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ORIGINAL RESEARCH

Plasma Electrolytes Response in Mud Catfish, *Clarias gariepinus* (Burchell 1822) exposed to Different Regimes of Salinity and pH

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Abstract:

Introduction: Aquatic organisms, especially fish, are highly sensitive to environmental changes that can profoundly affect their physiological processes essential for survival. Physico-chemical factors such as salinity and pH exert significant influences on osmoregulation and ion balance, which are critical for maintaining cellular homeostasis in fish.

Aims: This study aims to explore how varying levels of salinity and pH affect plasma electrolyte concentrations in *Clarias gariepinus*, focusing on potassium (K^+), sodium (Na^+), chloride (Cl^-), and bicarbonate (HCO_3^-) ions. The objective is to understand the physiological responses of *C. gariepinus* to these environmental factors and their potential as biomarkers for assessing aquatic ecosystem health.

Materials and Methods: Juvenile *C. gariepinus* was exposed to different salinity levels (4 ‰, 6 ‰, 8 ‰) and pH levels (4, 6, 8) over 21 days. Plasma electrolyte concentrations were measured using an SFRI ISE 6000 Electrolyte Analyser. Statistical analysis included ANOVA and DMRT to identify significant differences ($p < 0.05$) among experimental groups.

Results: The study found significant variations in K^+, Cl^-, Na^+, and HCO_3^- concentrations in *C. gariepinus* across different salinity and pH conditions. K^+ levels decreased with increasing salinity, indicating stress-induced responses, while pH variations had less pronounced effects on K^+ regulation. Cl^- concentrations increased with higher salinity levels, suggesting adaptive osmoregulatory strategies. Na^+ levels showed significant fluctuations across salinity and pH conditions, whereas HCO_3^- levels responded uniquely to changes in these parameters, demonstrating adaptive mechanisms in acid-base balance.

Conclusion: This research underscores the adaptive responses of *C. gariepinus* to fluctuations in salinity and pH, highlighting their ability to maintain plasma electrolyte balance under varying environmental conditions. These findings contribute to understanding the ecophysiological adaptations of *C. gariepinus* and suggest the potential utility of plasma electrolytes as biomarkers for assessing the health of aquatic ecosystems.

Keywords: *Clarias gariepinus*, plasma electrolytes, salinity, pH, aquatic ecosystems, Ion regulation

All co-authors agreed to have their names listed as authors.

1. INTRODUCTION

Aquatic organisms, especially fish, are very sensitive to environmental fluctuations that can profoundly affect their physiological processes essential for survival [1, 2]. Physico-chemical parameters such as temperature, pH, salinity, and dissolved oxygen play crucial roles in determining cellular responses in aquatic organisms, influencing functions like gas exchange, acid-base balance, and ion regulation [3, 4, 5, 6].

Salinity, expressed in parts per thousand (‰), is a critical environmental factor affecting aquatic ecosystems and chemical processes. It directly impacts the osmoregulation of fish, influencing their survival, metabolism, and distribution [7]. Similarly, pH levels in water are vital as they affect the physiology and metabolic activities of aquatic animals, with low pH impairing ionic regulation and reducing fish survival [8].

The use of biomarkers has been advocated as crucial for assessing the overall health of individual organisms in contaminated ecosystems [9]. Biomarkers are functional measures that indicate exposure to stressors and their effects, operating at molecular, cellular, physiological, or behavioral levels within organisms [10]. They serve as measurable indicators of biological responses to environmental stressors or chemicals, enabling early detection and prediction of stress-related consequences [11]. Spanning molecular, cellular, physiological, and behavioural changes, biomarkers offer valuable insights into the health status and ecological risks faced by organisms [12]. In aquatic research, biomarkers are pivotal for assessing how environmental stressors impact physiological processes, thereby supporting efforts to evaluate ecosystem health and develop conservation strategies.

Among the biomarkers, plasma electrolytes serve as important indicators of exposure to environmental stress. Electrolytes such as potassium (K^+), sodium (Na^+), chloride (Cl^-), and bicarbonate (HCO_3^-) play crucial roles in osmoregulation, acid-base balance, and cellular function in fish [13]. Changes in plasma electrolyte concentrations can lead to severe physiological dysfunctions.

Fish are commonly chosen for evaluating the impacts of environmental pollution on aquatic ecosystems [14]. They are often seen as indicator species for assessing the health of aquatic environments, responding to a range of substances resulting from human activities like higher vertebrates [15, 16]. The African mud catfish, *Clarias gariepinus* (Family: Clariidae), is extensively distributed throughout Africa, demonstrating significant adaptive capability to thrive in various environmental conditions [17]. This species possesses an accessory breathing organ, allowing it to breathe air when water conditions are challenging, and further enhancing its resilience in fluctuating habitats [18].

Several laboratory studies have examined the impact of toxic substances on the electrolyte response of *C. gariepinus*. These investigations include experiments on potassium permanganate exposure [6], pesticide diazinon [19], detergent effects from linear alkylbenzene sulfonate [20], and the correlation between body length, weight, and electrolyte levels in *C. gariepinus* [21].

This study aims to investigate the impact of different salinity and pH regimes on plasma electrolyte concentrations in *C. gariepinus*. Specifically, it focuses on understanding how variations in these environmental factors influence the levels of potassium, sodium, chloride, and bicarbonate ions in the plasma of the African mud catfish. By elucidating these responses, the study aims to contribute to a deeper understanding of the physiological adaptations of *C. gariepinus* to environmental stressors and provide insights into the potential biomarkers for assessing aquatic ecosystem health. Monitoring of fish plasma electrolyte levels may allow partial assessment of the ecophysiological status of the fish population and detect possible aquatic stress [22].

The investigation of plasma electrolyte responses in *C. gariepinus* under varying environmental conditions is significant for both ecological research and conservation efforts, offering valuable information on the health status and adaptability of this species in changing aquatic environments.

2. MATERIAL AND METHODS

2.1 Test Animals

Two hundred and forty (240) juvenile African Mud Catfish (*Clarias gariepinus*) with an average weight of 16.2 g and standard length of 9.5cm were sourced from Treasure Base Farms, Ikotun, Lagos State. They were transferred to the Aquatic Toxicological Laboratory, Department of Marine Sciences, University of Lagos, and acclimatized in well-aerated rectangular tanks (60 x 37 x 9cm) filled with dechlorinated water. Tanks were covered with 1mm mesh gauze to prevent fish escape and ensure maximum aeration. Fish were fed twice daily with Coppens® pellets (1 mm) at 4 % of their body weight. Tanks underwent daily water changes, with thorough cleaning to prevent fungal and parasite outbreaks, removing debris and excess feed.

2.2 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. Salinity and pH regimes used by [23] were adopted. Salinity regimes were prepared by dissolving 130 g, 236 g, and 320 g of NaCl in 25 liters of fresh water to achieve concentrations of 4 ‰, 6 ‰, and 8 ‰, respectively. pH regimes were established by dissolving measured quantities of NaOH and HNO_3 to adjust pH levels to 8, 6, and 4 using 25 liters of fresh water, with untreated water serving as the control (0 ‰).

2.3 Bioassay Procedure

A semi-static renewal system was employed with solutions refreshed every 48 hours over 21 days, housing 10 fish per tank. Each experimental condition was triplicated. Test solution concentrations were based on [23]. 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.

Two (2) fish were selected from each tank comprising salinity, pH regimes, and control groups, totaling 6 fish per concentration at 7-day intervals for blood collection. Blood was drawn from the caudal peduncle using a plastic disposable syringe and hypodermic needle pre-rinsed with heparin into lithium heparin bottles to prevent coagulation. Blood samples collected from each experimental group were taken to the Pathology Laboratory of the Lagos University Teaching Hospital (LUTH) Idi-Araba for further analysis.

2.4 Analysis of Plasma Electrolytes

In this study, electrolyte analysis was conducted using an SFRI ISE 6000 Electrolyte Analyzer. Each sample, consisting of three milliliters (3 ml) of blood was tested to measure Potassium (K^+), Sodium (Na^+), Chloride (Cl^-), and Bicarbonate (HCO_3^-) levels. Calibration and analysis procedures followed manufacturer guidelines and utilized commercial calibrators to ensure precise and accurate results. This standardized approach was crucial for maintaining reliability and consistency in the electrolyte measurements throughout the study.

2.5 Statistical Analysis

Data were presented as mean ± standard error and analysed using Microsoft Excel and SPSS version 17.0 statistical software. One-way analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) was carried out to determine whether the mean response to the test solutions was significantly different ($P< 0.05$) from the control group.

3. RESULTS

3.1 Physico-Chemical Parameters of the Test Solution

The values of physico-chemical parameters obtained were similar among the different treatments. Dissolved oxygen ranged from 6.20 mg/L – 12.78 mg/L, temperature ranged from 27.50 ^{o}C - 28.30 ^{o}C and total dissolved solid values ranged from 6.10 g/L – 8.53 g/L for each test solution.

3.2 Plasma Electrolyte Responses in Exposed *C. gariepinus*

3.2.1 Plasma potassium (K^+)

The potassium ion (K^+) activities in the plasma of *C. gariepinus* exposed to varying salinity and pH conditions are illustrated in Figures 1 and 2 respectively. The mean K^+ levels ranged from 7.86 - 23.36 mEq/L across different salinity regimes (Figure 1). The highest mean (23.36 mEq/L) was observed on day 7 in organisms exposed to 4 ‰ salinity, whereas the lowest mean (7.86 mEq/L) occurred on day 14 in organisms exposed to 6 ‰ salinity. Generally, K^+ activity showed a declining trend with increasing salinity and decreased over time until day 14, followed by a slight increase by day 21. Analysis of Variance (ANOVA) indicated significant differences ($P < 0.05$) in K^+ activity among salinity regimes on days 7, 14, and 21. Duncan Multiple Range Test (DMRT) further revealed significant differences ($P < 0.05$) between K^+ activity in *C. gariepinus* exposed to 4, 6, and 8 ‰ salinity compared to control fish at days 7 and 21.

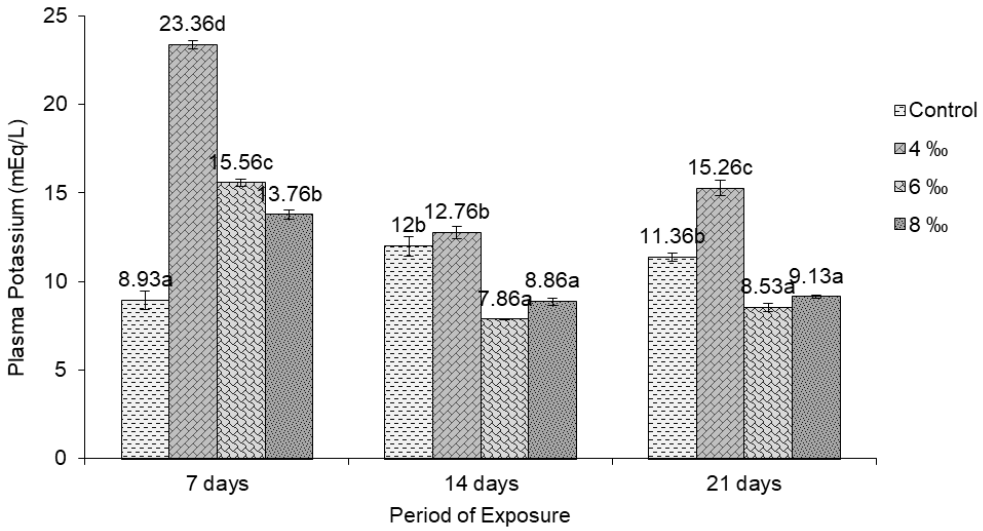

Figure 1: Mean ± S.E values (n=6) of K^+ activity in the plasma of *C. gariepinus* exposed to different salinity regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different ($P > 0.05$, DMRT)].

In the pH regimes (Figure 2), K^+ activity ranged from 8.70 - 15.20 mEq/L. The lowest mean (8.70 mEq/L) was recorded at pH 4 on day 7, while the highest mean (15.20 mEq/L) was observed at pH 6 on day 7. ANOVA demonstrated significant differences ($P < 0.05$) in K^+ levels among different pH regimes across days 7, 14, and 21. Post hoc DMRT analysis showed no significant difference ($P > 0.05$) between K^+ levels in *C. gariepinus* exposed to 4 ‰ salinity and the control solution at days 7 and 21, respectively.

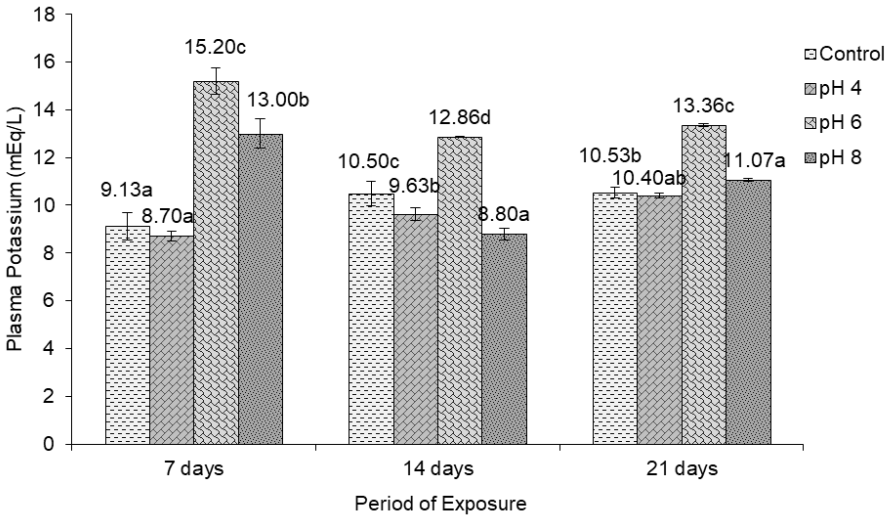

Figure 2: Mean ± S.E values (n=6) of K^+ activity in the plasma of *C. gariepinus* exposed to different pH regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)].

3.2.2 Plasma chloride (Cl^-)

Plasma chloride ion (Cl^-) activity in *C. gariepinus* exposed to various salinity and pH conditions is depicted in Figures 3 and 4. Across salinity regimes (Figure 3), mean Cl^- activity ranged from 115.33 - 183.00 mEq/L. The highest mean (183.00 mEq/L) occurred on day 21 in organisms exposed to 8 ‰ salinity, while the lowest mean (115.33 mEq/L) was noted on day 7 in the control solution. Cl^- activity generally increased with longer exposure duration at 4, 6, and 8 ‰ salinity levels. ANOVA revealed significant differences (P < 0.05) in Cl^- activity among salinity regimes on days 7, 14, and 21. DMRT analysis indicated no significant difference (P > 0.05) between Cl^- activity in *C. gariepinus* exposed to 4 ‰ salinity and the control solution at day 14.

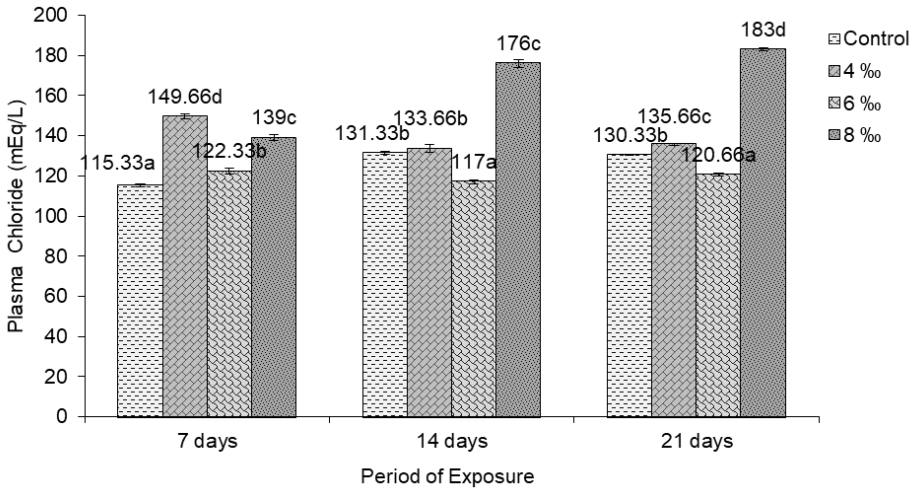

Figure 3: Mean ± S.E values (n=6) of Cl^- activity in *C. gariepinus* exposed to different salinity regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)].

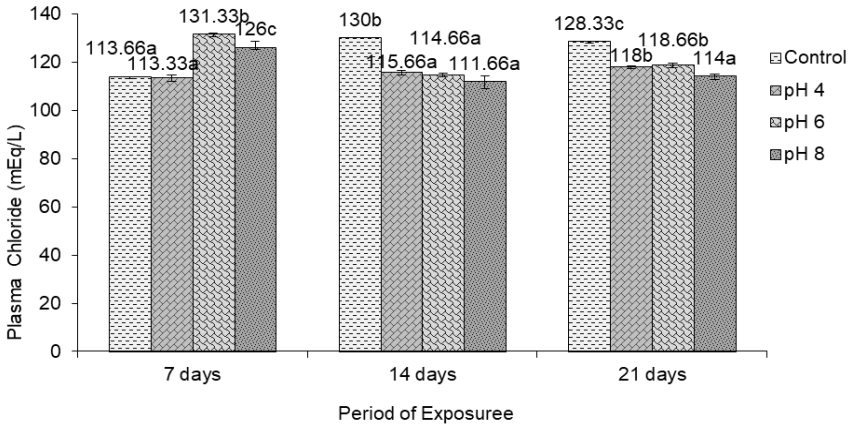

Figure 4: Mean ± S.E values (n=6) of Cl^- activity in *C. gariepinus* exposed to different pH regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)].

In pH regimes (Figure 4), mean Cl^- activity ranged from 111.6 - 131.33 mEq/L. The highest mean (131.33 mEq/L) was recorded at pH 6 on day 7, while the lowest mean (111.6 mEq/L) occurred at pH 8 on day 7. ANOVA indicated significant differences (P < 0.05) in Cl^- activity among pH regimes. Further DMRT analysis showed no significant difference (P > 0.05) between Cl^- activities in *C. gariepinus* exposed to pH 4, 6, and 8 and the control solution at day 7.

3.2.3 Plasma sodium (Na+)

Sodium ion (Na^+) activity in the plasma of *C. gariepinus* under different salinity and pH conditions is presented in Figures 5 and 6. Across salinity regimes (Figure 5), mean Na^+ activity ranged from 117.00 - 211.66 mEq/L. The highest mean (211.66 mEq/L) was observed on day 21 in organisms exposed to 8 ‰ salinity, while the lowest mean (117.00 mEq/L) occurred on day 14 in the control group. Na^+ activity increased with longer exposure duration at 4, 6, and 8 ‰ salinity levels. ANOVA revealed significant differences (P < 0.05) in Na+ activity among salinity regimes on days 7, 14, and 21. DMRT analysis showed no significant difference (P > 0.05) in Na^+ activity between *C. gariepinus* exposed to 4 ‰ and 6 ‰ salinity and the control solution at days 7 and 14, respectively.

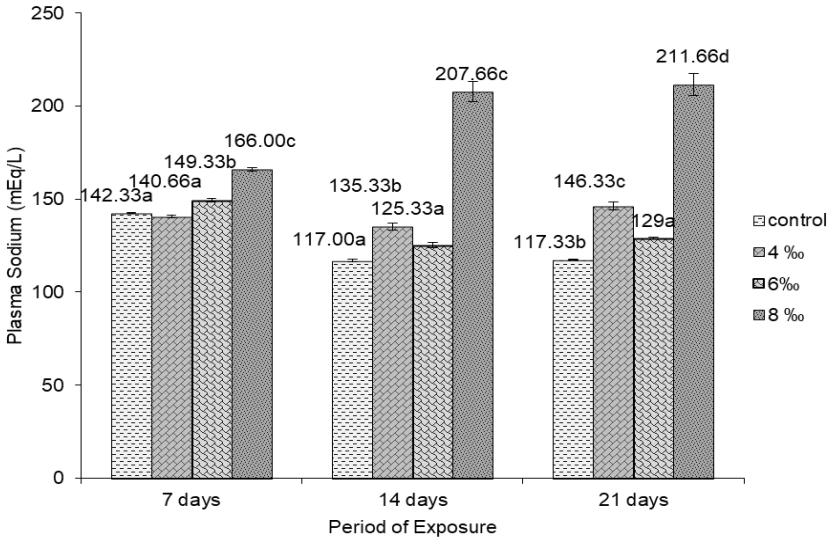

Figure 5: Mean ± S.E values (n=6) of Na^+ activity in *C. gariepinus* exposed to different salinity regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)]

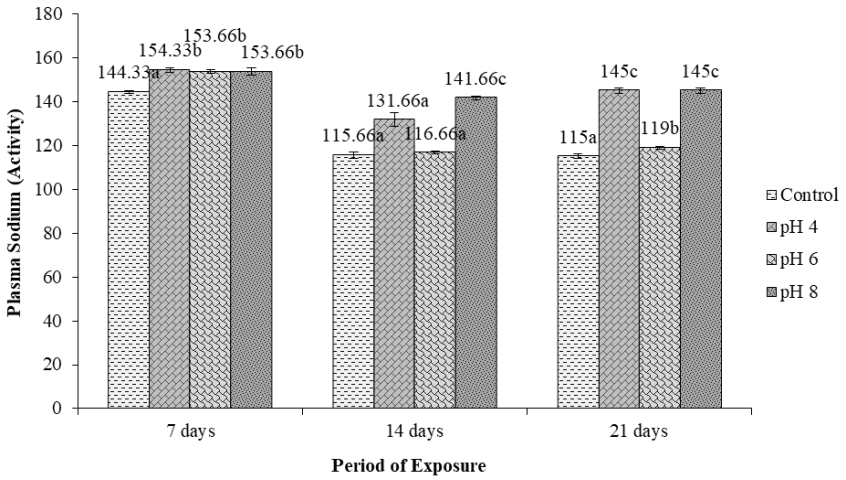

Figure 6: Mean ± S.E values (n=6) of Na^+ activity in *C. gariepinus* exposed to different pH regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)]

In pH regimes (Figure 6), mean Na^+ activity ranged from 115.33 - 154.33 mEq/L. The highest mean (154.33 mEq/L) was recorded at pH 4 on day 21, while the lowest mean (115.33 mEq/L) occurred at day 21 in the control group. ANOVA indicated significant differences ($P < 0.05$) in Na^+ activity among pH regimes. DMRT analysis revealed no significant difference ($P > 0.05$) in Na+ activity between *C. gariepinus* exposed to pH 4, and 6 and the control solution at day 14.

3.2.4 Plasma bicarbonate (HCO_3^-)

Bicarbonate ion (HCO_3^-) activity in the plasma of *C. gariepinus* under different salinity and pH conditions is presented in Figures 7 and 8. Across salinity regimes (Figure 7), mean HCO_3^- activity ranged from 6.66 - 24.66 mEq/L. The lowest mean (6.66 mEq/L) occurred on day 7 in organisms exposed to 6 ‰ salinity, while the highest mean (24.66 mEq/L) was observed on day 21 in organisms exposed to 8 ‰ salinity. HCO_3^- activity increased with longer exposure duration at 4, 6, and 8 ‰ salinity levels. ANOVA indicated significant differences ($P < 0.05$) in HCO_3^- activity among salinity regimes. DMRT analysis showed no significant difference ($P > 0.05$) in HCO_3^- activity among different salinity levels.

In pH regimes (Figure 8), mean HCO_3^- activity ranged from 8.66 to 15.66 mEq/L. The highest mean (15.66 mEq/L) was recorded on day 21 in organisms exposed to pH 6, while the lowest mean (8.66 mEq/L) occurred on day 7 in the control solution at pH 4 and 8. ANOVA indicated significant differences ($P < 0.05$) in HCO_3^- activity among pH regimes on days 7, 14, and 21. Post hoc DMRT analysis revealed no significant difference ($P > 0.05$) in HCO_3^- activity between *C. gariepinus* exposed to pH 4 and the control solution at day 14, as well as between those exposed to pH 4 and pH 8 at day 21.

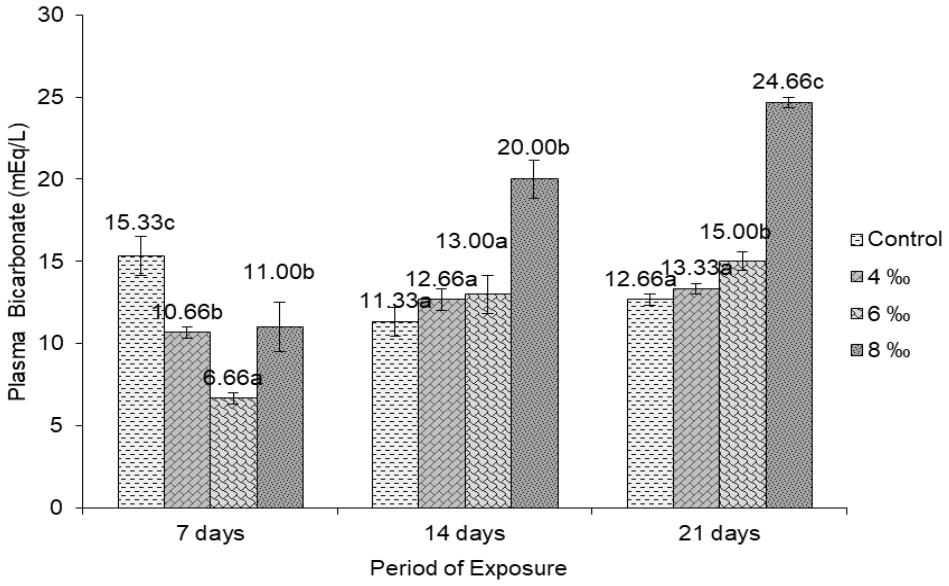

Figure 7: Mean ± S.E values (n=6) of (HCO_3^-) activity in *C. gariepinus* exposed to different salinity regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)].

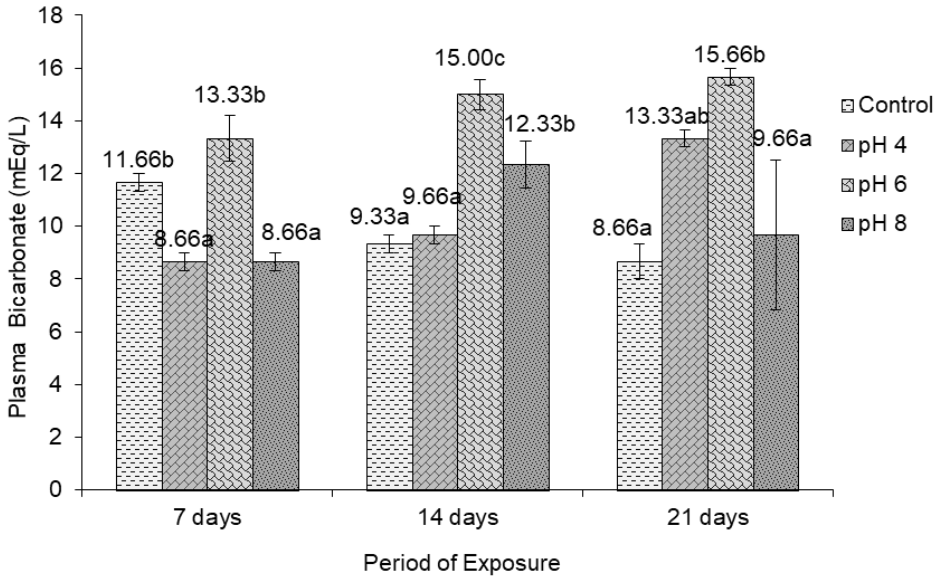

Figure 8: Mean ± S.E values (n=6) of (HCO_3^-) activity in *C. gariepinus* exposed to different pH regimes for 21 days. [Mean ± S.E bars with the same alphabet in each exposure period are not significantly different (P > 0.05, DMRT)].

4. DISCUSSION

The study on plasma electrolyte responses in *Clarias gariepinus* exposed to varying salinity and pH conditions provides valuable insights into the osmoregulatory mechanisms of this freshwater fish species. The findings highlight significant variations ($P < 0.05$) in potassium (K^+), chloride (Cl^-), sodium (Na^+), and bicarbonate (HCO_3^-) ion activities in response to environmental stressors, shedding light on how *C. gariepinus* maintains internal ion balance under changing aquatic conditions. [24] reported significant ($P < 0.05$) alterations in the plasma electrolytes such as sodium, potassium, and chloride levels of *Cyprinus carpio* exposed to low pH (5.0) for 7–35 days.

In this study, the potassium ion (K^+) activity in the plasma of *C. gariepinus* demonstrated varied responses to both salinity and pH changes. The observed decline in K^+ activity with increasing salinity aligns with the osmoregulatory challenges typically faced by freshwater organisms when exposed to higher salinity levels. This trend was particularly evident up to day 14, after which there was a slight recovery or stabilization by day 21. The significant differences ($P < 0.05$) across different salinity regimes at various time points underscore the sensitivity of K^+ regulation to environmental salinity changes. Notably, exposure to 4, 6, and 8 ‰ salinity resulted in significantly higher K^+ levels compared to the control group at days 7 and 21, suggesting a stress-induced response in potassium ion regulation. In contrast, pH variation showed less pronounced effects on K^+ levels, with no significant differences ($P > 0.05$) between the 4 ‰ salinity group and the control across the same time points. This indicates that while salinity poses greater challenges to K^+ homeostasis, pH fluctuations within the tested range do not significantly impact potassium ion regulation in *C. gariepinus* over short exposure durations. Increased levels (77.8%) of plasma K^+ have been reported in *C. carpio* exposed to low pH (5.0) during long-term (35 days) exposure periods [24].

Chloride ion (Cl^-) activity in the plasma of *C. gariepinus* exhibited distinct responses to salinity and pH alterations. The increase in Cl^- activity with increasing salinity levels reflects the osmoregulatory strategy of marine teleosts, where chloride ion uptake aids in maintaining osmotic balance in hyperosmotic environments [25]. Significant differences ($P < 0.05$) were observed among salinity regimes, highlighting the adaptive responses of *C. gariepinus* to varying salinity conditions over time. However, in pH regimes, although significant differences ($P < 0.05$) in Cl^- activity were detected among different pH levels, DMRT analysis did not reveal significant differences ($P > 0.05$) between the experimental pH groups and the control at day 7. This suggests that short-term exposure to pH variations within the tested range may not significantly alter chloride ion regulation in *C. gariepinus* [26].

Sodium ion (Na^+) activity in the plasma of *C. gariepinus* showed a clear trend of increasing levels with higher salinity exposure, indicative of osmoregulatory adjustments typical in marine environments [27]. The significant differences ($P < 0.05$) observed among salinity regimes underscore the sensitivity of Na^+ regulation to environmental salinity changes over time. Despite this no significant differences ($P > 0.05$) were found between the 4 ‰ and 6 ‰ salinity groups compared to the control at specific time points, indicating a regulatory capacity to maintain Na^+ homeostasis within certain salinity ranges. In contrast, pH variations showed significant differences ($P < 0.05$) in Na^+ activity among pH levels, yet post hoc analysis revealed no significant differences ($P > 0.05$) between pH 4, pH 6, and the control at day 14. This suggests that short-term exposure to pH changes within the studied range may not disrupt sodium ion regulation to a significant extent in *C. gariepinus*.

Bicarbonate ion (HCO_3^-) activity in the plasma of *C. gariepinus* responded to salinity and pH changes with notable variability. The observed increase in HCO_3^- levels with increasing salinity aligns with the compensatory mechanisms used by fish to regulate acid-base balance in saline environments [28]. Significant differences ($P < 0.05$) in HCO_3^- activity among salinity regimes indicate a robust response to prolonged exposure durations across different salinities. However, DMRT analysis did not reveal significant differences ($P > 0.05$) in HCO_3^- levels between different salinity groups, suggesting a certain level of adaptive stability over short exposure periods. In pH regimes, while significant differences ($P < 0.05$) in HCO_3^- activity were detected among pH levels, post hoc analysis showed no significant differences ($P > 0.05$) between pH 4 and the control at day 14, nor between pH 4 and pH 8 at day 21. This indicates that short-term exposure to pH fluctuations within the tested range may not disrupt bicarbonate ion regulation to a significant extent in C. *gariepinus*.

The findings highlight the intricate regulatory mechanisms employed by *C*. *gariepinus* to maintain plasma electrolyte homeostasis in response to environmental challenges posed by varying salinity and pH conditions. Salinity fluctuations exerted more pronounced effects on potassium, chloride, sodium, and bicarbonate ion activities compared to pH variations within the tested ranges. These responses underscore the species' ability to adapt to changing environmental conditions over short-term exposure durations, reflecting its adaptive plasticity in managing electrolyte balance in response to aquatic habitat changes [29, 30]. Further studies could explore longer exposure periods and additional environmental parameters to elucidate more comprehensive insights into the physiological adaptations of *C. gariepinus* in dynamic aquatic environments.

5. CONCLUSION

The findings highlight the intricate regulatory mechanisms employed by *C*. *gariepinus* to maintain plasma electrolyte homeostasis in response to environmental challenges posed by varying salinity and pH conditions. Salinity fluctuations exerted more pronounced effects on potassium, chloride, sodium, and bicarbonate ion activities compared to pH variations within the tested ranges. These responses underscore the species' ability to adapt to changing environmental conditions over short-term exposure durations, reflecting its adaptive plasticity in managing electrolyte balance in response to aquatic habitat changes [29, 30]. Further studies could explore longer exposure periods and additional environmental parameters to elucidate more comprehensive insights into the physiological adaptations of *C. gariepinus* in dynamic aquatic environments.

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COMPETING INTERESTS

There are no competing interests among the authors regarding the publication of this article in JRRS.

AUTHORS' CONTRIBUTIONS

Olusegun B. Samuel: Conceptualization, supervision and statistical analysis. Mulikat O. King and Olusegun B. Samuel: involved in the management of the experimental set-up, literature search, manuscript drafting and approval of the final manuscript

CONSENT

Not applicable

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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