Variations in Oxygen Carrying Capacity of Sarotherodon melanotheron Blood in Different Acclimation Media

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Abstract: Variations in Oxygen Carrying Capacity of Sarotherodon melanotheron was investigated. The fish were acclimated using three media (brackish water, gradual reduction in salinity and fresh water) for a period of seven days. The results indicated various levels of reduction (p<0.05) exhibited by both juveniles and adults, before and after acclimation in different media. For fish acclimated in brackish water, Oxygen Carrying Capacity for juveniles (OCC) reduced from 5.17±0.87 to 4.70±0.59, vol. % adult fish from 8.46±0.75 to 8.17±1.01 vol.%. While in gradual acclimation, juveniles from 5.11±0.95 to 3.83±0.7 vol. % adults from 8.44±0.72 to 6.02±0.73 vol. %. And in fresh water, juveniles 3.74±0.87 to 2.57±0.94 vol. %; adults fish from 8.64±0.66 to 5.87±0.70 vol.%. These variations in oxygen carrying capacity were more pronounced in fish acclimated in fresh water and least variation in fresh brackish water. Results from this study suggests that acclimation, a common procedure, in brackish water fish farming exerts some degree of influence oxygen carrying capacity of the fish and hence the need to reckon with then when reporting oxygen and metabolic related activities of this species.

Key words: Variations in oxygen, acclimagtion media, Sarotherodon melanothern, Nigeria

INTRODUCTION

Oxygen carrying capacity in fish is the amount of oxygen that can be maximally carried by unit volume of blood (Nikinmaa, 2001). The oxygen capacity of whole blood comprises of oxygen in solution in the blood plus oxygen in combination with haemoglobin (Bone et al., 2004). In general, more than 90% of oxygen transported from the respiratory epithelia to the tissue capillaries is transported by haemoglobin that is contained within the erythrocyte (Brauner and Randall, 1998). Aerobic metabolism requires a supply of oxygen to the tissues and adequate elimination of acid and products like carbon-dioxide to prevents undue acidification of tissues. According to Pelster (2001) metabolic and ventilatory activities are tightly coupled in most fishes. In addition, the low solubility of oxygen in body fluids is compensated by the presence of respiratory pigments like haemoglobin, which increase the oxygen-carrying

capacity of blood or haemolyph by one or two order of magnitude (Fago et al., 1997). The optimal use of these respiratory pigments often requires loading and unloading of the oxygen in a very narrow range of oxygen partial pressures (Pelster and Rendall, 1998). Studies on the structure and functions of the respiratory pigment haemoglobin have revealed a striking flexibility in its intrisinc oxygen binding characteristics even among closely related species, caused by replacements in various amino acid residues of the protein moiety of these pigments (Jensen et al., 1998). Also, the haemoglobin may show large variability in their sensitivity to effectors protons, organic phosphates, environmental conditions, internal organizations and various fish management procedures in aquaculture (Angelichs et al., 1987; Pelster, 2001).

One of the management techniques commonly used in aquaculture is acclimation, which is a prerequisite for stocking of ponds both for culture and experimental

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purposes (Akinrotimi, 2006). Acclimation involve 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, has been reported by Gabriel et al. (2007) to cause some distortions in blood parameters of fish. Alkahem et al. (1998) observed that alterations in natural and chemical composition of aquatic environment to alter the behaviour, biochemistry and physiology of fish which intrinsically may have effects on its haematological properties and invariably affects its oxygen carrying capacity. According to Nikinmaa (2001) the amount of oxygen carried by unit volume of blood is determined by the number of oxygen-binding properties the haemoglobin and the prevailing environmental conditions of the fish.

Since oxygen carrying capacity of fish reflects the metabolic conditions of fish more quickly then other commonly measured parameters and since they respond quickly to changes in environmental and handling procedures in aquaculture (Alkinson and Judd, 1978), they have been widely used for the description of fish health (Blaxhall, 1972); for monitoring stress responses (Soivio and Oikari, 1976) and for predicting systematic relationships and the physiological adaptations of fish (Atamanalp and Yanik, 2002).

Available information in the literature on the oxygen carrying capacity of fish under various circumstances, include that of Rainbow trout-salmo gairdneri (Smith and Jones, 1982; Nikinmaa, 1982; Cossins and Richardson, 1985; Primmet et al., 1986) Common Corp-cyprinus carpio (Holk, 1996) hag fish-myxine glutinosa (Muller et al., 2003). Striped bass-morone saxatilis (Nikinmaa et al., 1984) sea lamprey-lampetra fluviatilis (Nikinmaa and Mattsof, 1992) European eel-Anguilla anguilla (Wood and Johnse, 1973; Pelster, 1995), but none is available on S. melanotheron a popular culture fish in the brackish water zone of Nigeria.

MATERIALS AND METHODS

Three hundred and twenty adult *Sarotherodon melanotheron* (Mean length 13.14±0.38SD; mean weight 45.26±0.26SD) and 320 juveniles (mean length 9.22±0.245 and mean weight 28.12±0.345) were harvested during the low tide from the recruitment ponds of African Regional Aquaculture Centre Brackish water fish farm, Buguma, Rivers State, Nigeria. they were immediately transferred to the heathery unit, where they were acclimated in nine

rectangular tanks of 0.36 m³, using three different methods with three tanks each. Brackish water Gradual acclimation (reduction in salinity from 12%) and direct transfer to fresh water, for a period of seven days. The fish were stocked at the rate of 70 fish per tank and were fed twice daily at 1% body weight. Half of the water in the tanks containing brackish (control) and fresh water were exchanged on the third day. While in trails involving gradual acclimation the water was replaced daily with a view to reduce the salinity levels.

During the experiment the following water quality parameter were monitored temperature, hydrogen ion concentration (pH) Dissolved oxygen (Do) salinity, ammonia nitrogen and nitrite nitrogen temperature were taken using mercury in glass thermometer (°C). 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). Dissolve oxygen in the experimental tank were determined twice at the beginning and at the end of the experimental fish was sexed by the examination of the number of orifices (openings) in the genital papilla.

Blood was sampled from a total of 108 fish, 54 males and 54 females. After collection the sample were preserved in EDTA bottles and labeled for easy identification and transferred to the department of medical laboratory Rivers State University of Science and Technology Port Harcourt for haematological analysis.

The blood were analysed based on the methods of Blaxhall and Daisley (1973), Haemoglobin (Hb) was done by the cyanomethaemoglobin method while oxygen carrying capacity of the fish blood was calculated by multiplying the haemoglobin content by 1.25, oxygen combining power of Hb/g (Johansen, 1970).

RESULTS

The result of the physico-chemical parameters of water, indicated, no significant difference in the parameter, except in salinity values in gradual acclimation, which was reduced from 12.08±0.4 to 0.20±0.11 (Table 1). The mean values of oxygen carrying capacity of fish, before and after acclimation, showed a trend which indicated reduction, in all acclimation media, which was more observable in fish acclimated in fresh water, (Table 2). For each acclimation medium stage and sex of fish, there is generally a reduction in the value of their oxygen carrying capacity before and after acclimation, in brackish water juveniles males value decreased from

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

	Before acclimation			After acclimation			
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Parameters	BR	GR	FR	BR	GR	FR	
Temperature (°C)	28.11 ± 0.25	27.02 ± 0.72	27.46 ± 0.18	27.61 ± 0.38	27.68±0.56	27.49 ± 0.61	
pН	6.56 ± 0.18	6.72 ± 0.67	7.06 ± 0.46	6.50 ± 0.28	6.58 ± 0.31	6.94 ± 0.41	
$N-NH_3$ (mg L^{-1})	0.49 ± 0.08	0.45 ± 0.07	0.28 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.49 ± 0.03	
$N-NO_2$ (mg L^{-1})	0.0042 ± 0.02	0.004 ± 40.01	0.00 ± 10.01	0.0048 ± 0.06	0.0045 ± 0.02	0.0018 ± 0.11	
Dissolved oxygen (mg L ⁻¹)	5.97±0.35	5.95 ± 0.04	6.84 ± 0.36	4.99 ± 0.12	4.86 ± 0.14	5.12 ± 0.26	
Sulfide (mg L ⁻¹)	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	
Salinity (0/00)	12.01 ± 0.32	12.08±0.41	0.08 ± 0.36	12.21±0.66	0.20 ± 0.11	0.02 ± 0.01	

Key: BR-(Brackish water); Gr-(Gradual Reduction Insalinity) Fr-(Fresh Water)

Table 2: Mean values of oxygen carrying of Sarotherodon melanotheron in different acclimation media

		Before acclimati	on		After acclima	tion			
Acclimation Media	Fish size	Mean	Min	Max	Mean	Min	Max		
Brackish water	Juveniles	5.17±0.87	3.76	6.23	4.70 ± 0.59	3.64	5.76		
Brackish water	Adults	8.46 ± 0.75	6.76	9.38	8.17±1.01	5.01	9.15		
Fresh water	Juveniles	3.74 ± 0.87	3.78	6.24	2.57 ± 0.94	1.26	3.74		
Fresh water	Adults	8.64±0.66	6.81	9.37	5.87±0.70	4.27	6.93		
Gradual acclimation	Juveniles	5.11±0.95	3.38	6.25	3.83 ± 0.74	2.75	4.97		
Gradual acclimation	Adults	8.44±0.72	6.78	9.25	6.02 ± 0.73	4.26	7.01		

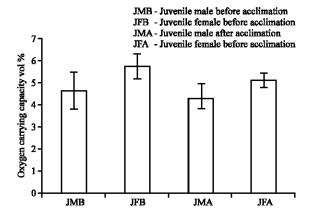


Fig. 1: Oxygen carrying capacity of Male and Female juvenile *Sarotherodon melanotheron* before and after acclimation in brackish water

AMB- Adult male before acclimation

AMA Adult male after acclimation

Oxygen carrying capacity vol %

4-3-

AMB

AFB - Adult female before acclimation

AFA - Adult female before acclimation

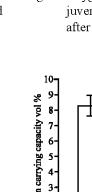


Fig. 2: Oxygen carrying capacity of Male and Female adult *Sarotherodon melanotheron* before and after acclimation in brackish water

AMA

AFA

AFB

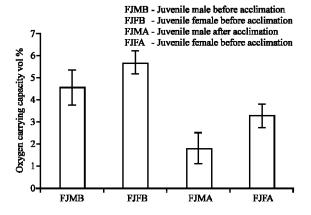


Fig. 3: Oxygen carrying capacity of Male and Female juvenile *Sarotherodon melanotheron* before and after acclimation in fresh water

FAMB - Adult male before acclimation

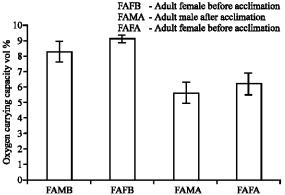


Fig. 4: Oxygen carrying capacity of Male and Female adult *Sarotherodon melanotheron* before and after acclimation in fresh water

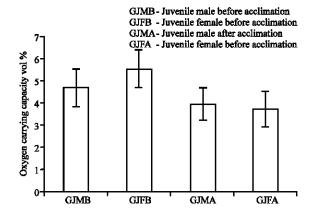


Fig. 5: Oxygen carrying capacity of Male and Female juvenile *Sarotherodon melanotheron* before and after gradual acclimation

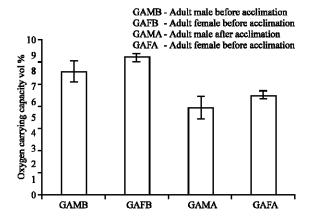


Fig. 6: Oxygen carrying capacity of Male and Female adult *Sarotherodon melanotheron* before and after gradual acclimation

 4.63 ± 0.81 to 4.29 ± 0.53 vol.%; juveniles females from 5.72 ± 0.54 to 5.12 ± 0.29 vol.% (Fig. 1), for adults; male 7.93 ± 0.69 to 6.93 ± 0.70 vol.%; female 9.00 ± 0.28 to 8.42 ± 1.23 vol. % (Fig. 2). In fresh water, the value of juvenile males decreased from 4.52 ± 0.49 vol. % (Fig. 3). While adults fish values in fresh water, indicated that in the males decreased from 8.20 ± 0.67 to 5.60 ± 0.61 vol.% females, 9.07 ± 0.21 to 6.15 ± 0.70 vol. % (Fig. 4). In gradual acclimation the value for juveniles male reduced from 4.693 ± 0.83 to 3.95 ± 0.71 vol. %, female from 5.53 ± 0.91 to 3.70 ± 0.79 vol. % (Fig. 5). And adults male value decreased from 7.95 ± 0.71 to 5.59 ± 0.79 ; a female adults reduced from 8.94 ± 0.22 to 6.46 ± 0.26 vol. % (Fig. 6).

DISCUSSION

Blood is a tissue which performs important roles in the organism, it transports oxygen and nutritive compounds (Bukowska et al., 2002). Fishes show an interesting diversity of approach to the problem of acquiring and transporting oxygen to the tissues in an aquatic environment. Fish are so intimately associated with their aqueous surrounding, in that any alterations in the physical and chemical changes in the environment reflected as measurable physiological rapidly changes in the fish (Musa and Omoregie, 1999). Therefore, blood is a pathophysiological reflector of the whole body and hence are important in diagnosing the structural, functional and metabolic of fish exposed to various degree of stress, as a result of handling, transportation, toxicants and acclimation (Adhikari et al., 2004; Akinrotimi et al., 2007; Anyanwu et al., 2007). Acclimation is the sum total of the adjustments which fish make to short and long term changes in their environment, the changes are in response to alterations in oxygen level, salinity, confinement and stocking density. The changes are complex mixtures of adjustments in hormones, metabolic pathways, enzymes and haematological parameters (Adeyemo et al., 2003).

Haematological parameters are very sensitive to stress of any kind. The decrease observed in the oxygen carrying capacity of S. melanotheron in all acclimation media, is as a results of confinement stress, similar observations was made by Gabriel et al. (2004) on Clarias gariepinus, subject to acclimation stress. The observed difference in oxygen carrying capacity between juveniles and adult fish may be due to red cell number and haemoglobin concentration which tends to increase with length, size and age of fish (Das, 1965). The pronounced reduction in the oxygen carrying capacity value, observed in the fish acclimated in fresh water, this is due to the sudden change in environment of the fish from brackish to fresh water which leads to impaired osmoregulation, reduction in the production of Red Blood Cell (RBC) and disruption of iron-synthesizing mechanism (Gabriel et al., 2007). The RBC are produced in the haemopoietic tissue, situated in the Spleen and Kidney (Health, 1991). According to Hibiya (1982) the most important function of RBC is the transport of haemoglobin which carries oxygen to all tissues in the body. Therefore, the reduction in the number of RBC automatically translates to a reduction in oxygen carrying capacity of S. melanotheron. This is in agreement with the findings of Adhikari et al. (2004) who observed a reduction in oxygen carrying capacity of Labeo rohita exposed to cypermethrin in the laboratory.

Kind *et al.* (2002) found that Australian lung fish *Neoceratodus forsteri* adjusted oxygen affinity during exposure to hypoxia, but not beyond the point where low oxygen in the water stimulated air breathing. Hence it is obvious that oxygen carrying capacity of fish, depends on external environment and internal organs of the fish.

CONCLUSION

In fish blood, oxygen is carried in physical solution and also in combination with haemoglobin, so physiologically, haemoglobin is crucial to the survival of S. melanotheron, as its role is directly related to the oxygen binding capacity of blood. This study suggests that drastic change in fish environment affects blood composition of the fish, which invariably affects its oxygen carrying capacity, which reduces its metabolic activity, leading to low productivity in aquaculture, this is because metabolic processes involve complex positional and temporal signals in which cells in the tissues communicate with each other, these processes are very precise and can therefore, be extremely sensitive to disruption by xenobiotic compounds and stressful environmental conditions. Therefore, the changes observed in oxygen carrying capacity of this species, in this study is a result of biological variability and sensitivity to the influences of acclimation.

REFERENCES

- Adeyemo, O.K., S.A. Agbede, A.O. Olaniyen and O.A. Shoogen, 2003. The haematological response of *Clarias gariepinus* to changes in acclimation temperature. Afr. J. Bio. Res., 6:105-108.
- Adhikari, Si., B. Sarkar, A. Chatterjee, C.T. Mahapatra and S. Ayyappan, 2004. Effects of cypermethrin and carbofuran on certain haematological parameters and prediction of their recovery in a freshwater teleost, *Labeo rohita* (Hamilton). Ecotoxi . Environ. Safety, 58: 220-226.
- Akinrontimi, O.A., 2006. Effects of Acclimation on haematological characteristics of Black-Chin Tilapia. Post Graduate Diploma Project Department of Fisheries, Rivers State University of Science and Technology Port Harcourt, pp. 65.
- Akinrotimi, O.A., U.U. Gabriel, P.E. Anyanwu and A.O. Anyanwu, 2007. Influence of sex, acclimation and period on haematological of *Sarotherodon melanotheron* (cichilidae). J. Fish Int., 2: 348-352.
- Alkahem, H.F., A.S. Ahmed, AI-Akel and M.J.K. Shams, 1998. Toxicity Bioassay and changes in Haemotological Parameters of *Oreochromis niloticus* induced by *trichlorofom* Arab Gulf. J. Soc. Res., 16: 581-593.
- Alkinson, J. and F.W. Judd, 1978 Comparative haematology of *Lepomis microlophus* and *chiclasoma cyanoguttarom*. Comp. Biol., 12: 23-237.

- Angelichs, P.F., Baudiro-LauRenein and P. Youinou, 1987. Stress in Rainbow trout. *Salmo gardner*. Effects upon phagocyte chemical luminescence circulating leucocytes and susceptibility to Aeromonas salmonicida. J. Fish Biol., 31: 113-122.
- Anyanwu, P.E., U.U. Gabriel, A.O. Anyanwo and A.O. Akinrotimi, 2007. Effect of salinity changes on Haematological Parameters of *Sarotherodon melanotheron* from Boguma Creek Niger Delta. J. Anim. Vet. Adu., 6: 658-662.
- APHA, 1985. Standard Method for the Examination of Water and Waste Water. (16th Edn.). Washington. Am. Pub. Health Assoc., pp. 260.
- Atamanalp, M. and T. Yanik, 2002. Alterations in Haematological Parameters of Rainbow trout *Oncurhynchus mykiss Exposed to mancozeb Turk*. J. Vet. Anim. Sci., 27: 1213-1217.
- Blaxhall, P.C., 1972. The haematological assessment of the health of fresh water fish: A review of selected literature. J. Fish. Biol., 4: 593-604.
- Blaxhall, P.C. and K.W. Daisley, 1973. Routine Haematological methods for use in fish blood. J. Fish Biol., 5: 771-781.
- Bone, Q., N.B. Marshall, J.H.S. Blaxter, 2004. Biology of Fishes. Garland Science/BIOS Scientific Publishers London, pp. 331.
- Brauner, C.J. and D.J. Randall, 1998. The Linkage Between Oxygen and Carbon Dioxide Transport. In: Penry, S.Y. and B.L. Tufts (Eds.) Fish Respiration. Acad. Press, San Diego, pp. 283-319.
- Bukowska, B.D., Pieniazek, W. Duda, 2002. Hemolysis and lipid peroxidation in human erythrocytes incubated with round-up current. Trop. Biol. Phys., 26: 245-249.
- Cossins, A.R. and P.A. Richardson, 1985. Adrenalin induced Na⁷/H⁺ exchange in trout erythrocytes and its effects upon oxygen carrying capacity. J. Exp. Biol., 118: 229-246.
- Das, B.C., 1965. Age-related trends in the blood chemistry and haematology of the Indian Carp (*Catla, catla*). Gerontologia, 10: 47-64.
- Fago, A., E. Bendixen, H. Malte and R.E. Weber, 1997. The anodic hemoglobin of *Anguilla* anguilla. Molecular basis for allasteric effects in a Root-effect hemoglobin. J. Biol. Chem., 272: 15628-15635.
- Gabriel, U.U., P.E. Anyanwu and A.O. Akinrotimi, 2007. Blood characteristics Associated. With confinement stress in Black Chin Tilapia Sarotherodon melanotheron J. Fish. Int., 2: 186-189.
- 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*. Afr. J. Biotech., 3: 463-437.

- Health, A.G., 1991. Water Pollution and Fish Physiology. (2nd Edn.), Lewis Publishers, Boca Raton, pp. 359.
- Hibiya, T., 1982. An atlas of fish histology-Normal and pathological features. Kodansha Ltd., Tokyo. *Stuttgart*, Gustav Fisher Verlog, pp. 147.
- Holk, K., 1996. Effects of isotonic swelling on the intracellular Bohr factor and the oxygen affinity of trout and carp blood. Fish. Physiol. Biochem., 15: 371-375.
- Jensen, F.B., A. Fago and R.E. Weber, 1998. Hemoglobin Structure and Function. In Fish Respiration. Perry, S.F. and B.L. Tufts (Eds.), San Diego, Acad., pp: 1-40.
- Johansen, K., 1970. Air-breathing fishes. In: Hoar, W.S. and D.T. Randall (Eds.). Fish Phy. Acad. Press, New York. London, Vol. IV, pp. 361-411.
- Kind, P.K., G.C. Grigg and D.T. Booth, 2002. Responses to prolonged hypoxia in the Australia long fish *Neoceratodus forsteri*. Res. Physiol. Neurobiol., 132: 179-190.
- Muller, G., A. Fago and R.E. Weber, 2003. Water regulates oxygen binding in hag fish (*myxine glutinosa*) haemaglobin. J. Exp. Biol., 206: 1389-1395.
- Musa, S.O. and E. Omoregie, 1999. Haematological Changes in the mudfish, *Clarias gariepinus* exposed to malachite green. J. Aquat. Sci., 14: 37-42.
- Nikinmaa, M., 1982. The effects of adrenaline on the oxygen transport properties of *Salmo gairdneris* blood Comp. Biochem. Physiol., 71: 353-356.
- 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.

- Nikinmaa, M., 2001. Haemoglobin function in vertebrates: evolutionary changes in cellular regulation in hypoxia. Respir. Physiol., 128: 317-329.
- Nikinmaa, M. and L. Mattsuff, 1992. Effects of oxygen saturation on the CO₂ transport properties of Lampetra red cells. Respir. Physiol., 87: 219-230.
- Pelster, B. and D.J. Randall, 1998. The physiology of the Root effect. In Fish Respiration edited by Perry S.F. and Tufts B.L. San Diego Academic, pp. 113-139.
- Pelster, B., 1995. Mechanisms of acid release in isolated gas gland cells of the European eel *Anguilla anguilla*. Am. J. Physical Regulatory Integrative Comp. Physiol., 269: 793-799.
- Pelster, B., 2001. The generation of hyperbaric oxygen tensions in fish. News Physiol. Sci., 16: 287-291.
- Primmett, D.R.N., D.J. Randall, M. Maze and R.C. Boutilier, 1986. The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow, trout (*Salmo gairdner*) during exercise. J. Exp. Biol., 122: 139-148.
- Smith, F.M. and D.R. Jones, 1982. The effect of changes in blood oxygen-carrying capacity on ventilation volume in the Rainbow trout *Salmo gairdneri*. J. Exp. Biol., 97: 325-334.
- Soivio, A. and A. Oikari, 1976. Haematological effects of stress on a teleost (*Esox lucius*). J. Fish. Biol., 8: 397-411.
- Wood, S.C. and K. Johansen, 1973. Organic phosphate metabolism in nucleated red cells: Influence of hypoxia on eel HbO₂ affainity. Neth. J. Sea Res., 7: 328-338.