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Influence of Sex, Acclimation Methods and Period on Haematology of *Sarotherodon melanotheron* (Cichilidae)

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Abstract: The influence of sex, methods and period of acclimation on haematological parameters of *S. melanotheron* was investigated. The fish were acclimated using three methods, (brackish water; gradual reduction in salinity and freshwater), for a period of 7 days. Pooled data for the trial indicate significant differences, in blood parameters before and after acclimation, in the values of haemoglobin ($p<0.001$), haematocrit ($p<0.05$), leucocrit ($p<0.05$), white blood cells ($p<0.05$), red blood count ($p<0.001$), lymphocytes ($p<0.001$) mean corpuscular haemoglobin ($p<0.01$), mean corpuscular volume ($p<0.01$) neutrophils ($p<0.001$) monocytes ($p<0.001$), while Mean Corpuscular Haemoglobin Concentration (MCHC) were not significant. Acclimation methods produced, various levels of interactions ($p<0.05$) in blood parameters, under consideration, with the exception of neutrophils and red blood count. The influence of sex ($p<0.05$) were involving haematocrit, haemoglobin, MCH platelests, neutrophils, lymphocytes and monocytes. Also, it was discovered that the female had higher values of blood parameter than the males. Results from this study suggests that sex methods and period of acclimation have some degrees of influence on the blood parameters of *S. melanotheron* and hence the need to take more cognizance of these when reporting haematological parameters of this species.

Key words: Sex, acclimation, methods, period, haematology

INTRODUCTION

Fish acclimation is a process of conditioning fish to new environment different from its natural habitat. (Akinrotimi, 2006). According to Diver (2005) it is an important aspect, of the gradual process involved in introducing fish to tanks, ponds or any culture medium. When fish is moved from its natural habitat, or from one location to another it experiences high levels of stress, which without proper acclimation makes it susceptible to a number unfavourable conditions, which include, impaired osmoregulation, stunted growth and mortality in extreme cases (Barton, 2002). For *S. melanotheron* to adjust, to new environment without much stress, it must properly, be acclimated. According to Balarin and Hatton (1979) stress reduces the rate of digestion in *S. melanotheron*, because improper handling of fish tends to reduce the acid concentration of the digestive juices which culminates in less digestion and absorption of feed leading to retardation of growth. Acclimation though time consuming and tedious is a key to healthy, vibrant and vigorous fish, for aquaculture development (Afonso, 2005).

Intensive culture of fish often involves the use of various handling and transportation procedures that cause stress resulting in disruption of haematological characteristics which ultimately leads to mortality.

According to Svobodova *et al.* (1994) study of haematological parameter are carried out on fish to ascertain the normal range of blood parameters, find out the variation with age, sex and season, determine the effect of disease condition on fish and the effect of certain chemical pollutants e.g., pesticides, heavy metals, petroleum products. The application of haematological techniques is therefore a valuable tool in fish biology, in the assessment of fish health and stress (Wedemeyer *et al*., 1983).

In recent years, haematological variables, have been used more often, when clinical diagnosis of fish physiology was applied, to determine the effects of

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external stressors (Ackerman, 2000). It has been illustrated that the use of haematological variables as indicators of stress, toxic substances as well as metals can provide information on the physiological response of fish to a changing external environment (Kori-Siakpere, 1985). This is as a result of the close association between the circulatory system of fish, gill and the external environment (Casillab and Smith, 1977).

Many authors have investigated the influence, sex and of acclimation on haematology of various species (Gabriel *et al*., 2004; Ezeri *et al*., 2004) but none is available on *S. melanotheron*, hence the need to undertake this study.

MATERIALS AND METHODS

Adults of *S. melanotheron* mean weight 40.12g±0.16SD and Mean Length 12.41cm + 0.18SD were harvested from the catching ponds of African Regional Aquaculture Centre, Brackish Water Research station Buguma, Rivers State. The fish were transferred to the hatchery units where they were acclimated in nine rectangular tanks of 0.36 m^3 using 3 different methods with 3 tanks each: Brackish water and gradual acclimation (reduction in salinity from 12ppt to opt) and direct transfer to fresh water over a period of 7 days. The fish were stocks 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 the gradual acclimation the water was replaced daily with a view to reduce the salinity.

During the study the following water quality parameters 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 H1 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, twice at the beginning and at the end of the experiment, by the Winkler method (APHA, 1985). The experimental fish was sexed by the examination of the number of Orifices (openings) in the genital papilla. There are two in females and one in males.

Blood was sampled from a total of 108 fish, consists 54 males and 54 females. After collection the blood 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 analysis.

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.

The blood were analysed based on the methods of Blaxhall and Daisley (1973).

RESULTS AND DISCUSSION

There was reduction in the value of Hb, Ht, Lct, RBC MCHC, Platelets and Lymphocytes and increase in the value of WBC, MCH, Neutrophils, with 5 units (Table 1). The values of haematological response of *S. melanotheron*, before and after acclimation were shown in (Table 1). It was observed that the females consistently had higher values of Hb, Ht, WBC, RBC, neutrophils, lymphocytes, MCH, MCHC and neutrophils than the males before and after acclimation while males had more Leucocrit count (Lct) and MCV than the females before acclimation but after acclimation the females had more. This shows that the males are more susceptible to stress of acclimation than the females.

Acclimation method, period, sex and interactions between them produced various levels of effects ($p<0.05$) in all the blood parameters except, interaction between acclimation method and sex (Table 2). However, acclimation period appeared to exert greater effect than sex of fish.

In the pooled data for Ht, mean separation showed significant difference at ($p<0.05$) at acclimation method and period only. While the other interactions levels are not significant, only the interactions between period, i.e., before and after acclimation, influenced significantly ($p<0.001$) the number of RBC (Table 2).

MCV values were increased in after acclimation significant differences ($p<0.001$) at acclimation method, ($p<0.01$) period and ($p<0.05$) for the interactions between acclimation method and period, while it was highly significant at acclimation method at ($p<0.001$) and also at the interactions between Acclimation method cum period ($p<0.01$, Table 2). There were significant differences ($p<0.001$) in the value of thrombocytes relative to

Table 1: Haematological response of *S. melanotheron* to acclimation, with respects to sex

	Male		Female	
Parameters	Before	After	Before	After
Hb	5.99±1.47[a]	4.81±1.93[b]	6.94±1.35[a]	5.69±1.67[b]
Ht	19.91±1.94[a]	17.13±3.14[b]	21.79±3.88[a]	18.53±3.33[b]
Lct	7.05±2.15[a]	3.35±2.08[b]	6.80±2.19[a]	6.45±2.54[b]
WBC	29.57±4.26[a]	27.60±8.49[b]	29.72±5.52[a]	31.35±3.53[b]
RBC	2.46±0.21[a]	2.03±1.32[b]	2.59±0.23[a]	1.88±0.48[b]
MCHC	30.48±7.72[a]	29.24±9.69[b]	32.35±6.35[a]	31.09±8.78[b]
MCH	24.62±6.27[a]	32.61±22.15[b]	26.69±5.40[a]	31.421±1.64[b]
MCV	81.20±9.69[a]	95.25±39.31[b]	81.11±16.39[a]	98.33±26.97[b]
Plt	176.32±27.11[a]	138.72±46.82[b]	171.24±23.07[a]	151.73±34.74[b]
Neutrophils	34.89±5.94[a]	39.11±5.48[b]	36.83±6.54[a]	41.51±5.37[b]
Lymphocytes	45.79±7.51[a]	40.98±9.36[b]	46.41±7.45[a]	42.53±8.23[b]
Monocytes	2.23±0.59[a]	2.95±0.59[b]	2.28±0.80[a]	3.27±0.59[b]

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells $\times 10^{12} L^{-1}$) MCV-Mean Corpuscular Volume (fl) WBC-White blood count (cells$\times 10^{9}$ L^{-1}); RBC-Red Blood Cells (Cell$\times 10^{6}$ L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). NB: Means within the row carrying the same superscript are not significant (p>0.05)

Table 2: Mean squares from ANOVA of haematological parameters of *Sarotherodon melanotheron* acclimated under different conditions and their level of interactions with period and sex

	Level of interactions					
Parameters	Accl method	Period	Sex	+ Accl and period	+Accl and sex	+ Period and sex
Hb	39.68***	38.76***	26.52**	7.02*	1.06[NS]	0.57[NS]
Ht	52.26*	232.36***	74.44**	2.46[NS]	1.84[NS]	0.28[NS]
Lct	38.98*	29.19*	4.18[NS]	1.81[NS]	1.00[NS]	11.69[NS]
WBC	75.34*	0.11*	83.86[N.S]	66.09[NS]	88.85[NS]	76.03[NS]
RBC	0.55[NS]	8.86***	0.01[NS]	0.84[NS]	0.93[NS]	0.47[NS]
MCHC	639.08***	41.04[NS]	133.18[NS]	94.36**	22.58[NS]	14.01[NS]
MCH	1781.59***	1050.22**	52.14[NS]	641.02*	240.44[NS]	3.18[NS]
MCV	2926.23***	6485.28**	240.08[NS]	2297.82*	871.12[NS]	293.85[NS]
Plt	14037.05***	22202.63***	1522.88*	9684.00***	1492.47[NS]	4378.58*
Neutrophils	30.89[N.S]	546.07***	113.41*	8.89[NS]	20.27[NS]	0.94[NS]
Lymphocytes	1452.49***	459.86***	47.76*	124.45*	43.04[NS]	38.66[NS]
Monocytes	1.500*	18.57***	1.03*	1.69[NS]	0.63[NS]	0.43[NS]

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells$\times 10^{12}$ L^{-1}) MCV-Mean Corpuscular Volume (fl) WBC-White blood count (cells$\times 10^{9}$ L^{-1}); RBC-Red Blood Cells (Cell$\times 10^{6}$ L^{-1}). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). NB: F-test significance ±Interactions - * Significant at p<0.05, ** Significant at p< 0.01, *** Significant at p<0.001, N.S-Not Significant

Table 3: Effect of acclimation period on haematological parameters of *Sarotherodon melanotheron*

	Acclimation period	
Parameters	Before	After
Hb	6.44±0.43[a]	5.29±0.44[b]
Ht	20.80±0.43[a]	17.9±0.44[b]
Lct	6.93±0.29[a]	5.45±0.32[b]
WBC	29.64±0.67[a]	31.64±0.872[b]
RBC	2.53±0.03[a]	1.94±0.12[b]
MCHC	31.36±0.98[a]	30.25±1.23[a]
MCH	25.59±0.81[a]	31.96±2.30[b]
MCV	81.16±1.80[b]	96.92±4.43[b]
Plt	175.92±3.45[a]	145.82±5.51[b]
Neutrophils	35.41±0.86[b]	40.42±0.74[a]
Lymphocytes	46.09±1.02[a]	41.83±1.17[b]
Monocytes	2.25±0.09[b]	3.12±0.81[a]

Key: Hb-Haemoglobin (g dL^{-1}), Ht-Haematocrit (%), Lct-Leucocrit (cells$\times 10^{12}$ L^{-1}) MCV-Mean Corpuscular Volume (fl) WBC-White Blood Count (cells$\times 10^{9}$ L^{-1}); RBC-Red Blood Cells (Cell$\times 10^{6} L^{-1}$). MCH-Mean Corpuscular Haemoglobin (pg). MCHC-Mean Corpuscular Haemoglobin Concentration (g dL^{-1}), Plt (platelets %) Neut-Neutrophils (%) Lymp-Lymphocytes (%) Mono-Monocytes (%). NB: Means within the row, carrying the same superscript are not significant (p>0.05)

acclimation method and period while significant differences (p<0.001) was observed in the number of monocytes vis-à-vis period, while for acclimation method and sex it was at (p<0.05) significant level (Table 2).

In acclimation methods and period the number of neutrophils increased (Table 3), which was more pronounced in male than female (Table 3). There was significant differences at (p<0.001) for period, while it was significant at (p<0.05) for sex. There were significant differences (p<0.001) in the number of thrombocytes in relation to acclimation method and period while for sex influence it was significant at (p<0.05, Table 2).

DISCUSSION

The overall results obtained, on the physiological indices as a result stress, reveal that acclimation of *S. melanotheron* leads to decrease in the values of

Haemoglobin, Haematocrit and RBC. This conforms to the results obtained by Srivastava and Mishra (1979) Mohamed (1995), Sikoki *et al.* (1989), Babatunde (1997), Seth and Saxena (2003), Yaji and Auta (2007) who observed similar decrease in various fish species exposed to various types of external stressor.

Males and females fish has been reported to differ in the way they respond to short-term stressor like handling, transportation, acclimation and trapping. According to Dacie and Lewis (1991) who believed, gender has a great influence on haematology of fish and included gender among factors influencing haematology, while Kori-Siakpere and Egor (1997) observed differences in haematology for different sexes of *C. buthupogon*. While Etim *et al.* (1999) did not observe any difference between male and female of *Chrysicthys nigrodigitatus*.

In this research, differences were observed in haematology of *S. melanotheron*, with the female had higher value, if Hb, Ht, Lct, WBC, MCHC, MCH, MCV, thrombocytes, neutrophils and monocytes then the males before and after acclimation. This is in agreement with the findings of Ezeri *et al.* (2004) who observed the same in *Clarias* under the influence of acclimation.

This sex-specific response in haematological status of fish, when exposed to any external stressor according to Barton (1997) may be linked to altered sex steroid hormones which acts as modulators of the stress response in fishes.

This was observed, in Rainbow trout from the wild, exposed to trapping and handling stress, the hormonal and haematological response in male was lower than that of females (Clement, 2002). Recently, exposure of juvenile Chinook Salmon to kraft mill effluent for 29 days has been shown to reduce the haemoglobin, RBC, Haematocrit and contisol levels in males, with no significant effect on the female. In contrast, only the females responded to this exposure at cellular levels, which is shown by increased hepatic HSP70 levels (Afonso, 2005).

Acclimation of these tilapias for seven days produced significant increase in WBC, neutrophils and monocytes but a significant reduction in the lymphocytes ($p<0.001$), this corroborates with the findings of Gabriel *et al.* (2004) on *Clarias gariepinus* under similar conditions. Changes in WBC indexes in population of fish under stressed conditions such as heavy metals (Musa and Omoregie 1999) handling (Angelidis *et al.*, 1987) and transport (Orji, 2005) is common. According to Harlow and Selye (1987) in the alarm reaction in fish, of which stress, unfavourable conditions and infections are major causes, there is actuation of the adrenal glands and corticotropic hormone which increase the WBC, causing a decrease in the peripheral lymphocytes and disintegration of lymphocytes in the lymphnodes. According to Gabriel *et al.* (2004) Reduced Hb, Ht, RBC, Thrombocytes and increased monocytes are good indictors of stress resulting from acclimation. All which have been consistently observed in this research.

CONCLUSION

This study has shown clearly, the influence of acclimation methods, period and sex on haematological parameters *S. melanotheron*. Also the importance of sex differences in modulating the haematological responses of fish under stress, this underscore the importance of taking sex of the fish into consideration when designing experiment for stress studies.

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