Advances in Bioresearch
Adv. Biores., Vol 8 (6) November 2017: 63-70
©2017 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.8.6.6370

Advances in Bioresearch

ORIGINAL ARTICLE

Analyses of Haematobiochemical Profile of Green Chromide – *Etroplus Suratensis* (Bloch, 1790)With Morphological Assessment of Blood Cells

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ABSTRACT
This study focuses on haematobiochemical parameters of Etroplus suratensis. Haemotobiochemical analyses were carried out to establish reference values for individuals and to assess sex-related changes as well as in early disease detection and diagnosis of this species. The results of this study confirmed that there are significant differences in blood parameters with respect to sex. The data of all stated parameters show higher value in males in comparison to females except the total leukocyte count (TLC) and globulin content. In this investigation we also observe different types of erythrocytes like, microcyte, macrocyte, tadpole cell, teardrop cell, sickle shaped cell, echinocyte, keratocyte and eliptocyte. The differences found in this study may be due to difference in sex or egg carrying stage in females. In conclusion, this is the most complete report regarding haematological parameters of this species. Haemotobiochemical values of fishes are largely influenced by sex and consideration of this factor will aid accurate diagnosis and therapeutic evaluation of fish diseases.
Keywords*: Blood cells, Haematology, Serum biochemistry, Etroplus suratensis.*

Received 14.05.2017 Revised 18.07.2017 Accepted 01.09.2017

How to cite this article:
Gayatri Acharya And Prafulla K. Mohanty. Analyses of Haematobiochemical Profile of Green Chromide –*Etroplus Suratensis* (Bloch, 1790)With Morphological Assessment of Blood Cells . Adv. Biores., Vol 8 [6] November 2017.63-70.

INTRODUCTION

The green chromide *Etroplus suratensis* is an euryhaline species, characterised by grey-green in colour with dark spot at the base of pectoral fin. This species inhabits in brackish water. It is a popular food fish despite the species being in high demand; the wild population of the species have not been given sufficient conservation attention. It is assessed as least concern in view of its distribution and is relatively declining trend in Kerala .In 2010 it is declared as a state fish of Kerala.This species is gradually endangered by many factors. Ecological and biochemical monitoring and measures may be needed to preserve them. Although several studies dealt with the phylogeography and evolutionary aspects of this species [1].Physiological studies of this species are inadequate. Hence the objectives of the present study are to analyses the haematological and biochemical profile of *Etroplus suratensis* as well as morphological assessment of blood cells. Haematological and biochemical analyses of blood are of great importance in studying the biology of a species and determining the health status of animal [2]. Fishes being poikilothermic vertebrates, can be used as bioindicators to investigate the presence of pollutants and mutagens in the environment. Moreover, by analyzing the haematology and blood biochemistry of fish, we can identify dehydration, anaemia, inflammatory diseases, parasitemia, haematopoietic disorders[3]. Haematological studies and the establishment of baseline ranges for *Etroplus suratensis* would be useful for understanding general aspects of this species and gives us information on the health status of this species. Therefore in this paper, we report data for haematological and blood chemical profiles, and also describe the morphology of circulating blood cells of adult *Etroplus suratensis* .This is the most complete report of haematological parameters of this fish species. An understanding of its haematology may indirectly improve aquaculture industry and prolong animal life span.

MATERIALS AND METHODS

The present study was undertaken with 37 (15 males and 22 females) healthy and adult individuals of *Etroplus suratensis* . Which were collected from the brackish water lagoon Chilika, Odisha from the month of March to June, 2015. Before collection of blood, specimens were weighed and their total length was measured. The males weights from 90 to 140 gm whereas females weighed 130gm. Male fish body length ranged from 11 to 20cm verses 14 to 23 cm among females.

The blood samples were taken from the caudal vein [4] using 22-25 guage needle in the morning hours to avoid diurnal variation. Special care was taken not to puncture the caudal lymph vessels during blood collection. Blood smears (three slides per individual fish) were made immediately after collection of blood to avoid morphological changes. The smeared slides were air dried, fixed with methanol and stained with Giemsa stain and kept for further morphological analyses of blood cells. The rest of the collected blood was quickly separated into two tubes. One contains Ethylenediaminetetraacetic acid (EDTA) for haematological parameter determination and other without EDTA for serum biochemical analyses.

Total red blood cell (RBC) and total white blood cell (WBC) were manually counted using a neubauer's haemocytometer, with dilution being performed by Hayem's solution for RBC and Turk's solution for WBC. The haemoglobin concentration was estimated by Sahli's haemometer [5] and expressed in gm/dl. Packed cell volume (PCV) was determined using the microhaematocrit method [6]. Erythrocytes indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated as per following formulae.

MCV=PCV/ Erythrocytes count ×10

MCH= Haemoglobin/ Erythrocytes count ×10

MCHC= Haemoglobin /PCV ×100

Blood smears were used for differential RBC and WBC counts following the nomenclature proposed by [4]. The differential RBC count was done by counting 1000 erythrocytes per slide (three slides per fish) which were classified as erythrocytes and other erythrocyte types, which included the micro and macrocytes, teardrop cell, tadpole cell, sickle shaped cell, echinocyte, keratocyte, spindle shaped cells and elliptocyte. For differential WBC count 100 leucocytes per slides (three slides per fish) were contend and classified as lymphocyte, neutrophil, monocyte and eosinophil. In order to obtain size of different blood cell types, 30 cells of each cell type for each individual fish were photographed with the help of Microscope Eyepiece Digital Camera (CatCam130 – 1.3 Mega Pixel (MP), Code No. CC130, Catalyst Biotech, Maharashtra, India ,attached to Hund Wetzlar Microscope GmbH, Wetzlar-Nauborn, Germany) and computer .

For biochemical analyses, The Eppendorf tubes with the blood samples were centrifuged for 5 minutes at 5000 rpm. The obtained serum was used to determine glucose, cholesterol, protein, albumin and globulin, which were measured using standard commercial kits (Crest Biosystem, India).

RESULTS

All fishes taken for this study were adults (length of 15.31± 0.57cm and weight of 106.2±3.96 gm and healthy. The number of fishes used was adjusted as per their availability. The present work is focuses on changes in red blood cell range depending upon sex of *Etroplus suratensis* (Table 1). Males showed significantly higher values than females for the haemoglobin, RBC and PCV count. Other haematological parameters are not influenced by sex. This investigation also represents size characteristics of the erythrocyte types found in blood of *Etroplus suratensis* (Fig.1a) (Table 2).The erythrocytes were oval in shape with ellipsoidal nucleus (Fig.1b).Occasionally, other erythrocyte types were observed under the same category. These consisted of microcyte (very small erythrocytes) (Fig.1c), macrocyte (unusually large erythrocytes) (Fig.1d),Tadpole cell (Fig.1e), tear drop cell (Fig.1f), sickle shaped cell (Fig.1g), echinocyte (thorny projection on cell membrane of erythrocytes) (Fig.1h), keratocyte (pair of spicules on cell membrane)(Fig.1i), and elliptical (Fig.1j). In a few cases, the erythrocytes were observed to be undergoing mitosis.

The total and differential white blood cell counts are given in Table 3. Females showed significantly higher WBC count than males. Lymphocytes were the most abundant of the WBC types, followed by neutrophil, monocyte and eosinophil. Males showed higher monocyte counts than females, while other measured parameters were not influenced by sex, while basophils were not observed during this study. The morphometric measurements of various types of leucocytes are represented in Table 4.Lymphocytes had a wider range of sizes than any other blood cell type. They mostly had large nucleus (Fig.2a). Neutrophils were spherical to slightly oval cells with a rounded eccentrically located nucleus (Fig.2b).

Eosinophils were rounded cells with bilobed nucleus (Fig.2c). Monocytes were rounded cells with kidney shaped nucleus with bluish cytoplasm (Fig.d).
Ranges established for the biochemical parameters in the blood of adult *Etroplus suratensis* with respect to sex (Table1). Females' showed significantly higher value for albumin than males'. Other biochemical parameters like, glucose, cholesterol, protein and globulin does not vary significantly.

Table 1 Red blood cell (RBC) range and biochemical parameters for adult *E.suratensis*

Parameters (Unit)	Males		Females		P-value
	Mean	Range	Mean	Range	
Haemoglobin (gm/dl)	7.9 ± 0.17	7-9	7± 0.15	6-8	0.002**
RBC (mllions/mm^3)	1.95 ±0.08	1.47-2.5	1.73± 0.96	1.38-2.34	0.004**
PCV(%)	24.8 ± 0.62	22-30	21.2± 0.63	17-26	0.004**
MCV(fl)	129.94± 5.86	114.28-149.65	128.36 ±8.67	95.23-176.47	0.84
MCH(pg)	41.31±0.89	36-47.61	42.20± 2.47	30.95-58.82	0.88
MCHC(%)	32.22±0.53	30-34.09	33.21± 0.69	30.76-36.11	0.32
Glucose(mg/dl)	115.93±4.07	91.15-152.74	114.75±5.61	83.53-165.34	0.52
Cholesterol(mg/dl)	289.46±10.55	242.74-362.59	280.22± 18.39	141.22-366.41	0.38
Protein(g/dl)	10.45±0.49	8.30-13.87	10.44± 0.59	6.99-13.63	0.97
Albumin(g/dl)	2.73±0.18	1.57-3.71	2.34 ±0.07	1.96-2.81	0.003**
Globulin(g/dl)	7.71±0.57	6.73-10.15	8.09 ±0.62	5.03-10.81	0.25

** Significant at P<0.01

Table 2 Size characteristics of the erythrocytes from *Etroplus suratensis*

Erythrocyte Type	Male				Female			
	Cell size (µm)		Nucleus size (µm)		Cell size (µm)		Nucleus size (µm)	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
Erythrocyte	9.90±0.08	8.21±0.08	6.01 ±0.10	4.57 ±0.08	10±0.08	8.23±0.08	6.2±0.10	4.77±0.09
Microcyte	7.44±0.16	5.54±0.30	4.28±0.28	2.78±0.18	6.88±0.24	5.87±0.15	4.06±0.21	3.85±0.98
Macrocyte	12.48±0.58	10.60±0.56	7.94±0.35	6.44±0.48	13.15±0.45	10.73±0.62	8.33±0.71	5.75±0.64
Tadpole cell	14.36±0.71	8.64±0.31	7.57±0.34	5.72±0.36	14.27±0.57	8.81±0.31	6.19±0.35	4.68±0.25
Teardrop cell	9.98±0.16	8.24±0.16	6.14±0.26	4.20±0.22	9.63±0.23	7.88±0.24	6.33±0.31	4.39±0.25
Sickle shaped cell	-	6.01±0.20	-	5.98±6.21	9.78±0.23	8.45±0.21	6.24±0.30	4.60±0.37
Echinocyte	9.93±0.25	8.23±0.20	6.83±0.23	4.81±0.24	9.78±0.23	8.45±0.21	6.24±0.30	4.60±0.37
Keratocyte	10.05±0.18	8.69±0.42	7.45±0.25	4.72±0.27	11.67±0.53	8.62±0.45	8.62±0.45	6.86±0.55
Eliptocyte	10.73±0.32	8.81±0.32	7.66±0.26	5.38±0.23	10.45±0.36	8.77±0.37	6.22±0.30	4.07±0.27

Table 3 White blood cell (WBC) range for adult *E.suratensis*

Parameters (Unit)	Males		Females		P-value
	Mean	Range	Mean	Range	
WBC(thousands/mm^3)	10.54±0.36	8.45-13.15	12.09-0.49	8.75-14.4	0.036*
Lymphocyte (%)	66.2±1.01	59-73	66.66±0.66	65-67	0.63
Neutrophil (%)	24.26±1.05	17-30	24.2±0.79	22-30	0.95
Eosinophil (%)	4.06±0.28	3-6	4.53±0.30	2-6	0.23
Monocyte (%)	5.46±0.37	4-8	4.6±0.36	4-7	0.23

*Significant at P<0.05

Table 4 Size characteristics of different leucocytes from *E.suratensis* blood (in micrometers)

Leucocyte Type	Male				Female			
	Cell size (µm)		Nucleus size (µm)		Cell size (µm)		Nucleus size (µm)	
	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth
Lymphocyte	9.91±0.26	7.75±0.2	6.12±0.22	4.50±0.20	10.44±0.28	8.53±0.28	6.17±0.25	4.38±0.27
Neutrophil	10.55±0.26	8.85±0.27	7.50±0.26	5.37±0.23	10.07±0.25	8.23±0.22	6.61±0.28	4.87±0.28
Monocyte	10.39±0.30	8.75±0.30	6.84±0.32	5.08±0.30	10.29±0.27	8.24±0.25	6.07±0.32	4.85±0.33
Eosinophil	10.46±0.18	8.90±0.19	6.54±0.21	5.47±0.34	9.69±0.21	8.20±0.22	6.14±0.24	4.67±0.27

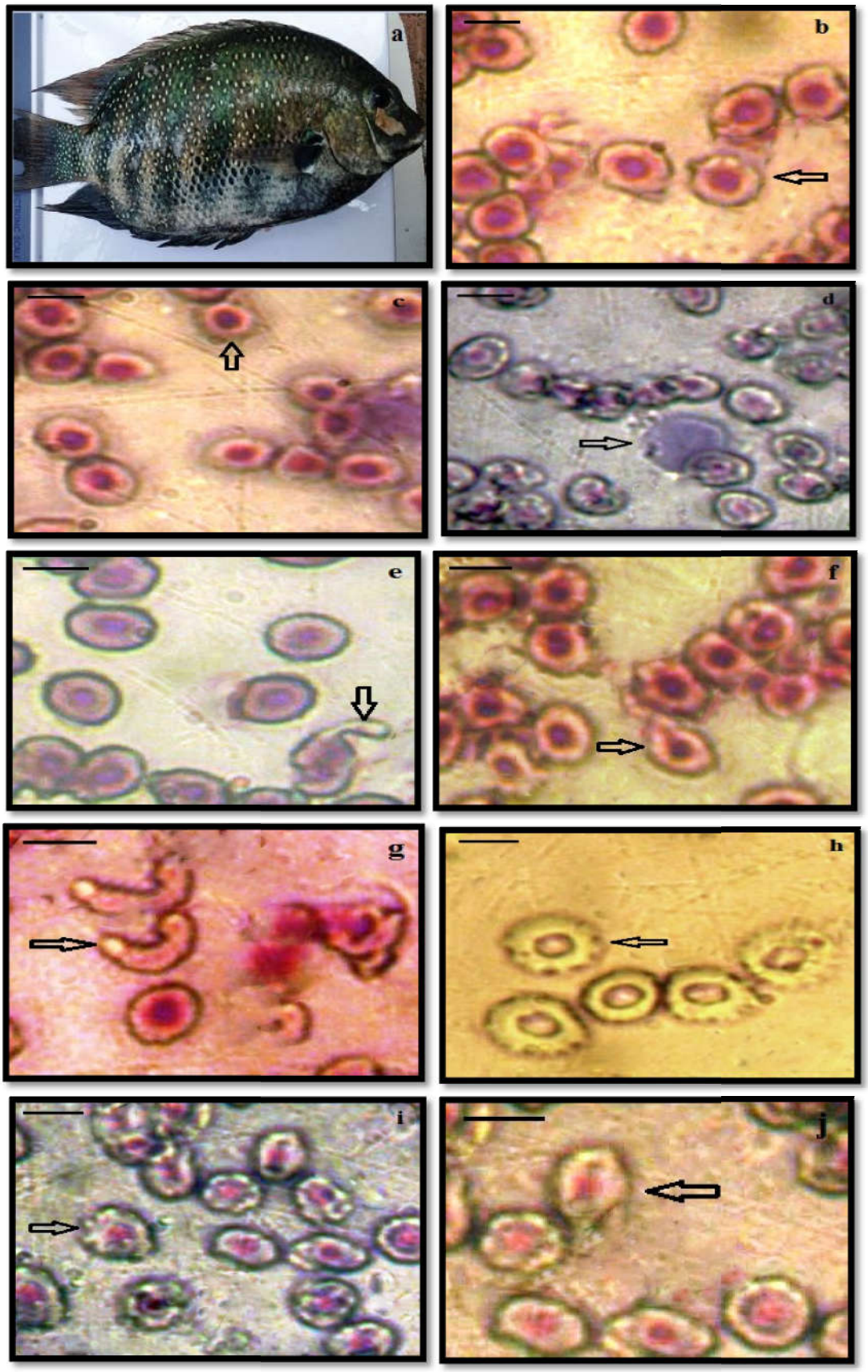

Fig.1 a Adult *Etroplus suratensis.* b Erythrocyte.c Microcyte. d Macrocyte. e Tadpole cell. f Tear drop cell. g Sickle shaped cell. h echinocyte.i keratocyte.J elliptical (bar=10 mm)

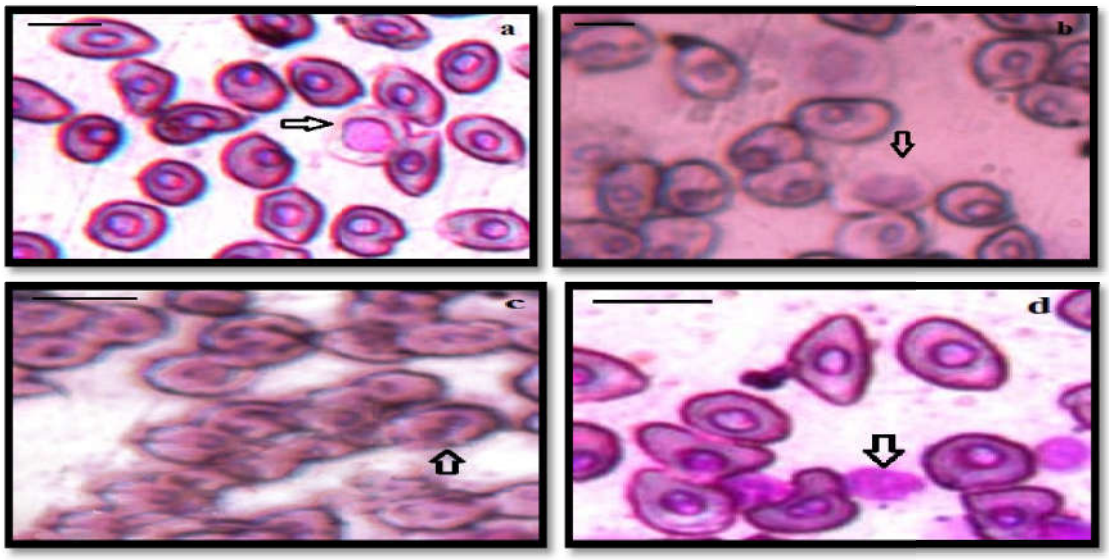

Fig. 2a Lymphocyte. b Neutrophil. C Eosinophil. D Monocyte. (bar=10 mm)

DISCUSSION

Haematological values of fish are determined for a variety of purposes such as for establishment of normal range of blood parameters, to assess environmental conditions and to investigate physiological status of fish. Very high level of haemoglobin in fishes should be due to the high temperature of the season .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. During the immature period Hb concentration was less may be due to metabolic rate. The haemoglobin content tend to increase with length and age of fish [7,8]. In the present study, the haemoglobin content was higher in the large sized specimens. There was a correlation between haemoglobin concentration and activity of fish [9]. The more active fishes tend to have high haemoglobin values than the sedentary ones. *Etroplus suratensis* being a relatively quite species has slightly lower haemoglobin concentration than the other more active species such as *Clarias batrachus* whose mean haemoglobin concentration is high [10]. In fish blood, oxygen is carried in physical solution and also in combination with haemoglobin. So physiologically, haemoglobin is crucial to the survival of fish as its role is directly related to oxygen binding capacity of blood. Erythrocytes count found in *Etroplus suratensis* is of 1.47 ± 2.5 x10^6 mm^3 ·Our finding is within the range described by [11]and similar observation was noted with another species such as *Tilapia niloticus* [12]. In the present study erythrocyte count were higher in the large sized specimens. Higher numbers of erythrocytes are needed for the high energy demands associated with gonadial maturation. In fishes where there is reduction below the normal range of value of erythrocyte numbers, there is correspondent reduction in haemoglobin values per cell [13]. According to the number of RBC, the cells become either larger or smaller in size, reciprocal relationship said to be existed between the size and number of RBC [14]. Haematocrit value used to detect anaemic condition in fishes [15]. Several reported values for fish haematocrit fall between 20% and 35% [15] and rarely values increases above 50 % been reported [16,17].The mean haematocrit values for *Etroplus suratensis* of all sizes falls within 22-30%. PCV is a major haematological characteristic that changes with fish activity. The reduction in PCV found in this study similar to the finding of on Indian Shad *Tenualosa* [18] .Differences in haematocrit value between the sexes are genetically determined [19] . Although some ichthyologists considered that the differences might be due to higher metabolic rate of males compared to females [20].

MCV values are important in the sense that, stress increases the value of MCV by swelling the erythrocytes [21]. The reduced MCV values were usually found during gonadial maturation as erythropoiesis at this time liberating small immature cells into the blood stream. MCV values derived in the present investigation are lower than those reported for *Tilapia* fishes [14] , this may be due to variation in habitat.

MCV gives an indication of the status or size of the red blood cells and reflects an abnormal/normal cell division during erythropoiesis. The increase in MCV may be attributed to the swelling of the erythrocytes resulting in macrocytic anaemia such an increase in RBC size is generally considered a response against stress. But MCV found to decrease with an increase in body weight in *Heteropneustess fossilis* [22]. MCH and MCHC do not vary significantly with respect to sex for *Etroplus Surantensis*. MCV and MCHC are

derived from Hb, MCH and MCHC were found to be directly related to Hb in both sexes. Erythrocyte indices such as MCV, MCH and MCHC are not differed between male and female fish [23]. Differences in the levels of some haematological parameters may be due to effects of body size and ecological conditions. Study of vertebrate blood cells including those of fishes particularly taleosts, attracted icthylogists attention since 18th centuary. It is reported that erythrocytes of fish are usually elliptical in their form [24] but it may vary from elliptical to circular in *Etroplus*. A few oval,elliptical and ablong cells were also found. Elliptical and circular cells are also found in *Heteropneusteus fossilis*[14,24] .Same shape also observed in the nuclei of 10 spices of teleosts fishes [25]. The mean values of the size of the mature erythrocytes were observed to vary greatly between species of fishes. 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 erythrocytes [26]. The size of the RBC reported for *Etroplus* are medium and this fish is not very active and they are not extremely sluggish. In this study, different types of erythrocytes were found in the blood of *Etroplus* such as microcyte , macrocyte, tadpole cell, teardrop cell, keratocyte, spindle shaped cell and sickle shaped. Basophils have not been described in fish blood by many workers.

Mechanisms of specific immunity in fishes are less developed and play markedly less important role than in birds or mammals [26,27]. In contrast, fishes have nonspecific resistance system, which provides defence against pathogenic and environmental factors [28,29]). Cellular and humoral immune system of fish activated with any infection by pathogens. This is followed by changes in circulating antibodies and percentage as well as absolute number of the different WBC [30].

Differences in the WBC counts may be due to age, season, maturity, sex, dissolved oxygen and in particular to stress [27,29]. Therefore, the difference demonstrated in this study may be related to sex.

WBC count was affected by water temperature and reproduction period. In this investigation *Etroplus suratensis* shows high WBC in females in comparison to males. This may be due to energy needed for reproduction and decreasing oxygen amount. The presence of leucocytes related to the health status in fish and in many cases, they are also helpful in the evaluation of immune system. Therefore, variations in the proportions of these defence cells in the blood are usually expected. In nonspecific defence mechanism leucocytes are involved [27,29]. Monocytes/ macrophages and lymphocytes are involved in the immune response, leading to the production of antibodies. Neutrophils are effector key cells in nonspecific immunity, as they migrate into the site of infection, to recognise, ingest and destroy the pathogens. In the present study one of the biochemical parameters like, albumin vary significantly with respect to sex but other parameters are not influenced significantly. The serum biochemical parameters vary from species to species and may be influenced by many biotic and abiotic factors such as water temperature, seasonal pattern, food, age and sex of the fish [31]. The concentration of plasma protein increases when the fishes are under starvation or any other stress condition [32]. In the present case, plasma protein concentration does not differ significantly between male and female. Plasma protein gives an index of the health status of the brood fish [33] and as indicator of nutritional status [34] .Albumin helps in transportation of lipid in fishes [35] and also helps in the general metabolism of fishes. The rise in albumin concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis [36] .In this study albumin content is higher in female than male but does not significantly vary between the species. Previous studies demonstrated that basal levels of glucose varied in ecologically-distinct species, in part influenced by environmental and non-environmental factors such as feeding habits and life mode of the fish, particularly related to locomotion. It is reported that glucose in blood serum is the best indicator of stress in fish [37]. The concentration of Cholesterol varies between the male and female fish because of variations in diet, activity and sexual development [38].

CONCLUSION

Haemocytological examinations and the correct interpretation of the results are being of increasing importance in fish culture activities. As haematological assessment is gradually becoming routine practice for diagnosing health of fish. Acquaculture needs accurate information for identification and control of stress situations in order to ensure health of fish. The evaluation of blood parameters may be the quickest way to detect these symptoms. Therefore, there is an urgent need of reliable normal database to be available for species of economic importance. In conclusion, the variation in methodology used for haemocytological studies, instant changes in physical and chemical properties of micro-environment in which fish lives makes it difficult in establishment of haematological data. Therefore, we purpose separate data collection and comparison from healthy and unhealthy fish to obtain further haematological data.

ACKNOWLEDGEMENTS

The authors would like to express their thanks to the Post-Graduate Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar,751 004, Odisha for providing facilities for carrying out current research programme. First author owe her thanks to the Department of Science and Technology towards Innovation in Science Pursuit for Inspired Research (DST- INSPIRE) Programme as the funding agency vide the letter number DST/INSPIRE Fellowship/ 2012, [146-2012]. There is no conflict of interest.

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