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Consequences of Direct Transfer to Fresh Water on the Blood Variables of *Sarotherodon melanotheron* (Rüppell, 1852)

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ABSTRACT

Alterations in blood variables of *Sarotherodon melanotheron* transmitted immediately from brackish water (salinity 13.71‰) to fresh water (salinity 0.12‰) were examined to evaluate the consequence of abrupt alterations in the environment on fish blood parameters. The results acquired revealed that significant reduction ($p < 0.05$) in mean values of Haemoglobin (Hb) from 7.37 ± 1.22 to $4.30 \pm 0.82 \text{ dl}^{-1}$; Packed Cell Volume (PCV) 23.48 ± 3.22 to $15.08 \pm 2.97\%$; Red Blood Cell (RBC) 5.99 ± 0.72 to $3.31 \pm 0.77 \text{ Cells} \times 10^6$; Mean Corpuscular Haemoglobin Concentration (MCHC) 31.68 ± 3.66 to $28.48 \pm 2.03 \text{ pg}$; and Platelets (PLT) 195.44 ± 16.77 to $138.99 \pm 9.05 \text{ } 10^3 \text{ uL}^{-1}$. However, notable elevation was recorded in the values of Erythrocyte Sedimentation Rate (ESR) from 4.89 ± 0.16 to $10.58 \pm 1.48 \text{ mm/hr}$; White Blood Cell (WBC) 27.03 ± 2.96 to $33.09 \pm 3.72 \times 10^9 \text{ L}^{-1}$; Mean Corpuscular Volume (MCV) 39.33 ± 3.07 to $45.88 \pm 7.03 \text{ fL}$ Neutrophils (NEUT) 41.71 ± 3.08 to $48.30 \pm 7.99\%$; Lymphocytes (LYMP) 54.60 ± 3.99 to $46.36 \pm 9.87\%$ and Monocytes (MON) 3.80 ± 1.04 to $5.97 \pm 1.99\%$. These variations in the blood parameters were more perceptible in adult than juvenile fish. Results from this study consequently imply that direct transfer of fish to a region of lower salinity may have damaging effect on the physiology of *S. melanotheron* as observed in this study.

1. Introduction

The specie, *Sarotherodon melanotheron* is considered widely distributed in the coastal areas of Niger Delta region of Nigeria,^[1] Adaptation to changes in environmental factors has been identified as one of the major area of research necessary for *S. melanotheron* culture development^[2]. In the wild, *S. melanotheron* are estuarine, found mostly in the coastal parts of the country. There is the need therefore to assess the possibility of culturing this specie in fresh water. Lemarie *et al.*^[3] recounted that the ability of tilapia

to inhabit diverse environment with irregular salinities depends on the species, mean individual weight, methods of transfer, feeding techniques for pre-acclimation, the physiological status of the fish and more commonly the consequence of environmental features.

Consistent changes in composition of aquatic environment have been observed to alter the behaviour, and physiology of fish^[4]. In aquaculture, the performance of cultured fish species is controlled by not only genetic potential and technological manipulation but also by its immediate environmental conditions^[5]. Sudden change in the

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environmental condition of fish, reportedly cause serious stress, which will eventually disrupt the haematological parameters of the fish. The disrupted blood components involved in maintaining or expanding the capacity of water, ion transport, and homeostasis are now limited in performing their functions, thus leading to immuno depression, low productivity disease outbreaks and ultimately mortality^[6]. Hence, proper knowledge of haematological response in fish to changes in environment will help in improving production of fish and control of stress in aquaculture. This work therefore examines the effect of direct transfer from brackish to fresh water on the blood indices of *S.melanotheron* a popular fish for culture among local farmers in the Niger Delta regions of Nigeria.

2. Materials and Methods

Experimental Fish and Procedure

One hundred and twenty *S.melanotheron* comprising of 60 juvenile sizes (mean length 9.00 cm±2.92SD; weight 37.61g±6.02SD) and 60 adult sizes (mean length 14.88 cm±5.99SD; mean weight 120.87 g±12.82SD) were collected from brackish water in Buguma creek at low tide. They were immediately conveyed to the outdoor hatchery where the initial blood samples were drawn from the fish and taken to laboratory for analysis. They were later kept at 20 each in three rectangular tanks (0.36 m³) filled to half capacity with fresh water for interlude of seven days for each experimental fish sizes. The fish was fed twice daily with crumblized pelleted feed (35% c.p) at 2% body weight. The mortality during the experimental period was ascertained by counting the number of the dead fish in each experimental tank for each size from the first day to the seventh day.

Evaluation of Physico-Chemical Parameters

Physico-chemical parameters namely temperature pH, ammonia nitrogen, nitrate, dissolved oxygen, sulfide and salinity in the creek (brackish water) and in the experimental tanks (fresh water) were monitored. Temperature was taken with mercury in glass thermometer; pH was taken with pH meter while Ammonia, nitrate, sulfide and dissolved oxygen were determined with Horiba U-7 water checker. And salinity was measured with hand held refractometer (Model HRN-2N Atago Products, Japan).

Blood Sampling and Analysis

Blood samples were collected from a total of 40 fish that is 20 each before and after transfer, comprising of 10

fish each for both juvenile and adult sizes. Blood samples were obtained with heparinized plastic syringe fitted with 21 gauge hypodermic needle and preserved in disodium salt of Ethylene Diamine Tetraacetic Acid (EDTA) bottles for analysis. The blood samples collected at the hatchery were labeled initial and those collected after seven days of transfer were labeled final samples. The values of Packed Cell Volume (PCV) were determined using microhaematocrit method of Snieszko^[7]. Haemoglobin (Hb) was done by cyanomethaemoglobin method^[8]. The Red Blood Cell (RBC) were enumerated in an improved Neubauer haemocytometer using Handricks diluting fluid, while the total White Blood Cell Counts (WBC) were similarly enumerated in an improved Neubauer haemocytometer using shaw's diluting fluid^[9]. The Erythrocyte Sedimentation Rate (ESR) was done by Wintrobe method. Also, Platelet (PLT) was done with Rees and Ecker Method^[10], while the Red Blood Cell indices; Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume were calculated from the equation given by Anderson and Klontz^[11]. The differential counts were done on blood film stained with may Grumwald-Giensa stain as described in Hrubec *et al.*,^[12].

Data Analysis

All data obtained were subjected to Analysis of variance (ANOVA) at 0.05% probability and differences among means were separated with the significant difference using SAS software^[13].

3. Results

The physico-chemical parameters of water in the creek (before transfer) and the experimental tanks (after transfer) was significant ($p < 0.05$) only at salinity (Table 1). After transfer the highest mortality (50.00 ± 8.03) was recorded in day 1 for adult fish while the lowest (3.00 ± 1.01) was observed in juvenile fish in day 7 (Table 2). The initial and final haematological parameters of juvenile and adult size of *S.melanotheron* transfer to fresh water environment were shown in Table 3 and 4. the results indicated a consistent reduction in the values of Hb, PCV, RBC, MCHC, MCH and PLT, while the values of ESR, WBC, MCV, Neutrophils, lymphocytes, and Monocytes increased significantly ($p < 0.05$) which was more pronounced in adult fish than the juvenile (Table 3 and 4). The pooled data for the variations in the blood variable of the transferred fish regardless of the size was presented in Table 5, the lowest value was observed in RBC, while PLT had the highest value.

Table 1. Physico-Chemical Parameters of Water before and after Trial

Parameters	Before Transfer (Brackish water)	After Transfer (Fresh Water)
pH	6.69±0.88 ^a	6.89±0.41 ^a
Temperature (°c)	28.97±1.80 ^a	28.77±1.74 ^a
Ammonia (mgL ⁻¹)	0.41±0.01 ^a	0.61±0.34 ^a
Nitrate (mgL ⁻¹)	0.0023±0.02 ^a	0.0042±0.01 ^a
Dissolve oxygen (mgL ⁻¹)	4.45±0.89 ^a	4.01±0.88 ^a
Sulfide (mgL ⁻¹)	0.04±0.01 ^a	0.02±0.01 ^a
Salinity (‰)	14.22±2.02 ^a	0.02±0.01 ^b

*Means with the same superscript within the row under before and after trial are not significantly different (p>0.05)

Table 2. Mortality (Mean ± SD) Observed in *S. melanotheron* Transfer to Fresh Water for a Period of Seven Days

Days	Fish Sizes	
	Juvenile	Adult
1	26.00±5.99 ^a	50.00±8.03 ^b
2	18.00±4.88 ^a	34.00±7.90 ^b
3	14.00±2.07 ^a	28.00±4.77 ^b
4	10.00±1.82 ^a	23.00±3.71 ^b
5	8.00±2.02 ^a	19.00±3.53 ^b
6	5.00±1.03 ^a	14.00±4.99 ^b
7	3.00±1.01 ^a	10.00±3.08 ^b

*Means with the same superscript within the row under before and after trial are not significantly different (p>0.05)

Table 3. Haematological (Mean±SD) in Juvenile size of *S. melanotheron* before and after transfer

Blood Parameters	Before Transfer	After Transfer
	(Brackish water)	(Fresh water)
Hb (g/dl)	6.77±0.92 ^a	4.03±0.92 ^b
PCV (%V)	20.02±3.77 ^a	15.09±3.99 ^b
ESR (mm/hr)	2.90±0.19 ^a	8.09±1.19 ^b
RBC (x10 ⁶ /mL)	4.99±0.66 ^a	3.59±0.92 ^b
WBC (x10 ⁹ /L)	24.99±7.90 ^a	29.20±4.88 ^b
MCHC (%)	33.81±3.88 ^a	26.70±2.72 ^b
MCH (g/dl)	13.57±2.02 ^a	11.23±2.90 ^b
MCV(fl)	40.12±4.77 ^a	42.03±4.88 ^b
PLT/(10 ⁸ /mL)	191.79±9.99 ^a	148.88±9.98 ^b
Neutrophils (%)	42.79±3.99 ^a	47.88±3.21 ^b
Lymphocytes (%)	52.85±2.92 ^a	46.38±13.12 ^b
Monocytes (%)	4.56±1.06 ^a	6.99±0.82 ^a

Mean within the row with different superscript are significant (P < 0.05)

Key: Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglobin PLT – Platelets.

Table 4. Haematological in Adult size of *S. melanotheron* before and after transfer

Blood Parameters	Before Transfer	After Transfer
	(Brackish water)	(Fresh water)
	Mean ± SD	Mean ± SD
Hb (g/dl)	7.96±0.94 ^a	4.56±0.99 ^b
PCV (%V)	26.94±1.09 ^a	15.07±6.66 ^b
ESR (mm/hr)	6.88±0.88 ^a	13.07±2.89 ^b
RBC (x10 ⁶ /mL)	6.99±0.83 ^a	3.03±0.03 ^b
WBC (x10 ⁹ /L)	29.02±3.77 ^a	36.99±3.88 ^b
MCHC (%)	29.54±3.07 ^a	30.26±3.99 ^b
MCH (pg)	11.39±1.77 ^a	15.04±3.77 ^b
MCV(fl)	38.54±5.04 ^a	49.74±5.88 ^b
PLT(10 ⁸ /mL)	199.88±9.99 ^a	128.88±6.00 ^b
Neutrophils (%)	40.62±3.41 ^a	48.73±8.07 ^b
Lymphocytes (%)	56.35±7.41 ^a	46.33±5.62 ^b
Monocytes (%)	3.03±0.77 ^a	4.94±0.77 ^b

Mean within the row with different superscript are significant (P < 0.05)

Key: Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglobin PLT – Platelets.

Table 5. Mean Values of Haematological Parameters of *S. melanotheron* Transfer Directly to Fresh water

Blood Parameters	Before Transfer	After Transfer
	(Brackish water)	(Fresh water)
	Mean ± SD	Mean ± SD
Hb (g/dl)	7.37±1.22 ^a	4.30±0.82 ^b
PCV (%V)	23.48±3.22 ^a	15.08±2.97 ^b
ESR (mm/hr)	4.89±0.16 ^a	10.58±1.48 ^b
RBC (x10 ⁶ /mL)	5.99±0.72 ^a	3.31±0.77 ^b
WBC (x10 ⁹ /L)	27.03±2.96 ^b	33.09±3.72 ^b
MCHC (pg)	31.68±3.66 ^b	28.48±2.03 ^b
MCH (g/dl)	12.48±3.11 ^a	13.14±4.71 ^a
MCV(fl)	39.33±3.07 ^b	45.88±7.03 ^a
PLT(10 ⁸ /mL)	195.44±16.77 ^a	138.99±9.05 ^b
Neutrophils (%)	41.71±3.08 ^b	48.30±7.99 ^a
Lymphocytes (%)	54.60±3.99 ^b	46.36±9.87 ^b
Monocytes (%)	3.80±1.04 ^b	5.97±1.99 ^a

Mean within the row with different superscript are significant (P < 0.05)

Key: Hb – Haemoglobin; PCV – Packed Cell Volume; ESR – Erythrocyte Sedimentation Rate; RBC – Red Blood Cell; WBC – White Blood Cell; MCHC – Mean Corpuscular Haemoglobin Concentration; Mean Corpuscular Haemoglobin PLT – Platelets.

4. Discussion

Understanding of the haematological characteristics in aquatic animal is an essential instrument in conjunction with the environmental factors that can deduce the health of fish population. Fish reacts to external stimuli in various ways, depending on the stressor involved, as observed by Martins *et al.*,^[14] and Gomes *et al.*,^[15]. According to Akinrotimi *et al.*,^[16], the haematological reaction of fish to stress is determined by the disposition of the stress itself, that is definite stress elicit a definite responses. According to Luskova^[17] and Gbore *et al.*,^[18] who reported that the importance of the haematological conditions of a fish, as a biological indication of stressful situation was established and a variety of haematological parameters were shown as very sensitive to intoxication with trace metals, micro contaminants, diseases, and changes in the environmental components.

The current trial revealed various degrees of haematological responses of *S.melanotheron* exposed to direct transfer from brackish to fresh water. The rapid change in the environment stimulates a significant ($P<0.03$) decrease in the following blood parameters: haemoglobin, packed cell volume, red blood cell, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin and platelets. The enumeration of old disintegrated erythrocyte was significantly higher in adult than the juvenile fish. Patterns of changes in these parameters found by other authors depended on the salinity level, duration of exposure, mode of transfer and fish species. The decrease in blood parameters as observed in this study agrees with the finding of Gabriel *et al.*,^[19] in *T.guineensis* where comparable decrease in blood parameters were reported. The results observed in this work may be due to the radical effect of stressor (change in environment) which leads to haemodilution, as a result of damaged erythropoiesis (production of erythrocytes) as a result of interrupted osmoregulation activities in the fish^[20]. Moreover, Zhiteneva *et al.*^[21] reported inhibition of erythrocyte production, splitting of the cytoplasm of erythrocytes in tilapia exposed to impulsive adjustment in environment.

Direct transfer of *S.melanotheron* into a new environment also reduces an increase in erythrocyte sedimentation rate, white blood cell, mean corpuscular volume, neutrophils, lymphocytes and monocytes. The erythrocyte sedimentation rate which evaluated the rate at which red cell settle out of their plasma, increased significantly, is an indicative of anaemia, due to fragility of the erythrocytes consequence of stress induced interference in the development and discharge of erythrocytes from haematopoeitic tissue^[22,23].

The present experiment established considerable

elevation in the amount of white blood cells (WBC), this is in the same opinion with the findings of Sardella *et al.*,^[24] who reported a notable increase in WBC in the Mozambique tilapia after exposure to new environment. Also, number of WBC increased tremendously in goldfish (*Carassius auratus*) after exposure of the fish to various categories of stressors^[25]. An increase in the quantity of WBC may be as a result of liberation of cells amassed in the spleen, when fish is exposed to stressors^[26]. The differential counts namely monocytes, neutrophils and lymphocytes increased to a large extent as observed in this study, this support the findings of Ginling *et al.*,^[27] in green back flounder (*Rhombosulea tapirina*) exposed to fresh water. The increased observed may be due to release of more of the white cells to combat the stressor^[28].

5. Conclusions

The present study confirmed that haematological parameters are very sensitive indicators of fish organism response to changes in the environmental factors. The study revealed that an abrupt and unexpected modification in the fish environment can distort fish physiological conditions and affects its performance in the culture medium. Hence, it is essential and important to culture fish in an appropriate environment for its adaptive and functional physiological mechanism. When there is the need to culture *S.melanotheron* in fresh water it should undergo gradual acclimation rather than direct transfer which may lead to decline in production capability of the fish and in some results in mortality.

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