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Comparative haematological and serum biochemical analysis of catfishes *Clarias batrachus* (Linnaeus, 1758) and *Heteropneustes fossilis* (Bloch, 1794) with respect to sex

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Abstract
Aim of this study was to investigate the haematological and serum biochemical parameters of two catfishes *Heteropneustes fossilis* (Bloch, 1794) and *Clarias batrachus* (Linnaeus, 1758) of Odisha. In this study, the estimated values were compared between the two species of catfishes as well as with respect to sex. 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 compared between and within the species as well as with respect to sex using One-Way Analysis of Variance (ANOVA) followed by Turkey's pair wise comparison tests. All haematological parameters differ significantly ($p<0.001$) between *C. batrachus* and *H. fossilis* except PCV and MCV. Except albumin all biochemical parameters differ significantly between *C. batrachus* and *H. fossilis*.

Keywords: *Clarias batrachus*, *Heteropneustes fossilis*, Blood, Serum, Haematological analysis, Biochemical analysis.

1. Introduction
The Asian catfish, *Clarias batrachus* locally known as 'magur' is a favourite edible fish in India and other Asian countries including Bangladesh, Thailand, Vietnam, Malaysia and Indonesia [1]. Indian catfish, *Heteropneustes fossilis* commonly known as 'singhi' is found in several Asian countries such as India, Bangladesh, Pakistan, Nepal, Sri Lanka, Myanmar and Indonesia [2]. Analyses of blood parameters have been carried out to determine the systematic relationship among certain species of fish [3-7]. Changes in haematological parameters depend upon the aquatic biotope, fish species, age, and sexual maturity and health status [8-10]. It is well known that blood comprises 1.3–7% of the total body weight of fish and it is one of the most active components that contribute to metabolic processes by ensuring gas exchange between the organism and the environment. For this reason, blood parameters are increasingly used as indicators of the physiological condition or sub-lethal stress response in fish to endogenous or exogenous changes [11-12]. The evaluation of physiological condition of fish depends on the availability of reference values. These should be as close as possible to normal values of various blood components considered as reliable descriptors of healthy fish under natural conditions [11]. It is clear that the environment in which fish live influences the metabolic content in blood [13]. Taking into account the long evolutionary history of fishes and the adaptation to different environment, it is obvious that no species can be used as a representative model for all fishes. For this study, two catfish species are considered which are typical fish of economic importance. The particular interest in studying the blood parameters of *C. batrachus* and *H. fossilis* lies in the fact that these species were proposed as a good model organism because of their commercial significance, economic importance and extensive consumption as a food source. Catfishes are easy to farm in warm climate, leading to inexpensive and safe food at local markets. Catfish is high in vitamin D. Farm-raised catfish contains low level of omega-3 fatty acids and a much higher proportion of omega-6 fatty acids. The catfishes are in great demand because of their therapeutic value in India .In view of this, the purpose of the present study was to compare the haematological and biochemical profiles between C. *batrachus* and *H. fossilis* based on sexual dimorphism.

The elucidation of their blood parameters may provide information about interspecies differences and influence of sex on blood.

2. Materials and Methods

Adult specimens of *C. batrachus and H. fossilis* used in this study were captured from freshwater pond (20º20'N; 85º 87' E) of Khordha District, Bhubaneswar, Odisha during November 2013 to March 2014. The collected fish was transported on the same day in a container filled with pond water to the laboratory and the analyses were carried out. A total of thirty adult specimens from each species were weighed and measured. *C. batrachus* (15 male, weight:113.46±4.49 g, length :27.75±0.53 cm and breadth 4.57±0.14 cm; 15 female, weight:124.93±6.89 g, length:27.66 ±0.66cm, breadth: 4.7±0.09 cm) and *H. fossilis* (15 male, weight: 107.57±8.76 g, length :26.07± 0.91 and breadth:5.04 ±0.25; 15 female, weight:111.92 ± 4.65, length:26.21± 0.63, breadth:5.10± 0.17) were kept in aquarium. Laboratory aquaria were well aerated and provided with external filtration and a layer of gravel on the bottom. Fishes were fed once a day with pelleted commercial food (Taiyo grow, Taiyo pet products (P) Ltd., Chennai). They were allowed to acclimate to captive condition for a month prior to collection of blood samples. Careful netting and handling were implemented to minimize stress. After sampling, fish were placed in separate tanks of freshwater for recovery. Within first 2h after each extraction, the blood samples were processed for Haemoglobin (Hb), total erythrocyte count (TEC/RBC), total leukocyte count (TLC/WBC) and packed cell volume (PCV) as follows: Haemoglobin concentration (Hb) was measured by Sahli's acid haematin method [14]. RBC and WBC were determined using a Neubauer haemocytometer. Haematocrit/PCV value was determined by the standard microhematocrit method and expressed in percentage. The following erythrocyte indices such as mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) was calculated according to [15]. The serum biochemical parameters like protein, albumin, globulin, glucose and cholesterol were estimated using standard kit (Crest Biosystem, India). The data were analysed and presented as mean± standard error (SE) by using statistical software Microsoft office excel 2007 and one way analysis of variance (ANOVA) followed by Turkey's pair wise comparison tests was used to assess the significance of differences between and within the species by the help of Paleontological Statistics (PAST) version 2.17.

3. Results

Results of haematological analyses are shown in Table 1.Mean value for haemoglobin (g/dl) differed significantly (P<0.001) between *C. batrachus* and *H. fossilis*. The findings of this study revealed the effect of sex on blood parameters of two different catfish species. The mean value of RBC differed significantly (p<0.001) between male *C. batrachus* and female *H. fossilis*. The value of RBC is higher in male than female in both the fishes but it shows significant difference in *H. fossilis* (Fig. 1).The higher value of RBC in male may be due to variation in sex. The value of Hb (g/dl) differed significantly with respect to sex as well as between the *C. batrachus* and H. *fossilis* (Fig. 2). Higher value of Hb found in *H. fossilis* as compared to *C. batrachus* indicates that *H. fossilis* is more active than *C. batrachus*. The value of WBC differed significantly with respect to sex as well as between the species (Fig. 3) and value of WBC was higher in female than male which indicates egg carriage stage**,** infection, or adverse condition in female [16]. PCV and MCV values were slightly higher in male, but those values were not statistically significant with respect to sex as well as between *C. batrachus* and *H. fossilis* (Fig. 4 and 5). MCH differed significantly between the two catfishes taken for this study. MCH shows slightly higher value in female than male but did not show significant difference (Fig. 6). High level of MCHC indicates more Hb in a unit of RBC [17] though the value is higher in male as compared to female but did not differ significantly. The value of MCHC differed significantly (p<0.001) between *H. fossilis* and *C. batrachus* (Fig. 7). The MCHC value was higher in *H. fossilis* than *C. batrachus* may be due to high amount of RBC.

Table 1: Haematological parameters of *Clarias batrachus* and *Heteropneustes fossilis*

Parameter	*Clarias batrachus*		*Heteropneustes fossilis*		F value
	Male(n=15)	Female(n=15)	Male (n=15)	Female(n=15)	
RBC(10^6mm^{-3})	2.97±0.07a	2.55±0.10	3.1± 0.25c	2.36±0.1b,d	5.177***
Hb (g/dl)	9.5 ±0.31a	8.26±0.38c	11.6±0.81b,d,e	9.13±0.5f	6.798***
WBC(10^3 mm^{-3})	7.63±0.35a,c,e	9.80±0.62b	9.84±0.48d	11.1±0.63f	7.287***
PCV (%)	30.2± 1.31	28.6 ±1.29	31.48±1.49	27.93±1.65	1.211NS
MCV (fl)	109.64 ± 4.53	115.53±5.08	110.46±5.07	111.7±3.82	1.466NS
MCH (pg)	33.25 ±0.74a,c	33.28±1.71e	38.05±1.4b	38.7±1.3d,f	6.159***
MCHC (%)	31.95±1.15a	29.38±1.4c	34.94±1.19b,d	34.73±0.65	6.441***

(Figures in parentheses indicate number of observations, *** significant at p<0.001. Consecutive letters in the superscript represents significant differences among the rows and NS represents not significant).

The blood biochemical profiles were estimated using the commercially available diagnostic kits. Except albumin all other biochemical parameters (Table 2) like protein, glucose, globulin and cholesterol differ significantly (p<0.001) between the *C. batrachus* and *H. fossilis* (Fig.8 to Fig.12). All biochemical parameters show higher value in female than male in both the catfishes used in this study but did not exhibit significant difference with respect to sex. The value of all biochemical parameters found were higher in *H. fossilis* than *C. batrachus* this may be due to highly nutritious value of *H. fossilis*.

Table 2: Biochemical parameters of *C. batrachus* and *H. fossilis.*

Parameters	*Clarias batrachus*		*Heteropneusteus fossilis*		F value
	Male (n=15)	Female (n=15)	Male (n=15)	Female (n=15)	
Protein(g/dl)	4.22±0.27a,c	4.46±0.32e,g	9.46±1.39b,f	11.25±1.25d,h	13.7***
Albumin(g/dl)	3.51±0.28	3.54±0.31	3.87±0.28	4.39±0.37	1.709NS
Globulin(g/dl)	0.60±0.14a,c	0.92±0.30e,g	5.59±1.48b,f	6.85±1.31d,h	10.13***
Glucose(mg/dl)	70.83±6.67a	82.88±8.76c	102.01±11.97	119.84±7.64b,d	5.742***
Cholesterol(mg/l)	210±28.75a,c	223.89±22.2e,g	343.66±29.72b,f	444.54±31.45d,h	15.24***

(Figures in parentheses indicate number of observations, *** significant at p<0.001. Consecutive letters in the superscript represents significant differences among the rows and NS represents not significant).

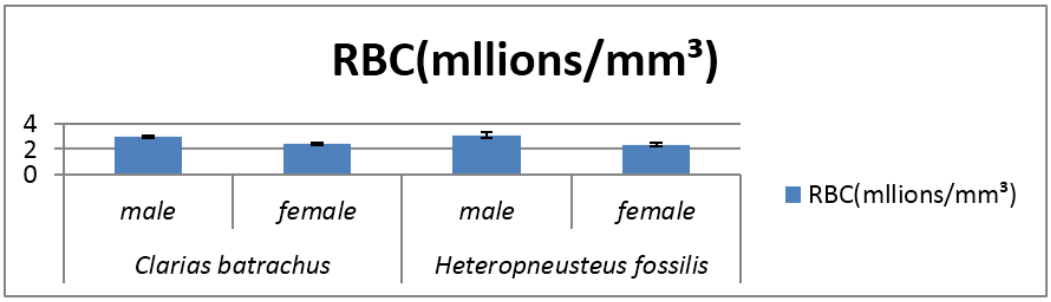

Fig 1: Comparision of RBC of *C. batrachus* and *H. fossilis*

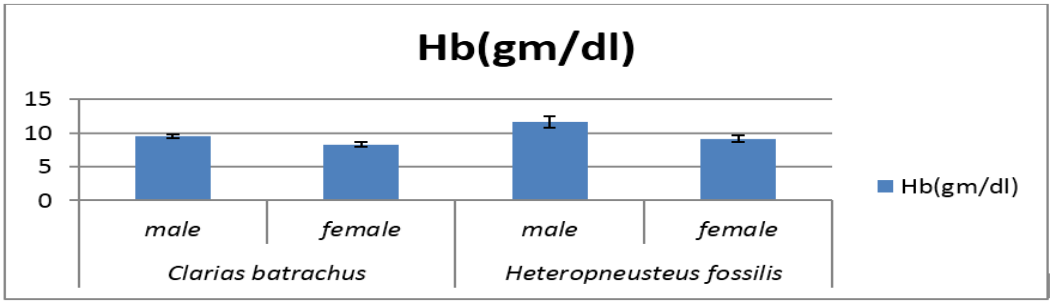

Fig 2: Comparision of Hb of *C. batrachus* and *H. fossilis*

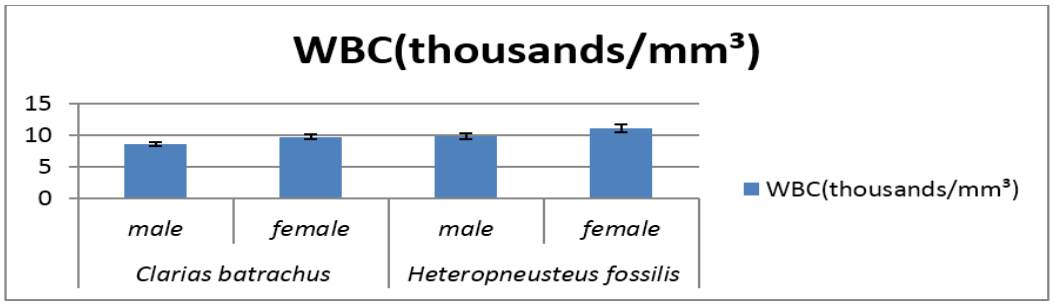

Fig 3: Comparision of WBC of C. *batrachus* and *H. fossilis*

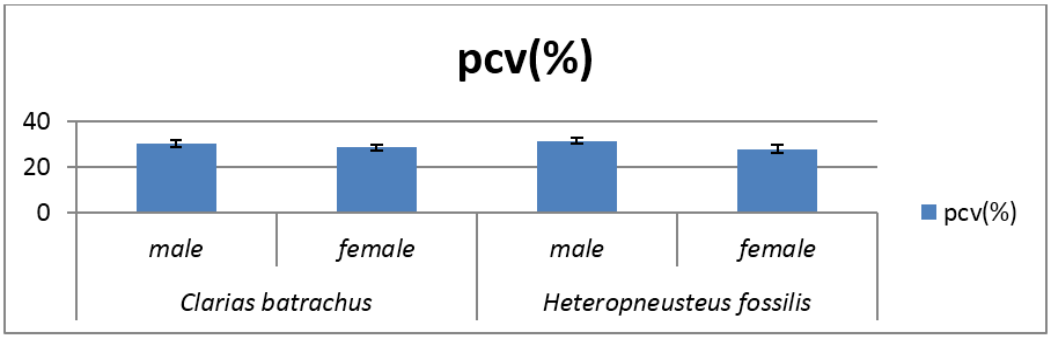

Fig 4: Comparision of PCV of *C. batrachus* and *H. fossilis*

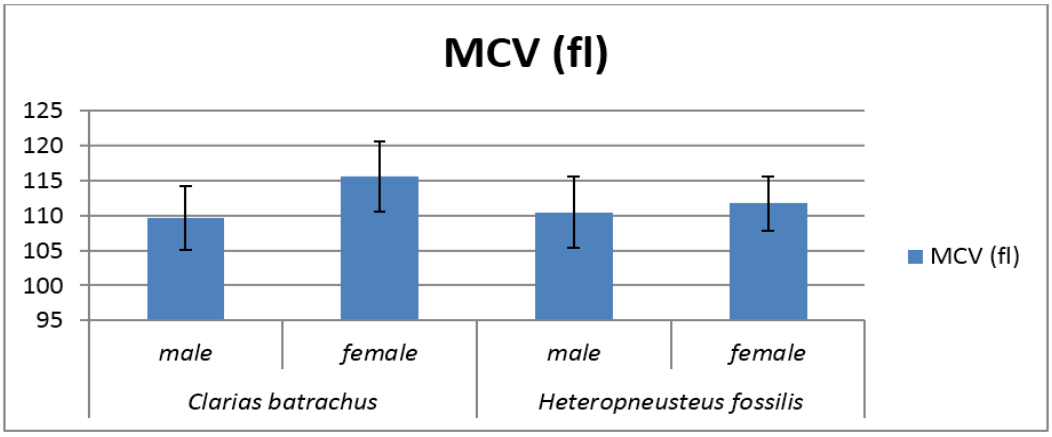

Fig 5: Comparision of MCV of *C. batrachus* and *H. fossilis*

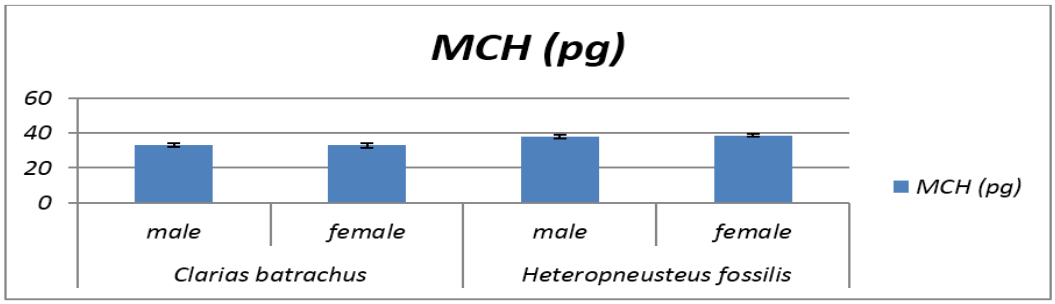

Fig 6: Comparision of MCH of *C. batrachus* and *H. fossilis*

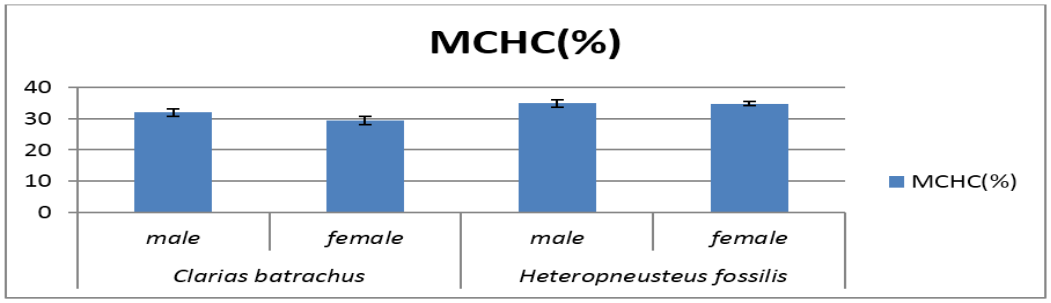

Fig 7: Comparision of MCHC of *C. batrachus* and *H .fossilis*

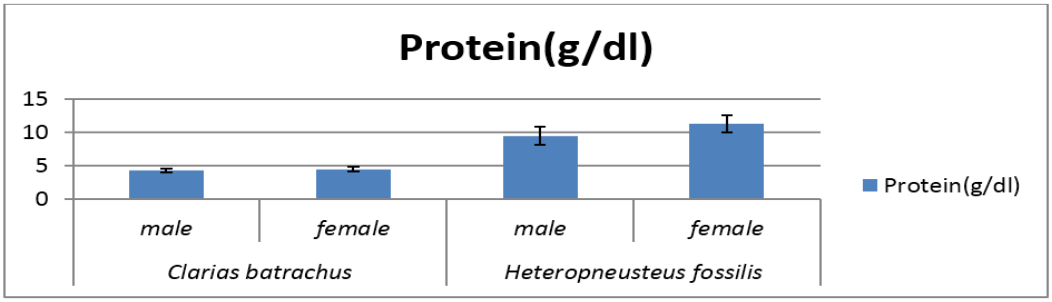

Fig 8: Comparision of Protein of *C. batrachus* and *H. fossilis*

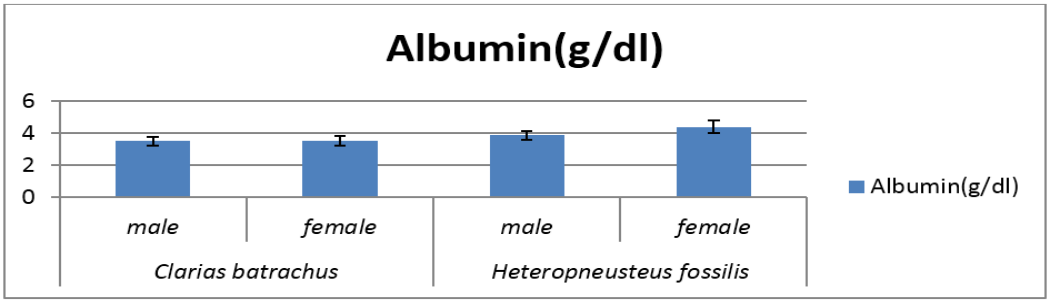

Fig 9: Comparision of Albumin of *C. batrachus* and *H. fossilis*

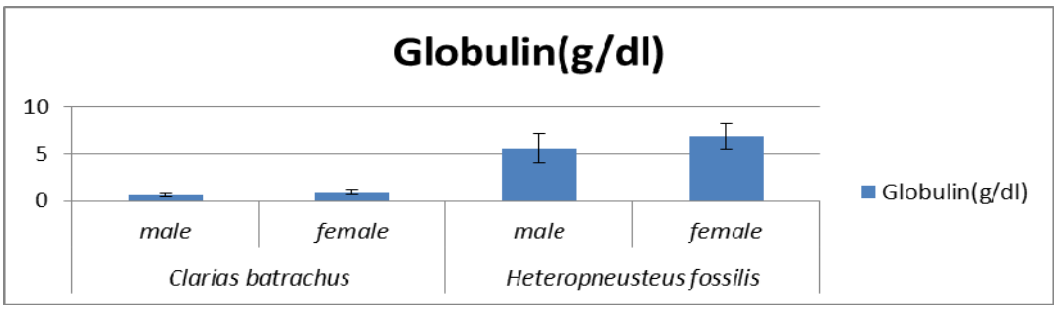

Fig 10: Comparision of Globulin of *C. batrachus* and *H. fossilis*

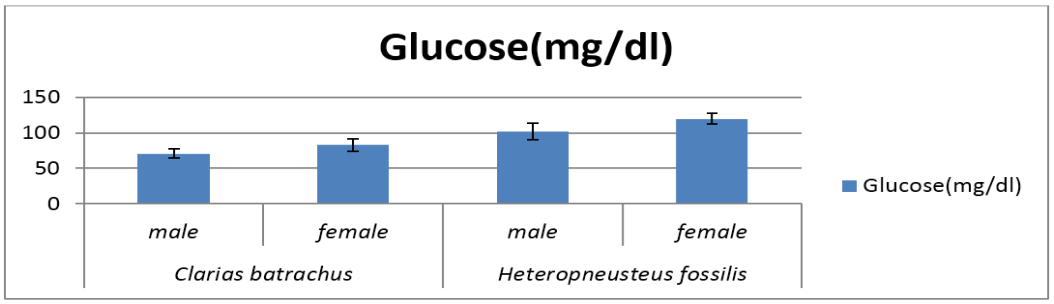

Fig 11: Comparision of Glucose of *C. batrachus* and *H. fossilis*

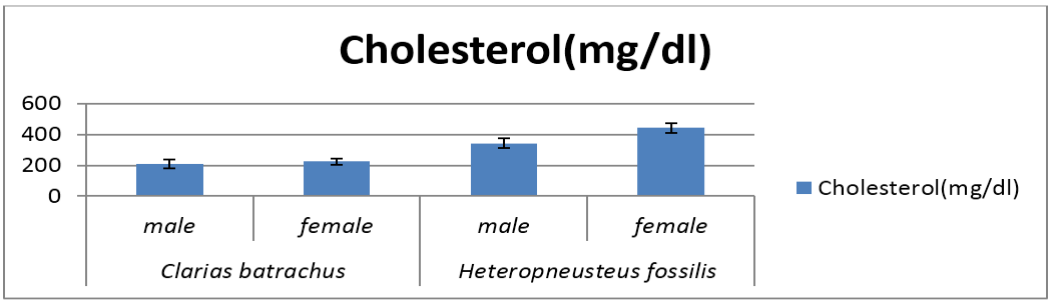

Fig 12: Comparision of Cholesterol of *C. batrachus* and *H. fossilis*

4. Discussion

Fisheries sector plays an important role in the economy of Odisha. As fish live in a very intimate contact with their environment, they are extremely dependent upon it [18]. Any changes in the environment would affect the physiology of the fishes. The blood is said to be a mirror in which all the vital processes taking place in the organisms are reflected therefore blood parameters are used in understanding the biological process taking place in fish species [19]. Fishes show abnormal effects due to food, habitat, temperature, pH, and many other elements of environment [20]. Fish blood is being studied in toxicological research and environmental monitoring as a possible indicator of physiological and pathological change in fishery management [21]. There is growing interest in the study of haematological and biochemical parameters and these parameters are regarded as important for aquaculture purposes. It, was, therefore considered desirable to evaluate the haematological and biochemical changes in the blood parameter of the predatory fish like *H. fossilis and C. batrachus* resulting from the sexual dimorphism. In the present study, significant variation in haematological parameters was found between *C. batrachus* and *H. fossilis* except PCV and MCV. The RBC of an organism determines the carrying capacity of dissolved oxygen. The RBC content of this investigation for male and female *C. batrachus* is 2.97±0.07 and 2.55±0.10 ($10^6 mm^{-3}$) and it is lower than that *H. fossilis* for both male and female are3.1± 0.25 and 2.36±0.1 ($10^6 mm^{-3}$) respectively. The reason for the differences may be due to differences in species or it may be due to environmental factors. Some reports demonstrate that variation in haematocrit value and other haematological parameters between sexes and their diversity might be due to the higher metabolic rates of male fish. Findings in the present work also support this theory, which is related to an increase in fish activity or with an increase in size. Considering the sex variations, many studies demonstrated that the male fish has higher values in almost all haematological parameters except in TLC [22]. These higher values in males may be attributed to their physiological activeness. In the present study, all the haematological parameters shows higher value in *H. fossilis* than *C. batrachus*, this may be due to different species or may be due to more activeness of *H. fossilis.*

Blood biochemical parameters with significant variations ($P<0.001$) were observed for the protein, globulin, glucose and cholesterol. The ranges of serum biochemistry 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 [23]. Plasma protein is the protein component of the blood and it increases with starvation or any other stress [24]. In the present case, plasma protein concentration does not differ significantly between male and female but shows highly significant difference between the two species of catfishes taken for this study. Plasma protein gives an index of the health status of the brood fish [25] and as

indicator of nutritional status [26]. Globulin differ significantly between the *C. batrachus* and *H. fossilis* but showed non-significant sexual difference. Albumin helps in transportation of lipid in fishes [27] 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 [28]. In this study albumin content is higher in female than male but does not significantly vary between and within 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 [29]. The higher level of glucose found in *H. fossilis* may be due to different species or due to stress. Cholesterol concentration varies both among and within fish species because of variations in diet, activity and sexual development [30].

In conclusion, this study has established a reference value regarding the selected haematological and serum biochemical parameters of two fresh water catfishes. Haematological and biochemical parameters regarded as valuable tool for monitoring fish health and also help in monitoring any changes in the quality of water. The range of normal values of the key haematological and biochemical parameters is still undefined for different species in different aquaculture conditions. Haematological studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution in aquatic ecosystem.

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