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Effects of Multiple Exposures of Nutmeg and Clove Seed Extracts on Some Electrolytes in Two Sizes of *Clarias gariepinus*

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Abstract: This study evaluated the effects of multiple exposures of nutmeg and clove seed extracts on some electrolytes in two sizes of Clarias gariepinus. A total of six hundred (600) apparently healthy C. gariepinus, consisting of 300, 12 weeks old juvenile fish (mean length 19.66cm ± 1.17SD; mean weight 350.88g ±1.07SD) and 300, 24 weeks old adult sizes (mean length 44.66cm ±5.99SD; mean weight 1011.77g ± 54.99SD) were exposed repeatedly three times at one week interval to nutmeg and clove extracts as anaestheticsv in the laboratory. This study was carried out to assess changes in the electrolytes of fish during exposure to these extracts. The results obtained, indicated that the two anaesthetics caused a significant alterations (P <0.05) in the electrolytes which include Calcium (Ca2+); Sodium (Na^+); Potassium (K^+); Chloride (Cl^-¬); and Bicarbonate ($HC0_{3-}$) in the plasma of the exposed fish. Values of Ca^{2+}, K^+, and Cl^-¬ increased significantly (P<0.05) across different concentrations of anaesthetics, while Na^+ and HCO_{3-} reduced. These alterations were more pronounced during the third exposure of C.gariepinus to these anaesthetics.

Keywords: Electrolytes, Fish, Stress, Anaesthetics, Aquaculture

INTRODUCTION

Aquaculture production all over the world is becoming more and more intensive, this involves manipulation of fish and other farm management procedures which include handling, liming confinement, fertilization, transportation and other operations from the hatchery to the final commercial stage (Angelids *et al.*, 1987; Gabriel *et al.*, 2007a). According to Pickering (1981) these management procedures as crucial as they are, produce some level of disturbances, which can elicit a stress response leading to decreased fish performance (Maule and Shreck, 1990), alterations of the peripheral leucocyte distribution, such as heterophilia and lymphocytopaenia (Ellsaesser and Clem 1986, Ainsworth *et al.*, 1991; Gabriel *et al.*, 2007b) increased susceptibility to diseases (Pickering and Pottinger 1985; Maule *et al.*, 1989) and in extreme, cases leads to mortality (Akinrotimi *et al.*, 2007a).

Stress in fish caused by physical disturbances encountered during fish farming activities, such as handling and transport, instigates a variety of responses that may be adaptive or detrimental (Barton and Iwama, 1991). Fish undergo a series of biochemical and physiological changes in an attempt to compensate for the challenge imposed upon them by stress (Sharma and Dash, 1991). According to Sharma and Dash (1991), handling stress is the most common factor that causes scale loss and skin damage, which subsequently leads to pathogen invasion. Handling stress physically removes mucus resulting in decreased chemical protection, osmoregulatory function and lubrication, hence, causing the fish to use more energy. This facilitates pathogens to invade.

Anaesthetics are commonly used to reduce mortality and stress during fish handling (King *et al.*, 2005; Ross and Ross, 2008).

Anesthesia is a biological reversible state induced by an external agent, which results in the partial or complete loss of sensation or loss of voluntary neuromotor control, through chemical or non chemical means (Summerfeld and Smith, 1990). Anesthesia is frequently applied in aquaculture being a valuable tool that helps to minimize fish stress and to prevent physical injuries to fish while handling them during routine practices, for example, Anesthesia is required for measuring or weighing fish, sorting and tagging, administrating vaccines, live transport, sampling for blood or gonadal biopsies and collecting of gametes, surgical procedures, to cite some of the main applications (Maricchiolo and Genovese, 2011). Knowledge about the ideal and optimum concentration of anesthetics for various fish species is necessary because inappropriate concentrations may lead to adverse effects such as stress; therefore, access to safe and effective fish sedatives is a critical need of fisheries researchers, managers, and culturists (Trushenski *et al.,* 2013). When choosing anesthetics, a number of considerations are important, such as efficacy, cost, availability and ease of use, as well as toxicity to fish, humans and the environment and the choice may also depend on the nature of the experiment and species of fish (Mylonas *et al.*, 2005).When the fish is removed from the anesthetic, the recovery should be rapid, the anesthetic should be effective at low doses (Coyle *et al.*, 2004).

Dry clove powder (Carnation flowers buds) is a common medicinal plant available in all medicinal plant stores, and is not expensive, thus, it is often used for fish anaesthetizing in research, biometry and injection instead of clove oil but to date, not enough information exists on its effects on stress response (Hoseini, 2011). It has several advantages over other anesthetic agents in fishery research, assessment studies and aquaculture applications. Clove is organic, hence no withdrawal period is required for fish intended for human consumption and another advantage of clove oil is that it does not pose a chemical health hazard to the user (Bressler and Ron, 2004). Anesthetic effect of the clove oil on some aquatic organisms was investigated in such cases as its use in the transfer of fish species used in the food sector (Ross and Ross, 2008).

Recently, nutmeg (*Myristica fragrans)* received favorable reviews as an alternative fish anesthetic for a variety of fish species as well as for crustaceans. Nutmeg has been used since time immemorial for medicinal purposes. Nutmeg (*Myristicafragrans*) is a member of the Myristicaceae family. It is a perennial tree found ecologically in the tropics and well distributed in the north-central region of Nigeria (Acker, 2000). Extracts from its nuts contains 70 – 90% myristidate4-21% beta-caryophyllene, 1-21% eugenyl acetate and 10 19% tannin (Acrete *et al.*, 2016).

In recent times appraisals of electrolytes in the blood of fish are great pointers of evaluating the wellbeing status of fish in ecotoxicology (Gabriel *et al.*, 2009). The electrolytes are substances which impact the circulation and maintenance of water around the arrangement of an individual (Gabriel and Edori, 2010). In view of this, Gabriel *et al.*, (2010) reported that sodium and potassium are the most significant osmotically viable electrolytes and consequently the most considered. Others of significant importance are chloride and calcium. Calcium is available in the body in larger amount than some other mineral component. It is available in the bones as stores of calcium phosphate. It is likewise present in little focuses in body liquids. The ionized calcium in the body assists in blood coagulation and keeping up the typical volatility of muscles. Changes in electrolyte levels show the activity of an assortment of homeostatic mechanisms in the system of the fish and assume an important job in osmoregulation and homeostasis (Akinrotimi *et al.,* 2011; Gabriel *et al.,* 2011). In spite of a lot aquaculture activities on *C. gariepinus* as a favourable fish for culture in Nigeria, the information on the use of naturally derived anaesthetics to manage both intentional and unintentional stress across its production chain is scarce, hence the need for this study. This study sheds more light on stress management in fish farming using plant extract as anaesthetics in Nigeria. Data concerning the repeated exposure of this specie to clove and nutmeg extracts on the electrolytes are scarce and incomplete Hence, the need for this study.

Materials And Methods

Project Location

This study was carried out at Alalibo Intergrated Fish Farms, Rumuolumeni , Port Harcourt, Rivers State, Nigeria. The unit consists of twenty 9m3 plastic tanks and 30 (50L) plastic tanks. The site is well ventilated and is completely protected from direct impact of sun light. The water supply to the unit is from a bore hole, free from any chemical pollution.

Sources of Experimental Fish

A total of six hundre (600) apparently healthy *C. gariepinus*, consisting of 300, 12 weeks old juvenile fish (mean length 19.66cm ± 1.17SD; mean weight 350.88g ±1.07SD) and 300, 24 weeks old adult sizes (mean length 44.66cm ±5.99SD; mean weight 1011.77g ± 54.99SD) were procured from African Regional Aquaculture Center (ARAC) Aluu, Port Harcourt, Rivers State, Nigeria.

Fish Acclimation

The fish were acclimated to laboratory conditions for a period of 14 days, following the method of Gabriel *et al.*, (2004), who recommended that fish for experimental purposes must be handled carefully and stocked in a well aerated holding tank, so as to reduce the incidence of stress to the barest minimum. During this period the fish were fed daily with Coppens Commercial feed (45% CP) at 5% body weight and the water in the holding tanks were renewed every two days.

Preparation of Plant Extracts

Nutmeg, *Myristica fragrans*, (Plate 1) and dried buds of clove plant, *Syzigium aromaticum* (Plate 2) were purchased from Choba Market in Obio Akpor Local Government Area of Rivers State. Plants authentication was done using the keys of Agbaje, (2008). These seeds were taken to the laboratory and ground into power using a kitchen blender (Model H_2, Ken Wood, Japan). The milled seeds were sieved using 0.1 micro nylon mesh to obtain the fine powder (Inoue *et al.*, 2003).

Experimental Design

The design of the experiment was Completely Randomized Design (CRD) having five treatments levels each with three replicates for each of the life stages in the two anaesthetic agent. A total of 60 plastic basins of dimension (70 x 44 x 34 cm^3) each were used for the experiments. The 60 basins were labeled based on life stage of the fish, treatment levels and replicates. Each basin was stocked with 10 fish per tank. A total of 600 (six hundred) fish were stocked.

Experimental Procedure

The powder was weighed into different concentrations (0.00, 100.00, 300.00, 500.00, and 700.00 mg/l) using a sensitive weighing balance. It was applied directly in three replicates into the water (20L) level in 40L experimental plastic aquaria, 30 in number (15each for juveniles and adult sizes). The mixtures were stirred vigorously to ensure homogenous mixture. The fish was weighed with 20 kg round top weighing scale (Model 1123HK, Digital Scales, Ltd, Beijing, China). While the length was measured with transparent meter rule. They were then introduced into prepared experimental aquaria, containing five concentrations of each of the powdered plant seeds. Another set of 30 aquaria were filled clean water for recovery purpose. The procedure was then repeated for the second anaesthetics. The entire procedures were repeated weekly for three consecutive weeks.

Plate 1: Nutmeg (*Myristica fragrans)*

Plate 2: Clove Seed (*Syzygium aromaticum*)

Blood Sample Collection and Preservation

A total of 180 fish were sampled for blood, 90 each from Juvenile and adult sizes in each of the anaesthetics for each sampling period. In each of the anaesthetics under consideration, 90 fish were sampled with 45 each for juvenile and adult sizes making it three fish in each experimental tank (in triplicates). Blood samples were taken at the deepest anaesthesia, when the fish was completely immobilized. However, blood samples for biochemical profiles analysis were preserved in heparinzed bottles (540 in numbers) according to Gabriel *et al.*, (2007c). Blood samples for biochemical indices were centrifuged (Crosby *et al.*, 2006) using a centrifuge (4,000rpm) Kantech International (Jianyxi) Co. Ltd, Beijing, China. The blood was drawn from caudal vein (Clark *et al.*, 1979). Fish were caught individually with a hand net. Blood samples were obtained with 5ml disposable syringes and 21-gauge hypodemic needle.

During collection the head of each fish was covered with a piece of cloth for physical restriction with minimal stress (Ayinla and Nwadukwe, 2003). The needle was inserted perpendicularly into the vertical surface of the fish at a point slightly above the openings in the genital papilla. As the needle pierced the vein, blood flowed easily into the syringe and 3ml of blood was taken before the needle was withdrawn. The needle was then detached from the syringe and the 1.5ml blood was transferred into EDTA and the remaining 1.5ml into herparinized bottles. The blood samples were analyzed at the Lively Stones Medical Laboratory, Rumukparali – Choba Road, Choba, Port Harcourt.

Determination of Blood Serum Electrolytes

At the end of each experimental period, 2 ml of fresh blood sample was collected by making a caudal puncture with the help of fine needle and poured in heparinized sample bottles. Serum was separated by centrifugation at 10,000rpm for 5-8 minutes in TG20-WS Tabletop High Speed Laboratory Centrifuge. Serum electrolytes such as Na^+, K^+, Ca^{2+} and Cl^- were determined by using Hitachi 902 automatic analyzer (Japan), following the method described by Gabriel [8]. All the tests were performed in triplicates.

Statistical Analysis

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using two way ANOVA and the two means were considered significant at 5% (P<0.05.

RESULTS

The level of electrolytes Ca^{2+} in both sizes of *C. gariepinus* subjected to two anaesthetics agents was shown in Figure 1. The results indicated that the maximum level (13.67) of Ca^{2+} was obtained in adult fish exposed to nutmeg extracts at the second week. and the minimum (6.13) in juveniles fish exposed to nutmeg at the third week. Generally, Ca^{2+} in adult fish were consistently higher than the juveniles in all experimental period (Figure 1). The level of electrolytes Na^+ in *C. gariepinus* exposed to two anaesthetics agents are shown in Figure 2. The result revealed that sodium ions in adult fish were generally higher than the juveniles in all experimental period. The comparative effects of two anaesthetic agents on the K^+ Packed were shown in Figure 3. The results indicated a consistent reduction pattern in the values of K^+ in both sizes with increasing experimental period. Changes in chloride ions in *C. gariepinus* exposed to clove and nutmeg extracts are presented in Figure 4. Significant elevations were observed in adult fish exposed to clove and juvenile exposed to nutmeg. While others were within the same range.The level of electrolytes HCO_3^- in *C. gariepinus* subjected to two anaesthetics agents was shown in Figure 5. The results indicated that the highest level (19.25) of $HCO_3^-$was obtained in adult fish exposed to nutmeg extracts at the first week. and the lowest (11.71) in juveniles fish exposed to nutmeg at the third week. Generally, HCO_3^- tends to decreased as the experimental period advances (Figure 5).

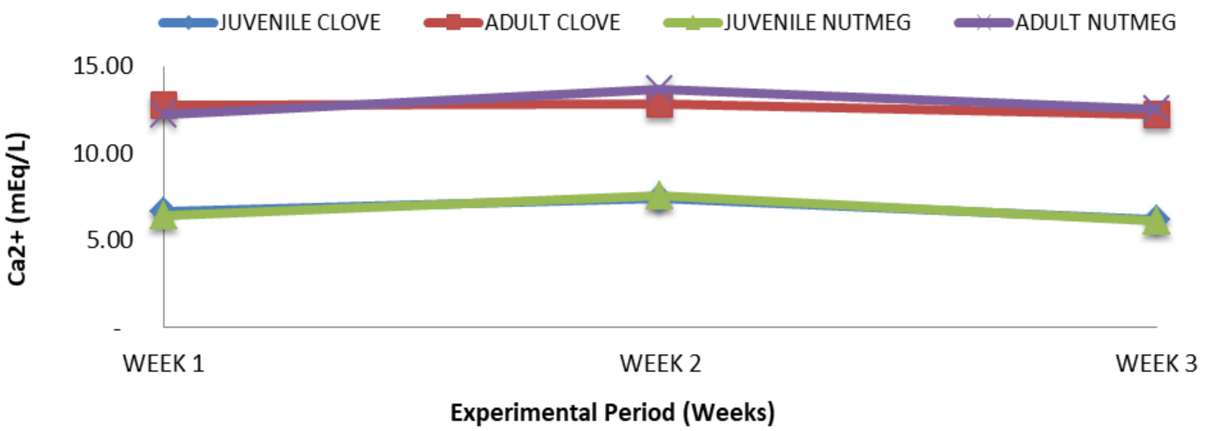

Figure 1: Changes in Calcium (Ca^{2+})Electrolytes in the Plasma of *C. gariepinus* exposed to Clove and Nutmeg Seed Extracts

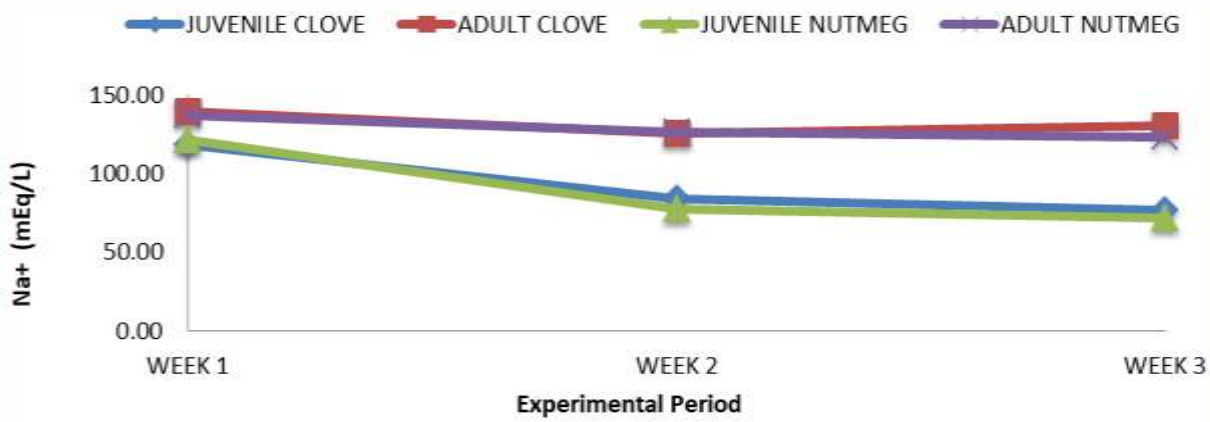

Figure 2: Changes in Sodium (Na)Electrolytes in the Plasma of C. gariepinus exposed to Clove and Nutmeg Seed Extracts

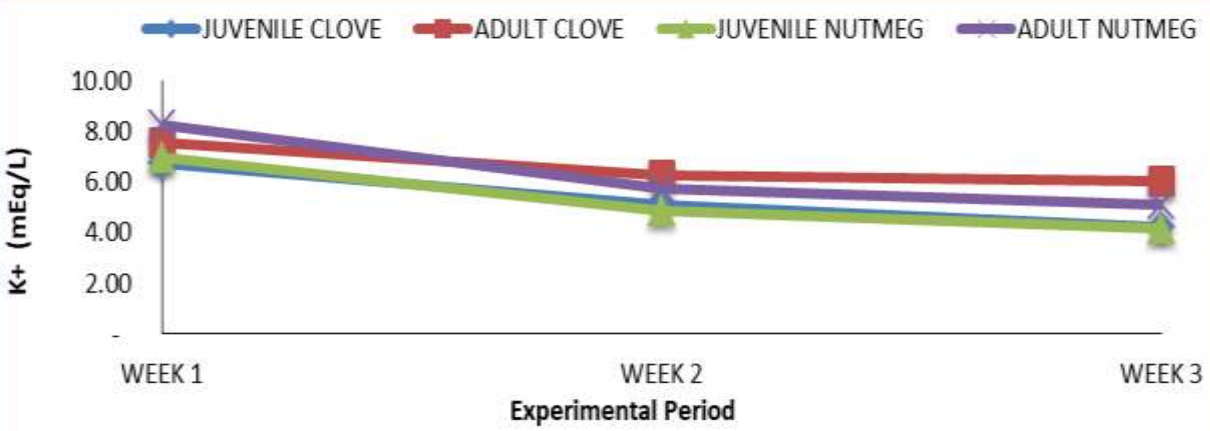

Figure 3: Changes in Pottassium (K^{+}) Electrolytes in the Plasma of *C. gariepinus* exposed to Clove and Nutmeg Seed Extracts

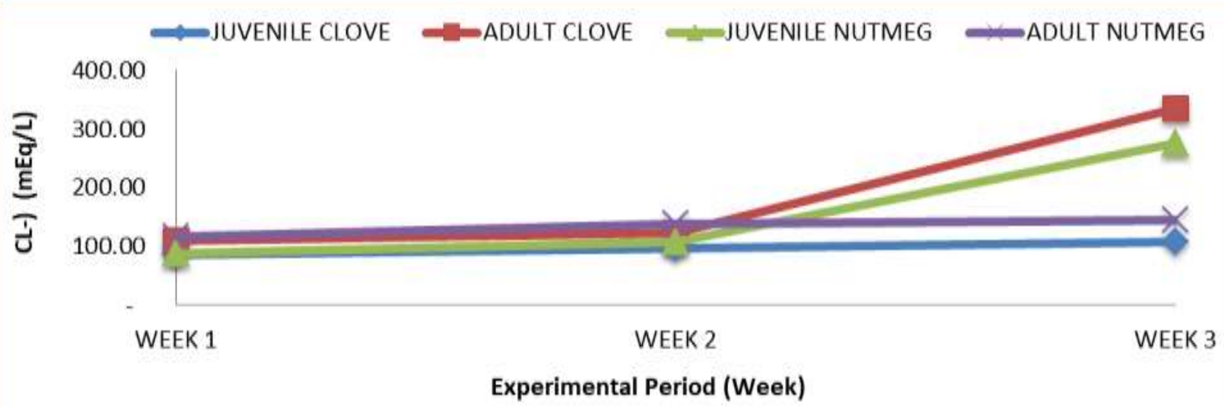

Figure 4: Changes in Chloride (CL)Electrolytes in the Plasma of *C. gariepinus* exposed to Clove Buds and Nutmeg Seed Extracts

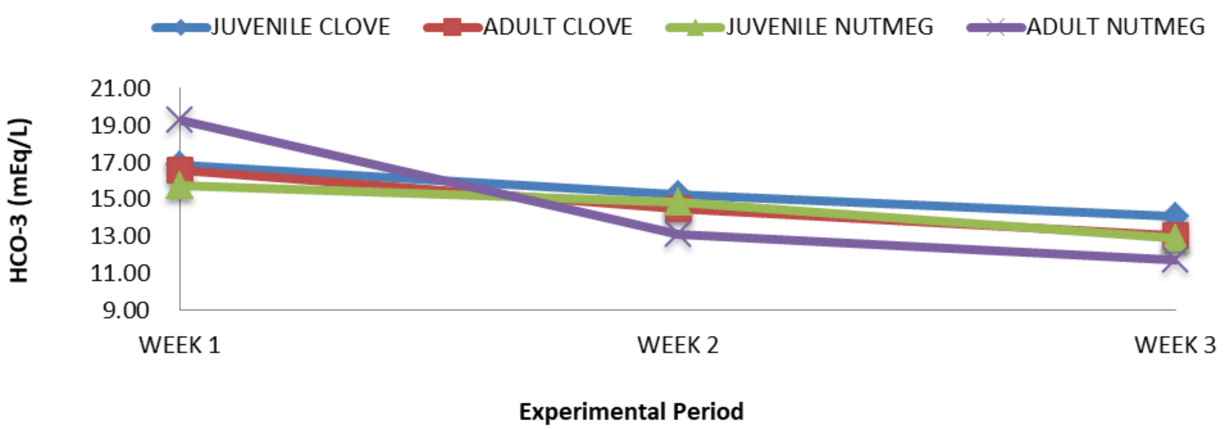

Figure 4.13: Bicarbonate Electrolytes in the Plasma of *C. gariepinus* exposed to Clove and Nutmeg Seed Extracts

DISCUSSION

Electrolytes are ions in solution which acquire the capacity to conduct electricity and their balance in the body of organism and it is essential for the normal function of cells and organs. According to Harper (1977) and Gabriel *et al.*, (2009), the basic function of electrolytes in the body lies in the control of fluid distribution, intracellular and extracellular acido-basic equilibrium which culminates in the proper maintenance of osmotic pressure of body fluids and normal neuro-muscular irritability. They can also be described as the salts in the body, and specific group of chemicals that must remain in balance for the body to function normally. Electrolytes exist as charged particles, called ions, and are responsible for the electrical communication required for many bodily functions including brain, muscle and nerve activity (Akram*et al.*, 2016). They are commonly used to determine the physiological characteristics, toxicity and health status of fish (Sara *et al.*, 2010). Monovalent ions namely, sodium (Na^+), and potassium (K^+) play an important role in osmoregulation and homeostasis. In vertebrates, the Na+ concentration in the extracellular fluid surpasses that in the cytosol whereas K^+ is higher in the intracellular fluid compared to the plasma. Thus, the levels of plasma electrolytes offer important knowledge concerning the health status of diseases of and impact of stress on fish (Ansari *et al.*, 2016).

Chloride is the major anion found outside the cell and the blood. It combines with sodium in the body to form sodium chloride (NaCl) and plays the role of helping the body to maintain normal fluid balance (Gabriel *et al.*, 2009). Significant increases in blood chloride concentrations were observed when fish were exposed to clove and nutmeg extracts. This could be attributed to retention of chloride ions by the gills to compensate for loss of hydrogen ions (Heisler, 1993), muscle contractions causing movement of chloride out of myocytes into plasma, release of catecholamines into the circulation, which stimulated chloride and hydrogen ions exchange in RBC to maintain constant pH. Sodium ion is the major ion (cation) in the extracellular fluid. Sodium plays a critical role in body functions. The brain, nervous system and muscle require the transmission of electrical signals for the organisms communication. The implication of the overproduction and underproduction can lead to cell malfunctioning and also indicates damage to the gills and the kidney (Ates *et al.*, 2015). However, reduction in this ion (Na^+) when compared to the control value, as observed in this study, is an indication of impairment of kidney function (Adedeji *et al.*, 2011). Sodium (chief cation of extracellular fluid) and potassium (chief cation of intracellular fluid) are the most important osmotically effective electrolytes.

Potassium ion (K^+) is the major positive ion in the intracellular fluid and it is essential for normal cell function and body regulation. The observed increase and decrease in potassium (K^+) ion in this study is as a result of stress in normal function of the blood. An increase or decrease in potassium (K^+) ion normally results in irregular heartbeats (arrhythmias) which can be fatal in extreme conditions (Gabriel *et al.*, 2009). Potassium is essential for the activity of many enzymes and is involved in ATP transport which participates in several metabolic processes. Na^+ and K^+ ATP are located in the membranes of cells and are responsible in the active transport of Na^+ and K^+ across cell membrane (Hamm *et al.*, 2016). Generally, the imbalance electrolytes in fish causes lateral line imbalance and hormonal disorder through affective endocrine organs through attack by pesticides (Tella, 2005; Gabriel and Edori, 2010). The changes observed in the electrolytes in the fish blood may have been caused by improper handling of fish in the culture medium.

One of the divalent ions, calcium (Ca^{2+}), serves a number of functions in fish. Calcium is present in the body in larger amounts than in any other mineral element. It is present in the bones as deposits of calcium phosphate in a soft fibrous matrix. It is also present in the small concentrations in body fluids. It combines with phosphorus (PO^+) for the deposition of bone. It is possible that bone serves as a reservoir of calcium for plasma and tissues. Additionally, Ca^{2+} appears to be important in the reproduction and mitochondrial functions. It is generally recognized that Ca^{2+} has an important role in osmoregulation (Cecik and Engin, 2005). Calcium taken up from the water in freshwater fish follows a transcellular, hormone-controlled pathway located in the chloride cells of the gills. In the present study calcium levels are within the same range and vary slightly with the control values in *C.gariepinus* exposed to anaesthetics. This result is in line with the one reported by Akinrotimi *et al.*, (2012), who reported a slight change in the electrolytes values of *C .gariepinus* exposed to eight anaesthetics in the laboratory. This is indication of good calcium profile of the fish. As the ionized calcium in the body plays an important role in blood coagulation and maintenance of normal excitability of muscles.

Bicarbonate is the second most abundant anion in the blood where it serves as part of the body's buffering system. This system, in conjunction with the respiratory system and kidney function, ensure that the pH (acidity) of the blood remains within a very narrow range. Bicarbonate also acts as a means of transportation for carbon dioxide from the tissues to the lungs where it is expired as a waste product(Giles, 1984) . The observed decrease of this ion in the plasma of *C.gariepinus* sampled in this study, agrees with the findings of Akinrotimi *et al* (2009), during exposure of juveniles and adult sizes of *C.gariepinus* to both synthetic and natural anaesthetics. The reduction of bicarbonates as observed in this work, may have resulted from a dehydration process in the fish and stomach (intestinal) secretions (Ashraf *et al.*, 2015). However, stomach diseases, over reactivity of the parathyroid glands and certain kidney diseases leads to increase of this ion in living organism (Gabriel *et al.*, 2010).

CONCLUSION

In this study, both anaesthetics had similar impact on the levels of the electrolytes in both fish sizes. Na^+ and $HC0_{3-}$ levels reduced continuously with increasing concentration and repeated exposure. Declining $HC0_{3-}$ levels may lead to metabolic acidosis or increasing acidity in the body. Declining Na^+ levels may be part of the direct impact of the onset of anaesthesia. (In Hyponatremia, the subjects become sluggish and confused, and if it worsens, they may have muscle twitches and seizures and become progressively unresponsive). Cl^- levels increased continuously with increasing concentration and repeated exposure. Hyperchloremia indicates dehydration or reduced kidney function.

REFERENCES

1. Acker, H. M. (2000). Comparative studies on the effect of water quality on haematological of *Orechromis niloticus* under culture condition. Ph.D. Thesis. Sudan University of Science and technology.
2. Acrete, M., Abdel-Ghany, A. E., & Ahmed, M. H. (2016). Effect of Diet supplementation on water quality, phytoplankton community structure and the growth of Nile tilapia *Orechromis niloticus* (L.), common crap, *Cyprinus carpio* (L.), and Silver carp, *Hypophthalmichithys molitrix* (V.), poly cultured in fertilized earthen ponds. *Journal of Aquatic Biology & Fisheries*,**5**(3): 17-30
3. Adedeji, O.B., Adedeji, O.A., Adeyemo, O.K. and Agbede, S.A. (2011). Acute toxicity of diazinon to the African catfish (*Clarias gariepinus*) *African Journal of Biotechnology*,**7**(5): 651-654.
4. Ainsworth, I., Pacheco, M., & Santos, M.A. (1991). Enzymatic and Non-enzymatic Antioxidants as an Adaptation to Phagocyte-induced Damage in *Anguilla anguilla* L. Following in Situ Harbor Water Exposure. *Ecotoxicology and Environmental Safety*, **3**(57): 290–302.
5. Akinrotmi, O.A., Ansa, E.J., Owhondaw, K.N., Onunkwo, D.N., Anyanwu, P.E., Edun, O.M., Oparaji, Y. and Cliff, P.T. (2007a). Effects of transportation stress on haematological parameters of blackchin tilapia Sarotherodon melanotherin. *Journal of Animal and Veterinary Advances*, **6**: 841-845.
6. Akinrotimi, O. A, Gabriel U. U, Anyanwu P.E., & Anyanwu.A.O.(2007b). Influence of sex, acclimation methods and period on haematology of *Sarotherodon .melanotheron*. *Research Journal of Biological Sciences*.2:348 -352.
7. Akinrotimi, O.A., Abu, O.M.G., Ansa, E.J., Edun, O.M., & George, O.S. (2009). Haematological Responses of Tilapia guineensis to acute stress. *International Journal of Natural and Applied Sciences*, **5**(3): 338-343.
8. Akinrotimi, O.A., Opara, J.Y., & Ibemere I.F. (2011). Effects of handling stress on immune functions of Black jaw tilapia, *Sarotherodon melanotheron.Nigerian Journal of Fisheries*,**8**(1): 154-158.
9. Akinrotimi, O.A., Agokei, E.O., & Aranyo, A.A. (2012). Changes in blood parameters of *Tilapia guineensis* exposed to different salinity levels. *Journal of Environmental Engineering and Technology*, **1**(2): 4-12
10. Akinrotimi, O.A., Gabriel U.U., & Edun,O. M. (2016). The efficacy of clove seed extracts as an anaesthetic agent and its effect on haematological parameters of African catfish (*Clarias*
11. Angelids, J.E., Kadri, S., Jewel, H., & Huntingford, F.A. (1987). The effects on demand
feeding on swimming speed and feeding responses in Atlantic salmon (*Salmo salar L.*), gilthead sea bream (*Spartus aurathus L.*) and European sea bass (*Dicentrarchus labrax L.*) in sea cages. *Aquaculture Research*,**33**: 501-507.
12. Ansari, B.A., Aslam, M., & Kumar, K. (2016). Diazinon toxicity:Activities of acetyl-cholinesteras and phosphatases in the nervous tissue of zebra fish, *Brachydanio rerio* (Cyprinidae). *Acta Hydrochim. Hydrobiology.*,**15**: 301-306.
13. Ashraf, W. (2015). Accumulation of heavy metals in kidney and heart tissues of *Epinephelus microdon* fish from the Arabian. *International Journal of Fisheries and Aquaculture*,**101**: 311–316.
14. Ates, M., Daniels, J., Arslan, Z., & Farah, I. O. (2015).Comparative study of accumulation, toxicity and trophic transfer of CuO and ZnO nanoparticles through waterborne and dietary exposure of goldfish (*Carassius auratus*) *Environmental Toxicology*,**30**:119–128.

15. Barton, B.A., & Iwama, J.R. (1991). Plasma cortisol and chloride stress responses in juvenile walleyes during capture, transport, and stocking procedures. *North American Journal of Aquaculture,* **65**: 210–219
16. Bressler, M., & Ron, B. (2004). Procalcitonin-guided Antibiotic Use vs A Standard Approach For Acute Respiratory Tract Infections in Primary Care. *Archives of Internal Medicine,* **168**(18): 2000-2007.
17. Coyle, S., Durbalov, M., & Tidwell, J. (2004). Anesthetic in aquaculture. Southern Regional Aquaculture Center publication, 3900, 1-6
18. Ellsaeesser, T., & Clem, D. (1986). The relationships between stocking density and welfare in farmed rainbowtrout. *Journal of Fish Biology,***61**: 493–531.
19. Gabriel, U.U., Ezeri, G.N.O., & Opabunmi, O.O. (2004). Influence of sex, source, health status and acclimatization on the haematology of *Clarias gariepinus* (Burchell 1822). *Afri. Journal of Biotecnology.*, **3**(9): 463-467.
20. Gabriel, U.U., Anyanwu, P.E. and Akinrotimi, A.O. (2007a). Haematological Profiles of Black Chinned tilapia (*Sarotherodon melantheron*) from Buguma Creek, Niger Delta. *Agriculture Journal,* **2**(3): 384 -387.
21. Gabriel, U.U., Anyanwu, P.E., & Akinrotimi, A.O. (2007b). Effect of Fresh water challenge on the blood characteristics of *sarotherodon melanotheron*. *Journal of Fisheries International,***4**: 112–120.
22. Gabriel, U. U., Jack I. R., Edori, O. S., & Egobueze, E. (2009). Electrolytes in selected tissues of *Heterobranchusbidorsalis*treated with sublethal levels of Cypermethrin. *Ethiopian Journal of Environmental Studies and Management,* **2**(3): 83-87.
23. Gabriel, U.U., & Edori, O.S. (2010). Mortality and behavioural responses of C*larias gariepinus* and its hybrid to acute levels of dichlobenil. *Journal League of Researchers in Nigeria,***11**(1):74-81.
24. Gabriel, U.U., Obomanu, F.G., & Edori, O.S. (2010). Biochemical changes in hybrid catfish (*Heterobranchus bidorsalis* × *Clarias gariepinus*) treated with nuracron. *Chinese Jounal of Applied and Environmental Biology,***16**(3): 353-357.
25. Gabriel, U.U., Akinrotimi, O.A., & Orluu, E.E. (2011). Haematological charactereistics of bloody cockle. *Anadara senitis* (L). from Andoni flats, Niger Delta, Nigeria. *Science World Journal,* **6**(1): 144-148.
26. Hamm, J.T., Wilson, B.W., & Hinton, D.E. (2016). Organophosphate-induced acetylcholinesterase inhibition and embryonic retinal cell necrosis in vivo in the teleost (*Oryzias latipes*). *Neurotoxicology,***19**: 853 -870.
27. Harper, H.A. (1977). Pfiehled fysiologické chemie. Avicenum, Praha, 639pp.
28. Heisler,W. (2005). Acute and prolonged toxicity to aquatic organisms of new and existing chemicals and pesticides. *Chemosphere,***31**: 2707-2726.
29. Hoseini, A.H. (2011). Components of the hematological response of fishes to environmental temperature change. *In* Environmental Biology of Fishes. pp. 241–298. NATO ASI Ser. A, Life Sciences, V. 35. Edited by M.A. Ali, Plenum Press, New York.
30. Maule, A.G., & Shreck, C.B. (1990). Changes in number of leucocytes in immune organs of juveniles Coho Salmon after acute stress of cortisol treatment. *Journal of Aquatic Animal Health,* **2**: 298-302.
31. Maule, A, Jasen G.M., & Syed, A.M. (1989). Effects of Stocking Density on Blood Cortisol, Glucose and Cholesterol Levels of Buluga Juveniles (Huso huso Linnaeus, 1758) Cultured in Subtropical climate. *International Journal of Fisheries and Aquatic Studies,* **4**(1): 400-404
32. Mariochiolo, A., & Genovese, M. (2011). Effects of stocking density on haemotological parameters on growth and fin erosion of great sturgeon juveniles (*Huso huso* Linnaeus 1758). *Research Opinions in Animaland Vetinery Sciences,* **39**: 1506-1513.
33. Mylonas, C. C., Cardinaletti, G., Sigelaki, I., & Polzonetti-Magni, A. (2005). Comparative efficacy of clove oil and 2-phenoxyethanol as anesthetics in the aquaculture of European sea bass

(Dicentrarchus labrax) and gilthead sea bream (Sparus aurata) at different temperatures. *Aquaculture*, *246*(1-4), 467-481.

34. Pickering, A.D. (1981). Acclimation of the internal tissues of brown trout. *Salmo trutta.L.* to chronic crowding stress. *Journal of Fish Biology*,**24**: 731-740.
35. Ross, L.G., & Ross, B.R. (2008) Anaesthetic and sedative techniques for aquatic animals. Blackwell Science, Oxford, United Kingdom
36. Sara, G., Scilipoti, D., Mazzola, A., & Modica, A. (2010). Effects of fish farming waste to sedimentary and particulate organic matter in a southern Mediterranean area (Gulf of Castellammare, Sicily): a multiple stable isotope study. *Aquaculture*, **234**: 199- 213.
37. Sharma, R., & Dash, M. (1991). Effect of Endosulfan to freshwater fish, *Ctenopharyn godonidella*, *J. Ecotoxicol. Environ. Monitor*,**15**: 113-116.
38. Tella, M. A. (2005). Serum electrolyte changes in West African dwarf (WAD) sheep with single or concurrent (*Bebesiaovis* and *Tympanosome Congolese*) infections. *African Journal of Biological Research*, **8**: 63-65.
39. Trushenski, K.S., Veeraiah, K., & Rao, D.K. (2013). Toxicity and bioaccumulation of Chlorpyrifos in Indian carp *Catla catla* (Hamilton), *Labeo rohita* (Hamilton), and *Cirrhinus mrigala* (Hamilton*), Bulletin of Environmental Contamination and Toxicology*,**73**(5): 933-941.