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Effect of Freshwater Challenge on the Blood Characteristics of *Sarotherodon melanotheron*

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Abstract: Study on haematological responses of *Sarotherodon melanotheron* transferred directly from brackish water (salinity $12^0/_{00}$) to fresh water of ($0.13^0/_{00}$), was carried out to asses the magnitude of the effect of sudden change in environment on fish haematology. The results indicated significant reduction ($p<0.05$) in mean values of heamoglobin from 5.51±0.51; to 3.95±0.49 g dL^{-1}; haematocrit 19.88±0.90 to 17.07±0.87%; leucocrit 6.74±0.54 to Red blood cells 2.48±0.06 to 1.89±0.12 Platelets 169.53±8.84 to 107.52±9.68%; Lymphocytes 41.97±1.61 to 36.57±0.92% and mean corpuscular haemoglobin concentration 27.57±2.28 to 24.51±2.50 g dL^{-1}. However, increment was recorded in the value of White Blood Cell from 29.42±0.88 to 31.37±0.80 x 109/l; mean morpuscular volume 78.33±3.96 to 79.78±5.80. mean corpuscular haemoglobin 21.64±1.94 to 29.94±2.21pg: Neutrophil 35.53±1.62 to 41.32±1.27% and monocytes 2.31±0.16 to 3.10±0.13. These variations in the blood parameters were more pronounced in male than female fish. Results from this study suggest that change in the salinity of the environment may impact negatively on the physiology of *S. melanotheron* as demonstrated in changes in the blood characteristics.

Key words: Haematology, black chin tilapia, sarotherodon melanotheron *brackishwater*, *direct transfer*, *freshwater*

INTRODUCTION

The black-chinned tilapias naturally occur in estuarine and lagoon ecosystems in Nigeria. The species, *Sarotherodon melanotheron* are particularly adapted to brackish water conditions, where they are randomly subjected to changes in environmental conditions that can lead to physiological stress (Panfili *et al*., 2004). Persistent alterations in natural chemical composition of aquatic environment have been confirmed to alter the behaviour, biochemistry and physiology of aquatic fauna (Alkahem *et al*., 1998). The performance of cultivated fish is governed by not only its genetic potential and technological manipulation but also by its immediate environmental conditions (Pickering, 1993). Sudden change in the environment of fish, reportedly cause serious stress, which will challenge internal homeostatic (Shreck, 1981).

Stress according to Barton (2002) is the response of the cell or organism to any demand placed on it such that it causes an extension of a physiological state beyond the normal resting state. Fishes are exposed to stressors in nature as well as in artificial conditions such as in aquaculture or in the laboratory. In aquaculture, the main sources of stress include transportation, handling and environmental alternations which are necessary components of modern intensive fish culture (Ackerman, 2000). Stress, has been linked as one of major factors of disease outbreaks, low productivity and mortality in aquaculture (Rottmann *et al*., 1992). Hence, good knowledge of fish response to various stressors will be of greater help in improving production of fish and in providing information on ways of effectively controlling and monitoring stress in aquaculture (Ajani *et al*., 2007).

This study examined the haematological response of *S. melanotheron* transfer directly from brackish to fresh water, a common practice among local farmers in Niger Delta.

MATERIALS AND METHODS

Two hundred and twenty-eight adults *S. melanotheron* (male and female, mean weight 30.12±0.32g SD; total length, 10.22cm ±0.15 SD) were collected from

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recruitment ponds of African Regional Aquaculture centre Brackish water fish farm during low tide. Blood samples were collected from 18 of the fish for analysis. After, the remaining fish were transferred to the hatchery where they were kept, 70 each in three circular tanks (0.36 m^3), filled to half capacity with fresh water, for a period of 7 days. The fish were fed crumblized pelleted feed (35% crude protein) at 2% body weight daily. The ration was divided into 2, dispensed at 0800 and 1700 h.

Physico-chemical parameters (temperature, pH, ammonia nitrogen, nitrite, DO and sulfide) in the ponds and fresh water were monitored with Horiba u-7 water checker. Salinity was determine by hand held refractometer (Model HRN-2N Atago Products, Japan). Water exchange in the tanks was done on the third day. Blood was collected from 18 fishes after transfer (9 males and 9 females) on the seventh day. The blood samples were analysed according to the methods of Blaxhall and Daisley (1973). The data obtained from the analyses were grouped under sex: male and female and Transfer (before and after) and each subjected to Analysis of Variance at 0.05% probability and differences among means were separated with the least significant using duncen multiple range test. The following indices: Mean Corpuscular Haemoglobin Concentration (MCHC) and Mean Corpuscular Volume (MCV) were calculated according to Brown (1980) leucocrit was done according to Wedemeyer *et al*. (1983). The data obtained from the analyses were grouped under sex: male and female and transfer (before and after) and subjected to anova at 0.05% probability. Differences among means were separated with the Duncan multiple range test.

RESULTS

Physico-chemical parameters of the water in the recruitment ponds and tanks were not significantly different except in salinity (Table 1). After transfer 20% mortality was recorded on the 2nd and 3rd 40% on the 4th to 6th day on the 7th day no mortality was recorded. Before transfer the values of Hb, Ht, MCHC, MCH, neutrophills, Lymphocytes and monocytes in female was higher than the males. But RBC, Lct, WBC, Plt, MCV values in the males were higher than the females. (Table 2 and 3).

Transfer to freshwater environment exerted some levels of change on the haematological parameters resulting in significant reduction ($p<0.05$) in mean values of Hb from 5.51± 0.51 g dL^{-1} to 3.95±0.49; Ht 19.88±0.90 to 17.07±0.87%; Lct 6.74±0.54 to 6.32±0.73×10^{12}/*l* RBC 2.48±0.06 to 1.89±0.12 cells×10^6/*l*, Plt 169.53±8.84 to 107.52±9.68 cell×10^9/*l*; Lymphocytes 41.97±1.61 to 36.57±0.92%, MCHC 27.57±2.28 to 24.51±2.50 g dL^{-1}. While the values of the other blood characteristics were raised: WBC from 29.42±0.88 to 31.37±0.80 x 10^9/*l*; MCV 78.33±3.96 to 79.78±5.80 f/*l*; MCH 21.64±1.94 to 29.94±2.21 pg; Neutrophils 35.53±1.62 to 41.32±1.27% and Monocytes 2.31±0.16 to 3.10±0.13 (Table4). These changes were more pronounced in males than females.

Table 1:Physico-Chemical Parameters of Recruitment Pond and Tanks

	Before transfer		After transfer
	BR	FR	FR
Temperature (°C)	27.05±0.25	26.93±0.15	27.49±0.61
pH	6.71±0.11	7.27±0.35	7.91±0.28
$N\text{-}NH_3$ (mg L^{-1})	0.47±0.01	0.25±0.02	0.49±0.03
$N\text{-}NO_2^-$(mg L^{-1})	0.0042±0.01	0.001±0.01	4.28±0.18
Dissolved oxygen (mg L^{-1})	4.27±0.35	6.34±0.36	4.28±0.18
Sulfide (mg L^{-1})	0.03±0.01	0.01±0.01	0.01±0.01
Salinity (ppt)	11.33±0.57	0.06±0.41	0.11±0.13

Key: FR-Fresh Water in tank; BR-Brackish water in recruitment ponds

Table 2: Blood characteristics of male *S. melanotheron* before and after transfer

	Before transfer			After transfer		
	Mean*	Min	Max.	Mean**	Min	Max.
Hb	5.28±2.24^a	1.00	7.50	2.67±1.84^b	1.10	6.40
Ht	19.76±2.21^a	16.20	23.10	15.73±3.64^b	11.70	21.20
Lct	7.43±2.13^a	3.60	11.20	4.38±2.15^b	2.80	8.80
WBC	29.80±2.90^a	26.00	35.00	30.55±4.21^b	24.00	36.60
RBC	2.46±0.26^a	1.90	2.80	1.87±0.56^b	0.90	2.60
MCHC	26.95±11.02^a	4.67	35.57	20.36±9.91^b	6.35	30.62
MCH	21.01±8.65^a	4.54	35.57	14.56±7.55^b	5.50	24.62
MCV	80.52±9.19^a	65.60	31.27	69.01±25.53^b	20.49	93.08
Platelets	171.00±39.85^a	78.00	220.00	81.00±44.49^b	54.00	180.00
Neutrophils	34.61±6.61^a	21.20	40.40	41.70±5.78^b	33.40	49.10
Lymphocytes	40.92±6.05^a	32.70	55.60	36.98±4.43^b	30.10	41.20
Monocytes	2.14±0.58^a	1.20	2.90	2.78±0.56^b	1.80	3.7

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells×10 L^{-1}) MCV-Mean Corpuscular Valume (fl) WBC-White blood count (cells×10^9 L^{-1}); RBC-Red Blood Cells (Cell×10^6 L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular haemoglobin concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono -Monocytes (%). Means in the row with asterisks (*, **) with different superscripts are not significantly different ($p<0.05$)

Table 3: Blood characteristics of female *S. melanotheron* before and after transfer

	Before transfer			After transfer		
	Mean*	Min	Max.	Mean**	Min	Max.
Hb	5.84±2.06^a	1.60	7.80	4.70±1.98^b	1.90	7.80
Ht	20.06±5.43^a	10.10	25.40	17.87±3.77^b	12.70	24.30
Lct	5.75±2.15^a	3.20	9.60	7.44±3.22^b	1.60	12.80
WBC	28.88±4.68^a	19.00	32.00	31.85±3.1^b 0	26.00	36.80
RBC	2.52±0.33^a	1.90	2.90	1.91±0.56^b	1.10	2.70
MCHC	28.45±7.25^a	15.84	38.32	26.93±11.06^b	11.65	44.28
MCH	22.56±7.55^a	8.42	31.20	23.08±9.59^b	9.05	38.75
MCV	75.22±23.77^a	38.33	97.69	86.07±23.97^b	55.65	145.72
Platelets	167.42±33.95^a	112.00	210.00	123.00±33.58^b	82.00	190.00
Neutrophils	36.86±7.04^a	28.60	48.60	41.11±5.66^b	29.80	48.20
Lymphocytes	43.47±7.57^a	34.80	55.40	36.34±3.93^b	30.40	43.80
Monocytes	2.55±0.79^a	1.20	3.60	3.28±0.57^b	1.90	3.9

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells×10^{12} L^{-1}) MCV-Mean Corpuscular Valume (fl) WBC-White blood count (cells×10^9 L^{-1}); RBC-Red Blood Cells (Cell×10^6 L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular haemoglobin concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp - Lymphocytes (%) Mono -Monocytes (%). Means in the row with asterisks (*, **) with different superscripts are not significantly different ($p<0.05$)

Table 4: Blood characteristics of male and female *S. melanotheron* acclimated in fresh water (n = 210; each data point = 6)

	Before transfer			After transfer		
	Mean	Male	Female	Mean	Male	Female
Hb	5.51±0.51	5.28±0.71^a	5.84±0.77^b	3.95±0.49	2.67±0.69^a	4.7±0.57^b
Ht	19.88±0.90	19.76±0.70^a	20.06±2.05^b	17.07±0.87	15.73±1.38^a	17.87±1.09^a
Lct	6.74±0.34	7.43±0.67^a	5.76±0.81^b	6.32±0.73	4.39±0.81^a	5.44±0.93^a
WBC	29.42±0.88	29.81±0.91^a	28.89±1.77^a	31.37±0.80	30.56±1.59^a	31.85±0.89^a
RBC	2.48±0.06	2.461±0.85^a	2.53±0.13^a	1.89±0.12	1.87±0.23^a	1.91±0.16^a
MCHC	25.57±2.28	26.95±3.49^a	28.46±2.74^b	24.51±2.50	20.36±3.74^a	26.93±3.19^b
MCH	21.67±1.94	21.01±2.74^a	22.56±2.85^a	29.94±2.21	24.56±2.86^a	23.07±2.77^a
MCV	78.33±3.96	80.52±2.90^a	75.22±8.99^b	79.78±5.80	81.02±9.64^a	86.07±6.92^b
Plt	169.53±8.84	171.00±12.60^a	167.43±12.83^b	107.52±9.68	81.00±16.81^a	123.00±9.69^b
Neutrophils	35.53±1.62	34.61±2.09^a	36.86±2.66^a	41.32±1.27	41.7±2.19^a	41.10±1.64^a
Lymphocytes	41.97±6.61	40.92±1.91^a	43.47±2.86^a	36.57±0.92	26.99±1.67^a	33.34±1.13^b
Monocytes	2.31±0.16	2.14±0.18^a	2.56±0.29^a	3.10±0.13	2.78±0.21^a	3.28±0.16^b

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells$\times10^{12}$ L^{-1}) MCV-Mean Corpuscular Valume (fl) WBC-White blood count (cells$\times10^9$ L^{-1}); RBC-Red Blood Cells (Cell$\times10^6$ L^{-1}). MCH – Mean Corpuscular Haemoglobin (pg). MCHC – Mean Corpuscular haemoglobin concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). Means with the same superscript in the same row under before or after transfer are not significant different ($p<0.05$). Means in the same row under before or after transfer with same superscripts are not significantly different ($p>0.05$)

DISCUSSION

The results recorded in this study corroborated the report of Mukittrick and Leatherland (1983) who stated that a sudden charge in the water quality characteristics specific to the area inhabited by fish population could affect their haemotological indices. Reduction in heamoglobin and red blood cell ($p<0.05$), was also recorded in both *Oreochromis niloticus* and *Chrysicthys auratus* on exposure to atrazine (Hussein *et al.*, 1996). This reduction in blood haemoglobin and red blood cell may be due to the presence of stressor (change in environment), which caused haemogilution to occur due to impaired osmoregulation (Rottman *et al.*, 1992) or erythropoiesis in the organs responsible for production of RBC.

The reduction observed in leucocrit, platelets and lymphocytes may due to the reaction of fish to the effect of the stress induced by new environment. Dick and Dixon (1985) reported a significant reduction in leucocyte and lymphocytes of rainbow trout (*Salmo gaidneri*) after acute exposure to copper for 24 h. This was attributed to a generalized stress response resulting from increased pituitary-interrenal activity. Also Alkahem (1994) also observed a significant decrease in total leucocrit of *O. niloticus* exposed to sub lethal levels of nickel. This was attributed to reduction in the numbers of circulating thrombocytes and lymphocytes due to a reduction in lymphocytes delivery to the circulatory system and a rapid destruction of cells, which leads to an increased rate of peripheral removal of lymphocytes. Moroad and Houston (1988) attributed such lymphoperias to the lysis of lymphocytes after exposure to stressors. The increase observed in WBC and monocytes, agreed with the finding of Davids *et al.* (2002) who reported increase in WBC and monocytes of *Tilapia guineensis* and *S. melanotheron* after exposure to industrial effluents. The increased observed in WBC and monocytes may due to recruitment of more cells to combat the stressor (Ajani *et al.*, 2007).

CONCLUSION

Sudden change in fish environment has been observed to affects the blood composition of the fish, Therefore it is very important to culture fish in suitable environment for its adaptive physiology. If there is any need to cultivate *S. melanotheron* in fresh water it should undergo gradual acclimation rather than direct transfer, as this may not only lead to mortality but may hamper fish from its growth thereby reducing its full potential in aquaculture production.

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