



Effects of water acidification on immune responses of the gercacinid, *Cardiosoma armatum* (Herklots, 1851)

A.O. Lawal-Are¹, R.O. Moruf^{2*}, I.O. Olaniyi¹ and D.S. Okafor¹

¹Department of Marine Sciences, University of Lagos, Akoka, Lagos State- Nigeria

²Department of Fisheries and Aquaculture, Bayero University, Kano, Kano State- Nigeria

Date Received: 29-05-2021

Date Accepted: 11-09-2021

DOI: <https://doi.org/10.48198/NJPAS/21.B01>

ABSTRACT

The biological response to chemical pollutants reflects the acid–base status of an aquatic ecosystem. The gercacinid, *Cardiosoma armatum* (75±0.1 g) was exposed to acidified waters to evaluate the effects on its immune parameters. The crabs were exposed to pH 4.0, 5.0, 6.0 and 7.8 (control) for 28 days. The hematological parameters of control crabs and crabs exposed to varied doses of acidified water indicated a marked reduction. Significant ($p < 0.05$) higher alkaline phosphatase and albumen were obtained in pH 4.0, 5.0 and 6.0 compared to control; other values were mostly similar to control. The highest superoxide dismutase (SOD) (252.61±0.06 min/mg pro) was recorded in control group, while highest CAT activity (2.08±0.16 min/mg protein) was recorded in crabs exposed to pH 4 treatment. Furthermore, the control group's SOD activity was significantly higher than the exposed groups. With a lower pH, the quantities of malondialdehyde increased substantially and were significantly different from the control group. While these findings demonstrate that changes in pH have limited impact on energy use, decreasing immune system conditions show that *C. armatum* is susceptible to pH variations and may be influenced in aquaculture, where a pH drop is more prominent.

Keywords: Crab, Oxidative stress, pH, Sulphur oxides, Serum.

Introduction

The phrase "acid rain" is used in environmental research to describe the mixing of acid contaminants with rainwater (Wang *et al.*, 2020). The mixing raises the acidity of rainwater by formation of acid following chemical reactions involving pollutant gases and water. Acid rain has been detected in Nigeria's industrialized areas, and its harmful effects are wreaking havoc on our ecosystem (Osu and Ekpo, 2013). Gas flaring is one of the primary causes of acid rain in these

areas. According to Nwaugo *et al.* (2006), gas flaring is the controlled burning of natural gases connected with oil production. These gases are then transformed to acid gas, which is then deposited in the form of rain. When any type of precipitation removes acids from the atmosphere and distributes them to the environment, this is known as wet deposition of acids. The chemical and pH balances in surface and domestic waters are immediately affected by acid rain. Acid neutralizing capacity (ANC) is a regularly used

Corresponding Author: R.O. Moruf

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

Phone: +2348022429983; Email: tunjimoruf@gmail.com



chemical indicator of surface water acidification for the estimation of critical loads because it provides a chemically and physiologically relevant robust assessment of the acid–base condition of fluids (Wu and Driscoll, 2009).

According to current knowledge, a critical load is a measurable estimate of an exposure to one or more pollutants, such as sulfate (SO_4^{2-}), nitrate (NO_3^-) and ammonium (NH_4^+), below which major negative effects on specified sensitive ecosystem elements do not occur (Burns *et al.*, 2008). The response of biological indicators to chemical pollutants reflects the acid–base state of an aquatic ecosystem. More importantly, biological indicators represent resources that resonate with the public. Fish and total zooplankton species richness decrease with decreases in ANC on the basis of spatial surveys (Lovett *et al.*, 2009).

Acid deposition can affect crabs, zooplankton and other biotic communities of aquatic ecosystems. The gercacinid (*Cardiosoma armatum*) is an effective sentinel, due to its biological and ecological characteristics (Lawal-Are *et al.*, 2019a). Similar to all other crabs, this species possesses a series of gills where the part of the carapace covering the gills is inflated and equipped with blood vessels. This organ extracts oxygen from the air, analogous to the vertebrate lungs (Rosenberg, 2001).

The effect of low pH on crabs has been documented by various authors (Meseck *et al.*, 2016; Tomasetti *et al.*, 2018). However, limited information is available on the effects of acid rain on haematological modulation in crabs. This research was therefore carried out to examine the effects of water acidification on the hematology, serum biochemistry and antioxidant enzyme activities of the gercacinid, *C. armatum*.

Materials and Methods

Experimental setup

Ninety-six (96) samples of active *C. armatum* (75 ± 0.1 g in weight) were obtained from the

mangrove area of University of Lagos Lagoon front (latitude $6^\circ 26' \text{N}$ and $6^\circ 39' \text{N}$ and longitude $3^\circ 39' \text{E}$ and $3^\circ 50' \text{E}$), western axis of the Lagos Lagoon (Moruf and Lawal-Are, 2015). The experiment was carried out at the Department of Marine Sciences of University of Lagos, Nigeria. The crabs were selected and randomly stocked into eight small plastic tank (length \times width \times depth = 8 m \times 8 m \times 1.5 m) at a 4 crabs per tank. They were first acclimatized under laboratory setting for 7 days before the start of the experiment. They were fed with trash fish (*Sardinella aurita*) once daily, the amount of which was approximately 2.2 % of the total weight of crabs held in the tank. However, feeding was terminated 24h prior to the range-finding toxicity test.

Bioassay procedure

Water was acidified using the same methods as Meseck *et al.* (2016). 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 pH at 4.0, 5.0, 6.0 and 7.8 (control) in triplicates with 5 L of lagoon water. The exposure period lasted for 28 days during which each plastic container were well aerated.

Analytical procedures

Crab haemolymph was drawn with a 23G Syringe 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 (THC) of haemocyte population were determined using an improved Neubauer haemocytometer according to methods described by Blaxhall and Daisley (1973). One of the aliquots of the haemolymph of individual crabs was transferred into the haemocytometer and

counted manually. Haemocyte morphotypes were identified and a total number of 100 cells from each slide were counted. The percentage of each counted cell type was calculated and multiplied by total haemocyte population count to obtain absolute count. The serum was assayed for transaminases such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and the phosphatase alkaline phosphatase (ALP) activities according to methods described by Coles (1986). Samples of excised muscle tissues of crabs stored at -20°C were later thawed and homogenized for the assays of reduced glutathione, catalase, superoxide dismutase and levels of proteins following the protocol described by Lushchaks *et al.* (2005) and Bertholdo-Vargas *et al.* (2009). Excised muscle tissues of the crabs stored at -20°C were later thawed and homogenized for the assays of SOD, CAT, MDA, and GSH following the protocol described by Lushchaks *et al.* (2005). Protein was determined spectrophotometrically using the Bio-Rad DC protein assay kit (Richmond, CA, USA) with bovine serum albumin (BSA) as a standard.

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.

Results

Hematological profile

Changes in hematological parameter such as haemoglobin (Hb), packed cell volume (PCV), total haemocyte count (THC), haemocyte sedimentation rate (HSR) and the haemocyte subpopulations in *C. armatum* exposed to varying levels of acidified water were presented in Table 1. The hematological analysis revealed a significant reduction ($P < 0.05$) in haemoglobin

from 1.80 ± 0.36 g/dl in the control crabs to 0.89 ± 0.16 g/dl in the pH 4 treatment. However, a significant increase was recorded in agranulocyte from 70.00 ± 1.06 % in control to 80.00 ± 0.4 % in the lowest pH treatment. There was no significant difference in HSR among the treatments while the lowest rate (2.00 ± 0.25 mm/hr) was observed in the treatment with the lowest pH.

Serum biochemical profile

In the serum biochemical profile of *C. armatum* exposed to varying levels of acidified water, significantly ($p < 0.05$) higher Alkaline phosphatase (ALP) and Albumen (ALB) values were obtained in pH 4.0, 5.0 and 6.0 compared to control (pH 7.8), other values were mostly similar to control (Table 2). Aspartate aminotransferase (AST) mean value in control group was recorded to be $2.39 \pm 0.31 \mu\text{I}^{-1}$ and decrease with decreasing pH.

Antioxidant Enzyme Activity

The antioxidant enzyme activity of *C. armatum* exposed to varying levels of acidified water can be seen in Table 3. Protein levels showed no significant difference among the treatments. The highest superoxide dismutase (SOD) (252.61 ± 0.06 min/mg pro) was recorded in control group, while highest CAT activity (2.08 ± 0.16 min/mg pro) was recorded in crabs exposed to pH 4 treatment. Furthermore, SOD activity in the control group was significantly higher than that of exposure groups. The Malondialdehyde (MDA) concentrations showed an appreciable increase with decreasing pH and were significantly different ($P < 0.05$) from the control group. The MDA content increased to 10.67 ± 0.12 (10.55-10.90), 10.63 ± 0.01 (10.62-10.64) and 9.35 ± 0.01 (9.33-9.37) in pH 4.0, pH 5.0 and pH 6.0 respectively. In contrast, there was an increase in glutathione (GSH) level in exposed crab groups (4.93 - $7.79 \mu\text{mol/ml}$) when compared to the control ($5.00 \pm 0.13 \mu\text{mol/ml}$).

Table 1: Hematological response of *Cardiosoma armatum* to water acidification

Parameters	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
Haemoglobin (g/dl)	(0.69-1.22) 0.89±0.16 ^b	(0.99-1.01) 1.00±0.01 ^b	(0.88-0.93) 0.90±0.02 ^b	(1.08-2.20) 1.80±0.36 ^a
Packed Cell Volume (%)	(2.90-3.11) 3.00±0.06 ^b	(2.99-3.01) 3.00±0.01 ^b	(2.99-3.02) 3.00±0.01 ^b	(3.38-4.40) 4.00±0.31 ^a
Total Haemocyte Count (mm³)	(1.98-3.60) 2.90±0.48 ^b	(3.07-3.94) 3.50±0.25 ^b	(4.19-4.72) 4.40±0.16 ^a	(4.58-5.00) 4.80±0.12 ^a
Haemocyte Sedimentation Rate (mm/hr)	(1.58-2.46) 2.00±0.25 ^a	(2.23-3.15) 3.00±0.4 ^a	(2.88-3.03) 3.00±0.12 ^a	(2.89-3.20) 3.01±0.06 ^a
Granulocyte (%)	(17.88-18.10) 18.00±0.06 ^b	(19.99-20.02) 20.00±0.01 ^b	(20.6-21.40) 21.00±0.23 ^b	(25.96-26.03) 26.00±0.02 ^a
Agranulocyte (%)	(79.23-80.55) 80.00±0.4 ^b	(75.58-76.46) 76.00±0.25 ^b	(75.9-76.10) 76.00±0.06 ^b	(67.89-71.10) 70.00±1.06 ^a
Monocyte (%)	(1.87-2.12) 2.00±0.07 ^b	(2.10-4.00) 3.00±0.55 ^b	(3.60-4.50) 4.00±0.26 ^a	(3.87-4.12) 4.00±0.07 ^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 *Cardiosoma armatum* to water acidification

Parameters	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
PRO(gl⁻¹)	(28.28-30.81) 28.49±1.16 ^a	(28.59-31.61) 29.6±0.01 ^a	(32.19-32.24) 32.21±0.02 ^a	(20.40-32.44) 27.12±3.55 ^a
AST(μl⁻¹)	(1.99-2.20) 2.09±0.06 ^a	(2.13-2.15) 2.14±0.01 ^a	(2.42-2.45) 2.33±0.01 ^a	(1.77-2.79) 2.39±0.31 ^a
ALT(μl⁻¹)	(7.15-7.68) 7.36±0.16 ^a	(5.22-8.36) 7.45±0.97 ^a	(7.17-8.04) 7.60±0.25 ^a	(6.92-7.34) 7.14±0.12 ^a
ALP(μl⁻¹)	(88.20-88.55) 88.32±0.12 ^a	(79.79-91.11) 85.56±3.27 ^a	(76.86-77.74) 77.28±0.25 ^a	(68.89-69.10) 69.00±0.06 ^b
ALB (gl⁻¹)	(2.35-2.38) 2.36±0.01 ^a	(3.15-3.37) 3.27±0.06 ^a	(3.51-4.31) 3.91±0.23 ^a	(1.56-1.63) 1.60±1.06 ^b

Keys: Keys: AST- Aspartate Aminotransferase, ALT- Alanine Aminotransferase, ALP - Alkaline phosphatase, ALB-Albumen. Range in bracket; mean±standard error; values with different superscripts across row are significantly different at (P < 0.05)

Table 3: Antioxidant enzymes activity of *Cardiosoma armatum* exposed to water acidification

Antioxidant enzymes	pH 4.0	pH 5.0	pH 6.0	pH 7.8 (Control)
PRO(g/L)	(25.28-28.81) 26.49±1.16 ^a	(26.49-29.7) 28.6±1.06 ^a	(31.21-33.31) 32.21±0.61 ^a	(20.4-32.44) 27.12±3.55 ^a
SOD (min/mg protein)	(209.34-212.18) 210.96±0.84 ^a	(240.89-245.79) 242.79±1.52 ^a	(249.95-250.20) 250.08±1.6 ^a	(252.51-252.72) 252.61±0.06 ^b
CAT (min/mg protein)	(1.87-2.40) 2.08±0.16 ^a	(1.37-1.79) 1.59±0.12 ^a	(1.39-1.44) 1.41±0.02 ^a	(1.34-1.36) 1.35±0.01 ^a
MDA (nmol/ml)	(10.55-10.90) 10.67±0.12 ^a	(10.62-10.64) 10.63±0.01 ^a	(9.33-9.37) 9.35±0.01 ^a	(8.04-8.25) 8.15±0.06 ^b
GSH(μmol/ml)	(4.03-4.06) 4.04±0.01 ^a	(5.43-8.17) 5.45±0.97 ^a	(5.88-8.33) 5.89±1.30 ^a	(6.08-6.15) 6.12±0.02 ^a

Keys: Range in bracket; mean±standard error; values with different superscripts across row are significantly different at (P < 0.05)

Discussion

Acidification has altered surface water chemistry, causing decreases in pH and increases in concentrations of inorganic monomeric pollutants, which could adversely impact sensitive species of fish and zooplankton (Driscoll *et al.*, 2001; Zhou *et al.*, 2015). Mustapha and Atolagbe, (2018) reported that organisms exhibit survival and behavioural responses to concentrated sulphuric acid when used for acidification. This elicits the potency and sensitivity of the gercacinid, *C. armatum* to the test chemical in this present study. The result of hematological parameters showed marked reduction between control crabs and the crabs exposed to different concentrations of pH, which is an indication of the deleterious effects of the chemical pollutant to the body fluid of *C. armatum*. According to Chen *et al.* (2015), simulated acid rain increased the proportion of heterotrophic respiration and had significant consequences, which could lead to a variety of physiological dysfunctions in organisms.

Low pH is particularly harmful to crustaceans, with pelagic larvae being the most vulnerable life stage (Tomasetti *et al.*, 2018). In the work of Rosenberg (2001), when crabs were exposed to lower pH, juvenile growth was slower, calcification was lower, and survival was lower compare to adult crabs. During the current study, significantly ($p < 0.05$) higher ALP and ALB were obtained in acidified water treatment crabs compared to control, other values were mostly similar to control. According to Sanni *et al.* (2020), 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. Serum albumin is the main protein of plasma; its main function is the regulation of the colloidal osmotic pressure of blood. Variations in serum parameters have been attributed to responses to a changed physiological and energetic

requirements and may be an early warning sign of stress before population declines are observed (Moruf and Lawal-Are, 2018; Okunade *et al.*, 2020). Melzner *et al.* (2016) 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 (2007) reported that Tanner Crab (*Chionoecetes tanneri*) exhibited an extracellular, compensatory response to extreme external acidification (7.08). The pH reported here is slightly higher than most marine crustaceans, but the pH is not unusual for marine organisms.

Most environmental factors (such as salinity, pH and dissolved oxygen) have been shown to influence blood protein concentration in crustaceans via influencing appetite and feeding behavior rather than directly affecting serum protein, thus higher serum protein concentrations have been linked to higher live wet weight and diet quality (Jesuniyi *et al.*, 2020). SOD is the first antioxidant enzyme, which scavenges superoxide radicals (Ugwu *et al.*, 2021). In this study, decrease in SOD activity were observed in *C. armatum* following exposure to acidified water. The decrease in superoxide anions and SOD activity were considered to result from the decreased activity of NADPH-oxidase responsible for the production of superoxide anions (Li and Chen, 2008). The greater activity of CAT in exposure group could be attributed to lesser production of superoxide anion radical. In the current study, MDA contents increased gradually as sulphuric acid concentrations decreased in crab. According to Lawal-Are *et al.* (2019b), MDA is a biomarker of oxidative damage that reflects the state of lipid peroxidation of the membranes of many living organisms. GSH anchors a vital role in the scavenging of cellular radical oxygen species as part of the cellular first layer defense system that

shields organisms from oxidative stress. An increase 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. According to Jacobo *et al.* (2016), when crabs are subjected to various stressors, their energy expenditure increases, resulting in an increase in metabolic rate to fuel the homeostatic regulation process.

Conclusion

The gecarcinid, *Cardisoma armatum* exhibits immune defense responses to concentrated sulphuric acid when used for acidification. This elicits the potency and sensitivity of the crab species to the test chemical. The result of hematological parameters showed marked reduction between control crabs and the crabs exposed to different concentrations of acidified water, which is an indication of the deleterious effects of the chemical pollutant to the body fluid of *C. armatum*.

Competing Interest

The authors declare no competing interests

References

- Bertholdo-Vargas, L.R., Martins, J.N., Bordin, D., Salvador, M., Schafer, A.L., Barros, N.M., Barbieri, L., Stirpe, F. and Carlini, C.R. (2009). Type 1 ribosome-inactivating proteinsentomotoxic, oxidative and genotoxic action on *Anticarsia gemmatalis* (Hubner) and *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). *Journal of Insect Physiology*, 55(1), 51-58.
- Blaxhall, P.C. and Daisley, K.W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, 5, 771-781.
- Burns, D.A., Blett, T., Haeuber, R. and Pardo, L. (2008). Critical loads as a policy tool for protecting ecosystems from the effects of air pollutants. *Front. Ecol. Environ.*, 6, 156–159.
- Chen, S., Zhang, X., Liu, Y., Hu, Z., Shen, X. and Ren, J. (2015). Simulated acid rain changed the proportion of heterotrophic respiration in soil respiration in a subtropical secondary forest. *Appl Soil Ecol.*, 86,148–157.
- Coles, E. H. (1986). *Veterinary Clinical Pathology*. W.B. Saunders, Philadelphia, PA, USA, 1-42.
- Driscoll, C.T., Lawrence, G.B., Bulger, A.J., Butler, T.J., Cronan, C.S., Eagar, C., Lambert, K., Likens, G.E., Stoddard, J.L. and Weathers, K.C. (2001). Acidic deposition in the northeastern United States: sources and inputs, ecosystem effects, and management strategies. *Bioscience*, 51, 180–198.
- Jacobo, L.L., Diaz, F., Re, A.D., Galindo-Sanchez, C.E., Sanchez-Lizarraga, A.L., Nunez- Moreno, L.A. and Moreno-Sierra, D. (2016). Physiological responses of the red rocky crab, *Cancer antennarius*, exposed to different concentrations of copper sulphate. *Revista de Biología Marina y Oceanografía*, 51 (2), 327–336.
- Jesuniyi, I.F., Moruf, R. O. and Lawal-Are, A.O. (2020). Immunomodulatory effect of *Moringa oleifera* Lam. aqueous extract on the burrowing crab, *Cardiosoma guanhumi* (Latreille, 1828). *Nigerian Veterinary Journal*, 41 (3), 264 – 273.
- Lawal-Are, A.O., Moruf, R.O., Akubueze, K.L. and Adewole, O. (2019a). Morphometric characteristics of two walking crabs, *Cardiosoma armatum* (Gecarcinidae) and *Goniopsis pelii* (Graspidae) from a tropical mangrove swamp in Lagos, Nigeria.

Nigerian Journal of Agriculture, Food and Environment, 15(1), 83-88.

Lawal-Are, A.O., Moruf, R.O., Oluseye-Are, S.O. and Isola, T.O. (2019b). Antioxidant defense system alternations in four crab species as a bio-Indicator of environmental contamination. *Bulletin UASVM Veterinary Medicine*, 76(1), 73-80.

Li, C.C. and Chen, J. C. (2008). The immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus* under low and high pH stress. *Fish & shellfish immunology*, 25(6), 701-709.

Lovett, G.M., Tear, T.H., Evers, D.C., Findlay, S.E.G., Cosby, B.J., Dunscomb, J.K., Driscoll, C.T. and Weathers, K.C. (2009). Effects of air pollution on ecosystems and biological diversity in the eastern United States. *Ann. N. Y. Acad. Sci.*, 1162, 99–135.

Lushchak, V. I., Bagnyukova, T. V., Huska, V.V., Luzhna, L. I., Lushchak, O.V. and Storey, K. B. (2005). Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in gold tissues. *International Journal of Biochemistry and Cell biology*, 37(8), 1670-1680.

Meseck, S.L., Alix, J.H., Swiney, K.M., Long, W.C., Wikfors, G.H. and Foy, R.J. (2016). Ocean acidification affects hemocyte physiology in the Tanner crab (*Chionoecetes bairdi*). *PloS one*, 11(2), e0148477.

Moruf, R.O. and Lawal-Are, A.O. (2015). Growth pattern, whorl and girth relationship of the periwinkle, *Tympanotonus fuscatus* var *radula* (Linnaeus, 1758) from a tropical estuarine lagoon, Lagos, Nigeria. *International*

Journal of Fisheries and Aquatic Studies, 3 (1), 111-115.

Moruf, R.O. and Lawal-Are, A.O. (2018). Haemato-Biochemical variations in estuarine crabs from a Lagoon ecosystem. *Journal of Applied Sciences and Environmental Management*, 22(12), 1899–19033.

Mustapha, M.K. and Atolagbe, S.D. (2018). Tolerance level of different life stages of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) to low pH and acidified waters. *The Journal of Basic and Applied Zoology*, 79(1), 1-6.

Nwaugo, V.O., Onyeagba, R.A. and Nwahunkwu, N.C. (2006). Effect of gas flaring on soil microbial spectrum in parts. *African journal of Biotechnology*, 5(19), 1824- 1826.

Okunade, G.F, Lawal, M.O., Uwadiae, R.E. and Moruf, R. O. (2020). Baseline serum biochemical profile of *Pachymelania fusca* (Gastropoda: Melanidae) from two tropical lagoon ecosystems. *African Journal of Agriculture, Technology and Environment*, 9(2), 141-149.

Osu, S.R. and Ekpo, M.O. (2013). Acid rain and environmental problems: Implications for the teaching of biology in schools in riverine communities. *Academic Journal of Interdisciplinary Studies*, 2(12): 101-108

Pane, E.F and Barry, J.P. (2007). Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Mar Ecol Prog Ser.* 334, 1–9.

Rosenberg, R. (2001). Marine benthic faunal successional stages and related sedimentary activity. *Scientia Marina*, 65(2), 107-119.

- Sanni, Z.A., Moruf, R.O. and Lawal-Are, A.O. (2020). Hemato-biochemical profiling of a burrowing crab exposed to polystyrene microplastic contaminant. *Federal University of Dutsin-Ma Journal of Science*, 4(2), 380-385.
- Tomasetti, S.J., Morrell, B.K., Merlo, L.R. and Gobler, C.J. (2018). Individual and combined effects of low dissolved oxygen and low pH on survival of early stage larval blue crabs, *Callinectes sapidus*. *PLoS one*, 13(12), e0208629.
- Ugwu, C.C., Moruf, R.O. and Lawal-Are, A.O. (2021). Sublethal effects of organophosphate chlorpyrifos on hemato-immunological parameters of the gercacinid crab, *Cardiosoma armatum* (Herklots, 1851). *Bio-Research*, 19 (1), 1185-1191.
- Wang, M.H.S., Wang, L.K. and Shammas, N.K. (2020). Glossary of acid rain management and environmental protection. *In Handbook of environment and waste management: Acid rain and greenhouse gas pollution control* (pp. 719-749).
- Wu, W. and Driscoll, C.T. (2009). Application of the PnET-BGC – an integrated biogeochemical model – to assess the surface water ANC recovery in the Adirondack region of New York under three multi-pollutant proposals. *Journal of Hydrology*, 378, 299–312.
- Zhou, Q., Driscoll, C.T. and Sullivan, T.J. (2015). Responses of 20 lake-watersheds in the Adirondack region of New York to historical and potential future acidic deposition. *Science of the Total Environment*, 511, 186-194.