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Abstract

Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly via the food chain which probably may cause variation in the way these pollutants affect different trophic level species. Blood parameters have been recognized as valuable tools for monitoring fish health. Haematological and serum biochemical parameters were studied and compared in different trophic level fish species of *Parachanna obscura* (carnivores), *Clarias gariepinus* (omnivores) and *Oreochromis niloticus* (herbivores). Blood parameter revealed low PCV in *Oreochreomis niloticus* (16.70±1.15%) which significantly varies (P<0.05) with value (23.40±0.58% and 21.33±1.76%) recorded for *Clarias gariepinus* and *Parachanna obscura* respectively. The highest values of Hb (7.90g/dl) were obtained in *Clarias gariepinus*, this also reflected on white blood cell and red blood cell count. There were significant differences in the values observed in MCH, MCHC and MCV for species. Most biochemical parameter increases along the feeding habit from carnivorous to omnivorous *i.e. Parachanna obscura* > *Clarias gariepinus* >*Oreochromis niloticus*. Based on these results, it appears that there are variations in the blood profile of fish as regard their feeding habit as well as physiological effects of anthropogenic activities on fish.

1. Introduction

Aquatic system are the ultimate sinks of both natural and anthropogenic inputs of contaminants into the environment which over time can have serious consequences for the aquatic wildlife that might not become apparent until changes occur at the population or ecosystem level **(Khallaf *et al.* 2010)**. Aquatic organisms, including fish, accumulate pollutants directly from contaminated water indirectly via the food chain **(Sasaki *et al.*, 1997)** which probably may cause variation in the way these pollutants affect different trophic levels species. Fish like any other aquatic organism live in direct contact with the aquatic environment where some changes are rapidly reflected as measurable physiological and pathological alterations in stressed fish **(Seth and Saxena, 2003).** The analysis of fish blood indices is a valuable guide to assess the condition of aquatic organisms in response to stress, pollutants and nutrition as well as ecological and physiological conditions.

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Major changes occur in the fish blood compositions such as hormone levels, protein, sugar, cholesterol and other basis components could be indicative of response of fish to various pollutants accumulate through the food chain.

The study of the physiological and haematological characteristics of cultured fish species is an important tool in the development of aquaculture system, particularly in regard to the use of detection of health from diseases or stressed animal **(Ranzani – Paiva et al., 2000; O'Neal and Weirich, 2001)**.

The analysis of blood indices has proven to be valuable approaches for analysing the health status of farmed animals as these indices provide reliable information on metabolic disorders deficiencies and chronic stress status before they are present in clinical setting **(Bahmani *et al.*, 2001)**. Blood biochemistry parameters can also be used to detect the health of fish **(De Pedro *et al.*, 2005)**. The increasing spates of water pollution have continued to be a major problem in Nigeria and other developing countries **(Adelegan, 2008)**. **Ayodele and Abubakar (2001) and Sani (2011)** have reported the pollution of Jebba dam, Nigeria. However, there is dearth of information on the effects of such pollution on the haematological and biochemical profile of the fish species in the dam. Haematology assessment of wild fish is an important tool in evaluating fish health. They can be induced by the presence of pollutants and factors such as temperature, salinity, PH, dissolved oxygen concentration carbon dioxide and inadequate management **(Ranzani-Paiva and Silva-Souza, 2004)**. Blood tissue reflected physical and chemical changes occurring in organism therefore detailed information can be obtained on general metabolism and physiological status of fish indifferent grouping age and habitat **(Kocabatmazve and Ekingen, 1978).**

During recent years considerable attention has been focused on the fates of metals and their derivatives in the aquatic environment. Human activities and increased use of metal containing fertilizers in agriculture could lead to continued rise in the concentration of metal pollutants in fresh water reservoirs as result of water runoff, thereby representing the greatest hazard to human consumers of fish **(Gutenman *et al.*, 1988).** Environmental pollution has become one of the most important problems in the world **(Chandran *et al.*, 2005)**. Exogenous factors, such as management (**Svobodova *et al.*, 2008)**, pollution **(Gaber *et al.*, 2013)**, diseases **(Chen *et al.*, 2005)** and stress **(Cnaani *et al.*, 2004)**, always induced major changes in fish health while basic ecological factors such as trophic level also have a direct influence on certain blood indices. In recent times, Upper Jebba Basin has been subjected to various forms of degradation due to pollution **(Adelakun, 2013; Oyewale and Musa, 2006)**. The resultant effect is that the associated fishery, the biota and the ecosystem upon which people depend for a living are in danger.

According to **De pedro *et al.* (2005)** blood indices can be used to detect the health status of fish in an ecosystem. However, little or no information is available on the comparative blood indices between different feeding behaviour of fish that share the ecological zone. This work therefore investigates the blood parameters of an herbivorous, omnivorous and carnivorous fish species (*Oreochormis niloticus*, *Clarias gariepinus* and *Parachanna obscura* respectively) as bio-indicator of environmental pollution which could serves as an early warning marker of habitat contamination.

2. Materials and Methods

Study Area

Upper Jebba basin is the part of Jebba dam constructed on River Niger situated between latitude 9006' and 9055' north and longitude 4002' and 4045' east. Its tributaries include Awun, Eku, Moshi and Oli rivers. It falls within the savanna zone but specifically Guinea savanna. The predicted fish catch potential using primary productivity and morphoedaphic factors of Jebba lake was estimated at 909 – 1818 tons/annum **(Kainji Lake Research Institute, 1983).**

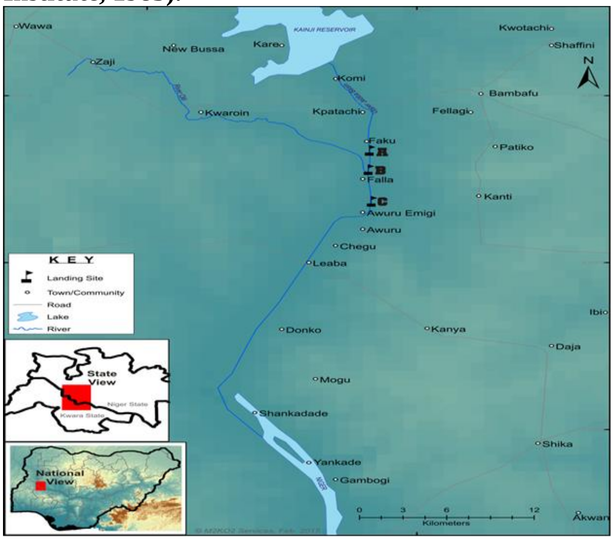

Figure 1: Map of the study areas on Jebba Basin (Adelakun et al., 2016).

Fish sample collection

Fifteen healthy live sub-adult freshwater fishes, five each of Oreochromis niloticus (weight: 50 – 100g), Clarias gariepinus (300 – 500g) and Parachanna obscura/Chryscthys nigrodigitatus (150 - 400g) were collected monthly between February and May, 2014 from commercial fishermen in Awuru landing site of Upper Jebba Basin, Nigeria. This size range avoided different state of reproductive physiology and possible error due to size differences. The fishes were transported alive to the laboratory of Federal College of Wildlife, New Bussa, in white plastic aquaria filled with quality water for subsequent analysis.

Blood collection

Blood samples were taken from the caudal vein of the fish species using a 22-gauge needle and syringe. The blood was immediately divided into labelled heparinized tube for whole blood preparation and tube without anticoagulant for serum preparation. All samples immediately subjected to laboratory analysis.

Haematological analysis

Heparinized samples were used for haematological parameters. The parameters analysed include: Packed cell volume (PCV), red blood cell count (RBC) and hemoglobin concentration (Hb) were conducted immediately. Blood smears made and stained with Giemsa, were used to determine the white blood cell count (WBC) **(Stoskopf, 1993).** PCV was determined by spinning blood samples contained in heparinized capillary tubes in a microhematocrit centrifuge (Hawksley, England). The RBC count was carried out in a modified

Neubauer chamber as described by **Baker and Silverton (1985)**, while Hb was determined by the cyano-methaemoglobin method **(Hesser, 1960)**. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from previously obtained RBC, PCV and Hb values.

Biochemical analysis

Serum samples were used for blood biochemistry examinations within 24hours of collection after serum was prepared by centrifugation. The parameters determined were: values of total serum protein using a modified Weichselbaum buiret method **(Weichselbaum, 1946)**, glucose through glucose-oxidase method **(Marks, 1996)**, cholesterol by enzymatic methods **(Bergmeyer and Grassl, 1983)**, uric acid by the uricase method **(Bauer, 1982)** while bromocresol green and picrate method **(Cheesbrough, 2005)** were used for albumin and creatinine respectively. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were carried out by the method of Reitman and **Frankel (1957)** whilst sodium and potassium were measured using flame photometer method as described by **Tietz (1986).**

Data analysis

Both descriptive and inferential statistical tools were used in analyzing the data obtained from the study. Results were presented as means ± SEM, where n equals the number of fish samples from which blood were collected. Results from all the specimens were compared using ANOVA and P < 0.05 were considered to indicate statistical significance while the means were compared using Duncan's Multiple Range Test (DMRT) **(Steele and Torrie, 1980)**.

3. Results

Description of fish's and its feeding behaviour

Table 1 reveals the feeding behaviour of the three species of fish i.e Oreochromis niloticus as an herbivores which feeds on phytoplankton in the water **(Ugwumba and Ugwumba, 2007),** Clarias gariepinus as an omnivores which feeds on both plants and animal inside the water **(Idodo-Umeh, 2003)** while Parachanna Obscura as a carnivores which feeds on the young fishes inside the river and also exhibit cannibalism **(Egwui et al., 2013)**.

Table 1: Description of fish's and its feeding behavior

NAME OF SPECIES	FAMILY	FEEDING BEHAVIOUR	REFERENCE
O. niloticus	Cichlidae	Herbivores	Ugwumba and Ugwumba, 2007
C. gariepinus	Claridae	Omnivores	Idodo-Umeh, 2003
P. obscura	Channidae	Carnivores	Egwei et al., 2013

The haematology indices of different feeding behaviour fish from Upper Jebba basin are presented in Table 2. Low PCV was observed in Oreochreomis niloticus (16.70±1.15%) which significantly varies (P<0.05) with value (23.40±0.58% and 21.33±1.76%) recorded for Clarias gariepinus and Parachanna obscura respectively. The highest values of Hb (7.90g/dl) were obtained in Clarias gariepinus, this is also reflected on white blood cell and red blood cell count. There were significant differences in the values observed in MCH, MCHC and MCV for the species (Table 2).

Table 2: Haematological parameters of P. obscura, O. niloticus and C. gariepinus from the study area

Parameters	*Parachanna obscura*	*Oreochromis niloticus*	*Clarias gariepinus*
PCV (%)	**21.33±1.76^b**	**16.70±1.15^a**	**23.40±0.58^b**
HB(g/100ml)	**5.30±0.53^a**	**5.60±0.12^a**	**7.90±0.23^b**
WBC (10^3/mm^3)	**4.77±0.06^b**	**3.56±0.24^a**	**6.59±0.05^c**
RBC (10^6/mm^3)	**2.90±0.29^b**	**2.40±0.17^a**	**3.80±0.23^c**
MCH(pg)	**18.28±0.58^a**	**23.33±0.59^c**	**20.78±0.12^b**
MCHC (g/dl)	**24.85±1.77^a**	**33.53±1.67^b**	**33.76±0.08^b**
MCV (fl)	**73.55±0.59^c**	**69.58±0.20^b**	**61.58±0.23^a**

Note: Values are mean ± standard error. Means in a row with the same superscript are not significant different (p<0.05).

Blood biochemical parameters of P. obscura, O. niloticus and C. gariepinus from the study area

Parachaana obscura. Protein and Albumin recorded varies significantly (p<0.05) from species to species along the food chain with carnivores fish Parachanna obscura recording the highest value (32.00±1.16mg/dl protein and 29.00±1.73mg/dl Albumin) while Oreochromis niloticus, an herbivores fish having a mean of 25.00±1.16mg/dl protein and 19.00±0.58mg/dl Albumin. Creatinine and glucose of 43.00±1.16mg/dl and 3.65±0.06mg/dl recorded for Clarias gariepinus were the highest while 34.00±1.73mg/dl and 3.28±0.14mg/l documented for Parachanna obscura and 24.00±1.16mg/dl and 2.56±0.03mg/dl observed for Oreochormis niloticus were all showing significant variation (p<0.05) within species.

Urea and Cholesterol also increases along the feeding habit i.e. Oreochromis niloticus > Clarias gariepinus > Parachanna obscura. For the cations, with exception of calcium, the variation shows relationship with feeding habit. The carnivores (P. obscura) recorded the highest value (16.88±0.60μ/l) of ALT which correlate with 15.77±1.16 μ/l recorded for O. niloticus while 8.34±1.17μ/l recorded for C. gariepinus was the lowest within the species and significantly varied (p<0.05). For AST, 16.78±0.64 μ/l and 14.45±1.74 μ/l were recorded for P. obscura and C. gariepinus respectively while significantly lower 10.22±0.64µ/l was observed in O. niloticus.

Table 3: Blood biochemical parameters of O. niloticus, C. gariepinus and P. obscura from the study area

Parameters	*P. obscura*	*O. niloticus*	*C. gariepinus*
protein(mg/dl)	**32.00±1.16[b]**	**25.00±1.16[a]**	**28.00±2.31[a]**
Albumin (mg/dl)	**29.00±1.73[c]**	**19.00±0.58[a]**	**23.00±1.15[b]**
Creatinine (mg/dl)	**34.00±1.73[b]**	**24.00±1.16[a]**	**43.00±1.16[c]**
Urea (nmol)	**3.85±0.18[c]**	**2.28±0.07[a]**	**3.56. ±0.03[b]**
Glucose(mmol 1^{-1})	**3.28±0.14[b]**	**2.56±0.03[a]**	**3.65±0.06[c]**
Chol. (mmol 1^{-1})	**4.75±0.16[b]**	**3.85±0.06[a]**	**3.99±0.29[a]**
Calc. (mmol 1^{-1})	**2.09±0.05[a]**	**2.02±0.02[a]**	**2.25±0.06[b]**
Potassium (mmol 1^{-1})	**23.33±1.16[b]**	**12.44±0.59[a]**	**22.89±1.16[b]**
Sodium(mmol 1^{-1})	**133.78±12.74[b]**	**92.56±1.15[a]**	**129.88±1.19[b]**
ALT (µ/l)	**16.88±0.60[b]**	**15.77±1.16[b]**	**8.34±1.17[a]**
AST (µ/l)	**16.78±0.64[c]**	**10.22±0.64[a]**	**14.45±1.74[b]**

Note: Values are mean ± standard error. Means in a row with the same superscript are not significant different (p<0.05).

4. Discussion

The Pack Cell Volume (PCV) of the three species was lower to the 38.75% recorded by **Gaber et al. (2013)** for Clarias gariepinus from El-Rahawy Drainage Canal in Egypt and 38-44.70% in culture system as reported by **Agbabiaka et al. (2013)**, but higher than the value recorded for Oreochromis niloticus from Egyptian River Nile **(Ibrahim, 2013)**. It has been reported that 20% to 35% is the reference range for non anaemic fish **(Pietse et al., 1981)**. The low PCV in O. niloticus implies that the fish are less active **(Satheeshkumar et al., 2011)** which could be consequence of stressed environment caused by pollution **(El-Naggar et al., 1998)**. The stress could be as a result of toxic effect of some heavy metal accumulated in fish tissues in the study area as earlier reported **(Adelakun 2013; Oyewale and Musa, 2006)**. This may catalyse reactions that generate reactive oxidative species which resulted to environmental oxidative stress. Heamoglobin (HB) values in the present study were also lower as compared to 6.32-13.15g/dl reported for O. niloticus **(Ibrahim, 2013)**, C. gariepinus (8.70 g/dl) **(Sowunmi, 2003)** and 5.70 g/dl obtained for P. obscura **(Kori-Siakpere et al. 2005)**. Similar results were reported by **Rambhaskar and Srinivasa-Rao (1986)**. Low PCV and haemoglobin could cause reduction in oxygen carrying capacity of blood, influencing anaemic disorder **(Gaber et al., 2013)** in P. obscura and O niloticus in the study. The wide variation among species noticed in WBC and its differentials is also similar to the observations of **Anderson (1996)**. This may be due to the different ways individuals respond to stress. Elevated WBC in all C. gariepinus shows high immunological reactions to fight against infection which could be resultant of polluted environment **(Douglas and Jane, 2010; Tayel, 2007)** while low values recorded for both the carnivorous and herbivorous species probably show their low immunity response to the same environment.

RBC is essential for the transport of oxygen in animals. The peculiarity of RBC noted in the blood of studied species is close to with the observation reported in many different feeding behaviour fish from Vellar estuary **(Satheeshkumar et al., 2011)**. RBC count for herbivorous fish in the study is higher than 1.76× 106mm^3 in same O. niloticus from Egyptian waters **(Ibrahim, 2013)** as well as 1.77×106mm^3 reported for C. gariepinus in Eleiyele reservoir **(Sowunmi 2003)** but is comparatively lower to the value observed for the same omnivorous fish in this study. The pattern display of RBC confirms report of **Clark et al. (1979)** who reported that RBC generally shows inter- and intra-species differences in the same or different environment. In this present study, high RBCs count in C. gariepinus; an omnivorous fish is usually associated with fast moving and highly active with streamlined bodied fish **(Rambhaskar and Srinivasa-Rao, 1986)**. The specie ability to withstand stress and its low oxygen tolerant nature could also be contributing factors.

MCH in the present study shows inverse relationship with RBC as low MCH show high RBC for all species. This is in agreement to the report of **Adedeji et al. (2000)** which report the correlation between both indices. Significant decreased MCH and MCHC reported for P. obscura is probably due to low haemoglobin combination which affected transportation of oxygen in the blood and can be attributed to effect of exposure to toxicant **(Adedeji and Adegbile, 2011).**

The range of mean corpuscular volume (MCV) were lower than the result obtained from similar work **(Fazio et al., 2013)** for both herbivorous and carnivorous wild fish. This may be due to low oxygen consumption rates and mobility of fish **(Stillwell and Benfey, 1995)**. Significant lower MCV reported for C. gariepinus in the study is in consonance with report from **Satheeshkumar et al. (2011)** which also reported the lowest MCV for omnivorous fish. This connection may be due to selection of the physiological state of fishes **(Docan et al., 2010)**.

For biochemical parameters, Protein, albumin, urea, cholesterol, potassium, sodium decreases down the food chain (from carnivores to herbivores) Parachanna obscura > Clarias gariepinus> Oreochromis niloticus. The value observed is within the range documented for tropical fishes **(Owolabi, 2010; Kori-Siakpere et al., 2005; Fagbenro et al.,**

2000). Increase blood protein and glucose has also being observed in carnivorous fish **(Satheeshkumar et al. 2011)**.

High blood protein, glucose and cholesterol observed in carnivorous and omnivorous could be related to abundance of available food to the fishes indicating that the fish are not under nutritional stress **(Yousafzai and Skakoori, 2011)** but rather presence of pollutant seem to have hindered metabolism of protein which is usually high in these fish food hence the glucose levels is increased as a an alternative source of energy for sequestering the effect of variety of energy of pollutants in the water body. This corroborates **Bano (1985)** observation of an increase in the serum cholesterol level after introduction of pesticides in a study. Low serum albumin, protein, glucose and minerals (Calcium, potassium and sodium) concentration in herbivorous fish (O. niloticus) may be a sign of toxicant contaminated available diet leading to loath food intake. **Adelakun (2013)** reported that the study area received agricultural effluents including herbicide and pesticides from the riverine communities. It is also possible that the dietary consumed contains toxin constituent of crude oil due to oil waste received from the lubrication and insulation used for auxiliary services on the turbine floor of the dam off-stream. This may lead to reduced albumin by preventing liver from manufacturing enough albumin for its release into the serum **(Tietz, 1986)**. Significant high blood urea level documented in secondary consumers (omnivorous and carnivorous) is likely to be a sign of stress **(Borges et al. 2007)** such as high ammonia concentration and low oxygen in the body tissue that cause failing of osmotic pressure **(Allen et al., 2005; Walsh et al., 2003 and Shen et al., 1991).**

Liver enzymes Aspartate aminotransaferase (ALT) and Alanine aminotransferase (AST) are serum transaminase activity use frequently for testing enzymes in fish for indication of toxicity. Elevation of serum levels of enzymes can occur with states of altered hepatocellular membrane permeability. Increasing serum enzymes were observed in common carp exposed to natural cyanobacterial population **(Kopp and Heteša, 2000)**. In this study, the values recorded for ALT were relatively higher than the value documented from an unpolluted canal but fall within range for fish from polluted lakes in Egypt **(Elghobashy et al., 2001)** and Synodontis membranacae from Jebba Lake; downstream of the study area **(Owolabi, 2010)**. Increased ALT concentration of fish is attributed to damage of hepatocytes; a consequence of action of heavy metals **(Zahra et al., 2001 and Zaghloul, 2000)** causing increased enzyme production to counter the damage caused by toxicants or permeability of cell membranes and eventual release of enzyme into the serum (**Elghobashy et al., 2001).** For AST, concentration level for O. niloticus was comparatively low and also lower than the value reported by **Elghobashy et al. (2001)** from both polluted and unpolluted lakes. Decrease in this AST in the study may be attributed to low synthesis of the enzyme leading to inability of hepatic cells to release enzymes into the circulatory system **(Tencalla et al., 1994).**

Conclusion

Upper Jebba Basin has definitely heavy metal which has been related to different anthropogenic activities occurring in the surrounding area of the river basin including artisanal mining, variety of pesticides and herbicides eroded from surrounding agricultural farms, component of crude oil from the maintenance of Hydro Electric Power facilities. The presence of these pollutants is toxic and could affect the survival and metabolism of inhabiting fish population differently as evident from the study. Probably the significant reduction in the values of RBCs and haemoglobin may be confirmed incident of anaemia in carnivorous and omnivorous fishes in the study. Elevation or fall of various bichemical parameters in comparison to literature exhibits effect of a variety of pollutants in this aquatic habitat. Nevertheless, obtained responses may vary among fish species and may be influenced by the type of stimulus, age, class and sex. The result obtained in this study emphasizes the need for more study on large number of fish population considering the aforementioned factors. Hence, the results of our research provide a contribution to the knowledge of the characteristics of blood parameters of different stressed trophic freshwater fishes. Based on these results, it appears that there are variations in the blood profile of fish as regard their feeding habit as well as physiological effects of anthropogenic activities on fish. Thus, plans which will identify the actions required for elimination or controlling sources of this pollution should be adopted as well as awareness to the riverine communities on the need for good water practice and guidelines to safeguard good water quality and preventing losses to fish in this aquatic environment.

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