International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Impact Factor (2012): 3.358

Haematological and Serum Biochemical Parameters in different Sexes of Walking Cat Fish, *Clarias batrachus* (Linnaeus, 1758)

Gayatri Acharya[1], Prafulla K. Mohanty[2]

[1]Ph.D. Research Scholar, P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar - 751 004, Odisha, India
[2]Professor, P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar - 751 004, Odisha, India

Abstract: ***A study was designed to evaluate the influence of sex on haematological and biochemical profile of Clarias batrachus. Haematological and serum biochemical studies on fishes have assumed to be greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of natural aquatic bodies. Changes in haematological and biochemical parameters depend on the fish species, age, sex and health condition .These parameters also changes due to environmental stress and fish size .The aim of the present study was to investigate the reference ranges for haematological and serum biochemical values of male and female Clarias batrachus. A total of 60 mature fish (30 males and 30 females) were collected. Blood samples were then taken from the caudal vein of fishes. Haematological parameters such as haemoglobin (Hb), total erythrocyte count(TEC), total leucocyte count(TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin(MCH), and mean corpuscular haemoglobin concentration(MCHC) and the serum biochemical parameters like protein, albumin, globulin, glucose and cholesterol were measured and each parameter was expressed as mean±SE in both male and female fish and compared according to sex using t-test statistical method. In haematological findings, four parameters namely RBC, Hb ($p<0.01$) and WBC, MCV ($p<0.05$) revealed significant difference between male and female fish. However, no significant difference was observed in PCV, MCH and MCHC. The value of biochemical parameters were higher in female compared to male but the differences are insignificant. The findings would be helpful to establish a baseline value and to draw conclusive remarks against the health status of Clarias batrachus, showing deviation from the normal parameters.***

Keywords: *Clarias batrachus*, Blood, Haematological parameters, Serum, Biochemical parameters.

1. Introduction

Haematological and biochemical test has not widely applied for diagnosis of piscine medicine but these tests could be a suitable diagnostic tool for monitoring physiological and pathological changes in fishes. Different factors such as season [1] water contamination [2], physicochemical parameters of the water [3], stressors [4], age and sex [5], and fish species [7] affect the haematological parameters. The serum biochemical analyses provide information about internal organs, electrolytes, proteins, and nutritional and metabolic parameters [8]. One of the difficulties in assessing the state of health of natural fish population has been the lack of reliable references of the normal condition. In pursuant to this goal, many fish physiologists have turned to studies of haematology, probably because it has provided a valuable diagnostic tool in evaluating human health. Determination of haematological parameters can provide substantial diagnostic information, once standardized reference values are established [9]. Moreover, recent attention has been given to the biochemical characterization of fish blood as an internal index [10]. Blood biochemistry parameters can also be used to detect the health of fish [11]. Due to handling and hypoxic stress, fluctuations were detected in the concentration of cortisol, glucose, cholesterol and other basic components in response [12]. Despite advances in fish medicine in recent years, interpretation of fish haematology is often troubled by a lack of meaningful reference values. Only a few normal values for a small number of haematological parameters have been established for some teleosts, but these values range widely due to the lack of standardized collecting and measuring techniques. Since the haematological studies on Siluriformes have not been attempted till now, this paper focuses on *Clarias batrachus* that belongs to Siluriformes order. *Clarias batrachus* commonly known as "magur", is the most popular and delicious edible fish in Asia including India, which is rich in protein content. This study finds out the baseline reference values of haematological and biochemical parameters of the walking cat fish based on sexual difference .This analysis could help in understanding the better health condition of this fish in natural habitat. Since information on haematological investigations of adult *C .batrachus* adults are extremely inadequate, the present work was carried out to arrive at some baseline values.

2. Materials and Methods

2.1 Fish samples

For haematology the fish samples were collected from a freshwater pond (20°20`N; 85° 87` E) of Khordha District, Bhubaneswar, Odisha during the period November 2013 to March 2014. The numbers of fishes caught were transported on the same day in a container filled with pond water to the laboratory and the analysis was carried out. A total of thirty adult specimens from each sex of the species having mean length 28.14±0.40 cm, breadth 4.63±0.08 cm and weight 119.2±4.18 g were utilized in the present investigation.

2.2 Blood Collection

Fishes were kept in aquarium at room temperature. Laboratory aquarium was aerated and provided with external s iltration and a layer of gravels on the bottom. Fishes were fed with pelleted commercial food (Taiyo grow, Taiyo pet products (P) Ltd., Chennai) procured from the market. They were allowed to acclimate to captive condition prior to experimentation. Careful handling was implemented to minimize the stress. Then blood was collected from live fish putting it on a a tray. A damp cloth was used to cover the fish head. A small sample of whole blood was drawn from the caudal vein. Blood was collected in the morning hours to avoid diurnal variation. Collected blood was transferred from the syringe into a anticoagulant, Ethylenediaminetetraacetic acid (EDTA) containing vials, for haematological studies and some blood into Eppendorf tube which was kept undisturbed for clotting. After following retraction of clot, the supernatant serums was pipette into labelled vials and were stored at -80 until analyzed.

2.3 Haematological Analyses

All the haematological parameters were determined by using the standard techniques .The haematological parameters included, haemoglobin (Hb), total erythrocyte count (TEC/RBC), Total Leukocyte Count (TLC/WBC) and Packed Cell Volume (PCV).

Haemoglobin concentration (Hb) was measured by Sahli's acid hematin method[13].

Red blood cell counts and Total leucocytes counts were carried out with a Haemocytometer method.

packed cell volume (PCV) was determined by means of a Microhaematocrit method [14].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

2.4 Biochemical analyses

The serum biochemical parameters like protein, albumin, globulin, glucose and cholesterol were estimated using standard kit (Crest Biosystem, India).

3. Statistical Analysis

Haematological and Biochemical parameters were expressed as mean±SE in both male and female fish and compared according to sex using t-test. All these statistical analyses were performed using the Microsoft Office Excel 2007.

4. Results

The present work focuses is on changes in blood value depending upon sex of *Clarias batrachus* (Table 1).

Table 1: Mean ±SE Haematological parameters results of the male and female *Clarias batrachus* (n=30 per sex)

Haematological Parameters	*Male Mean ±SE*	*Female Mean ±SE*
RBC(10^6mm^{-3})	2.89±0.08*	2.41±0.07*
Hb (g/dl)	9.6±0.24*	8.48±0.24*
WBC(10^3 mm^{-3})	8.59±0.27**	9.71±0.43**
PCV (%)	29.5±0.89	28.3±1.04
MCV (fl)	104.98±2.82**	119.25±5.58**
MCH (pg)	33.37±0.60	34.12±1.15
MCHC(%)	32.79±0.59	32.41±0.4

*Shows significance ($p<0.01$) between samples
**Shows significance ($p<0.05$) between samples

Total erythrocyte count and Hb show highly significant difference ($p<0.01$) between male and female. Male and female *Clarias batrachus* show significant difference ($p<0.05$) in case of WBC and MCV. PCV, MCH and MCHC do not show significant difference with respect to sex in walking cat fish.

The biochemical parameters were higher in female than male but they are not significantly different (Table 2).

Table 2: Mean ±SE Biochemical parameters results of the male and female *Clarias batrachus* (n=10 per sex)

Biochemical Parameters	*Male Mean ±SE*	*Female Mean ±SE*
Protein(g/dl)	4.22±0.27	4.46±0.32
Albumin(g/dl)	3.51±0.28	3.54±0.31
Globulin(g/dl)	0.60±0.14	0.92±0.30
Glucose(mg/dl)	70.83±6.67	82.88±8.76
Cholesterol(mg/dl)	210±28.75	223.89±22.2

5. Discussion

Haematological analyses of fish are important as these are linked to the health of fish. Fish species are impacted with different factors such as pollution, water quality, and microorganisms in natural habitat. They adapt somewhat to these adverse conditions by changing their physiological activities. Findings of reference value for fish species will help to establish and identify the causes of disease in fish which presents challenge for the ichthyologist. The RBC of fish determines the dissolved oxygen carrying capacity. The result of the RBC count of this work for male and female *Clarias batrachus* is 2.89±0.08 x10 ^{6} /mm^{3}and 2.41±0.07x10 ^{6} /mm^3 respectively which is higher than the unsexed *Clarias batrachus* RBC value 1.77±0.014 x10 ^{6} /mm^3 as reported [15] and it is lower than that *Heterobranchus bidorsalis* male and female 5.05 ± 0.17x10 ^{6} /mm^3 5.2 ± 0.26x10 ^{6} /mm^{3}as reported by [16]. st may be due to change in habitat or may be due to two different species. Present study showed higher RBC count in males than females which denote sex variation. The observed higher RBC counts in males are possibly because

of their activeness compared to the females. RBC counts have proven to be a highly variable blood parameter among fishes and showed a remarkable difference in their sex [1].The result of Hb obtained of this work for male was 9.6±0.24g/dl but in female it was 8.48±0.24g/dl. Higher Hb concentration found in males compared to females may be due to sex differences or males are more active and aggressive than the females. The result of WBC found in this study for male and female is 8.59±0.27x10 ^{3}/mm ^{3}and9.71±0.43x10 ^{3}/mm of blood, respectively. WBCs in female were significantly higher than the levels measured in the males, which indicate egg carriage stage**,** infection, or adverse condition in female [18].PCV is higher in males than that of females but is not significantly different. The result of PCV of this study for male and female are 29.5±0.89% and 28.3±1.04 %respectively .Males have higher value of PCV than females which is perhaps because of relatively higher RBC counts in males. Another reason could be sex difference.MCV value reflects the size of red blood cells by expressing the volume occupied by a single red blood cell. The present study shows significantly higher value of MCV in female compared to male which may be due to lower PCV.MCH value observed in this study for male and female is 33.37±0.60pg and 34.12±1.15pg respectively. Higher MCH value in female than male indicates higher likelihood of occurrence of macrocytic anaemia in females than in males [19].There was no significant difference in MCHC between male and female. The highest was 32.79±0.59 in male and lowest was 32.41±0.4 in female. High level of MCHC indicates more Hb in a unit of RBCs [19].

The results of the biochemical profile of *Clarias batrachus* (Table 2) are used as a tool to assess the health status of fishes. These parameters can change with the fish species, age, and the cycle of sexual maturity and health conditions. Through biochemical constituents of the fish blood, the metabolic disturbances of fishes could easily be assessed [20]. Sex of a fish has a great differentiation in various components of the blood of fish, depending upon the quantum of metabolic activities of the organism [21].In the present study, biochemical indices such as Protein, Glucose, Albumin, Globulin and Cholesterol show higher value in female than male but the differences were insignificant ($p>0.05$).The present study revealed insignificant differences in serum protein levels with respect to sex. Serum protein of *Clarias batrachus* is the protein component of the blood and is vulnerable to increase with starvation or any other stress .In the present case, plasma protein concentration in male and female fish is 4.22±0.27g/dl and4.46±0.32dl respectively. Plasma protein gives an index of the health status of the brood fish [22] and as indicator of nutritional status [23].

Albumin in fish blood performs the transportation of lipids [24] and helps in the general metabolism of fish. The rise in albumin concentration in animals due to loss through urine or faeces or through break down may result in impaired synthesis [25]. [22] Reported higher albumin concentration in *Lebeo rohita* at the time of reproduction. In our study, total albumin levels of male and female *Clarias batrachus* was .51±0.28and3.54±0.31 (g/dl) respectively. Serum protein and globulin are associated with stronger innate response in fish .In this study, globulin level was found higher in female than male0.60±0.14 and 0.92±0.30 (mg/dl) respectively but the difference is insignificant. [26] Reported that glucose in blood serum is the best indicator of stress in fish. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma [26].The present study reveals higher value of glucose in female compared to male fish which is 70.83±6.67mg/dl and 82.88±8.76mg/dl respectively. Similar to our finding [27] the higher glucose level is found in *Caspian kutum* female serum. The liver plays a major role in cholesterol homeostasis [28] by regulating plasma lipoprotein metabolism and lipid output in bile [29]. The cholesterol concentration in this study was 210.34±28.75 mg/dl and 223.89±22.2 mg/dl respectively. Cholesterol concentration varies both among and within fish species because of variations in diet activity and sexual development [30].

6. Conclusion

The present findings revealed differences in blood profile of *Clarias batrachus*, owing to the sexual differentiation of the fish. The results of the present investigation serve as a baseline value for haematological and biochemical parameters to draw conclusive remarks against the health status of *Clarias batrachus*, showing deviation from the normal parameters.

References

[1] C.L. Mahajan , J.S. Dheer, " Seasonal variations in the blood constituents of an air-breathing fish, *Channa punctatus* Bloch," Journal of Fish Biology,14,pp.413–417, 1979.
[2] M. Witeska, "Changes in selected blood indices of common carp after acute exposure to cadmium", Acta Veterinaria Brno, 67, pp.289–293, 1998.
[3] B.D. Joshi, "Circannual fluctuation in some blood componentsof the fish *Rita rita*, in relation to certain eco-physiological conditions",Uttar Pradesh Journal of Zoology, 2,pp. 62–66,1982 .
[4] M. Nikoo, Falahatkar, B. Falahatkar, M. Alekhorshid, B.N. Haghi, A. Asadollahpour, M.Z. Dangsareki, H.F. Langroudi, "Physiological stress responses in kutum *Rutilus frisii kutum*subjected to captivity", International Aquatic Research, 2,pp. 55–60,2010.
[5] A. Svetina, Z. Matasin, A. Tofant, M. Vucemilo, N. Fijan,"Haematology and some blood chemical parameters of young carp till the age of three years", Acta Veterinaria Hungarica. 50, pp.459–467, 2002.
[6] A.L. Langston, R.Hoare, M. Stefansson, R. Fitzgerald, H. Wergeland, M. Mulcahy, "The effect of temperature on non species defense parameters of three strains of juvenile Atlantic halibut *Hippoglossus hippoglossus* L", Fish & Shellfish Immunology. 12, pp.61–76, 2002.
[7] M. Nikoo, B. Falahatkar, H. Rahmani," Blood parameters of Southern Caspian kutum, *Rutilus kutum*," Journal of Applied Ichthyology, 28, pp.293–295, 2012.

[8] S.H.Newman, J.F.Piatt, J.White, "Hematological and plasma biochemical reference ranges of Alaskan seabirds: their ecological significance and clinical importance, *Waterbirds,*" 20(3), pp. 492–504, 1997.
[9] H.R. Jamalzadeh, S. Oryan, M. R. Ghomi, "Haematological characteristics and correlations of diploid and triploid Caspian salmon (*Salmo trutta caspius*) in juvenile stage", Journal of Cell and Animal Biology. 2, pp. 195–198, 2008.
[10] C.C. Edsall, "A blood chemistry profile of lake trout," Journal of Aquatic Animal Health, 11, pp.81–86, 1999.
[11] N. De Pedro, A.E. Guijarro, M.A. Lopez-Patino, R. Marinez-Alvarez, M. Delgado, "Daily and seasonal variation in haematological and blood biochemical parameters in tench (*Tinca tinca*)", Aquaculture Research. 36, pp.85–96, 2005.
[12] P.O. Skjervold, S.O. Fjaera , P.B. Ostby , O . Einen, "Live-chilling and crowding stress before slaughter of Atlantic salmon (*Salmo salar*)", Aquaculture 192, pp.265–280, 2001.
[13] H. Sahil, "*Lehrbuch d.klin. Untersuchungen Methode*", 5th edn. Leipsic. P.846, 1909.
[14] R.A. Mcinroy, "A micro-haematocrit for determining the packed cell volume and haemoglobin concentration on capillary blood. Journal of clinical pathology", 7, pp. 32-36, 1953.
[15] R. Maheswaran, A. Devapaul, S. Muralidharan , B. Velmurugan, S. Ignacimuthu, "Haematological studies of fresh water fish, *Clarias batrachus* exposed to mercuric chloride", International Journal of Integrative Biology.2(1), pp. 49-54,2008.
[16] L.U. Onyia, K.G. Michael, B. Ekoto, "Haematological profile, blood group and genotype of *heterobranchus bidorsalis*", Journal of Agricultural Science, 1(2), pp.69-72, 2013.
[17] H. Svobodova, H. Kroupova, H. Modra, M. Flajshans, T. Randak, L.V. Savina, Gela. D, "Haematological profile of common carp spawners of various breeds", Journal of Applied Ichthyology, 24, pp.55-59, 2008.
[18] L.S. Smith , "Introduction of fish physiology", Indian Edition by Narendra Publishing House, Delhi, India, pp .322-333, 1986.
[19] S.L. Robbins, R.S. Cotran, V. Kumar,"Pathologic basis of disease", 5th edn. W.B. Saunders Company, pp583-615, 1974.
[20] H.R. Jamalzadeh, A. Keyvan, M.R. Ghomi, F. Gheradi," Comparison of Blood Indices in Healthy and Fungal Infected Caspian Salmon (*Salmo trutta caspius*)", African Journal of Biotechnology. 8, pp.319-322, 2009.
[21] M. Svoboda, J. Kouril , J. Hamackova , P. Kalab, L. Savina, Z. Svobodova , B. Vykusova , "Biochemical profile of blood plasma of tench (*Tinca tinca* L.) during pre- and post spawning period", Acta Veterinaria Brno. 70, pp.259–268, 2001.
[22] P. Swain, S. Dash, P.K. Sahoo, P. Routray , S.K. Sahoo, S.D. Gupta, P.K. Meher, N. Sarangi, "Nonspecific Immune Parameters of Brood Indian Major Carp *Labeo Rohita* and Their Seasonal Variations", Fish & Shellfish Immunology. 22, pp.38-43, 2007.
[23] D.H. McCarthy, J.P. Stevenson, M.S. Roberts, "Some Blood Parameters of the Rainbow Trout (*Salmo gairdneri* Richardson)",Journal of Fish Biology, 5,pp 1-8. 1973.
[24] A.M. Andreeva, "Structural and Functional Organization of the Blood Albumin System in Fish", Vopr. Ikhtiol. 39, pp.825-832, 1999.
[25] H.T. Nguyen, "Transport Proteins. The Clinical Chemistry of Laboratory Animals", 2nd Edn. Taylor and Francis, Philadelphia, PA, USA, pp309-335, 1999.
[26] F.Percin, S. Konyalioglu, "Serum Biochemical Profiles of Captive and Wild Northern Bluefin Tuna (*Thunnus thynnus* L. 1758) in the Eastern Mediterranean", Aquaculture Research, 39, pp.945-953, 2008.
[27] F. Farid , A. Zeynab, R. Hossain, K.K. Mohammad, "A comparative study of some blood factors in male and female Caspian kutum(*Rutilus frisii kutum*) brood stock from the southern basin of the Caspian Sea", Turkish Journal of Veterinary and Animal Sciences 37, pp.320-325,2013.
[28] S.I. Teshima," Sterol Metabolism. Mem. Fac. Fish Kagoshima Univ"21, pp. 69-147, 1972
[29] M.P. Marzolo, A. Rigotti, F. Nervi, "Secretion of Biliary Lipids from the Hepatocyte", Hepatology, 12, pp.134-142, 1990.
[30] D.G. McDonald, C.L. Milligan, "Chemical Properties of the Blood", In: W.S. Hoar, D.J. Randall and A.P. Farell (Edn). Fish Phy Academic Press Inc, San Deigo. pp 55-133, 1992

Author Profile

Gayatri Acharya, M. Sc Gold Medallist and currently pursuing Ph.D. (DST INSPIRE Fellow) in P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

Prof. Prafulla K. Mohanty is serving as a Professor and Head of the P.G. Department of Zoology, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India. He has authored three research books, one monograph, one dictionary and 52 research papers. He has already guided 19 Ph.D. scholars and at present 08 Ph.D. scholars are undertaking research under his supervision.