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HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF *Sarotherodon melanotheron* FROM A SLUM NEIGHBOURHOOD ENVIRONMENT

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Abstract:

A total of 18 live specimens of the black jaw tilapia, *Sarotherodon melanotheron*, mean weight (82.95 ±3.27g) and mean length (13.1 ±0.35cm), were sampled bimonthly for three months from the Makoko area, a slum neighbourhood, of Lagos Lagoon, to establish the haematological and biochemical indices of the fish species which will serve as baseline for further studies and ascertain the health of the environment. The mean value and standard error of mean of some of the haematological parameters analyzed were: Hb: 89.65 ±3.63g/l, PCV: 27.47 ±0.58%, RBC: 3.05 ±0.06T/L, WBC: 10.02 ±0.40g/L, MCV: 86.87 ±1.39fl, MCH: 29.64±0.62pg, MCHC: 33.29 ±0.13g/dl, Neutrophils: 22.89 ±0.63%, Lymphocyte: 79.67 ±1.49% and Monocyte: 0.72 ±0.25%. No Eosinophils and Basophils were recorded in this study. While the mean and standard error of mean of the biochemical enzymes analyzed in IU/L, were: AST (76.84 ±4.08), ALT (23.06 ±2.12) and ALP (77.74 ±6.12). There was statistical significance ($p<0.05$) in the following haematological parameters: Hb, WBC, MCV, Neutrophils and Lymphocytes. There was statistical significance ($p<0.05$) in all the biochemical indices. It was established that the water was polluted with heavy metals, sewage and other organic pollutants such as wood wastes, and that these pollutants have altered the haematology and biochemistry of the fish species.

Keywords: Makoko, Lagos Lagoon, Pollutants, haematological, biochemical indices, *Sarotherodon melanotheron*

Introduction

Fishes are highly sought after today because of their high nutritive values. According to Ademola-Aremu *et al* (2009), fish constitute a major source of animal protein to a large number of Nigerians, particularly in the Lagos environs; they contain a high level of protein (17 - 20%) with an amino-acid profile similar to that of meat in ruminants and fowl, and when compared to beef, mutton, chicken and bush meat, fish tissues are less tough and more digestible. Today, these fishes are being threatened by pollution. Emmanuel and Ogunwenmo (2010), on their study of the Macrobenthos and the Fishes of a Tropical Estuarine Creek in Lagos, South-Western Nigeria, reported a low density of species for both macrobenthos and fish species. They attributed this decline to an unstable physically controlled environment resulting from anthropogenic induced stressors. As observed in this present study in Makoko Area of Lagos Lagoon, these anthropogenic stressors include, among others, discharges from: industrial effluents, urban wastes, agricultural runoff, untreated or partially treated sewage, oil and smoke from the engine and exhaust pipes of motorized boats and vehicles plying the third mainland bridge. Fishes are being used as biosensors to monitor the health of aquatic ecosystems, because of their sensitivity to water pollution. This point is supported by that of Summarwar and Deepali (2013) who stated that fish are relatively sensitive to changes in their surrounding environment, including an increase in pollution; and that fish health may as a result reflect, and give a good indication of the health status of the aquatic ecosystem in which the fish occurs. They further stated that the initial toxic effects of the pollution may, however, only be evident on cellular or tissue level before significant changes can be identified in fish behavior or external appearance. Thus the health of an ecosystem is thus often reflected by the health of its fauna (Summarwar and Deepali, 2013). According to Nte and Akinrotimi (2011), the effects of exposure of fishes to sub lethal levels of pollutants can be measured in terms of their biochemical and physiological responses. The physical and chemical changes reflect in the blood component of the fish. Blood is therefore recognized as a potential index of fish response to water quality (Hickey, 1982). Biomarkers are measurements in body fluids, cells or tissues indicating biochemical or cellular modifications due to the presence and magnitude of toxicants, or of host response (NRC, 1987). Fish haematological and biochemical indices have been described by Nte and Akinrotimi (2011) as biomarkers used to ascertain the health status of the fish and that of the aquatic ecosystem where the fish is gotten from. Variations in blood indices in response to environmental conditions have been a major biomarker of stress in organisms. Alteration in enzymes activities of the exposed fish is one of the major biomarker indicating the level of changes consequent of pollutants in the tissues, the organs and body fluid of the fish that can be recognized and associated with established health impairment process (Akinrotimi *et al.,* 2009). However, Gabriel and Akinrotimi (2011) noted that biomarker can also be used to confirm and assess fish exposure to toxicants, providing a link between external exposure and internal structure and degree of responses to toxicant exposure observed between different individuals. The most compelling reason for using biomarkers is that they can give information on the biological effects of pollutants rather than a mere quantification of their environmental levels (Olakolu *et al.*, 2012).

The use of haematological parameters as fish health indicators was proposed by Hesser (1960) and has since been used as an index of fish health status in a number of fish species to detect physiological changes as a result of exposure to different stressful conditions such as handling, pollutants, metals, hypoxia, anaesthetics and acclimation (Duthie and Tort, 1985; Ogbulie and Okpowasili, 1999; Alwan *et al.,* 2009). Haematological indices are essential parameters for the evaluation of fish physiological status. Early diagnosis is possible when evaluating haematological data (Folmar, 1993; Golovina, 1996; Luskova, 1997). In addition, analysis of blood parameters will reveal conditions within the body of the fish long before there is any outward manifestation of disease or effects of unfavourable environmental factors such as stress (Sampath *et al*., 1993, Musa and Omoregie 1999). Thus haematological analysis of fish can be used for monitoring the aquatic ecosystem, where the fish lives, for early detection of pollution.

Enzymes are chemical substances (proteins) that help speed up a chemical reaction in the body (Ramalingam, 2011). Biochemical or liver function test also helps to determine the health status of fish as well as that of its environment. Biochemical constituents and some enzymes in the blood are used as biomarkers as they are very sensitive, less variable and conserved between species (Owolabi, 2011). Changes in the activities of these enzymes could be an indication of tissue injury, environmental stress or diseased condition (Kori-Siakpere *et al*., 2010). An increase in enzymatic activity in the extracellular fluid or organs is a sensitive indicator of even minor cellular damage, since the levels of these enzymes within the cell exceed those in the extracellular fluid by more than three orders of magnitude (Das *et al.*, 2004). On the other hand, toxicants can also inhibit the activity or synthesis of enzymes (Jung *et al.*, 2003), resulting in decreased activities in the organs. This present study sought to establish baseline values for the haematological and biochemical parameters of *Sarotherodon melanotheron* (black jaw Tilapia) from the Makoko area – a slum neighbourhood on the Lagos Lagoon.

This present study sought to determine the impact of pollution of the Makoko Area of the Lagos Lagoon on the fish species, through the determination of the alterations in the haematology and biochemistry of the Black-jawed Tilapia (*Sarotherodon melanotheron*) caught from the area.

Materials and Methods

Study site: Makoko (Figure 1) is a slum neighbourhood community located on the eastern part of Lagos Lagoon, Nigeria. It lies approximately within latitude 6°29′46″N and longitude 3°23′16″E within the Lagos Lagoon. Majority of the people live in floating shanties built on the Lagoon and they engage in fishing as means of livelihood. Over a thousand fishing boats (paddled and motorized) could be located at a time. The water was observed to be darkly coloured, with offensive odour and human waste (faeces) was commonly observed floating on the water surface. The most discernible floras observed were the floating aquatic macrophytes; *Vossia cuspidata* and *Eichhornia crassipes* (water hyacinth).

Fish species:

Sarotherodon melanotheron (Ruppell 1852) is a cichlid that occurs commonly in West Africa and supports a major fishery in the lagoons. This status earns it the common name 'West African lagoon tilapia' (Eyeson, 1979). Pauly (1976) had earlier mentioned the species as a possible candidate for aquaculture. *S. melanotheron* is a demersal (bottom-associated) species inhabiting fresh to brackish water where it occurs. It is a tropical West African native occurring from Senegal to Zaire and southern Cameroon (Trewevas 1983, Robbins *et al.* 1991). The species is common in quiet muddy backwater habitats where aquatic vegetation is abundant (Jennings and Williams 1992). *S. melanotheron,* the blackchin (or black jaw) tilapia, is a pale (variable light blue, orange, golden yellow) cichlid whose common name refers to the dark pigmentation usually (but not always) concentrated on the underside of the head (the chin) in adult animals. Melanie pigmentation is usually also present on the posterior edge of the gill (the cleithrum) and on the tips of the soft dorsal rays. Irregular bars, spots or splotches on the body are also typical. The mouth is small and filled with up to several hundred very small teeth arranged in 3-6 rows (Trewevas 1983). In the Makoko area, the various species of Tilapia, including *S. melanotheron*, are the most landed fish species. The fishes were landed through the assistance help of the local fisher men using cast nets of various mesh sizes. The weight of specimens used in this present ranged between 47.9 – 96 g while the length ranged from 10 – 15cm.

Blood Sampling:

Bi-monthly collection of 18 live specimens of *S. melanotheron* was carried out for three months (May – July, 2013). The fishes were carefully netted and handled to minimize stress. They were put in large container (containing the lagoon water) for 2hrs prior to collection of blood samples. Collection of blood samples was done on the field to avoid stress due to transportation. Approximately 2ml of blood was collected using 2ml sterile plastic disposable syringes fitted with 0.8×38-mm hypodermic needles. The recovered blood was expressed into a vial containing dried or powdered potassium salt of ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood sample was rocked gently in the vial to allow thorough mixing of its contents. A further 2ml was taken with Lithium heparinised bottle and used to prepare blood films and for the determination of serum biochemical levels. The blood samples were taken in the morning time (between 08:00 and 10:00 hours) and held on ice chest until all samples were collected.

Figure 1. Map of Lagos Lagoon showing the Makoko Slum Area

Haematological and biochemical techniques

Haemoglobin (Hb) count was done with the cyanomethaemoglobin method; Packed Cell Volume (PCV) by micro haematocrit method. Red blood cell (RBC) and total white blood cell (WBC) counts were done using the Neubauer haemocytometer. Differential counts (neutrophils, monocytes and lymphocytes) were done on blood film stained with May Grumwald-Giensa stain. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the data using standard formulae.

Serum was separated from the cellular blood components by centrifugation for 5 min at 14,000 rev/min. Blood alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) were determined using a portable automated chemical analyzer following the procedure and using the reagents recommended and as described by the manufacturer (RANDOX Laboratories Ltd, UK; AST/ALT (Cat. No. Sc 2643) and ALP multi-sera level 2 (Cat. No. 1530) and level 3 (Cat. No.1532)), assay kits. All blood analyses were carried out within 48 hours of collection.

Haematological parameters and biochemical indices were analysed using one-way analysis of variance (ANOVA) at 5% level of significance. While post-hoc comparison of significance of variance result gotten from ANOVA was done using Duncan Multiple Range Test (DMRT).

Results and Discussion

Haematological parameters

Table 1 showed the mean and standard error of mean for the haematological parameters. The analysis of variance (ANOVA) for the haematological parameters showed that only Hb, WBC, MCV, NEUT, and LYMP are significant ($P<0.05$). Post-Hoc analysis using DMRT (Duncan Multiple Range Test) showed that there was significant difference ($P<0.05$) in Hb, WBC, MCV, MCH, NEUT. and LYMP.

Biochemical parameters

Table 2 showed the mean and standard error of mean for the biochemical parameters. ANOVA showed that there was significant difference ($P<0.05$) in the three biochemical parameters.

But DMRT showed that there was significant difference in: AST for weeks 1, 2, 3 and 5; ALT for weeks 1, 2, 4, 5 and 6; ALP for weeks 1, 2, 4, 5 and 6.

In this present study, the analysis of the physicochemical parameters of the water showed that the Makoko Area is polluted with heavy metals and organic pollutants – sewage and wood wastes, and oil film. These lead to low/depletion of the oxygen level. All these, in turn, cause physiological stress to the fish. This could be responsible for the low RBC and Hb and the comparable higher WBC recorded in this study. This view conforms to that of Ugwu *et al.* (2006) who reported significant changes in blood haemoglobin and neutrophil concentrations of *Heterobranchus bidorsalis* and concluded that the changes reflect the responses to the effects of stress caused by toxicants - crude oil and its fractions. According to Witeska and Kosciuk (2003), this results in cell swelling deformation and damage. According to Atamanalp and Yanık (2002) the low Hb levels may impair oxygen supply to the various tissues and result in slow metabolic rate and low energy production. Long term exposures of fish to effluents and sewages have been reported to alter haematological parameters by disrupting haematopoiesis, consequently resulting in anaemia condition (Nikinmaa and Oikari, 1992; Ellis *et al.*, 2003). Landman *et al.,* (2006) also observed changes in blood profiles of *Cyprinus carpio* and attributed it to the effect of effluents from paper mill industries.

Table 1. Mean and S.E for Haematology of *Sarotherodon melanotheron* caught at the Makoko area of the Lagos Lagoon

PARAMETER	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	GRAND MEAN
Hb(g/L)	94.57 ± 3.35^{ab}	90.17 ± 10.62^{ab}	84.30 ± 7.54^{a}	83.90 ± 4.91^{a}	111.33 ± 6.77^{b}	73.63 ± 3.82^{a}	89.65±3.63
PCV(%)	27.00 ± 1.53^{a}	29.67 ± 0.55^{a}	26.67 ± 2.03^{a}	25.67 ± 1.45^{a}	29.47 ± 0.64^{a}	26.37 ± 0.86^{a}	27.47±0.58
RBC(T/L)	3.06 ± 0.03^{a}	3.18 ± 0.11^{a}	3.24 ± 0.18^{a}	2.87 ± 0.20^{a}	3.07 ± 0.07^{a}	2.87 ± 0.07^{a}	3.05±0.06
WBC(g/L)	10.14 ± 0.88^{ab}	8.96 ± 1.43^{a}	11.93 ± 0.41^{b}	8.29 ± 0.65^{a}	10.77 ± 0.45^{ab}	10.03 ± 0.50^{ab}	10.02±0.40
MCV(fl)	91.21 ± 1.06^{b}	89.88 ± 1.24^{b}	78.87 ± 2.63^{a}	89.83 ± 1.30^{b}	90.74 ± 1.20^{b}	80.67 ± 3.04^{a}	86.87±1.39
MCH(pg)	32.18 ± 0.04^{b}	30.10 ± 0.55^{ab}	26.73 ± 1.23^{a}	29.60 ± 0.46^{ab}	30.67 ± 1.0^{ab}	28.57 ± 2.78^{ab}	29.64±0.62
MCHC(g/dL)	33.59 ± 0.48^{a}	33.10 ± 0.06^{a}	32.93 ± 0.12^{a}	33.10 ± 0.45^{a}	33.10 ± 0.15^{a}	33.90 ± 0.21^{a}	33.29±0.13
NEUT(%)	23.33 ± 0.88^{ab}	20.67 ± 1.76^{a}	23.00 ± 1.15^{a}	26.67 ± 0.67^{b}	23.33 ± 0.88^{ab}	20.33 ± 0.88^{a}	22.89±0.63
LYMP(%)	78.33 ± 1.45^{b}	88.00 ± 1.15^{c}	78.00 ± 1.73^{b}	70.33 ± 0.88^{a}	78.33 ± 1.86^{b}	85.00 ± 2.52^{c}	79.67±1.49
MONO(%)	0.33 ± 0.33^{a}	0.67 ± 0.67^{a}	0.33 ± 0.33^{a}	1.67 ± 0.88^{a}	0.33 ± 0.33^{a}	1.00 ± 1.00^{a}	0.72±0.25
EOS(%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
BAS(%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Table 2. Biochemical Parameters of *Sarotherodon melanotheron* caught at the Makoko area of the Lagos Lagoon

PERIODS	PARAMETER		
	AST(IU/L)	**ALT(IU/L)**	**ALP(IU/L)**
Week1	76.52 ± 9.84^{ab}	22.67 ± 0.67^{c}	63.48 ± 5.52^{b}
Week2	74.73 ± 13.63^{ab}	22.56 ± 1.39^{c}	74.52 ± 1.59^{bc}
Week3	94.93 ± 1.85^{b}	11.14 ± 2.57^{a}	45.08 ± 5.12^{a}
Week4	66.11 ± 0.77^{a}	26.33 ± 0.38^{c}	73.62 ± 2.43^{bc}
Week5	92.49 ± 0.62^{b}	16.94 ± 0.91^{b}	125.12 ± 6.44^{d}
Week6	56.24 ± 1.98^{a}	38.71 ± 1.34^{d}	84.64 ± 3.31^{c}
GRAND MEAN	76.84±4.08	23.06±2.12	77.74±6.12

NB: Mean frequencies (mean ± S.E, Standard Error) with different superscript letters in a row are significantly different in the DMRT ($p<0.05$)

In the present study, the lymphocytes, mean value 79.67 ±1.49, were observed to be numerous than any other differential cells, this is typical of most fishes (Owolabi, 2011). The abundance of neutrophils and monophils in this fish species is also typical of most fishes (Owolabi, 2011). No eosinophils and basophils were recorded in this study; this is also typical of most fish species and could be attributed to the influence of the heavy metals especially cadmium. It has been evidenced that cadmium influences the differential blood count (Gill and Eple 1993).

In this present study, there are significant differences (P<0.05) in the values of the serum enzymes; AST, ALT and ALP. This could be an indication that the water contains some stressors that cause damage to some organs and tissues such as the liver and the cardiovascular tissues of the fish, leading to the liberation of these enzymes into the blood. Gul *et al.* (2004) considered enzymes as sensitive biochemical indicators of toxicity in organs of fish. Blood is an active transport medium in higher animals, especially in vertebrates (Ramalingam, 2011). It constantly bathes all the organs and tissues of the body, enabling exchange of materials between the internal and external environment of these organs and tissues (Ramalingam, 2011). Therefore, a biochemical analysis of the fish blood should confirm the level of these enzymes – and this should be an indicative of the pollution status of the environment they are caught from. This view is confirmed by Parma *et al.* (2007) who stated that the enzymes AST and ALT are transaminases which are basically intercellular enzymes found in most organs of fish.

In Makoko area of Lagos Lagoon, raw sewage is commonly seen floating on the water surface.

This sewage could act as stressor and cause distress to fish. According to Ortiz *et al.* (2003), distress is one of the early symptoms of sewage poisoning in fish. Distress in fish leads to damage in cells and tissues of the organs, which, in turn, elicits the liberation of these enzymes into the blood stream; prompting their high levels in the blood as recorded in this present study. This view is supported by that of Nte *et al* (2011) who also recorded high levels of these enzymes in the organs of *S. melanotheron* exposed to different concentrations of industrial effluents. They noticed that the levels of the enzymes increased as the concentration of the effluents increased. In this present study, the levels of the enzymes are relatively constant throughout the period under survey; this could be due to continual introduction of pollutants into the Lagos lagoon all year round, causing their consistency in concentration.

The significant difference (P<0.05) in the ALT mean values of the *S. melanotheron* could be due to several reasons such as differences in mode of feeding, age and species. This view is supported by the report of WHO (1989) and Hunn *et al* (1993) who observed that effluents acute toxicity varies widely depending on the species, age and formulation.

Conclusion

This study has shown that the Makoko Area of Lagos Lagoon is polluted. The various pollutants are inducing serious physiological stress on the commercial feral fish species. This has resulted in alterations in the haematology and biochemical indices of these fish species, as shown by the relative high values recorded in this present study. There is, therefore, need to abate pollution of the area, to prevent decline in the commercial fish species, such as *S. melanotheron*, that serve as rich source of protein to the inhabitants of Lagos and its environs.

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