Alternations in Enzyme Activities of *Clarias gariepinus* Infected with *Aeromonas hydrophila* and *Pseudomonas aeruginosa*

ABSTRACT

Clarias gariepinus were infected with *Aeromonas hydrophila* and *Pseudomonas aeruginosa*, and blood samples were collected weekly for biochemical analysis to ~~analyse~~ evaluate the~~re~~ir enzyme activities and pathogenesis for four weeks. The enzymes include~~s~~d: aspartate aminotransferase (AST), alkaline phosphastase (ALP), acid phosphastase (ACP) and lactate – dehydrogenase (LDH). The fish were distributed in three different groups in triplicates as: control (C_1 C_2 C_3), *A .hydrophila* (A_1, A_2, A_3) and *P. aeruginosa* (P_1, P_2, P_3). After two weeks of acclimatization, A_1 – A_3 were injected with 1.5ml of 10^6 cfu/ml of *A. hydrophila*, P_1-P_3 were injected with 1.5ml of 10^6 cfu/ml of *P. aeruginosa*, while C_1-C_3 were pathogen free. At the end of the experiment, it was observed that there was a constant increase, in the enzyme activities of the infected fish, indicating increase in virulence with respect to weeks of exposure –but *P. aeruginosa* had higher pathogenicity compared to *A. hydrophila*.

Keywords: *P.aeruginosa, A.hydrophila,* Enzyme, Virulence, Pathogenicity.

Introduction

Aquaculture remains the fastest growing food industry in the world (FAO, 2013), because of the high demand for protein by man and other animals. The importance of aquaculture by man can never be over emphasized. The high demand for aquacultural products have led to employment opportunities in both developed and developing societies (Ukwe *et al.*, 2018a). Water is an essential factor in aquaculture, because the physcio-chemical parameters of the aquatic environment determines the success of aquaculture in that environment. The source of water for the practice of aquaculture plays a key role and the biological or industrial activities in the area of practice affects the water quality (Obianeme and Obire, 2017; Otene and Ukwe, 2018).

Formatted: Font: Italic

Formatted: Font: Italic

Aquacultural products such as fish are open to a wide range of bacterial pathogens (Schmidt *et al*, 2000), which have the capacity to cause diseases. These pathogens can only cause infections, disease and death if the fish is immunosuppressedive as a result of nutritional imbalance or stress, arising from ill practice (Anderson, 1995).

Diseases are the major causes of mortality in aquaculture. Of all the disease causing micro organisms, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* are known to cause high mortality in farms, with common symptoms such as skin ulcers, fin rot, haemorrhages, absess etc. (Austin and Austin, 1987; Iglewski, 1996). The presence of these organisms in farms have led to severe economic loses and reduction in productivity (Khahil *et al.*, 2010). Thersse effects are mostly in fish organs, immune system and blood parameters (Banerjee *et al.*, 2016; Nandi *et al.*, 2016). Though the type of feed administered to fish promotes the growth and survival of some micro organisms in the fish environment (Ukwe *et al.*, 2018b), with good pond management and farm practice, the rate of disease occurrence and economic loses in aquaculture can be drastically reduced.

This research is not only focused on the effect of the pathogens on the fish, but also on the pathogenesis/pathogenecity.

3. Materials and Method

3.1 Fish

A total of 90 (ninety) *Clarias gariepinus* weighing between 110 – 120g were purchased from IDI- ONYANA farm along Ahoada – Abua Road in Rivers State, Nigeria. They were transported

Comment [U1]: What informed the sample size?

to the project site in Port Harcourt by the use of anaeshetics. They were acclimatized for two weeks (14 days) to ascertain their health status.

3.2 Feeding and Experimental Set-up

Feeding with commercial feeds (coppens) started twenty four (24) hours after stocking. After two weeks of feeding, ten (10) fish per tank were randomly distributed for *A. hydrophila, P. aeruginosa* infections, and control in triplicates (A_1, A_2, A_3 and P_1, P_2, P_3. and C_1, C_2, C_3).

3.3 Bacterial Challenge

The bacterial pathogens were purchased from the National Veterinary Research Institute, Vom, in Jos, Plateau State in Nigeria. 1.5ml of 10^6cfu/ml of an overnight grown bacterial pathogen were injected intraperitoneally into the fish in each tank accordingly, using 2ml injection syringe, but the control was not injected with pathogen.

Feeding continued after the bacterial challenge for four weeks. The fish were physically examined weekly for any effects.

3.4 Biochemical Test for Enzymes

At the end of each week, blood samples were randomly collected from the fish in each tank via caudal venous puncture method, using 5ml injection syringe. The collected blood samples were transferred into LITHUM HEPARIN tube and sent to the laboratory for biochemical analysis within twelve (12) hours. They were assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) and lactate-dehydrogenase (LDH)

Comment [U2]: How were the activities of the enzymes scored?

4. Statistical Analysis

One way analysis of variance (ANOVA) was used to analyse the results, while Durcan multiple range test was used to evaluate differences between treatments.

5. Results

No mortality was recorded at the end of the experiment. Physical Examination of the fish showed serious ulceration on the fish's skin between the first and the second week of the *A. hydroplinla* infected fish (figure 1), but recovery process started from the third week. The fish infected with *P. aeruginosa* had red blood patches on the skin and reduction in weight progressed to the end of the experiment (figure 2).

Figure 1

There were significant differences in all the enzymes activities of the experimental fish compared to the control in all the weeks (tables 1-4).

Enzyme activities were constant in the control groups, but they were increased as the period of infection of the bacteria increased, though at different rates in the infected fish.

Figure 2

The pathogenecity of the bacteria in all the treated fish is shown in figures 3-6. It indicates the rate of virulence of the bacteria on the infected fish.

Comment [U3]: Captions for Figures 1 and 2 are not included, what do the figures show?

Comment [U4]: How was the rate of virulence measured?

Table 1: Changes in Enzymes Activities in *C. gariepinus* Challenged with *Aeromonas* and *Pseudomonas Spp* Bacteria in the First Week of Exposure

Enzymes (µ/l)	Control Fish	*Aeromonas* challenged fish	*Pseudomonas* challenged fish
AST	84.56±4.16[a]	110.00±2.00[b]	107.33±13.61[b]
ALT	9.00±2.00[a]	15.66±3.21[b]	26.33±3.05[c]
ALP	7.66±4.16[a]	19.35±1.52[b]	38.66 ± 6.65[c]
ACP	62.00 ± 12.16[a]	75.35 ± 5.68[b]	85.35±7.25[c]
LDH	220.61± 12.16[a]	269.71± 16.50[b]	295.71± 15.86[c]

Means within the same row with different superscripts are significantly different (P<0.05)

Table 2: Changes in Enzymes Activities in *C. gariepinus* Challenged with *Aeromonas* and *Pseudomonas Spp* Bacteria in the Second Week of Exposure

Enzymes (µ/l)	Control Fish	*Aeromonas* challenged fish	*Pseudomonas* challenged fish
AST	86.33±3.78[a]	121.17±8.08[b]	130.15±15.27[b]
ALT	9.66±2.18[a]	25.00±4.00[b]	31.67± 4.16[c]
ALP	8.00±3.26[a]	30.66±5.13[b]	48.68 ± 8.07[c]
ACP	63.60 ± 16.09[a]	85.66 ± 11.13[b]	104.00±11.11[c]
LDH	221.61± 28.18[a]	299.11± 12.41[b]	327.19± 13.54[c]

Means within the same row with different superscripts are significantly different (P<0.05)

Table 3: Changes in Enzymes Activities in *C. gariepinus* Challenged with *Aeromonas* and *Pseudomonas Spp* Bacteria in the Third Week of Exposure

Enzymes (µ/l)	Control Fish	*Aeromonas* challenged fish	*Pseudomonas* challenged fish
AST	87.66±2.51[a]	131.67±5.16[b]	143.33±3.78[b]
ALT	10.06±2.51[a]	30.00±2.64[b]	40.66± 1.52[c]
ALP	9.66±3.21[a]	37.33±8.62[b]	65.67 ± 2.51[c]
ACP	63.01 ± 18.12[a]	92.68 ± 17.00[b]	119.00±17.32[c]
LDH	221.67± 28.96[a]	335.00± 51.91[b]	376.00± 10.04[c]

Means within the same row with different superscripts are significantly different (P<0.05)

Table 4: Changes in Enzymes Activities in *C. gariepinus* Challenged with *Aeromonas* and *Pseudomonas Spp* Bacteria in the Fourth Week of Exposure

Enzymes (µ/l)	Control Fish	Aeromonas challenged fish	Pseudomonas challenged fish
AST	88.33±3.78[a]	142.00±6.00[b]	160.33±4.16[b]
ALT	11.00±3.60[a]	38.33±3.05[b]	64.33± 6.42[c]
ALP	10.00±2.64[a]	41.33±8.02[b]	77.33 ± 7.66[c]
ACP	62.66 ± 12.22[a]	104. ± 14.74[b]	148.00±16.82[c]
LDH	222.71± 37.23[a]	366.78± 29.67[b]	446.14± 42.01[c]

Means within the same row with different superscripts are significantly different (P<0.05)

Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic
Formatted: Font: Italic

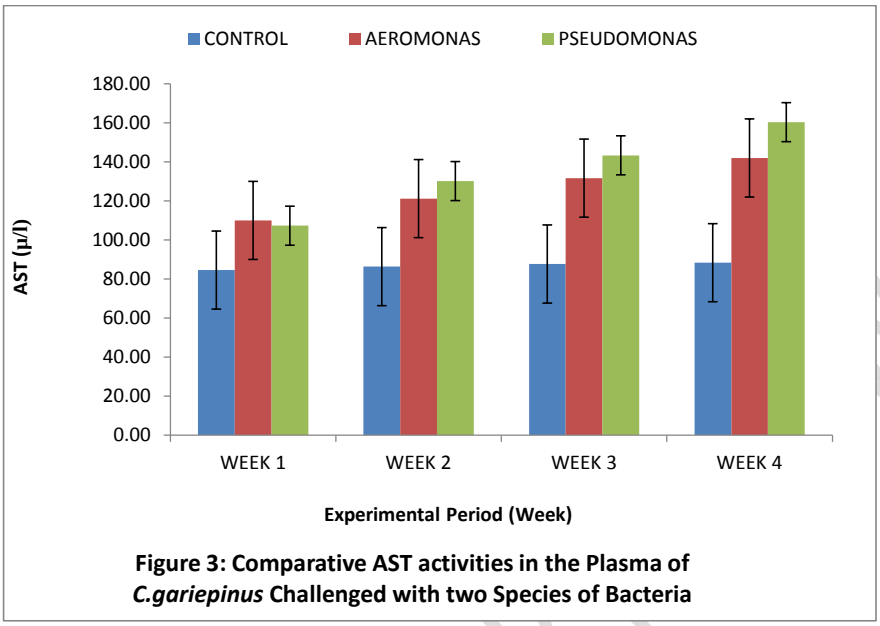

Figure 3: Comparative AST activities in the Plasma of *C.gariepinus* Challenged with two Species of Bacteria

121

122

123

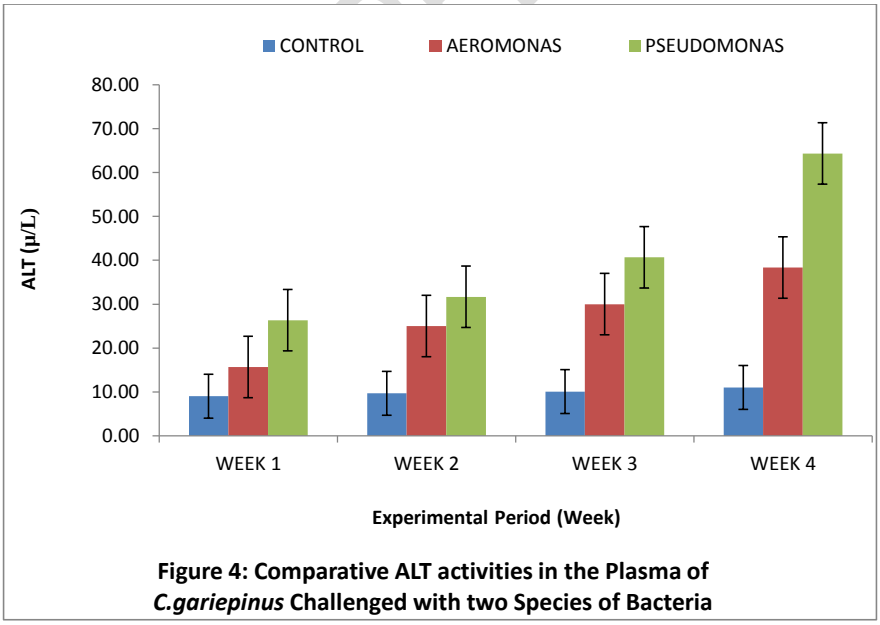

Figure 4: Comparative ALT activities in the Plasma of *C.gariepinus* Challenged with two Species of Bacteria

124

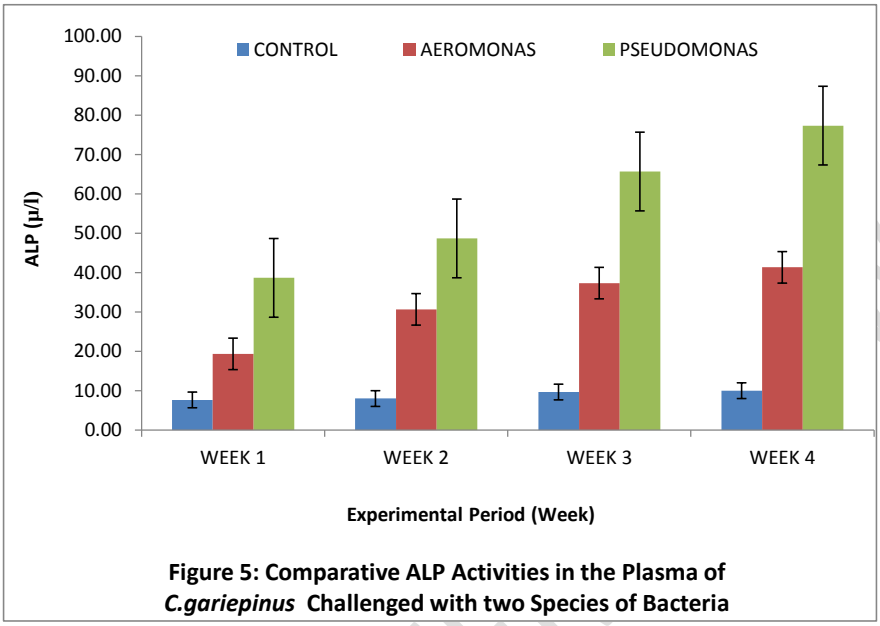

Figure 5: Comparative ALP Activities in the Plasma of *C.gariepinus* Challenged with two Species of Bacteria

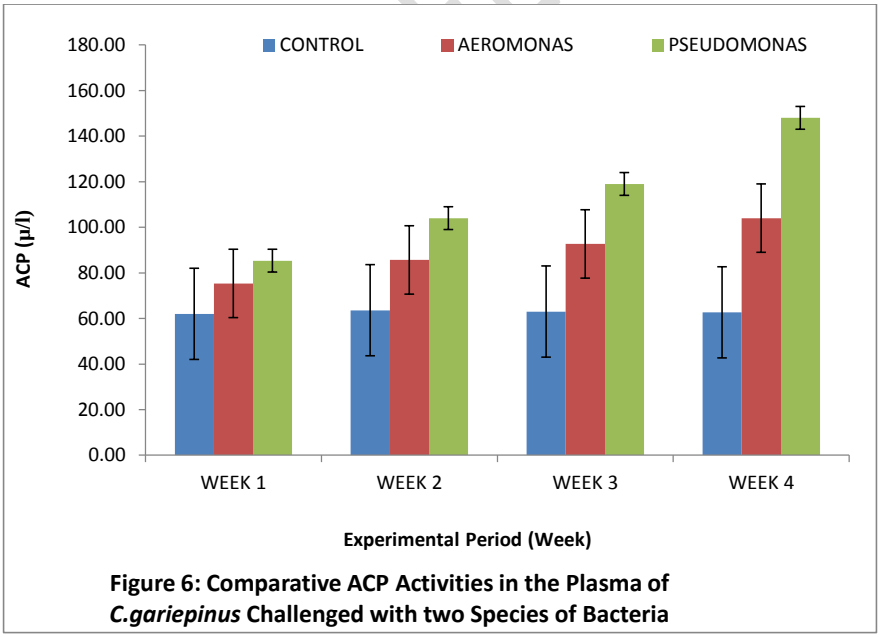

Figure 6: Comparative ACP Activities in the Plasma of *C.gariepinus* Challenged with two Species of Bacteria

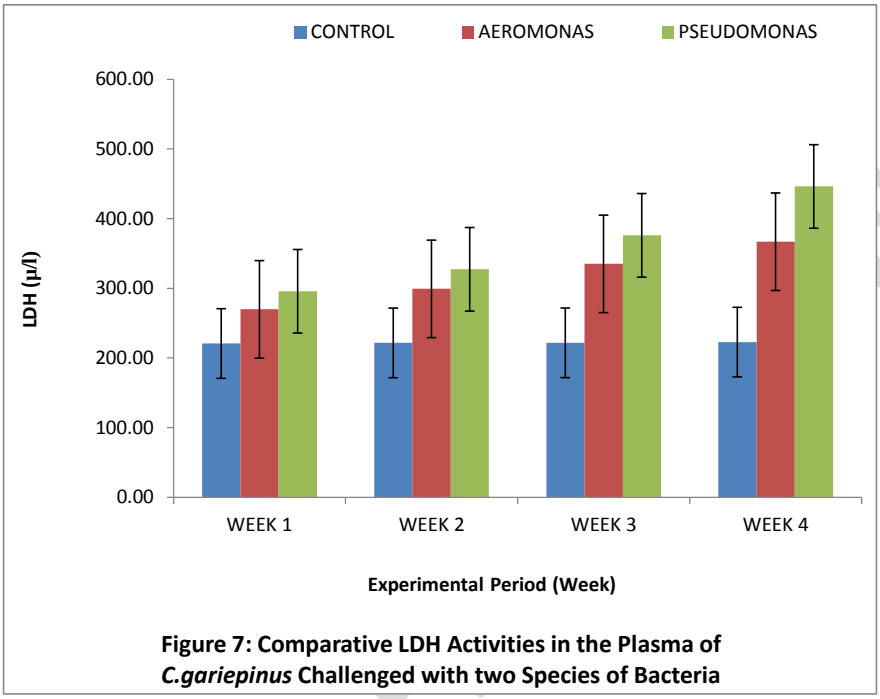

Figure 7: Comparative LDH Activities in the Plasma of *C.gariepinus* Challenged with two Species of Bacteria

Discussion

Physical observation of the fish infected with *Pseudomonas aeruginosa and Aeromonas hydrophila* showed severe hemorrhage and ulceration on the skin and fins of the fish, this is in agreement with previous results and reports (Amrevuawho, *et al.*, 2014; Austine and Austine, 2007), it also confirms the findings of Fadi *et al.*, 2013, who reported skin hemorrhage, deep ulcers and fin rot, when Nile tilapia was infected with *A. hydrophila.* Fish exhibits non-specific responses to checkmate disturbances or stress and maintain physiological balance (Barton, 2002). But the fish's health is usually affected negatively, if the said stress is sustained. Some of

these stress caused by contaminants, pollutants, pathogens etc can be detected in the blood, by analyzing some of its components for disease conditions and metabolic alterations in the fish (Celik, 2004). AST, ALT, ACP and ALP are known to be biomarkers in assessing the level of damage to body organs and health status of animals (Pari and Amali, 2005; Zarki *et al*, 2007). Some of the conditions that leads to the increase in the LDH of animals includes pulmonary infarction, hypatic dysfunction, haemolysis and myopathy (Steensma and Wtitziz, 2011). The increase in LDH values is also an indication of acute cell damage that leads to its presence in the blood (Najeeb and Aziz, 2013.)

The increase in the AST, ALT, ALP, ACP and LDH activities in the A. *hydrophilla* and *P.aeruginosa* infected fish, compared to the control indicates that the effects of the pathogens on the infected fishes stimulated the activities of AST and ALT enzymes. This may be due to hepatic cells injury or increased synthesis of the enzymes by the liver. (Yang & Chen 2003). Aspartate aminotransferase catalyzes the reversible transfer of a L-amino group between aspartate and glutamate thereby making it an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Serum AST level, serum ALT level, and their ratio (AST/ALT) are commonly measured clinically, as biomarkers for liver health. Alanine aminotransferase is found in plasma and in various bodily tissues, but is most commonly associated with the liver. It catalyzes the transfer of an amino group from L-alanine to α-Ketoglutarate, the products of this reversible transamination reaction being pyriuvate and L-glutamate. Elevated levels of ALT often suggest the existence of other medical problems such as viral hepatitis, diabetes, liver damage, bile duct problems, congestive heart failure, infectious mononucleosis or myopathy (Rashannasab *et al*., 2016).

Comment [U5]: infection?

Acid phosphatase is an enzyme that acts to liberate phosphate under acidic conditions and is made in the liver, spleen, bone marrow and prostate gland. Elevations are usually due to infections, injury or cancer of the prostate. The increase observed here is as a result of the effect of the pathogens (*A. hydrophilla and P. aeroginossa*).

Damaged or injurious fishes release more LDH into the blood stream. It is increased in liver disease, heart attack, anaemia, trauma, bone fracture, cancer and infections such as meningitis and encephalitis (Itolmes & Goldberg, 2009). The LDH enzyme catalyzes the conversion of lactate to pyriuvate. This is an important step in energy production in cells, heart, kidneys, liver and muscle. It is increased when cells are damaged or destroyed in lymphoma, lenkaemia, testicular or ovanian cells, and also in non cancerous cells such as heart, lungs or kidney disease. High levels cause acute or chronic cell damage according to Schurene *et al.*, 2014. The organs of the fish infected with *P. aeruginosa* and *A. hydrophilla* were highly damaged, this is in agreement with several authors concerning bacterial infection (Adsaid *et al.*, 2015). It is also in agreement with Khalil *et al.*, (2011) who observed that increase in enzymatic activities of the plasma was associated with organs damage in *Aguilla Anguilla* infected with *vibro anguillarum.*

The increase in the LDH, AST, ALP could be as a result of damages to the heart, liver, brain, blood cells and lungs (Najeeb and Aziz, 2013). Though both pathogens showed damaging effects on the organs of fish as revealed by the enzymatic activities, in this research work, the results show that A. *hydrophila* caused the production of more AST in the first week of infection compared to *P. aeroginosa*, but the *P. aeruginosa* became more virulent from the second to the forth week of the experiment. For the other enzymes, (ALT, ALP, ACP, and LDH), the rate of increase in their production was higher in *P. aeroginosa* and A. *hydrophila* throughout the experiment.

Formatted: Font: Italic

Comment [U6]: No evidence is provided to support this; may state that the organs were probably highly damaged.

Formatted: Font: Italic

Conclusion

P. aeroginosa and *A. hydrophila* have been observed as infectious bacteria causing diseases such as ulcers and hemorrhage in fresh water fish. This experiment showed that these bacteria increase the enzymatic activities of some plasma enzymes, which is an indication of organ damage in the fish. Though both pathogens have been confirmed to be harmful to the fish, *Pseudomonas aeruginosa* is seen in this experiment to be more virulent with higher pathogencity in *Clarias gariepinus*, when compared with *Aeromonas hydrophila*.

Formatted: Font: Italic

REFERENCES

Alsaid, M. Abuseliana, A. F., Daud, H. H., Mustapha, N. U., Bejo, S. K., Abdelhadi, Y. M. & Hardam, L. H. (2015). Haematological, biochemical and clinical signs changes following experimental infection of *streptococcus agalactiae* in red hybrid tilapia *(oreochromus sp). Basic Research Journal of Agricultrual Science and Review*, 4(9):289-295.

Anderson, D. P. (1998). Novel techniques for fish disease diagnosis. In Diseases in Asian Aquaculture II. Eds Shariff M, Arther J.R. Subasinghe R. P. Fish Health Section. *Asian Fisheries Society. Manillan*, Pp. 23 – 39.

Austin, B. & Aystin, D. a. (1987). Bacterial fish pathogens. Disease in Farmed and wild fish. Ellish Horwood Ltd. West Sussex, England, pp. 3 – 350.

Banerijee, G., Nandi, A. & Ray, A. K. (2016). Assessment of hemolytic activity, enzyme production and bacteriocin characterization of *Bacillus subtilis* LRI isolate from gastrointestinal track of fish. *Archives of microbiology*, 199: 115-124.

Barton, A. B. (2002). Stress in Fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. Integ and comp. Biology, 42: 517 – 525.

Celik, E. S. (20004). Blood Chemistry (electrolytes, Iipoproteins and Enzymes) Values of black scorpion fish (*scorpaena porcus)* in the Dardanelles. *Turkey Journal of Biological Science*, 4(6):716-719.

Itolmes, R. S & Goldberg, E. (2000). Computational analyses of mammalian lactate dehyorogenase: human, mouse, opossum and platy pus LDITS. Computational Biology and Chemistry. 33(5):379-385.

Fadi, S. E., Barakat, M. & Elgohary, M. (2013). Biochemical studying of anabaena (*Cyanobacterial)* on Nile Tilapia. *Alexandria Journal of Veterinary Science*, 3991-104.

Khalil, RD. H., Hana, R. E. & Nadia, B. (2011). Contribution to vibrosis in cultured eels (*Anguilla Amguill) Journal of American Society*, 7(12): 101-110.

Khalil. S. A., Khalil, R. H., Sand, T. T. & Safara, M. H. (2010). Studies on *Pseudomonas septicemia* among cultured *Oreochomis niloticus. Journal of the Arabian Aquaculture society*, 5(1).

Najeeb, Q. & Azize, R. (2013). Comparism of Alkaline phosphate, lactate Dehydrogenase and Acid phosphatase levels in serum and synovial fluid between patients with Rheumatoid Arthritis and Osteoarthritis. *International Journal of Science and Research*, 4 (4): 1-4.

Nandi, A., Banerjee, G., Dan, S. K., Gosh, K. & Ray, A. K. C (2016). Potentiality of probiotic strain Baucillius sp. In labeo rolita challenged by Aeromonas hydrophila: assessment of oxidative stress, hemato-biochemcial parameters and mumum responses. Aquaculture Research. Dol:10.IIII/are.13255.

Obianeme, N. E. & Obire, O. (2017). Microbiological and Physico-chemical characteristics of Fish Ponds in Port Harcourt. *Current studies in Comparative Education, Science and Technology*, 4,126-139.

Otene, B. B. & Ukwe, O. I. K., (2018). Evaluation of Heavy metal Accumulation in Water and Sediment from Elechi Creek, Port Harcourt, Nigeria. International Journal of Geography and Environmental Management, ISSN, 2504-8821, 4(1).

Pari, L & Amali D.R. (2005). Protection role of tetrahydrocurcumim (THC) an active principle of rats Journal of Pharmacy and Pharmatological Science 8 : 115-127.

Rashannasab, A., Afsharmanesh, S., Rahimi, R. and Sharifian, I. (2016). Alternations in the liver enzymatic activity of common carp, cyprinus carpio in response to parasites. Dactylogynis spp. And Gyrodactyhis spp. Journal of Parastitic Diseases. 40(4): 1146-1149.

Schmdit, A. S., Bruun, M. S., Dalsaguard, L., Pedersen, K., & Larsen, J. L. (2000). Occurrence OF Antimicrobial Resistance in Fish Pathogenic and Environmental Bacteria Associated with four Danish Rainbow Trout Farms. *Applied Environmental Microbiology*, 66 (11): 4909 – 4915.

Schueren, F., Lingner, T. George, R., Haflins, J. Gartner, J., Thoms, S. (2014). Peroxisiomal Lactate delydrogenase is generated by translational read through in mammals. Elife.3:eo3640.

Steensma, P. D. & Witzig, T. E. (2001). Elevated serum LDH in Patients with non-Hodgkins lymphoria: not always an ominous sign. *British Journal of haematology*, 107(2):1365-2141.

Ukwe, O. I. K., Edun, O. M. & Akinrotimi, O. A. (2018a). Growth and microbial indices in African Catfish (*Clarias gariepinus)* Larvae fed formulated and commercial diets. *Journal of Fisheries Science com.* 12(2):001-008.

Ukwe, O. I. K., Edun, O. M. & Akinrotimi, O. A. (2018b). AQUACULTURE AND FISHERIES: A Recipy for Job Creation and Health Challenges. *International Journal for Research under literal Access*, 1(4).

Yang, J., & Chen, H. (2003). Serum Metabolic enzyme activities and hepatocyte ultra structure of common carp after gallium exposure. Zoological studies. 42(3):455-461.

Zaki, M. S. Sharaf, N. E & Osfor, H. M. (2007). Effect of Vanadium toxicity on biochemical. Haematological and clinicopathological changes in clarias lazera present in River Nile. American – Eurasian Journal of Agricultural Environment, 2:741-745.