Original Research Article

Journal of Biological Research & Biotechnology

Bio-Research Vol. 20 No.2; pp. 1546-1551 (2022). ISSN (print):1596-7409; eISSN (online):2705-3822

Growth and immunological profile of gercacinid crab at different sublethal salinity regimes

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Abstract

Salinity is the most variable ecological parameter in the lagoon with daily and seasonal variations. The changes in growth, serum biochemistry and antioxidant enzymes activities of *Cardisoma armatum* were examined during 84-day exposure to five different sublethal salinities (0, 5, 10, 15 and 20 ppt). At the start of the trial, the crab initial average body weight was not significantly different ($P > 0.05$). The highest weight gain was recorded in 15 ppt (47.40±1.01 g), followed by 20 ppt, 10 ppt and 5 ppt with no significant differences between them. At the end of 84 days experiment, the crab exhibited the lowest body weight growth (48.29 %) at 0 ppt and the highest (81.04±1.08%) at 10 ppt. The 10 ppt treatment had the highest specific growth rates (0.31±0.11 %/day), followed by 5 ppt, 20 ppt, 15 ppt, and then 0 ppt treatments. Aspartate and alanine aminotransferases significantly decreased after 0 ppt, but mean serum protein value increased with salinity increase. Greater activities of catalase, superoxide dismutase and malondialdehyde were recorded in 5-20 ppt. All the antioxidant enzyme activities (with the exception of glutathione) showed significant differences. Thus, deviation from the brackish water condition adversely affects the growth and immune functions of the gecarcinid, resulting in population decline in reclaimed wetlands.

Keywords: Antioxidant Enzymes, Biochemical, Crab, Immune Response, Salinity.

Received March 18, 2022**; Revised** June 4, 2022**; Accepted** June 5, 2022

https://dx.doi.org/10.4314/br.v20i2.5
Journal Homepage: **http://www.bioresearch.com.ng.**
Publisher***: Faculty of Biological Sciences, University of Nigeria, Nsukka, Nigeria.***

INTRODUCTION

One of the most elusive crab species around the brackish water environment in Nigeria is the gecarcinid (*Cardisoma armatun*, Herklots, 1851), which inhabits almost every microhabitat in the mangrove ecosystem (Moruf *et al*., 2021). In the Lagos Lagoon mangrove swamps, *Cardisoma sp.* coexists with graspid crabs, which are gathered by locals for domestic markets. The Gercacinid is an effective sentinel due to its biological and ecological characteristics (Sanni *et al*., 2020; Lawal-Are *et al*., 2021). *C. armatun* is an euryhaline species that can survive in both freshwater and brackish environment, making it a powerful osmoregulator. This is due to the presence of a well-developed osmoregulatory system (Jia *et al*., 2012). Meanwhile, salinity change can affect

osmoregulation in aquatic species by altering the osmotic pressure between medium and body fluid. According to Long *et al*. (2017), the activity of immune enzymes in crustaceans is altered in response to salt stress. Thus, crab immune systems may be affected by salinity adaptation.

Many aquatic organisms are affected by salinity, which is one of the most critical environmental factors impacting their survival, growth and dispersal (Chand *et al*., 2015). Salinity changes have a profound impact on an ecosystem biodiversity and functioning (Carrasco and Perissinotto, 2012; Thabet *et al*., 2017). Osmoregulation during salinity acclimation may cause alterations in digestive enzyme activity in euryhaline aquatic species (Vargas-Chacoff *et al*., 2015). A possible explanation is that aquatic creatures' metabolisms reorganize to meet the increased energy demands caused by changes in salinity (Wang *et al*., 2013). Crustaceans face death if they do not have optimal biochemical and physiological adaptations, and species may go extinct if they do not have physiological acclimatization or genetic evolution (Reusch, 2014; Sara´ *et al*., 2014; Thabet *et al*., 2017). As a result, crabs evolved unique adaptations to cope with salt stress by evolving several molecular, physiological, ecological and behavioral responses (Gama *et al*., 2011). Salinity levels that are optimal for growth, survival and productivity are often species-specific (Chand *et al*., 2015). As a result, determining the optimal salinity level for commercial crab species in a suggested culture system where salinity may be adjusted to suit the species. Crab survival, growth and immunological responses have all been studied in relation to short-term salinity acclimation or rapid salinity shifts (Lawal-Are and Kusemiju, 2010; Long *et al*., 2017; Lv *et al*., 2022). In Nigeria, however, there is dearth of information on the effects of high salinity on adult gercacinid crab. Hence, the present research aimed to examine salinity effect on growth and immunological profile of the gercacinid, *C. armatum* from mangrove swamp of the Lagos Lagoon.

MATERIALS AND METHODS

Experimental procedure

A total of forty-six (46) samples of active *C. armatum* (68±0.6g average weight) were obtained from the mangrove swamp of Lagos Lagoon with Latitude 6°26°N and Longitude 3°39°E. The experiment was carried out at the Department of Marine Sciences of University of Lagos, Nigeria. The crabs were selected and randomly stocked into each of the four 5 litres bowls having different salinity regimes at a 4 crabs per bowl. Dilution of brackish water collected from the Lagos Lagoon with freshwater supplied from a borehole yielded the varied salinity levels. The salinity of the various bowls was determined using a refractometer after serial dilution of water to 5, 10, 15 and 20 ppt. To reduce the influence of direct sunshine, black colored holding bowls were used for the experiment.

Each of the experimental bowls had its water temperature, dissolved oxygen, and pH monitored using digital water analysis instrument (HANNA, HI 9828, Germany). Crabs were fed trash fish (*Sardinella aurita*) once a day, which accounted for 2.2 % of the total weight of the crabs in the bowl. The feeding was stopped 24 hours before the range-finding test. Every 48 hours or when needed, the water in each bowl was changed. The experiment lasted 12 weeks and was replicated. The weight gained was monitored fortnightly and average daily growth (ADG), body weight gain (BWG) and specific growth rate (SGR) were calculated according to Brown (1957) and Hopkins (1992) using the below formulae:

$$\mathrm{ADG} = \frac{(\mathrm{Wf} - \mathrm{Wi})}{t} \quad (1)$$

$$\mathrm{BWG} = \frac{(\mathrm{Wf} - \mathrm{Wi})}{Wi} \mathrm{X}\ 100 \quad (2)$$

$$\mathrm{SGR} = \frac{(\mathrm{LnWf} - \mathrm{LnWt})}{t} \mathrm{X}\ 100 \quad (3)$$

Where Wf = final wet weight,
Wi= initial wet weight
t= duration in day
Ln= natural log of individual wet weight.

Analytical procedure

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 1 mL 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 Neubaeur heamocytometer 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 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 catalase (CAT), malondialdehyde (MDA), glutathione (GSH), superoxide dismutase

(SOD) and levels of proteins following the protocol described by Lushchaks *et al.* (2005) and Bertholdo-Vargas *et al.* (2009).

Statistical analysis

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

RESULTS AND DISCUSSION

Growth at different sublethal salinities

The growth performance of gercacinid at different sublethal salinity regimes is presented in Table 1. The crabs initial average body weight was not significantly different ($P > 0.05$) at the commencement of the experiment. Highest weight gain was recorded in 15 ppt (47.40±1.01 g), then 20 ppt, 10 ppt and 5 ppt with no significant differences between them. The 0 ppt group had the lowest weight gain (26.90±0.23 g), which was statistically different ($P < 0.05$) from the other treatments. The highest average daily growth of 0.56±1.06 g was obtained at the 15 ppt salinity. The lowest body weight growth (48.29 %) was recorded at 0 ppt while the highest (81.04±1.08 %) at10 ppt. Furthermore, highest specific growth rate of 0.31±0.11 %/day was recorded in 10 ppt treatment, then 5 ppt, 20 ppt, 15 ppt and 0 ppt with no significant difference. In agreement to the present study, Chand *et al*. (2015) reported the lowest final average weight for prawn at 20 ppt salinity and highest at 10 ppt. Similarly, Lawal-Are and Kusemiju (2010) reported highest specific growth rate (1.98 %/day) for the Lagoon Crab (*Callinectes amnicola*) in 15 ppt group while the lowest (-0.28 %/day) in 35 ppt group.

Table 1: Mean Growth Performance of *Cardiosoma armatum* at Different Salinity Levels

Growth	Salinity (ppt) concentration				
	0	5	10	15	20
Initial weight (g)	55.70±0.05^{a}	47.80±0.03^{a}	50.10±0.05^{a}	71.70±0.12^{a}	62.20±0.15^{a}
Final weight (g)	82.60±0.15^{a}	83.30±0.05^{a}	90.70±0.07^{b}	119.10±0.03^{b}	105.40±0.27^{b}
Weight gain per crab (g)	26.90±0.23^{a}	35.50±1.05^{b}	40.60±0.26^{b}	47.40±1.01^{b}	43.20±2.06^{b}
Average daily growth (g)	0.32±0.05^{a}	0.42±2.03^{a}	0.48±0.06^{a}	0.56±1.06^{a}	0.51±1.06^{a}
Body weight gain (%)	48.29±0.12^{a}	74.27±0.24^{b}	81.04±1.08^{b}	66.11±0.15^{b}	69.45±0.55^{b}
Specific Growth Rate (%/day)	0.20±0.70^{a}	0.29±0.23^{a}	0.31±0.11^{a}	0.26±0.52^{a}	0.27±0.13^{a}

Immunological profile of gercacinid at different sublethal salinities

Table 2 shows serum biochemical profile obtained from *C. armatum* on the 84th day at different sublethal salinities. AST and ALT significantly decreased after 0 ppt treatment while mean serum protein value increased as salinity increases without significant difference. Highest value of AST (81.88±30.73 μl^{-1}), ALT (37.68±2.83 μl^{-1}) and ALP (76.73±5.31 μl^{-1}) were obtained at 0 ppt (freshwater condition) while the lowest values of 35.23±0.51 μl^{-1}, 16.34±0.42 μl^{-1} and 56.32±1.22 μl^{-1} were recorded at 5 ppt for AST, ALT and ALP respectively. Higher AST in the freshwater condition (0 ppt) compared to the brackish water condition (5-20 ppt) may be due to disruption in the crab immune system which resulted in cell damage or a response mechanism to pollution exposure. ALP is a multi-functional enzyme that plays a role in immunological responses as well as foreign protein, carbohydrate, and lipid breakdown (Sui *et al*., 2016). The present finding is confirmed by Fang *et al*. (2014), who discovered that ALP activity in the gill and kidney of the juvenile tongue sole *Cynoglossus semilaevis* do not change significantly between low and high salinity treatments. Lv *et al*. (2022) predicted that the immune functions of estuarine organisms, such as crab that are adversely affected by reduced salinity could lead to population decline in wetland habitat. According to Long *et al.* (2017), brackish water increases osmolality and ionic concentrations in hemolymph while decreasing the activity of Na^{+} /K^{+}- ATPase and its mRNA expression in adult crab posterior gill. Most environmental factors (such as salinity, pH and dissolved oxygen) are known to impact serum protein level in crustaceans via influencing appetite and feeding behavior than directly affecting serum protein. As a result, higher serum protein concentrations have been associated to increased live wet weight and improved diet quality (Jesuniyi *et al*., 2020).

Table 2: Serum Biochemical Profile of *Cardiosoma armatum* at Different Salinity Levels

Parameters	Salinity (ppt) concentration				
	0	5	10	15	20
PRO(g/l)	30.14±4.62[a]	30.25±2.41[a]	31.12±0.23[a]	33.21±2.11[a]	33.37±4.46[a]
AST (μl^{-1})	81.88±30.73[a]	35.23±0.51[b]	37.12±20.31[b]	40.33±13.02[b]	45.96±9.28[b]
ALT (μl^{-1})	37.68±2.83[a]	16.34±0.42[b]	16.68±2.83[b]	18.11±1.05[b]	35.28±8.30[ab]
ALP (μl^{-1})	76.73±5.31[a]	56.32±1.22[a]	59.01±0.31[a]	64.92±1.90[a]	69.55±14.91[a]

Key: PRO: protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase. Means having different alphabets in the same row are significantly different.

Antioxidant enzyme activity of *C. armatum* exposed to different levels of salinity can be seen in Table 3. The highest values for CAT (8.72±0.52 min/mg pro), SOD (46.20±08.01 min/mg pro), MDA (16.12±0.15 nmol/ml) and GSH (7.72±0.21 µmol/ml) were recorded in 5 ppt group, while the lowest values of 1.27±0.35 min/mg pro, 29.10±39.04 min/mg pro and 8.01±0.83 nmol/ml were recorded at 0 ppt for CAT, SOD and MDA respectively. The 20 ppt group had the lowest mean value of GSH with 2.73±1.14 nmol/ml. Furthermore, the enzyme activities (with exception of GSH) were significantly different between the freshwater condition and the other treatments. The decreased activity of NADPH-oxidase, which is responsible for the formation of superoxide anions, could be linked to the decrease in superoxide anions and SOD activity in freshwater (Li and Chen, 2008). Also, the greater activities of CAT in 5-20 ppt group might be due to lesser production of superoxide anion radical. According to Lawal-Are *et al.* (2019), MDA is an oxidative damage biomarker that represents the condition of lipid peroxidation in membranes of many organisms. As part of the cellular first layer defense system that protects organisms from oxidative stress, GSH anchors a critical function in the scavenging of cellular reactive oxygen species (Ugwu *et al*., 2021). A rise often indicates the commencement of cellular defense mechanisms in response to an increase in free radical concentration in the cell, whereas a reduction may signal an antioxidant defense. When crabs are exposed to various stressors, their energy expenditure increases, resulting in an increase in metabolic rate to fuel the homeostatic control process (Jacobo *et al*., 2016).

Table 3: Antioxidant Enzyme Activity of *Cardiosoma armatum* at Different Salinity Levels

Parameters	Salinity (ppt) concentration				
	0	5	10	15	20
CAT(min/mgprotein)	1.27±0.35[a]	8.72±0.52[b]	7.21±0.11[b]	7.04±0.05[b]	1.76±0.61[a]
SOD(min/mg(protein)	29.10±39.04[a]	46.20±08.01[b]	43.91±07.11[b]	41.87±82.41[b]	39.53±45.02[ab]
MDA (nmol/ml)	8.01±0.83[a]	16.12±0.15[b]	14.24±0.83[b]	13.06±1.02[b]	9.64±0.87[ab]
GSH(µmol/ml)	2.87±1.66[a]	7.72±0.21[a]	6.01±0.16[a]	4.11±2.31[a]	2.73±1.14[a]

Key: CAT: Catalase, MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase. Means having different alphabets in the same row are significantly different.

CONCLUSION

The gercacinid crab, *Cardisoma armatum* naturally occurs in the mangrove wetland of the Lagos Lagoon and has adapted to a continually changing salinity condition. The findings in this study revealed that, aside from initial body weight and average daily growth, brackish water conditions (5-20 ppt) have significantly greater growth parameters than freshwater conditions (0 ppt). Aspartate, alanine aminotransferases and alkaline phosphatase decreased after an increase in salinity at 0 ppt, but mean serum protein value increased with an increase in salinity. Greater activities of catalase, superoxide dismutase and malondialdehyde were recorded in 5-20 ppt. All the antioxidant enzyme activities (with the exception of glutathione) showed significant differences. Thus, deviation from the brackish water condition could alter the growth performance and immune processes of gercacinids.

Conflict of interest

Authors have no conflict of interest to declare

Author contributions

LAO, MRO and AVS: Conceptualization, sampling, and manuscript writing. SAM and AH: Methodology, statistical analysis, and manuscript review. All authors funded the study and approved the final draft of the manuscript.

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