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Biochemical and histological studies on juvenile tilapia, *Oreochromis mossambicus* exposed to salinity stress

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Abstract
The secondary stress response of juvenile Tilapia, *Oreochromis mossambicus* after direct transfer to saline water was evaluated by assessing the levels of plasma glucose, plasma protein, hepatosomatic index and liver histology. After an acclimation period of 6 days, fishes were transferred directly to different experimental salinities (2, 4, 8 and 10 ppt) for a period of 5 days. Plasma glucose increased, plasma protein and hepatosomatic index showed slight decrease. However, liver histology did not exhibit any marked variation from that of the control. These alterations at the molecular and biochemical levels are measures of processes that are a normal component of an organism's attempt to maintain a constant internal environment, and may be regarded as biological markers of exposure to salinity stress. Considering the parameters measured in this study *Oreochromis mossambicus* appeared to exhibit a stress response to direct transfer to saline water, nevertheless, the magnitude of stress response was related to salinity level.

Keywords: Tlapia, stress, hematology, hepatosomatic index, histology

Introduction
The salinity as a stress on fish will induce a series of biochemical and physiological changes as an attempt to compensate the challenge imposed on it. The objective of this study was to evaluate the secondary stress response of Mozambique tilapia, *Oreochromis mossambicus* after direct transfer to different salinities for a short-term.

Stress in fish has been shown to cause a primary response, involving neuro-hormonal stimulation, resulting in an increase in corticosteroid and catecholamine secretions. In turn, these primary effects cause a number of physiological changes known as 'secondary effects'. Secondary effects comprises the various biochemical and physiological effects associated with stress such as alterations in metabolic pathways, hematology. One of the most common indicators of metabolic effects due to stress is the increase in plasma glucose concentration, which assists the animal by providing energy substrates to tissues such as brain, gills and muscles to cope with the increased energy demand. The tertiary response represents whole animal and population level changes associated with stress. Failure to acclimate or adapt to the stressor results in decreased reproductive capacity, decreased growth and even death.

Apart from hematological changes, stress also induces histopathological changes mainly in gill and liver causing distortion in the normal architecture. The liver is a complex organ, one that is uniquely positioned in the body such that all harmful effects of stressors are primarily exerted within the liver cells. Although the liver has the ability to detoxify most compounds it may be overwhelmed by elevated levels of harmful substances and become vulnerable to and eventually harmed by them. The hepatocyctes or liver cells that make up the bulk of the liver thus can be used as biomarkers in the assessment of aquatic stress in *Oreochromis mossambicus*. Hepatosomatic index, which is the ratio of liver weight to body weight, is affected by change in salinity. Hepatosomatic index is considered as an effective tool for assessing the general condition of fish exposed to various concentrations of salinity.

The study of chemical biology of fishes is fast gaining importance in the field of fishery biology and ichthyology. Blood parameters are useful and sensitive for diagnosis of diseases and monitoring of the physiological status of fish exposed to stress (Adhikari *et al*., 2004) [1].

Studies involving tilapia in saline waters include basic research on the physiology of salinity stress in fish as well as more practical research on aquaculture practices.

Materials and Methods

Fifty healthy individual fishes weighing between 35-45g were reared in 10 circular plastic tanks of 50L capacity. After an acclimation of 6 days, individual fish were randomly divided into 5 groups (10 fish for each group) and redistributed in tanks (5 each for a tank). The treatments were 2 ppt, 4 ppt, 6 ppt (control), 8 ppt and 10 ppt salinities respectively with replicates. Fishes from each replicates were randomly collected and sacrificed after the study period of 5 days. After termination of the trial, individual fishes were sampled randomly from each tank. Blood was collected from the caudal vein of the individual fish after anaesthetization.

Glucose was estimated by Random Blood Sampling method using plasma (quantitative) and total serum protein was quantitatively determined by Biuret method. Preparations of liver sections were made and examined under microscope and hepatosomatic index was studied. Data on levels of plasma glucose, plasma protein, and hepatosomatic index were subjected to One Way Analysis of Variance (ANOVA) followed by Duncan's multiple range t tests for comparisons. All data were analyzed using the STATISTICA 8.0 package for Windows.

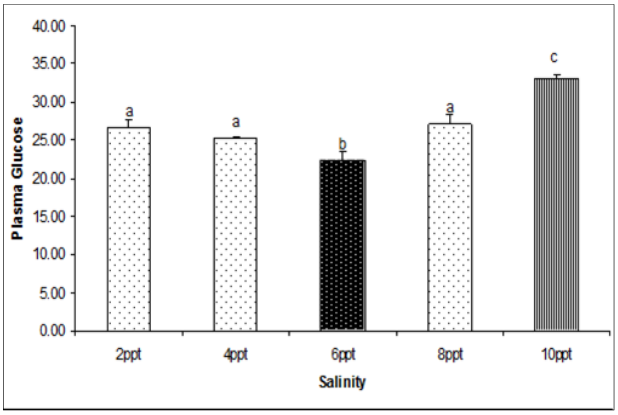
Results

Significant differences were noted in the biochemical response of juvenile tilapia to salinity stress. Compared to the control treatment (6ppt), fishes subjected to 10 ppt salinity showed very high plasma glucose concentrations indicating that they were under considerable stress (Figure 1). Notable differences in plasma protein (Figures 2) also indicated that fishes subjected to higher and lower salinity levels were under varying degrees of stress when compared to the control. Similar differences were also noted in Hepatosomatic Index among stressed groups (2ppt, 4ppt, 8ppt and 10ppt) (Figures 3). The histological studies of liver revealed that there was no significant difference between the normal architecture of control and that of after the treatment (Figure 4 & 5).

Results indicate that juvenile Tilapia, *Oreochromis mossambicus* subjected to 2, 4, 8 and 10ppt salinities were under varying degrees of stress as noted by changes in plasma levels of plasma glucose, plasma protein and hepatosomatic index.

Table 1: Plasma glucose levels (mg/dl) in relation to salinity stress in *Oreochromis mosambicus.*

Salinity (ppt)	Glucose(mg/dl)	S.D	t Test
2	26.55000	1.202082	a
4	25.15000	0.212132	a
6	22.37000	1.088944	b
8	27.15000	1.202082	a
10	33.00000	0.565685	c

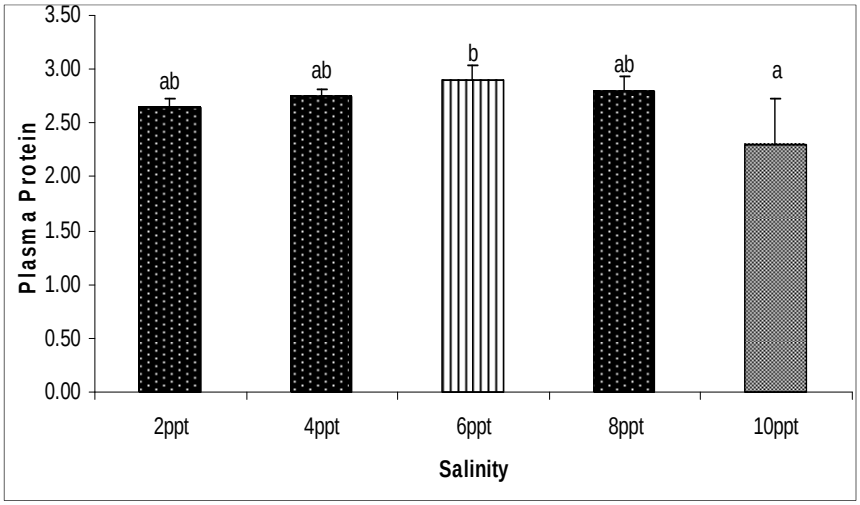


Values are mean ± SE. Different superscript letters denotes differences between treatments

Fig 1: Plasma Glucose Levels (mg/dl) in relation to salinity stress in *Oreochromis mossambicus*

Table 2: Plasma protein levels (g/dl) in relation to salinity stress in *Oreochromis mossambicus*.

Salinity (ppt)	Plasma protein(g/dl)	S.D	t Test
2	2.650000	0.070711	ab
4	2.750000	0.070711	ab
6	2.900000	0.141421	b
8	2.800000	0.141421	ab
10	2.300000	0.424264	a

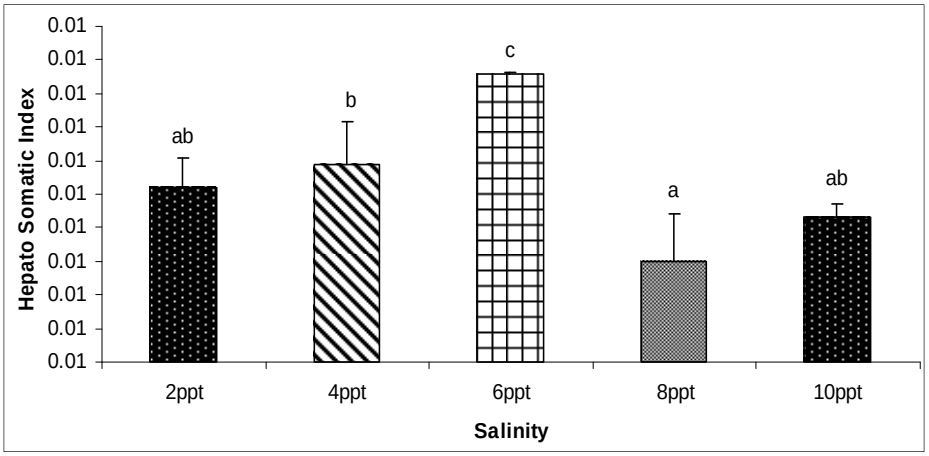


Values are mean ± SE. Different superscript letters denotes differences between treatments

Fig 2: Plasma Protein levels (g/dl) in relation to salinity stress in *Oreochromis mossambicus*

Table 3: Hepatosomatic index in relation to salinity stress in *Oreochromis mossambicus*.

Salinity (ppt)	H.S.I	S.D	t Test
2	0.007621	0.000086	ab
4	0.00769	0.000127	b
6	0.007958	0.000003	c
8	0.0074	0.000141	a
10	0.00753	0.000042	ab



Values are mean ± SE. Different superscript letters denotes differences between treatments

Fig 3: Hepato Somatic Index (HSI) in relation to salinity stress in *Oreochromis mossambicus*

Discussion

The stress response of Mozambique tilapia to salinity was evaluated using the levels of plasma glucose and total serum protein as indicators. Secondary stress might be indicated by a significant decrease or increase in some biochemical variables in the blood. Changes at this level can reflect loss of homeostasis (e.g. decreased blood electrolytes) and demonstrate a compensatory response (eg. elevated blood glucose) as reported by (Schreck, 1990) [8].

In the experimental protocol used here, the exposure of the tilapia to salinity stress, dramatically disturbed their osmo-ionic homeostasis, and their responses demonstrated osmotic stress and non-adaptation to the challenge imposed (Martínez-Álvarez *et al.*, 2002) [5]. In the current study activities of the parameter i.e., plasma glucose progressively increased on exposure to salinity stress. The biological significance of the elevated glucose levels in fish is probably related to an increased demand for metabolic substrates after exposure to a stress stimulus. Alterations in the plasma glucose levels may also be due to the disruption of glycogen homeostasis resulting from protein phosphatase inhibition (Malbrouck *et al*., 2003) [4]. Most of the studies on carbohydrate breakdown during stress and infection are restricted to glucose levels in circulatory fluid.

The seasonal variations in the total plasma protein concentrations are studied by Sano (1960) [7] in rainbow trout. Booke (1964) [3] has reviewed the effects of exogenous and endogenous factors on serum protein levels of fishes. Many euryhaline species respond to changes in salinity of the medium by altering free amino acid (FAA) content while its inorganic ion content and osmolality of circulating fluid are being modified. In the current study the amount of total serum protein of the stressed groups showed slight decrease from that of the unstressed groups. It is generally assumed that the proteins undergo hydrolysis to maintain the pool of free amino acids which is required for several vital functions like maintaining the osmotic balance. The decrease in protein may also be due to the use of protein as a source of energy. However the pathway of pyruvate to glucose is retarded and even if protein is converted to pyruvate, energy is not produced due to less production of glucose. Final shifts out of the plasma protein are caused by an osmotic imbalance between the extra cellular and intracellular and extra cellular compartments; and any stress that induces such an imbalance can lead to variations in plasma protein.

Histopathological changes in animal tissues are powerful indicators of prior exposure to environmental stressors and are the net result of adverse biochemical and physiological changes in an organism. Microscopic study of liver sections revealed that in the control group, the liver exhibited a normal architecture and there were no pathological abnormalities, with polygonal hepatocytes presenting a homogenous cytoplasm and a large central or sub central spherical nucleus. Sections after termination of treatment showed no significant difference from that of the control.

The current study showed a decrease in hepatosomatic index, which is the ratio of liver weight to body weight. These transitory changes in liver size are largely due to variations in glycogen and fat content; Barton *et al.,* (2002). Its was concluded that the exposure of the animal to salinity stress resulted in hyperglycemia and hypoproteinemia and the magnitude of stress response was related to salinity level.

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