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Enzymes activities in juveniles and adults of *Clarias gariepinus* reared in earthen ponds and concrete tanks

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Abstract
Clarias gariepinus is the most commonly cultivated fish species in Nigeria, making the country the largest producer in Africa and third in the world, after Thailand and Indonesia. Level of enzyme activities in the plasma of *Clarias gariepinus* were used to detect tissue damage caused by stress as a result of poor management practices. Sixty-four blood samples were collected from juveniles and adults of catfish reared in six randomly selected fish farms in Lagos State and analyzed with Randox test kits. Results obtained indicated that alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST) were significantly elevated (p<0.05) in experimental fish farms when compared to control farms. The study established that amino transaminase and aspartate amino transaminase were higher in fish reared in earthen ponds than concrete tanks as a result of stress, thus indicating renal-hepatic malfunctions.

Keywords: Aquaculture, catfish, physiology, stress

Introduction
The African catfish, *Clarias gariepinus* is the most widely cultured species in Nigeria and in many countries of Africa tropical sub region [1]. The specie is widely distributed in Africa, from Nile to West Africa and from Algeria to South Africa. The African catfish has many desirable characteristics which include fast growth rate, hardiness and ability to convert feed to carcass weight [2]. In culture medium, various handling procedures may cause stress in the system of the fish, without necessarily leading to death [3]. Stress response in fish is characterized by biochemical and physiological changes which may be manifested in changes induced by culture systems [4, 5]. The disruption of the biochemical and physiological integrity is assessable by the changes in the enzyme activities in plasma of the fish [6].

Enzymes are biochemical macromolecules that control the metabolic process of organisms, thus a slight variation in enzyme activities would affect the organism's metabolic integrity [7]. They are indispensable for signal transmission and cell regulation, often via kinases and phosphates. The activities of alkaline phosphatase, alanine aminotransferase, aspartate and aminotransferase, are useful marker enzymes of damage to the system of the fish [8] [9]. Transamination is one of the principal pathways for the synthesis and deamination of amino acids, enabling carbohydrate and protein metabolism during fluctuating energy demands of the fish under various adaptive conditions [10]. Maintenance of internal homeostasis through biochemical processes in the Kreb's cycle may be reflected by variation in the levels of the enzymes AST, ALT, ALP in the plasma of the fish, triggered by cellular damage in the functional organs such as liver, heart, gill, muscles and kidney as they are generally found in the tissues of these organs [11]. Both serum AST and ALT activities in the cell of an organism are raised when infections affects cell integrity [12]. The complex of unspecified biochemical indicators of blood and organs reveals the general effect of pollutants and toxin on fish makes it possible to forecast the consequences of the long-term exposure to chemical pollutants [13]. Moreover, evaluation of blood biochemistry was considered as a useful tool for the diagnosis of diseases and assessing the physiological status of fish [14].

Several authors have carried out alteration studies on numerous physiological and biochemical indices induced by environmental conditions and the presence of contaminants [15] [16] [17]. The biochemical parameters in fish are essential for physio-pathological assessment and are highly sensitive for detecting potential adverse effects and relatively early events of stress [18] [19]. Insignificant consideration has been given to the study of enzymatic effects of different culture

systems on the African catfish *C. gariepinus*. Hence, the present study was carried out to evaluate the effects of two different culture systems (earthen ponds and concrete tanks) on the plasma enzymes of African catfish *C. gariepinus* which is widely cultured in different parts of Nigeria.

Materials and methods

Experimental Location

The experiment was carried out in three agricultural zones of Lagos State, Nigeria (Figure 1). A total of six experimental and two control fish farms were selected for the experiment based on their culture systems (earthen pond and concrete tank). For earthen pond: TRA (eastern zone), BLU (western zone), MOY (far eastern zone). While concrete tanks experimental fish farms are: TEM (eastern zone), TIM (western zone), CHA (far eastern zone), and control farm comprising of earthen ponds and concrete tanks, located in Nigeria Institute for Oceanography and Marine Research, Badore Lagos.

Note: TRA, BLU, MOY, TEM, TIM and CHA are acronyms of the experimental fish farms, for ethical grounds and non-disclosure agreement

Collection of blood samples

Blood samples were collected at 7th week (juveniles) and 16th week (adults) of rearing period. Each blood collection was completed within 5 minutes of fish removal from the culture system. 5ml samples were drawn once and poured into Eppendorf tubes containing 500U of sodium heparin used as an anticoagulant. The blood samples were put in ice chest box and transported within 6 hours of collection to biochemistry laboratory for analysis.

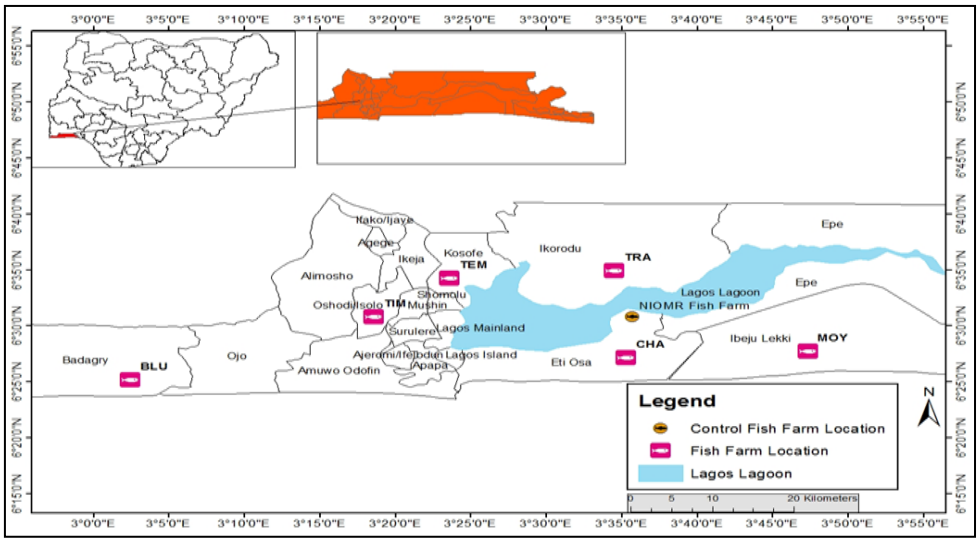

Fig 1: Map of Lagos showing the experimental fish farms

Analytical procedure

Blood samples were centrifuged immediately for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20 °C until assayed [20]. The results were read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405).

Separation of plasma

The 8ml blood samples collected with heparin tubes were transferred into clean, dry centrifuge tubes and later centrifuged at 5000 rpm for 10 min at controlled temperature of 4 °C, to obtain plasma. Plasma was pipetted into Eppendorf tubes and later stored in refrigerator at -20 °C until analyzed [20]. All blood samples were analyzed in triplicates read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405).

Determination of Alkaline phosphatase (ALP)

The concentration of alkaline phosphatase in plasma was determined spectrophotometrically according to [21, 22]. Three cuvettes marked Macro, Semi micro and Micro was arranged in a rack. 0.05ml of plasma sample was pipetted into Macro cuvette, 0.02 ml sample was pipetted into semi-micro and 0.01 ml sample was pipetted into micro cuvette. 3.00ml reagents were pipetted into macro cuvette, 1.00 ml of the reagents were pipetted into semi-micro cuvette and 0.50 ml of the reagents was pipetted into the micro cuvette. The solution was mixed and the initial absorbance was read at Hg 405 nm at a temperature of 37 ° C. It was read again after 1, 2, and 3 minutes. (Timer was set to run simultaneously).

ALP concentration was calculated using the following formular:

$$U/l = \frac{2760 \times \text{Absorbance 405 nm}}{\text{Minute}}$$

Determination of Aspartate aminotransferase (AST)

The concentration of aspartate aminotransferase in plasma was determined spectrophotometrically. This method was carried out according to [23, 24]. AST was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2, 4dinitrophenylhydrazine. Two test tubes were labeled blank (B) and sample (S). In the reagent blank test tube was pipetted 0.5ml of buffer (reagent 1) followed by 0.1ml distilled water, while the sample test tube labeled (S) was pipetted 0.1ml plasma sample and 0.5ml of buffer. The mixture was incubated for exactly 30 minutes at 37 °C. Later 0.5 ml of 2, 4-dinitrophenylhydrazine (reagent 2) was pipetted into the two test tubes, mixed and allowed to stand for exactly 20 minutes at 25 °C. Later, 5.0 ml of Sodium hydroxide was pipetted into the two test tubes. The solution was mixed and the absorbance of the sample was read at 546 nm against the reagent blank after 5 minutes. The activity was extrapolated from the standard curve.

Determination of Alanine aminotransferase (ALT)

Alanine amino transferase was measured spectrophotometrically according to the method of [23] [24]. This was done by monitoring the concentration of Pyruvate hydrazone formed with 2, 4 dinitrophenyl hydrazine. Two test tubes were labeled Reagent blank (B) and Sample (S). 0.5 ml of 100 mmol/l phosphate buffers and 0.5 ml of 200 mmol of L- alanine and 0.1 ml of distilled water was pipetted into reagent blank test tube. 0.1 ml of plasma sample, 0.5 ml of 100 mmol/l phosphate buffers and 0.5 ml of 200 mmol of L-alanine was pipetted into test tube (S). The solution in the three tubes was mixed, incubated for exactly 30 minutes at 37 ^{o}C. Later, 0.5ml of 2.0 mmol/l 2, 4-dinitrophenylhydrazine was pipetted into reagent blank tube and sample test tubes. The solution was mixed, incubated for exactly 20 minutes at 25 ^{o}C. Lastly, 5.0 ml of Sodium hydroxide was pipetted into reagent test tube and sample test tube. The solution was mixed and the sample absorbance was read at 578 nm against the reagent after 5 minutes. The activity was extrapolated from the standard curve.

Statistical analysis

Indices of oxidative stress were analyzed using one-way analysis of variance, (ANOVA) at 5% level of significance. Post-hoc comparison of significance of variance results gotten from ANOVA was done using DMRT (Duncan Multiple Range Test) tests. These analyses were carried out based on a computer programme SPSS 10.0 designed and implemented by Ge Le Pattaurel.

Results

The enzyme activities in the plasma of *C. gariepinus* juveniles reared in grow-out earthen ponds and concrete tanks in two production cycles are presented in Figures 1-3. Juveniles in earthen ponds in experimental farms showed significant difference (p<0.05) comparable to control farm in alkaline phosphatase (ALP) and alanine transaminase (ALT), while aspartate transaminase (AST) were within the same range (11.06-16.66) with no significant difference (p<0.05) however, the highest values (411.11±25.01, 20.00±3.00 and 16.66±1.15U/l for ALP, ALT and AST respectively were recorded in MOY farm while the lowest values were observed in BLU. The adults in earthen ponds recorded highest value of ALP (409.40±45.60U/l) in MOY farm and lowest value (383.40±45.60) in BLU (Figure 1). The values of ALT were within the same range (11.33-13.00), however a higher value (20.33±12.22) was obtained in MOY (Figure 2). AST showed no significant difference (p>0.05) in all the farms, however, the highest value (43.66±4.61) was observed in MOY fish farm (Figure 3)

In concrete tank farms, the values of ALP, ALT and AST were elevated compared to the control values with the highest values of 332.73±82.04, 14.66±1.52 and 25.33±3.14U/l for ALP, ALT and AST respectively. The enzymes activities for adult *C. gariepinus* raised in concrete tanks revealed that ALP varied significantly (p<0.05) in all the farms with the highest value (566.10±68.20) recorded in TEM fish farm and the lowest (297.54±71.41) recorded in CHA fish farms under the control value of 154.25±24.98 (Figure 1). The comparative values of ALP in both sizes of *C. gariepinus* raised in concrete and earthen ponds indicated that the values of ALP were higher in TEM fish farms than other farms. The adult fish recorded more ALT than the juveniles fish except in TRA; however the highest values were recorded in MOY while the lowest was recorded in CHA. (Figure 2). AST in both sizes of *C. gariepinus* indicated that the adult had higher values of AST, in all the farms however TRA fish farms recorded the highest values (Figure 3).

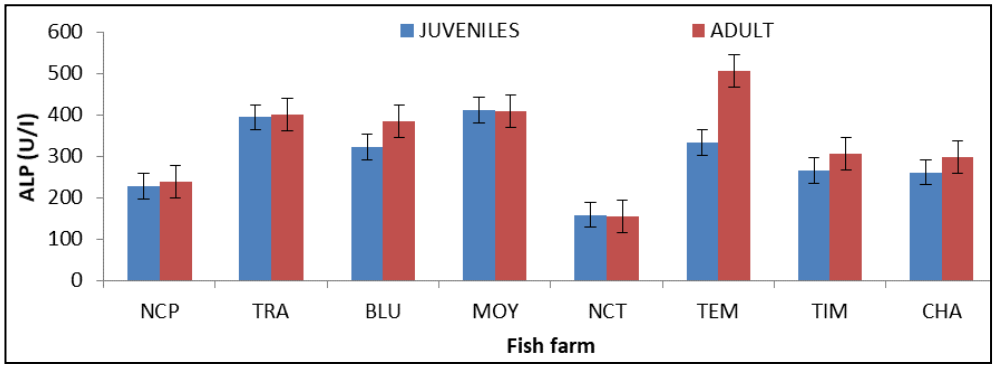

Fig 1: Values of ALP in *C. gariepinus* reared in earthen ponds and concrete tanks

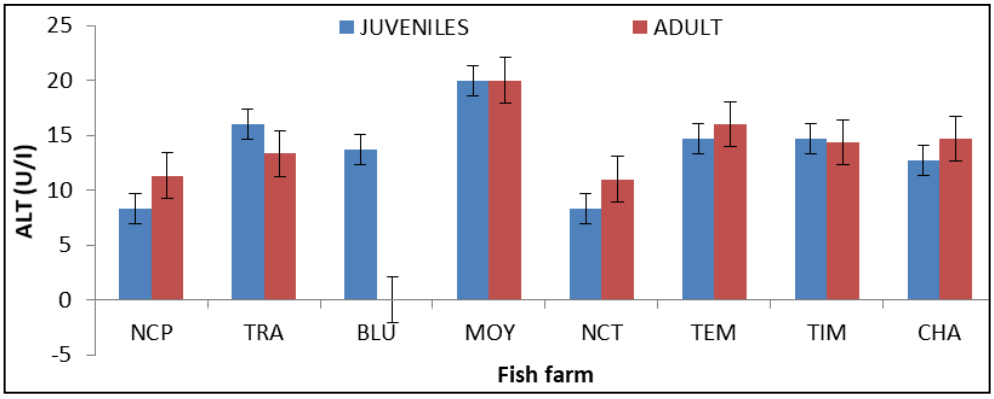

Fig 2: Values of ALT in *C. gariepinus* reared in earthen ponds and concrete tanks

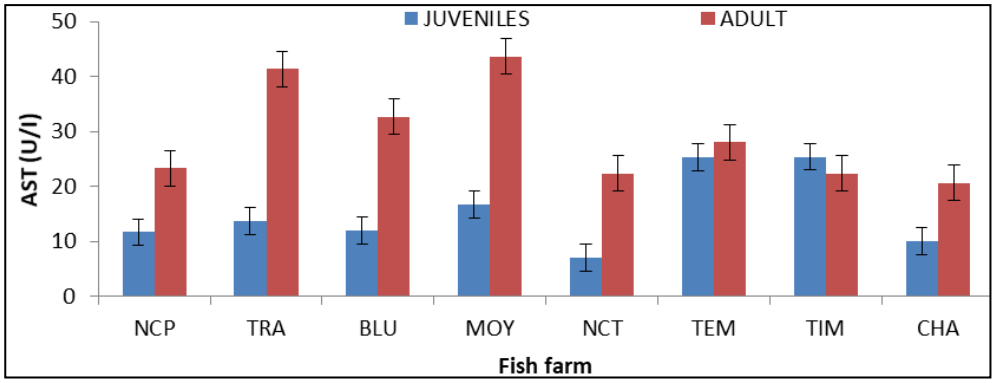

Fig 3: Values of AST in *C. gariepinus* reared in earthen ponds and concrete tanks

Discussion

Stressful aquaculture practices have been reported to affect enzyme profiles in fish [25, 26] observed that an increase of ALT, ALP and AST activities in fish stressed by low DO and high stocking density may reflect the use of excess hydrocarbons from amino acids to supply energetic demands. The rise in ALT and AST in Stressed fish may indicate use of dietary amino-acids for growth as well as compensatory for energy demand as a response to the stressor. These enzymes provide information about hepatic state and disorders. These disorders could be as a result of injury or liver disease. The injury could be caused by reactive metabolites, resulting from xenobiotic metabolism in the liver [27]. Also, the observed elevated levels of ALP may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, which may result to increased detoxification effects of the kidney and thus a possible stress on the kidney membrane that could cause cell injury [28]. The increases could also result in a shift in biosynthesis, mixed-function oxidase and energy metabolism pathways [29].

In the present study slight increases were observed in the values of ALP, ALT and AST in the plasma of *Clarias gariepinus* reared in two culture systems. Both AST and ALT increased significantly under the effect of stressful conditions of poor water quality. The rise in ALT and AST in stressed fish may indicate use of dietary amino-acids for growth as well as compensatory for energy demand as a response to a stressor. Also, the observed elevated levels of ALP may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, which may result to increased detoxification effects of the kidney and thus a possible stress on the kidney membrane that could cause cell injury [30]. Similar results were however observed in *Catla catla* [31], *Labeo rohita* [32] *Sarotherodon mossambicus* [33]. Moreover, increased activities of ALP, AST and ALT were observed in plasma of *Cyprinus carpio* [34] exposed to cypermethrin pesticides.

Conclusion

The study established that amino transaminase and aspartate amino transaminase were found to be higher in fish reared in earthen ponds than concrete tanks as a result of stress which resulted from poor water quality. Thus indicating renal-hepatic malfunctioning in fish reared in earthen ponds. Therefore, various handling procedures and management practices in fish farms that affect fish physiology should be reckoned with when reporting incidence of culture systems in aquaculture.

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