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# SEASONAL INFLUENCE ON HAEMATOLOGICAL PARAMETERS OF MALE AND FEMALE *HETEROBRANCHUS BIDORSALIS* (GEOFFROY SAINT-HILAIRE, 1809) IN RIVER RIMA SOKOTO, NIGERIA

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**ABSTRACT:** *Seasonal variations usually affect aquatic life in one way or another. This study evaluated the influence of season on haematological parameters of male and female Heterobranchus bidorsalis. Fish samples were collected in two seasons; rainy and dry season in the months of July, August, September (rainy season) and November, December, January (dry season). Collection was made from three different stations (A, B and C), landing sites along River Rima of Sokoto State, Nigeria. Haematological parameters were analyzed automatically using Erma Particle Counter. The results obtained in respect to stations and seasons were; Haematological parameters; WBC, LY, LY%, RBC, Hgb, HCT, MCV, MCH, MCHC and MPV show significant difference at P <0.05 in some parameters. But all the results indicated that the Heterobranchus bidorsalis was healthy.*

**KEYWORDS:** heterobranchus bidorsalis, heamatological parameters, sex, season

## INTRODUCTION

*Heterobranchus bidorsalis* is a highly economic species that gain more acceptance from the consumers, farmers and researchers because it performs better than any other species in the family clariidae due to its fast growth, ability to resist diseases, high palatability and its ability to tolerate adverse environmental conditions (Owodeinde *et al*., 2011). It is easily identified from other members of its family due to the presence of longer dorsal fin when compared to its adipose fin, with absence of black spot at its tail end in matured individuals (Olaniyi and Omitogun, 2014). It grows well in captivity by attaining maturity in 10-12 months of domestic rearing but two to three years in the wild (Adebayo and Fabenro, 2004).

Haematological parameters of fish have been widely known/used to be the most valuable tools for monitoring its health status (Satheeshkumar *et al*., 2012). Many environmental and physiological factors are known to influence fish haematology. These include stress due to capturing, transportation, sampling, age and sex (Hamid *et al*., 2013). The aquatic environment, where fish and other aquatic organisms live, is subjected to different type of pollutants which are introduced into water bodies through industrial and domestic activities (Sheikh and Ahmed, 2016; Diyaware *et al*., 2009).

Stress is a general and non-specific response to any factors disturbing homeostasis and its reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Hamid *et al*., 2013). As such, information on the haematological characteristics has been regarded as the most important, effective and sensitive tool that can be used to monitor the physiological changes in fishes (Zhou *et al*., 2009). Observation on deviation of certain blood parameters from their normal ranges could be a guide for diagnosis or differential of a disease condition (Sripad *et al*., 2014).

The present study was carried out to evaluate the effects of season and gender on the haematological parameters of *H. bidorsalis* at River Rima in Sokoto, Nigeria

## MATERIALS AND METHODS

### Study area

The River Rima of Sokoto is located in the North-Western region of Nigeria, Sokoto is found between Latitude 12° N and 13°58´ N and Longitude 4° 8`E and 6° 5`E. It covers a land area of approximately 131,600 km$^2$ and share borders with Niger Republic to the north, and covers Sokoto, Kebbi, Zamfara and large part of Katsina State to the East, it also bordered to Niger State to the South-east, and Benin Republic to the west. The whole basin can be described as Sudan and Sahel Savanna, and it extends beyond the border to Niger Republic and the northern part of Benin Republic. The basin topography consists of a vast floodplain (fadama wetland) and rich alluvial soils that is suitable for the cultivation of different type of crops. There are also isolated hills (inselberg) and hill ranges scattered all over the area (Ekpoh and Nsa, 2011). Temperature is generally extreme, with average daily minimum of 16°C during colder months of January and December, and in the hottest months of April to June, an average maximum of 38°C with a minimum of 24°C. Throughout the year, the average maximum used to be 36°C and the average daily minimum being 21°C. River Rima flows in a south western direction of over 100km and joins the major River Sokoto to form the Sokoto-Rima River system. It flows North-west passing through Gusau in Zamfara State, where the Gusau Dam forms a reservoir that supplies the city with water. The average annual rainfall for 35 years is about 470mm and most of the rain falls between the months of May to September, while the dry season months are between October to April.

### Sample Collection

### Collection of Fish Samples

The samples of *H. bidorsalis* used for the study were obtained from three landing sites (Kwalkwalawa station “A”, Hayi station “B” and Kagara-Rima station “C”) labeled as A, B and C respectively. Kwalkwalawa village is surrounded by farm lands and the soil type is sandy loam. Irrigation of crops such as onions, tomatoes and rice take place in the dry season, with rice and other crops cultivation in the rainy season. Domestic activities such as washing of cars, bicycles and clothes takes place at the river bank. At each landing sites, six (6) adult fish (ranging 300g and above), comprising three male and three female were collected every month for two seasons from July to September, 2015 (for rainy season) and November and December, 2015 and January 2016 (for dry season). The fish samples were caught by the local fishermen using gill, clap and trap nets.

### Blood Sample Collection

Before blood collection, the fish surface was carefully wiped-dried with tissue and hand towel was used to hold the fish in position. A very sharp knife was used for head ablation and tranquilized with 150mg L$^{-1}$ solution of tricane methanesulphate (ms-222). Two ml disposable sterile syringe with 21-G needle was inserted at right angle to the surface of the fish and pushed in gently to draw 1ml of blood. The blood sample was quickly transferred into sample bottles coated with anti-coagulant tripotassium salt of ethylene diamine tetra-acetic acid ($K_3EDTA$) in accordance with Whitman (2004) method. The blood sample was immediately taken to haematology Laboratory for haematological investigation.

### Determination of Heamatological Parameters

Determination of heamatological parameters was done using Heamatology autoanalyser Erma particle counter, PCE-210 model. The haematological parameters analysed were; White Blood Cell count (WBC $10^9$/L), Lymphocyte count (LY $10^9$/L), Lymphocyte percent (LY %) Red Blood Cell count (RBC $10^{12}$/L), Haematocrite or PCV (HCT %), Mean Cell Volume (MCV fl), Hemoglobin (Hgb g/dL), Mean cell haemoglobin concentration (MCHC g/dL), Mean cell haemoglobin (MCH pg), Mean platelet volume (MPV fL) all were analyzed according to Jain (1986).

### Statistical Analysis

The data obtained for the haematological parameters of both male and female *H. bidorsalis* were subjected to Analysis of Variance (ANOVA) using SAS (2002). Stat view statistical package. Means were separated with least significant difference (LSD) at 5% confidence limit.

## RESULTS

The results of heamatological parameters for *Heterobranchus bidorsalis* at the three stations were presented in Table 1. The highest value of white blood cell (WBC) was found in station A (67.747 ± 2.941), followed by station B (65.103 ± 3.108) and lastly station C (57.736 ± 4.413). Significant difference ($p < 0.05$) was in station A and C, lymphocytes and lymphocytes percent (LY %) values showed no significant difference between the stations ($P > 0.05$%). Effect on station was also checked on red cells indices, in which all the parameters; RBC, Hgb, HCT, MCV, MCH, MCHC and MPV did not show significant difference ($p < 0.05$) at all the stations (Table 1).

Table 1. Hematological Parameters of *Heterobranchus bidorsalis* Obtained at Three Stations of River Rima, Sokoto.

| Parameters | Station A | Station B | Station C |
|---|---|---|---|
| WBC ($10^9$/L) | 67.747± 2.941$^a$ | 65.103± 3.108$^{ab}$ | 57.736± 4.413$^b$ |
| LY ($10^9$/L) | 47.792 ±3.332$^a$ | 45.664 ± 3.370$^a$ | 43.897 ±4.102$^a$ |
| RBC ($10^{12}$/L) | 2.025 ± 0.087$^a$ | 1.945 ± 0.103$^a$ | 1.875 ± 0.104$^a$ |
| LY (%) | 63.989 ±3.863$^a$ | 64.178 ± 3.875$^a$ | 59.992 ±4.514$^a$ |
| Hgb (g/dl) | 6.775 ± 0.213$^a$ | 6.814 ± 0.251$^a$ | 6.494 ± 0.241$^a$ |
| HCT (%) | 20.794± 0.562$^a$ | 20.511 ± 1.116$^a$ | 20.183 ±1.309$^a$ |
| MCV (fl) | 103.589± 3.686$^a$ | 109.250± 3.055$^a$ | 104.364± 3.041$^a$ |
| MCH (pg) | 35.919 ± 0.908$^a$ | 36.836 ± 1.247$^a$ | 37.811 ±0.835$^a$ |
| MCHC (g/dl) | 34.764 ± 0.777$^a$ | 35.144 ± 1.073$^a$ | 37.242 ± 0.786$^a$ |
| MPV (fl) | 6.550 ± 0.276$^a$ | 6.542 ± 0.280$^a$ | 6.194 ± 0.261$^a$ |

**Footnote**: Means followed by the same letter across the row are not significantly difference at 5% level of significance, using least significance difference (LSD) ($p>0.05$) A= Kwalkwalawa, B= Hayi, C= Kagara-Rima, WBC= White Blood Cells, LY= Lymphocytes, LY %= Lymphocyte's percent, RBC= Red Blood Cells, Hgb= Haemoglobin, HCT= Hematocrit, MCV= Mean Cell Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, MPV= Mean Platelets Volume.

The influence of season on hematological profile of *Heterobranchus bidorsalis* was obtained as shown in Table 2 below. The WBC had the higher mean value in rainy season (65.235 ± 3.537 x $10^9$/L) and the least mean value in dry season (61.822 ± 2.167 x $10^9$/L) but there was no significant difference (P > 0.05%). The effect of season on lymphocytes and LY% was also evaluated. Lymphocytes and LY% had the highest mean values in dry season (49.539 ± 1.963 %), (69.470 ± 2.529 %) respectively with significant difference between the two seasons in LY% only. Red cell indices; RBC, Hgb and HCT showed no significant difference (P > 0.05) also. The highest mean value for RBC was recorded in dry season (2.017 ± 0.081 x $10^{12}$/L) followed by rainy season (1.879 ± 0.079 x $10^{12}$/L), the same observation was recorded for values of Hgb in which highest values were recorded in dry season (6.711 ± 0.184 g/dl), followed by rainy season (6.678 ± 0.200 g/dl). Hematocrit was observed to have highest value in dry season also, (20.533 ± 0.501 %), but all the above parameters show no significant difference (P > 0.05%) statistically. Significant difference was found in mean corpuscular hemoglobin (MCH) with its highest value in dry season (38.265 ± 0.795 pg) followed by least value in rainy season (35.446 ± 0.819 pg). Mean corpuscular hemoglobin concentration (MCHC) also show no significant difference (P > 0.05%) in which highest value was recorded in rainy season 36.354 ± 0.567 g/dl as indicated in Table 2. There was also no much difference in all hematological parameters in terms of gender as shown in Table 3 below.

Table 2. Seasonal Variations in Hematological parameters of *Heterobranchus bidorsalis* at River Rima, Sokoto.

| Parameters | Rainy season | Dry season |
|---|---|---|
| WBC ($10^9$/L) | $65.235 \pm 3.537^a$ | $61.822 \pm 2.167^a$ |
| LY ($10^9$/L) | $42.030 \pm 3.607^a$ | $49.539 \pm 1.963^a$ |
| LY (%) | $55.969 \pm 3.765^a$ | $69.470 \pm 2.529^b$ |
| RBC ($10^{12}$/L) | $1.879 \pm 0.079^a$ | $2.017 \pm 0.081^a$ |
| Hgb (g/dl) | $6.678 \pm 0.200^a$ | $6.711 \pm 0.184^a$ |
| HTC (%) | $20.459 \pm 1.092^a$ | $20.533 \pm 0.501^a$ |
| MCV (fl) | $95.372 \pm 2.724^a$ | $116.092 \pm 1.711^b$ |
| MCH (pg) | $35.446 \pm 0.819^a$ | $38.265 \pm 0.795^b$ |
| MCHC (g/dl) | $36.354 \pm 0.567^a$ | $35.080 \pm 0.868^a$ |
| MPV (fl) | $6.222 \pm 0.255^a$ | $6.635 \pm 0.180^b$ |

**Footnote**: Means followed by different superscript across the rows are statistically significance at 5% level of significance, using least significance difference (LSD). A= Kwalkwalawa, B= Hayi, C= Kagara-Rima, WBC= White Blood Cells, LY= Lymphocytes, LY %= Lymphocyte's percent, RBC= Red Blood Cells, Hgb= Haemoglobin, HCT= Hematocrit, MCV= Mean Cell Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, MPV= Mean Platelets Volume.

Table 3. *Heterobranchus bidorsalis* Hematological Parameters based on Gender at Sampling Stations on River Rima

| Parameters | Station A | | Station B | | Station C | |
|---|---|---|---|---|---|---|
| | Male A | Female A | Male B | Female B | Male C | Female C |
| WBC ($10^9$/L) | 67.411±4.080[ac] | 68.083 ±4.354[a] | 66.622±4.421 [ad] | 63.583±4.468s[ab] | 63.156 ±5.440[a] | 52.317 ±6.866 [b] |
| LY ($10^9$/L) | 50.329±4.058[a] | 45.256±5.337[a] | 46.228 ±4.694[a] | 45.100 ±4.971[a] | 45.817 ±5.796[a] | 41.978 ±5.939[a] |
| LY (%) | 68.500±5.528[a] | 59.478±5.338[a] | 63.350 ±5.010[a] | 65.006 ±6.053[a] | 67.461 ±5.223[a] | 52.522 ±7.076[a] |
| RBC ($10^{12}$/L) | 1.953 ±0.097[a] | 2.097 ±0.145[a] | 2.004 ±0.151[a] | 1.885 ±0.144[a] | 1.935 ±0.173[a] | 1.814 ±0.120[a] |
| Hgb (g/dl) | 6.894 ±0.298[a] | 6.656 ±0.310[a] | 6.939 ±0.345[a] | 6.689 ±0.373[a] | 6.717 ±0.386[a] | 6.272 ±0.291[a] |
| HCT (%) | 21.050±0.597[a] | 20.539±0.967[a] | 19.839 ±1.422[a] | 21.183 ±1.747[a] | 21.850 ±2.381[a] | 18.517 ±1.030[a] |
| MCV (fl) | 105.489±6.13[a] | 101.689±4.23[a] | 105.900 ±4.653[a] | 112.600±3.932[a] | 103.856±4.849[a] | 104.872±3.814[a] |
| MCH (pg) | 37.022±1.056[a] | 34.817±1.461[a] | 34.783 ±1.598[a] | 38.889 ±1.833[a] | 36.844 ±1.241[a] | 38.778 ±1.104[a] |
| MCHC (g/dl) | 34.411 ±1.175[a] | 35.117 ±1.045[a] | 34.228 ±1.145[a] | 36.061 ±1.826[a] | 36.528 ±1.193[a] | 37.956 ±1.030[a] |
| MPV (fl) | 6.556 ±0.000 [a] | 6.544 ±0.000[a] | 6.894 ±0.000[a] | 6.189 ±0.000[a] | 6.533 ±0.000[a] | 5.856 ±0.000[a] |

**Footnote**: Means followed by the same superscript across the row are not superscript across the row are not significantly difference at superscript across the row are not significantly difference at 5% level of significance, using least significance difference (LSD).

## DISCUSSION

Hematological parameters of fish are important as they are linked to fish health (Akinwumi, 2015). The result of RBC count of station A was higher than that of B and C, regarding the differences in the stations which could be due to low dissolved oxygen in station B and C caused by the pollution level of the water. Fish species are impacted with different factors such as pollution, water quality and microorganism in natural habitat (Sheikh and Ahmed, 2016; Seriani *et al*., 2013) they adapt to these adverse conditions by changing their physiological activities. The RBC count of this work for station A 2.025 ± 0.087 ($10^{12}$/L), station B 1.945±0.103 ($10^{12}$/L) and station C 1.875 ± 0.104 ($10^{12}$/L) was higher than the sexed *Clarias batrachus* RBC values 1.77 ± 0.014 ($10^{12}$/L) reported by Maheswaran *et al*. (2008) and lower than that of *Heterobranchus bidorsalis;* male - 5.05 ± 0.17 ($10^{12}$/L) and female- 5.2 ± 0.26 ($10^{12}$/L) as reported by Onyia *et al*. (2013) which could be due to changes in their habitat.

Hemoglobin and Hematocrit are related to the oxygen binding ability and change in both values is related to reactions to stress. Generally, values for these two parameters decrease when a fish is exposed to stress (Diyaware *et al*., 2012; Akinwumi, 2015; Sabullah *et al*., 2013). Hgb and HCT count for both the stations were not significant (Table 1), which may be likely because the fish was brought early in the morning before they undergo any stress. Hgb value in the present study ranged from 6.494 to 6.814 g/dl. Similar results were obtained by Rambhaskar and Srinivasa (Rambhaskar and Srinivasa, 1987). Hgb values for this study were lower than those recorded by Diyawere *et al.* (2012), 9.00- 10.33 g/dl. RBC and Hgb concentration were observed to increase with length and age of the fishes.

The level of White Blood Cell (WBC) recorded for the three stations (A, B and C) during this research (Table 1) were lower than those reported by Divaware *et al.* (2012) for Juveniles and their pure lines in North Eastern Nigeria (181.53 ± 3.57- 193.70 ± 3.26 × $10^9$/L), the low level of WBC in this work could be due to less environmental stress, in the stations when compared to drastic fluctuations in the weather conditions of the North Western Nigeria. Almost all the values of heamatological parameters except for WBC (Table 2), were found to be higher in dry season than rainy season, the least value of WBC observed in dry season could be attributed to the least concentration of heavy metals in the fish organs ref. The WBCs are the defensive cells of the body, their levels have implication for immune responses and the ability of the animal to fight infections (Divaware *et al.*, 2012). Species with higher levels of WBC will be able to fight infection more effectively than other species (Divaware *et al.*, 2012).

The MCH recorded for these studies in respect of the three stations A, B & C (Table 1) were higher than earlier reports 24.24 pg *C. gariepinus* juveniles by Omitoyin (2006), 33.10pg Ochang *et al*. (2007) and 21.86 and 18.83 for male and female *H. bidorsali* (Onyia *et al*., 2013). This explains that the red cell enlargement is probably due to nutritional deficiency of folic acid or vitamin B12, in another word higher MCH indicates good volume of hemoglobin which indicates good oxygen transport.

The MCV is an estimate of the volume of red blood cells, the mean MVC values for this study (103.589, 109.250 and 104.364 fl) for the three stations A, B, C respectively are lower than 240.18 fl recorded for juvenile hybrid African catfish reported by Kori-Siakpere and Ubugo, (2008) for juvenile *Heteroclarias* and 200.93 fl for *C. gariepinus* fingerlings (Gbore *et al*., 2006). It is higher than 96.62 fl for *C. gariepinus* fingerlings (Ochang *et al*., 2007). However, the high value of the MCV could be due to the high concentration of hemoglobin in the red blood cell. The study also revealed that the higher Lymphocytes observed for both effect on station (Table 1) and effect on season (Table 2) suggested immunity for this fish, compared to other results recorded for juveniles *C. gariepinus* 33.00% Adeyemo (2007) and hence it has the potentials of thriving well in the harsh conditions of North Western Nigeria.

The current study also shows that the mean corpuscular hemoglobin concentration (MCHC) values varied between 36.354 and 35.080 g/dl for rainy and dry season which is higher than that of *C. gariepinus* with 33.33 and 33.33 g/dl for male and female reported by Omitoyin (2006) and it is lower than the result obtained by Onyia *et al*. (2013) which has 196.51 and 196.29 g/dl for male and female, the differences could be due to differences in environmental factors.

## CONCLUSION

The haematological parameters of *Heterobranchus bidorsalis* recorded in the present study for the two seasons may serve as reference values for respective *H. bidorsalis* in River Rima of Sokoto, Sokoto state, Nigeria. It also shows how healthy the species is in terms of haematological status.

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