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Comparative Effects of Different Acclimation Media on Haematological Characteristics of Brackishwater Tilapia, *Sarotherodon melanotheron* (Rupell, 1852)

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Abstract: A study was carried out to compare the effects of different acclimation media (brackish water, gradual reduction in salinity and direct transfer to fresh water) on haemotological parameters of *Sarotherodon melanotheron*. Six hundred and thirty *S. melanotheron* (mean weight 30.12 g±0.32 SD; mean total length 10.22 cm 0.14 SD) were sampled from recruitment ponds of African Regional Aquaculture (ARAC) Brackish Water Fish Farm, Buguma and Rivers State and acclimated in three different methods for seven days. Before acclimation the blood sample were taken, with mean values of Haemoglobin (Hb) 6.44±0.43 g L^{-1}; Haematocrit (Ht) 20.80±0.43%; Leucocrit (Lct) 6.93±0.29%; white blood cells 29.84±0.07×10^{9} cell L^{-1}, Red Blood Cell (RBC) 2.53±0.03×10^{12} cell L^{-1}; Mean Corpuscular Haemoglobin Concentration (MCHC) 31.36±0.98 g L^{-1}; Mean Corpuscular Haemoglobin (MCH) 25.60±0.81pg; Mean Corpuscular Volume (MCV) 81.16±1.80 f L; Platelets 173.43±3.46%; Neutrophils (Neut) 35.81±0.85% Lymphocytes (Lymp) 46.09±1.01%; Monocyte (Mon) 2.25±0.01%. Differences between male and female ($p<0.05$) before acclimation were recorded in Hb, Ht, RBC, Plt. The values of the blood parameters in the female were consistently higher than that of the male. There was reduction ($p<0.05$) in Hb, Ht, Lct, RBC, Plt and Lymp after while WBC, MCHC, MCH, MCV, Neut and Mon increased. These variations were more in males and more pronounced in *S. melanotheron* acclimated in fresh water. The study suggests that method of acclimation affects the blood characteristics of *S. melanotheron* and that where the fish is to be cultured in or acclimated to fresh water environment the best method should be by gradual salinity reduction. The latter method seemed to allow the fish system to adjust gradually to fresh water challenge with minimal impact on the blood characteristics.

Key words: *Sarotherodon melanotheron*, haematological characteristics, gradual transfer, direct transfer

INTRODUCTION

One of the production and management techniques used in brackish water species is acclimation, which is a prerequisite for stocking of ponds, both for culture and experimental purposes (Akinrotimi, 2006). Acclimation is the modification of biological functions, especially those of physiological structures to maintain or minimize deviation from homeostasis despite change in some environmental quality such as temperature, salinity, light, pH, hardness or toxicant concentration (Gabriel *et al*., 2001). Distinction therefore be made between acclimatization, a shifts taking place under natural conditions and acclimation Shifts taking place in artificial conditions (Newsman, 2003). It is a recommended practice to subject fish species to be used in laboratory experiments to acclimation period of at least seven days (Gabriel *et al*., 2004). Generally it is believed that during this period the fish may manifest symptoms of diseases that may assist in the separation of apparently healthy fish from sick individuals thereby ensuring that only healthy fish are used for any trial or culture. This ensures that results thus obtained are not influenced by the state of health of the fish.

Alterations in natural and chemical composition of aquatic environment have been confirmed to alter the behaviour, biochemistry and physiology of aquatic fauna (Alkahen *et al*., 1998). During the acclimation period the fish may undergo some degree of stress similar to that characteristic of high density stocking in intensive

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culture systems (Gabriel *et al*., 2004). Such overcrowding conditions have the likelihood of increasing the incidence of diseases that may produce a number of measurable changes in the physiological processes of the fish. Such changes include levels of serum enzymes, stress hormones and haematological parameters (Health, 1987). The use of haematological parameters in diagnosing the health of fish stocked in fish farms is gaining ground as a tool in the management of the health of farmed fish. According to Fernades and Mason (2003) haematological parameters are closely related to the response of the animal to the environment, an indication that the environment where fish lives could exert some influence on the blood characteristics.

Changes in the blood characteristics of *Clarias gariepinus* caused by stress because of exposure to environmental pollutants, diseases or attack by pathogens have been studied by a number of authors. (Onusiriuka and Ufodike, 2000; Ezeri, 2001). Also influence of acclimation on haemotological parameters of *clarias gariepinus* have be studied (Gabriel *et al*., 2004; Ezeri, 2004). However, report on the effects of acclimation, a common practise in brackish water aquaculture, on the blood parameters of fishes including black chinned tilapia are non-existent. The objective of the study was to assess the effect various acclimation media on the blood characteristics of the fish species.

MATERIALS AND METHODS

Six hundred and thirty adult, male and female *S.melanotheron* (mean length 10.22 cm±0.14 SD; weight 30.12 g±0.32 SD) were sampled from ARAC fish farm recruitment ponds at low tide. They were weighed and sexed. Blood samples were collected from 54 fishes (27 males and 27 females) before acclimation and same number were sampled for blood after acclimation, bringing the total fish of 108. The fish were moved in a separate container to the hatchery unit, where they were acclimated separately in tanks 0.36 m^3 with an effective water depth of 0.3 m for seven days. They were stocked 70 fish per tank. The fish were acclimated in three different media. The first, brackish water; the second, gradual reduction in salinity from 12 to 0 ppt, with two units per day over a period of 7 days, while the third, grown was acclimated in freshwater. Half of the water in the brackish and fresh water were renewed on the 3 day.

The fish were fed pelleted feed (35% crude protein) at 1% body weight daily. Physico-chemical parameters (pH, water temperature, dissolve oxygen, salinity, ammonia nitrogen and nitrite nitrogen) were taken using mercury in glass thermometer °C. pH was determined by use of a pH meter (Model HI 9812, Hannah Products, Portugal). Salinity was measured by hand held refractometer (Model HRN-2N Atago Products, Japan). Dissolve oxygen levels in the experimental tank were determined by Winkler method (APHA, 1985).

Standard haematological procedures described by Blaxhall and Daisley (1973) were employed in the assessment of the various blood parameter. Haemoglobin (Hb) was done by the cyanometaemoglobin method, Ht by micro haemotocrit method by the micro-wintrobe method WBC was determined with the improved Neubacuer counter; differential count was done on blood film stained with May-Grum-wald sa stain. Platelet count was done with methods in Brown. Lct was assessed according to Wedemeyer *et al.* RBC was estimated using the relationship between Hb and PCV. The following indices MCH; MCHC; and MCV were calculated according to Seiverd (1964).

Data obtained from the experimental fish were subjected to statistical analysis of ANOVA at 0.05% probability. Ducan multiple range were used to separate the means.

RESULTS

There were no obvious signs of disease or abnormality in the physical conditions and the behaviour of the experimental fish during the period of acclimation. A 25% mortality was recorded in fish acclimated in fresh water, 10% in the gradual acclimation treatment and 5% for experimental fish in brackish water. The result of the physico-chemical parameters of water indicated that the pH, were higher in fresh water compared to others; whereas DO level were lower, salinity was 12.10-12.33 (Table 1). The response pattern of Hb, Ht in all the acclimation methods were similar with the values for the female being consistently higher than that of the male before and after acclimation (Table 2 and 3). The values recorded in both sexes transferred directly to fresh water were low, compared to brackish and gradual acclimation. After acclimation the Hb and Ht gradually declined from brackish to gradual reduction in salinity and freshwater.

There were significant differences ($p<0.05$) in the values of leucocrit in relation to Acclimation method and period (Table 3). There was an increase in the number of WBC in all the acclimation method, in both sexes (Table 4). WBC values increased from 29.89±1.90, 28.89±1.77 unit to 30.56±1.59 unit and 31.85±0.89 unit for male and female, respectively for fish acclimated in brackish water; while for fish acclimated in fresh water, it was from 29.81±0.91 units, 28.89±1.77 units to 30.56±1.59 units and 31.85±0.89 units for male and female, respectively.

Table 1: Physico-chemical parameters of tanks in which *S. melanotheron* were acclimated for 7 days

	Before acclimation			After acclimation		
	BR	GR	FR	BR	GR	FR
Temperature (°C)	27.06±0.25	27.13±0.55	26.93±0.15	27.51±0.57	27.67±0.56	27.49±0.61
pH	6.71±0.11	6.64±0.14	7.27±0.35	6.51±0.11	7.51±0.36	7.91±0.28
N-NH_3 (mg L^{-1})	0.47±0.01	0.46±0.01	0.25±0.02	0.57±0.02	0.53±0.46	0.49±0.03
N-NO_2^-(mg L^{-1})	0.0042±0.01	0.0044±0.01	0.001±0.01	0.0048±0.08	0.0045±0.03	0.0017±0.12
Dissolved oxygen (mg L^{-1})	4.27±0.35	4.15±0.04	6.34±0.36	3.98±0.04	4.00±0.03	4.28±0.18
Sulfide (mg L^{-1})	0.03±0.01	0.03±0.02	0.01±0.01	0.039±0.12	0.01±0.02	0.01±0.01
Salinity (ppt)	11.33±0.57	12.1+0.95	0.06±0.41	12.14±0.61	0.34±0.12	0.11±0.13

Key: BR-(Brackish Water) GR (Gradual Reduction in Salinity) FR (Fresh Water)

Table 2: Haematological response of male *S. melanotheron* in different acclimation media

		After acclimation		
		Brackish water	Gradual acclimation	Fresh water
Hb	5.99±1.47^a	5.92±0.25^a	4.81±0.32^b	2.67±0.69^c
Ht	19.91±1.94^a	18.86±0.90^b	16.50±0.86^c	15.72±1.37^d
Lct	7.05±2.15^a	6.70±0.69^b	4.74±0.45^c	4.39±0.81^c
WBC	29.57±4.26^a	21.51±3.65^b	31.4±1.39^c	30.55±1.59^c
RBC	2.46±0.21^a	2.31±0.66^b	1.80±0.14^c	1.47±0.22^d
MCHC	30.47±7.72^a	33.82±1.71^a	31.56±3.04^a	20.36±3.74^b
MCH	24.61±6.27^a	32.68±2.98	46.59±9.68	14.56±2.85
MCV	81.20±9.69^a	92.09±12.61^b	118.82±12.81^c	69.02±9.65^d
Platelets	176.32±27.11^a	163.67±7.82^b	158.67±7.29^c	81.00±16.81^d
Neutrophils	34.89±5.94^a	36.81±1.94^a	39.4±1.48^b	41.70±2.19^b
Lymphocytes	45.79±7.51^a	50.48±1.89^b	34.60±2.47^c	36.98±1.68^c
Monocytes	2.28±0.80^a	3.02±0.16^b	3.01±0.24^b	2.78±0.21^c

Key: Hb-Haemoglobin (g dL^{-1}) Ht-Haematocrit (%) Lct-Leucocrit (%) MCV-Mean Corpuscular Valume (fl) WBC-White Blood Count (cells×10^9 L^{-1}) RBC-Red Blood Cells (Cell×10^{12} L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}) Plt-Platelets (×10^9cells L^{-1}) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). Means within the row, carrying the same superscript are not significant ($p>0.05$)

Table 3: Haematological response of female *S. melanotheron* in different acclimation media

		After acclimation		
		Brackish water	Gradual acclimation	Fresh water
Hb	6.94±1.35^a	6.83±0.18^a	5.86±0.40^b	4.70±0.57^c
Ht	21.79±3.88^a	20.18±1.10^a	17.78±0.78^b	17.68±1.08^b
Lct	6.80±2.19^a	6.40±0.66^b	4.95±0.36^c	3.44±0.93^d
WBC	29.73±5.53^a	31.11±0.89^b	30.91±1.64^c	31.85±0.89^b
RBC	2.59±0.23^a	1.92±0.10^b	1.91±0.17^b	1.90±0.16
MCHC	32.35±6.35^a	34.68±1.53^b	33.07±2.24^c	26.94±3.19^d
MCH	26.69±5.39^a	38.81±2.57^b	35.17±3.70^c	23.08±2.77^d
MCV	81.12±16.40^a	112.49±7.29	100.51±10.06	86.06±6.92
Platelets	171.24±23.07^a	161.00±5.25^b	156.77±7.53^c	123.0±09.69^d
Neutrophils	36.83±6.54^a	40.90±1.70^b	42.64±1.89^b	41.10±1.63^c
Lymphocytes	46.41±7.45^a	42.89±1.33^a	39.43±2.37^b	36.34±1.13^c
Monocytes	2.28±0.80^a	3.25±0.20^b	3.25±0.22^b	3.28±0.16^b

Key: Hb-Haemoglobin (g dL^{-1}) Ht-Haematocrit (%) Lct-Leucocrit (%) MCV-Mean Corpuscular Valume (fl) WBC-White Blood Count (cells×10^9 L^{-1}); RBC-Red Blood Cells (Cell×10^{12} L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}) Plt-Platelets (×10^9cells L^{-1}) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). Means within the row, carrying the same superscript are not significant ($p>0.05$). F-test significance:+Interactions- *$p<0.05$, **$p<0.01$, ***$p<0.001$, N.S-Not Significant

There was a decrease in the value of RBC in all acclimation methods which was more pronounced in fresh water (Table 4). MCV values were increased in all acclimation methods, with higher values in female more than the males (Table 2 and 3). Also, MCH increased in all the acclimation method.The highest increase was recorded in brackish water while the fresh water recorded the lowest (Table 4). MCHC value increased for both male and female in brackish water, but the MCHC reverse was the case in fish acclimated to fresh and gradual decrease in salinity (Table 2-4).

The number of thrombocytes decreased in all acclimation method, but was more noticeable in fresh water. The decrease in the number of thrombocytes was more in male than the female for all treatments. There was general increase in the number of monocytes and neutrophils in all the treatments. Increase in monocytes was more in female then male, but the reverse was the case with neutrophils. Also the number of lymphocytes throughout the experimental period decreased considerably with lowest value recorded in freshwater (Table 4). Values in the male were higher than that of the female (Table 2 and 3).

Table 4: Comparative effects of various acclimation media on haematological parameters of *S. melanotheron*

		After acclimation		
		Brackish water	Gradual acclimation	Fresh water
Hb	6.44±0.43[a]	6.25±0.15[a]	5.33±0.28[b]	3.93±0.48[c]
Ht	20.80±0.43[a]	19.52±0.71[b]	17.14±0.58[c]	17.07±0.86[c]
Lct	6.93±0.29[a]	6.67±0.46[a]	4.85±0.28[b]	4.31±0.73[c]
WBC	29.64±0.67[a]	26.31±2.16[a]	31.15±1.04[b]	31.37±0.80[c]
RBC	2.53±0.03[a]	2.25±0.34[a]	1.71±0.12[b]	1.89±0.12[b]
MCHC	31.36±0.98[a]	34.25±1.12[b]	32.31±1.84[b]	24.51±2.49[c]
MCH	25.60±0.81[a]	35.75±2.05[b]	40.88±5.21[b]	20.79±2.21[c]
MCV	81.16±1.81[a]	102.29±7.48[b]	109.66±8.20[b]	79.78±5.80[c]
Platelets	173.93±3.46[a]	167.33±4.66[b]	164.72±5.30[b]	107.53±9.68[c]
Neutrophils	35.81±0.85[a]	38.85±1.34[b]	41.02±1.22[c]	41.32±1.27[c]
Lymphocytes	46.09±1.01[a]	51.18±1.13[b]	38.02±1.85[c]	36.57±0.92[d]
Monocytes	2.25±0.09[a]	3.14±0.12[b]	3.13±0.16[b]	3.10±0.13[b]

Key: Hb-Haemoglobin (g dL^{-1}) Ht-Haematocrit (%) Lct-Leucocrit (%,) MCV-Mean Corpuscular Valume (fl) WBC-White Blood Count (cells$\times 10^9$ L^{-1}); RBC-Red Blood Cells (Cell$\times 10^{12}$ L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}) Plt-Platelets ($\times 10^9$cells L^{-1}) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). Means within the row, carrying the same superscript are not significant ($p>0.05$)

DISCUSSION

There were no significant differences between the physico-chemical parameters of the water in all the three acclimation method with the exception of salinity (Table 1) an indication that acclimation do not have adverse effects on the water parameters as since half of the similar observations was made by Gabriel *et al.* (2004) who studied the water was changed daily acclimation of *Clarias gariepinus* under similar conditions. The mortality recorded during the course of the trial could be have been attributed to handling stress and sudden transfer to fresh water due to disrupted homeostasis caused by salinity change (Newsman, 2003).

Summerfelt (1967) observed that males of rainbow trout consistently had significantly higher haematocrit values than the females and suggested the need to separate blood component data on the basis of sex to avoid attributing sex differences to other factors. The higher values of Hb, Ht, Lct, WBC, MCHC, MCH, MCV, thrombocytes, neutrophils and monocytes in the female than the males agrees with the finding of Ezeri (2001) in acclimated *Clarias gariepinus* the observed result according to Kubokawa (1999) may be due to the fact that females have higher resting plasma cortisol levels than do males and they do increase in Hb, WBC, Thrombocytes, levels after the confinement stress while the male have very low resting plasma cortisol level.

A significantly different ($p<0.05$) was observed in Thrombocyte in all acclimation, this corroborates the findings of Angelids *et al.* (1987) that the effects of confinement stress in rainbow trout, was significant, the females having platelets values than males. Acclimation caused reduction in the number of RBC, the acclimation methods and this was more visible in fish transferred directly to fresh water (Table 4) as was supported by Sharp *et al.* (1998) in juveniles of Chinook Salmon, during confinement stress. Did Sharp study effect of sex. The reduction in RBC according to Balm (1994) is due to the action of circulating stress hormones, catecholamines and corticosteroids, which elevate the plasma glucose levels and WBC indices thereby leading to reduction in the levels of RBC.

The lack of difference between the values of Lct, RBC, MCHC, MCH, MCV, Plt and neutrophil in brackish water and gradual acclimation methods may be that the system of the fish may have gradually adjusted to the shock and stress confirming the observations of Nikinmaa *et al.* (1984) who observed that gradual adjustment of striped bass to fresh/salt water challenge in the laboratory did not have a significant effect on its blood characteristics. Wide significant differences observed between fish acclimated in brackish and fresh water, might be due to deviation from homeostasis, which is dependent on species, stage of development, degree of salinity and temperature as was observed in Atlantic salmon, transferred abruptly into fresh water (Smith, 1999).

The study suggests that method of acclimation affects the blood characteristics of *S. melanotheron* and that where the fish is to be cultured in or acclimated to fresh water environment for the best method should be by gradual salinity reduction. This seem to allow the fish system to adjust gradually to fresh water challenge with minimal impact on the blood characteristics.

REFERENCES

Akinrotimi, O.A., 2007. Effects of Acclimation on Haematological parameters of *Sarotherondon melanotheron*; Post Graduate Diploma Project, Rivers State, University of Science and Technology, pp: 62.

Alkahan, H.F., Z. Ahmed, A.S. Al-Akel and M.J.K. Shams, 1998. Toxicity Bioassay and Changes in Haematological Parameters of *Oreochromis niloticus* induced by *trichlorofon. Arab Gulf* J. Societies Res., 16: 581-593.

Angelidis, P., F. Baudin-Lau Rencin and P. Youinou, 1987. Stress in rainbow trout, *salmo gairdneri.* Effects upon phagocyte chemicaluminescence, circulating leucocytes and susceptibility to *Aeromonas salmonicida*. J. Fish Biol., 31: 113-122.

APHA, 1985. Standard Method for the examination of water and waste waters (16th Edn.), Washington. Am. Pub. Health Assoc., pp: 260.

Balm, P.H.M., 1994. Adeno corticotropic hormone in relation to interregnal function during stress in tilapia (*Oreochromis mossambicus*). Gen. Comp. Endocrinol., 96: 347-351.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine Haemotological Methods for use in Fish Blood. J. Fish Biol., 5: 771-781.

Ezeri, G.N.O., 2001. Heamotological responses of *Clarias gariepinus* to bacterial Infection and Prophylactic Treatment with Antibiotic. J. Aqu. Sci., 16: 22-24.

Ezeri, G.N.O., U.U. Gabriel and O.O. Opabunmi, 2004. Heamotological Response of Cultures and Wild *Clarias Gariepinus* to Acclimation. Environ. Ecol., 22: 628-632.

Fernandes, M.N. and A.F. Mazon, 2003. Environmental Pollution and Fish Gill Morphology. Pp. 203-231. In: Fish Adaptation (Val, AL, Kapour, B.G. Eds.) Sciences Publication Enfield, U.S.A.

Gabriel, U.U., G.N.O. Ezeri and O.O. Opabunmi, 2004. Influence of Sex, Source, Health Status and Acclimation on the Heamotology of *Clarias garriepirus.* Afr. J. Biotech., 3: 463-437.

Heath, A.G., 1987. Water Pollution and Fish Physiology. CRC Press Boca Raton, Florida, pp: 245.

Kubokawa, K., 1999. Effects of acute stress on plasma cortiscol. Sex-steroid hormone and glucose levels in male and female sockeye salmon during the breeding season. Aquaculture, 172: 336-338.

Newsman, M.J., 2003. An evaluation factor in sensing and transfer of respiratory gases at the fish fill. J. Exp., 293: 449-463.

Nikinmaa, M., J.J. Coch and M. McEnroe, 1984. Blood oxygen transport in stressed stripped bass role of beta-and renergic responses. J. Comp. Physiol., 154: 366-369.

Onusiriuka, B.C. and E.B.C. Udodike, 2000. Effects of Sublethal Concentrations of Akee Apple, *Bligha sapida* and Sausage Plant, *Kigelia africana* on Tissue Chemistry of the African Catfish Clarias Gariepinus J. Aqu. Sci., 15: 47-49.

Seiverd, C.E., 1964. Haemotology for medical Technologists. Lea and Febiger Philadelphia, pp: 946.

Sharp, C.S., D.A. Thompson, A.L. Blenkership and C.B. Shreek, 1998. Effect of routine tagging stress responses in juvenile Chinook Salmon. Progr. Fish. Cult., 60: 81-87.

Smith, T.R., 1999. Characterization of Atlantic Salmon *Salmon salar* transferred into fresh water. Fish Physiol. Biochem. 20: 279-281.

Summerfelt, I.F., 1967. The haematological assessment of the health of fish, J. Fish Biol., 6: 218-225.