Response of *Sarotherodon melanotheron* Rüppell (1852) in the Niger Delta wetland, Nigeria to changes in pH

Respuesta del pez óseo común *Sarotherodon melanotheron* Rüppell (1852) en los humedales del Delta del Níger, Nigeria a los cambios de pH

Alex Chuks CHINDAH ✉[1], Amabaraye Solomon BRAIDE[1] and Olisa ORANYE[2]

[1]Institute of Pollution Studies. Rivers State University of Science and Technology. P M B 5080, Port Harcourt, Rivers State, Nigeria and [2]Department of Petroleum Resources. Nigerian National Petroleum Corporation, Moscow Road, Port Harcourt. E-mails: E-mails: alexchindah@yahoo.com and alexchindah@hotmail.co.uk
✉ Corresponding author

Received: 04/24/2008
First review received: 10/16/2008
First reviewing ending: 06/20/2008
Accepted: 10/27/2008

ABSTRACT

The response of a common Niger Delta wetland Cichlid (*Sarotherodon melanotheron* Rüppell) to changes in pH was assessed under renewal static assssy in the laboratory using physical attributes such as swimming and body movement (including opercular and fin movement), mucus deposition at the inner opercular cover in addition to hematological parameters such as erythrocyte and leucocyte numbers and hemotocrit values of the fish. Fishes were exposed to varying adjusted pH regimes of 3.6*,* 4.0, 5.0, 6.0, 7.0 and 8.0 by acidification and liming employing recommended standard procedures. The result demonstrated that the fishes surfaced to the top of the water column regular in erratic and unsteady manner with increased acid of the water. Fish's responses to different pH through hematological parameters as blood glucose, red blood cells and hematocrit) are also discussed.

Key words: *Sarotherodon melanotheron,* pH changes, hematological parameters, hematocrit

RESUMEN

La respuesta del pez *Sarotherodon melanotheron* (Rüppell) a cambios en el pH se determinaron bajo condición semi estática en el laboratorio usando atributos físicos tales como movimientos de natación y del cuerpo (incluyendo movimiento opercular y de aletas), deposición de moco en la cubierta opercular interna en adición a los caracteres hematológicos tales como número de eritrocitos y leucocitos y valores de hematocritos del pez. *S. melanotheron* se expuso a los regímenes de pH de 3,6; 4,0; 5,0; 6,0; 7,0 y 8,0 empleando procedimientos estándares recomendados. El resultó demostró que los peces estuvieron en el tope de la columna de agua de una manera errática e inestable con el incremento de la acidez. La respuesta de los peces a las variaciones del pH a través del fluido corporal (caracteres hematológicos –glucosa en la sangre, glóbulos rojos y hematocritos) es también discutida.

Palabras claves: *Sarotherodon melanothero*, cambios de pH, caracteres hematológicos, hematocritos

INTRODUCTION

Anthropologic activities change the environment quality. Magnitude of the resultant effects varies depending on the type, extent and quality of impacting conditions. The alterations have threatened functional attributes and the existence of aquatic organisms especially fish (FAO, 1997, Chindah and Hart 2000).

Activities such as construction, clearing of vegetation, dumping of solid wastes, industrial and municipal effluents especially in the wetlands acidify of the water body. Other common industrial activities in the Niger Delta region such as gas flaring amongst others yield combustion products such as CO_2, NO_2, CO, water vapour and soot or carbon particles, heavy metals and incombustibles in the atmosphere that are ionized and become chemically reactive as free radicals (Ibiebele, 1987). These chemicals and particles in presence of rainwater and water vapour, readily form acids (and other corrosive chemical compounds), which build up in the atmosphere and are eventually washed out as acid rain, altering the pH of the recipient medium. The presence of several industrial plants such as refineries, flow stations, Petrochemical, Liquefied Natural Gas and Fertilizer Plants in the region with their respective flare stacks

deposit large volumes of gas into the atmosphere. In addition, the effluent arising from these industrial activities is discharged into surrounding water bodies thus contributing significantly to the alteration of the pH of the aqueous medium (Spiff and Horsefall, 1998).

Changes in the pH and redox-potential of the aquatic environment are of great concern to all stake holders such as the Industries (IDS), Community Based Organizations (CBO), Academia (AC), Governmental Agencies (GA) and Non Governmental Agencies (NGO) following the declining catch of fin and non-fin fish species which had often times been attributed to altered water quality especially changes in pH (Spiff and Horsefall 1998). Some studies have implicated nutrient enrichment, increased heavy metals, and presence of pesticides to the reduced pH of the aquatic medium (FAO 1997; Brown *et al*, 1984; Sadler and Lynam, 1987). Physical (movement of body, fins, opercular bones) and physiological (hematological parameters) attributes of fishes have been used as indicator of fish responses to its externalities (Casillas and Smith, 1977). It is consequently crucial to use these attributes of fish in the monitoring of fishes responses to increasingly acidic pH levels.

Despite the threat posed by changes in pH in the aquatic systems of the Niger Delta region, little has been reported on its effect on fishes (Spiff and Horsefall 1998).

In an attempt to bridge the existing gap on the effects of reduced pH on fish, physical and hematological parameters were considered. In order to achieve this, *Sarotherodon melanotheron* a freshwater species was exposed to low pH regimes, to determine changes in hematological parameters (erythrocyte, leucocyte, and hematocrit values).

MATERIALS AND METHODS

Description of test species

The tested fish species is a fresh water type of the family Cichlidae - *Sarotherodon melanotheron* (Ruppel, 1852) that is commonly found in waters of the Niger Delta contributing in a high percentage to the artisanal fisheries of Southern Nigeria as their oily flesh tissue is greatly relished by most local people (Akiri, 1987 and Pudo *et al*.,1990). This species is characterized by deep pre-orbital bones, paternal mouth brooding habit and preference for brackish water environment as against the species such as *Tilapia zilli* (Trewavas, 1983). Colouration varies with location, sexual activity and changes with environmental background indicting a form of mimicry of the immediate habitat. The black spots on the chin and throat vary considerably both within and among populations. Mature males often have a proportionately large head caused by mouth brood (Akiri, 1987 and Pudo *et al*.,1990).

Sample collection

Sarotherodon melanotheron of almost uniform length (5.7 ± 0.5cm) and weight 3.6± 0.4g) were collected with drag-nets from freshwater fishpond at African Regional Aquacultural Centre Aluu, Portharcourt. Samples were sorted to different size classes using standard length (cm) and weight (using a OHAUS Triple Beam Balance - g) and sex of the fish not accounted for during the experiment. In the field, fishes considered healthy on the basis of their appearance and absence of obvious signs of stress were transferred to large holding tanks for immediate transportation to the laboratory (Kori-Siakpere, 1985).

Experimentation

Acclimatization of test species laboratory conditions

In the laboratory, 750 individuals collected were transferred and equally distributed using portable hand net into twenty five (25) 80-litre capacity glass tanks (i.e. 30 fishes in each) with each tank measuring 65 cm x 35 cm x 35 cm and filled with 50 L of water from the natural environment. Portable aerating pumps were connected to each tank for oxygenation. A 1.3 KVA Honda generating set was on standby as alternative power supply source, of. A 1.91 cm nylon mesh was carefully positioned at the top of each tank to prevent fish escape as a result of jumping. Fishes were observed daily and any dead, injured or morbid ones were removed immediately. They were fed twice daily between 0900 hrs and 1000 hrs, and between 1500hrs and 1600hrs on a special diet of 30% crude protein marshed fish feed and kept in this condition for 2 weeks (Kori-Siakpere, 1985). These fish formed the ready stock for the 96 hr LC_{50} and the treatment schedule.

96 hr LC_{50} test

A 96 hr LC_{50} test was carried out for the selected fish species within an acute toxicity range of pH 2.5 to 4.0. The test was to serve as a guide in determining the lower-limit pH value for the study (Chindah *et. al* 2004).

Twenty of each already acclimatized samples were introduced into each of the 15 tanks containing 50 litres of fresh water. Tanks were maintained at five pH values - 2.5, 3.0, 3.3, 3.6, and 4.0 by adding concentrated H_2SO_4 (BDH, GR grade). The tank for each pH value was setup in triplicate. The acid dropping system (Dheer *et al*., 1987) was done for all tanks to ensure constant pH during the 96-hour exposure period.

Concentrated H_2SO_4 (96% Stock) was dropped from a 2ml pipette into a beaker containing one litre of natural water and then using a CORNING pH meter model 7, the desired pH for the volume of water was attained. The volume of H_2SO_4 required to adjusting 50 litres of water was applied and thoroughly stirred for few seconds and re-measured with a pH meter to ensure the desired pH value.

The tanks were maintained for 96 hours. They were cleaned and water changed after 2-day interval when the concentration of H_2SO_4 (or alkali) was adjusted to counteract the pH drift due to release of excretory products and other metabolites. Continuous aeration was maintained throughout the experimental period to avoid, the building up of any free CO_2 which is toxic and capable of altering the pH in the tank. Observations were made every 24 hours and numbers of dead and live fishes were recorded. Fishes were considered dead when they lost their equilibrium, floated with ventral sides up and did not respond to touch and they were promptly removed.

The arithmetic graphic method was employed in determining the 96-hr LC_{50}. Percentage mortality after 96 hours was calculated and plotted on the ordinate axis against the pH value on the abscissa. Each point was then plotted and connected graphically. A horizontal line was drawn from the 50% survival point to intersect the plot from which point, a vertical line is dropped to the abscissa. This intersection point on the abscissa corresponded to the 96 hr LC_{50}. This was done for all three replicates for each pH value and the mean determined. Safe pH level used as lower limit for the selected range was determined using an application factor of 1.03 based on the work of Reish and Oshida (1986).

Hematological analysis on each treatment were conducted on weekly basis by sacrificing 2 fish species and blood samples collected using insulin syringe and needle rinsed with EDTA to determine the various hematological parameters (Wedemeyer and Yasutake, 1977). The significant differences among means were tested with 2 –way analysis of variance (ANOVA, 0.05) (Zar, 1984).

Analysis of physicochemical parameters

Water samples were analysed regularly to ensure that the expected water quality were maintained. The analyses for the water quality were conducted using standard procedures as indicated in APHA (1998)

RESULTS

Physical-chemical quality of waters

The results of the physico chemical analysis of the water surface water of the treatment and control tanks are presented in Table 1.

96hr L.C_{50} test

Data on 96hr exposure of twenty (20) samples each of *S. melanotheron* at different pH levels are presented in Table 2. The test showed increased mortality with increased acidity. With the arithmetic graphic method, the 96hr LC_{50} for *S. melanotheron* was 3.68 (Figure 1).

Effect of pH on *Sarotherodon melanotheron*

Behavioural Changes

S. melanotheron samples were observed to exhibit very erratic and disturbed movement, which increased at low pH levels (Table 3). While the response was immediate at pH 3.8 (commencing in day 1), it was delayed at pH 4.0 and 5.0, commencing in days 3 and 6 respectively. Between weeks 2 and 3, the intensity of behaviour was reduced considerably when compared to normal at pH 3.8, 4.0 and 5.0 as was observed in the control. At weeks 4 and 5 movement was slow and lethargic in fish maintained at pH 3.8 and 4.0 respectively. Fish

Table 1. The range and mean of the water quality (physico-chemical) of the treatment and control tanks of water samples in the Niger Delta wetland, Nigeria.

Treatment	Feature	Temperature (°C)	pH	Conductivity µS/cm	Dissolved oxygen (mg/l)	Biochemical oxygen demand BOD_5 (mg/l)
3.6	Range	23.6-24.1	3.6	36.0-38.0	3.0-3.7	0.58-0.66
	Mean	23.8	3.6	37.0	3.21	0.64
4.0	Range	23.5-24.2	4.0	36.0-40.0	3.32-3.8	0.56-0.64
	Mean	23.7	4.0	38.2	3.4	0.62
5.0	Range	23.4-24.2	5.0	36.0-38.5	3.1-3.8	0.59-0.69
	Mean	23.8	5.0	37.1	3.5	0.64
6.0	Range	23.4-24.6	6.0	36.2-39.3	3.1-3.8	0.57-0.68
	Mean	23.9	6.0	37.7	3.5	0.63
7.0	Range	23.6-24.3	7.0	36.6-38.8	3.2-3.8	0.57-0.66
	Mean	23.8	7.0	37.7	3.5	0.63
Control	Range	23.5-24.1	6.3-6.5	36.8-39.0	3.1-3.8	0.58-0.71
	Mean	23.8	6.4	37.2	3.5	0.65

Table 2. Mean mortality after 96hrs exposure of *Seratherodon melanotheron* to different pH in the Niger Delta wetland, Nigeria.

	Mortality/Duration				Total	Percentage
pH	24 hrs	48 hrs	72 hrs	96 hrs	mortality	Mortality
2.5	20	N.M	N.M	N.M	20	100
3.0	8	6	3	1	18	90
3.3	7	4	2	2	15	75
3.6	5	4	2	1	12	60
4.0	N.M	N.M	N.M	N.M	N.M	0

NM: No mortality

maintained at pH 6.0, 7.0, 8.0 and control did not show any abnormal pattern in fish movement.

Neither shoaling nor surfacing for atmospheric air was observed in the different tanks. Mucus secretion was high at low pH levels of 3.8, 4.0 and 5.0 at weeks 3, 4 and 5 respectively. Secretion was normal in the other tanks throughout the test period. While mortality exceeded 50% at pH 3.8, and 4.0 at weeks 5 and 6 respectively, pH 5.0 and 6.0 recorded low mortality rates of less than 50%. Survival of 100% was observed in fish kept at pH 7.0, 8.0 and control tanks.

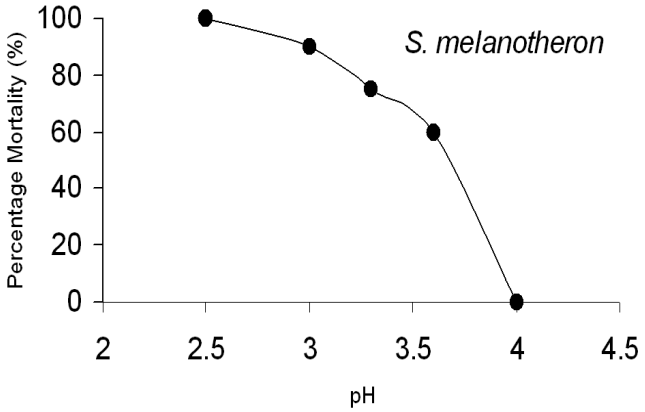

Figure 1. 96hr Percentage mortality against pH of *Seratherodon melanotheron* in the Niger Delta wetland, Nigeria.

Blood Glucose

Blood glucose levels increased with acid level (Figure 2). At pH 3.8 and 4.0 the glucose levels showed exponential increases over time. At pH 5.0, increase in values with time was also observed

except for a decline in week 3. Similar pattern was exhibited at pH 6.0 though at lower values. At pH 7.0, the glucose level indicated initial increases to week 3 but fluctuated thereafter. Glucose level fluctuated at pH 8.0 to week 5 but stabilized at week 6. In fish in the control tanks (pH), the blood glucose remained relatively uniform value throughout the experimental period (Figure 2). The values showed significant differences in the blood glucose between the treatments [F cal = 74.05 > P (2.60) $_{0.05}$] and with exposure time [F cal= 14.50 > P (2.60) $_{0.05}$].

Table 3. Behavioural changes in *Sarotherodon melanotheron* at different pH levels in the Niger Delta wetland, Nigeria.

Indices	pH						
	3.8	4.0	5.0	6.0	7.0	8.0	Control
	Fast and Very Erratic /Day 1	Fast and Very Erratic /Day 3	Fast and Very Erratic / Day 6	Fast and Erratic /Day 1	Fast and Erratic/ Day 1	Fast and Erratic/ Day 3	Fast and Erratic/ Day 1
Movement Intensity/ Onset	Fast and erratic/ weeks 2 and 3	Fast and erratic/ weeks 2 and 3	Fast and Erratic/ Week 2				
	Slow and Lethargic /week 4	Slow and Lethargic/ week 5					
Mucus Secretion: Intensity/ Onset	+++/WK3	+++/WK4	+++/WK5	+/WK1	+/WK1	+/WK1	+/WK1
Mortality	60% Wk 5	55% Wk 6	55% Wk 8	0	0	0	0

+++ : High; ++ : Medium; + : Normal and - : Did not occur

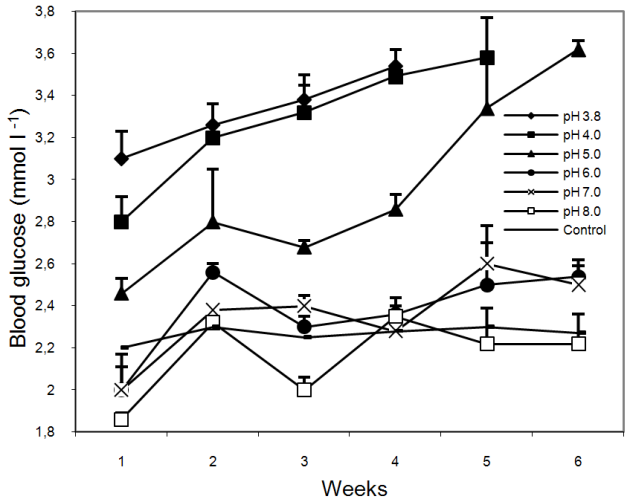

Figure 2. Effect of pH on number of erythrocytes (x10^6 mm^{-3}) of *Sarotherodon melanotheron* each week in the Niger Delta wetland, Nigeria.

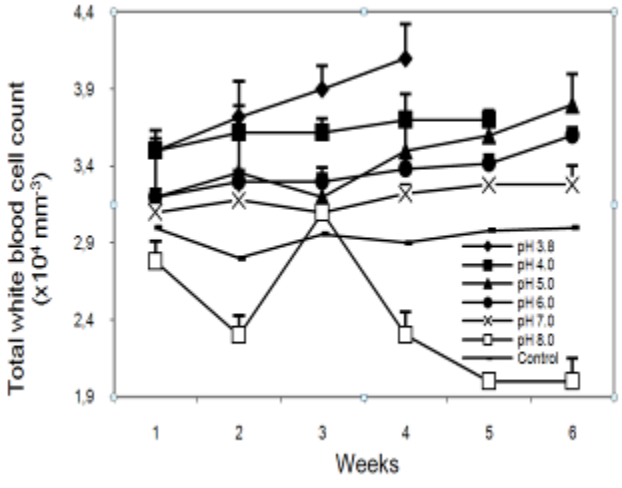

Figure 3. Effect of pH levels on total white blood cell count (x10^4 mm^{-3}) of *Sarotherodon melanotheron* each week in the Niger Delta wetland, Nigeria.

Total white blood cell count

The total white blood cell count of *S. melanotheron* at different pH levels is presented in Figure 3. *S. melanotheron* exposed to pH 3.8, 4.0, 5.0 and 6.0 showed a gradual increase in the count over time except for a slight decline at week 3 for pH 5.0 (Figure 3). At pH 7.0, values were observed to change marginally throughout the exposure time. At pH 8.0, the white blood cell count showed more pronounced fluctuation; it declined in week 2, rose in weeks 3 and then declined steadily through the remaining weeks. Ovoid-shaped leucocytes with eccentric nuclei were observed under the microscope. Differential count showed that these cells occurred mostly as lymphocytes and neutrophils. Monocytes occurred in very low percentages (Table 4).

Statistical analysis of the changes recorded showed significant differences in the effect of pH [F cal =21.68 > P (2.60) $_{0.05}$]. However there was no statistically significant difference in cell count with exposure period [F cal = 0.533 < P (2.60) $_{0.05}$].

Red blood cell count

The red blood cell count of *S. melanotheron* at different pH levels are presented in Figure 4. The changes at pH 3.8, 4.0 and 5.0 are consistent with those observed with other parameters; a sharp rise with exposure time, the rise being proportional with the acid stress (Figure 4).

The changes in red blood cell count did not appear appreciable at pH 6.0 whereas at pH 7.0, values rose gradually to week 3 and stabilized in the remaining weeks. At pH 8.0, no definite pattern was

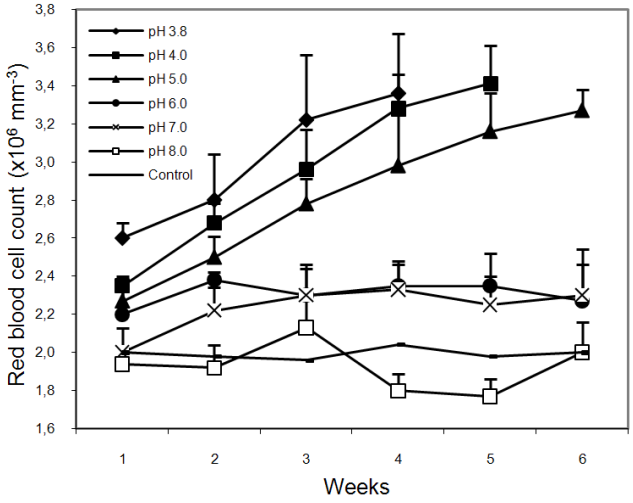

Figure 4. Effect of pH on Mean Red blood cell count (x10^6 mm^{-3}) of *Seratherodon melanotheron* each week in the Niger Delta wetland, Nigeria.

Table 4. Mean values of differential Leucocyte Count (%) of *Sarotherodon melanotheron* exposed to different pH levels and weeks in the Niger Delta wetland, Nigeria.

Weeks	1			2			3		
pH	Lym	Mn	Nt	Lym	Mn	Nt	Lym	Mn	Nt
3.8	77.58	2.86	19.56	75.00	2.97	22.03	76.50	3.08	20.42
4.0	80.00	2.92	17.08	82.75	3.36	13.89	85.50	3.48	11.02
5.0	83.00	2.92	14.08	82.18	3.40	14.42	80.95	3.62	15.43
6.0	83.24	2.98	13.78	81.00	3.72	15.28	82.00	3.80	14.20
7.0	85.00	3.60	11.40	83.23	3.75	13.02	83.55	3.88	12.57
8.0	85.00	3.50	11.50	87.10	3.75	9.15	88.20	3.84	7.96
Control	83.50	3.50	13.00	85.00	3.50	11.50	85.00	3.00	12. 00

Weeks	4			5			6		
pH	Lym	Mn	Nt	Lym	Mn	Nt	Lym	Mn	Nt
3.8	74.80	2.80	22.40	75.00	2.86	22.14	75.00	2.80	22.20
4.0	84.00	3.60	12.40	84.00	3.10	12.90	85.37	3.00	11.63
5.0	80.00	3.66	16.34	81.22	3.56	15.22	81.80	3.50	14.70
6.0	82.00	3.82	14.18	82.66	3.90	13.44	83.00	3.90	13.10
7.0	81.60	3.84	14.56	81.00	3.76	15.24	81.80	3.90	14.30
8.0	85.60	3.90	10.50	85.00	3.90	11.10	87.00	3.88	9.12
Control	88.50	2.00	9.50	84.00	2.50	13.50	85.00	3.50	11.50

Lym: Lymphocytes; Mn: Monocytes and Nt: Neutrophils

observed in the changes in RBC count. Control pH maintained relatively steady values throughout the period with nucleated and non-nucleated cells also observed.

Statistical analysis of data showed that calculated F is greater than the critical F for effects due to both pH and exposure time. Hence, there is a significant difference in the recorded changes due to pH effect; [F =29.62 > P (2.60) $_{0.05}$] and exposure time, [F (5.93) > P (2.60) $_{0.05}$].

Hematocrit

The hematocrit values of *S. melanotheron* at different pH levels are presented in Figure 5. At pH 3.8, 4.0 and 5.0, the hematocrit values increased throughout the experimental period except for a very slight decline in week 2 at pH 5.0. At pH 6.0 values increased steeply up to week 3 after which the increases became more gradual. At pH 7.0, value increased gradually up to week 4 before gradually declining in the remaining period. Values at pH 8.0 rose up to week 3, declined in week 4 and continued its rise in weeks 5 and 6. Hematocrit values at control pH were steady throughout the experiment.

DISCUSSION

The physicochemical parameters of the fish examined in this study showed values characteristic of freshwater environment. The pH of the surrounding medium was slightly acidic (6.4) and dissolved oxygen concentration was well as other attributes measured were adequate to support freshwater aquatic life.

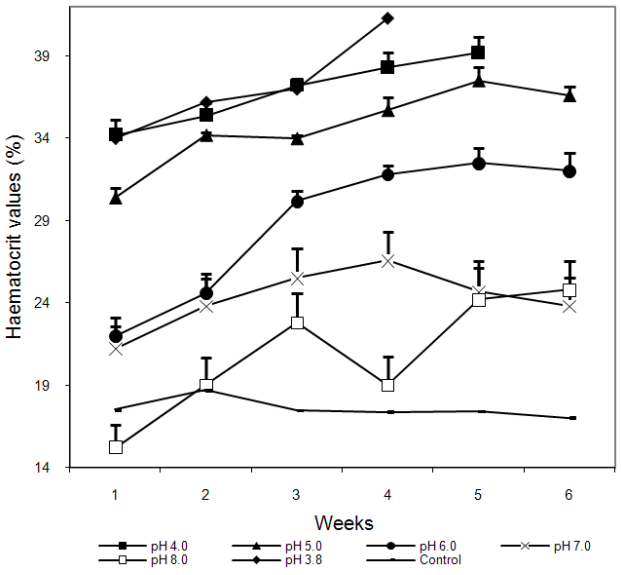

Figure 5. Effect of pH on Mean Hematocrit Values (%) of *S. melanotheron* each week in the Niger Delta wetland, Nigeria.

The erratic and abnormal movement of the fish such as regular surfacing at the water column especially at acidic pH of 3.8 and 4 is evidence discomfort implying a measure stress on the physiological function of the fish species which was not observed on fishes exposed to elevated pH (6.0, 7.0 and , 8.0). The importance of this observation is that fishes exposed to low pH conditions either in the natural habitat or reared in aquaculture pond will suffer similar stress condition and this may induce growth retardation, reproductive failure and eventual lead to the mortality of fishes.

In addition, the progressive increase in values of plasma glucose observed point to the fact that the fish (*S. melanotheron)* demonstrated obvious hyperglycemic response during the exposure to sublethal pH regimes. This signifies that acidic pH conditions may prevent the complete metabolism of blood sugar to glycogen. This significant change in blood glucose level with pH suggests a stress response with tendency of enhancing negative osmoregulatory status in the fish. Wood (1991), environmental acidification from anthropogenic sources has been identified as a major factor affecting salmonid populations

Chindah *et al.* (2004) observed similar hyperglycaemic response on a common Niger Delta wetland catfish (*Clarias buthopogon*). Omoregie *et al*, (1990) reported that this incomplete metabolism could induce impaired osmoregulation. The observed plasma glucose levels in the *S. melanotheron* are in consistent with the works of Wedemeyer (1973), Mcleay and Brown (1975), Krishnamurthy *et al*. (1981), Dheer *et al*. (1987), Omoregie *et al*, (1994) and Omoregie, (1998). The increased blood glucose level in fishes suggests the presence of the stress hormones such as catecholamines and corticosteroids, in the peripheral blood (Fager, 1967; Selye, 1973) and this scenario demands for increased energy requirement in order for the fish to withstand the acid stress condition. The secretion of these hormones induces marked changes in carbohydrate reserves which according to Oguri and Nace (1966) is responsible for the hyperglycemias. Although glycogen reserves were not monitored, it is probable that the reported lethargy before death may be

associated with reduction in muscle glycogen (Duncan and Klaverkamp, 1983).

These significant increases in values for hematological and mucus secretion of the gills attributes between treatments of the test species (*S. melanotheron*) are associated with the low acidic condition. The observed secretion of mucus by the gills is an evidence suggesting irritation due to stress conditions (Omoregie *et al*, 1994 and Omoregie, 1998). This mucus cover of the gill surface may possibly impair its functions in oxygen exchange. This development, could lead to dehydration and enhance reduction in the blood oxygen level to which the fish homeostatic system responded to by the observed increases in the erythrocytes, lymphocytes and hematocrit levels in order to increase the efficiency of transporting the reduced oxygen in the blood. This observed increase in erythrocytes, lymphocytes and hematocrit levels contrasted with the works of Sikoki *et al*. (1989), Omoregie *et al*. (1990) and Omoregie *et al*. (1994) all of whom reported decreases in values of these parameters in juveniles of *Clarias garienpinus* and *Oreochromis niloticus* when exposed to sublethal concentrations of other stress factors (heavy metals, crude oil and formalin). However, our result is in consonance with those of Vaala and Mitchell (1970) and Vaala (1972), which independently reported that fish subjected to acid stress, may experience a decrease in arterial oxygen level and respond to this hypoxemia by increasing the oxygen-carrying capacity of the circulating blood. This development is manifested in those parameters associated with oxygen transport – erythrocytes, hematocrit and hemoglobin (Neville, 1979; Spry *et al*. 1981; Milligan and Wood, 1982). Wedemeyer and Mcleay (1981) also reported that the high values of erythrocytes, leucocytes and hematocrit indicate hemoconcentration possibly due to gill damage and dehydration.

The more active nature of *S. melanotheron*, depicts its hematological requirements of high oxygen demand to meet the requirements of a high metabolic rate, hence the significantly higher hemoglobin and hematocrit values at acid stress levels reported for *S. melanotheron* in this study. The high values recorded for these parameters in *S. melanotheron* may also be due to their blood rich gills exposed almost directly to the oxygen in the water column thus limiting the effect of unfavourable aquatic pH on respiration and energy demand. This is consistent with earlier observations in comparative hematology (Engel and Davis, 1964; Larsson *et al*, 1976). Mavares and Perez (1984), Rambhaskar and Srinivasa (1986) and Chindah *et al*. (2000) also reported that active fish also have higher values of erythrocyte in addition to high hematocrit and hemoglobin levels.

Mature red blood cells are usually nucleated. The observation that non-nucleated cells were also seen indicate that fishes respond to maintain homeostasis in the peripheral blood cell population by facilitating the quick transfer into the blood stream, of non-nucleated red blood cells which occur in their penultimate stage of development. The observed mean RBC of $1.99 \times 10^6 mm^{-3}$ at control pH for *S. melanotheron* is higher than values reported by Etim *et al*. (1994) in similar studies for *Chrysichthys nigrodigitatus* ($1.77 \times 106mm^{-3}$), *Chrysichthys furcatus* ($1.98 \times 106mm^{-3}$), *Ictalurus nebulosus* ($1.2 \times 106mm^{-3}$), and *Ictalurus punctatus* ($2.16x106mm^{-3}$).

The hematocrit and hemoglobin values at their control pH were recorded as 17.8% and 6.3g/dl for *S. melanotheron.* These values support results of earlier studies by Clark *et al.* cited by Oranye, (2002) that reported fish hematocrit values of between 20-35% scarcely attaining values higher than 50% while Larsson *et al*. (1976) actually reported hematocrit values of 51.3% and 52.3% for *Clupea harengus* and *Scomber scrombrus* respectively and hemoglobin values of 14.0g/dl and 12.7g/dl.

The results of leucocyte counts (2.94×10^4 mm^{-3}) are lower than values reported for *C. nigrodigitatus* and *C. furcatus* (5.82×10^4 mm-3 and 3.1×10^4 mm^{-3}) respectively (Etim *et al*,1994). The increase in leucocyte counts with time in both species depicts an attempt at enhancing the body's defense mechanism arising from increasing stress levels. This appears also to be associated with the observed high mucus secretion at stress levels indicative of disease condition.

It is worthy of note that the changes in the leucocyte counts for the fish species points to the occurrence of lymphocytes, monocytes and neutrophils. Thrombocytes, known to be the critical cells involved in fish blood coagulation, as with other vertebrates, were not detected, yet the rapidity with which blood clotted during the sampling procedure when insufficient anti-coagulant was used indicated substantial presence of these cells. It is probable that failure to detect these cells is a reflection of an

increase in their fragility such that when a blood smear is prepared; the cytoplasm is stripped away leaving denuded nuclei which often appear as lymphocytes. Ellis (1977) argued that only occasionally can the entire thrombocyte population appear as undisrupted cells and be differentiated from lymphocytes. A more accurate determination of thromobocytes population may be done using the immuno-fluorescent technique which stains only the lymphocytes. The number of lymphocytes in fish can vary widely between individuals of even a single species. Nonetheless, the very high percentage of lymphocytes recorded in this study alongside the fact that thrombocytic cells were not seen seems to indicate that the thrombocytes must have appeared as lymphocytes as reported by Ellis (1977).

It is therefore concluded that *S. melanotheron* responded negatively to low acidic levels which generates unfavourable physiological conditions affecting body fluids, physiological functioning of the body, and perhaps may degenerate further to cause reproductive failure and mortality.

LITERATURE CITED

American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water and Waste water. p. 16 and 75-427.

Akiri, P. J. 1987. Taxonomy of the Genus *Clarias* (Pisces: Siluriformes) in Rivers State, Nigeria and the Ecology of its species in relation to selected freshwater habitats. Ph. D Thesis. Rivers State University of Science and Technology (Department of Biological Science). p. 33, 67 and 145.

Brown, D. J. A.; R. Morris and S. A. Goldthorpe. 1984. Sublethal effects of acid water. In Stress and Fish. A. D. Pickering (ed.). Academic Press. London. U. K. 562 p.

Casillas, E. and L. S. Smith. 1977. Effect of stress on blood coagulation and haematology in rainbow trout (*Salmo gairdneri*). Journal of Fish Biology 10 (5): 481-491.

Chindah, A. C.; F. D. Sikoki and I. Vincent-Akpu. 2000. Toxicity of Cypermethrin to *Tilapia guineensis* Jurveniles. J. Agric. Biotech. Environ. 2 (1&2): 60-66.

Chindah, A. C.; S. A. Braide and R. O. Oranye. 2004. Response of a common Niger Delta Wetland Catfish to changes in pH. Niger Delta Biologia 4 (2): 56-65.

Chindah, A. C. and A. I. Hart. 2000. Occurrence and distribution of epifauna and infauna community in shallow mangrove wet land in the tropical West African Region. Afri. J. Environmental Studies. 1: (1&2): 76-83.

Dheer, J. M. S.; T. R. Dheer and C. L. Mahajan. 1987. Haematological and haematopoietic response to acid stress in an air-breathing freshwater fish, *Channa punctatus* Bloch. Journal of Fish Biology 30 (5): 577-588.

Duncan, D. A. and J. F. Klaverkamp. 1983. Tolerance and resistance to cadmium in white suckers, *Catastomas commersani* previously exposed to cadmium, mercury, zinc and selenium. Canadian Journal of Fisheries and Aquatic Science. 40: 128-138.

Ellis, A. E. 1977. The leucocytes of fish: A review. Journal of Fish Biology 14: 453-491.

Engel, D. M. and E. M. Davis. 1964. Relationship between activity and blood composition in certain marine teleosts. Copeia 1964 (3): 586-587.

Etim, L.; S. B. Ekanem and A. Utin. 1994. Haematological profile of 2 species of Catfish, *Chrysichthys nigroditalus* Lacepede and *Chrysichthys furcatus* Gunther from the great Kwa Rivers, Nigeria. Global Journal of Pure and Applied Science 5 (1): 262-268.

Fager, U. H. M. 1967. Plasma cortisol concentration in relation to stress in adult Sockeye Salmon during freshwater stage of life cycle. Gen. Comp. Endocrin. 8: 197-200.

FAO Fisheries Report No. 587. 1997. Working party on pollution and fisheries committee for inland fisheries of Africa. p. 7, 9 and 10.

Ibiebele, D. D. 1987. Oshika Oil Spill incident. A case study four years after spill. Proceedings of the Petroleum Industry and the Nigerian Environment.

Krishnamurthy, V.; P. Reddanna and S. Govindappa.

1981. Hepatic carbohydrate metabolism in *Tilapia mossambica* (Peters) acclimated to low environmental pH. Can. J. Zool. 59: 400-402.

Larsson, A.; M. L. Johansson-Sjabeck and R. Fange. 1976. Comparative study of some haematological and biochemical blood parameters in fishes from Skagerak. Journal of Fish Biology 9: 425-430.

Mavares, R. N. and J. E. Perez. 1984. Blood adaptations to marine and fresh water environments in fish of the family Sciaenidae (Perciformes). Journal of Fish Biology 25: 657-659.

McLeay, D. J. and D. A. Brown. 1975. Effects of acute exposure to bleached Kraft pulpmill effluent on carbohydrate metabolism of juvenile Coho salmon (*Oncorhynchus kisutch*) during rest and exercise. Journal of the Fisheries Research Board of Canada 32:753-760.

Milligan, C. L. and C. M. Wood. 1981. Branchial and renal acid and ion fluxes in the rainbow trout, *Salmo gairdneri,* at low environmental pH. J. Exp. Biol. 93: 101-107.

Neville, C. M. 1979. Sublethal effects of environmental acidification on rainbow trout, *Salmo gairdneri*. Journal of the Fisheries Research Board of Canada 36: 84-85.

Oguri, M. and P. F. Nace. 1966. Blood sugar and adrenal histology of the gold fish after treatment with mammalian adreno-corticotrophic hormone. Chesaspeake Science 9: 198-199.

Omoregie, E.; E. B. C. Ufodike and I. R. Keke. 1990. Tissue chemistry of *Oreochromis niloticus* exposed to sublethal concentrations of gammalin 20 Actellic 25 EC. J. Aquatic Science 5: 33-36.

Omoregie, E.; T. G. Eseyin and P. C. Ofojekwu. 1994. Chronic effects of formalin on erythrocyte counts and plasma glucose of Nile tilapia *Oreochromis niloticus.* Asian Fisheries Science 7: 1-6.

Omoregie, E. 1998. Changes in the haematology of the Nile tilapia *Oreochromis niloticus* Trewavas under the effect of crude oil. J. Acta Hydrobiologica. 4: 287-392.

Oranye, R. O. 2002. Haematological responses to acid stress in *Clarias buthopogon* and *Sarotherodon melanotheron*. M.Phil Thesis, Institute of Geoscience and Space technology Rivers State University of Science and Technology Port Harcourt. I-XI. 287 p.

Pudo, J.; A. Lysak and J. F. Afred-Ockiya. 1990. The interrelationship of phytoplankton and fish species in tropical brackish water fish ponds of Southern Nigeria. Acta Hydrobiol. 32(1/2): 227-235.

Rambhaskar, B. and R. K. Srinivasa. 1986. Comparative haematology of ten species of marine fish form Visakhapatnam Coast. J. Fish Biol. 30: 59-62.

Reish, D. J and P. S. Oshida. 1986. Manual of methods in aquatic environment research. Part 10. Short term static bioassays. FAO Fish Technical Paper (247) 22 p.

Sadler, K. and S. Lynam. 1987. Some effects on the growth of brown trout from exposure to aluminum at different pH levels. Journal of Fish Biology 31: 209-219.

Selye, H. 1973. The evolution of the stress concept. Am. Science. 61: 692- 699.

Kori-Siakpere, O. 1985. Haematological characteristics of *Clarias isheriensis* Sydenham. Journal of Fish Biology 27 (3): 259-263.

Sikoki, F. D.; A. I. Ciroma and C. Ejike. 1989. Haematological changes in *Clarias geriepinus* following exposure to sublethal concentrations of zinc, lead and cadmium. In: Onyia, A.D. and G.N. Asala (Eds.). Proceedings of the 7th Annual Conference of Fisheries Society of Nigeria, FISON, Bukuru, Jos, Nigeria. p: 20-26.

Spiff, A. I. and M. N. Horsefall. 1998. Principles of environmental chemistry. Metroprints Ltd. Port Harcourt. 82 p.

Spry, D. J.; C. M. Wood and P. V. Hodson. 1981. The effects of environmental acid on freshwater fish with particular reference to the soft water lakes in Ontario and the modifying effects of heavy metals. A literature review. Can. Tech. Rep. Fish. Aquat. Sci. 999: 144 pp.

Trewavas, E. 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. London.

Vaala, S. S. and R. B. Mitchell. 1970. Blood oxygen tension changes in acid exposed brook trout. Proceedings of Pennsylvania Academy of Science 44: 41- 44.

Vaala, S. S. 1972. Erythrocytic indices of stress in brook trout *(Salvelinus fontinalis)* exposed to sublethal levels of acidity. Proceedings of Pennsylvania Academy of Science 45: 110-112.

Wedemeyer, G. A. 1973. Some physiological aspects of sublethal heat stress in the juvenile steelhead trout (*Salmo gairdneri)* and Coho salmon (*Oncorhynchus kisutch*) Journal of the Fisheries Research Board of Canada 30: 831-834.

Wedemeyer, G. A. and W. T. Yasutake. 1977. Clinical methods for the assessment of the effects of environmental stress on fish health. Technical Paper of the U.S. Fish and Wildlife Service. Washington D. C. 89. p. 19-21.

Wedemeyer, G. A. and D. J. Mcleay. 1981. Methods for determining the tolerance of fishes to environmental stressors: *In* Stress and Fish. A. D. Pickering (ed). Academic Press. London, U. K. p. 247-275.

Wood, C. M. 1991. Acid-base and ion balance, metabolism and their interactions after exhaustive exercise in fish. J. Exp. Biol. 160: 285-308.

Zar, H. J. 1984. Biostatistical analysis. 2nd Edition. Prentice Hall, England Cliffs. p. 328 and 334.