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 Niger, Nigeria a los cambios de pH

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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

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 stakeholders 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 indicating 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 LC50 and the treatment schedule.
96 hr LC₅₀ test

A 96 hr LC₅₀ 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 H₂SO₄ (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 H₂SO₄ (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 H₂SO₄ 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 H₂SO₄ (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 CO₂ 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 LC₅₀. 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 LC₅₀. 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.C₅₀ 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 LC₅₀ 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
Chindah et al. Response of *Sarotherodon melanotheron* in the Niger Delta wetland, Nigeria to changes in pH

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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feature</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Conductivity (µS/cm)</th>
<th>Dissolved oxygen (mg/l)</th>
<th>Biochemical oxygen demand BOD₅ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6</td>
<td>Range</td>
<td>23.6-24.1</td>
<td>3.6</td>
<td>36.0-38.0</td>
<td>3.0-3.7</td>
<td>0.58-0.66</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.8</td>
<td>3.6</td>
<td>37.0</td>
<td>3.21</td>
<td>0.64</td>
</tr>
<tr>
<td>4.0</td>
<td>Range</td>
<td>23.5-24.2</td>
<td>4.0</td>
<td>36.0-40.0</td>
<td>3.32-3.8</td>
<td>0.56-0.64</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.7</td>
<td>4.0</td>
<td>38.2</td>
<td>3.4</td>
<td>0.62</td>
</tr>
<tr>
<td>5.0</td>
<td>Range</td>
<td>23.4-24.2</td>
<td>5.0</td>
<td>36.0-38.5</td>
<td>3.1-3.8</td>
<td>0.59-0.69</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.8</td>
<td>5.0</td>
<td>37.1</td>
<td>3.5</td>
<td>0.64</td>
</tr>
<tr>
<td>6.0</td>
<td>Range</td>
<td>23.4-24.6</td>
<td>6.0</td>
<td>36.2-39.3</td>
<td>3.1-3.8</td>
<td>0.57-0.68</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.9</td>
<td>6.0</td>
<td>37.7</td>
<td>3.5</td>
<td>0.63</td>
</tr>
<tr>
<td>7.0</td>
<td>Range</td>
<td>23.6-24.3</td>
<td>7.0</td>
<td>36.6-38.8</td>
<td>3.2-3.8</td>
<td>0.57-0.66</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.8</td>
<td>7.0</td>
<td>37.7</td>
<td>3.5</td>
<td>0.63</td>
</tr>
<tr>
<td>Control</td>
<td>Range</td>
<td>23.5-24.1</td>
<td>6.3-6.5</td>
<td>36.8-39.0</td>
<td>3.1-3.8</td>
<td>0.58-0.71</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>23.8</td>
<td>6.4</td>
<td>37.2</td>
<td>3.5</td>
<td>0.65</td>
</tr>
</tbody>
</table>

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

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

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.

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.

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

<table>
<thead>
<tr>
<th>Indices</th>
<th>pH 3.8</th>
<th>pH 4.0</th>
<th>pH 5.0</th>
<th>pH 6.0</th>
<th>pH 7.0</th>
<th>pH 8.0</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow and Lethargic</td>
<td>+++/WK3</td>
<td>+++/WK4</td>
<td>+++/WK5</td>
<td>+/WK1</td>
<td>+/WK1</td>
<td>+/WK1</td>
<td>+/WK1</td>
</tr>
<tr>
<td>Mortality</td>
<td>60%</td>
<td>55%</td>
<td>55%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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<table>
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<tr>
<th>Indices</th>
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<th>pH 4.0</th>
<th>pH 5.0</th>
<th>pH 6.0</th>
<th>pH 7.0</th>
<th>pH 8.0</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow and Lethargic</td>
<td>+++/WK3</td>
<td>+++/WK4</td>
<td>+++/WK5</td>
<td>+/WK1</td>
<td>+/WK1</td>
<td>+/WK1</td>
<td>+/WK1</td>
</tr>
<tr>
<td>Mortality</td>
<td>60%</td>
<td>55%</td>
<td>55%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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

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

Figure 3. Effect of pH levels on total white blood cell count \((x10^4 \text{ mm}^{-3})\) 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

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

<table>
<thead>
<tr>
<th>Weeks</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Lym</td>
<td>Mn</td>
<td>Nt</td>
</tr>
<tr>
<td>3.8</td>
<td>77.58</td>
<td>2.86</td>
<td>19.56</td>
</tr>
<tr>
<td>4.0</td>
<td>80.00</td>
<td>2.92</td>
<td>17.08</td>
</tr>
<tr>
<td>5.0</td>
<td>83.00</td>
<td>2.92</td>
<td>14.08</td>
</tr>
<tr>
<td>6.0</td>
<td>83.24</td>
<td>2.98</td>
<td>13.78</td>
</tr>
<tr>
<td>7.0</td>
<td>85.00</td>
<td>3.60</td>
<td>11.40</td>
</tr>
<tr>
<td>8.0</td>
<td>85.00</td>
<td>3.50</td>
<td>11.50</td>
</tr>
<tr>
<td>Control</td>
<td>83.50</td>
<td>3.50</td>
<td>13.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Weeks</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Lym</td>
<td>Mn</td>
<td>Nt</td>
</tr>
<tr>
<td>3.8</td>
<td>74.80</td>
<td>2.80</td>
<td>22.40</td>
</tr>
<tr>
<td>4.0</td>
<td>84.00</td>
<td>3.60</td>
<td>12.40</td>
</tr>
<tr>
<td>5.0</td>
<td>80.00</td>
<td>3.66</td>
<td>16.34</td>
</tr>
<tr>
<td>6.0</td>
<td>82.00</td>
<td>3.82</td>
<td>14.18</td>
</tr>
<tr>
<td>7.0</td>
<td>81.60</td>
<td>3.84</td>
<td>14.56</td>
</tr>
<tr>
<td>8.0</td>
<td>85.60</td>
<td>3.90</td>
<td>10.50</td>
</tr>
<tr>
<td>Control</td>
<td>88.50</td>
<td>2.00</td>
<td>9.50</td>
</tr>
</tbody>
</table>

Lym: Lymphocytes; Mn: Monocytes and Nt: Neutrophils

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Figure 4. Effect of pH on Mean Red blood cell count (x10^6 mm^-3) of *Sarotherodon melanotheron* each week in the Niger Delta wetland, Nigeria.
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; $[F = 29.62 > \text{P (2.60) } 0.05]$ and exposure time, $[F (5.93) > \text{P (2.60) } 0.05]$.

**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.

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*). Omorogie *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), Omorogie *et al.*, (1994) and Omorogie, (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 Omorogie, 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), Omorogie et al. (1990) and Omorogie et al. (1994) all of whom reported decreases in values of these parameters in juveniles of Clarias gariepinus 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$^-3$, for S. melanotheron is higher than values reported by Etim et al. (1994) in similar studies for Chrysiichthys nigrodigitatus ($1.77 \times 10^6$ mm$^-3$), Chrysiichthys furcatus ($1.98 \times 10^6$ mm$^-3$), Ictalurus nebulosus ($1.2 \times 10^6$ mm$^-3$), and Ictalurus punctatus ($2.16 \times 10^6$ mm$^-3$).

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 ($2.94 \times 10^4$ mm$^-3$) are lower than values reported for C. nigrodigitatus and C. furcatus ($5.82 \times 10^4$ mm$^-3$ and $3.1 \times 10^4$ 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 thrombocytes 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**


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


Chindah et al. Response of *Sarotherodon melanotheron* in the Niger Delta wetland, Nigeria to changes in pH


