


Plasma Biochemical Responses in Fishes (*Oreochromis niloticus* and *Clarias gariepinus*) exposed to Different Regimes of Salinity and pH

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ABSTRACT

Aquatic organisms often undergo marked blood biochemical responses triggered by exposure to environmental or physiological stressors. The present study investigated the effect of environmental stress due to different regimes of salinity and pH on the enzymes of liver function [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and Lactate dehydrogenase (LDH)] activities in the plasma of Nile tilapia, *Oreochromis niloticus* and African mud catfish, *Clarias gariepinus*. Fishes (*O. niloticus* and *C. gariepinus*) were exposed singly to 4, 6, and 8 ‰ regimes of salinity and 4, 6, and 8 pH regimes in a semi-static renewal system for a period of 7, 14 and 21 days. Significant differences ($p < 0.05$) in the biochemical responses (AST, ALT, ALP and LDH) of *O. niloticus* and *C. gariepinus* to different regimes of salinity and pH compared to the control at days 7, 14, and 21 were found in most experimental groups. The results obtained showed that the biochemical responses increased significantly with concentration and duration of exposure thus reflecting the effect of stress as a result of changes in salinity and pH on the plasma biochemical composition of the fishes. The study confirmed that the activities and expression levels of AST, ALT, ALP and LDH can be used as biomarkers to indicate potential damages triggered by stress related environmental factors.

Keywords: Biomarkers, Enzyme, Fish, pH, Plasma, Salinity, Stress

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INTRODUCTION

Aquatic environments are regarded as places of great biodiversity due to the presence of numerous and diverse organisms that spreads from the prokaryotes to the higher vertebrates (Almroth, 2008). These environments are great deposits for a wide range of anthropogenic pollutants, as a result of industrialization, urbanization and increase in the human population. Increase in anthropogenic carbon dioxide emissions dissolved in aquatic environments can decrease water pH and this may cause acidification overtime (Hoegh-Guldberg *et al.*, 2007).

Anthropogenic climate change leading to sea level rise can induce flooding and saltwater intrusion of coastal areas and freshwaters (Horton *et al.*, 2014). It may also cause climatic shifts and variations in seasonal patterns of evaporation and precipitation (Talley *et al.*, 2011). These factors cause radical changes to water quality parameters such as

temperature, salinity, pH, turbidity, and colour thereby causing stress to organisms and may result in lethal and sub-lethal effects on aquatic organisms (Choi *et al.*, 2013).

The levels of dissolved salts in fluid control the biochemical process of osmosis and many metabolic processes (Kultz, 2015). In the aquatic environment, high salinity has been found to inhibit the survival, growth and reproduction of fish and engender many physiological and biochemical defects in them. Studies have shown that environmental salinity influences the growth and metabolism of tilapia (Kucuk *et al.*, 2013). Salinity regimes at 9‰ and 10‰ are lethal in *Oreochromis niloticus* (Lawson & Anetekhai, 2011).

Acidic conditions that are lethal to fish have resulted from exposure to low levels of pH (pH 4 and below) (Ikuta *et al.*, 1992). Effects of sublethal acidification induce various physiological and ecological problems in fish, due to the stress of

acidification (Ikuta *et al.*, 1996). Studies revealed that decreasing pH resulted in decreased haematocrit and haemoglobin values (El-Sheriff *et al.*, 2009). Stress resulting from elevated pH levels (9.0 and above) in fish have been found to induce tissue damage and excessive mucus secretion (Serafy & Harrel, 1993).

The levels of plasma enzymes in fish have been proposed to act as good biomarkers of extreme stress and organ dysfunction (Li *et al.*, 2010). The study by Boyd (1983), also observed that tissue injury, environmental stress, or a diseased condition can result from changes in plasma enzyme activity. However, the rate at which the plasma enzyme activity increases is dependent on the cellular enzyme concentration, the rate of leakage due to injury and the rate of enzyme removal from the plasma. The analysis of Aspartate transaminase (AST) and Alanine transaminase (ALT) in blood plasma can be used to indicate the liver function or toxicant-induced hepatotoxicity (Roy & Bhattacharya, 2005; Datta *et al.*, 2007; Sopinka *et al.*, 2016). The determination of Alkaline Phosphatase (ALP) activity is important for the detection of liver disease. This is because damaged liver cells release greater concentrations of ALP into the bloodstream. Studies by Kori-Siakpere *et al.* (2010) observed that the means of plasma ALP levels in *Clarias gariepinus* exposed to KMnO_4 increased significantly as the concentration increased ($p < 0.05$). Lactate Dehydrogenase is usually determined to detect damage in tissues (Granchi *et al.*, 2010). Sangiao-Alvarellos *et al.* (2003) study on the Gilthead seabream (*Sparus aurata*) observed an increase in the glucose and lactate levels in the plasma, kidneys, and liver after acclimatization to different salinity conditions.

The Nile Tilapia, *Oreochromis niloticus* (Linnaeus 1758) is an ecologically important and commercially valued fish. This fish is frequently and widely cultured in ponds and it occurs freely in natural tropical fresh waters (Samuel & Ojikutu, 2020). The fish is used increasingly for intensive aquaculture which may result in it being the most important cultured fish in the 21st century (Lim *et al.*, 2005). Mortality in juveniles of *O. niloticus* is reported to increase in waters with salinity > 7 ‰ and pH < 3.0 (Ross, 2000; Jumah & Traifalger, 2015). The African mud catfish, *Clarias gariepinus* (Burchell 1822), inhabits freshwater environments and is known to survive in a pH range of 6.5-8.0 (Gunder, 2004). It is also of great importance because it is one of the most widely cultured and consumed fish in Nigeria and can adapt to very extreme environmental conditions.

This study investigated the effect of environmental stress due to different regimes of salinity and pH on

the activity of enzymes of liver function (Aspartate transaminase, Alanine transaminase, Alkaline phosphatase and Lactate dehydrogenase) in the plasma of *Oreochromis niloticus* and *Clarias gariepinus*.

MATERIALS AND METHODS

Test Organism Collection and Acclimatization

Juveniles of *Oreochromis niloticus*, (Family: Cichlidae) with mean weight of 15.7 ± 1.2 g and mean standard length of 8.5 ± 0.31 cm and *Clarias gariepinus*, (Family: Clariidae) with mean weight of 19.12 ± 1.50 g and mean standard length of 12.18 ± 0.52 cm were obtained from the Treasure Base Farms, Ikotun, Lagos State and transferred to the Aquatic Toxicology and Ecophysiology Laboratory, Department of Marine Sciences, University of Lagos. The fishes were transferred into well aerated rectangular tanks ($60 \times 37 \times 9 \text{ cm}^3$) containing dechlorinated water for two weeks in order to allow for acclimatization.

Test Chemicals

Sigma-Aldrich Analar grades of Sodium chloride (NaCl) 99.0% salt, Nitric acid (HNO_3) 90.0% liquid and Sodium hydroxide (NaOH) 97.0% pellet used for this study were obtained from the Department of Marine Sciences, University of Lagos.

Experimental Design

The semi-static system was adopted in which the test solutions were renewed every 48 hours for a period of 21 days at a stocking density of 10 fish per tank after the acclimatization period. Preliminary screening was done to ascertain the suitable range of concentration for the test solutions as described by (Bartram & Balance, 1996). Preparation of the salinity and pH regimes was done by dilution of the test chemicals accordingly. There were three replicates for each of the treatments; Salinity regimes (4, 6, and 8) ‰; pH regimes (4, 6, and 8) and Control (0 ‰ and neutral pH). Other water quality parameters: dissolved oxygen, temperature, and total dissolved solids were determined at intervals of 7 days using the Horiba U-50 to ensure salinity and pH are kept constant and maintain good water quality of the test solutions.

At each interval of 7 days for the 21-day exposure period, three (3) fish were randomly selected from the different pH and salinity regimes, including the replicates and control. Each fish was placed belly upwards and blood was taken from the heart with the

aid of a 2 ml heparinized disposable plastic syringe until about 0.5 ml was obtained from each of the selected fishes under aspiration. Thereafter, the blood was pooled together, thoroughly mixed and gently transferred into special tubes containing potassium ethylenediaminetetraacetic acid (EDTA) as an anticoagulant agent and then stored in the refrigerator at 2-8°C prior to biochemical analysis. This procedure was carried out separately on the juveniles of *O. niloticus* and *C. gariepinus*.

Biochemical Analysis

The determination of Aspartate transaminase (AST) activity was carried out by measuring the concentration of oxaloacetate hydrazine formed with 2,4- dinitrophenylhydrazine while the Alanine transaminase (ALT) activity was determined by measuring the concentration of pyruvate hydrazone produced with 2,4-dinitrophenylhydrazine (Reitman & Frankel, 1957). The Alkaline phosphatase activity was obtained using the Qualitative Comparative Analysis (QCA) method. A reagent kit containing a chromogenic substrate, a colour developer and a standard solution of Alkaline phosphatase in water/ethanol was used for the analysis (Kind & King, 1954). The determination of Lactate dehydrogenase (LDH) activity was obtained with the use of the Randox reagent as described by Weisshaar *et al.* (1975).

Statistical Analysis

One way analysis of variance (ANOVA) and Duncan multiple range test (DMRT) were used to test for statistical differences (5% level) in the mean plasma biochemical responses of *O. niloticus* and *C. gariepinus* to different regimes of salinity and pH at 7, 14 and 21 days of exposure. Analysis (mean, ANOVA and DMRT) were carried out using SPSS 17.0 for windows.

RESULTS

The results of the physico-chemical parameters of the test solutions recorded throughout the duration of exposure are shown in (Table 1). The results of the statistical analysis of the biochemical responses on the enzyme activity [Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH)] in the plasma of *Oreochromis niloticus* and *Clarias gariepinus* revealed significant differences ($p < 0.05$) when

exposed to different regimes of salinity and pH at days 7, 14, and 21. The enzyme levels varied with increase in the regimes and the duration of exposure. However, highest enzyme activity obtained was in the order: LDH>AST>ALT>ALP.

In *O. niloticus* exposed to the different salinity regimes (Table 2), the AST activity ranged from 80.99 IU/L – 261.12 IU/L while the ALT activity ranged from 20.16 IU/L – 255.67 IU/L. Further analysis showed no significant differences ($p > 0.05$) in the mean AST activity of *O. niloticus* exposed to 4 ‰ (day 14), as well as in the mean ALT activity at 6 ‰ (day 7), compared to the control. The ALP activity in the plasma of *O. niloticus* exposed to different salinity regimes ranged from 24.33 IU/L – 238.33 IU/L while the LDH activity ranged from 73.00 IU/L – 314.00 IU/L. There was no significant difference ($p > 0.05$) found in the mean ALP activity in the plasma of *O. niloticus* exposed to 4‰ and control at day 7.

The AST activity of *C. gariepinus* exposed to the different salinity regimes ranged from (91.79 – 159.70) IU/L while the ALT activity ranged from (30.63 – 101.14) IU/L (Table 3). Further analysis comparing treatments with the control indicated no significant differences ($p > 0.05$) in the AST activity in organisms exposed to 4 ‰ at day 7; 6 ‰ and 8 ‰ at day 14. Similar results were found in the ALT activity which showed no significant differences ($p > 0.05$) in all treatments compared to the control except at day 21. The ALP and LDH activities ranged from (21.63 – 188.63) IU/L and (77.00 – 312.98) IU/L respectively. There were no significant differences ($p > 0.05$) in the mean ALP (day 14) and mean LDH (day 7) activity in the exposed organisms to 4 ‰ compared to the control.

For regimes of pH (Table 4), the AST and ALT activity in the plasma of *O. niloticus*, ranged from 101.53 IU/L – 249.69 IU/L and 36.19 IU/L – 162.47 IU/L, respectively. In the pH regimes, further analysis showed no significant difference ($p > 0.05$) in the mean AST activity of *O. niloticus* exposed to pH of 6 compared to the control at day 21. The ALP activity in the plasma of *O. niloticus* ranged from 25.00 IU/L – 245.67 IU/L and the LDH activity ranged from 92.33 IU/L – 314.67 IU/L. However, comparing treatments with the control revealed no significant difference ($p > 0.05$) in the mean LDH activity of *O. niloticus* exposed to pH of 8 at days 14 and 21.

In *C. gariepinus* exposed to the different pH regimes (Table 5), the AST activity ranged from 101.80 – 192.18 IU/L while the ALT activity ranged from 30.80 – 84.60 IU/L. However, further analysis showed no significant differences ($p > 0.05$) in the mean AST activity of *C. gariepinus* exposed to pH of 4 and

6 (day 7), as well as in the mean ALT activity at pH of 8 (day 14), compared to the control. The ALP activity in the plasma of *C. gariepinus* exposed to different pH regimes ranged 25.13 – 74.44 IU/L while the LDH activity ranged 92.83 – 383.75 IU/L. There was no

significant difference ($p>0.05$) in the mean ALP activity in the plasma of *C. gariepinus* exposed to pH of 6 and 8 (day 7) and pH of 4 and 6 (day 14), compared to the control.

Table 1: The results of water quality parameters through the duration of exposure

Parameters	Salinity regimes	pH regimes
Temperature (°C)	27.92±0.28	28.07±0.51
Dissolved Oxygen (mg/l)	8.04±2.24	10.64±2.45
Total Dissolved Salts (g/l)	4.98±3.31	0.13±0.02
Salinity	NA*	6.99±0.16
pH	0.1±0.00	NA**

Data are presented as mean ± SD of 3 replicates; SD=Standard deviation; NA: Not applicable, * indicates the salinity regimes: (4‰, 6‰, & 8‰) while ** indicates the pH regimes: (4, 6, & 8)

Table 2: Enzyme activity (IU/L) in the plasma of *Oreochromis niloticus* exposed to different salinity regimes for 21 days.

Salinity (‰)	Days of Exposure (<i>Oreochromis niloticus</i>)		
	7	14	21
Aspartate aminotransferase (AST)			
0 (Control)	124.42±0.64 ^a	130.07±0.54 ^a	105.33±0.88 ^b
4	178.83±0.90 ^b	126.38±0.87 ^a	175.33±2.60 ^c
6	252.23±0.73 ^c	233.68±1.22 ^b	80.99±1.36 ^a
8	256.62±0.27 ^d	261.12±1.05 ^c	87.90±0.62 ^a
Alanine aminotransferase (ALT)			
0 (Control)	37.39±2.89 ^b	58.07±0.80 ^b	131.33±1.45 ^b
4	20.16±1.22 ^a	79.03±1.48 ^c	99.77±0.79 ^a
6	38.91±1.45 ^b	84.89±1.45 ^a	236.33±0.88 ^c
8	137.61±1.25 ^c	122.78±1.25 ^d	255.67±1.45 ^d
Alkaline Phosphatase (ALP)			
0 (Control)	39.00±3.46 ^a	24.33±1.33 ^a	238.33±1.76 ^d
4	36.33±1.20 ^a	129.04±1.23 ^b	214.67±1.20 ^c
6	51.67±3.53 ^b	135.56±0.83 ^c	167.67±1.45 ^a
8	220.67±1.45 ^c	160.00±1.15 ^d	187.67±1.45 ^b
Lactate Dehydrogenase (LDH)			
0 (Control)	181.33±1.20 ^b	73.00±1.15 ^a	198.67±1.66 ^d
4	111.00±1.00 ^a	125.33±1.20 ^b	116.67±1.20 ^a
6	114.33±2.33 ^a	190.67±0.88 ^c	132.33±1.76 ^b
8	119.33±2.60 ^a	314.00±1.15 ^d	152.33±1.76 ^c

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ($p<0.05$) in the Duncan test.

Table 3: Enzyme activity (IU/L) in the plasma of *Clarias gariepinus* exposed to different salinity regimes for 21 days.

Salinity (‰)	Days of Exposure (<i>Clarias gariepinus</i>)		
	7	14	21
Aspartate aminotransferase (AST)			
0 (Control)	119.56±3.21 ^a	136.18±1.24 ^b	135.39±1.26 ^c
4	124.20±2.91 ^a	123.73±4.12 ^a	91.79±0.83 ^a
6	150.79±3.51 ^b	130.20±0.54 ^{ab}	111.72±1.08 ^b
8	159.70±3.75 ^c	106.71±0.86 ^b	146.73±0.80 ^d
Alanine aminotransferase (ALT)			
0 (Control)	45.21±4.05 ^{ab}	53.39±0.66 ^{ab}	53.39±0.66 ^b
4	30.63±2.87 ^a	48.15±2.96 ^a	45.89±1.20 ^a
6	42.72±3.48 ^{ab}	56.98±0.90 ^{bc}	84.03±0.92 ^c
8	63.34±5.32 ^b	63.50±0.74 ^c	101.14±1.16 ^d
Alkaline Phosphatase (ALP)			
0 (Control)	29.70±3.68 ^a	24.02±0.99 ^a	24.32±0.94 ^a
4	43.32±1.59 ^b	21.63±2.06 ^a	35.03±1.16 ^b
6	51.83±1.39 ^{bc}	32.83±1.56 ^b	65.91±0.88 ^c
8	61.63±3.62 ^c	43.95±0.21 ^c	188.63±0.79 ^d
Lactate Dehydrogenase (LDH)			
0 (Control)	116.53±4.11 ^b	103.30±1.18 ^a	103.68±1.01 ^b
4	127.28±2.78 ^b	132.74±76.64 ^b	143.98±2.25 ^d
6	312.98±3.93 ^c	142.86±2.08 ^c	77.00±0.72 ^a
8	85.60±1.66 ^a	126.38±1.52 ^b	121.03±1.66 ^c

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ($p < 0.05$) in the Duncan test.

Table 4: Enzyme activity (IU/L) in the plasma of *Oreochromis niloticus* exposed to different pH regimes for 21 days.

pH	Days of Exposure (<i>Oreochromis niloticus</i>)		
	7	14	21
Aspartate aminotransferase (AST)			
0 (Control)	124.30±0.62 ^a	133.33±0.33 ^a	114.67±1.20 ^b
4	152.86±1.47 ^b	191.35±0.91 ^b	101.53±1.64 ^a
6	185.30±0.98 ^c	205.13±1.64 ^c	116.67±1.45 ^{bc}
8	185.80±0.83 ^c	249.69±1.17 ^d	122.33±0.88 ^c
Alanine aminotransferase (ALT)			
0 (Control)	36.19±1.99 ^a	55.27±1.47 ^a	136.67±1.20 ^a
4	52.68±1.15 ^b	101.57±0.95 ^b	150.33±1.10 ^b
6	110.04±1.56 ^c	104.39±1.57 ^b	157.49±0.86 ^c
8	111.22±2.27 ^c	110.48±4.82 ^b	162.47±0.74 ^d
Alkaline Phosphatase (ALP)			
0 (Control)	37.00±2.31 ^a	25.00±1.73 ^a	245.67±0.67 ^d
4	81.67±1.86 ^b	95.00±1.15 ^d	184.08±0.92 ^a
6	93.00±1.00 ^c	51.00±1.73 ^c	192.07±1.58 ^b
8	95.33±2.03 ^c	32.00±1.33 ^b	203.17±1.59 ^c
Lactate Dehydrogenase (LDH)			
0 (Control)	181.33±1.20 ^a	102.67±0.88 ^b	212.33±0.88 ^c
4	284.33±1.20 ^b	170.00±1.00 ^c	192.14±1.32 ^a
6	314.67±2.02 ^c	92.33±2.03 ^a	200.23±2.06 ^b
8	314.00±1.73 ^c	101.67±2.03 ^b	211.67±1.20 ^c

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ($p < 0.05$) in the Duncan test.

Table 5: Enzyme activity (IU/L) in the plasma of *Clarias gariepinus* exposed to different pH regimes for 21 days.

pH	Days of Exposure (<i>Clarias gariepinus</i>)		
	7	14	21
Aspartate aminotransferase (AST)			
0 (Control)	158.87±2.93 ^b	117.15±1.70 ^a	118.90±1.37 ^a
4	167.29±1.11 ^b	124.40±0.70 ^b	163.20±0.04 ^d
6	157.78±3.60 ^b	192.18±1.20 ^c	147.83±0.36 ^c
8	101.80±4.36 ^a	118.54±0.78 ^a	125.34±0.64 ^b
Alanine aminotransferase (ALT)			
0 (Control)	69.17±3.93 ^{bc}	73.08±1.14 ^c	72.20±1.21 ^b
4	81.12±4.17 ^c	84.60±4.25 ^d	72.47±0.45 ^b
6	57.81±4.25 ^b	49.52±1.39 ^b	68.80±0.52 ^b
8	34.93±4.78 ^a	30.81±2.08 ^a	48.60±1.74 ^a
Alkaline Phosphatase (ALP)			
0 (Control)	36.16±3.57 ^{ab}	42.66±1.48 ^b	43.49±0.65 ^a
4	50.28±1.14 ^c	47.90±1.83 ^b	74.26±1.12 ^c
6	37.98±3.37 ^b	48.13±1.55 ^b	60.81±1.48 ^b
8	25.13±1.88 ^a	35.03±1.29 ^a	74.43±2.21 ^c
Lactate Dehydrogenase (LDH)			
0 (Control)	207.63±3.60 ^a	122.19±1.88 ^d	122.86±1.79 ^a
4	383.75±4.83 ^d	111.95±1.44 ^c	181.24±1.08 ^c
6	296.64±4.37 ^c	102.49±1.80 ^b	160.10±1.66 ^b
8	260.30±4.80 ^b	92.83±1.92 ^a	210.84±1.97 ^d

Means (± S.E, standard error) with same superscript letter in a column are not significantly different ($p < 0.05$) in the Duncan test.

DISCUSSION

In this study, there was a general decline in the AST activity in both fishes (*O. niloticus* and *C. gariepinus*) exposed to the salinity and pH regimes whereas, the ALT activity increased with increase in the salinity regimes and fluctuated with the increase in the pH regimes as the duration of exposure progressed. However, AST was considerably higher than the ALT activity in both fishes. This elevation and inhibition of AST and ALT activities in fish indicate cellular damage that could arise as a result of exposure to the regimes of salinity and pH resulting in the release of these enzymes to the plasma. According to Philip *et al.* (1995) and McGill (2016), the transaminases, AST and ALT are very important in the amino-acid metabolism as they serve to retain the amino groups, thus an increase in the activities of both transaminases would indicate an increase in transamination processes while a decline can be as a result of inactive transamination and oxidative deamination. Kaya *et al.* (2016) also pinpointed that fluctuating enzyme activity which is in line with this study could indicate adaptation mechanisms to stress conditions in the exposed fishes. Similar findings by Brett (2009) and Alkatrani *et al.* (2018) on increased AST and ALT activities owed it to enzymes released into the cytoplasm and mitochondria which increased metabolic rate. This is

also similar to studies by Kumari *et al.* (2011) who reported significant increases in the transaminases (ALT and AST) activity in the fish *Labeo rohita* most likely as a result of damages to the plasma membrane thereby causing enzyme leakage. Jumah and Traifalger (2015) also revealed a significant ($p < 0.01$) increase of the aminotransferases affecting the survival of *O. niloticus* due to exposure to high salinity which could induce increased stress responses and amino enzymes catabolism. The reduction in enzyme activity is captured by Kaoud *et al.* (2011) with the study on *O. niloticus* exposed to Cadmium stating that a reduction in the AST activity in the plasma may be possible, in a case of leakage and/or inhibition of liver enzymes as a result of liver necrosis.

The mean ALP activity observed in this study showed the lowest biochemical responses with fluctuating levels increasing with the duration of exposure. Increased ALP activity can be associated with the adverse effects of liver impairment, kidney dysfunction and bone diseases. This agrees with the findings of Kori-Siakpere *et al.* (2010), Kumari *et al.* (2011) and Akani & Gabriel (2016). The low ALP activity can be ascribed to the effects of stressors causing an alteration of the phosphate pathway, leading to lowered metabolic demand, and/or inhibition to the growth and increase of cells. The lowered ALP activity in plasma of the exposed fishes

was similar to that obtained by Mohammed *et al.* (2019) who reported decreased ALP activity in the liver of *Tilapia zilli* and *Mugil capitol* due to metal contamination. Abdel-Tawwab *et al.* (2004) also recorded a significant reduction in ALP activity in the liver and kidney of *O. niloticus* after mercury contamination possibly due to liver damage and dysfunction. Similarly, Sarma *et al.* (2013), observed less growth and survival of *Clarias batrachus* exposed to high salinity, indicated by a significant decrease ($p < 0.01$) in the biochemical responses, ALP and adenosine triphosphatase (ATPase) activity in the liver and muscle tissues as salinity increased ($0 < 4 < 8$) ‰.

The concentrations of LDH enzyme in both fishes exposed to the salinity and pH regimes showed varying fluctuations with an increase in the duration of exposure. The increase in plasma LDH activity might be due to the destruction of the tissues causing a release of the enzymes. It may also take root from an increase in the dependence of the exposed fish on anaerobic carbohydrate metabolism. Similar studies by Tkachenko and Grudniewska (2016), revealed active glycolysis in *Oncorhynchus mykiss* exposed to formalin as a result of increased LDH activity. The significant reduction of LDH activity in *O. niloticus* blood plasma further suggests a lower metabolic rate on exposure to environmental stress (salinity and pH) leading to a decline in the glycolytic process. Similar findings have been reported in the plasma of *Oncorhynchus mykiss* on acute exposure to lindane by Strmac and Braunbeck (2002) and in the tissues of *C. gariepinus* exposed to oil wastewater in the work of Akani and Gabriel (2016).

The results obtained indicate that the biochemical responses varied with concentration and duration of exposure which reflects the effect of stress as a result of changes in salinity and pH in the plasma biochemical composition of the juveniles. It can be concluded that the enzyme activities of Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase and Lactate Dehydrogenase can be used as biomarkers to determine the effects of environmental stress in *Oreochromis niloticus* and *Clarias gariepinus* and for biological monitoring of unacceptable levels of environmental contamination, and changes in seasonal and/or climatic patterns.

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Conflicts of Interest: The authors declare that no conflicts of interest exist in respect to publishing these research findings.

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