

Effect of Salinity Changes on Haematological Parameters of *Sarotherodon melanotheron* from Buguma Creek, Niger Delta

¹P.E. Anyanwu, ²U.U. Gabriel, ¹A.O. Anyanwu and ³A.O. Akinrotimi

¹Nigerian Institute for Oceanography and Marine Research,
 P.M.B. 12729, Victoria Island, Lagos, Nigeria

²Department of Fisheries and Aquatic Environment,
 Rivers State University and Technology, P.M.B. 5080, Port Harcourt, Nigeria

³Nigerian Institute for Oceanography and Marine Research, African Regional,
 Aquaculture Centre Aluu, P.M.B. 5122, Port Harcourt, Nigeria

Abstract: The effects of changes in salinity levels on some haematological indices of *S. melanotheron* were studied. The fish were sampled from the wild of salinity 12‰ and transferred to laboratory where the salinity which was level gradually reduced by 2 units everyday to 0.34‰ over a period of 7 days. This caused a consistent reduction ($p < 0.05$) in the blood characteristics with the value of haemoglobin 6.38 ± 0.71 to 4.81 ± 0.98 (g dL⁻¹); packed cell volume 19.03 ± 1.38 to $16.50 \pm 2.59\%$, leucocrit 5.60 ± 1.27 to $4.74 \pm 1.36\%$, red blood cell 2.50 ± 0.14 to 1.50 ± 0.44 ($\times 10^{12}$ cells), mean corpuscular haemoglobin concentration 33.83 ± 5.31 to 31.56 ± 9.13 g dL⁻¹ thrombocytes 177.22 ± 19.54 to 158.67 ± 21.87 ($\times 10^9$ cells) and lymphocytes 44.63 ± 6.67 to $34.60 \pm 7.40\%$. However, the other parameters in the treated group had an increment ($p < 0.05$) in the values white blood cells 30.00 ± 4.30 to 31.40 ± 4.17 ($\times 10^9$ cells); neutrophils 34.52 ± 4.57 to $39.40 \pm 4.42\%$, mean corpuscular volume 76.26 ± 6.06 to 118.82 ± 38.45 (pg); mean corpuscular volume 76.27 ± 6.08 to 118.82 ± 38.45 (fl) and monocytes 2.53 ± 0.63 to $3.01 \pm 0.72\%$. This study shows that gradual reduction in salinity levels exerts some degree of stress in the blood characteristics of *S. melanotheron* and that such changes could either be a reduction or an increase in the values depending on the parameters.

Key words: Salinity changes, haematology, osmoregulation, *Sarotherodon melanotheron*

INTRODUCTION

Tilapiine fishes (cichlidae), endemic to Africa, are widely distributed in tropical areas and have colonized a wide range of inland waters as natural or introduced species (Vannuccin, 2003). Among them the genus *Sarotherodon* whose numerous species account for a large part of catches in estuaries and lagoons (Panfili *et al.*, 2004). The black chinned tilapia, *Sarotherodon melanotheron* characteristic of estuarine and lagoon ecosystems in West-Africa (Blaber, 1997) are adapted to brackish-water bodies where they are regularly subjected to fluctuations in salinity levels (Fagade and Olaniyan, 1974; Trewavas, 1982; Whitefield and Elliot, 2002). Fluctuations in salinity undoubtedly impose stress on the physiology of the exposed fish population and can modify their structure (Leveque and Paugy, 1999). According to Albaret (1999) seasonal variation of salinity levels in the estuaries have direct effects on life-history traits of fish and particularly on reproductive traits. The need to respond to salinity change may be rapid such as

during tidal cycles or rapid movement of water bodies. It has been noted that changes in blood characteristics and plasma levels serve as primary link between environmental change and physiological response (McCormick, 2001).

An understanding of salinity effects on haematological characteristics *S. melanotheron* is essential to predict responses of fish populations to rapid environmental change. Haematological parameter have been recognised as valuable tools for the monitoring of fish health (Bhaskar and Rao, 1984; Schutt *et al.*, 1997) and in helping fish biologists interpret physiological responses to stress, imposed by the environmental characteristics of estuaries and lagoons. It has been observed that blood parameters such as haematocrit, haemoglobin concentration and RBC are related to environmental factors such as water temperature and salinity (Graham, 1997).

In spite of the vast numbers of reports on the haematology of the different species of fish, only a few studies have investigated the effects of salinity on fish

haematology (Sherrer, 1984; Hwang, 1989; Hiroi *et al.*, 1998; Uchida *et al.*, 2000; McCormick, 2003). None is available on the effects of salinity on haematological characteristics of *S. melanotheron* which prompted this study.

MATERIALS AND METHODS

Two hundred and forty healthy adults of *S. melanotheron* (mean weight 34.14g±0.36 SD and mean length 11.01 cm±0.16 SD) were sampled from Buguma creek, at low tide, they were immediately transferred to the hatchery unit of African Regional Brackish water Fish Farm at Buguma, Rivers State, Nigeria. They were stocked 100 fish each in three concrete tanks of 0.6×0.6×1 m. The tanks were half filled with water from the creek (salinity 12‰). The salinity was gradually reduced by 2 units daily to 0.13‰ over a period of seven days. The fish were fed pelleted feed (35% crude protein) at 1% body weight daily. The ration was split into two and dispensed at 0800 and 1700 h.

During the study physico chemical parameters were monitored. Measurements of temperature were taken at the beginning and the end of the experimental period using mercury in glass thermometer. Hydrogen ion concentration (pH) was determined by the use of a pH meter (model HI 9812, Hannah products, Portugal). Salinity was measured by hand held refractometer (Model HRN-2N. Atago products, Japan). Dissolved oxygen, Ammonite Nitrite and sulfide were determined using APHA (1985). Blood sample was collected before and after the trial by kidney puncture using heparinised 2 cm³ disposable syringes and 21-gauge hypodermic needle.

The blood samples were analysed according to the methods of Blaxhall and Daisly (1973) and Brown (1980).

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

The physico-chemical parameters from the wild and in the experimental tanks were not significantly different except in salinity (Table 1). The haematological parameter of male *S. melanotheron* before and after trial shown in Table 2 indicated that there was more pronounced reduction in the RBC compared to the other parameters. Differences in blood variables before and after trial for females (Table 3) were recorded in all the parameters the level of change recorded, was the lowest in RBC as in the

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

Parameters	Before trial Pond	After trial Tank
Temperature (°C)	27.13±0.55 ^a	27.67±0.56 ^a
pH	6.64±0.14 ^a	7.51±0.36 ^a
N-NH ₃ (mg L ⁻¹)	0.46±0.01 ^a	0.53±0.46 ^a
N-NO ₂ (mg L ⁻¹)	0.0044±0.01 ^a	0.0045±0.03 ^a
Dissolved oxygen (mg L ⁻¹)	4.15±0.04 ^a	4.00±0.03 ^a
Sulfide (mg L ⁻¹)	0.03±0.02 ^a	0.01±0.02 ^a
Salinity (ppt)	12.1±0.95 ^b	0.34±0.12 ^c

Table 2: Haematological parameter of male *S. melanotheron* before and after trial

Parameter	Before trial			After trial		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Hb (g dL ⁻¹)	6.38±0.71 ^a	5.40	7.60	4.81±0.98 ^b	3.80	6.10
PCV (%)	19.03±1.38 ^a	16.40	20.80	16.50±2.59 ^b	12.30	20.40
Lct (%)	5.60±1.27 ^a	3.80	7.60	4.74±1.36 ^b	2.90	6.80
WBC (×10 ⁹ cells)	30.00±4.30 ^a	25.00	40.00	31.40±4.17 ^b	26.10	38.00
RBC (×10 ¹² cells)	2.50±0.14 ^a	2.30	2.70	1.50±0.44 ^b	1.00	2.20
MCHC (pg)	33.83±5.31 ^a	27.41	41.46	31.56±9.13 ^b	20.43	49.17
MCH (g dL ⁻¹)	25.53±2.24 ^a	22.00	28.33	46.59±29.07 ^b	21.82	105.00
MCV (fl)	76.27±6.08 ^a	68.33	85.65	118.82±38.45 ^b	66.61	170.00
Thromb (×10 ⁹)	177.22±19.54 ^a	140.00	200.00	158.67±21.87 ^b	131.00	186.00
Neut (%)	34.52±4.57 ^a	27.40	41.20	39.40±4.42 ^b	29.60	44.20
Lymp (%)	44.63±6.67 ^a	33.70	54.50	34.60±7.40 ^b	20.10	41.20
Monocyte (%)	2.53±0.63 ^a	1.70	3.60	3.01±0.72 ^b	1.60	3.90

Means with the same superscript in the same row under before and after transfer are not significantly different (p>0.05)

Table 3: Haematological parameter of female *S. melanotheron* before and after trial

Parameter	Before trial			After trial		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Hb(g dL ⁻¹)	7.44±0.54 ^a	6.20	7.90	5.87±1.20 ^b	4.10	7.80
PCV(%)	22.07±2.90 ^a	17.60	27.30	17.78±2.36 ^b	14.10	21.60
Lct (%)	6.11±1.31 ^a	4.10	7.80	4.95±1.08 ^b	3.80	6.90
WBC (×10 ⁹ cells)	30.00±4.09 ^a	24.00	36.00	30.91±4.92 ^b	23.00	38.00
RBC (×10 ⁶ cells)	2.66±0.22 ^a	2.30	2.90	1.91±0.54 ^b	0.90	2.40
MCHC (pg)	35.02±5.35 ^a	28.70	44.31	33.06±6.74 ^b	22.98	46.42
MCH(g dL ⁻¹)	27.88±3.49 ^a	21.37	33.91	35.17±11.10 ^b	17.08	53.33
MCV (fl)	78.51±12.60 ^a	55.61	94.40	100.50±30.18 ^b	72.50	156.67
Thromb (×10 ⁹)	184.00±9.51 ^a	170.00	201.00	170.77±22.59 ^b	130.00	197.00
Neut (%)	38.74±4.79 ^a	28.40	46.20	42.64±5.69 ^b	32.70	50.50
Lymph (%)	45.26±6.53 ^a	33.70	53.60	41.43±7.13 ^b	31.20	54.20
Monocyte (%)	2.73±0.64 ^a	1.70	3.70	3.26±0.68 ^b	2.10	4.10

Means with the same superscript in the same row under before and after transfer are not significantly different (p>0.05)

Table 4: Mean values of *S. melanotheron* before and after trial (mean±SD)

Parameter	Before trial			After trial		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Hb(g dL ⁻¹)	6.92±0.82 ^a	5.40	7.90	5.33±1.19 ^b	3.80	7.80
PCV(%)	20.56±2.70 ^a	16.40	27.30	17.13±2.49 ^b	12.30	21.60
Lct (%)	5.86±1.28 ^a	3.80	7.80	4.85±1.19 ^b	2.90	6.90
WBC (×10 ⁹ cells)	30.00±4.07 ^a	24.00	40.00	31.15±4.43 ^b	23.00	38.00
RBC (×10 ⁶ cells)	2.57±0.19 ^a	2.30	2.90	1.70±0.52 ^b	0.90	2.40
MCHC (pg)	34.43±5.20 ^a	27.41	44.31	32.31±7.82 ^b	20.43	49.17
MCH(g dL ⁻¹)	26.71±3.09 ^a	21.37	33.91	40.88±22.14 ^b	17.08	105.00
MCV (fl)	77.39±9.66 ^a	55.61	94.40	109.66±34.82 ^b	66.61	170.00
Thromb (×10 ⁹)	180.6±115.31 ^a	140.00	201.00	164.72±22.45 ^b	130.00	197.00
Neut (%)	36.63±5.04 ^a	27.40	46.20	41.02±5.21 ^b	29.60	50.50
Lymph (%)	44.95±6.41 ^a	33.70	54.50	38.02±7.88 ^b	20.10	54.20
Monocyte (%)	2.63±0.63 ^a	1.70	3.70	3.13±0.69 ^b	1.60	4.10

Means with the same superscript in the same row under before and after transfer are not significantly different (p>0.05)

case for males. There were no significant differences in all the parameters between males and female, before and after the trial. The mean values of effects of salinity on haematology of *S. melanotheron* (Table 4) indicated reduction (p<0.05) in the values of haemoglobin packed cell volume, RBC, thrombocytes and lymphocytes, while the value of WBC, neutrophils and monocytes increased (p<0.05).

DISCUSSION

The haematological parameter of some fish species have been investigated with the purpose of establishing normal value ranges and any deviation from it may indicated a disruption in the internal physiology of fish (Rainza-Paiva *et al.*, 2000; Gabriel *et al.*, 2004). Haematological studies in fish can provide important information on the effect of the external environment on the internal physiology of fish (Masopust, 2000). Numerous phases have been described in fish haematological response regarding salinity changes. Zhiteneva (1999) described these phases as a period of rapid and significant change leading up to stabilization phase in which homeostatic mechanisms regulate internal conditions to a stable value within the tolerance range.

In this study haematological responses recorded in *S. melanotheron* following gradual reduction in salinity level from 0.12 to 0.13‰ indicate a crisis phase and compare favourably with studies on eels (Kirsch and Mayer, 1973) tilapia guineensis (Hwang *et al.*, 1989) barramundi sp. (Almendras, 1996). The reduction in the value of Hb and RBC is similar to the one recorded on Juvenile cobia *Rachycentron canadum*, exposed to various degrees of salinity (Denson *et al.*, 2003). This may be attributed to salinity induced osmo-regulatory dysfunction (Weirich and Tommasso, 1991). According to Putman and Freel (1978) different rates of fish activity demand different levels of metabolic activity, such activity requires several physiological adjustments, these includes haematological parameters. Packed Cell Volume (PCV) is a major haematological characteristics that changes with fish activity. The reduction in PCV, in this study corroborates the finding of Jawad *et al.* (2004) on India Shad *Tenuulosa ilisha* and the degree of change may be due to changes in water balance which will cause increase in blood volume and in the red blood cell resulting in decreased PCV (Cameron, 1970). The decrease in the value of lymphocytes and thrombocytes, in fresh water acclimated *S. melanotheron* is similar to that recorded in Atlantic salmon *Salmo gairdneri* and *oreochromis*

niloticus by Matushima and Mariano (1996) which suggested a suppression of production from the Haemopoetic organs. Reduced lymphocytes and thrombocytes indicate a weakened defense and delay clothing in the event of an injury to the fish in the new environment.

The increase observed after the trial in the values of WBC, neutrophils and monocytes is due to a non specific immune response to stress as a result of interaction of prolactin and cortisol hormone to restore ion balance in isosmotic salinity. This supports the findings of Eckert *et al.* (2001) who observed similar increase in channel catfish (*Ictalurus punctatus*) acclimated to different salinities. The variations in haematological parameters obtained in this study due to gradual reduction in salinity emphasizes the fact that changes in blood characteristics are an important indices in monitoring the effects of habitat changes on the physiology of *S. melanotheron*.

REFERENCES

- Albaret, J.J., 1999. Les Peuplements des Estuaries et des Lagones. In: Leveque C. Paugu D. (Eds.), Les Poisons des Caux Continentales Africaines, Editions de IIRD, Paris, pp: 326-349.
- Almendras, J.M.E., 1996. Plasma osmolality and chloride regulation in the sea bass, *Lates calcarifer*. *Bamigdeh*, 48: 28-34.
- APHA, 1985. Standard Method for the Examination of Water and Waste Waters (16th Edn.), Washington. Am. Public Hlth. Assoc., pp: 360.
- Bhaskar, B.R. and K.S. Rao, 1984. Influence of environmental variables on haematological ranges of milkfish, *Chanos chanos* (Forsk), in brackish-water culture. *Aquaculture*, 83: 123-136.
- Blaber, S.J.M., 1997. Fish and Fisheries of tropical estuaries. Chapman and Hall, London, UK., pp: 10-15.
- Blaxhall, P. and K.W. Daisley, 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.*, 5: 771-781.
- Brown, B.A., 1980. Haematology. Principles and Procedure. Lea and Fabiger, Philadelphia. (3rd Edn.), pp: 356.
- Cameron, J.N., 1970. The influence of environmental variables on the haematology of pinfish, *Lagodon rhomboids* and striped mullet, *mugil cephalus* comp. *Biochem Physiol.*, 32: 175-192.
- Denson, M.R., K.R. Stuart and T.I.J. Smith, 2003. Effects of salinity on growth, survival and selected Haematological Parameters of Juvenile *cobi-Rachycentron canadum* *J. W. Aqu.*, 34: 496-503.
- Eckert, S.E., T. Yadal, K.L. Stephand, M.H. Stetson, T. Hirano and E.G. Grau, 2001. Hormonal Control of Osmo regulation in the channel catfish, *Ictalurus punctatus* Gen. Comp. Endocrinol., 65: 201-234.
- Ezeri, G.N.O., 2001. Haematological response of *Clarias gariepinus* to bacteria infection and prophylactic treatment with antibiotics. *J. Aqu. Sci.*, 16: 22-24.
- Fagade, S.O. and C.I.O. Olaniyan, 1974. Seasonal distribution of the fish fauna in Lagos Lagoon. *J. Bull. F.A.N.*, 36: 245-252.
- Gabriel, U.U., G.N.O. Ezeri and O.O. Opabunmi, 2004. Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). *Afr. J. Biotech.*, 3: 463-407.
- Graham, J.B., 1997. Air-breathing Fishes: Evolution diversity and adaptation Academic Press, San Diego, pp: 10-26.
- Hiroi, J., T. Kaneko, K. Uchida, S. Hasegawa and M. Tanaka, 1998. Immunolocalization of vacuolar-type H⁺-ATPase in the the yolk-sac membrane of tilapia (*Oreochromis mossambicus*) larvae. *Zool. Sci.*, 15: 447-453.
- Hwang, P.P.; C.M. Sun and M. Wus, 1989. Changes of plasma osmolality, chloride concentration and gill Na-K-ATPase activity in tilapia *Oreochromis mossambicus* during sea-water acclimation: *Mar. Biol.*, 100: 295-299.
- Jawad, L.A., M.A. Al-mukhtar and H.K. Ahmed, 2004. The relationship between haematocrit and some biological parameters of the Indian Shad, *Tenulosa ilisha*. *Anim. Biol. Conservat.*, 27: 47-52.
- Kirsch, R. and N. Mayer-Gastan, 1973. Kinetics of water and chloride exchanges during adaptation of the European eel to sea-water. *J. Exp. Biol.*, 58: 105-121.
- Kori-Siakpere, P., 1985. Haematological characteristics, of *Clarias isheriensis*. *J. Fish Biol.*, 27: 259-263.
- Leveque, C. and D. Paugy, 1999. Les des poisons des caux continentales africaines Editions de 1 IIRD, Paris, France, pp: 200.
- Masopust, J.C., 2000. Clinical Biochemistry (in Czech) Karolinum, Prague, pp: 832.
- McCormick, S.D., 2001. Endocrine Control of Osmoregulation in Teleost. *Fish. Am. Zool.*, 41: 781-794.
- McCormick, S.D., 2003. Cortisol directly stimulates differentiation of chloride cells in tilapia opercular membrane. *Am. J. Physiol.*, 259: 857-863.
- Mctushima, E.R. and M. Mariano, 1996. Kinetics of the inflammatory reaction induced by carregenin in the swim bladder of *Oreochromis niloticus* (Nile Tilapia). *Braz. J. Vet. Res. Anim. Sci.*, 33: 5-10.

- Omoregie, E., 1998. Change in the haematology of the Nile Tilapia, *Oreochromis niloticus* under the effect of crude oil Hydrobiologia, 40: 287-292.
- Panfili, J., A. Mbow, J. Durand, K. Diop, K. Diouf, D. Thior, P. Ndiaye and R. Lae, 2004. Influence of salinity on the life-history of the West African black chinned tilapia (*Sarotherodon melanotheron*): Comparison between the Gambia and Saloum estuaries. *Aqu. Liv. Resour.*, 17: 65-74.
- Putman, W. and R.W. Freel, 1978. Haematological parameters of five species of marine fishes. *Comp. Biochem. Physiol.*, 61: 585-588.
- Rainza-Paiva, M.J.T., C.M. Ishikawa, A.A. DasEiras and N.N. Felizando, 2000. Haematological analysis of 'Chara' *Pseudoplatysoma Fasciatum* in captivity. *Aqua 2000. Responsible aquaculture in the new millennium: Nice, France. Eur. Aqu. Soc. Special Pub.*, 28: 590-592.
- Scherrer, P., 1984. Temperature and salinity influences on the growth and oxygen consumption of juveniles turbot *Scophthalmus maximus*. Brest University Thesis, pp: 160.
- Schuett, D.A., J. Lehmann, R. Guerlich and R. Hamers, 1997. Haematology of Swondtail Xiphiphorus helleri 1: Blood parameters and light microscopy of blood cells. *J. Applied Ichthyol.*, 13: 83-89.
- Trewavas, E., 1982. Generic groupings of Tilapini used in Aquaculture. *Aquaculture*, 27: 79-81.
- Uchida, K., J. Kaneko, H. Miyazaki, S. Hasegawa and T. Hirano, 2000. Excellent salinity tolerance of Mozambique tilapia (*Oreochromis mossambicus*): Elevated chloride cell activity in the branchial and opercular epithelia of the fish adapted to concentrated sea water. *Sciences*, 13: 219-227.
- Vannuccin, S., 2003. Overview of fish production, utilization, consumption and trade based on 2001 data. FAO, Fishery Information, Data and Statistic Unit, Rome, Italy, pp: 200.
- Weirich, C.R. and J.R. Tomasso, 1991. Confinement and transport-induces stress in white bass x striped bass hybrids: Effects of calcium and salinity. *J. World Aqu.*, 23: 49-57.
- Wedemeyer, G.A., R.W. Gould and W.T. Yasutake, 1983. Some potentials and limits of the leucocrit test as fish health assessment method. *J. Fish Biol.*, 23: 711-716.
- Whitefield, A.K. and M. Elliot, 2002. Fishes as indicators of environmental and ecological changes within estuaries; a review of progress and some suggestion for the future. *J. Fish Biol.*, 61: 229-250.
- Zhiteneva, L.D., 1999. Naturally occurring ecological influence on ichthyo-haematology. *Bull. Azov. Sci. Res. Inst. Fish Indus.*, A2: 1-53.