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Comparative study on haematological characteristics of both sexes of catfish *Clarias gariepinus* (Burchell 1822) (Pisces: Clariidae) in Cross River, Cross River State. Nigeria

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Abstract
The comparative haematological characteristics of both sexes of African Catfish *Clarias gariepinus* in Cross River, Cross River state was determined. The blood parameters determine includes Red and White Blood cells counts, Haemoglobin, Haematocrit, Mean Cell Haemoglobin Concentration, Mean Cell Volume, Mean Cell Haemoglobin, Protein, Sodium, Potassium, Lymphocyte, Platelet, Red Cell Distribution with standard size, Red Cell distribution with Corpuscular Volume and Mean Cell Haemoglobin Concentration. The blood cell count were higher in male (WBC, RBC, HGB, HCT, MCH, PLT, LYM, MCHC, RCD-WSS and RCD-WCV) with mean values (1.60±24.71, 1.28±8.27, 9.38±4.02, 17.75±8.59, 47.78±9.09, 3.44±29310.43, 91.14±9.70, 46.20±12.55, 46.81±12.46 and 20.52±17.16) except MCV which was higher in female than male with mean value (98.54±42.09). The red and white blood cell counts did not positively correlate with the physical parameters length and weight. There were significant correlations between weight of female and Male weight (at $p< 0.05$), Red Blood Cell of female negatively correlated with Male Haemoglobin (at $p<-0.05$), white blood cell of female negatively correlated with male Platelet (at $p <0.05$), female fibre did not correlate with any of the male parameters. The result reveals that all the haematological and biochemical parameters of the male were higher than the female except the electrochemical parameters which were examined to be the same. The result will serves as baseline information for disease and pollution assessment in a fisheries management activities.

Keywords: haematological, biochemical, electrochemical, *clarias gariepinus and* Cross River

Introduction

Haematology is the study of blood. Blood is recognized as a potential index of fish response to water quality (Hickey, 1982) [12], as it can be used to ascertain the effect of pollutants in the environment. Blood parameters have been commonly used to observe and follow fish health, since variations in blood tissues of fish are caused by environmental stress (Shad and Altindag, 2005) [19]. Blood parameters in fish have been studied to elucidate physiological adaptation and assess the health of fishes (Vazquez and Guerrore, 2007). Bouk and Ball (1966) [6] stated that haematology may be a useful tool in monitoring stress levels of aquatic pollution on fish. Haematological characteristic is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish and other animals both in water and on land. The blood composition of fish could be used as one of the indices to indicate their well-being. Environmental factors, seasonal conditions (Joshi and Tondon, 1977), different period of reproductive cycle of chemical stress. Blaxhall and Daisley (1973) [5] have reported the possibility of using haematocrit, packed cell volume (PVC) and haemoglobin values as tools for checking anaemic condition in fisheries management and aquaculture. Serum protein, urea, uric acid, cholesterol and glucose concentration are accepted as indicators of nutritional status (Garcia – Rodriguez *et al*., 1987) [10], reported that, white blood cell (WBC) is useful as indicator of diseased condition or extent of infection and elevated values are obtained. Blood glucose and PCV as well as electrolytes balance are considered to be valuable tools for the identification of secondary stress condition in fish (Barton and Iwama, 1991) [3]. The analysis of blood indices has proven to be a valuable approach for analyzing the health status of fishes as these indices will provide reliable information on metabolic disorder, deficiencies and chronic stress status before they are present in clinical setting (Bahmani *et al*., 2001) [2]. The fluctuations in the blood constituents in fishes are subjected to change in temperature, ecological condition, and food habits, chemical and environmental stress (Benarjee *et al*., 2009) [4].

African catfish *Clarias gariepinus* is a fresh water fish which can be tolerant to wide range of environmental conditions like low dissolved oxygen, high and low temperatures, turbidity, acidic alkaline and pH. *Clarias gariepinus* are successful and the most desirable fish for culture from the mid70s. It has mostly been used as 'police fish' to control breeding in mixed-sex tilapia culture in earthen ponds. The inability of this fish to detect and respond to chemical stimulus is as a result of significant effect of water pollutants, which is capable of posing serious adverse effect on the feeding, growth and physiological performances within a polluted aquatic ecosystem. This polluted habitat may also result in mass mortality or failure to breed in it environment. *Clarias gariepinus* (African catfish) is one of the most important fish species from the family claridae currently being cultured both inside and outside its natural range of tropical and subtropical environments and it has fast growth potential, efficient use of natural aquatic food, propensity to consume a variety of supplemental feed, omnivorous food habits, resistance to disease, ease of reproduction in captivity and handling. Adewolu *et al*., (2008) [1], and Chepkirui-Boit, (2011) [7]. Positive attributes such as resistance to diseases, high fecundity, and ease of larval production in captivity make it of commercial importance in aquaculture (Kestemont *et al.* 2007) [15]. Different research work has been carried out on the

haematological characteristics of *Clarias gariepinus* (African catfish), but no work has been done on the comparative haematological study on both sexes (male and female) *C. gariepinus*. This work will establish the normal haematological profile of both sexes of Catfish *Clarias gariepinus* juvenile and the results will serve as baseline information for the assessment of disease and environmental toxicological study.

Materials and Method

This experiment was carried out in the Department of Fisheries and Aquatic Science wet Laboratory, Faculty of Agriculture and Forestry, Cross River University of Technology, Calabar, Obubra Campus**,** Nigeria**.**

Collection of Experimental Fish

50 live juvenile's catfish *Clarias gariepinus* (25 male and 25 female) were identified using a taxonomic key of Reed *et al.*, (1967) [18]. The fresh fish were purchased from fresh artisanal fishermen landing at the Cross River in Obubra Local Government Area, Cross River State. They were batch weighed to the nearest grams using the electronic scale (E.K 5.350), the length measurement were made to the nearest centimeters using measuring board. The fish were not fed for 12 hours before and during transportation, to prevent defecation. The fish was transported in an aerated plastic container from the landing site at Obubra to the laboratory.

Method of Fish Blood Sampling

Blood (1-2ml) was collected from the vertebral caudal blood vessel according to Schmit *et al*., (1999), using disposable 5ml syringe and needle. The blood was emptied into the heparinized blood bottle treated with Ethyl Diamine Tetracetic Acid (EDTA). A blood sample was centrifuge (1500 rpm for 7mins) to obtain the blood plasma. Plasma samples were stored at (-20^{0}C) for the electrochemical and biochemical analysis.

Method of Blood Analysis

Computerized method employing System KX-2IN^{TM} Automated Hematology Analyzer was used in blood analysis, the KX-2IN is an ideal hematology analyzer for a clinical satellite laboratory or research testing. Spectrophotometric method was used for biochemical analysis as described by Svobodova *et al.*, (2003) [22]. While The plasma electrolytes were determined using corning 400 flamephotometer. Other metals was determined using (a back) Model 200A flame of the Atomic Absoption Spectrophotometer (AAS).

Statistical Analysis

The data that was obtained from this study was subjected to descriptive statistics, one way analysis of variance and Pearson's correlation. The differences in the means between both sexes was assessed with Duncan multiple range test Using SPSS version 16 at P<0.05 significant level.

Results

The results of comparative haematological parameters of juvenile catfish *Clarias gariepinus* are presented in table 1. This shows that, there was no significant differences between the mean values of male and female in the blood cell count (WBC, RBC, Haematocript, platelet and RCD-WCV) with a significant differences in (RCD-WSS, MCHC, Lymphocyte, MCH and MCV). The results of comparative biochemical parameters of male and female catfish juvenile *C. gariepinus* are presented in table 2. This shows that, there was no significant difference between the mean values of male and female (Ash, Ether Extract, Protein, Nitrogen Free Extract, Energy, weight, standard length. The comparative electrochemical parameters of male and female catfish juvenile *C. gariepinus* are presented in table 3. Which shows that, there was no difference between the mean values of male and female catfish juvenile *C.gariepinus.*

Table 1: Comparative Haematological Characteristic of Male and Female Catfish *Clarias gariepinus.*

MALE			Female		
Parameters	**Mean ±S.D**	**Range**	**Parameters**	**Mea ± S.D**	**Range**
White blood cell (WBC) 10^3mm^4	1.6 ± 24.71	100.00-193.70	White blood cell(WBC) 10^3mm^4	1.24 ± 73.99	0.00-193.70
Red blood cell(RBC) 10^3mm^6	1.28 ± 8.27	16200-2.62	Red blood cell(RBC) 10^3mm^6	1.20 ± 8.65	0.00-2.62
Haemoglobin (g/dl) (Hb)	9.38 ± 4.02	5.30-23.00	Haemoglobin (g/dl) (Hb)	7.35 ± 3.74	0.00-13.90
Haematocript(%)	17.75±8.59	0.10-29.40	Haematocript(%)	17.05±0.41	0.00-29.40
Mean cell volume(MCV)fl	1.12±15.36	56.90-132.20	Mean cell volume(MCV)fl	98.54±42.09	0.00-132.20
Mean cell haemoglobin (MCH)pg	47.78±9.09	35.00-77.70	Mean cell haemoglobin (MCH)pg	37.12±22.60	0.00-77.70
Platelet (ul)	3.44±29310.43	3000.00-1.15	Platelet (ul)	3.12±29127.37	0.00-1.15
Lymphocyte (%)	91.14±9.70	56.00-98.20	Lymphocyte (%)	72.33±41.55	0.00-98.20
Mean cell haemoglobin concentration (MCHC) u/l	46.20±12.55	34.60-78.00	Mean cell haemoglobin concentration (MCHC) u/l	31.77±19.81	0.00-72.80
Red cell dist. With std size(fl)	46.20±12.46	28.30-65.00	Red cell dist. With std size(fl)	31.34±22.61	0.00-65.00
Red cell dist. With corpuscular volume (%)	20.52±17.16	0.00-69.00	Red cell dist. With corpuscular volume (%)	19.52±17.74	0.00-69.00

Table 2: Comparative Biochemical Characteristics of Male and Female Catfish *Clarias gariepinus.*

Male			Female		
Parameters	Mean± S.D	Range	Parameters	Mean± S.D	Range
Moisture (%)	68.99±4.80	56.97-86.15	Moisture (%)	68.99±4.79	56.97-85.15
Fibre (%)	0.00±.0.00	0.00-0.00	Fibre (%)	0.00±0.00	0.00-0.00
Protein (%)	26.73±2.64	16.50-30.43	Protein (%)	27.02±0.89	25.12-29.12
Ether extract (%)	3.36±0.64	2.12-5.12	Ether extract (%)	3.17±0.42	2.12-4.11
Ash (%)	0.85±0.20	0.00-0.98	Ash (%)	0.83±0.20	0.00-0.98
Nitrogen Free Extract (%)	0.43±0.17	0.21-0.89	Nitrogen Free Extract (%)	0.43±0.17	0.21-0.89
Energy (%)	1.32±27.20	14.77-146.31	Energy (%)	1.32±27.20	14.77-146.31

Weight (gm)	62.74±5.72	52.60-73.60	Weight (gm)	62.74±5.72	52.60-73.60
Standard length (cm)	19.34±1.12	17.78-22.60	Standard length (cm)	19.14±1.12	17.78-22.60

Table 3: Comparative Electrochemical Characteristics of Male and Female Catfish *Clarias gariepinus*.

Male			Female		
Parameters	Mean± S.D	Range	Parameters	Mean± S.D	Range
Sodium Na. (Mmol)	79.58±34.31	27.90-195.20	Sodium Na. (Mmol)	79.58±34.31	27.90-195.20
Potassium k. (Mmol)	35.52±18.72	12.30-107.30	Potassium k. (Mmol)	35.52±18.72	12.30-107.30

Table 4: Correlation between haematological characteristics of male and female catfish *Clarias gariepinus* Juvenile.

WBC (ul)	RBC (ul)	HGB (g/dl)	HCT (%)	MCV (fl)	MCV (pg)	PLT (ul)	PLT (%)	MCHC (u/l)	RCDWS (fl)	RCDWV (%)	FWBC (ul)	FRBC (ul)	FHGB (g/dl)	FHCT (%)	FMCV (fl)	FMCH (pg)	FPLT (ul)	FLYM (%)	FMCHC (u/l)	FRCDWSS (fl)	FRCDWCV (%)
WBC	1																				
RBC	0.309	1																			
HGB	-0.151	-0.228	1																		
HCT	0.581	0.595	-0.144	1																	
MCV	0.319	0.418	-0.662	0.608	1																
MCH	0.167	0.268	-0.064	0.155	0.334	1															
PLT	0.258	0.128	-0.175	-0.023	-0.011	0.614	1														
LYM	0.231	0.436	-0.242	0.793	0.395	-0.268	-0.177	1													
MCHC	-0.262	-0.452	0.546	-0.698	-0.784	0.180	0.410	-0.633	1												
RCDWS	-0.008	-0.206	-0.167	-0.395	0.089	0.457	0.500	-0549	0.380	1											
RCDWV 0.193	-0.166	-0.546	-0.299	-0.095	-0.107	0.563	-0.111	0.272	0.375	1											
FWBC	0.486	0.573	-0.047	0.931	0.551	0.206	-0.050	-0.765	-0.579	-0.345	-0.289	1									
FRBC	0.251	0.900	-0.098	0.583	0.314	0.171	0.036	0.487	-0.355	-0.262	-0.126	-0.100	1								
FHGB	0.570	0.446	-0.101	0.791	0.415	0.307	0.316	0.618	-0.292	-0.170	0.094	0.878	0.619	1							
FHCT	0.507	0.516	-0.043	0.942	0.502	0.081	-0.087	0.793	-0.593	-0.418	-0.254	0.977	0.653	0.885	1						
FMCV	0.250	0.364	-0.471	0.649	0.572	-0.006	0.066	0.735	-0.521	-0.183	0.233	0.763	0.570	0.814	0.774	1					
FMCH	0.222	0.542	-0.063	0.767	0.568	0.483	0.163	0.612	-0.390	-0.123	-0.240	0.903	0.668	0.846	0.830	0.756	1				
FPLT	0.201	0.070	-0.033	0.000	-0.104	0.498	0.869	-0.085	0.481	0.406	0.580	0.123	0.204	0.533	0.105	0.323	0.336	1			
FLYM	0.264	0.568	-0.155	0.876	0.635	0.175	-0.129	0.789	-0.656	-0.357	-0.320	0.964	0.691	0.802	0.930	0.818	0.9205	0.051	1		
FMCHC 0.257	0.558	-0.027	0.759	0.521	0.517	0.220	0.583	-0.333	-0.120	-0.220	0.896	0.680	0.862	0.820	0.726	0.995	0.387	0.900	1		
FRCDWS0.061	0.414	-0.240	0.641	0.588	0.018	-0.171	0.629	-0.637	0.037	-0.235	0.702	0.500	0.501	0.678	0.678	0.6678	-0.041	0.771	0.622	1	
FRCDWV0.159	-0.185	-0.450	-0.275	-0.137	-0.149	0.486	-0.062	0.301	0.317	0.968	-0.189	-0.035	0.203	-0.146	0.351	-0.139	0.631	-0.216	-0.122	-0.160	1

Table 5: Correlation between Biochemical and electrochemical characteristics of male and female catfish *Clarias gariepinus* Juvenile.

MOI (%)	FBR (%)	PRO (%)	EE (%)	ASH (%)	NFE (%)	EGY Kc/100g	Na Mmol	K Mmol	WGT (gm)	STDL (cm)	F MOI (%)	F FBR (%)	FPRO (%)	FEE (%)	FASH (%)	FNFE (%)	FEGY Kc/100g	FNa Mmol	FK Mmol	FWGT (gm)	FSTDL (cm)	
MOI	1																					
FBR	0.00	1																				
PRO	-0.095	0.00	1																			
EE	0.194	0.00	-0.175	1																		
ASH	0.132	0.00	-0.045	-0.190	1																	
NFE	0.027	0.00	-0.224	-0.290	-0.108	1																
EGY	0.031	0.00	-0.073	0.078	-0.024	0.054	1															
Na	0.262	0.00	-0.221	0.187	-0.086	-0.091	0.168	1														
K	0.049	0.00	-0.085	0.152	-0.298	-0.112	0.052	-0.012	1													
WGT	0.050	0.00	0.218	-0.143	-0.134	0.179	0.442	0.087	-0.064	1												
STDL	0.109	0.00	0.035	-0.173	0.059	-0.052	0.219	0.039	0.050	0.721	1											
F MOI	1.000	0.00	-0.095	0.194	0.132	0.027	0.031	0.262	0.049	0.050	0.109	1										
F FBR	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1									
FPRO	-0.412	0.00	0.227	-0.301	0.025	-0004	-0.018	-0.163	-0.064	-0.076	-0.092	-0.412	0.00	1								
FEE	0.360	0.00	-0.034	0.436	-0.509	0.047	0.025	0.098	0.337	-0.194	-0.170	0.360	0.00	0.124	1							
FASH	0.132	0.00	-0.045	-0.190	1.000	-0.108	-0.024	-0.086	-0.298	-0.134	0.059	0.132	0.00	0.025	-0.509	1						
FNFE	0.027	0.00	-0.224	-0.290	-0.108	1.000	0.054	-0.091	-0.112	0.179	-0.052	0.027	0.00	0.004	0.047	-0.108	1					
FEGY	0.031	0.00	-0.073	0.078	-0.024	0.054	1.000	0.168	0.052	0.442	0.219	0.031	0.00	-0.018	0.025	-0.024	0.054	1				
FNa	0.262	0.00	-0.221	0.187	-0.086	-0.091	0.168	1.000	-0.012	0.087	0.037	0.262	0.00	-0.168	0.098	-0.086	-0.091	0.168	1			
FK	0.049	0.00	-0.085	0.152	-0.298	-0112	0.052	-0.012	1.000	-0.064	0.050	0.049	0.00	-0.064	0.337	-0.298	-0.112	0.050	-0.012	1		
FWGT	0.050	0.00	0.218	-0.143	-0.134	0.179	0.442	0.087	-0.064	1.000	0.721	0.050	0.00	-0.076	-0.194	-0.134	0.179	0.442	0.087	-0.064	1	
FSTDL	0.109	0.00	0.035	-0.173	0.059	-0.052	0.219	0.037	0.050	0.721	1.000	0.109	0.00	-0.092	-0.170	0.059	-0.052	0.219	0.037	0.050	0.721	1

Discussion

The haematological characteristics of male and female catfish *C. gariepinus* have been investigated with the aim of establishing normal blood values and range with respect to sex, age and environmental and physiological conditions. The result of comparative haematological examination presented in Table 1 shows that the number of blood count: WBC, RBC, HCT, PLT, and RCD-WCV of male parameters were not significantly different with mean values (1.60±24.71, 1.28±8.27, 9.38±4.02, 17.75±8.59, 47.78±9.09, 3.44±2.93, 91.14±9.70, 46.20±12.55, 46.81±12.46 and 20.52±17.16) from all the female parameter with significant difference in (RCD-WSS, MCHC, LYM, MCH and MCV) except MCV which was higher in female with mean value (98.54±42.09) than the male with mean value of 1.12±15.36, (P>0.05) The WBCs are the defensive cell of the body and it was higher in male than female. According to Douglass and Jane (2010), their levels have implications for immune response and the ability of the animal to fight infection more effectively than other species.

In *Clarias gariepinus*, the platelet count is 172450±10661 and *Chrysichthys nigrodigitatus* is 173000±70958. The value of platelet in female *Clarias gariepinus* is lower than the male *Clarias griepinus* and has more ability to maintain heamostasis during blood lost. The species with higher value of circulating lymphocyte will be able to defend itself from invading pathogen both by cell-mediated and humoral - mediated responses Douglas and Jane 2010, thus from this present study, the male *Clarias gariepinus* with higher value will be able to mount adequate immune responses better than female catfish.

The result of this present work is similar to the work of Kori-Siakpere 1985; Sowunmi 2003; Gabriel *et al.*, 2007, who carried out comparative studies on blood parameters of fish to determine the systematic relationship among certain species. The Mean Cell Haemoglobin was 47.78±9.70 for male and 37.12±22.60 for female; It was higher than that of 26.45±14.58gdl^{-1} and 26.62±15.8 for male and female

Synodontis membranacea as reported by Owolabi, (2008). The haematocrit value of 17.75±8.59 for male and 17.05±9.41 recorded in the present study was lower than that of 48.33±0.144%, 45.33±0.015%, 36.31±0.010% and 33.33±0.013% for *M. tengara, H. fossilis, L. bata*, and *L. cursa* (Goel *et al*., 1981). It was also lower than those of 28.12 ±2.98% for *Clarias isheriensis*, Kori-siakpere (1985), and 27.16±3.72%) for *Labeo sp.* (Ayotunde and Ochang, 2006). The mean cell volume value for male and female *Clarias gariepinus* was 1.12±15.36 and 98.54±42.09 respectively in the present study. It was higher than that of 78.30±37.89% for male *Synodontis membranacea* (Owolabi, 2008).

The result of Jawad *et al*., (2004) is similar to the result of the present study which found that, values of RBC, Hct, and Hb increased with increased fish size. In this study, values of RBC and Hb were higher in male which have the highest biometric parameters. The result of biochemical parameters presented in Table 2 show that there were no significant differences in male and female parameter (moisture, protein EE, Ash, NFE, energy, weight and standard length). The value for protein in this study was 26.73± 2.64 for male, 27.02± 0.89 for female and it was lower than that of 40.24± 7.56mgdl^{-1} for *S. membranacae* Owolabi (2008) which does not agree with Siakpere (1985) who reported very low mean value of 7.63±0.11 of plasma protein for *clarias isheriensis*.

The plasma electrolyte in Table 3 showed no significant difference in male (Sodium and Potassium) with mean values (79.58±34.31 and 35.52±18.72) and female sodium and potassium with mean values (79.58±34.31 and 35.52±18.72) respectively. The value of sodium was lower than that of 139.86±22.63mol l^{-1} for *Synodontis membraneacea* Owolabi (2008), the value for potassium was also higher than that of 13.24±3.37mol l^{-1} for *S. membranacae* Owolabi (2008). The study shows positive and negative correlation between the male and female parameters. The correlation between male and female catfish juvenile C. *gariepinus* shows Tables 4 and 5 that there was a positive correlation between the female moisture and male moisture, protein, EE, ash, NFE, energy, sodium, potassium, weight, standard length, MCV and LYM (at P<0.05), while there was a negative correlation between the female moisture and male WBC, RBC, HGB, MCH, PLT, MCHC, RCD-WSS and RCD-WCV (at p>-0.05 and p<-0.05). There was no correlation between the female fibre and all the male parameters.There was a positive correlation between the female protein and male ash RBC, HGB, HCT (at p<0.05) and protein, LYM, MCHC (at p<0.05). Other are negatively correlated (at p<-0.05 and p>-0.05).

These results show that the male and female catfish species *C. gariepinus* in Cross River were in good health. These results of this study produce the baseline data on blood profile of male and female catfish *C. gariepinus* in Cross River, Cross River State, Nigeria.

Conclusion

Haematological studies contributes to an understanding of the relationship between blood characteristics and habitat and the adaptability of the species to the environment, so there is need to establish normal haematological values in different species and sexes of fish. The haematological parameters of the male Catfish *Clarias gariepinus* from Cross River, were higher than the female parameters. The biochemical parameters of the both sexes showed no significant differences while the electrochemical parameters showed no differences between the both sexes. Therefore, the haematology of the fish is influenced by the environment in which it lives. The blood is the most important fluid in the body and its composition often reflects the total physiological condition of an organism. Blood parameters can be used for assessing the quality of the fish's environment. Haematological parameters are nowadays not only used for clinical diagnosis of physiology but also help in addressing the effects of toxic substances in the fish. The establishment of the comparative study of haematological parameters of *C. gariepinus* in Cross River aimed at providing the baseline result and knowledge of blood parameters of the male and female catfish in Cross River State. However, the blood parameters of both the male and female catfish were within the normal range values. Therefore this work shows that, the catfishes in Cross River are in good health, so therefore there is need to abort the idea of water pollution.

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