

Changes in Blood Parameters of *Tilapia Guineensis* Exposed to Different Salinity Levels

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Abstract -The effects of different salinity levels on some blood parameters of *Tilapia guineensis* were tested. Adult and juvenile sizes were sampled from the wild of salinity 15‰ and transferred to the laboratory where they were exposed to different salinities level of 15‰, 10‰, 5‰, 0‰ for a period of 7 days. The exposure result in significant reduction ($P < 0.05$) in the values of haemoglobin (Hb), Red blood cell (RBC), Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), and Thrombocytes (THMB); while the mean values of White Blood Cell (WBC), Erythrocyte sedimentation Rate (EST), Neutrophils (NEUT), Lymphocytes (LYMPH) and Monocytes (MON) increased significantly ($P < 0.05$). These alterations were more pronounced in the adult than the juvenile fish. The results from this study, therefore suggest that variation in the salinity levels of the environment may impact negatively on the physiological and metabolic activities of *T. guineensis* as revealed in the changes observed in these blood parameters.

Index Terms – Blood; salinity; *Tilapia*; environment; brackish water

I. INTRODUCTION

Tilapiine fishes (Cichlidae family), endemic to Africa are widely distributed in tropical areas and have colonized a wide range of inland water as natural or introduced species (Panfili et al., 2004). *Tilapia guineensis* naturally occurs in estuaries and

they are subjected to changes in environmental conditions that can lead to environmental stress (Akinrotimi et al., 2007a). According to Francis et al. (2007), salinity is one of the most fluctuating water quality parameters in brackish water environment. And Leveque and Paugy (1999), observed that fluctuations in salinity levels undoubtedly impose stress, on the physiology of the exposed fish population and can modify their structures.

In the surrounding water, the fish homeostatic system is continuously affected by the changes of the level of salinity, temperature, pH, oxygen concentration as well as anthropogenic substances (Imslaad et al., 2008). Magil and Sayes (2004), reported that the ability of fish to tolerate or show behavioural adaptation to such conditions is essential as the physiological challenge presented by fluctuating salinities may compromise survival and fitness of the inhabiting individuals. In recent years, there has been an increase in the commercial aquaculture potential of *T. guineensis* especially in the Niger Delta region of Nigeria. (Akinrotimi et al., 2009). The majority of brackish water aquaculture systems operate in relatively shallow coastal areas, where there are constant perturbations in temperature and salinity. And cultured fish have no means of avoiding potentially stressful water conditions, unlike wild individuals, therefore, a fuller understanding of how fish are physiologically challenged in fluctuating physico-chemical environment may benefit future farming practices.

The physiological response to reduction in salinity levels in an aquatic environment has been investigated in a number of fresh and marine water species (Woo and Wu, 1982; Hutchinson and Hawkins, 1990; Proverches *et al.*, 1993, Kelly and Woo 1999; Foss *et al.*, 2001). In addition, limited information exists with respect to the effects of salinity on physiology of this species, of all parameters available to researcher in assessing environmental effects on fish physiology, haematological studies seems to be more reliable (Katalog and Parlak, 2004). This is because, haematology is an indicator of water balance nutritional status and overall health condition of fish

Manuscript received January 9th, 2012

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lagoon ecosystem in Nigeria. This specie is particularly adapted to brackish water areas, where

(Chang and Hor 1999; Denson *et al.*, 2003), previous studies has shown haematology to be affected by salinity (Gabriel *et al.* 2007). Also, Jawad *et al.* (2004), noted that studies in fish blood lies in the possibility that the blood will reveal conditions within the body of the fish long before there is any outward manifestation of diseases.

This study therefore examined the haematological response of *T. guineensis* exposed to different (low) salinity levels. The comparisons were made between juveniles and adult fish in order to highlight any significant ontogenetic shifts in tolerance.

II. MATERIALS AND METHODS

A total of 240 *T. guineensis* comprising 120 adult size (mean total length 21.24cm \pm 3.24SD; mean weight 388.42g \pm 32.71SD) and 120 juvenile size (mean length 12.60 \pm 3.21; mean weight 44.74 \pm 5.88) were collected from recruitment ponds of African Regional Aquaculture Centre (ARAC) brackish water fish farm, Buguma, Rivers State, Nigeria during low tide. (These ponds were directly linked to the creek, where fish comes in during high tide, and were trapped for collection at low tide). They were immediately taken to the hatchery where they were exposed to different levels of salinity. 15‰ (control); 10‰; 5‰ and 0‰ for a period of seven days. The different levels of salinity concentrations used were achieved by serial dilution of brackish water (15‰) with fresh water, to get the desired concentration.

The fish were stocked 10 fish per tank of 0.6m x 0.6m dimension with an effective water depth 0.6m, using four treatments (15, 10, 5 and 0‰) with three replicates for each experimental fish sizes. They were fed with pelleted feed (33% crude protein) at 1% body weight twice daily. Physico-chemical characteristics of the water were monitored. Measurements of water parameters were taken on the first and the last day of the experimental period, Hydrogen ion concentration (pH) was determined by the use of a pH meter (model HI 9812, Hannah Products, Portugal). Temperature was taken with mercury in glass thermometer. The salinity was measured using hand held refractometer (Model HRN – 2N, Adago Products, Japan). While the values of dissolved oxygen, ammonia nitrite and sulfide were evaluated using the methods described by APHA (1985). The mortality of the fish during the experiment in the tank were taken and the percentage mortality calculated.

Standard haematological analysis procedures described by Blaxhall and Daisley (1973), were employed in the assessment of the various blood

parameters, Haemoglobin (Hb) was done with the cyanomethaemoglobin method. Packed Cell Volume (PCV) by micro haematocrit method, Erythrocyte sedimentation Rate (ESR), was estimated using micro wintrobe method, White Blood Cell (WBC) was determined with the improve Neubauer Counter, differential counts (neutrophils, monocytes and lymphocytes) were done on blood film stained with may Grunwald-Giensa stain. Red blood cell (RBC) was estimated using the relationship between Hb and PCV (Miale, 1982). The red blood indices; mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular Volume (MCV) were calculated according to Brown (1980). The values of thrombocytes were determined using the Rees and Becker method (Seiverd, 1983).

Data obtained from the experiment were subjected to analysis with the General Linear Model (GLM) of ANOVA at 0.05 probability and difference among means were separated using Duncan multiple range test (Zar, 1999).

III. RESULTS

The physico-chemical parameters in the experimental tanks were not significantly different except in the values of salinity (Table 1). The highest percentage mortality (75.0%) was observed in the adult *T. guineensis* exposed to 0.00ppt, while the lowest (6.0%) was recorded in juveniles fish in the control 15‰ (Table 2).

Exposure of *T. guineensis* to different salinity levels exerts some level of change on the blood parameters resulting in the reduction of the mean values of Haemoglobin (Hb), Packed Cell Volume (PCV), and Red Blood Cell (RBC) which was more noticeable at salinity level of 0.00‰ with significant difference ($P > 0.05$) between juvenile and adult fish (Fig. 1,2,3). The erythrocyte sedimentation rate (ESR) increased significantly ($P > 0.05$) with the highest value observed in the fish exposed to 0.00‰ also significant difference were equally observed between juvenile and adult fish at 10.00, 5.00 and 0.00‰ salinity levels (Fig. 4). The number of white blood cell slightly increased with no significant different ($P > 0.05$) in all the concentration levels (Fig. 5). The mean corpuscular haemoglobin concentration (MCHC) obtained were within the same range except in 15.00‰ salinity (Fig. 6). The values of mean corpuscular haemoglobin (MCH), did not show any significant different between juveniles and adult fish in all the salinity concentration levels except 0.00‰ (Fig. 7). Changes observed in the values of mean corpuscular

volume and thrombocytes were only significant at 0.00‰ level (Fig. 8 and 9). The values of neutrophils increased significantly ($P < 0.05$) from the 15.00‰ (Control) to 0.00‰ with the values of adult fish higher than the juveniles (Fig. 10). The

values of lymphocytes recorded were all within the same range with no significant difference (Fig. 11), while the values of monocytes were higher in the lower salinity levels (Fig. 12).

Table 1: Physico-Chemical Parameters of Experimental Tanks during the trial
Different salinity concentration

Parameter	Different salinity concentration			
	0‰ Mean ± SD	5‰ Mean ± SD	10‰ Mean ± SD	15‰ Mean ± SD
Ph	6.64±0.24 ^a	6.62±0.32 ^a	6.63±0.14 ^a	6.61±0.11 ^a
Temperature (°C)	27.14±1.21 ^a	27.12±1.16 ^a	27.14±1.17 ^a	27.13±0.36 ^a
N – NH ₃ (mg ^{L-1})	0.43±0.02 ^a	0.46±0.01 ^a	0.44±0.02 ^a	0.41±0.01 ^a
N – NO ₂ (mg ^{L-1})	0.0047±0.02 ^a	0.0046±0.81 ^a	0.0047±0.02 ^a	0.0048±0.01 ^a
Dissolved oxygen (mg ^{L-1})	4.41±1.21 ^a	4.64±1.01 ^a	4.54±1.11 ^a	4.63±1.21 ^a
Sulfide (mg ^{L-1})	0.07±0.01 ^a	0.012±0.01 ^a	0.02±0.01 ^a	0.02±0.01 ^a
Salinity (‰)	0.00±0.00 ^a	5.00±0.05 ^b	10.00±2.21 ^c	15.00±3.22 ^{ab}

Means with different superscript within the row are significant

Table 2: Percentage Mortality in *T. guineensis* exposed to different levels of salinity

Salinity concentration (‰)	Sizes of <i>T. guineensis</i>	
	Juvenile (%)	Adult (%)
0.00	60.00	75.00
5.00	42.00	58.00
10.00	19.00	35.00
15.00	6.00	9.00

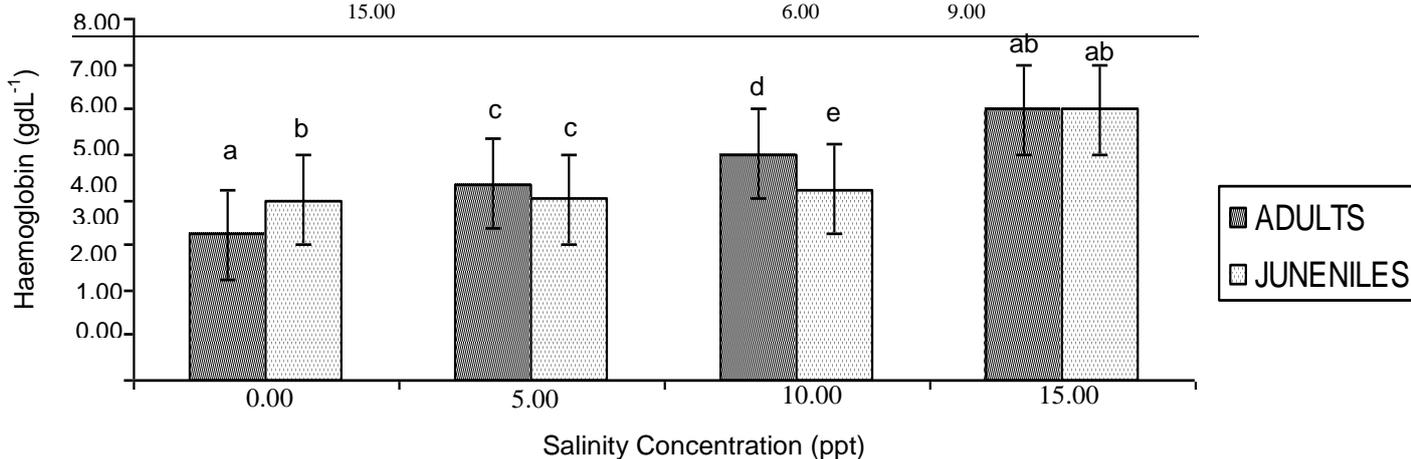


Fig. 1: Effects of different salinity levels on haemoglobin of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

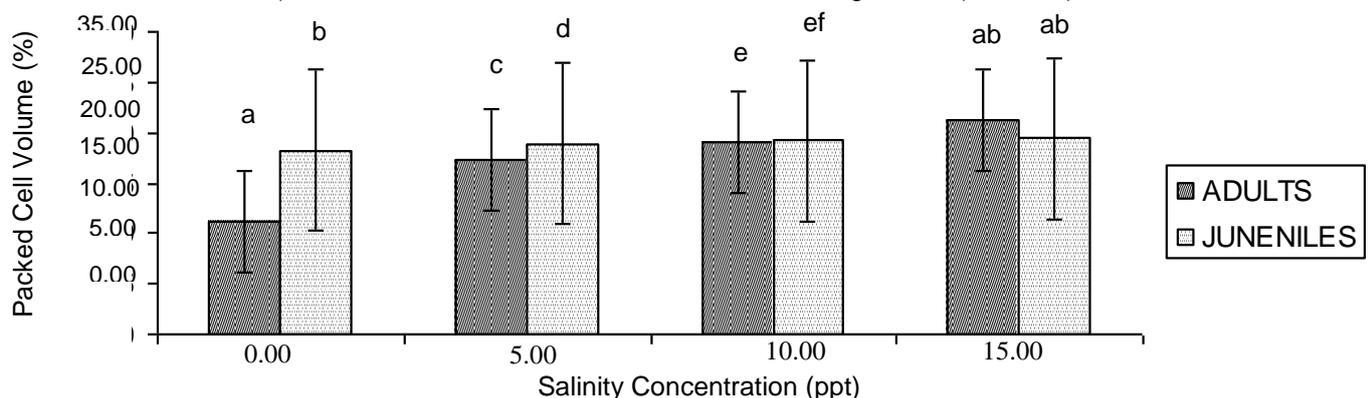


Fig. 2: Effects of different salinity levels on Packed Cell Volume of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

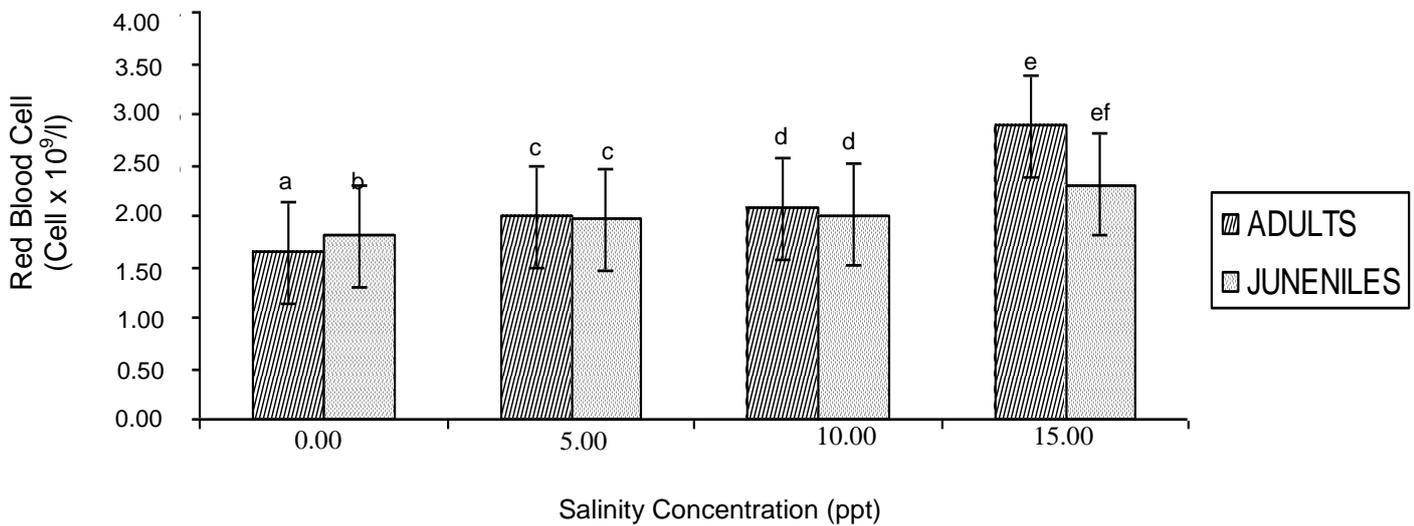


Fig. 3: Effects of different salinity levels on Red Blood Cell of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

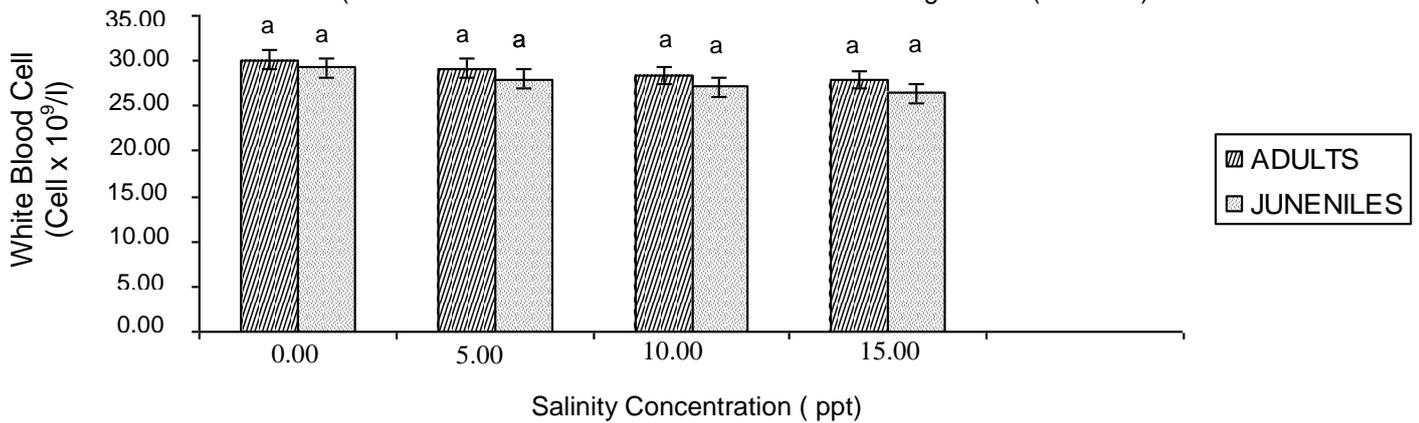


Fig. 5: Effects of different salinity levels on White Blood Cell of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

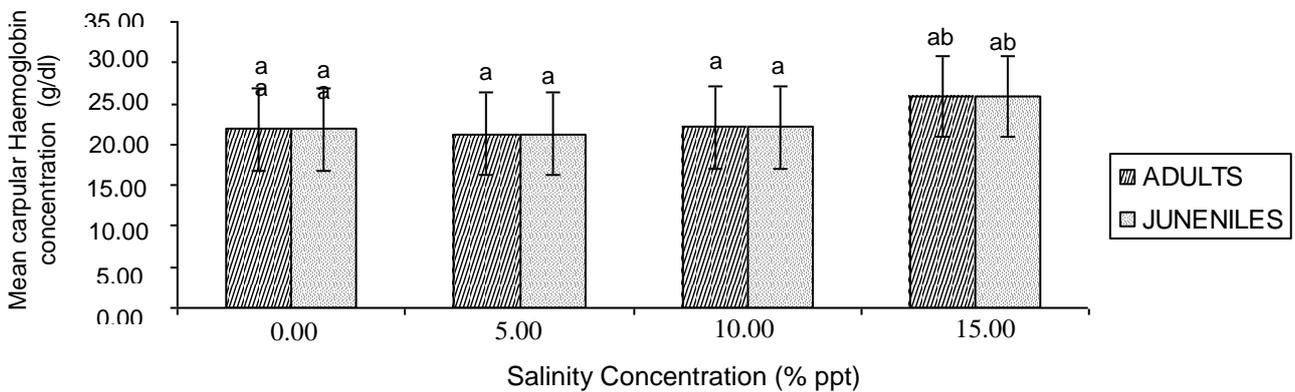


Fig. 6: Effects of different salinity levels on Mean corpuscular haemoglobin concentration of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

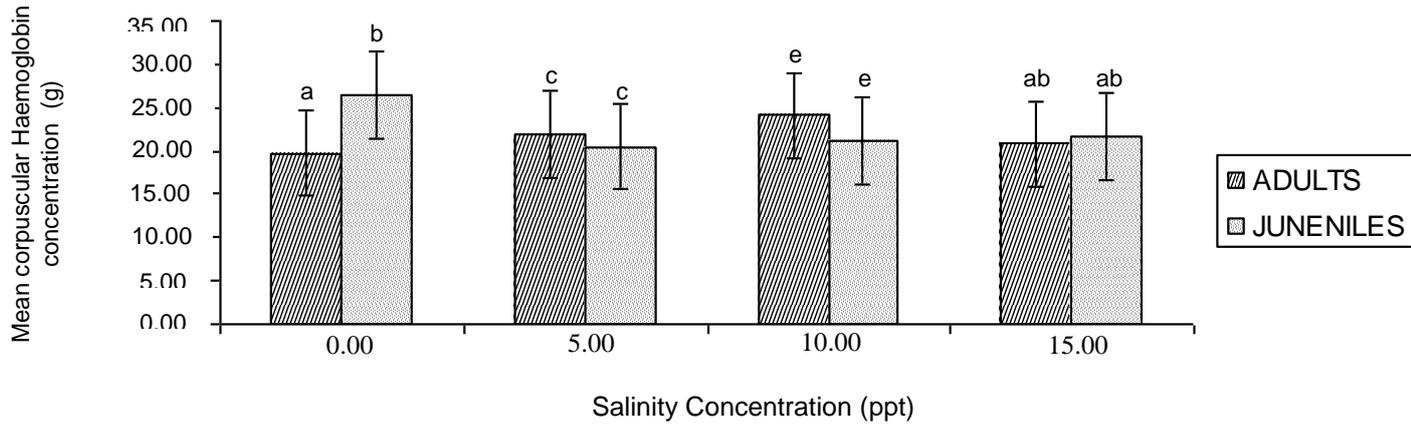


Fig. 7: Effects of different salinity levels on Mean corpuscular haemoglobin of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

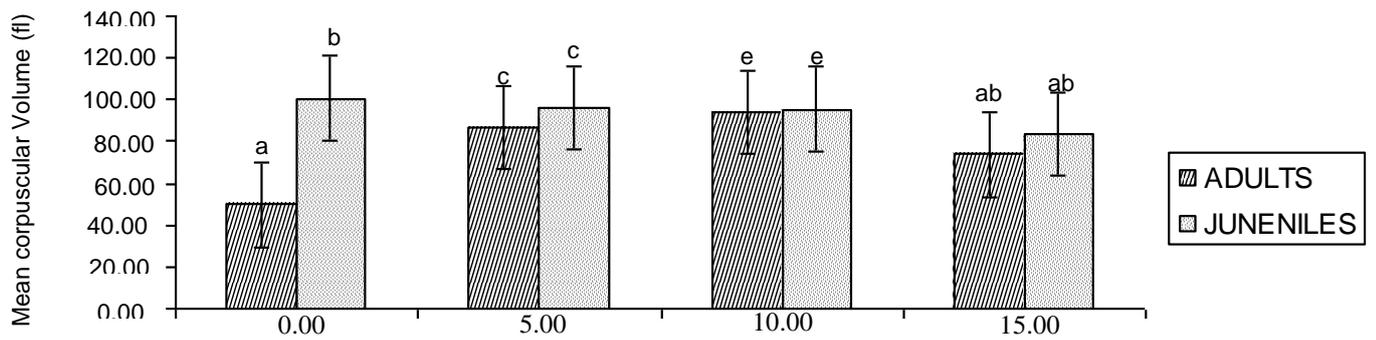


Fig. 8: Effects of different salinity levels on Mean corpuscular Volume of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

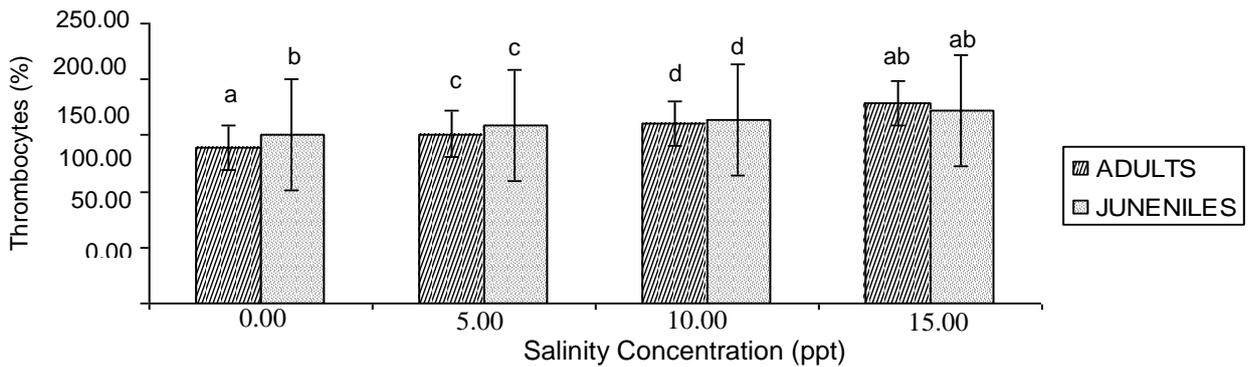


Fig. 9: Effects of different salinity levels on Thrombocytes of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

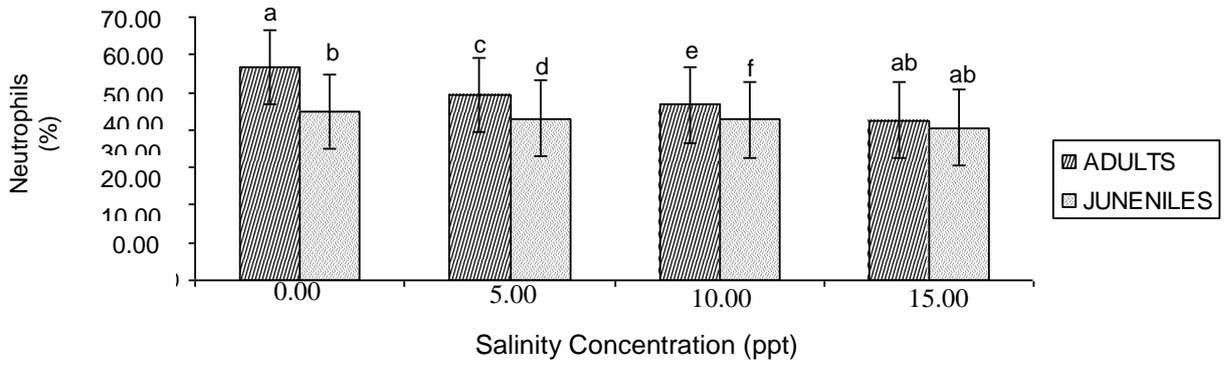


Fig. 10: Effects of different salinity levels on Neutrophils of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

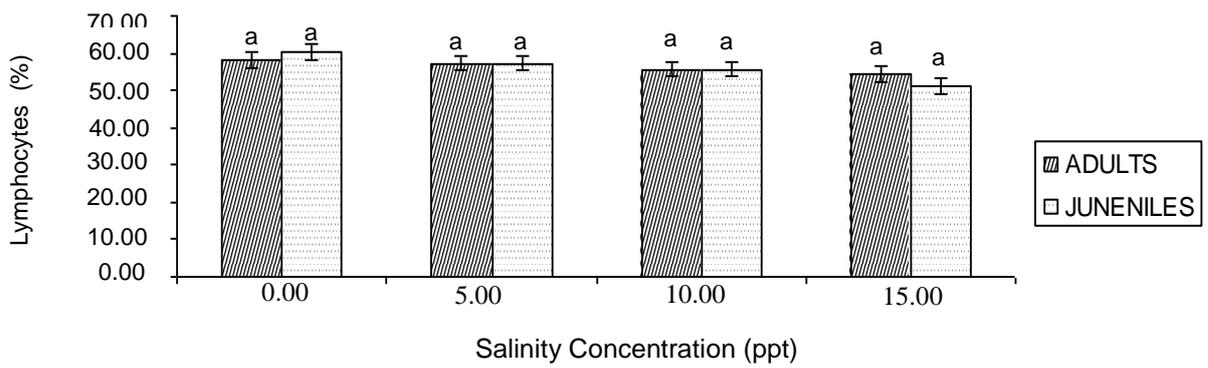


Fig. 11: Effects of different salinity levels on Lymphocytes of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

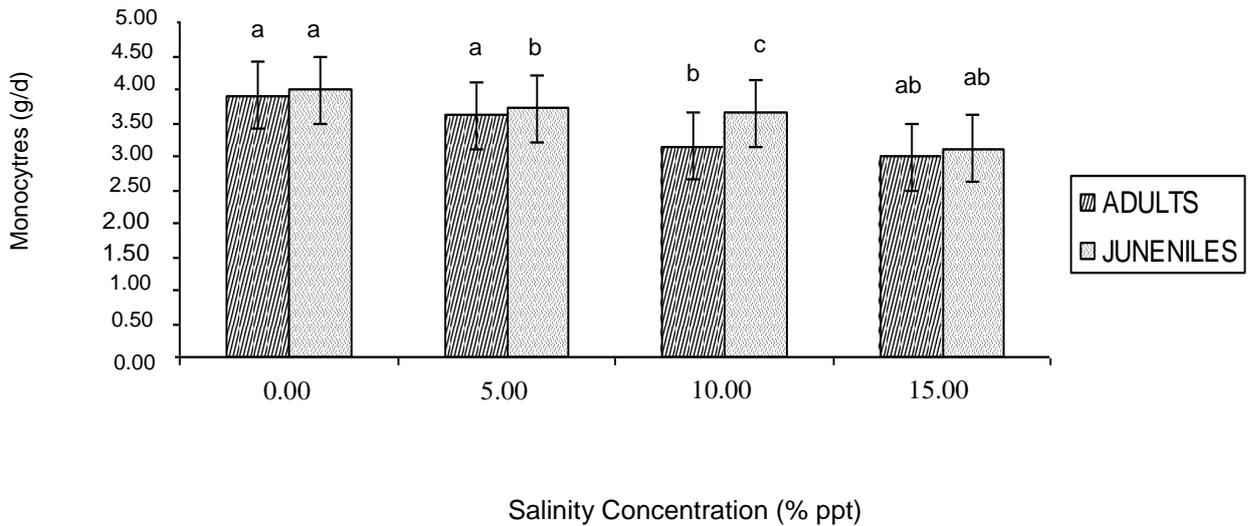


Fig. 12: Effects of different salinity levels on Monocytes of *T. guineensis* (Different letters on the bars indicate statistical significant ($P < 0.05$))

IV. DISCUSSION

Aquaculture urges for more accurate information on stress control, in order to be assured of good health status of fish, especially those transferred to a new environment. Haematological parameters can be of great importance for fish farmers serving as indicators of the physiological status, helping in the prevention and control of pathologies related to stress as a result of changes in the environmental components (Aldrin *et al.*, 1982; Tavares – Dias, 2001, Imsland *et al.*; 2008). Numerous phases have been described in fish following salinity challenges. Houston (1959), was the first to describe these phases, defining the crisis phase, as a period of rapid and significant change, leading to the stabilization phase in which homeostatic mechanisms regulate internal conditions to a stable value within the tolerance range.

In this study, there was no significant differences between the physico-chemical parameters of the water in all the experimental tanks with the exception of salinity, which varies significantly in all the treatment groups. These parameters were within tolerable range for fish culture (Akinrotimi *et al.*, 2007b). The mortality of fish observed in this study indicated that the percentage mortality of fish increased significantly as the salinity decreases with the highest mortality observed in the fish exposed to zero salinity level. This supports the findings of Anyanwu *et al.* (2007), who observed similar results in black chin tilapia. *Sarotherodon melanotheron* exposed to different salinity levels. This may be due to stress related activities as a result of drastic change in salinity from 15.0ppt to 0.0ppt, which disrupt its internal homeostasis and consequently leads to death (Newsman, 2003).

Stress due to salinity changes has been reported to alter the standard haematological characteristic of teleosts, elevating plasma corticosteroids (Barton and Iwama, 1991; Yada and Nakanishi, 2002) reducing the levels of some blood parameters (Martin *et al.* 2002) and also increasing the values of some blood components (Martins *et al.*, 2004). These changes can affect both oxygen transports in the blood and transfer across the gills. According to Akinrotimi *et al.* (2007c), these alterations depends on the species, age, sex, environment and the nature of the stressor.

The blood physiology of juveniles and adult of *T. guineensis* was investigated in the study with respect to the changes in salinity. The

results obtained, indicated fluctuations in levels of blood characteristics with significant reduction in the values of haemoglobin, packed cell volume, and red blood cell. This compared favourably with studies on Atlantic halibut *Hippoglossus hippoglossus* (Imsland *et al.*, 2008), channel catfish *Ictalurus punctatus* (Eliasaesser and Clem, 1986) and black chin tilapia *Sarotherodon melanotheron* (Anyanwu *et al.* 2007). This reduction, which is more pronounced in the adult fish than the juvenile is similar to the one reported in Atlantic cod *Gadus morhua* exposed to different salinity levels (Magil and Sayer, 2004). This observation may be attributed to low salinity osmo-regulatory dysfunction which leads to erythrocyte fragility (Montero *et al.*, 1999; Girling *et al.*; 2003).

When the red cells are allowed to settle out from their plasma the speed of their fall is known as erythrocyte sedimentation rate (ESR). In this work the ESR increased as the salinity reduces, this corroborates the findings of Gabriel *et al.*, (2007) in black jaw tilapia *Sarotherodon melanotheron* exposed to low salinity. The increase in the value of ESR may be due to deviation of plasma proteins such as albumin globulin and fibrinogen from their normal ratio, as a result of salinity induced stress (Denson *et al.*, 2003).

In *T. guineensis* subjected to varying salinity stress, the values of neutrophils and monocytes increased significantly ($P < 0.05$) and those of white blood cell (WBC) and lymphocytes exhibited a slight alteration. This is in line with the results of Tavares – Dais *et al.* (2001) in tambaqui *Colossoma macropomum*. The increase in white blood cells and differential counts was caused by an increase in leukocyte migration from the blood circulation (Gomes *et al.*, 2003).

The mean corpuscular values namely mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are concerned with the volume of an average erythrocyte, weight and concentration of haemoglobin in the average erythrocyte respectively. The values obtained in this study reduced as the value of salinity reduces, in the experimental tanks. This is in agreement with the results of Jawad *et al.*, (2001) in Indian Shad *Tenuulosa, illisha*, but contradicts that of Brown *et al.*, (2001), in pike perch *Atizostedion lucioperca*, who noted increase with an increased salinity level. The low values of these indices as observed in this study may be due to catecholamine – induced erythrocyte shrinking

consequent of variations in the S. values of salinities which ultimately leads to reduction in the value of these indices (Jensen *et al.*, 1998).

V. CONCLUSION

The present study assessed the osmoregulatory abilities of *T. guineensis*, so as to determine the accessibility of culturing this species in fresh water environment, as the species is particularly endemic to brackish water. However, in the estuarine environment more gradual changes in salinity are likely and may involve tidal cycles. The experiment which aimed to mimic tidal cycles of fluctuating salinities, has shown that short-term exposure of fish to salinities as low as zero led to extreme physiological disturbances as revealed in changes observed in their blood characteristics. Therefore, for this specie to be cultured in fresh water, it need to be acclimated gradually to low salinities, so as to minimize the impact of sudden change in the environment on the physiology of the fish and enhances its performance in the fresh water medium.

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