



## COMPARATIVE STUDIES OF HEMATOLOGICAL PROFILES OF CULTURED AND WILD CLARIID CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822) IN SOUTH-EAST NIGERIA

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**ABSTRACT:** *The study compared the hematological profiles of cultured and wild *Clarias gariepinus* from selected fish farms and rivers in Southeast Nigeria. Wild fish were collected from Anambra, Imo, Otamiri and Cross Rivers while farmed fish were sampled from Awka, Umuahia, Owerri and Abakaliki farms. 15 *C. gariepinus* were collected monthly from each of the rivers and farms for 12 months (January – December 2023). The overall mean weight of wild fish and farmed fish examined were  $339.14 \pm 2.84$  and  $345.87 \pm 3.06$ g respectively. There were no significant differences ( $p < 0.05$ ) in packed cell volume between the wild and cultured fish. Hemoglobin, red blood cell count, mean cell hemoglobin, mean cell hemoglobin concentration, white blood cell and monocytes counts in the wild population were significantly higher ( $p < 0.05$ ) than that in the cultured type. Furthermore, mean cell volume, neutrophils, lymphocytes and platelet count in cultured individuals were significantly lower ( $p < 0.05$ ) than that in wild catfish. The introduction of regular checks of the blood profiles in wild and farmed fish is highly recommended, since blood collection for analytical objectives need not kill the fish and can be applied repeatedly to the same individuals.*

**KEYWORDS:** Cultured and wild fish, Hematology, *Clarias gariepinus*, South-East Nigeria, RBC, WBC.



## INTRODUCTION

Hematological parameters are valuable tools for the monitoring of fish health, detecting illness and following the progress of disease and response to therapy (Jawad *et al.*, 2004). The use of hematological parameters is acquiring acceptance worldwide, as a tool in the management of fish farms and they are affected by many endogenous and exogenous factors such as water temperature, reproduction cycle and metabolic rate (Svoboda, 2001; Kavadias *et al.*, 2003; Bayir, 2005). Since hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters, and since they respond quickly to changes in environmental conditions, they have been widely used for the description of healthy fish, monitoring stress responses and predicting systematic relationships and the physiological adaptations of animals (Atamanalp & Yanik, 2003). Furthermore, since a change or lack of change in the blood picture is a fundamental characteristic of practically every physiologic or pathologic state, hematological findings are among the most valuable and most generally useful of all laboratory diagnostic aid (Satheeshkumar *et al.*, 2012). The simplicity of most blood sampling techniques accounts for the widespread use of blood studies as a means of assessing the state of health of fish. Regular monitoring of the hematological parameters of farmed fish can be used to enhance fish production (Adebayo *et al.*, 2007).

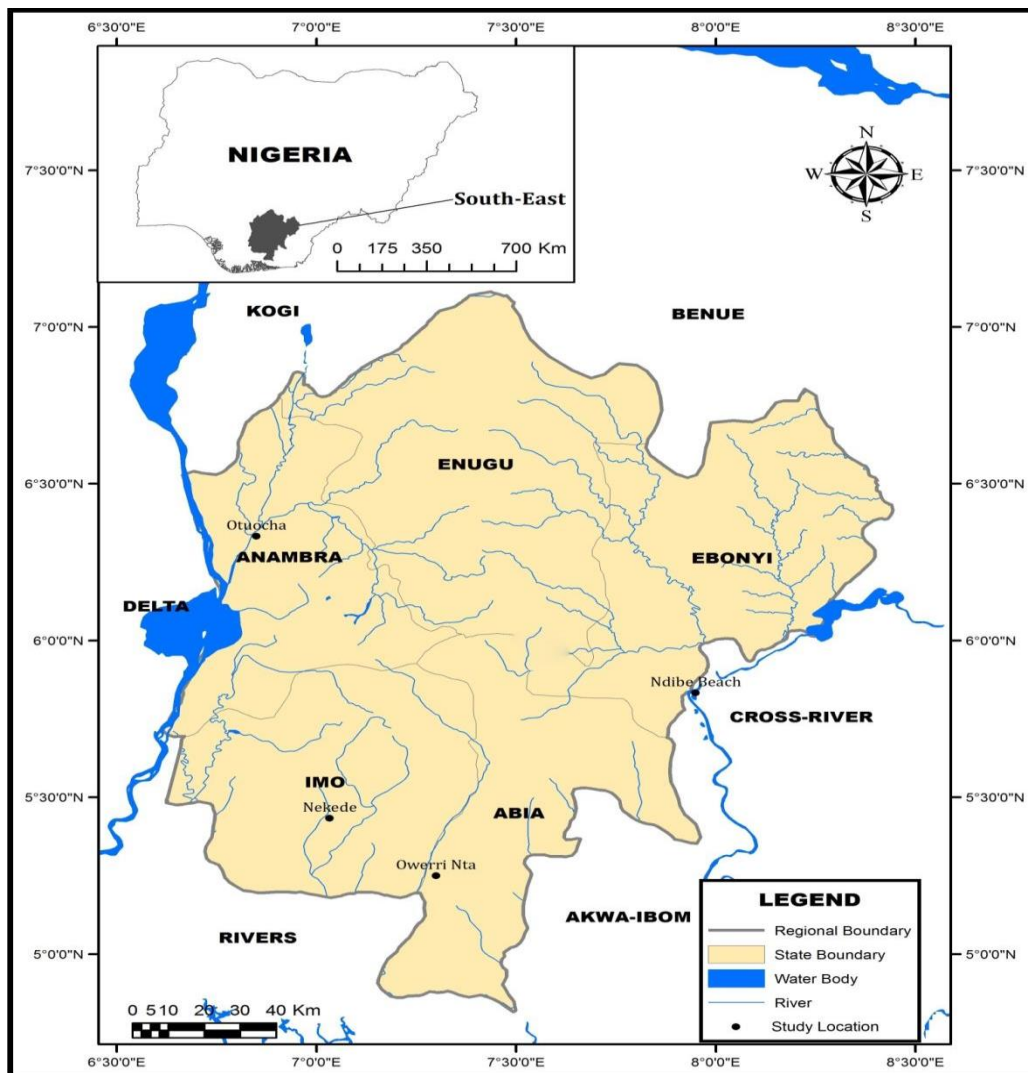
*Clarias gariepinus* is an indigenous species in Africa. It naturally inhabits tropical swamps, lakes, rivers and floodplains, some of which are subject to seasonal drying (Idodo-Umeh, 2003). In Nigeria, *C. gariepinus* is an indigenous fish occurring in freshwater throughout the country. It is estimated that apart from tilapia, *C. gariepinus* is the most abundant cultivated fish species in Nigeria (Erhunmwunse & Aineura, 2013). The fish is extremely popular on account of its tasty flesh, unparalleled hardness, its rapid growth and high marketability (Idodo-Umeh, 2003).

Data on hematology of the clariid catfish, *C. gariepinus* is of interest due to its commercial significance, economic importance and extensive consumption as a food source, especially in the South Eastern part of Nigeria. Therefore, the present study is crucial to fill the gap in the current knowledge on the hematology of farm-raised and wild *C. gariepinus* in Southeast Nigeria, in order to provide baseline information for stock management and assessments.

## MATERIALS AND METHODS

### Study Area

The experimental sites comprised four major rivers and four fish farms in the South-East geopolitical zone of Nigeria. The map of South-East Nigeria showing the study locations is presented in Figure 1.



**Figure 1:** Map of South-East Nigeria showing the study locations

Anambra River lies between latitude  $6^{\circ} 10'$  and  $7^{\circ} 20'N$ , longitude  $6^{\circ} 35'$  and  $7^{\circ} 40'E$ , east of the Niger (Awachie & Hare, 1977). The river has a southward course crossing the Kogi/Enugu State boundary, and then meanders through Ogurugu to Otuocha from where it flows down to its confluence with the Niger at Onitsha (Odo *et al.*, 2007). The main river channel is approximately 207.4 km to 210 km in length (Azugo, 1978).

The Imo River lies between latitude  $5^{\circ} 00'$  to  $5^{\circ} 45'N$  and longitude  $6^{\circ} 50'$  to  $7^{\circ} 45'E$ , and covers an area of about  $2256 \text{ km}^2$  (Ekwe *et al.*, 2006). The Imo River drains three states, namely: Imo State, Abia State and Rivers State (Okoro *et al.*, 2014). It takes its course from the Okigwe/Awka uplands and runs through the area underlain by the Imo Shale and the coastal plain sands down to the coast in Rivers State (Ibe *et al.*, 1991). The Imo River at Owerri-Nta is located within longitude  $7^{\circ} 17'E$  and latitude  $5^{\circ} 18'N$  (Ukagwu *et al.*, 2012).

Otamiri River lies between latitude  $5^{\circ} 30'$  to  $7^{\circ} 30'N$  and longitude  $5^{\circ} 39'$  to  $5^{\circ} 42'E$  (Agbabiaka, 2012). The Otamiri watershed covers an approximate area of  $1719.25 \text{ km}^2$  (Amangabara, 2015) with annual rainfall of 2250 – 2500 mm (Umunnakwe & Nnaji, 2011). Otamiri River starts as a first order stream at its source at Egbu, captures Nworie River at Nekede (Anyanwu, 2009),



and flows for about 30 km to confluence with the Oraminiukwa River at Emeabiam (Ofulume & Amadi, 2011).

Cross River, a floodplain river located in the South Eastern part of Nigeria lies between latitudes 5° 04' to 6° 36'N and longitudes 7° 27' to 8° 48'E (Nganje *et al.*, 2015). The study area is the lower part of the Cross River Basin located at Ndibe in Afikpo North Local Government Area of Ebonyi State. It is about 5 km eastwards away from Afikpo main town. The Cross River Basin forms the border between Ebonyi State and Cross River State.

*C. gariepinus* were obtained from Dozzy Farms, Awka, Pioneer Fish Farms, Umuahia, TonyPhil Fisheries and Integrated Farms, Owerri, and Endurance Integrated Farms, Abakaliki.

### Fish Sampling

15 fish samples (*C. gariepinus*) were collected monthly for 12 months (January – December 2023) from four rivers, namely: Anambra River at Otuocha, Imo River at Owerri-Nta, Otamiri River at Nekede, Cross River at Afikpo; and four reputable fish farms at Awka, Umuahia, Owerri and Abakaliki, all in South-East Nigeria. The size of the fishes ranged from 223.3 to 570.7 g in weight and 30.0 to 48.7 cm in body length. The overall mean weight of wild fish and farmed fish examined were  $339.14 \pm 2.84$  and  $345.87 \pm 3.06$ g respectively. Fish from the rivers were obtained using gill nets, scoop nets, fishing baskets, and cast nets from hired artisanal fishermen. They were purchased from fishers from the landing site in the mornings at about 0800 hrs. Farmed fish in the same cohort stocked in concrete ponds were caught using scoop nets. They were acclimatized in large plastic tanks. A total number of 1440 fish were sampled from the wild and farms.

### Blood Sampling

Blood samples were collected from the fish's caudal artery using 5 ml plastic disposable syringe and needle treated with anticoagulant into glass tubes containing EDTA because it allows the best preservation of cellular components and morphology of blood cells. The collected blood samples were transferred into sample bottles and processed within 2 hours for blood indices estimation.

### Haematological Examination

Hemoglobin estimation (Hb) was determined using cyano-methemoglobin method as described by Sood (2006). Exactly 5 ml of Drabkin's solution was placed in clean test tubes, followed by 0.02 ml of blood sample. The solution was mixed thoroughly and allowed to stand for 20 minutes at room temperature. The absorbance of the solution was read against reagent blank using a spectrophotometer at 540 nm and the hemoglobin concentration calculated from the reference curve.

Haematocrit (packed cell volume) estimation was determined by the microhematocrit method as described by Coles (1986). Packed cell volume was calculated using the formula:

$$PCV (\%) = \frac{\text{Height of red cell (mm)}}{\text{Total height (mm)}} \times 100$$

Red blood cell (RBC) count was estimated using the method of Baker *et al.* (1975). The number of cells counted for each sample was calculated using the formula:



$$RBC (mm^3) = \frac{\text{cell counted} \times \text{dilution factor}}{\text{volume counted in } mm^3}$$

Red blood cell indices *i.e.* mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated from the red blood cell count, hemoglobin (Hb) content and packed cell volume (PCV) according to Baker *et al.* (2001) using the formula:

$$MCV (\mu^3) = \frac{PCV (\%) \times 10}{RBC /mm^3 \text{ in millions}}$$

$$MCH (pg Hb) = \frac{Hb g /100 ml \times 10}{RBC /mm^3 \text{ in millions}}$$

$$MCHC [\%(g/100 ml)] = \frac{Hb g /100 ml \times 100}{PCV}$$

White blood cell (WBC) count was determined using the method described by Baker *et al.* (1975). The number of cells counted for each sample was calculated using the formula:

$$WBC (mm^3) = \frac{\text{cell counted} \times \text{dilution factor}}{\text{volume counted in } mm^3}$$

Differential white cell count was determined from the total white blood cell (WBC) count. The number of differential WBC was counted in 100 fields and recorded.

Platelet count was determined using the method of Cheesbrough (2005). The number of platelets in 1 liter of blood *i.e.* the actual number of platelets counted  $\times 10^9$  was calculated as follows:

$$\text{Platelet count per liter} = \frac{\text{cells counted} \times 20^* \times 10^6}{0.2^T \times 0.1^\omega}$$

where: \* = 1 in 20 dilution of blood,  $T = 0.2 \text{ mm}^2$  area counted,  $\omega = 0.1 \text{ mm}$  depth of chamber

### Statistical Analysis

Data was analyzed in R version 4.2.0 (R Core Team, 2022). Generalized linear model (GLM) was fitted to estimate disparities in parameters of interest between wild and farmed *C. gariepinus*. This was followed by Analysis of Variance (ANOVA). All the models were full factorial. The four-way ANOVA was followed by Tukey HSD *post-Hoc* test and adjusted p-values reported. The level of significance was set at 95% probability ( $p < 0.05$ ).

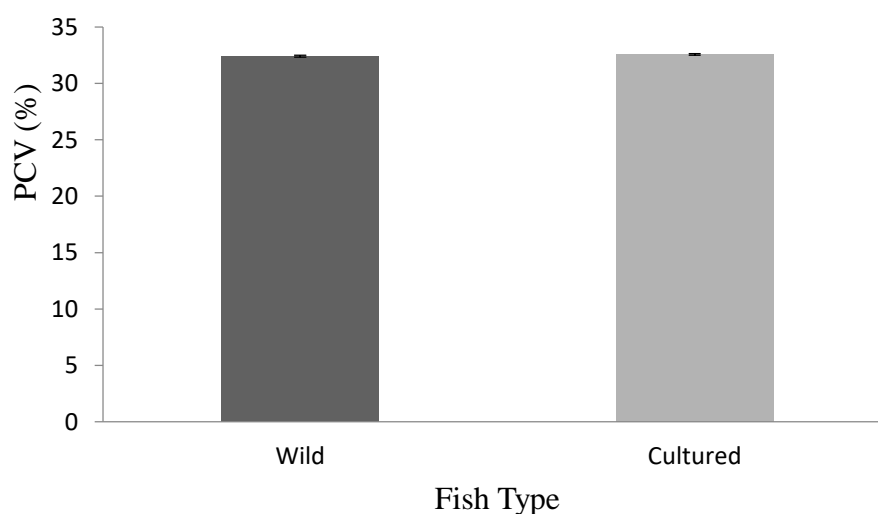
## RESULTS

**Table 1: Hematological parameters of wild and cultured *Clarias gariepinus* in South-East Nigeria**

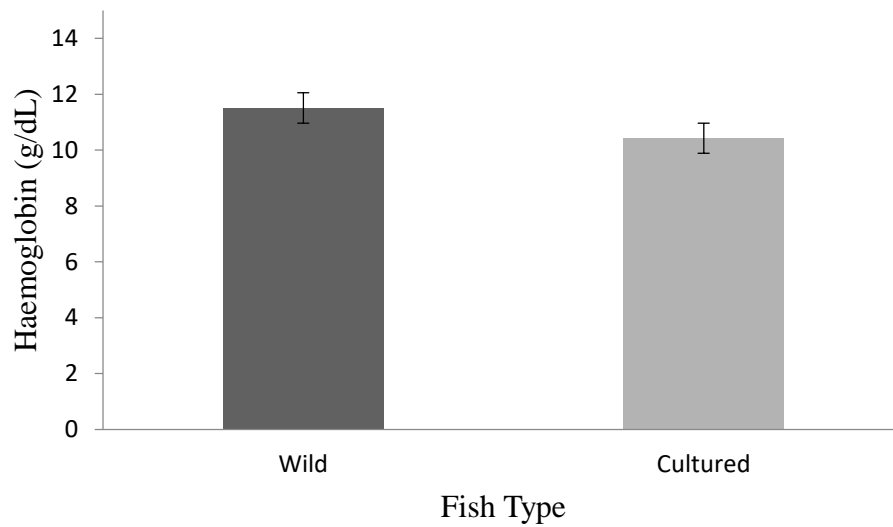
Parameters	Wild	Cultured	P-value
Hemoglobin (g/dL)	11.51 ± 0.08	10.43 ± 0.05	< 0.0001
Packed cell volume (%)	32.41 ± 0.17	32.57 ± 0.17	0.393
Red blood cell (x 10 <sup>12</sup> /L)	5.72 ± 0.04	5.59 ± 0.04	0.017
Mean cell volume (fL)	58.53 ± 0.51	59.98 ± 0.50	0.026
Mean cell hemoglobin (pg)	20.79 ± 0.20	19.17 ± 0.15	< 0.0001
Mean cell hemoglobin concentration (g/dL)	35.88 ± 0.24	32.59 ± 0.22	< 0.0001
White blood cell (x 10 <sup>9</sup> /L)	20.98 ± 0.09	20.40 ± 0.10	< 0.0001
Neutrophils (%)	33.50 ± 0.07	33.67 ± 0.07	0.006
Lymphocytes (%)	60.53 ± 0.07	60.70 ± 0.06	0.0008
Monocytes (%)	2.54 ± 0.01	2.48 ± 0.01	< 0.0001
Platelet count (x 10 <sup>9</sup> /L)	115.46 ± 0.42	117.66 ± 0.46	0.0002

Values as mean ± standard error.

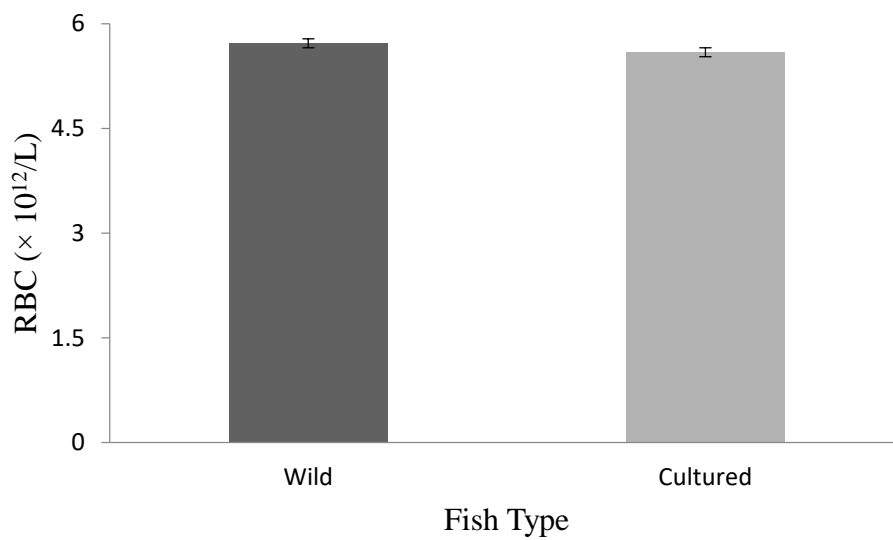
The mean values, standard errors and p-values of the haematological parameters are summarized in Table 1. Statistical analysis revealed non-significant difference ( $p < 0.05$ ) in packed cell volume between the wild and cultured fish (Figure 2). The PCV of the fish ranged from 30.23 to 34.82%, with an average of  $32.41 \pm 0.17\%$  in the wild fish and ranged from 31.20 to 33.58%, with an average of  $32.57 \pm 0.17\%$ , in the cultured type. There were significant differences ( $p < 0.05$ ) in the overall mean of the other hematological indices examined between the two ecotypes. Hemoglobin ( $11.51 \pm 0.08$  g/dL) (Figure 3), RBC ( $5.72 \pm 0.04 \times 10^{12}/L$ ) (Figure 4), MCH ( $20.79 \pm 0.20$  pg), MCHC ( $35.88 \pm 0.24$  g/dL), WBC ( $20.98 \pm 0.09 \times 10^9/L$ ) (Figure 5) and monocytes count ( $2.54 \pm 0.01\%$ ) in the wild population were significantly higher ( $p < 0.05$ ) than that in the cultured type. Conversely, the MCV ( $58.53 \pm 0.51$  fL), neutrophils ( $33.50 \pm 0.07\%$ ), lymphocytes ( $60.53 \pm 0.07\%$ ) and platelet count ( $115.46 \pm 0.42 \times 10^9/L$ ) (Figure 6) in cultured individuals were significantly lower ( $p < 0.05$ ) than that in wild catfish.



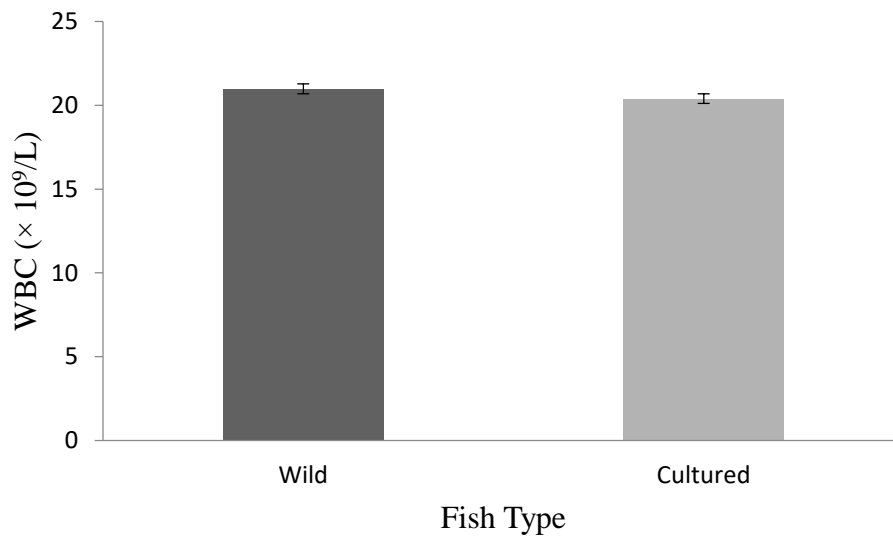
**Figure 2: Packed cell volume (%) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



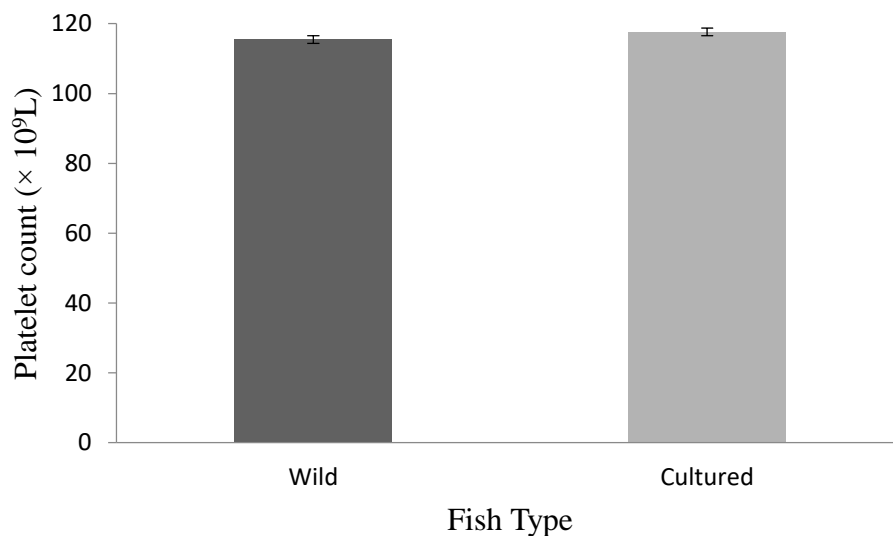
**Figure 3: Hemoglobin concentration (g/dL) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



**Figure 4: Red blood cell count ( $\times 10^{12}/L$ ) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



**Figure 5: White blood cell count ( $\times 10^9/L$ ) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



**Figure 6: Platelet count ( $\times 10^9/L$ ) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**

The PCV of fish in each of the rivers and farms was not different in magnitude between the dry and rainy season. However, overall PCV of fish was significantly higher in dry than rainy season ( $p < 0.05$ ) (Figure 7). The fish from Imo Farm had the highest PCV while those from Abia farm had the least. The Hb of fish from Otamiri River and Cross River were significantly higher compared to fish from the other water bodies. Fish from Anambra Farm and Imo Farm





had the least Hb. There were no seasonal differences in Hb of fish in each of the river and farm samples (Figure 8).

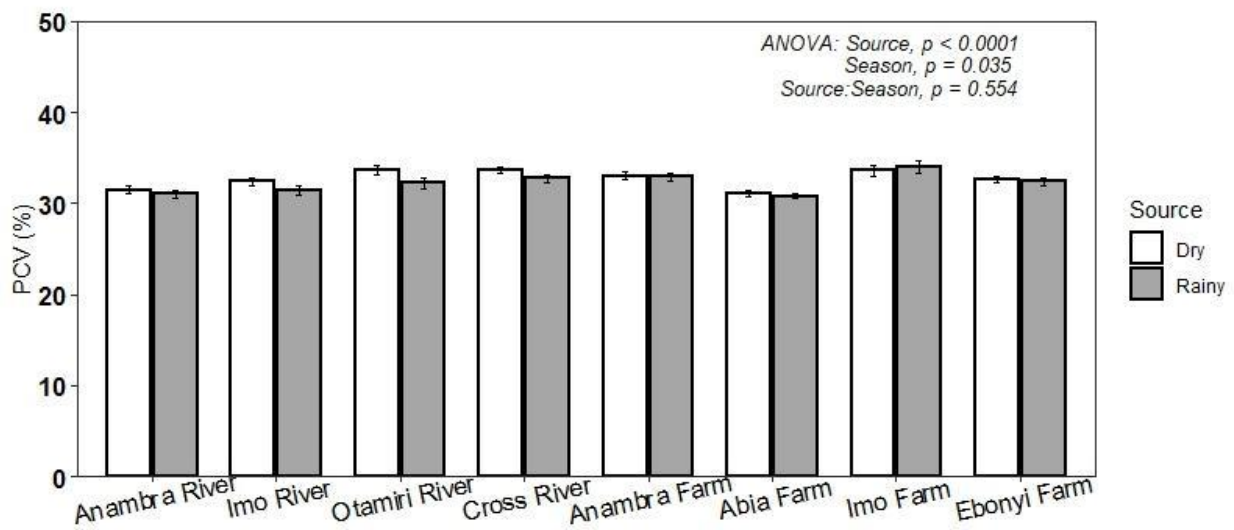
The total RBC of wild fish compared to farmed fish was significantly higher ( $p < 0.05$ ). Fish in Imo River had higher RBC counts than their counterparts in Ebonyi Farm ( $p < 0.05$ ). RBC of the fish was not different between the dry and rainy season within each river and farm and overall (Figure 9).

The MCV of the fish was not different between the dry and rainy season within each river and farm and overall. Imo Farm and Ebonyi farmed fish had the highest MCV while Imo River and Abia Farm had the least. The difference in MCV between those with the highest levels and those with the lowest were significant. There was a wide variation in MCH of fish across the water bodies sampled. Fish in Cross River had the highest MCH while those from Anambra Farm had the least. There was no difference in dry and rainy season MCHC of fish within each of the four rivers and four farms sampled. The MCHC of fish from rivers was higher than the MCHC of fish from farms. Fish from Cross River had the highest MCHC, followed by Otamiri River and then Anambra River and Imo River.

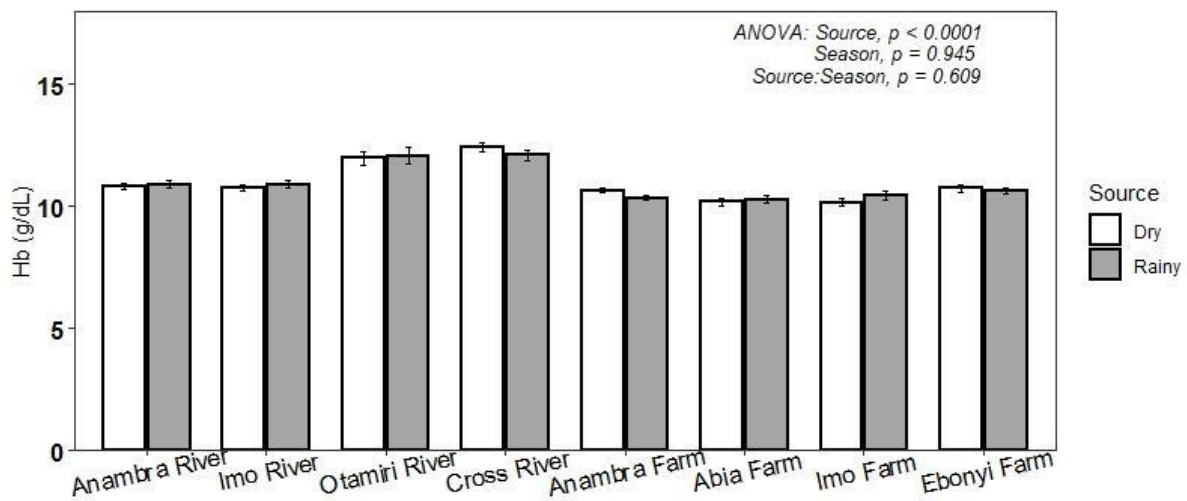
Mean WBC counts were highest in fish from Anambra River and Abia Farm and lowest in those from Imo Farm and Ebonyi Farm. The difference between the highest and lowest WBC counts were significant. Also, WBC counts in fish from Imo River, Otamiri River, Cross River and Anambra Farm were not different from one another, but were significantly low compared to WBC of fish in Anambra River and Abia Farm. There were no statistically significant differences in WBC between rainy and dry seasons within each river and farm (Figure 10).

There were significant monthly disparities in neutrophils counts between wild and farmed fish. Apart from April and November, the neutrophil counts were significantly different between wild and farmed fish from January to December. Neutrophil counts in fish from the four rivers (Anambra River, Imo River, Otamiri River and Cross River) were significantly higher in the rainy season unlike the farms where there were no seasonal differences. Rainy season neutrophil counts in fish were significantly higher overall.

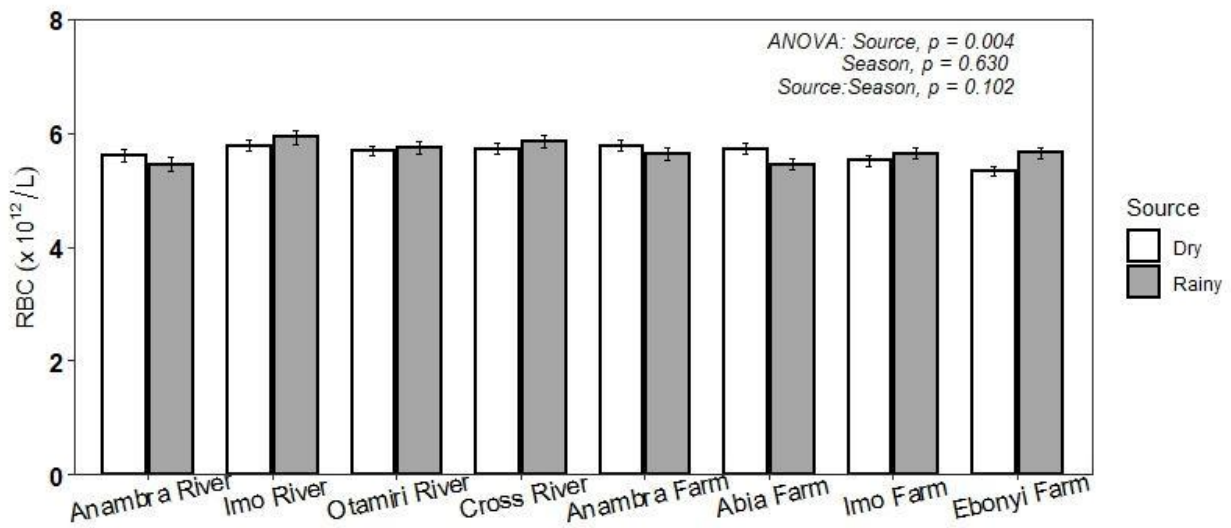
The mean lymphocyte counts were significantly different ( $p < 0.05$ ) between wild and farmed fish in all the months from January to December, except April and November. However, fish in the four rivers sampled, had significantly higher lymphocyte counts during the rainy than the dry season. In the months of March, April, May, September, October, November and December there were significant differences between wild and farmed fish in the values of monocyte counts. The monocyte counts of fish in each of the rivers and farms were not different between dry and rainy seasons. Fish in Otamiri River had the least platelet count followed by those in Imo River. Mean platelet counts in fish from Anambra River, Cross River, Anambra Farm, Abia Farm, Imo Farm and Ebonyi Farm were significantly high compared to mean platelet count in fish from Otamiri River. There were statistically significant differences in the farmed and wild fish except in January, June, July and October, the other months had similar magnitudes of platelet counts (Figure 11).



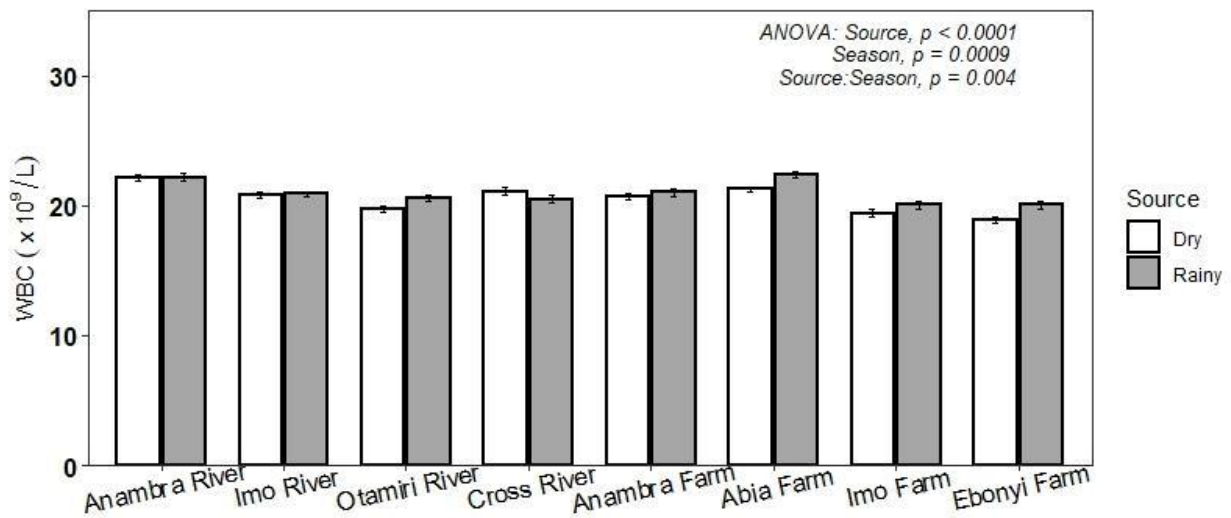
**Figure 7: Seasonal packed cell volume (%) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



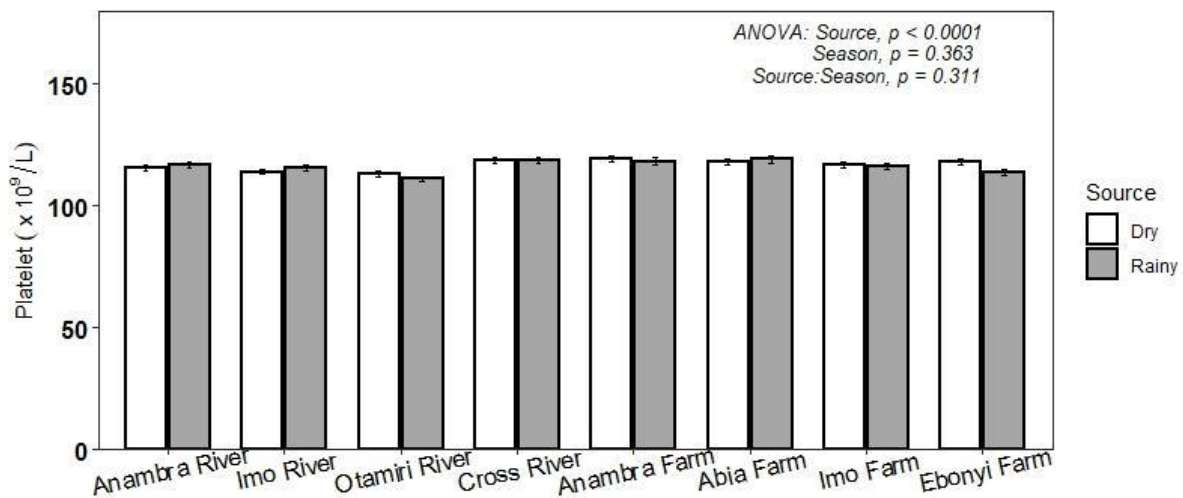
**Figure 8: Seasonal hemoglobin concentration (g/dL) of wild and cultured *Clarias gariepinus* in Southeast, Nigeria.**



**Figure 9: Seasonal red blood cell count (x 10<sup>12</sup>/L) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



**Figure 10: Seasonal white blood cell count (x 10<sup>9</sup>/L) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**



**Figure 11: Seasonal platelet count (x 10<sup>9</sup>/L) of wild and cultured *Clarias gariepinus* in South-East, Nigeria**

## DISCUSSION

Analysis of blood indices has proven to be a valuable approach for analyzing the health status of animals as these indices provide reliable information on metabolic disorders, deficiencies, and chronic stress status before they are present in a clinical setting (Bahmani *et al.*, 2001). Exogenous factors such as management (Svobodova *et al.*, 2008), diseases (Chen *et al.*, 2005), and stress always induce major changes in blood composition.

Erythrocytes are the dominant cell type in the blood of a vast majority of fish species; it is widely accepted that fishes are like most other vertebrates. The high erythrocyte number was associated with fast movement, omnivorous nature, and high activity with streamlined bodies (Rambhaskar & Srinivasa Rao, 1986). However, slight differences in the values of erythrocyte volumes between wild and farmed *Clarias gariepinus* ( $5.72 \pm 0.04$  and  $5.59 \pm 0.04$ ) were observed in this study, suggesting that, although elevated RBC counts and HB concentrations are responses to higher metabolic demand, these have no impact on erythrocyte volume. RBC and HB concentration tend to increase with length and age (Das, 1965). The indifferences observed between the wild and farmed fish in RBC, Hb, PCV, WBC, erythrocytic indices (MCV, MCH, MCHC) and leukocytes differential counts signify the fish's ability to survive in different environments due to its hardiness, without compromising their physiological functions such as oxygen transportation to vital organs, the capability to defend the body against foreign materials, and health status negatively. The increased number of RBC in the wild fish indicates oxygen demand to meet higher oxygen requirements at higher metabolic rates (Engel & Davis, 1964). This is in contrast with Zhou *et al.* (2009) who reported higher values for RBC in cultured *Misgurnus anguillicaudatus* than in wild fish. Furthermore, the RBC, Hb, and PCV values recorded for the wild and farmed fish were within the range reported by other researchers for *C. gariepinus* (Taufek *et al.*, 2016; Okore *et al.*, 2016; Adeoye *et al.*, 2020; Fawole *et al.*, 2020). However, lower RBC values were recorded by Satheeshkumar *et*



*al.* (2010a) for *Lates calcarifer*, Satheeshkumar *et al.* (2010b) for *Arius subrostratus*, and Bindu (2011) for *Etrophus maculatus*. High RBC counts perhaps lessen the requirement for a large number of WBC. Since erythrocyte characteristics partly determine the efficiency of oxygen transport from respiratory systems to tissues (Nespolo & Rosenmann, 2002), increments in their number could be related to the high respiratory demand in intensive living conditions (Shen *et al.*, 1991). Nevertheless, other factors, such as food quality, dissolved oxygen concentrations and regular management also strongly influence the blood attributes (Xu & Cao, 1989; Domezain *et al.*, 1997; Bahmani *et al.*, 2001).

The results indicated that the mean hemoglobin values of the wild and farmed catfish were still in the range of normal for catfish. Normal catfish (*Clarias* sp.) hemoglobin concentrations range from 10 – 14 g% (Oluah *et al.*, 2020). HB functions to bind oxygen, which will then be used for catabolism to produce energy. The ability to bind oxygen in the blood depends on the amount of hemoglobin (Alamanda *et al.*, 2007). Decreased blood HB may be a consequence of changes in the numbers of circulating erythrocytes and indicates abnormalities in fish health (Yanuhar *et al.*, 2021). Low feed protein content reduces the hemoglobin content thereby causing infection.

Hematocrit is used to define the ratio of erythrocytes to plasma. The range of 30 - 45% is considered as normal for fish in general (Adams *et al.*, 1993). Hematocrit level was found in the range between 30.23% and 34.82%. Blaxhall and Daisely (1973) have reported the possibility of using HCT as a tool in aquaculture and fishery management for checking the anemic condition. Fish HCT values were usually between 20% and 35% and rarely attained >50% (Clark *et al.*, 1976). Differences in HCT level between this and previous studies in *C. gariepinus* may be due to age/body size differences and environmental factors such as salinity. Yekeen and Fawole (2011) and Al-Deghayem *et al.* (2017) recorded lower haematocrit values for *C. gariepinus*.

The overall mean corpuscular volume (MCV) of farmed fish was higher than that of wild fish, while mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of wild fish was higher than that of farmed fish. This may be due to differences in the overall oxygen consumption rates and swimming performance in the different environments. Adeoye *et al.* (2020) recorded similar MCHC values and increased values for MCV and MCH.

In fish, as in mammals, blood cells including WBC are frequently used as indicators of health status in fish because WBC are key components of innate immune defense and leukocytes are involved in the regulation of immunological function in the organism (Duthie & Tort, 1985; Gallardo *et al.*, 2003; Ballarin *et al.*, 2004). It seems that salinity exposure can stimulate the production of leukocytes, especially lymphocytes (Satheeshkumar *et al.*, 2010a). However, these changes in blood parameters can be different among species based on genetic makeup, life history, nutritional status, and the fishes' environment (Satheeshkumar *et al.*, 2010b). The WBC count of wild fish was higher than that of farmed fish. This is in consonance with the report of Zhou *et al.* (2009) on cultured and wild Dojo loach, *Misgurnus anguillicaudatus*. Fawole *et al.* (2020) reported similar WBC values for *C. gariepinus*. There were differences between the differential white cell counts. Similar values for the differentials were recorded by Adeoye *et al.* (2020) and Fawole *et al.* (2020).



## CONCLUSION AND RECOMMENDATION

Changes in observed values of these parameters may reflect the responses of animals to changes in their environment. The results of this research provide a contribution to the knowledge of the characteristics of hematological parameters of the African catfish, *Clarias gariepinus*. These studies on fishes have assumed greater significance due to the increasing emphasis on pisciculture and greater awareness of the pollution of the aquatic ecosystem. The introduction of regular checks of the blood profiles in wild and farmed fish is highly recommended, since blood collection for analytical objectives need not kill the fish and can be applied repeatedly to the same individuals. The authors are grateful to the management and staff of the Department of Zoology, Nnamdi Azikiwe University, Awka, Nigeria and University of Nigeria, Nsukka, for access to the laboratory facility.

**Availability of data and materials:** The datasets are available on request from the corresponding author.

**Declarations Ethical approval:** The handling of the fish samples were in accordance with the ARRIVE Guidelines 2.0 and also in line with the principles of laboratory animal care as put in place by the ethical committee on the use of experimental animals in the Department of Zoology and Environmental Biology, University of Nigeria Nsukka, Nigeria.

**Consent for publication:** All authors approved for the publication of the manuscript if accepted.

**Competing interests:** The authors declare no competing interests.

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