EFFECTS OF HANDLING STRESS ON IMMUNE FUNCTIONS OF BLACKJAW TILAPIA *Sarotherodon melanotheron*

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ABSTRACT
Immunological responses in blackjaw tilapia Sarotherodon melanotheron exposed to capture and handling stress were evaluated. The result obtained indicated a significant ($P < 0.05$) increase in the values of White blood cell (WBC), from 18.66 ± 3.81 to 24.32 ± 6.88 x $10^9 L^{-1}$ and the differential counts neutrophils 35.13± 9.34 to 43.41± 11.21%; monocytes 2.40± 1.01 to 3.78± 1.64% and eosinophils 1.23 ± 0.88 to 2.14 ± 1.11%. There was significant ($P < 0.05$) reduction in the lymphocytes number from 61.24 ± 12.31 to 49.67 ± 10.74%. There was a positive correlation ($P < 0.01$) between WBC and the differential counts; an indication that handling procedures in aquaculture can impair fish immunological properties.
Keywords: immune-suppressive activity, stimuli, fish manipulation

INTRODUCTION

Aquaculture production in Nigeria has increased tremendously in recent times, as a result of increasing demand for fish and steady reduction in fish production from the wild (Akinrotimi *et al.,* 2007a). The intensive production of fish, coupled with different methods of fish manipulation and handling has exposed the cultured fish to various degrees of stress in the culture medium (Akinrotimi *et al.,* 2007b). According to Barton (2002), repeated or prolonged exposure of fish to stressful stimuli can result in reduced growth, impaired reproductive performance and suppression of immunological functions.

The stress response in fish is characterized by the activation of the sympathetic – chromaffin axis and the hypothalamic – pituitary – interrenal (HPI) axis, resulting in various physiological and metabolical changes (Wendelaar Bonga, 1997; Perry and Bernier, 1999), which include alterations of immune system (Maule *et al.,* 1987), susceptibility to infections and pathogens (Hoeger *et al.,* 2004). Reddy and Leatherland (1994), observed that there is high relationship between environmental stressors and outbreak of disease in aquaculture. This immuno suppressive activities, based on the report of Barton and Iwama (1991), is generally linked to the stress induced elevations in plasma artisol levels, which plays a significant role through membrane stabilization actions. A correlation between susceptibility of fish to diseases and stress, consequent of cortisol release was established by some authors (Wiik *et al.,* 1989; Fe volden *et al.,* 1993).

Impairment of immune mechanism in aquatic organisms due to stress can be found on different immune cell types. A decrease in total number of blood leucocytes or lymphocytes can be observed following exposure to stressors in mammals as well as in fish (Maule and Shreck, 1990; Sunyer *et al.,* 1995). Despite the growing interest in the field of fish immunotoxicology, basic knowledge about effects, mechanism of actions and imports of stress on the fish immune system, especially for tropical fish species is scanty, thus necessitating the need to carry out this work using *S. melanotheron*.

Black jaw tilapia (*S. melanotheron*), is a euryhaline species found in the tropical waters in West Africa. According to Akinrotimi (2006), it is the most common species of tilapia in the brackish water zone of Nigeria, especially in the Niger Delta. Culture of this species now dominates aquaculture production in the brackish water environment (Akinrotimi *et al.,* 2007c).

The objective of the present study was to determine the effects of various handling stress, a necessity in aquaculture production, on immune systems of *S. melanotheron* as this information will be of tremendous assistance to aquaculturists in effective handling of this tilapia in the culture medium.

MATERIALS AND METHODS

A total of fifty *S. melanotheron* specimens (mean length 16.61 ± 3.66 cm and mean weight 85.89 ± 12.61 g) were collected during low tide from the recruitment ponds of African Regional Aquaculture Centre, Brackish Water Research Fish Farm, Buguma, Rivers state, Nigeria. Blood was rapidly collected from the caudal vein of each fish with 2 ml syringes and 21 G needles. After collection, the sample was transferred into Ethylene Diamine Textracetic Acid (EDTA) bottles for analysis. After the first blood sampling, fish were kept in 30 m^3 concrete tanks and fed at 5% body weight for 72 hours. After this period all the fish (50) were captured individually using a small scoop

net and subjected to stress, by allowing them to struggle out of the water for 40 seconds (Barton and Zitzow, 1995; Davis and Shreck, 1997; Tavares-Dias, 2001). Another round of blood sampling similar to the first one was performed again.

The water in the experimental tanks was renewed daily, and parameters such pH, temperature, dissolved oxygen, Salinity and Nitrate were monitored before and after a stress procedures, using the methods described by APHA (1998).

The blood parameters were analyzed using standard procedures. White blood cells (WBC) were counted in Neubauer chamber after blood dilution in sodium chloride solution (0.65%) with gentian violet (1%) and red neutral. Blood smears were stained using May-Gruenwald/Giemsa staining (Rosenfield, 1947), for estimation of differential counts of leukocyte under microscopic examination. Blood cell population was expressed as percentage of total cells counted (Tavare – Dias *et al.,* 2002).

Data from the experiment were subjected to one way analysis of variance (ANOVA) test at 0.05% probability and difference among means were determined by Tukey's multiple comparison test. Also the data were equally submitted to Pearson linear correlation (Zar, 1996).

RESULTS

The values (Mean ± SD) of physico-chemical parameters before and after stress (Table 1) were within the same range, with no significant different ($P > 0.05$), except the values of dissolved oxygen which reduced from 5.81 ± 3.14 to 4.60 ± 1.71 mgL^{-1}.

After stress there was significant ($P < 0.05$) increase in the values of white blood cells (WBC) from 18.66 ± 3.81 to 24.32 ± 6.88 x 10^9 L^{-1}; neutrophils 35.13 ± 9.34 to 43.41 ± 11.21%; monocytes 2.40 ± 1.01 to 3.78 ± 1.64% and eosinophils 1.23 ± 0.88 to 2.14 ± 1.11% (Table 2). While the values of lymphocytes significantly ($P < 0.05$) reduced from 61.24 ± 12.31 to 49.67 ± 10.74% (Table 2).

Significant linear correlation ($P < 0.01$) between WBC and differential counts were shown in Table 3. The correlation between the differentials counts indicated that monocytes and eosinophils were not significant ($P > 0.05$).

Table 1: Physico-Chemical Parameters in the Experimental Tanks before and after stress (Mean ± SD)

Parameters	Before Stress	After Stress
pH	6.55 ± 1.44^a	6.56 ± 1.21^a
Temp (°C)	28.61 ± 6.22^a	28.74 ± 2.46^a
Dissolved Oxygen (mg L^{-1})	5.81 ± 3.41^a	4.60 ± 1.71^b
Salinity (‰)	12.71 ± 3.44^a	12.76 ± 3.66^a
Nitrate (Mg L^{-1})	0.0037 ± 0.01^a	0.0041 ± 0.02^a

Means within the row with same alphabets are not significant ($P > 0.05$)

Table 2: Values (mean±SD) of leukocytes count in *S. melanotheron* before and after handling stress

Parameters	Before Stress	After Stress
WBC (x 10^9 L^{-1})	18.66 ± 3.81^a	24.32 ± 6.88^a
Lymphocytes (%)	61.24 ± 12.31^a	49.67 ± 10.74^b
Neutrophils (%)	35.13 ± 9.34^a	43.41 ± 11.21^b
Monocytes (%)	2.40 ± 1.01^a	3.78 ± 1.64^a
Eosinophils (%)	1.23 ± 0.88^a	2.14 ± 1.11^b

Means within the row with same alphabets are not significant ($P > 0.05$)

Table 3: Pearson Linear Correlations among the leucocytes count of *S. melanotheron* after handling stress

Parameters	WBC	Lymphocytes	Neutrophils	Monocytes	Eosinophils
WBC (x$10^9$$L^{-1}$)	1.00	0.42^{**}	0.84^{*}	0.50^{**}	0.42^{**}
Lymphocyte (%)	-	1.00	0.56^{*}	0.62^{*}	0.41^{*}
Neutrophils (%)	-	-	1.00	0.36^{*}	0.29^{*}
Monocytes (%)	-	-	-	1.00	0.38^{NS}
Eosinophils (%)	-	-	-	-	1.00^{NS}

Key NS – not significant (P > 0.05) * - significant (P < 0.05) ** - Significant (P < 0.01)

DISCUSSION

Evaluation of haematological parameters might be very useful in the diagnosis of fish pathologies and physiological status (Stoskopf 1993). Changes in the blood components might be indicative of unfavourable environmental conditions. In this study the water parameters were within the same range that is suitable for fish culture (Boyd, 1992).

The before stress (basal) WBC level, found in the present work (18.66 ± 3.81) was similar to the basal level found previously in the same species (Akinrotimi et al. 2007d), suggesting acceptable culture conditions preceeding the experimental procedure. The increase observed in WBC after stress is similar to the other fish species as observed for *Limanda limanda* (Pulsford *et al*., 1994), *Clarias gariepinus* (Gabriel *et al*., 2010) and *Tilapia guineensis* (Akinrotimi *et al*., 2009). The increase observed may be due to release of white blood cells from the spleen to the blood stream to combat the stress (Barcellos *et al*., 2004).

The lymphocyte values in the non-stressed *S. melanotheron* in this work (61.24 ± 12.31%), indicated a higher value than that found by Gabriel *et al*. (2007a) for the same species. However, this variation may be as a result of culture system, season, life stage, and physiological status (Akinrotimi, 2008).

The reduction in the circulatory lymphocytes (lymphopenia) in stressed *S. melanotheron* was comparable to that found in other teleost fish, like *Tilapia guineensis* (Akinrotimi *et al*., 2010), *Clarias gariepinus* (Gabriel *et al*. 2010), *Ictalurus punctatus* (Ellsaesser and Clem, 1987) *Pagrus parus* (Fanouraki *et al*. 2007), *Salmo trutta* (Morgan *et al*. 1993), *Oncorhynchus kisutch* (Maule and Shreck, 1990). This confirms lymphopenia to be one of the main stress induced phenomenon that occurs during stressful conditions in fish. This may vary from one species to another depending on the rate and intensity of the stress. The lymphopenia observed in this work might be connected to cell re-distribution to lymphoid tissues as reported by Engelema *et al*. (2003) and to cortisol induced apoptosis in B cells and eventual clearance from the blood stream after stressful conditions (Weyts *et al*., 1998; Verburg – Van *et al*., 1999).

After stress the values of monocytes, neutrophils and eosinophils increased significantly, this corroborates the findings of Gabriel *et al*. (2007b) in the same species transferred abruptly to fresh water from brackish water of 15‰ salinity. This may be due to mobilization of segmented cells from the bone marrow to the blood stream (Barcellos *et al*., 2004).

CONCLUSION

The results from the study indicated that handling stress in day to day aquacultural practices could impair the immune function of *S. melanotheron* through a reduction in the value of lymphocytes which ultimately will reduce the ability of the fish to resist pathogens and disease causing organisms. There is therefore the need for proper handling of *S. melanotheron* such that the fish should not be left without water for too long and should not be made to struggle for optimum performance in the culture medium.

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