Covenant Journal of Physical & Life Sciences (CJPL) Vol. 9 No. 2, Dec 2021
ISSN: *print 2354 – 3574; electronics 2354-3485 DOI:*

An Open Access Journal Available Online

Effect of Short-Term Exposure of Simulated Acid Rain on the Hemato-Immunological Parameters of the Purple Mangrove Crab *Goniopsis pelii* (Herklots, 1851)

Rasheed Olatunji Moruf

Department of Fisheries and Aquaculture, Bayero University, Kano, Kano State, Nigeria

E-mail: tunjimoruf@gmail.com
Telephone: +2348022429983
ORCID: https://orcid.org/0000-0002-0459-0621

Received: 05.06.2021; Accepted: 21.11.2021
Date of Publication: December, 2021

***Abstract*:** Acid rain is a broad term to describe the mixing of acid contaminant with rainwater. It has been detected in Nigeria's industrialized areas with effects on aquatic organisms. The effect of pH under rising stimulated acidic condition on the immune parameters of Purple Mangrove Crab (*Goniopsis pelii*) was investigated. The crabs were subjected to four experimental pH at 4.0, 5.0, 6.0 and 7.8 (control) in triplicates with 5 L of lagoon water. The short-term exposure period lasted for 14 days during which each plastic container was well aerated. Results showed that *G. pelii* exhibited marked reduction in hematological parameters between control and the exposed crab groups. There was a significant ($P<0.05$) reduction in haemoglobin from 2.63±1.52 g/dl in the control crabs to 0.95±0.01 g/dl in the pH 4.0 treatment. Significantly, higher alkaline phosphatase (70.92±3.24-75.44±4.01 μI^{-1}) and albumen (3.21±0.06-6.44±0.09 gl^{-1}) values were obtained in the treatments, increased with decrease in pH. The highest superoxide dismutase (260.93±6.11 min/mg pro) and catalase activities (2.447±1.25 min/mg pro) were recorded in the crabs exposed to pH 4.0. The malondialdehyde concentrations showed an increase from 10.41±1.91 nmol/ml to 10.77±2.02 nmol/ml, with decreasing pH and were significantly different from the 8.98±2.05 nmol/ml mean value of the control group. This finding suggests that decreasing pH is more detrimental to *G. pelii* survival under acidic condition. Hence, this pH-dependent phenomenon should be taken into consideration for optimal conservation of *G. pelii*.
Keywords: Acid rain, crab, hematology, oxidative stress, serum biochemistry.

1.0 Introduction

Acid rain is a broad term that includes any form of precipitation with acidic components, such as sulfuric or nitric acid. When rain is contaminated, its pH falls below 5.6 [1]. The high concentrations of HNO_3 and H_2SO_4 in acid rain are due to atmospheric oxidation of NO_x and SO_2 emitted by fossil fuel combustion [2]. Most sulfuric acids are consumed in manufacturing processes or recovered and re used. Sulfuric acid, H_2SO_4 can make its way into the environment through unintentional releases (spills) and industrial or consumer

discharges. Some coastal ecosystems already experience pH values lower than those predicted to occur in the open ocean by 2100 [3], and the acidification of these systems will be intensified by the climate change-associated acidification of the future [4].

Cases of acid deposition in most aquatic habitats and its effect on crabs have been documented [5, 6, 7]. One of the effects of acid rain on water bodies is the lowering of the pH levels, which consequently affect crabs negatively. Adult blue crabs are generally considered tolerant of acidification because of their ability to regulate HCO_3^- [8], and increase high oxygen-affinity respiratory pigments through a hypoxia-stimulated structural change in their blood [9]. Despite their ability to regulate hemolymph chemistry, exposure to low pH can impair the immune response, slowing the rate of clearance of bacteria from the hemolymph [10]. Additionally, low pH conditions can significantly reduce the activity (up to 70%) of the terminal enzyme of the prophenoloxidase activating system, a major defence system of the blue crab [5].

Organisms able to withstand periods of low pH may experience sublethal stress in the form of reduced growth, feeding, locomotion, or fecundity [11, 12]. Furthermore, altered physiology or behaviour in response to lowered pH can make some organisms more vulnerable to disease [12] as well as other co-stressors [13, 14]. Survey of the biological implications of water acidification have primarily focused on calcifying marine organisms [15–17] because of their physiological dependence on the availability of CO_3^{2-} ions. Results of a meta-analysis among calcifying organisms indicate negative effects on reproduction, growth, calcification, and survival that vary across taxa and life stage [18]. Despite this refined understanding established by previous studies, there are no published studies investigating the effect of short-term exposure of simulated acid rain on the hemato-immunological parameters of mangrove crabs. Studies regarding the effects of stressors generally involve short-term exposure in the laboratory [19]. Hence, the purpose of this study is to document the effect of short-term exposure of simulated acid rain on hematology, serum biochemistry and antioxidant enzyme activities of the Purple Mangrove Crab *Goniopsis pelii* (Herklots 1851).

2.0 Materials and Methods

Experimental setup and bioassay procedure.

Forty-eight (48) *G. pelii* samples weighing 21.70 ± 3.1 g were obtained from the mangrove area of University of Lagos Lagoon front (Latitude 6°26°N and 6°39°N and Longitude 3°39°E and 3°50°E), the Lagos Lagoon's western axis [20]. The experiment was carried out at the Department of Marine Sciences of University of Lagos, Nigeria. The crabs were selected and randomly stocked into four small plastic tank (length × width × depth = 8 m × 8 m × 1.5 m) at 4 crabs per tank. Prior to the start of the experiment, they were acclimatized for 7 days in a laboratory setting. Once a day, they were given trash fish (*Sardinella aurita*), which accounted for around 2.2 percent of the total weight of crabs in the tank. The feeding was stopped 24 hours before the range-finding toxicity test.

Stimulated acid rain was prepared using the same methods as Meseck et al. [6]. Since our low pH levels were chosen to represent eutrophic (coastal systems), ambient lagoon water was mixed with the acidified water. The crabs were subjected to four experimental pHs at 4.0, 5.0, 6.0 and 7.8 (control) in triplicates with 5 L of lagoon water. The short-term exposure period lasted

for 14 days during which each plastic container was well aerated.

Analytical procedures

With a 23G Syringe, crab haemolymph was collected from the juncture between the bases of the ischium of the fifth walking leg. The haemolymph was collected into a syringe flushed with 1mL of anticoagulant (0.3 M NaCl, 0.1 M glucose, 30 mM Sodium citrate and 26 mM Citric acid), transferred into a 5 mL lithium heparin bottle kept in an ice chest for immediate analysis. Total haemocyte counts of haemocyte population were determined using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley [21]. Individual crab haemolymph aliquots were transferred to the haemocytometer and manually counted. The morphotypes of haemocytes were determined, and 100 cells from each slide were counted. To achieve an absolute count, the percentage of each counted cell type was computed and multiplied by the overall haemocyte population count. The serum was assayed for transaminases according to methods described by Coles [22]. Following the protocol described by Lushchaks et al. [23] and Bertholdo-Vargas et al. [24], samples of excised muscle tissues of crabs were thawed and homogenized for the assays of reduced glutathione, catalase, superoxide dismutase, malondialdehyde and protein levels.

Statistical analysis

Analysis of variance (ANOVA) and Duncan multiple post hoc tests were used to compare the differences between means at $p < 0.05$ level of significance. All statistical analyses were conducted using SPSS version 17.

3.0 Result

Hematological profile

The percentage haemocyte subpopulation in *G. pelii* exposed to simulated acid rain can be seen in Figure 1. The control (pH 7.8) has the highest granulocyte (30 %) and monocyte (30 %) but lowest agranulocyte subpopulation (67 %). The differences in the mean values among the treatments and the control were not significant. Changes in hematological parameter such as haemoglobin (Hb), packed cell volume (PCV), total haemocyte count (THC) and haemocyte sedimentation rate (HSR) in *G. pelii* exposed to simulated acid rain is also shown in Table 1. The hematological analysis revealed a significant reduction ($P<0.05$) in haemoglobin from 2.63±1.52 g/dl in the control crabs to 0.95±0.01 g/dl in the pH 4 treatment. Similar trend was observed in THC from 5.09±0.50 mm^3 in the control crabs to 3.51±0.01 mm^3 in the pH 4 treatment. The lowest percentage of PCV (3.00±0.12 %) can be seen in treatment with pH 5. However, the non-significant values of HSR ranging from 2.00±0.05 mm/hr to 3.00±0.01 mm/hr was obtained in the experiment.

Serum biochemical profile

Table 2 shows the serum biochemical profile of *G. pelii* exposed to simulated acid rain. Control group had the lowest values of aspartate aminotransferase (AST) (2.98±0.06 μI^{-1}) and alanine aminotransferase (ALT) (4.53±1.21 μI^{-1}), which were not significantly different from the values obtained in the various treatment groups. However, significantly ($p<0.05$) higher alkaline phosphatase (ALP) (70.92±3.24-75.44±4.01 μI^{-1}) and albumen (ALB) (3.21±0.06-6.44±0.09 gl^{-1}) values were obtained in the treatments, increased with decrease in pH.

Antioxidant enzyme activity

Table 3 shows the antioxidant enzyme activity of *G. pelii* exposed to simulated acid rain. Protein levels ranging between 20.57-31.89 g/L (control) and 27.11-37.38 g/L (treatments) showed no significant difference across the study groups.

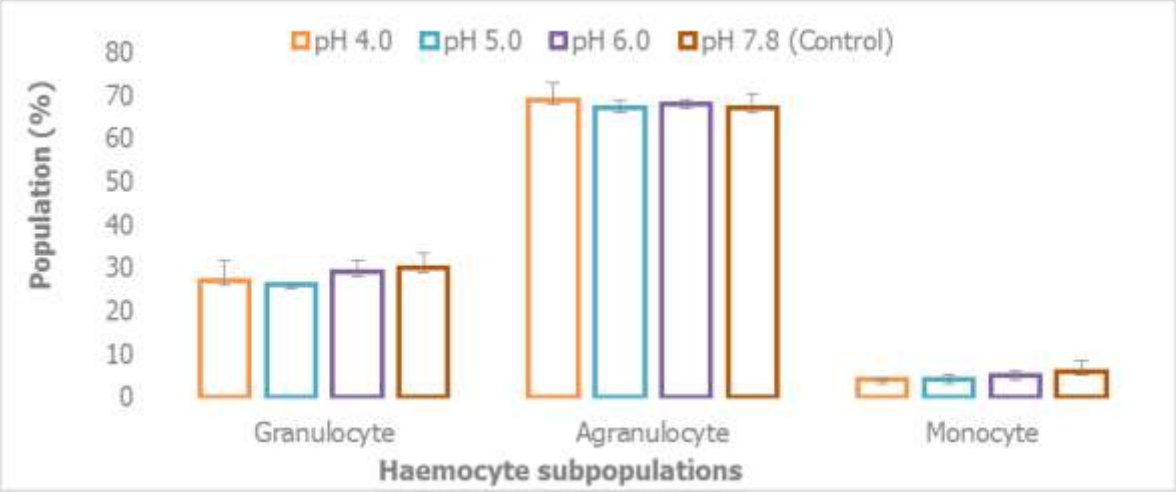

Figure 1: Haemocyte subpopulation in *Goniopsis pelii* exposed simulated acid rain

Table 1: Hematological response of *Goniopsis pelii* exposed to simulated acid rain

Parameters	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
Haemoglobin (g/dl)	(0.69-1.26) 0.95±0.01[a]	(1.60-2.50) 1.90±0.01[a]	(1.75-2.60) 2.0±0.70[a]	(2.29-2.83) 2.63±1.52[b]
Total Haemocyte Count (mm^3)	(2.35-4.52) 3.51±0.01[a]	(3.26-4.05) 3.78±0.02[a]	(3.17-5.34) 4.23±0.01[a]	(3.72-7.67) 5.09±0.50[b]
Packed Cell Volume (%)	(5.90-6.10) 6.00±0.02[a]	(2.20-3.90) 3.00±0.12[a]	(4.87-8.12) 7.00±0.06[a]	(8.89-14.23) 11.00±0.12[b]
Haemocyte Sedimentation Rate (mm/hr)	(2.02-2.45) 2.00±0.05[a]	(2.76-4.31) 3.00±0.01[a]	(2.67-4.37) 3.00±0.04[a]	(2.70-4.40) 3.00±1.01[a]

Keys: Range in bracket. Mean ± Standard Error; Values with different superscripts across row are significantly different at $P < 0.05$

Table 2: Serum biochemical response of *Goniopsis pelii* exposed to simulated acid rain

Parameters	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
AST (μl^{-1})	(3.05-3.09) 3.06±0.58[a]	(3.06-3.093) 3.07±0.09[a]	(1.35-4.47) 3.07±1.12[a]	(1.36-4.20) 2.98±0.06[a]
ALT (μl^{-1})	(4.49-7.70) 6.60±0.96[a]	(5.42-7.85) 6.53±2.00[a]	(4.62-7.74) 6.34±2.02[a]	(2.52-6.97) 4.53±1.21[a]
ALP (μl^{-1})	(72.31-76.45) 75.44±4.01[a]	(73.4-73.75) 73.53±0.12[a]	(56.5-97.38) 70.92±3.24[a]	(64.87-65.08) 64.98±1.11[b]
ALB (gl^{-1})	(6.43-6.45) 6.44±0.09[a]	(4.94-5.01) 4.98±0.61[a]	(3.2-3.3) 3.21±0.06[a]	(1.66-5.46) 3.06±0.07[b]

Keys: Aspartate (AST); Alanine aminotransferase (ALT); Alkaline phosphatase (ALP); Albumen (ALB). Range in bracket, Mean ± Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

The highest superoxide dismutase (SOD) (260.93±6.11 min/mg pro) and catalase activities (2.447±1.25 min/mg pro) were recorded in the crabs exposed to pH 4.0. The malondialdehyde (MDA) concentrations showed an appreciable increase from 10.41±1.91 nmol/ml to 10.77±2.02 nmol/ml, with decreasing pH and were significantly different ($P<0.05$) from the 8.98±2.05 nmol/ml mean value of the control group. In contrast, higher glutathione (GSH) value was observed in the control (5.8±3.25 µmol/ml) compared to exposed crab groups (4.45±2.12 - 5.55±0.91 µmol/ml) with no significant difference.

Table 3: Antioxidant enzyme activity of *Goniopsis pelii* exposed to simulated acid rain

Parameters	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
PRO(g/L)	(25.34-37.38)	(27.02-30.23)	(27.11-29.21)	(20.57-31.89)
	32.06±2.62[a]	29.13±4.01[a]	28.11±1.91[a]	26.34±2.20[a]
SOD	(255.31- 65.15)	(224.98-229.88)	(204.11-09.36)	(165.14-74.26)
(min/mg protein)	260.93±6.11[a]	226.88±5.03[a]	207.24±3.71[a]	169.15±4.11[a]
Catalase	(2.43-2.48)	(1.588-3.708)	(2.176-2.20)	(1.449-2.879)
(min/mg protein)	2.447±1.25[a]	2.31±0.02[a]	2.19±0.52[a]	1.559±0.06[a]
MDA (nmol/ml)	(10.65-11)	(10.47-10.68)	(10.39-10.43)	(8.97-9.00)
	10.77±2.02[a]	10.58±2.23[a]	10.41±1.91[a]	8.98±2.05[b]
GSH (µmol/ml)	(2.53-5.67)	(2.09-8.54)	(5.54-5.57)	(3.69-6.90)
	4.45±2.12[a]	5.10±1.26[a]	5.55±0.91[a]	5.8±3.25[a]

Keys: Protein (PRO); Superoxide dismutase (SOD); Malondialdehyde (MDA), and Glutathione (GSH). Range in bracket. Mean ± Standard Error; Values with different superscripts across row are significantly different at ($P < 0.05$)

4.0 Discussion

The short-term experiment tends to examine if and how quickly an organism is able to regulate physiology when challenged with acid rain; whereas, long-term experiments tend to provide more information about the general health status of an organism living with acid rain conditions [6]. An example of a short-term experiment was conducted with the Tanner crab *Chionoecetes bairdi* exposed to varying pH [6]. It was discovered that the energetic costs of responding to acid rain and maintaining defense mechanisms in Tanner crab may divert energy from other physiological processes, such as reproduction.

Despite the acclimatization of different crab species, the effect of the acidic media is strongly felt in terms of immune response. Lower pH (more acidic) may cause physiological stress because pH affects how molecules move across the gills. In the present study, the result of hematological parameters showed marked reduction between control crabs and the crabs exposed to different levels of pH, which is an indication of the deleterious effects of the acidic chemical to the body fluid of *G. pelii*. According to Chen et al. [25], simulated acid rain increased the proportion of heterotrophic respiration and had significant consequences, which could lead to a variety of physiological dysfunctions in organisms.

During the present study, significantly ($p<0.05$) higher ALP and ALB were obtained in exposure crabs compared to control while other serum biochemicals were similar. Serum albumin is the main protein of plasma, its main function is the regulation of the colloidal osmotic pressure of blood. According to Sanni et al. [26], changes in AST could be linked to interference in the crab's immune system, resulting in cell damage, or a mechanism in which the crabs react to pollutant exposure. Variations in serum parameters have been attributed to responses to a changed physiological and energetic requirement and may be an early warning sign of stress before population declines are observed [27, 28]. Melzner et al. [6] predicted that highly mobile organisms, such as crabs that are capable of controlling hemolymph pH through active ion transport would be more tolerant of water acidification than organisms without strong ion-regulating mechanisms. Pane and Barry [29] reported that Tanner crab (*C. tanneri*) exhibited an extracellular, compensatory response to extreme external acidification (7.08) and was able to only slightly recovered pH after 24 hrs exposure to stimulated acid rain.

Antioxidant defense enzymes analyzed in the present study are generally involved in cellular protection against peroxidation damage and maintained redox equilibrium within the cell [30]. Enhanced SOD activity at elevated/ decreased pH conditions indicates higher detoxification of ROS radical to the toxic acid chemical, which can be degraded by the concomitant increased catalase activity [31, 32]. Likewise, catalase activity was enhanced at lower pH, indicating direct involvement in scavenging the toxic chemical involved [32]. Similar increased catalase activity associated with lower pH content has been reported in the macroalga, agarophyton vermiculophyllum [33]. These results suggest that enhanced activities of SOD and catalase at lower pH values are crucial for *G. pelii* survival under acidic condition.

In the present study, MDA contents increased gradually as sulphuric acid concentrations decreased in crab. According to Lawal-Are et al. [33], MDA is a biomarker of oxidative damage that reflects the state of lipid peroxidation of the membrane of many living organisms. Furthermore, an increased GSH is often indicative of initiation of cellular defense mechanism in response to increasing concentration of free radical in the cell while a decrease may indicate an overrun of the antioxidant defense.

5.0 Conclusion

The result of the hematological parameters showed marked reduction between control crabs and the crabs exposed to different acidic pH, which is an indication of the deleterious effects of the acidic chemical to the body fluid of *G. pelii*. Additionally, the immediate increases of SOD and catalase at lower pH under stronger acidic condition are to cope with oxidative stress as defense mechanisms. This finding suggests that acidic pH is detrimental to *G. pelii* survival. Finally, this pH-dependent phenomenon should be taken into consideration for optimal conservation of *G. pelii*. Moreover, future studies are needed on long-term basis to understand the tolerance mechanism under this changing pH due to waste disposal into the mangrove swamps.

Acknowledgements

The author would like to acknowledge Mrs Ife O. Olaniyi, a postgraduate student of the Department of Marine Sciences, University of Lagos for her efforts in the failed preliminary experiment. The author would also like to acknowledge the editorial assistance rendered by Hajia Hawwau Moruf (CLN) of the Department of Library

and Information Science, Federal University Dutsin- Ma, Katsina State, Nigeria.

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