes in the temperature. The highest value of MCHC in the stage III coincided with maximum temperature in April. A linearity existed between the patterns of changes in photoperiod and rainfall and MCHC for the first three stages. Then it was lost.

MCHC in female showed a general trend similar to that of male when considering the effects of temperature. It increased when there was high temperature in March - April months and then decreased with a lowering

of temperature. With a lengthening of the period of day length and rate of rainfall, MCHC increased during the stage II in February and March and the high level was maintained in April too. Then the MCHC decreased even-though rainfall and photoperiod continued to be increased in the next two stages.

6.4.3.6.2 Clarias batrachus

MCHC in Clarias male showed a positive correlation with temperature irrespective of GSI. Maximum value of MCHC in the maturing stage III in April corresponded to the highest environmental temperature. During the first three stages of maturity, MCHC increased parallel to an increase in photoperiod and rainfall. The linear relationship was lost after that.

MCHC in female Clarias followed the trend in male. Maximum MCHC corresponded to the highest temperature in April. Rise and fall in temperature were well simulated by the changes in MCH. Just like male, for the first three stages from January to April, similarity existed between the changing pattern of photoperiod and rainfall and MCHC. Then the patterns changed.

6.4.4. Haematology in relation to gonadial cycle

In accordance with the changes in maturity stages, the haematological parameters were found to change in both cat fishes.

6.4.4.1 RBC Count

6.4.4.1.1 Heteropneustes fossilis (Tables 18 and 19 ; Figures 13a and 13b)

In all six reproductive stages of H. fossilis male, the RBC number was found to be varying. The highest number of RBC was found during the

Table 18. Haematological parameters in male Heteropneustes fossilis from I to VI maturity stages (Number of values 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
RBC	Mean	248.00	427.40	392.20	305.20	324.00	275.20
	+ SD	12.79	20.33	17.52	14.45	8.15	10.08
x $10^4/mm^3$	Range	245.00-260.00	425.00-450.00	375.00-418.00	282.00-317.00	310.00-331.00	260.00-286.00
Hb	Mean	8.50	13.30	14.19	9.82	10.14	8.22
	+ SD	0.38	0.35	0.27	0.63	0.01	0.60
g/dl	Range	8.10-8.90	12.90-13.80	13.80-14.50	9.10-10.50	10-10.25	7.20- 8.70
PCV	Mean	34.71	46.38	43.90	38.15	38.69	37.85
	+ SD	1.63	2.18	1.78	1.33	1.04	1.47
%	Range	32.00-36.10	43.00-48.82	42.00-46.50	36.80-40.34	37.00-39.50	35.59-39.30
MCV	Mean	140.03	108.52	111.95	125.10	119.40	137.59
	+ SD	40.06	1.33	0.84	4.00	1.08	3.97
μ^3	Range	135.08-146.33	106.33-109.93	111.24-113.36	123.70-130.50	117.79-120.43	131.12- 141.37
MCH	Mean	34.30	31.18	36.22	32.25	31.31	29.85
	+ SD	0.93	1.03	1.25	2.84	0.99	1.63
pg	Range	32.93-35.53	30.05-32.66	34.69-37.27	29.02-35.99	30.49-32.90	27.69- 31.37
MCHC	Mean	24.51	28.73	32.35	25.76	26.23	21.69
	+ SD	0.96	0.90	0.98	1.70	0.87	0.86
%	Range	23.43-25.54	27.57-30.00	31.18-33.41	24.21-27.58	25.32-27.57	20.23-22.44

Table 19. Haematological parameters in female Heteropneustes fossilis from I to VI maturity stages (Number of values 5, for each mean)

	Maturity Stages	I	II	III	IV	V	VI
RBC	Mean	237.00	379.40	454.40	323.40	284.80	269.80
	+ SD	12.00	14.98	31.81	20.82	13.33	7.66
x $10^4/mm^3$	Range	222.00-250.00	362.00-395.00	411.00-493.00	310.00-359.00	270.00-304.00	260.00-280.00
Hb	Mean	8.10	13.71	14.78	11.11	8.98	7.96
	+ SD	0.20	0.27	1.10	0.65	0.70	0.19
g/dl	Range	7.80-8.30	13.40-14.10	14.10-16.50	10.00-11.65	8.50-10.20	7.70-8.20
PCV	Mean	33.55	43.08	48.73	42.73	34.00	37.88
	+ SD	1.11	1.79	3.22	2.08	0.79	1.21
%	Range	32.50-35.00	41.50-45.15	46.12-53.33	41.01-46.32	33.10-35.00	36.30-39.50
MCV	Mean	141.69	113.59	107.37	132.25	119.48	140.39
	+ SD	3.69	3.26	5.40	2.67	2.96	0.54
μ^3	Range	137.73-146.40	108.23-116.57	99.18-114.18	129.03-135.05	115.13-122.59	139.62-141.07
MCH	Mean	34.23	36.32	32.53	34.98	31.51	29.48
	+ SD	1.57	1.14	0.74	1.47	1.25	0.83
pg	Range	33.20-36.94	34.81-37.00	31.83-33.47	32.45-36.22	30.53-33.55	28.21-30.19
MCHC	Mean	24.15	31.86	30.34	26.47	26.39	21.00
	+ SD	0.67	1.38	1.26	0.89	1.55	0.65
%	Range	23.64-25.23	30.56-33.84	28.99-32.09	25.25-27.55	25.37-29.14	20.00-21.63

Table 20. Haematological parameters in male Clarias batrachus from I to VI maturity (Number of values 3 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
RBC	Mean	324.67	390.67	395.66	300.33	313.33	243.33
	+ SD	27.30	10.02	6.02	5.51	4.16	10.41
x $10^4/mm^3$	Range	300.00-354.00	383.00-402.00	390.00-402.00	295.00-306.00	310.00-318.00	235.00-255.00
Hb	Mean	11.53	13.40	14.20	10.52	10.77	7.15
	+ SD	0.55	0.26	0.15	0.29	0.25	0.13
g/dl	Range	11.00-12.50	13.20-13.70	14.05-14.35	10.25-10.82	10.50-11.00	7.00-7.25
PCV	Mean	45.40	49.83	49.17	39.29	39.83	33.50
	+ SD	3.12	0.29	0.76	0.73	0.76	1.50
%	Range	42.50-48.70	49.50-50.00	48.50-50.00	38.75-40.12	39.00-40.50	32.00-35.00
MCV	Mean	139.96	127.61	124.27	130.82	127.13	137.67
	+ SD	2.13	3.09	1.44	0.72	1.22	1.74
μ^3	Range	137.57-141.67	124.38-130.54	122.78-125.66	130.00-131.36	125.81-128.21	136.17-139.58
MCH	Mean	35.60	34.31	35.89	35.03	34.36	29.41
	+ SD	1.28	0.37	0.17	0.76	0.42	0.85
pg	Range	34.18-36.67	34.08-34.73	35.70-36.03	34.17-35.59	33.87-34.61	28.43-30.00
MCHC	Mean	25.75	26.89	28.88	26.78	27.02	21.36
	+ SD	0.17	0.44	0.35	0.44	0.12	0.60
%	Range	25.56-25.88	26.60-27.40	28.66-28.28	26.28-27.10	26.92-27.16	20.71-21.88

Table 21. Haematological parameters in female Clarias batrachus from I to VI maturity stages (Number of values 3 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
RBC	Mean	321.00	409.33	423.67	308.67	293.33	232.33
	+ SD	20.07	26.63	9.29	8.08	10.40	14.19
x $10^4/mm^3$	Range	300.00-340.00	380.00-432.00	413.00-430.00	300.00-316.00	285.00-305.00	217.00-245.00
Hb	Mean	11.47	13.47	14.89	10.53	9.71	6.75
	+ SD	0.45	0.31	0.34	0.45	0.28	0.48
g/dl	Range	11.00-11.90	13.12-13.65	14.50-15.06	10.10-10.50	9.45-10.00	6.20-7.10
PCV	Mean	47.00	51.43	51.33	42.03	36.33	32.33
	+ SD	3.61	4.00	1.61	1.57	1.04	2.25
%	Range	43.00-50.00	47.00-54.79	49.50-52.50	40.80-43.80	35.50-37.50	30.00-34.50
MCV	Mean	146.44	125.98	121.15	136.16	123.65	139.12
	+ SD	7.32	2.01	1.42	2.37	0.62	1.47
μ^3	Range	141.18-154.80	123.68-127.43	119.85-122.66	133.87-138.61	122.95-124.14	138.25-140.82
MCH	Mean	35.76	32.98	35.13	34.13	33.11	29.03
	+ SD	0.85	1.47	0.14	1.35	0.74	0.50
pg	Range	35.00-36.67	31.60-34.53	35.01-35.28	32.58-35.00	32.59-33.96	28.57-29.57
MCHC	Mean	24.46	26.27	29.88	25.06	26.73	20.87
	+ SD	1.32	1.52	0.26	0.70	0.51	0.44
%	Range	23.00-25.58	24.91-27.91	29.61-30.12	24.34-25.74	26.25-27.27	20.58-21.38

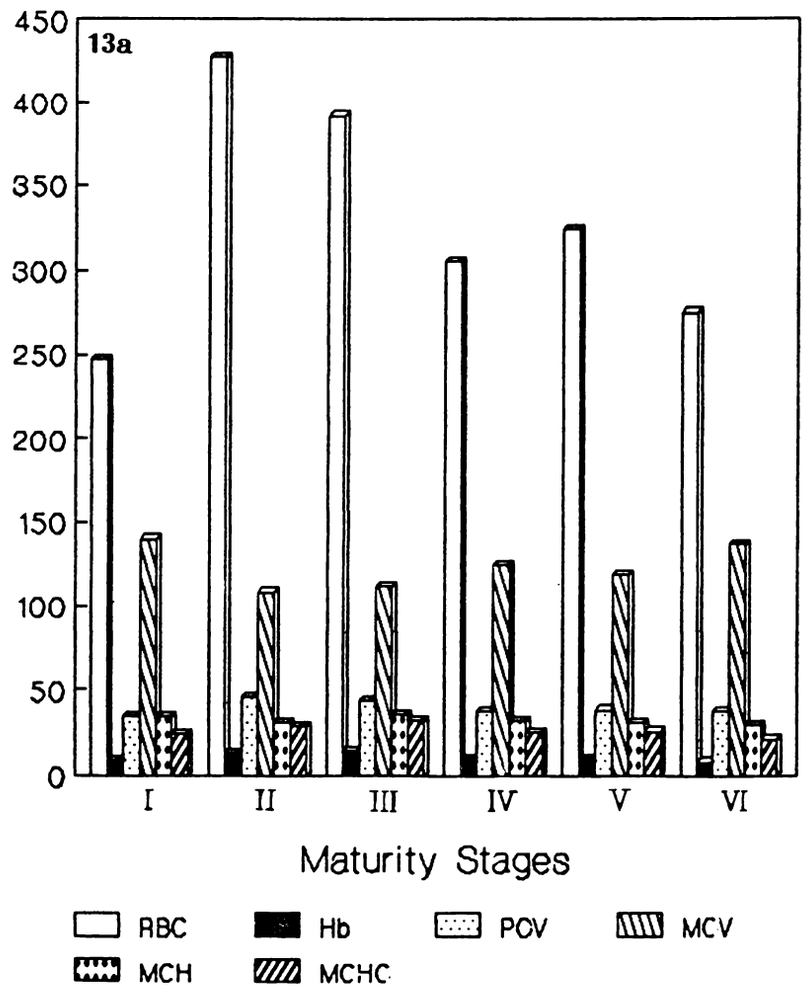


Fig.13a. Haematological parameters in male H. fossilis from I to VI maturity stages.

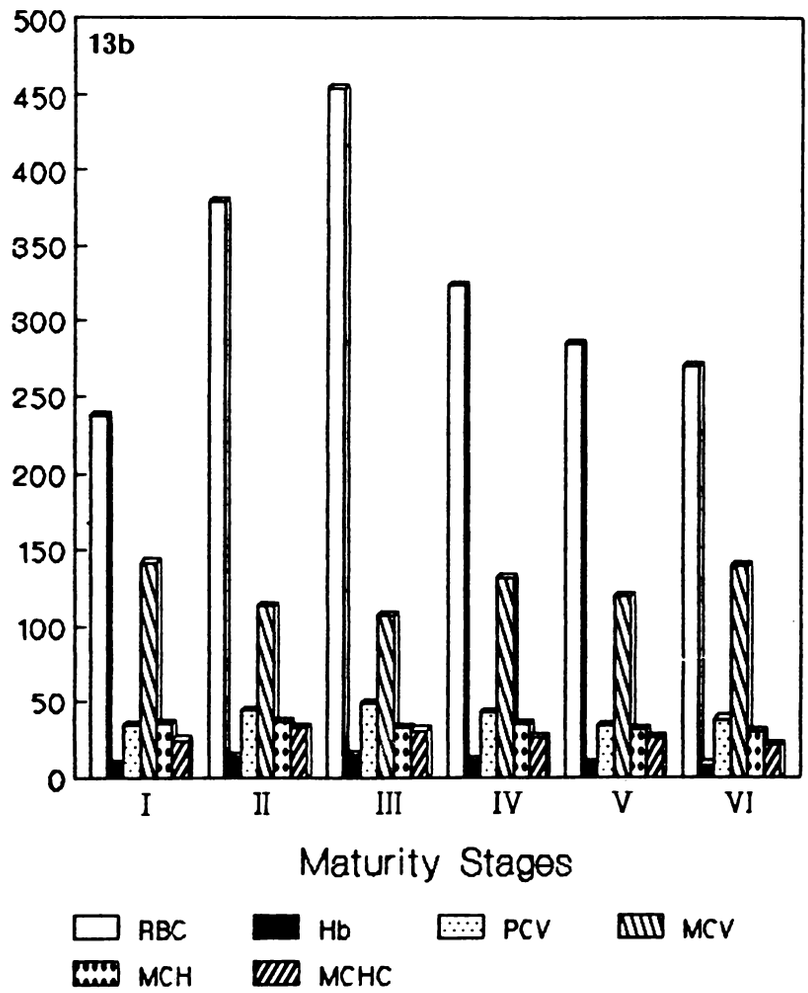


Fig.13b. Haematological parameters in female H. fossilis from I to VI maturity stages.

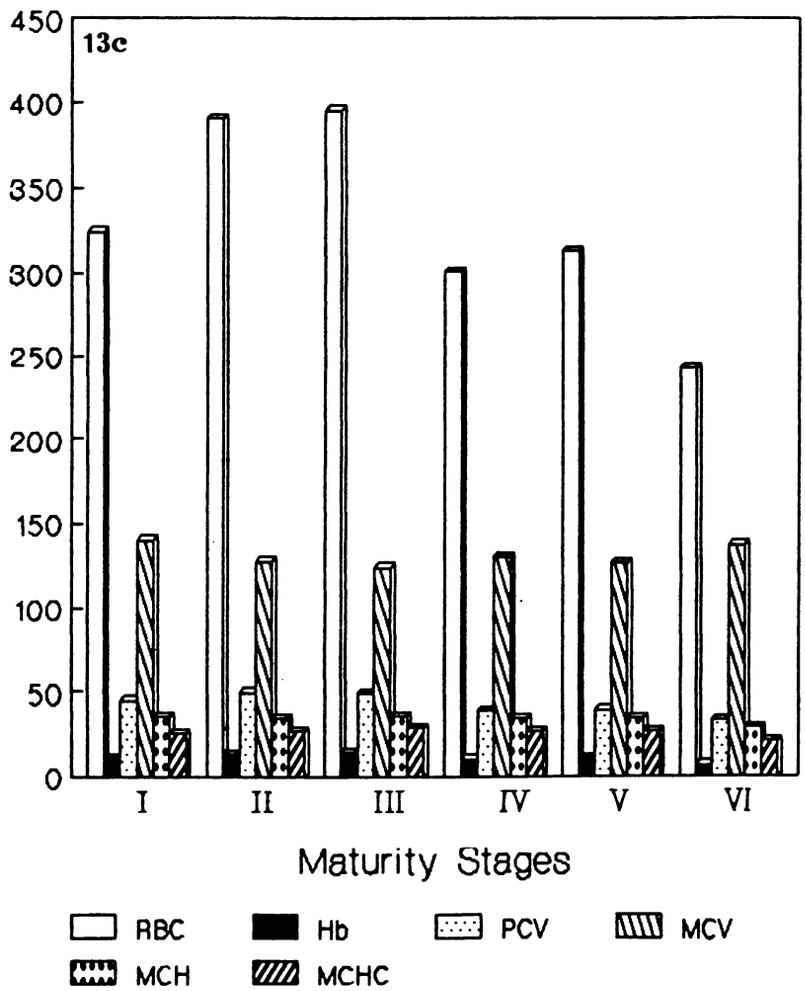


Fig.13c. Haematological parameters in male C. batrachus from I to VI maturity stages.

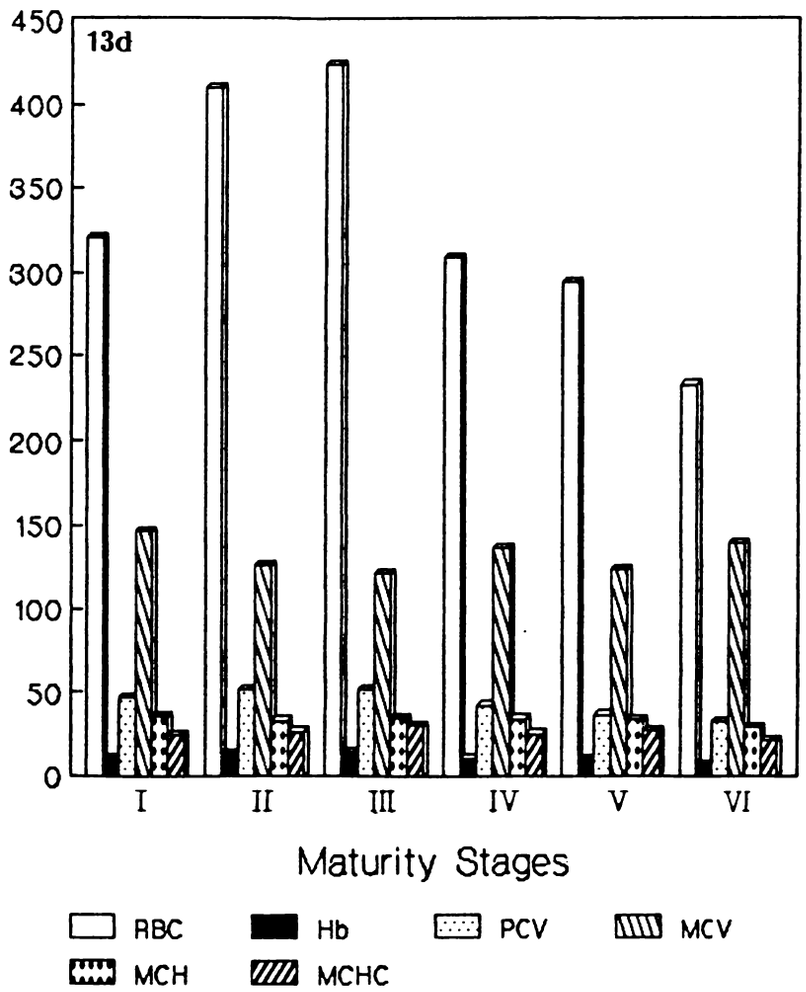


Fig.13d. Haematological parameters in female C. batrachus from I to VI maturity stages.

stage II. In the stage III, high level of RBC was maintained. It decreased during the stage IV to be a little raised by the spawning time (stage VI). In the spent, the RBC count was considerably lower than that of the developing and mature stages. Erythrocyte number and GSI were positively related only in the first two stages.

In the female H. fossilis, unlike the male, the RBC count was highest in the stage III. Then it gradually decreased through the stages IV and V to reach a low value in the spent fish. Upto the stage III, RBC count increased parallel to GSI and after that the RBC decreased with an increase in GSI.

When Two Way Analysis of Variance (ANOVA) was done, there was no significant difference between sex at 5% level. But there was significant difference between stages and the interaction between sex and stages at 1% level ($P < 0.01$).

6.4.4.1.2 Clarias batrachus (Tables 20 and 21 ; Figures 13c and 13d)

In both sexes of C. batrachus, RBC count in the first stage was found to be higher than that of H. fossilis.

In male Clarias, the highest count was found during the stages II and III. Then it decreased to reach a low level in the spent fish, which is strikingly lower than that of the immature fish.

The female fish followed a similar trend as that of the male. The highest number of RBC was found in the stages II and III and then decreased to reach the lowest level in the spent fish. RBC count in both sexes increased parallel to GSI upto the third stage and then declined.

When Two Way Analysis of Variance was done, there was no significant difference between sex at 5% level. But stages showed a significant difference at 1% level ($P < 0.01$). The interaction between sex and stages was not significant at 5% level.

6.4.4.2 Hb concentration

6.4.4.2.1 Heteropneustes fossilis (Tables 18 and 19 ; Figures 13a and 13b)

During the testicular cycle of the male, the highest amount of haemoglobin was found in the stage III (maturing). In the immature fish, it was only 8.50 g/dl which increased to the notable of 14.19 g/dl in the maturing stage. Hb decreased during the stages IV and V to attain the least value during the whole of the gonadial cycle in the spent fish.

The variations in Hb content in H. fossilis during the ovarian cycle was more or less similar to that of male fish. Hb suddenly increased during the developing period to reach the maximum value in the maturing stage. It then lowered during the stages IV and V to reach the least value of 7.96 g/dl in the spent fish.

In both sexes, Hb increased parallel to the GSI upto the maturing stage, then it decreased against an increase in GSI.

When Two Way Analysis of Variance was performed, there was no significant difference between sex at 5% level. But stages showed a significant difference ($P < 0.01$). The interaction between sex and stages was significant at 1% level ($P < 0.01$).

6.4.4.2.2 Clarias batrachus (Tables 20 and 21 ; Figures 13c and 13d)

In the male, Hb increased from the immature to the maturing stage.

Then it decreased to reach the lowest level in the spent fish. General trend is similar to that of male H. fossilis. Hb in the spent fish is considerably lower than that of the immature fish. Maximum level of Hb was found in the stage III.

In the female, the general trend is similar to that of H. fossilis and C. batrachus male. The highest amount of Hb was found in the stage III and the lowest in the stage VI. When compared to the stages IV, V and VI, Hb in the immature fish was high in both sexes.

In C. batrachus in general, GSI and Hb showed positive correlation upto the stage III. In the stages IV and V the correlation was negative. When Two Way Analysis of Variance (ANOVA) was done there was no significant difference between sex at 5% level of significance. But between stages and the interaction between sex and stages showed significant difference at 1% level ($P < 0.01$).

6.4.4.3 PCV

6.4.4.3.1 Heteropneustes fossilis (Tables 18 and 19 ; Figures 13a and 13b)

PCV followed the same trend as that of RBC during testicular cycle. The highest value was found during the maturing stage II. The high level of PCV was maintained in the stage III too. But in the following three maturity stages, PCV were low, but higher than that of the immature fish.

During the ovarian recrudescence, the highest PCV was found in the stage III just as in the case of RBC. From the values it can be seen that a close positive correlation exists between RBC and PCV upto the mature

stage. But in the next stage (V) compared to RBC, PCV was low and in the stage VI the trend was just the opposite.

Just as it appeared in the results of RBC and Hb, between GSI and PCV there was a positive correlation upto the stage III, and then PCV decreased with an increase in GSI.

When Two Way Analysis of Variance (ANOVA) was done, there was no significant difference between sex at 5% level. But between stages and the interaction between sex and stages showed significant difference at 1% level ($P < 0.01$).

6.4.4.3.2 Clarias batrachus (Tables 20 and 21 ; Figures 13c and 13d)

Compared to H. fossilis, PCV in immature fish was high and the changes due to maturity was not as drastic.

In male, high level of PCV was found in the maturity stages II and III. Compared to first four stages, PCV at the stage V was low and the lowest level of PCV was found in the spent fish.

In the female too, the highest level of PCV was found in the stages II and III and the lowest level in the spent fish. The trend in changes in the packed cell volume resembled the one shown by male C. batrachus.

Between GSI and PCV, there was a positive correlation upto the stage III. Then PCV lowered though GSI increased.

When Two Way Analysis of Variance (ANOVA) was done, there was no significant difference between sex and the interaction between sex and stages. But between stages showed significant difference at 1% level ($P<0.01$).

6.4.4.4 MCV

6.4.4.4.1 Heteropneustes fossilis (Tables 18 and 19 ; Figures 13a and 13b)

In male H. fossilis in the stage II, MCV was drastically reduced and the reduction was maintained during the stage III. The raised MCV during the stage IV was followed by a diminishing trend in the spawning time. MCV was again increased in the spent fish.

In the female MCV suddenly lowered in the stage II to reach the least volume in the stage III. During the stage IV, MCV reached a higher level to be depressed again during the spawning time. In the spent, MCV was raised almost similar to the initial volume.

A negative correlation was found between MCV and GSI in the first three stages. When Two Way Analysis of Variance (ANOVA) was done, there was no significant difference between sexes. But between stages and the interaction between sex and stages showed significant difference at 1% level ($P < 0.01$).

6.4.4.4.2 Clarias batrachus (Tables 20 and 21 ; Figures 13c and 13d)

Compared to H. fossilis, MCV range was comparatively low at different stages in C. batrachus.

During testicular cycle, the lowest MCV was found in the stage III. In the stages II and IV too, MCV was comparatively low. Elevated MCV was found in the stages IV and VI when compared to stages II, III and V. Stage I registered high MCV values.

With an increase in GSI, MCV decreased during stages II and III. When there was the maximum GSI in the spawning condition, the MCV was low.

During the ovarian cycle, lowest MCV was noted in the stage III. Stages II and V too registered low MCV. During the stages IV and V, MCV was elevated. Immature fish showed high MCV. As in the male, MCV was negatively correlated with GSI upto stage III. It was low during the highest period of GSI.

When Two Way Analysis of Variance (ANOVA) was done, there was no significance between sexes and the interaction between stages and sex at 5% level. But between stages showed significant difference at 1% level ($P < 0.01$).

6.4.4.5 MCH

6.4.4.5.1 Heteropneustes fossilis (Tables 18 and 19 ; Figures 13a and 13b)

Compared to immature fish, MCH was low in the stage II. Maximum MCH was registered during the stage III. Then it gradually decreased through stages IV and V to reach the lowest level in the spent fish.

In the female, the highest level was found in the stage II to be decreased during stage III. It again increased in the stage IV to decrease during the stage V to reach the lowest level in the spent fish. The relation between GSI and MCH was found to be erratic.

When Two Way Analysis of Variance was done, between sexes no significant difference was found at 5% level. But between stages and the interaction between sex and stages were found to be significant at 1% level ($P < 0.01$).

6.4.4.5.2 Clarias batrachus (Tables 20 and 21 ; Figures 13c and 13d)

In male, the range of difference in MCH between stages was very

narrow except for the spent fish. In the stages II and V, a slight lowering of MCH was observed. During stages I, III and IV, MCH was more or less the same. But in the spent fish, MCH was definitely low.

In the female, during stages II and V, MCH was low. The lowest level was recorded in the spent fish. For the stages I and III, MCH was almost same. In the stage IV, MCH was a little lower than the stages I and III. The relation between GSI and MCH was found to be erratic in both sexes.

When the Two Way Analysis of Variance was done, between sexes and interaction between sex and stages were not significant at 5% level. Between stages was significant at 1% level ($P<0.01$).

6.4.4.6 MCHC

6.4.4.6.1 MCHC in H. fossilis

In H. fossilis male, the highest MCHC was found in the stage III and the lowest in the stage VI. MCHC during the stage II was higher than that of other stages except the stage III. Upto the stage III, MCHC changes closely followed rise in GSI and from the stage IV onwards it became erratic.

In the female, the highest MCHC was found in the stage II and the lowest in the stage VI. Stage III too registered high levels of MCHC. MCHC was more or less similar during stages IV and V. The immature fish registered low MCHC compared to other stages except the spent fish. The relation between GSI and MCHC was found to be erratic in the female.

When Two Way Analysis of Variance was done, between sexes was not significant at 5% level. Between stages and interaction between sex

Table 24. ANOVA TABLE - Two Way Analysis of Variance of haematological parameters in male and female Heteropneustes fossilis

	Source of variation s	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
RBC	Total	292653.7333	59		
	Sex	224.2666	1	224.2666	0.81
	Stages	258927.7333	5	51785.5500	187.62**
	Sex x Stages	20252.9334	5	4050.5900	14.67**
	Error	13248.8000	48	276.0200	
Hb	Total	384.2865	59		
	Sex	0.0882	1	0.0882	0.31
	Stages	361.4190	5	72.2838	256.96**
	Sex x Stages	9.2753	5	1.8551	6.59**
	Error	13.5040	48	0.2813	
PCV	Total	1491.6041	59		
	Sex	0.0380	1	0.0380	0.01
	Stages	1147.9826	5	229.5965	74.79**
	Sex x Stages	196.2403	5	39.2481	12.79**
	Error	147.3432	48	3.0697	
MCV	Total	9970.0451	59		
	Sex	61.9354	1	61.9400	6.13
	Stages	9213.4096	5	1842.6800	182.26**
	Sex x Stages	209.2486	5	41.8500	4.14**
	Error	485.4515	48	10.1100	
MCH	Total	394.2147	59		
	Sex	6.4289	1	6.4289	3.22
	Stages	179.2773	5	35.8555	17.98**
	Sex x Stages	112.7891	5	22.5578	11.31**
	Error	95.7194	48	1.9942	
MCHC	Total	788.7462	59		
	Sex	0.3729	1	0.3729	0.31
	Stages	692.6169	5	138.5234	113.33**
	Sex x Stages	37.0866	5	7.4173	6.07**
	Error	58.6698	48	1.2223	

Level of Significance : *P < 0.05 **P < 0.01

Table 25. ANOVA TABLE - Two Way Analysis of Variance of haematological parameters in male and female Clarias batrachus

	Source of variations	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
RBC	Total	134190.2222	35		
	Sex	93.4444	1	93.4444	0.433
	Stages	126371.8889	5	25274.3778	117.1164**
	Sex x Stages	2545.5556	5	509.1111	2.3591
	Error	5179.3333	24	215.8056	
Hb	Total	219.8508	35		
	Sex	0.1419	1	0.1419	1.1509
	Stages	214.2540	5	42.8508	347.5328**
	Sex x Stages	2.4950	5	0.4990	4.0470**
	Error	2.9599	24	0.1233	
PCV	Total	1668.3795	35		
	Sex	2.9584	1	2.9584	0.6570
	Stages	1513.8993	5	302.7799	67.2381**
	Sex x Stages	43.4471	5	8.6894	1.9296
	Error	108.0747	24	4.5031	
MCV	Total	2323.1167	35		
	Sex	6.3504	1	6.3504	0.6545
	Stages	1940.0757	5	388.0151	39.9888**
	Sex x Stages	143.8169	5	28.7634	2.9644
	Error	232.8737	24	9.7031	
MCH	Total	213.5080	35		
	Sex	4.7379	1	4.7379	2.9078
	Stages	167.3052	5	33.4610	20.5358**
	Sex x Stages	2.3587	5	0.4717	0.2895
	Error	39.1062	24	1.6294	
MCHC	Total	242.5927	35		
	Sex	2.6353	1	2.6353	5.3003*
	Stages	221.9976	5	44.3995	89.2991**
	Sex x Stages	6.0279	5	1.2056	2.4248
	Error	11.9319	24	0.4972	

Level of Significance : *$P < 0.05$ **$P < 0.01$

and stages were significant at 1% level ($P < 0.01$).

6.4.4.6.2 MCHC in C. batrachus (Tables 20 and 21 ; Figures 13c and 13d)

During the testicular cycle, the highest MCHC was noted in the maturing stage III and the lowest in the spent. MCHC was more or less same during stages II, IV and V. Immature fish registered low MCHC when compared to other stages except the stage VI. In the male, MCHC increased with GSI upto the stage III and then the relationship was found to be erratic.

During the ovarian cycle, the trend in changes in MCHC was more or less similar to the male. Highest and lowest MCHC were found during the stages III and VI respectively. In the stages II and V, almost similar MCHC were recorded. MCHC in the stage I was only higher than the spent fish. In female MCHC increased parallel to GSI upto the stage III, then their relationship was erratic.

When Two Way Analysis of Variance (ANOVA) was done, between sexes showed significant difference at 5% level ($P < 0.05$). Between stages showed significance at 1% level ($P < 0.01$). But the interaction between sex and stages was not significant at 5% level.

6.5 DISCUSSION

As the haematological parameters were found to be affected by both environmental parameters as well as gonadial cycle in both H. fossilis and C. batrachus, the discussion is laid out separately for them under separate headings.

6.5.1 Haematological changes in relation to environmental factors

Temperature increase imposes increase in standard metabolism of fishes, manifested by their oxygen requirements. At the haematological level, temperature induced changes are visualized by the increase or decrease in RBC count, haemoglobin content, PCV and the derived factors like MCV, MCH and MCHC. According to syrove (1970) the RBC and Hb values were higher in summer months and lower in winter months.

As is shown in the data for environmental parameters, the environmental temperature was increased during the February and March months of 1989. Then the O_2 consumption in H. fossilis and C. batrachus might have increased needing an amplification in the Hb level. As the Hb is contained in the RBC in vertebrates, erythropoiesis happened in both fishes causing an increase in RBC number, Hb and PCV. The drastic reduction in MCV at this period can be attributed to two reasons. One is the presence of smaller immature cells in the blood stream due to erythropoiesis and the other is the reduction in mean red cell volume. Reductions in MCV offer some advantage in a high temperature environment since the rate of oxygen combination with Hb varies inversely with red cell volume (Holland, 1970). In Cyprinus carpio, Houston and De Wilde (1968) and Houston and Smeda (1979) found increase in Hb, PCV and RBC with an increase in temperature, MCV decreased with temperature. In the goldfish, Carassius auratus, Houston and Cyr (1974) observed an increase in Hb and PCV with a rise in temperature. Pandey (1977) reported that an increase in almost all blood parameters except MCV occur at high temperature.

It does not seem that photoperiod or rainfall exert any direct effect on any of the haematological parameters. Period of day length increased parallel to an increase in temperature from January to April, 1989. During this period there was a slight, but steady increase in rainfall too though generally rainfall was scanty. Then the RBC, Hb and PCV also increased.

But in June, when the day length and rainfall were the highest, all the haematological parameters were low. It may be that the effect of temperature was enhanced by an increase or decrease in photoperiod and rainfall instead of exerting direct effects. This assumption is supported by the observations of Wolfson (1964).

Another reason for high RBC, Hb and PCV during the summer months may be the drying up of ponds and rivers in which the air breathing fishes live. Then H. fossilis and C. batrachus will depend more on air breathing needing added amount of Hb which in turn might cause erythropoiesis.

During April, when the atmospheric temperature was the highest, occurred the highest level of RBC and lowest level of MCV in the female H. fossilis and C. batrachus. It indicates the continuation of erythropoiesis. The cause of the highest MCH and MCHC at this time in the male H. fossilis might be due to the maturing of RBC in circulation. In the female H. fossilis, the lowering of MCH and MCHC than in the preceding period might be due to the continuing recruitment of immature RBC into the blood.

In C. batrachus of both sexes, the high temperature induced changes were comparatively lower. Specimen size has considerable bearing on both the occurrence and extent of haematological response (Houston, 1980). Smaller carp exhibits substantially greater increases in haemoglobin levels and red

cell number than do larger animals exposed to the same temperature conditions, coupling with this proportionally larger reductions in erythrocyte volume (Smeda and Houston, 1979). So it is only natural that C. batrachus which come under the size group of 100-130 gms show only moderate or relatively smaller changes in relation to the temperature when compared to H. fossilis coming under the size group of 40-65 gms.

The sudden drop in erythrocyte number followed by a significant reduction in Hb during May, June and July in both species should be due to fall in temperature enhanced by increased rainfall. The decrease in temperature might have lowered standard metabolism resulting in reduced oxygen consumption. So naturally erythropoiesis will be stopped followed by a reduction in RBC number and associated factors. Molnar et al. (1960) suggested that temperature seems to be the main factor regulating the number of erythrocytes in Abramis brama (L) and Lucioperca lucioperca (L). Bhatt and Singh (1981) found in a hill stream fish Schizothorax richardsonii where in the month of January (temperature 8-10°C) the Hb concentration and TEC values were poor while the same values were higher during the summer months. Bhatt and Singh (1985) observed marked lowering of RBC count, Hb, PCV and MCHC when fishes of the species Schizothorax plagiostomus were transferred from 20.5 to 17°C. Joshi (1982b) also reported a fall in blood values following the fall in ambient temperature in the catfish Rita rita (Ham).

Haematology of the spent fishes of both species can't be correlated with temperature, photoperiod or rainfall. The cause of its depletion should be sought elsewhere.

6.5.2 Haematological changes in relation to gonadial cycle

Eventhough haematological picture of H. fossilis and C. batrachus were modified by environmental parameters especially temperature during the summer months, many of the changes in the blood picture can be fully explained only by attributing them to gonadial recrudescence and associated physiological changes occurring in the fishes concerned.

In the immature condition, the occurrence of comparatively low RBC count, Hb, PCV and MCHC in H. fossilis should be due to its low metabolic rate. The air breathing cat fishes are comparatively sluggish, their energy being spent only for an occassional trip to the surface for air breathing and for foraging at the bottom of pond or river.

With an increase in size group, RBC, Hb and PCV are increased (Pandey et al., 1976; Smith, 1977; Chaudhuri et al., 1986c; Ruparelia et al., 1986). So that should be the reason for high initial values of RBC, Hb and PCV in C. batrachus which come under a higher size group.

During the developing stage, in H. fossilis fish started intense feeding as indicated by their feeding in the lab and by the appearance of stomach of fishes at this stage in nature. For the catabolic and anabolic precesses in association with the build up of gonads and feeding, energy is needed. So standard metabolism was automatically raised increasing the oxygen demand, hence the increase in RBC, Hb and PCV. Schlicher (1927) opined that the number of erythrocytes of some fresh water fishes didn't depend on temperature, salinity and feeding, but exclusively on physiological processes connected with spawning cycles. At this time, the MCH in male H. fossilis was low

due to the immature RBC in circulation. But in the female, the highest MCH was found at this time. As the female had to build up more gonadial material, the haemopoietic centres might have released more mature RBC containing higher amount of Hb resulting in high MCH. This assumption is made plausible by the MCV and MCHC levels in female H. fossilis of developing stage. But in C. batrachus, except for RBC number, only smaller changes in Hb, PCV, MCV, MCH and MCHC were found. With an increase in size, the gonadial changes may bring about only minor changes in larger fishes.

During the maturing stage III, the highest red cell number, Hb, and PCV were found in the female H. fossilis and C. batrachus. Sex differences in PCV may be particularly relevant when dealing with mature fish(Munkittrick and Leatherland, 1983). Higher mobilization of metabolites as shown by a notably increased GSI in female at this stage might have caused the higher level of erythropoiesis in the female. The highest MCH and MCHC in the male H. fossilis at this stage, may be due to the maturing of immature RBC in the blood stream.

In the mature fish, the GSI had reached almost the peak level. The feeding rate also should be reduced by this time. So the oxygen requirement diminished resulting in the lowering of the levels of RBC, Hb and PCV in the stage IV. Another cause for reduction in haematological factors at this period should be haemodilution due to the flooding of body with the external medium. As protein is removed from the body muscle for gonad build up, the water content of body rises steadily and this acts as a useful index of depletion of fish (Love, 1960, 1962).

Some authors have reported a sexual difference in RBC, Hb or PCV in sexually mature salmonids with males having higher values than the females (Sano, 1960; Poston, 1966; Lane, 1979; Pickering, 1986). In salmonids, during the long spawning migration, males frequently fight among themselves. This might have increased their haematological values. But in H. fossilis, RBC, Hb and Ht were a little higher in the female than in the male in mature stage IV fish. Here, two factors to be taken into account are the increased body weight of the female due to mature ovary and the water content of the body causing more haemodilution in female which lose more protein for the oocyte build up. In the female, eventhough most of the metabolite input into the ovary was almost stopped by the stage IV, the vitellogenin (the serum yolk precursor) build up and transport from the liver to the gonad will continue until ovulation (Lamba et al., 1983). To cope with this increased metabolic need, respiratory gas carriers RBC and Hb should be increased, hence the increased rate of RBC and Hb in the female H. fossilis. It should be due to the higher haemodilution in the female at this time that the difference between the male and female was not so pronounced. The increased haemodilution might have caused erythrocyte swelling as evinced from the higher MCV in the females of both species, although erythrocyte swelling also occurred in the male.

With an increase in age, the depletion of body tissues will be higher especially in the female (Love 1970) resulting in highly diluted plasma. That may be the reason why only smaller differences in haematological values were found between the male and female of C. batrachus.

At the time of spawning, both species of cat fishes migrate for spawning from their natural habitat to low lying adjacent places which are flooded during rains. For the spawning site selection and for the selection of mate, the male might show aggression which is known to occur in fishes (Pickering, 1986). C. batrachus male is known to make holes in the embankment for the deposition of eggs. For the additional energy needed, mobilization of metabolites might occur resulting in increased oxygen demand; hence the increase in RBC number, Hb content and PCV in the male of both species.

At this time, the PCV and MCH of both species of fishes were low. Low MCV during the spawning period was also reported by Pandey (1977) and Joshi (1982b). During the monsoon rains, the food availability is poor due to high rainfall, turbidity, low transparency and fast flow of water. So a temporary anaemia may happen in spawning female fishes which is of the microcytic type as reported by Woodall et al. (1964) and Kawatsu (1972). Here a question may arise, whether the haemodilution which is possible to happen at spawning due to high body water content won't cause erythrocyte swelling? It may be that after the water content of the plasma increased to a certain level, osmotic regulation would start either by active uptake of minerals through gills, increased secretion of hyposmotic urine or by the absorption of water into various body tissues. Corticosteroids have been shown to increase the uptake of sodium by the gills of fresh water teleosts (Maetz, 1964; Maetz and Morel, 1965; Chester Jones and Henderson, 1965) and also elevate plasma osmolarity of the hypophysectomized fish (Chan et al., 1968; Parwez and Goswami, 1985). Dharmamba and Maetz (1972) have suggested that corticosteroids may be important to the fresh water fish in maintaining an active sodium pump. Stress arising from various factors

during reproduction could increase plasma titres of 11 oxygenated corticosteroids in some species of fishes (Wingfield and Grimm, 1977). Pickering and Christie (1981) demonstrated rise in the plasma cortisol levels of the mature female brown trout at a time coincident with the onset of ovulation. Lamba et al. (1983) reported increased plasma cortisol content during spawning in H. fossilis. So to combat with the flooding of water sodium uptake, plasma osmolarity and urine secretion might have increased resulting in increased osmotic pressure of plasma causing shrinking of RBC or reduced MCV.

In the spent fish of both species anaemia was evident from the very low RBC and Hb. The MCH and MCHC too declined. The production of eggs or sperm always depletes a fish (Love, 1970) which in its turn will lower the production of RBC and Hb. Depletion due to starvation caused reduction in RBC, Hb or PCV in Ophiocephalus maculatus (Woo and Cheung, 1980), Channa punctatus (Mahajan and Dheer, 1983) and Noemacheilus montanus (Bhatt and Singh, 1984). Love was of the opinion that many of the phenomena described during maturation process can be duplicated by straightforward depletion. In the laboratory, fishes at this time was found to take in food. But may be, more than utilizing the energy from food for erythropoiesis, it was used for the gradual building up of lost body constituents.

Spent fishes showed an increase in their MCV. It should be due to the further haemodilution occurring in the depleted fish and the removal of the effect of cortisol, the secretion of which was low by this time (Lamba et al., 1983).

Considering both species of fishes, C. batrachus is the more depleted their haematological values being definitely lower than that in the immature

fish. This should be due to their larger size causing increased exhaustion due to depletion.

Thus the controlling processes of the haematology of the blood of the cat fishes Clarias batrachus and Heteropneustes fossilis are very complex during the spawning season. The fluctuation in each parameter is determined by multiple factors. Temperature, food availability and maturity stage along with steroid hormones seem to control the erythropoiesis and associated factors in these fishes. The GSI values in both male and female of both sexes closely followed photoperiod and to some extent temperature. During the later period of development, rainfall also exerted its effect. Ovulation occurred only with the highest rainfall. From the present data, it can be assumed that with an increase in temperature and photoperiod which cues gonadotropins, the gonadial recrudescence starts along with synthesis of steriods. A concomitant increase in haematological parameters occurs to provide oxygen for increased energy demands due to the high metabolic turn over. Increase in GSI was closely followed by haematological parameters upto the stage III when RBC, Hb and PCV reached their maximum corresponding to the highest mobilization and transport of biochemical components in blood. The environmental temperature was the maximum at this period. Full maturity of gonads reached when the environmental temperature lowered, due to intermittant rainfall. Then the haematological parameters also lowered, due to the lowering of temperature and body metabolism and change in osmoregulation. Spawning occurred only with the highest rainfall during south-west monsoon. In H. fossilis and C. batrachus both sexes show anaemia due to depletion in the spent stage.

CHAPTER - VII

BIOCHEMISTRY OF BLOOD DURING THE GONADIAL CYCLE

7. 1 INTRODUCTION

The initiation of breeding season in oviparous teleosts is characterized by the mobilization of organic materials needed for gametogenesis. The production of reproductive elements stresses a fish by depleting it. The depletion is closely linked with carbohydrate, protein and fat metabolism. Glycogen plays a part in the accumulation of energy in the body of fishes. Eventhough its content in the body is negligible, the fact that it is a readily mobilizing substance which can liberate energy under anaerobic conditions makes it of special value. Spawning determines the dynamics of the major vital processes in the organism including that of fat. The fat deposited in the body is the main source of energy for the synthesis of generative tissue. It is partially transferred to the gonads and is included as nutritive material in the yolk of oocytes. The sterol, cholesterol is utilized for the synthesis of testicular, ovarian and interrenal hormones which are steroids and for the building of the basic membrane structure of the generative tissue. By the study of the protein composition of blood plasma during the spawning season, useful data on protein metabolism can be obtained.

Very little is known about nitrogen metabolism during spawning season when the maximum amount of catabolic and anabolic processes take place in the body of fishes. Then the nitrogen turn over will be high and it would be interesting from the physiological point of view to know whether urea is formed more than the normal rates in fishes during the breeding period.

7.2 REVIEW OF LITERATURE

Maturity and spawning bring about changes in the blood chemistry of teleosts. There are many reports indicating that dramatic changes occur in liver and plasma through out the reproductive cycle of the fish and that these changes are often closely correlated to gonadial development (Korsgaard and Petersen, 1979; Van Bohemen and Lambert, 1980; Dindo and MacGregor, 1981). Elevated glucose levels during spawning season have been observed in teleosts (Yanni, 1961; Mackay and Beatty, 1968; Tandon and Joshi, 1974). Yanni (1961) found that Clarias lazera increased glucose levels from a low 14.3 mg% during November to a peak of 34.5 mg% during June in spawning season. Pickford (1953) observed differences in liver size between fish captured in spring and fall. But in Myxine glutinosa, no difference was detected between the blood glucose contents of egg bearing and non egg bearing females (Falkmer and Winbladh, 1964). But Bentley and Follett (1965) found a fall in glucose level in Lampetra fluviatilis. Venugopalan (1962) correlated the fluctuations in the blood carbohydrate levels with that of oogenesis in ophiocephalus striatus.

The fluctuations in serum proteins during gonad maturation and spawning have been discussed in detail by Shul'man (1974). Aida et al. (1973) studied the sexual difference in the plasma proteins of ayu during gonadial maturation. Fourie and Hattingh (1976) found a winter maxima for plasma protein which is during the prespawning period.

According to Blaxter and Holliday (1963), maturation and spawning consume 90% of fat reserves in Salmonidae, 80% in Acipenseridae, 60% in smelt, 50% in pike perch and Baltic herring and 15% in herring. There are large differences between males and females in the expenditure of energy

for gonad. Lipids and lipoproteins act as fundamental substances of egg yolk synthesis. They are produced in the liver and delivered to gonads via the plasma (Takashima et al., 1972). Mature females therefore have increased plasma levels of total lipids as well as an additional plasma lipoprotein, the vitellogenin which is evident on the electrophoretic pattern and immunologically identical to the major protein component of eggs (Skinner and Rogie, 1977; Lamba et al., 1983).

Idler and Isuyuki (1958) measured plasma cholesterol and electrolyte levels in sockeye salmon during spawning migration. Mohan and Singh (1987) suggested massive use of hepatic store in the reproduction of Clarias batrachus. High levels of lipid in plasma and low levels in testes with simultaneous unapparent change in muscular lipid was observed. In Cyprinus carpio, plasma cholesterol may be the potent source of steroidogenesis which is regulated by pituitary gonadotropin (Guha and Mukherjee, 1987).

There is a scarcity of literature on blood urea levels in teleosts. Idler and Isuyuki (1958) estimated blood urea N of sockeye salmon.

High serum cortisol levels and increased interrenal activity were observed in teleosts during spawning season (Yaron, 1970; Johnson, 1972; Peter et al., 1978). Cortisol is known to affect carbohydrate metabolism during spawning season (Robertson et al., 1961). The sex steroid estrogen elevates the plasma content of calcium and protein (Hori et al., 1979; Tinsley, 1985). Estradiol injections affect lipid metabolism in Gadus morhua (Plack et al., 1971).

7.3 MATERIALS AND METHODS

7.3.1 Collection, transportation and maintenance of fishes

Collection of fishes was done as in 6.3.1. Transportation and maintenance of fishes were done as described in 3.1 and 3.2.

7.3.2 Sampling procedure and separation of plasma

Sampling of blood was done as given in 3.3. Whole blood was used for the determination of blood urea content. For the separation of plasma, heparinized blood was centrifuged for 15 minutes at 2000 rpm. The supernatant yellowish fluid, the plasma was used for the estimation of plasma sugar, plasma protein and plasma cholesterol. All spectrophotometric readings were done in duplicate in a Hitachi Model UV - Vis Spectrophotometer U-2000.

7.3.3 Determination of maturity and GSI

After blood sampling, the abdominal cavity of fishes were opened ventrally and the maturity stages were determined as described in 3.4 and gonadosomatic index as in 3.5.

7.3.4 Estimation of plasma glucose

O-toluidine method of Hyvarinen and Nikkila (1962) was used. It is a very sensitive method and gave good results.

Glucose in blood plasma reacted with orthotoluidine in glacial acetic acid to produce a blue green colour.

Glucose standard was prepared by dissolving 100 mg reagent grade glucose in 100 ml of water in a volumetric flask. It was kept refrigerated. Ortho-toluidine colour reagent was prepared by adding 6 ml of O-toluidine to 94ml glacial acetic acid.

0.05 ml plasma, glucose standard and water for blank were taken in separate test tubes containing 3.5 ml colour reagent. All tubes were heated in a boiling water bath for 10 minutes. It was removed and cooled to room temperature. The optical density of plasma and glucose standards were read at 635 nm. The concentration of glucose was calculated by the formula

Plasma glucose (mg/100 ml)

$$= \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

7.3.5 Estimation of plasma protein

Total protein present in plasma was estimated by the Biuret method (Gornall et al., 1949).

Two carbamyl groups present in protein molecules combine with copper and potassium of the biuret reagent to form a blue coloured copper - potassium - biuret compound. The colour formed is proportional to the amount of carbamyl groups present in the protein.

1 N NaOH and biuret reagent were used. Biuret reagent contains copper sulphate, sodium potassium tartrate and NaOH.

0.05 ml plasma was taken in a test tube and 1.95 ml of 1 N NaOH was added to it. After shaking well, 8 ml of biuret reagent was added. It was allowed to stand for 30 minutes and the optical density was measured at 540 nm against the reagent blank. Protein standard was prepared by dissolving 25 mg bovine serum albumin in 1 N NaOH and made upto 5 ml in a standard flask. For calculations, a standard graph was prepared using protein solutions of known concentrations and from it the concentration of sample was found out in grams per 100 ml of blood.

Biuret method is thought to be less sensitive than the Folin-Ciocalteu method (Bailey, 1967). But the latter method is more susceptible to interfering substances than biuret, possibly due to the mechanism of colour reaction (Subhashini and Ravindranath, 1981). In Biuret method, copper binds with carbamyl groups of protein and gives colour. It is directly estimated. But in Folin-Ciocalteu method, it is not only the copper-protein complex that reduce the phosphomolybdate, but also the tyrosil residues present in the protein, the amount of which differs from one protein to another. So the colour reaction in this method differs from one protein to another (Young, 1963).

For the above mentioned reasons, Biuret method was selected for the protein estimation in the present investigation.

7.3.6. Estimation of plasma cholesterol

The method of Zak (1957) was followed for the estimation of plasma cholesterol.

Stock ferric chloride was prepared by dissolving 840 mg $FeCl_3 . 6 H_2O$ in glacial acetic acid and made upto 100 ml. 10 ml of stock ferric chloride solution was diluted to 100 ml with glacial acetic acid to form precipitating reagent. 85 ml of precipitating reagent was made upto 100 ml with glacial acetic acid to form the diluting reagent. Concentrated sulphuric acid is another reagent used.

Into 3 small test tubes, pipetted out 0.05 ml of serum for test, water for blank and standard cholesterol solution for standard. To the serum added 2 ml of precipitating reagent; to the blank and standard added

2 ml of diluting reagent. All tubes were stoppered and mixed thoroughly on a vortex mixer or by inversion. It was centrifuged for 30 seconds. 1.5 ml of each supernatant was transferred to a clean colorimeter tube. To all the tubes 1 ml concentrated H_2SO_4 was added, mixed and kept in a water bath at $80^{o}C$ for 5 minutes. They were then removed to a rack and cooled to room temperature. The optical density was measured at 550 nm. From the readings, concentration of the sample was calculated using the formula

Cholesterol (mg/100 ml)

$$= \frac{\text{Absorbance of unknown x 200}}{\text{Absorbance of standard}}$$

7.3.7 Determination of blood urea content

The diacetyl monoxime method deviced by Natelson (Varley, 1975) was used for these investigations for better sensitivity.

The principle of this method is that when urea is heated with substances such as diacetyl containing two adjacent carbonyl groups, coloured compounds are formed. The colour thus developed is measured colorimetrically. This standard method was found to yield good results.

0.1 ml of whole blood was taken in a micropipette and washed into 3.3 ml of water in a test tube. To this was added 0.3 ml 10% sodium tungstate and 0.3 ml 2/3 N H_2SO_4. It was mixed well and centrifuged. To 1 ml of the supernatant fluid was added 1 ml of water, 0.4 ml 2% diacetyl monoxime and 1.6 ml of sulphuric acid - phosphoric acid mixture. It was placed in a boiling water bath for 30 minutes for colour development; then cooled and read against a water blank at 480 millimicrons. In the

same way colour was developed from 1 ml of two urea standards each containing 0.025 mg urea/ml and 0.015 mg urea/ml of water. The two standards were included with each batch of samples for urea determination. The unit for blood urea is mg/100 ml.

7.3.8 Computation and presentation of data

Data for all parameters were expressed as mean ± standard deviation (SD). All values for individual parameters were subjected to Two Way Analysis of Variance (ANOVA). Data are presented in the form of tables and line graphs.

7.4. RESULTS

The results presented here deal with GSI, plasma sugar, plasma protein, plasma cholesterol and blood urea in six arbitrary reproductive stages of both male and female H. fossilis and C. batrachus.

7.4.1 Maturity stages and GSI

7.4.1.1 Heteropneustes fossilis (Table 26 ; Figure 14a)

In male during the immature stage, the lowest GSI was found. It increased a little during the stage III of maturity. During stage III, a sudden increase in GSI was found. The maximum GSI occurred in June and July during the stage V. After spawning, it lowered in the spent fish.

In the female, ovaries were very small during the immature period. During the developing period (stage II) it increased in size due to the development of eggs. In the stage III, a sudden increase in GSI of the female occurred. Notable increase occurred in stage IV to reach its maximum in stage V. After the shedding of eggs during spawning, GSI was low in the spent fish.

Table 26. GSI in Heteropneustes fossilis from I to VI maturity stages (Number of values 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Male	Mean	0.115	0.260	0.388	0.514	0.620	0.288
	± SD	0.017	0.028	0.020	0.023	0.025	0.104
	Range	0.095-0.125	0.242-0.269	0.360-0.391	0.495-0.550	0.605-0.635	0.167-0.325
Female	Mean	0.375	0.868	5.325	15.305	17.515	3.590
	± SD	0.051	0.075	0.397	0.397	1.201	0.950
	Range	0.329-0.395	0.778-0.955	4.545-5.759	14.910-15.760	16.196-18.935	2.315-3.840

Table 27. GSI in Clarias batrachus from I to VI maturity stages (Number of values 3 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Male	Mean	0.112	0.260	0.386	0.554	0.632	0.203
	± SD	0.012	0.018	0.022	0.010	0.015	0.019
	Range	0.105-0.120	0.250-0.275	0.370-0.415	0.542-0.565	0.618-0.645	0.195-0.215
Female	Mean	0.250	0.825	3.850	14.512	15.575	0.925
	± SD	0.038	0.049	0.650	0.481	0.590	0.108
	Range	0.212-0.270	0.790-0.880	3.270-4.642	13.760-14.951	15.010-16.150	0.813-1.098

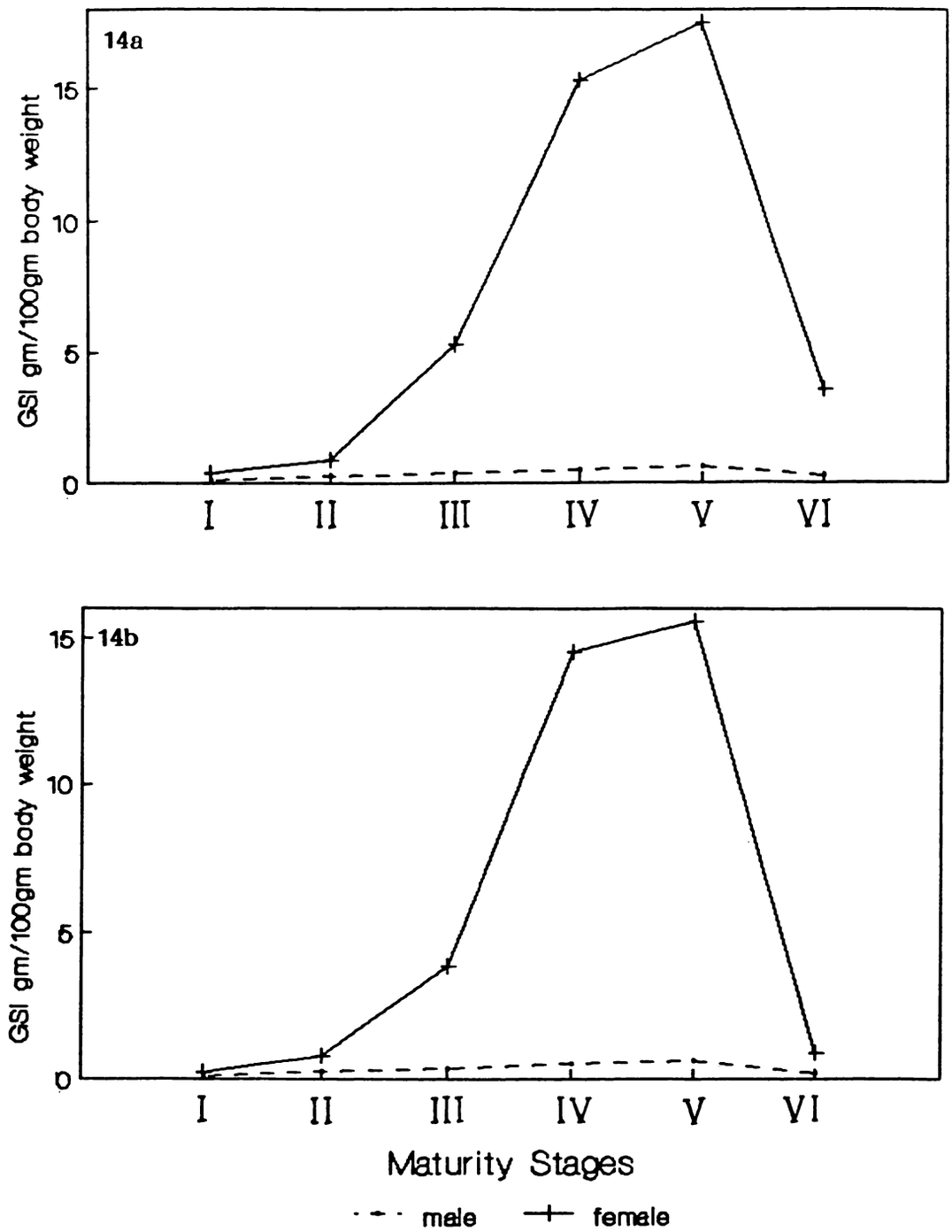


Fig.14. GSI from I to VI maturity stages
14a. H. fossilis 14b. C. batrachus

7.4.1.2 Clarias batrachus (Table 27 ; Figure 14b)

The changes in maturity stages and GSI closely followed that of H. fossilis.

The lowest GSI was found in the immature male (stage I). The developing of testes occurred in stage II. After the sudden increase in GSI during stage III,the testes continued to increase in weight until stage V. Then it decreased significantly in the spent fish.

In the female too, the pattern is the same as that of male fish. In the immature fish the GSI was the minimum. The increase in GSI occurred from the stage II to IV. The maximum GSI was found in the stage V. The spawning also occurred in the same stage. Spawning was completed in a single spurt by the female, because only very few eggs remained in the ovary of the spent fish collected during the post-spawning period.

7.4.2 Plasma glucose

7.4.2.1 Heteropnuestes fossilis (Tables 28 and 29 ; Figure 15a)

Variation of plasma glucose in the blood of male H. fossilis shows a close relationship with the mobilization of other metabolites. As plasma sugar is an indicator of carbohydrate metabolism, a low level of plasma sugar shows the relatively low dependence of the organism on the carbohydrate metabolism.

In H. fossilis in the male, the lowest level of plasma glucose was found in the immature fish. As the gonadial recrudescence started, it increased to reach the peak level in the stage III , to be diminished in the IV. During spawning, glucose was raised a little to be further increased

Table 28. Blood chemistry in male Heteropneustes fossilis from I to VI maturity stages (Number of values 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Plasma	Mean	29.82	45.26	71.39	36.36	42.20	49.86
sugar	± SD	3.93	3.19	2.10	2.53	2.04	2.70
mg/100ml	Range	25.50-34.15	41.20-49.75	68.50-74.15	32.95-39.80	40.38-45.50	46.80-53.75
Plasma	Mean	3.519	4.355	5.590	4.104	4.001	3.824
protein	± SD	0.287	0.255	0.262	0.115	0.208	0.198
g/100ml	Range	3.313-3.857	4.063-4.688	5.313-5.950	4.000-4.260	3.715-4.291	3.50-4.00
Plasma	Mean	485.24	345.95	624.87	643.22	360.32	508.77
cholesterol	± SD	39.59	23.51	37.48	19.15	14.93	76.65
mg/100ml	Range	420.00-520.75	316.71-380.85	610.18-670.75	620.50-661.75	344.97-376.92	403.08-567.69
Blood	Mean	2.296	2.136	6.689	3.911	1.973	1.638
urea	± SD	0.232	0.969	0.532	0.882	0.201	0.274
mg%	Range	1.975-2.615	0.893-3.57	5.983-7.125	3.127-5.173	1.786-2.235	1.345-1.963

Table 29. Blood chemistry in female Heteropneustes fossilis from I to VI maturity stages (Number of values 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Plasma	Mean	25.44	49.45	75.46	49.01	27.63	53.29
sugar	± SD	6.23	6.42	4.22	5.59	6.40	6.74
mg/100ml	Range	18.11-35.1	42.98-59.70	69.20-79.92	41.72-55.15	21.05-35.56	42.50-59.80
Plasma	Mean	3.600	5.426	6.865	4.483	3.963	3.443
protein	± SD	0.307	0.493	0.507	0.578	0.224	0.228
g/100ml	Range	3.227-3.897	4.975-6.125	6.250-7.500	3.750-5.000	3.625-4.250	3.112-3.688
Plasma	Mean	536.26	443.12	845.90	347.69	377.88	582.39
cholesterol	± SD	26.98	36.43	91.67	11.56	14.51	27.56
mg/100ml	Range	495.00-565.23	400.01-482.75	761.34-961.54	330.15-355.71	360.35-392.50	560.15-610.77
Blood	Mean	2.062	1.761	7.149	3.433	1.860	1.602
urea	± SD	0.126	0.476	1.567	1.330	0.298	0.341
mg%	Range	1.89-2.21	1.051-2.225	6.552-8.929	2.143-5.357	1.586-2.225	1.217-2.060

Table 30. Blood chemistry in male Clarias batrachus from I to VI maturity stages (Number of values 3 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Plasma	Mean	24.24	47.30	68.62	30.17	51.72	20.53
sugar	± SD	4.65	5.67	3.31	7.18	5.53	2.05
mg/100ml	Range	20.71-29.51	40.93-51.80	65.00-71.50	23.68-37.88	45.80-56.75	18.70- 22.75
Plasma	Mean	3.688	4.378	5.260	4.484	4.037	3.144
protein	± SD	0.166	0.190	0.517	0.168	0.194	0.116
g/100 ml	Range	3.500-3.813	4.190-4.570	4.690-5.700	4.290-4.591	3.815-4.175	3.021- 3.252
Plasma	Mean	400.24	462.54	612.53	589.58	330.90	261.84
cholesterol	± SD	28.50	46.90	27.11	37.60	26.48	17.20
mg/100 ml	Range	371.50-428.50	423.08-514.39	581.37-630.72	547.82-620.75	300.75-350.37	245.50- 279.79
Blood	Mean	2.072	1.814	7.053	3.446	2.272	1.635
urea	± SD	0.112	0.856	0.643	0.862	0.276	0.291
mg%	Range	1.943-2.143	0.893-2.586	6.500-7.759	2.586-4.310	2.069-2.586	1.413-1.964

Table 31. Blood chemistry of female Clarias batrachus from I to VI maturity stages (Number of values 5 for each mean)

	Maturity Stages	I	II	III	IV	V	VI
Plasma	Mean	30.71	57.12	77.46	38.50	34.12	19.86
sugar	± SD	4.71	11.00	5.12	9.98	8.83	0.96
mg/100ml	Range	25.80-35.20	49.18-69.67	71.90-81.97	31.38-49.90	25.50-43.15	18.75-20.50
Plasma	Mean	3.771	5.052	6.767	5.295	4.002	3.088
protein	± SD	0.095	0.530	0.525	0.367	0.291	0.054
g/100ml	Range	3.688-3.875	4.580-5.625	6.250-7.300	4.900-5.625	3.750-4.320	3.050-3.150
Plasma	Mean	402.67	468.40	765.74	333.34	351.64	266.73
cholesterol	± SD	28.67	37.51	37.77	11.39	13.48	15.18
mg/100ml	Range	371.54-428.00	427.69-501.55	730.77-805.80	323.08-345.59	338.62-365.54	250.45-280.50
Blood	Mean	2.143	2.399	8.433	3.152	2.411	1.400
urea	± SD	0.175	0.853	0.611	1.003	0.908	0.140
mg%	Range	1.964-2.321	1.724-3.357	7.750-8.929	2.586-4.310	1.724-3.441	1.250-1.527

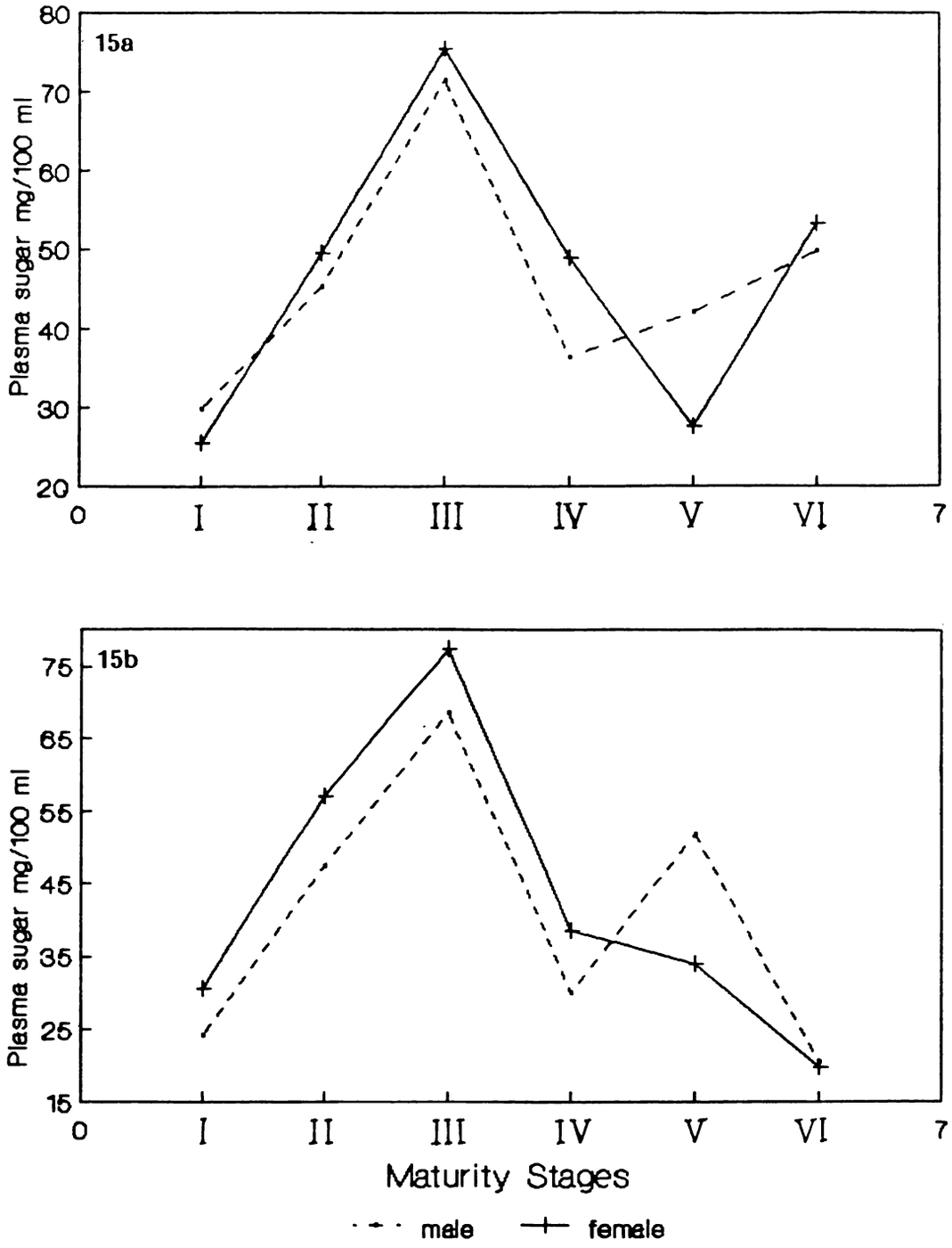


Fig.15. Plasma glucose from I to VI maturity stages
15a. H. fossilis 15b. C. batrachus

in the spent fish. With an increase in GSI, sugar increased upto the IIIrd stage. Then the pattern was irratic.

In the female H. fossilis, the pattern was similar to that of the male except for the spawning fish. The plasma sugar content in the female showed an elevation when compared to that of the male. The peak glucose level in female was found in the stage III when the GSI suddenly increased; after that the amount of glucose in blood decreased to be raised again in the spent. The GSI and plasma sugar elevated parallel to each other, upto the maturing condition; then the positive relationship was lost.

When Two Way Analysis of Variance (ANOVA) was done on the plasma sugar of both male and female, between sexes no significant difference was found; between stages and the interaction between sex and stages were significant at 1% level ($p < 0.01$).

7.4.2.2 Clarias batrachus (Tables 30 and 31 ; Figure 15b)

Plasma glucose in male C. batrachus closely followed the pattern of changes in male H. fossilis from I to V stages. The peak value was found in the stage III ; then the glucose level declined. The reduction in value was rectified in the spawning fish. But in the spent male, unlike in H. fossilis, the plasma glucose diminished to a very low value. Increased in plasma glucose in C. batrachus male was linear to GSI from I to III stages; then the pattern changed.

In the female C. batrachus, plasma glucose variations closely followed the changes in that of female H. fossilis except for the spent fish. Peak value was found in the maturing fish. It decreased through the stages IV and V to reach the lowest value in the stage VI. Plasma sugar

elevated along with GSI until the maturing period; then it declined eventhough GSI continued to be increased until spawning time.

When Two Way Analysis of Variance was performed, between sexes no significant difference was found. Difference between stages and the interaction between sex and stages were significant at 1% level ($p < .01$).

7.4.3 Plasma protein

Protein is the most important generative tissue in germ cell production. Mobilized protein from body tissues will be converted to plasma proteins to be transported through blood to the gonad during the reproductive cycle.

7.4.3.1 Heteropneustes fossilis (Tables 28 and 29 ; Figure 16a)

In H. fossilis male, the highest values in plasma protein were found during the maturing of testes. Then it gradually lowered to have low values in the stage VI. The lowest values were found for the first and last stages. Plasma protein and GSI showed a positive correlation upto the third stage in the male. Then the relationship was lost.

In the female H. fossilis, the maximum value was found in the maturing fish which was higher than that of male. The trend in changes were similar to that of male. Plasma protein was positively correlated to GSI from I to III maturity stages. Then the linearity in increase disappeared due to the changing pattern of both parameters.

When Two Way Analysis of Variance was performed on the plasma proteins of both male and female H. fossilis, the difference between sexes, between stages and the interaction between sex and stages were significant at 1% level ($p < .01$).

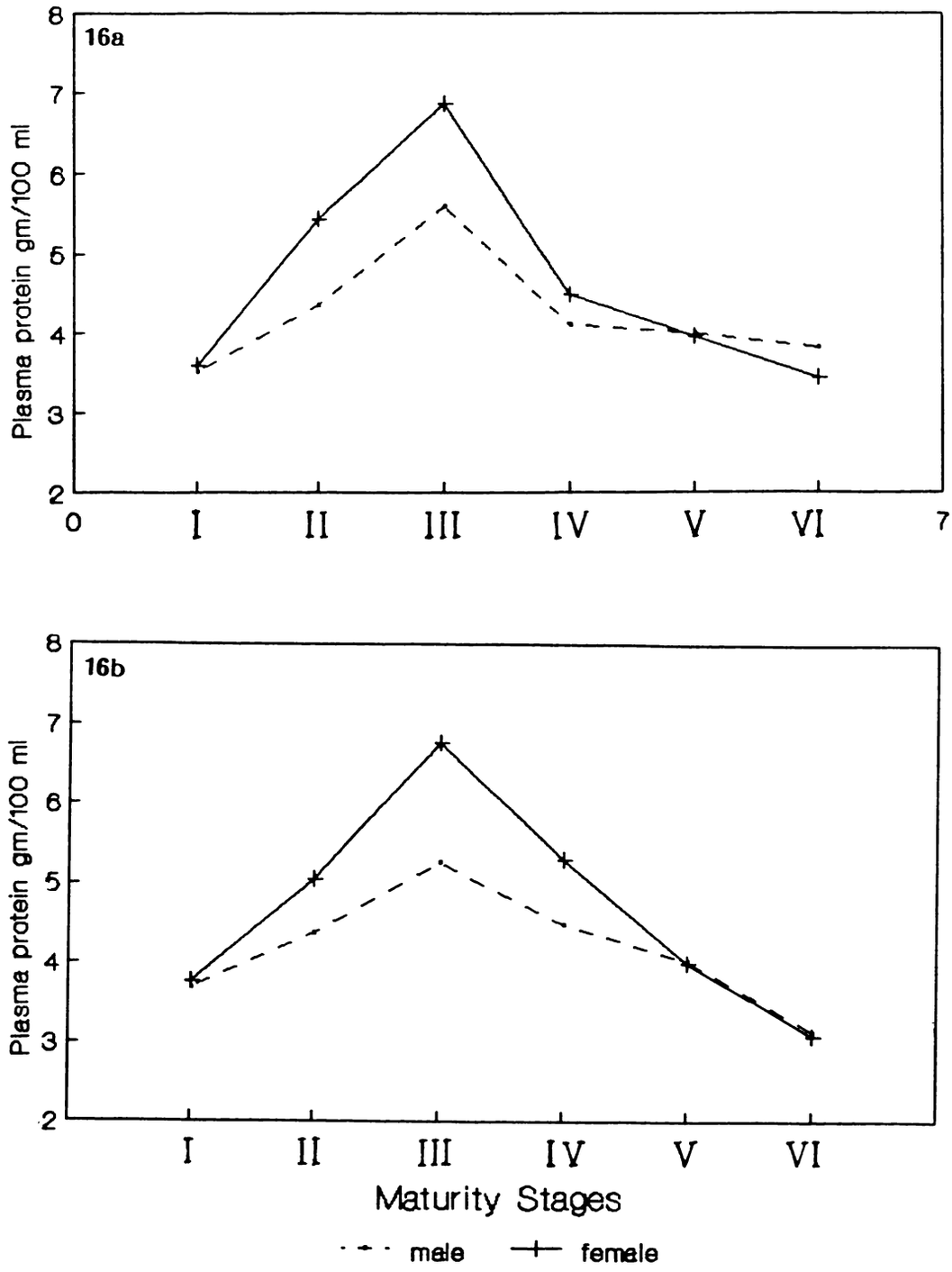


Fig.16. Plasma protein from I to VI maturity stages
16a. H. fossilis 16b. C. batrachus

7.4.3.2 Clarias batrachus (Tables 30 and 31 ; Figure 16b)

In C. batrachus, during the testicular cycle in male, plasma protein concentration was elevated from the low value in the immature fish to the highest value in the stage III; then it declined gradually and in the spent fishes, plasma protein levels were low. GSI increased parallel to plasma protein upto maturing period. Then GSI continued to be increased until the spawning time, but plasma protein declined in values.

In the female C. batrachus, plasma protein levels were higher than in the male in the stages II, III and IV. But the trend in changes were similar to that of male. Plasma protein was positively related to GSI and increased linear to it upto the maturing period; then the linearity disappeared due to the changing pattern of both.

On performing Two Way Analysis of Variance on plasma protein values of both sexes of C. batrachus, the difference between sexes, between stages and the interaction between sex and stages were significant at 1% level ($P < 0.01$).

7.4.4 Plasma cholesterol

It is one of the parameters which experienced wide variations in both sexes in both species.

7.4.4.1 Heteropneustes fossilis (Tables 28 and 29 ; Figure 17a)

In H. fossilis, the male registered initial high values for plasma cholesterol in the immature stage. It decreased in the next stage to be elevated again during the stages III and IV. A second decline was found in the spawning fish to be increased in the spent. No direct correlation

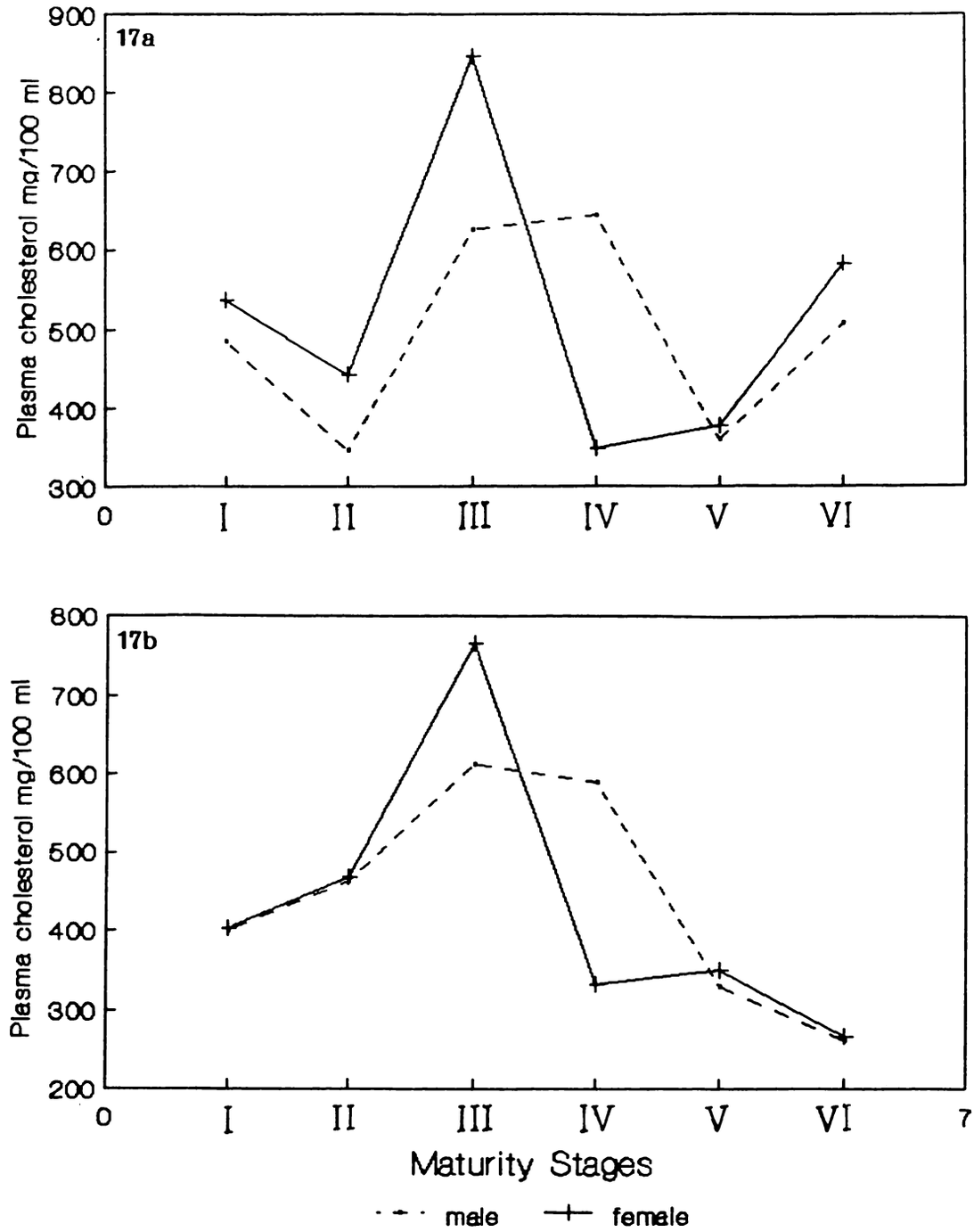


Fig.17. Plasma cholesterol from I to VI maturity stages
17a. H. fossilis 17b. C. batrachus

with GSI was found for plasma cholesterol except for the stages III and V.

Female H. fossilis, showed higher values in the immature, developing, maturing and spent fishes than in the male. But the trend of changes in both sexes was almost the same, except that the male maintained a higher level of cholesterol in the stage IV. Except for stages I and VI, GSI and plasma cholesterol were found to be positively related.

When Two Way Analysis of Variance was performed on the plasma cholesterol of both sexes of H. fossilis, difference between sex was found to be significant at 5% level ($P < 0.05$). Between stages and the interaction between sex and stages were significant at 1% level ($P < 0.01$).

7.4.4.2 Clarias batrachus (Tables 30 and 31; Figure 17b)

In C. batrachus, the pattern of plasma cholesterol in male registered a close similarity to that of male H. fossilis except for the developing and spent fishes. The lowest cholesterol level during testicular cycle was found in the spent fish. Initial levels of cholesterol increased in the developing stage II, to reach its maximum in the maturing stage III, when the GSI suddenly increased. The high level of cholesterol in the stage III in male was maintained during the stage IV. But it declined during the stage V to register the lowest values in the stage VI. A direct relationship existed between GSI and plasma cholesterol except for the stages IV and V.

During the ovarian cycle, the cholesterol values in C. batrachus followed the trend in changes in the plasma cholesterol values of female H. fossilis except for the stages II and VI. It increased from the first through second stages to reach the peak values in the stage III. It declined in the stage IV, but became a little higher in the stage V to register the lowest values

in the spent fish. Cholesterol values changed parallel to GSI except for the stages IV and V.

When Two Way Analysis of Variance was performed in both sexes of C. batrachus on plasma cholesterol, between sexes no significant difference was found. But between stages and interaction between sex and stages were found to be significant at 1% level ($P < 0.01$).

7.4.5 Blood urea

Blood urea is a measure of nitrogen metabolism taking place in the body. The pattern of urea metabolism was similar to that of other metabolites in both fishes.

7.4.5.1 Heteropneustes fossilis (Tables 28 and 29 ; Figure 18a)

In H. fossilis, during the testicular cycle, low blood urea levels were found during the stages I and II. It suddenly elevated to a very high level in the stage III. Then it lowered gradually through the stages IV and V to reach the lowest level in the stage VI. Just like its relation to other metabolites, GSI values didn't show any relationship with blood urea levels except for the stages III and VI.

During ovarian recrudescence in the female H. fossilis, blood urea was found to be low in the immature and developing fish. In the maturity stage III it reached the highest level, higher than that of male at the same stage. Then a sudden decrease in blood urea was found in the stage IV, which continued until the last stage. GSI values changed linear to blood urea levels only for the stages III and VI.

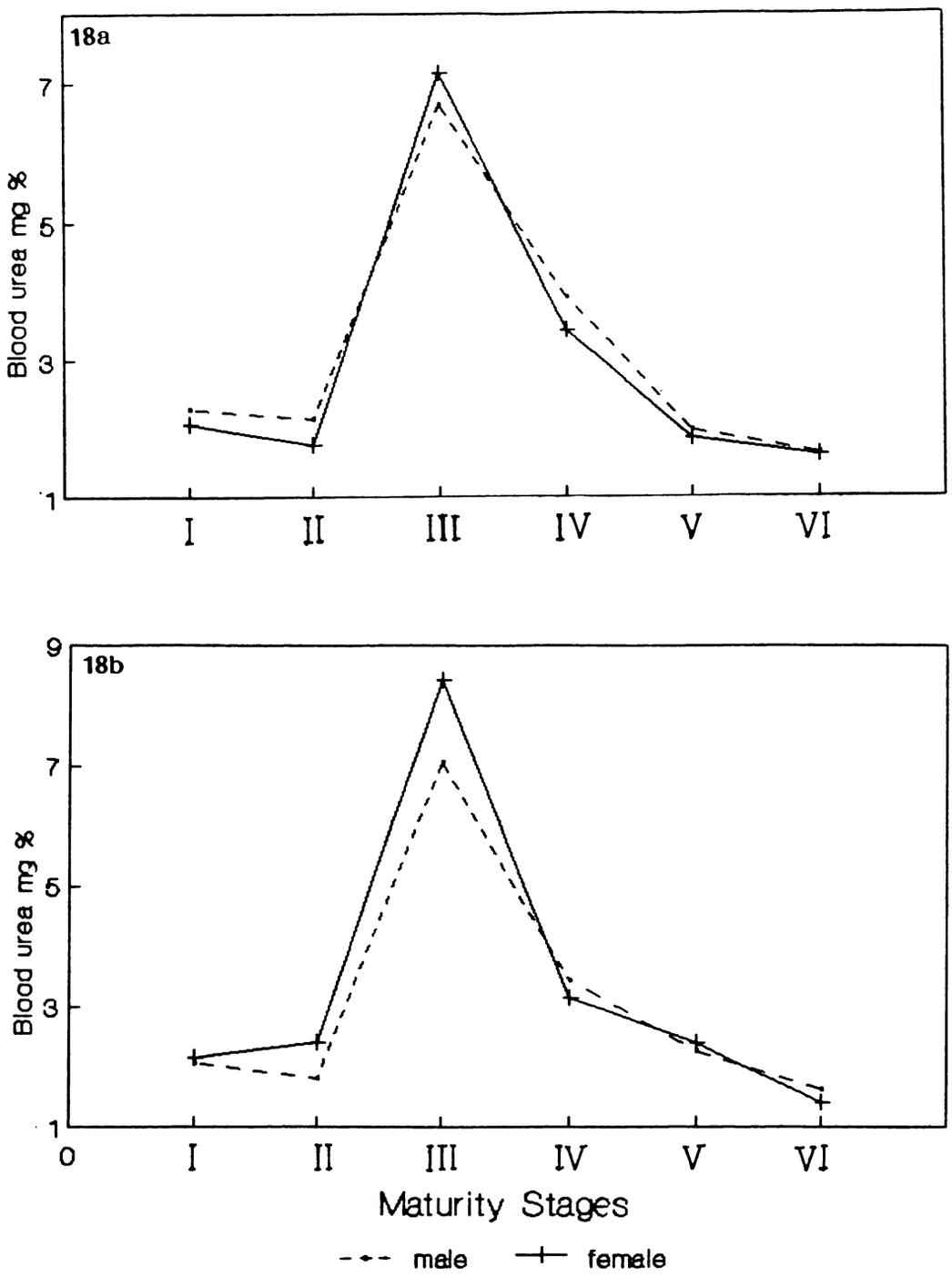


Fig.18. Blood urea from I to VI maturity stages
18a. H. fossilis 18b. C. batrachus

Table 32. ANOVA TABLE - Two Way Analysis of Variance of biochemical factors in the blood of male and female Heteropneustes fossilis

	Source of variation s	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
Plasma glucose	Total	14705.1968	59		
	Sex	12.0961	1	12.0961	0.55
	Stages	12558.5264	5	2511.7053	114.45**
	Sex x Stages	1081.1424	5	216.2285	9.85**
	Error	1053.4319	48	21.9465	
Plasma protein	Total	63.5889	59		
	Sex	2.3737	1	2.3737	21.23**
	Stages	50.5521	5	10.1104	90.43**
	Sex x Stages	5.2970	5	1.0594	9.48**
	Error	5.3661	48	0.1118	
Plasma cholesterol	Total	1324934.5285	59		
	Sex	11161.6120	1	11161.6120	6.263*
	Stages	854904.5037	5	170980.9007	95.946**
	Sex x Stages	373329.4810	5	74665.8962	41.899**
	Error	85538.9318	48	1782.0611	
Blood urea	Total	230.4858	59		
	Sex	0.7896	1	0.7896	1.65
	Stages	177.2549	5	35.4510	73.90**
	Sex x Stages	29.4163	5	5.8833	12.26**
	Error	23.0250	48	0.4797	

Level of Significance : *P < 0.05 **P < 0.01

Table 33. ANOVA TABLE - Two Way Analysis of Variance of the biochemical parameters in the blood of male and female Clarias batrachus

	Source of variation s	Sum of Squares ss	Degrees of freedom df	Mean Square ms	Variance ratio F
Plasma sugar	Total	12770.2469	35		
	Sex	57.6081	1	57.6081	1.3856
	Stages	10878.5023	5	2175.7005	52.3318**
	Sex x Stages	836.3339	5	167.2668	4.0232**
	Error	997.8026	24	41.5751	
Plasma protein	Total	38.5898	35		
	Sex	2.2241	1	2.2241	22.2633**
	Stages	31.1042	5	6.2208	62.2703**
	Sex x Stages	2.8647	5	0.5729	5.7347**
	Error	2.3968	24	0.0999	
Plasma cholesterol	Total	787243.8578	35		
	Sex	1209.1847	1	1209.1847	1.3591
	Stages	631240.8639	5	126248.1728	141.8989**
	Sex x Stages	133440.8952	5	26688.1790	29.9967**
	Error	21352.9140	24	889.7048	
Blood urea	Total	173.3135	35		
	Sex	0.6850	1	0.6850	1.6337
	Stages	159.6409	5	31.9282	76.1464**
	Sex x Stages	2.9250	5	0.5850	1.3952
	Error	10.0626	24	0.4193	

Level of Significance : *P$<$0.05 **P$<$0.01

The results of Two Way Analysis of Variance performed on the blood urea values of both sexes showed that between sexes no significant difference existed. Between stages and the interaction between sex and stages showed significance at 1% level ($P < 0.01$).

7.4.5.2 Clarias batrachus (Tables 30 and 31 ; Figure 18b)

In C. batrachus, in the male, during the stages I and II, blood urea levels were low. Then a sudden increase was registered in the stage III, the highest value during the testicular cycle. During the stage IV, a sudden dip in values were found which continued and showed the lowest value in the spent. Between GSI and blood urea, the only positive relationship were found in the stages III and VI.

In the female C. batrachus, the maximum level of blood urea in the stage III was higher than that of male in the same stage. Initial low blood values during the first two stages were followed by the highest blood urea content in the stage III to be decreased through the stages IV and V to reach the lowest level in the stage VI. A definite positive relationship existed between GSI and blood urea levels upto the stage III.

The results of Two Way Analysis of Variance performed on the blood urea levels in both sexes of C. batrachus showed no significant difference between sexes and the interaction between sex and stages. Between stages showed significance at 1% level ($P < 0.01$).

7.5 DISCUSSION

7.5.1 Plasma glucose

In H. fossilis and C. batrachus, blood glucose is one of the investigated factors which showed maximum fluctuations during the spawning season. Initial low plasma sugar in both species might be due to the fact that these bottom dwelling cat fishes are comparatively inactive and they are mainly carnivores. In the developing stage, in both species high blood glucose levels were observed. This might have been caused by two factors, either due to the intense feeding of fishes at this time or by the mobilization of already stored glycogen. In H. fossilis increased feeding activity was noted in the laboratory alongwith fat deposits in both species just under the skin and in the abdominal cavity. Glycogen and glucose have been reported to accumulate in the ovary during maturation by Greene as quoted by Love (1970) in Oncorhynchus tschaaytscha, Chang and Idler (1960) in Oncorhynchus nerka and Yanni (1961) in Clarias lazera.

When the gonads reached the maturing stage in H. fossilis and C. batrachus, blood glucose reached its maximal level in both male and female. Joshi (1982b) in the cat fish Rita rita (Ham) and Joseph (1987) in Mugil cephalus males obtained higher blood glucose levels during the maturing of gonads.

Beamish (1964) has shown that both Salvelinus fontinalis and Salmo trutta exhibited maximum rate of standard metabolism during the spawning season. A similar finding was reported to occur in the pumpkin seed fish, Lepomis gibbosus (Burns, 1975). An increase in overall metabolic processes

require mobilization of energy reserves to supply necessary substrates. It is also possible that glucose may be functioning directly in the formation of reproductive products. In male fish glucose may be utilized to aid in fat deposition in maturing testes (Mackay and Beatty, 1968).

Billiard and Jalabert (1973) reported the active deposition of alpha and beta glycogen particles in the sertoli cells and spermatids right from the beginning of gametogenesis in the guppy. Chaturvedi et al. (1976) found that the amount of glycogen and glucose in the gonads of the male H. fossilis were much higher than that of the female in the prespawning period. It is at this time that the vitellogenesis is reported to start in full scale (Lamba et al., 1983) in the female. So the energy demands for steroid synthesis and for the incorporation of vitellogenin into the oocyte might be higher in the female. Some part of this energy might be derived from glycolysis and hence the low level of both glycogen and glucose in the gonads of the female. In the male, the higher amount of energy stored in the form of glycogen and glucose in the testes might be utilized for spermiation at the time of spawning. According to Yanni, (1961) hyperglycemia in Clarias lazera might be contributed by liver glycogen. In Fundulus heteroclitus too, it was supposed to be the same case (Leach and Taylor, 1976). Pickford (1953) observed differences in liver size between fish captured in spring and fall.

An increase in interrenal activity might contribute to spawning caused hyperglycemia through its influence on gluconeogenic processes. This is observed in salmonoid fishes where marked changes in cortisol levels have been reported and correlated with serum glucose elevations (Robertson et al., 1961). Eddy (1984) opined that the secretion of cortisol influences

carbohydrate metabolism in fish and is stimulated by stresses such as handling, temperature changes, exercise and spawning. The finding of Peter et al. (1978) that serum cortisol increases during pre-spawning period in fishes agrees well with the work of Lamba et al. (1983) in H. fossilis.

So in H. fossilis and C. batrachus with an increase in temperature which elevates metabolism, at the time of maturing of gonads when the maximum turn over of organic materials happened, by the action of cortisol, glycogenolysing might be happening in the body tissues liberating increased amount of glucose into the blood to be deposited in the gonads for the immediate mobilization of energy as and when the need occured.

The gradual decrease in blood glucose during the mature and spawning period is due to the lowering of body reserves of glycogen and glucose as well as lowered body metabolism, resulting from low temperature. Reduced feeding rate is another reason for lower flood glucose level at this period. In the male, a little elevation in blood glucose happened during spawning, which might be due to energy needed for spawning behaviour like site selection, aggression etc. which are usually found in male teleosts.

In the spent fish, both in male and female, elevation of blood glucose was observed. The spawned fish might have started intense feeding again to compensate for the drained body reserves. Hence the higher plasma glucose level. Post spawning increase in plasma glucose level is also reported in male white sucker and female northern pike by Mackay and Beatty (1968) and in Channa punctatus (Bloch) by Khanna and Singh (1971).

7.5.2 Plasma protein during gonadial cycle

Plasma protein is an index of the turn over of protein metabolism in the somatic tissues. According to Shul'man (1974), the most characteristic feature of pre-spawning period is the intensive protein synthesis, associated with differentiation and growth of generative tissue. The results obtained in the present investigation agree with this observation. In H. fossilis as well as in C. batrachus, plasma protein gradually increased with an increase in the weight of the gonad and reached its maximum during the IIIrd, maturing period of gonad. Then it gradually declined through the succeeding stages. The low level of plasma protein in the Ist stage in January might be due to the low level of metabolism in both species during this period as shown by low haematological parameters in the preceeding chapter. With the gonadial recrudescence intense feeding alongwith mobilization of already stored protein might have started during February - March months, hence the increase in plasma protein values during the IInd stage in both sexes of both species.

Love and Robertson (1967) and Iles (1974) found that the protein synthesized and accumulated in the somatic tissues during the prematuration period is utilized for gamete formation in addition to the growth of fish. Korzenko (1966) and Love (1970) suggested that certain amino acids from the muscle may be mobilized for the production of sex products. MC Bride et al. (1960) have opined that much of the gonad tissue is built from protein from the muscles. Ando and Hatano (1986) observed that total protein and sarcoplasmic protein in chum salmon markedly decreased during spawning migration.

Emerson et al. (1979) found that higher doses of estradiol mobilized liver protein in male flounder Platychthys flesus. When male crucian carp was injected with estradiol 17β , along with phosphoprotein and calcium, plasma protein was found to be elevated (Tinsley, 1985). In H. fossilis female, maximum level of estradiol 17β coincided with the prespawning period (Lamba et al., 1983) when the peak level of plasma protein was detected. So, etradiol 17β , the female sex steroid might have acted on somatic tissues mobilizing the protein which then appeared in the plasma while carried to the gonads.

In H. fossilis vitellogenin is continued to be transported to the gonads until spawning of the fish (Lamba et al., 1983). The maximum level of vitellogenin in female fish was found during the spawning period when in H. fossilis and C. batrachus females plasma proteins were low. So in the case of these two species of fishes, plasma protein levels can't be taken as an index of exogenous vitellogenesis. Tinsley (1985) stated that the considerable amount of calcium and protein in male crucian carp plasma occured as a result of injection of estradiol, reflect the environment of these constituents in biological processes other than exogenous vitellogenesis. So he says that plasma total calcium and plasma total protein levels are non-specific indices of exogenous vitellogenesis.

The highest level of plasma protein both in male and female during the IIIrd stage might be the result of protein mobilized from the somatic tissues like muscle and liver by the action of the female steroid estradiol 17β . A part of the liver protein thus mobilized and all of the muscle protein might be carried to the gonads for the building up of the structural gonadial tissue and the rest of the liver protein utilized in the synthesis of serum

phospholipoprotein complex, vitellogenin. Joshi (1982b) also obtained the highest values of plasma protein during the early pre-spawning period which is equivalent to the maturing stage, in the cat fish Rita rita (Ham).

Kulikova in 1962, as quoted by Shul'man (1974) found that the protein content of the serum dropped sharply in the period before spawning in small and large horse mackeral on account of the consumption of reserve protein of the serum for the formation of genital products. The protein concentration in the round goby too declined on maturation of gonads. In the male and female of both H. fossilis and C. batrachus the plasma protein decreased during the mature period (IVth stage) when the gonadial tissue achieved a very high weight increase. It is apparent that all the available mobilized proteins in the form of plasma proteins in both species are utilized for the enlargement of gonads. Joshi (1982b) reached the same conclusion for plasma protein reduction in Rita rita.

In H. fossilis and C. brachus the diminishing protein levels continued through spawning to spent. This leads to the inference that the spent fish, which will start to feed intensively during the post spawning period should have high plasma protein levels. But the draining of protein from the somatic tissues might have been so high that all the absorbed protein from the digested food was immediately transported to the muscle and liver leaving the blood low in values.

The results reveal highly significant difference between the male and female plasma protein values. As is evident from the GSI, while the testes achieved even in its fully mature condition only below 1% of the weight of male, the ovary made about 18% of the body weight in H. fossilis and

15% of body weight in C. batrachus. Such a provocatively asymmetric distribution of generative tissue in different sexes itself point out the cause of high level protein in female.

Another important factor observed is the correlation between the environmental temperature and protein metabolism. In both sexes of both species the plasma protein levels follow the rise and fall of temperature. Maximum levels of both were found during the IIIrd stage in April when the maximum metabolism of both sexes of both species occurred as evinced from the values for haematological and biochemical parameters. As water temperature rises, the protein requirement increases because of accelerated fish growth. Falling water temperatures decrease growth rates and so less protein is required by the fish (Phillips, Jr., 1969). So the protein metabolism in H. fossilis and C. batrachus is affected by temperature alongwith endogenous factors.

7.5.3 Plasma Cholesterol during gonadial cycle

Fat is the main source of energy for fish. The principal sources of energy for protein synthesis during the prespawning period are the large fat reserves and energy derived from food. The absolute and relative fat content of fish declines during the mobilization of energy, the decline being especially marked in the first stages of gonad development (Shul'man, 1974). In addition to the utilization of fat as an energy source, it is also used for the formation of generative tissue and for the synthesis of steroid hormones. Cholesterol, one of the lipids is the parent sterol and accepted precursor of steroids including estrogens, androgens and corticosteroids (Awapara, 1974; Colombo et al., 1977; Rishi and Kaul, 1984).

Higher plasma Cholesterol levels were found in the immature stage in H. fossilis when compared to that of C. batrachus. It reflects the gradation in the feeding rate and the fat stores during the resting months. During the IInd stage, in H. fossilis, lowering of plasma cholesterol occured. Serum cholesterol might have been utilized for the building up of gonadial tissue as well as for energy purposes before the full mobilization of fat stores started. But in C. batrachus the mobilization of cholesterol might have started in the second stage itself, otherwise comparatively high values of cholesterol wouldn't have occurred at this time.

In both species, surprisingly elevated values of cholesterol was reached in the maturing condition. In the females, the cholesterol levels were significantly higher than the males in both species. Lamba et al. (1983) reported peak estradiol 17β levels and the larger peak of cortisol in female H. fossilis and Singh and Singh (1987) observed the highest levels of estradiol 17β and estrone in C. batrachus female during this period. In addition to its participation in vitellogenin synthesis, estrogens regulate lipid metabolism (Plack and Pritchard, 1968; de Vlaming et al., 1977a,b; Korsgaard and Petersen, 1979; Sand et al., 1980; Dasmahapatra and Medda, 1982). They probably control lipid mobilization from fat stores (de Vlaming et al., 1977a). Salmon gonadotropin SG-G100 (Donaldson et al., 1972) increases the plasma concentrations of triglycerides and total cholesterol in gold fish with small ovaries. The lipid mobilization may be mediated by estrogen produced by the ovary under gonadotropin stimulation (Wiegand and Peter, 1980). In the male H. fossilis, the highest levels of cholesterol was observed during the prespawning period (stage IV) when peak serum testosterone occurred (Lamba et al.,

1983). So just like estrogens in female, testosterone too may have a lipid mobilizing effect.

In striped mullet, Dindo and Mac Gregor (1981) reported two peaks for blood cholesterol, the first larger one during intensive feeding and the second smaller peak before spawning. But Joshi (1982b)found the maximum level of blood cholesterol during the early prespawning period in the cat fish Rita rita (Ham) agreeing with the present observation.

Plasma cholesterol in the females of both species fell during stages IV and V . Lesser need of cholesterol for the synthesis of estrogens may be one of the reasons for this. A reduction in the synthesis of estradiol 17β at this period in trout was observed (Fostier et al., 1978). Low levels of estrogen at the time of spawning have been confirmed in rainbow trout (Scott et al., 1980), striped mullet (Dindo and Mac Gregor, 1981),King mackerel (Mac Gregor et al., 1981) and the white spotted char (Kagawa et al., 1981). Some of the cholesterol mobilized might be incorporated into vitellogenin, the synthesis and transportation of which accelerated during this period (Lamba et al., 1983). The high levels of serum cholesterol in the males of both species during the stage VI should be due to the continued mobilization of cholesterol for the synthesis of testosterone, as high testosterone at this stage was reported by Lamba et al. (1983). In the spent, in both sexes of H. fossilis high cholesterol levels were found. This might be due to the intense feeding of fishes to renew their lost fat reserves. McCartney (1967) also reported peak values of serum cholesterol during the post spawning months in Salmo trutta. But in C. batrachus, the fishes were so much physically exhausted after spawning that they might not be able to renew intense feeding for sometime resulting in low plasma cholesterol values.

During the breeding season, significant sex difference in plasma cholesterol content occured between male and female H.fossilis and C.batrachus. In male, cholesterol is mainly utilized for the synthesis of sex steroids and corticosteroids, but in female in addition to act as the precursor of estrogens, it is also found in serum vitellogenin and egg yolk proteolipid complex (Riazi and Fremont, 1988). So the accumulation of cholesterol from the food and its mobilization from the liver will be higher than those in the male. Temperature or other environmental factors doesn't seem to have an effect on cholesterol.

7.5.4 Blood urea during gonadial cycle

Various fishes have two main path ways of nitrogen elemination, leading to the production of ammonia on the one hand and to the synthesis of urea on the other. The ultimate source of nitrogen in both cases is presumably the amino and amide groups of aminoacids (Forster and Goldstein, 1969).

Idler and Isuyuki in 1958 reported fluctuations in the blood urea. N of sock eye salmon during spawning migration. In H. fossilis and C. batrachus there is a gradual increase in blood urea reaching the peak level in the IIIrd stage to be decreased gradually to end in the lowest level in the spent fish. The maximum amount of blood urea coincided with the peak catabolic activities taking place in the body during the maturing of gonads, due to the mobilization of body reserve materials.

Metabolism of arginine is associated with its breakdown by arginase and with the production of urea. Dietary arginine could be a source of urea, but it is unlikely that the degradation of an essential amino acid which is

not synthesized in the body could provide more than a minor fraction of the nitrogen excreted as urea (Forster and Goldstein, 1969).

Yakovenko et al. (1982) reported that arginine in the muscles of carp practically disappeared after 40 days of starvation. Grubinko et al. (1987) observed high arginase activity in the liver and intestine and very high urea content in the muscle, liver, intestine, gills and blood in carp as a result of starvation. During the maturation of gonads, there is a chance of reduced feeding in H. fossilis and C. batrachus due to the diminished food availability and the reduced space in the abdominal cavity resulting from the enlargement of gonads. So there is a possibility of starvation which may cause arginine breakdown forming large quantity of blood urea. But in the laboratory, the two species of fishes were found to take in food during the breeding season. During maturation period, arginine in the gonad of the male and female Clupea sprattus (Petrenko and Karasikova, 1958) and in the testes of the male Oncorhynchus keta increased. Eventhough during the intense feeding period of the fish, dietary arginine is concentrated in increased amounts in the body, it might be transported to the gonads to maintain the high arginine levels. In teleost fishes, the enzymes in the ornithine-urea cycle is incomplete or lacking (Manderscheid, 1933; Brown and Cohen, 1960). So the only available pathway for the production of urea is purine degradation Aminoacids could be funneled into the pathway during formation of the purine ring and then converted to uric acid eventually to be transformed to urea. Creelman and Tomlinson in 1959 observed a decrease in RNA in the whole fish of both sexes in Oncorhynchus nerka during spawning run. There are reports of

decreased aminoacids in the gonads of the male and female teleosts (Korzhenko, 1966; Petrenko and Karasikova, 1958). In H. fossilis and C. batrachus plasma protein and blood urea has got a very high positive correlation during the breeding period. So the most possible explanation for increased blood urea during gonadial maturation is the funnelling of large quantity of aminoacids (resulting from the break down of tissue proteins to form blood protein) which were not needed for the formation of gonadial tissue, through the purine pathway to be converted to uric acid and finally to urea.

Another probable explanation lies with the evolutionary adaptation of live fishes. Periodic exposure of air breathing fishes to desiccation due to drying up of shallow puddles and pools in the tropics happens regularly. Then the air breathing fishes will take refuge in soft mud (Dehadrai and Tripathi, 1976). This happens during the summer months when there is no rainfall and temperature is at it's highest. In such an inimical environment, as n evolutionary adaptation for survival, these air breathing fishes, H. fossilis and C. batrachus might have resorted to increased ureotelism due to the absence of enough water, to remove the amino and amide nitrogen resulting from the high protein metabolism during gonadial maturation.

After the stage III, blood urea decreased and reached very low levels in the spent stage might be due to the low protein metabolism in the spent fish. Even though the spent fish fed, it might again resort to ammonotelism which required comparatively lower level of energy expenditure than ureotelism and at this period the rainfall was so high that there was no scarcity of water.

In addition to the above mentioned factors, blood dilution due to the flooding of body tissues with the medium might be one of the reasons for the lowering of blood components during stages IV, V and VI. It should be only slight because in H. fossilis in the spent stage when the maximum flooding of body tissues happen, high levels of plasma glucose and cholesterol occurred.

When the trend in the biochemistry of blood of H. fossilis during the reproductive period is closely followed, several interesting factors could be understood. All the four parameters investigated, the plasma sugar, plasma protein, plasma cholesterol and blood urea were found to be affected by various maturity stages. They reached their maxima during April when the exogenous vitellogenesis started in full scale as can be assumed from the suddenly increased GSI. This should be due to the influence of steroid hormones. From the stage IV, all biochemical values lowered. So it may be assumed that the plasma sugar, cholesterol and protein are needed in large quantities only upto the stage III. The maximum nitrogen metabolism takes place in the stage III as can be seen from the highest blood urea values. Corresponding to this stage, from literature it is known that on the stage IV the major biochemical process taking place is the transport of vitellogenin from liver and in the stage V eggs absorb only water. So in these two stages in gonad, no synthesis of generative tissue should be taking place. In the stage VI in H. fossilis, the spent fish starts intense feeding again as explained by its high plasma cholesterol and sugar contents. But C. batrachus, which is the larger fish, became physically exhausted so much at the end of breeding that it showed very low level of biochemical components.

CHAPTER - VIII

SUMMARY

The first chapter is general introduction. The importance of the culture of air breathing fresh water fishes in India, the food habits of two culturable air breathing fishes, Heteropneustes fossilis and Clarias batrachus, culture of these two fishes in India and other countries and the relevance of blood studies in aquaculture system are discussed in this chapter.

The second chapter is a general review of literature on the haematology and blood chemistry of teleost fishes. Selected works on normal erythrocyte count, haemoglobin content, haemoglobin polymorphism and packed cell volume are reviewed. Review of literature on normal levels of blood glucose, plasma protein content, lipid and blood urea are also included. The origin and development of erythrocytes and leucocytes are reviewed in detail. Selected works on blood constituents in relation to growth and size, sex and season, diseases and pollution are also reviewed.

The third chapter contains materials and methods which are used in more than one chapter. In this chapter the collection, transport and maintenance of fishes, their feeding regime and the treatment for diseases are described. The method used for the collection of blood and the analysis of haematological factors are also described in this chapter. The method of determination of maturity of gonad is also given.

The collection sites of fishes utilized for the present investigation were the ponds and rivers of Mavelikara in Alleppey District and Panangadu, Edappally and Irimpanam in Ernakulam District, Kerala State, India. They were acclimatized in fibre glass tanks for one week near the collection sites

before transportation to avoid mortality. The fishes were transported in covered plastic buckets with provision for air circulation. Fishes were maintained under laboratory conditions for the study of normal values in haematology as well as for the leucocyte studies. For investigations in blood regarding reproduction, they were kept in out door tanks to provide natural environmental temperature and photoperiod. Feeding was done at fixed times using a balanced diet and natural foods like earthworms and beef liver. For treating skin diseases of fishes kept in captivity, methylene blue was found to be the most effective medicine.

Fishes were immobilized with a hard blow on head before the collection of blood. Blood was collected in heparinized vials by severing the caudal peduncle. The Red Blood Corpuscle (RBC) count was done using a haemocytometer with improved 'Neubaur ruling'. Haemoglobin (Hb) concentration was estimated by cyanmethemoglobin method and Packed Cell Volume (PCV) by microhaematocrit method. From these values, the erythrocyte constants, MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration) were calculated. For blood studies in relation to gonadial cycle, six arbitrary maturity stages were found out from the appearance of gonads. GSI (Gonadosomatic Index) was calculated from the weight of gonads and body weight of fish.

In the fourth chapter, the morphology of the RBCs present in the blood and the variation in normal haematological parameters of both H. fossilis and C. batrachus during the year are described. The weight related variation in haematology in male and female of both species were investigated. Immature and mature RBCs were found in the blood of both fishes. They were also reported in some other cat fishes. So it may be concluded that the presence

of immature RBCs in the blood circulation is a characteristic feature of cat fish blood. RBCs without nucleus were seen in the blood smears of H. fossilis and C. batrachus. They are called erythroplastids. They were found in cat fish blood during the breeding season.

In H. fossilis, most of the erythrocytes are elliptical in shape. In C. batrachus the most common form is circular. Between H. fossilis and C. batrachus, no great variation in the size of the erythrocytes are found. The reason may be that these two fishes are placed very close phylogenetically and they do belong to the same habitat.

The RBC, Hb and PCV values are high in H. fossilis and C. batrachus. This should be due to the air breathing nature of the fishes under investigation. Seasonal variations do occur in normal values in all the haematological parameters studied. The maximum values are seen in summer months in both species when the gonads mature. The minimum values are found in immature stage in H. fossilis, when the temperature was low. But in C. batrachus, the lowest values are found during the post-monsoon period caused by spawning depletion. The erythrocyte constants are also affected by variation in seasons. All the haematological parameters and derived values in both sexes of both species generally increase in relation to weight except MCV. All haematological parameters are positively correlated to weight and the relationships are highly significant. With an increase in weight, the oxygen requirements of the body increases necessitating increased number of RBC along with an increase in respiratory pigment Hb and PCV. The MCV decreases with an increase in weight. It may be that to cope with the increased need of oxygen, blood cells might have reduced their size. MCH and MCHC values closely

follows the changes in Hb as these two are derived values from Hb. So it is apparent from the observations that before establishing normal haematological values in H. fossilis and C. batrachus, the body weight, physiological condition of fish, temperature and season should be taken into account. Sex of the fish doesn't seem to have much effect except for some stages during breeding season. Another factor to be considered is intraspecific variations existing in the haematological parameters of H. fossilis and C. batrachus occuring in geographically distant places.

The fifth chapter deals with the identification and characterization of leucocytes and related cells. Leucocytes were identified using morphological, ontogenic and functional characteristics. Giemsa stained blood smears are used for morphological studies. Ontogeny of blood cells was studied from imprints of haemopoietic organs. By injecting particulate carbon and bacteria into the peritoneal cavity of fish, the phagocytosing capacity of various blood cells were observed. Various cytochemical methods were used to identify PAS positive substances, Sudan B positivity and the presence of enzymes like acid and alkaline phosphatase and peroxidase. Six types of cells were identified; lymphocytes, thrombocytes, monocytes, macrophages, plasma cells and one type of granulocyte, the neutrophil. Three types of lymphocytes are found, small medium and large. They are weakly positive for only PAS and acid phosphatase. They do not phagocytose any material. Lymphoblasts are found in kidney imprints. So just like mammalian lymphocytes these should be immunocompetent cells. Four types of thrombocytes are observed; tear drop, spindle, oval and round. They are positive for PAS and acid phosphatase. Small particles of particulate carbon are found in them

when injected with carbon. So they should possess a limited amount of phagocytosing capacity. The precursor cells of thrombocytes are not found. Monocytes are positive for all cytochemical tests. They phagocytose both carbon and bacteria. Monoblasts are found in kidney imprints and in circulation when the fishes are stressed. Three types of macrophages are identified; the circulating, melano and peritoneal. The circulating type is found in circulation when the fishes are stressed and during breeding period. Melano-macrophages appear in circulation when the fishes are injected with bacteria or parasitized by trypanosomes. The peritoneal macrophages are found in the peritoneal fluid in large numbers when foreign bodies are injected into the peritoneal cavity. All of them are positive for PAS, acid and alkaline phosphatase. The macrophages are found to avidly phagocytose both carbon and bacteria. The melano-macrophage contained melanin granules. Two types of melano-macrophages are found and it is suggested that they should be functionally different. Neutrophils are the only kind of granulocyte found in H. fossilis and C. batrachus. They are strongly positive for all cytochemical tests. They phagocytose both carbon and bacteria. Their younger stages are found in kidney imprints. It can be concluded that a well developed phagocytosing system is present in both H. fossilis and C. batrachus and the use of morphological characters alone is inadequate for the identification of cells in these two fishes.

The sixth chapter contains haematological changes in relation to environmental factors and reproduction. All the three environmental factors examined (temperature, photoperiod and rainfall) are found to have a direct effect

on GSI. But only temperature is positively related to haematological factors. The other two parameters indirectly enhance the effects of temperature. With an increase in environmental temperature, haematological factors were found to increase. At the same time gonadial changes also affect haematology. The maximum values for RBC, Hb and PCV were found generally in the maturing fish when the temperature was the maximum. But MCV was the minimum during this period. The reduction in the volume of RBC and erythropoiesis are two reasons attributed for this. In the mature fish haematological values lower due to a lowering in temperature. The GSI reaches almost the peak level at this time. The feeding rate is also diminished. So the oxygen requirement is reduced resulting in the lowering of the levels of RBC, Hb and PCV. Another cause is the haemodilution due to the flooding of body with the external medium. Post-spawning depletion is found in both fishes. But it is comparatively higher in C. batrachus. Multiple factors are responsible for the haematological changes during the gonadial cycle. In addition to the temperature changes and increased energy demand for the deposition of generative tissue, the osmoregulatory activities and hormones like estrogen and cortisol are also involved.

The seventh chapter describes the changes in four biochemical factors in blood (plasma glucose, plasma protein, plasma cholesterol and blood urea) during the gonadial cycle. The maximum values for all these factors were found during the maturing stage. It is at this time a sudden increase in the weight of gonad happened. So the increase in glucose, protein and cholesterol in plasma should be for the synthesis of gonadial material. The

increase in blood urea should be either due to the change in mode of excretion or due to the breakdown of aminoacids released during protein catabolism. Temperature seems to affect protein metabolism. The female gonadial hormone estrogen and cortisol are also seemed to be involved in these processes. During the mature period biochemical factors suddenly decreased due to their increased utilization. Another reason should be the dilution of blood by osmoregulatory changes. Post-spawning depletion is smaller in H. fossilis may be due to their smaller size and intense feeding. But spawning stress is comparatively high in C. batrachus, the larger fish.

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