BIOMETRIC, HAEMATOLOGICAL AND BIOCHEMICAL INDICES OF THREE BRACHYURAN CRABS FROM MANGROVE MUDFLATS OF THE LAGOS LAGOON

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ABSTRACT
Over the years, uncontrolled discharges from increasing anthropogenic perturbations has resulted in the contamination of several coastal ecosystems. The study assesses the health status of three brachyuran crab species, cardiosoma armatum, sersama huzardi and goniopsis pelii from mangrove mudflats of the Lagos lagoon. Length weight relationship, haematic and biochemical indices of crab heamolymph were evaluated following standard methods and procedures. the results showed that the mean carapace length of examined crabs ranged from 2.20 to 4.30 cm, 2.30 to 2.60 cm and 1.90 to 2.50 cm while the mean total weight ranged from 29.46 to 97.29g, 18.04 to 35.18g and 22.76 to 39.34g for cardiosoma armatum, sesarma huzardi and goniopsis pelii respectively. Relatively low mean total haemocyte count (2.2× 10^7 cell/ml) was recorded for goniopsis pelii when compared to cardiosoma armatum (4.80x10^7cell/ml) and sesarma huzardi (2.28 x 10^7 cell/ml). the haemocytic subpopulation obtained from all three species was dominated by granulocytes (18, 29 and 51%), hyalinocytes (39, 36 and 23%) and semigranulocytes (49, 32 % and 18 %). Generally, aminotransaminases ast and alt showed lower activities in cardiosoma armatum (19.76 and 38.05ui) as compared to the levels in sesarma huzardi (32.26 and 50.09ui) and goniopsis pelii (51.08 and 54.93ui). The recorded higher aminotransaminase ast and alt levels in sesarma huzardi and goniopsis pelii suggest possible incidence of liver tissue damage in response to habitat condition. This calls for continuous monitoring and effective regulation for the protection of ecosystem and human health in the area.

Keywords: *Haematology, Biochemical profile, Brachyuran crabs, Mangrove mudflats*.

INTRODUCTION

Over the past few decades, increasing anthropogenic perturbations have resulted in the contamination and pollution of several estuarine and coastal ecosystems in many regions of the world. A steady increase in pollutants of priority concern from urban runoffs, agricultural, industrial and domestic wastes have also been recorded in most littoral and inshore coastal waters, wetlands, tidal creeks and mudflats of lagoons adjacent to city centres and metropolis. The uncontrolled discharges from these sources and related human activities have the potential to affect aquatic ecosystem health and the survival of socioeconomically important biota. According to Connon *et al.* (2009), the effects are more often subtle and may ultimately modulate organism's fitness.

Crab, a major food item in the marine, coastal and Lagoon fishery has been shown to occur commonly in West Africa (Lawal-Are and Nwankwo, 2011). Adeogun *et al.* (2015) and Boschi, (2000) observed that most of the ecologically and economically relevant crab species are found throughout the tropical and subtropical regions of the world. Generally, the Brachyuran crabs, true crabs of the infra order Brachyura in the Order Decapoda has been reported as the best known and most intensely studied groups (Josileen, 2015). In the mangrove habitats of the Lagos Lagoon, several crab species are found where they are distributed up to the tidal limit within the Lagoon. The population characteristics (Abowei and George, 2010; Udoh and Nlewadim, 2011), mating behavior and recruitment (Lawal-Are and Kusemiju, 2010) and parasite fauna (Ekanem *et al.*, 2013) of economically important crabs species have been well documented in Nigeria. However, most of the studies in literature focused on taxonomy and distribution, nutritional composition, ecology, morphometrics, food and feeding habits of these crabs (Ezekiel and Bernard, 2014; Adeogun *et al.*, 2015).

Recently, crabs have been used as bioindicators of ecosystems health and evidence of environmental features such as salinity, sediment types and content. Brachyuran crab species have also been used as pollution indicators in most aquatic ecosystems. In a study by Bartolini *et al.* (2009), the behaviors of fiddler crab species *Uca annulipes* and *Uca inversa* strongly affected by domestic sewage dumping was used as bioindicator. Studies have also shown that hemocytes circulating in hemolymph play a key role in the innate immune response of crustaceans as the first line of defense against internal pathogens such as viruses, bacteria and parasites (Bauchau, 1981). Additionally, haematological profiles often used as an index of physiological condition in organisms also provide information about the health status of local populations (Handy and Depledge, 1999; Petri *et al.*, 2006). Earlier, Olakolu *et al.* (2012) observed the induction of oxidative stress in muscle and gills of the blue crabs *Callinectes amnicola* from the Lagos Lagoon. Recently, Adeogun *et al.* (2015), observed a great dependence of the haematological and serum biochemical profile of the blue crab, *Callinectes amnicola* from Lagos and Epe Lagoon in Southwest Nigeria on habitat quality and the organisms' response to the varying habitat conditions. However, most of the studies fail to establish a link with prevailing environmental and habitat conditions. Furthermore, there is limited information on the biochemical and haematological responses or alterations in populations of brachyuran crabs inhabiting contaminated ecosystems in Nigeria. Evaluating the health of the environment using sentinel organisms like crabs can predict the impact of pollutants present in the environment and provide important data for environmental managers and other stakeholders. Such assessments might serve as a good reflection of the ecosystem health and early warning indicators of ecosystem failure. Therefore, this study was carried out to investigate the impact of increasing contaminants on socioeconomically important biota inhabiting the Lagos Lagoon mangrove mudflats.

MATERIALS AND METHODS

Description of Study Sites

The study was carried out in the mangrove mudflats of the Lagos lagoon bordering the University of Lagos, a habitat for diversity of brachyuran crabs (Onadeko *et al.*, 2015). The mangrove mudflat is connected to the Lagos lagoon by tidal creek and exhibit a clear gradient of environmental contamination that increases from the western axis. Three sampling stations (Site A, B and C) were established based on the nature of anthropogenic waste and the procedure of Onadeko *et al.* (2015). Site A located on Latitude 6^0 31.228'N and Longitude 3^0 24.044'E is characterized by a little vegetation and a few trees with some segment extending about 5m into the Lagoon. Site B located on Latitude 6^0 30'N and Longitude 3^0 24'E was a swampy land area on the periphery of Abule-Agege creek while Site C located on Latitude 6^0 31.015'N and Longitude $3^0$23.948'E was also a swampy area.

Collection of Crab Samples and Determination of Biometric Indices

Sixty samples of crabs, *Cardiosoma armatum* (n=20), *Sesarma huzardi* (n=20) and *Goniopsis pelii* (n=20) were caught weekly using traps with bait at night for 5 weeks. The trapped crabs were then hand-picked with protective rubber gloves (Plate 1). The live crab samples were stored in a plastic container and transported to the Aquatic Toxicology and Ecophysiology Laboratory located at the Department of Marine Sciences, University of Lagos for processing and further analysis. The width and length of the crab's carapace was measured using Vernier calliper to the nearest 0.1cm. The width was taken when the carapace was measured dorso-laterally from right side to left side where the 1^{st} walking legs protrudes and at the base of the largest spines. The length was measured along the midline of the shell from the edge of the carapace between the rostrums to the posterior edge of the carapace. In order to determine the condition factor, the relationship between the carapace length and weight of the crab was expressed by the equation given by Parson (1988).

Cardiosoma armatum (Land crab) *Sesarma huzardi* (Purple mangrove crab) *Goniopsis pelii* (Hairy mangrove crab)

Plate 1:-Bracyuran crabs collected from the Lagos Lagoon mudflats.

Collection of Haemolymph from Crabs

Samples of crabs *Cardiosoma armatum, Sesarma huzardi* and *Goniopsis pelii* were anaesthetized on ice for 10 mins and haemolymph were drawn with a 23G syringe from the juncture between the basis of the ischium (the joint connecting the fifth walking leg to the carapace) of the fifth walking leg according the procedure described by Söderhäll and Smith (1983). 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) and transferred into a 5mL lithium heparin bottle kept in an ice chest. The total haemolymph obtained from individual crabs species were used for haematological and biochemical analyses.

Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC) analysis in Crabs

Total haemocyte count (THC) and differential count (DHC) of haemocyte population were determined according to methods described by Blaxhall and Daisley (1973) using an improved Neubaeur haemocytometer. To determine the total hemocyte number per mL (THC), one aliquot of individual crab haemolymph, was examined in hemocytometer chamber. Differential counts were performed on slides prepared with 100 µl of a diluted cell suspension ($3x10^6$ cells). 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 (Celi *et al*., 2013; Adeogun *et al.*, 2015).

Biochemical analysis

Haemolymph samples were centrifuged at 4000rpm for 10mins and 1ml of the supernatant and the serum derived was stored at -20 ^{o}C for further analysis. The serum was assayed for Glucose (GLUC-) PAP, Total protein (TP), Cholesterol (CHOL), and Lactate Dehydrogenase (LDH), Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities according to methods described by Coles (1986). Total protein was determined by the Buiret method, albumin levels were determined with a Boehringer Mannheim's albumin reagent while globulin was determined by subtracting the concentration of albumin from the total protein (Coles, 1986).

Data analysis

Statistical analyses were carried out using SPSS version 10.0. The serum biochemical profile and haematological parameters data were subjected to one-way analysis of variance (ANOVA) between the different sample station means. Significant difference was determined at 5% confidence level ($P<0.05$).

RESULTS

Biometric indices of *Cardiosoma armatum, Sesarma huzardi* and *Goniopsis pelii* from the Lagos Lagoon mudflats

In the present study, the size composition of sixty crabs, *Cardiosoma armatum, Sesarma huzadii* and *Goniopsis pelii* was used as representative samples to evaluate the health status of crabs inhabiting the mangrove mudflats of the Lagos lagoon. There were morphological variations in examined crab species especially in the mean body weight (Table 1). The result obtained for mean body weight of crabs in the order *Cardiosoma armatum* > *Goniopsis pelii* > *Sesarma huzardi* indicated that *Cardiosoma armatum* with mean body weight of 65.98 ± 2.48 g and carapace length of 3.27 ± 0.24 had fairly higher biometric dimensions. *Sesarma huzardi* with mean weight of 28.85 ± 2.02 and carapace length of 2.46 ± 0.13 cm was the smallest when compared to other crab species examined (Table 1). Relatively higher condition factor was also recorded for *Cardiosom armatum* and *Sesarma huzardi* when compared to *Goniopsis pelii* (Table 1).

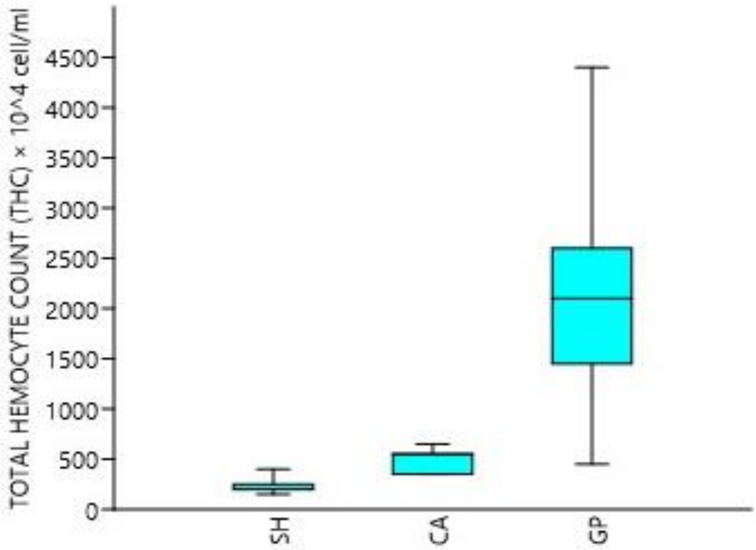
Table 1.Length weight relationship in Bracyuran crabs from the mangrove mudflats of Lagos Lagoon

Species	Body Weight (g)	Carapace Length (cm)	Condition factor (K)
C. armatum	65.98±2.48	3.27±0.24	21.92 ±0.24
G. pelii	33.39±3.45	2.27±0.30	29. 01 ±0.30
S. huzardi	28.85±2.02	2.46±0.13	18. 56 ±0.13

Haematological Indices of Bracyuran Crabs

Total Haemocyte Count (THC) and Differential Haemocyte (DHC) sub-population of *Cardiosoma armatum, Sesarma huzardi* and *Goniopsis pelii*

The total haemocyte counts (THC) of examined crabs was observed in the range of 150.3 ± 0.4 x 10^4 to 400 ± 0.4 x 10^4 cell/ml, 350 ± 0.4 x 10^4 to 650 ± 0.4 x 10^4 cell / ml and 4600 ± 0.4 x 10^4 cell / ml to 450 ± 0.4 x 10^4 for *Sesarma huzardi, Cardiosoma armatum* and *Goniopsis pelli* respectively (Fig. 1). Considerable variation was observed in circulating haemocyte of individual crabs for hyalinocyte, semi granulocyte and granulocyte sub-population. Granulocytes, constituting 38.47% of total haemocytes count was the most abundant haemocyte type recorded while the percentages of semi granulocyte and hyalinocytes were obtained as 24.59% and 36.93% respectively in all examined crab heamolymph during the study. The results also showed that mean total hyalinocytes sub-population was highest in the circulating haemocytes of *Cardiosoma armatum* (43.70 ± 1.01) and lowest in *Goniopsis pelii* (25.90 ±2.21). The semi granulocyte sub- population of crab were also highest in *Cardiosoma armatum* and lowest in *Goniopsis pelii* (13.30 ± 0.83) (Table 2). In contrast, granulocyte population in circulating haemocyte recorded highest mean values in *Goniopsis pelii* (59.30 ± 1.67) and lowest mean values in *Cardiosoma armatum* (21.00±0.80) as shown in Table 2.



SH- *Sesarma huzardi;* CA-*Cardiosoma armatum;* GP- *Goniopsis pelii*

Fig. 1. Total Hemocyte count in Bracyuran Crabs from mangrove mudflats of the Lagos Lagoon

Table 2. Haematological Indices of Bracyuran Crabs from Mangrove mudflats of the Lagos Lagoon

Crab Species	Total count (THC) cells / mL	Hyalinocyte %	Semi granulocytes %	Granulocytes %
Cardiosoma armatum	65.98±2.48	43.70 ±1.01	36.40 ± 1.29	21.00±0.80
Goniopsis pelii	33.39±3.45	25.90 ±2.21	13.30 ± 0.83	59.30 ± 1.67
Sesarma huzardi	28.85±2.02	40.30±0.84	23.50 ± 0.94	34.20 ± 1.57

Biochemical Indices of Bracyuran Crabs from Mangrove Mudflats of the Lagos Lagoon

The results of serum biochemistry of crabs C*ardiosoma armatum, Sesarma huzardii* and *Goniopsis pelii* from Lagos Lagoon is presented in Table 3. Aminotransaminases AST and ALT showed lowest activities in C*ardiosoma armatum* (50.00 ± 1.81 IU and 38.05 ± 0.86 UI) followed by *Sesarm*a *huzardi* (32.26 ±2.19 IU and 50.00 ± 2.88 UI). Relatively higher Aminotransaminases AST (51.08 ± 4.86) and ALT (54.00 ± 5.34) activities were observed in *Goniopsis* *pelii* (Table 3). The phosphatase ALP recorded lowest values in *Sesarma huzardi* (28.82 ± 1.67 IU) when compared to other crabs as shown in Table 3. On the contrary, mean glucose levels were significantly higher ($p < 0.05$) in *Sesarma huzardi* (50.58 ± 3.55 mg/dL) as compared to C*ardiosoma armatum* (42.55 ± 3.11 mg/dL) and *Goniopsis pelii* (36.54 ± 1.70 mg/dL). Lactate dehydrogenase (LDH) levels were generally high in all crabs; however, C*ardiosoma armatum* recorded significantly higher ($p < 0.5$) level than other crabs examined. Mean total protein was higher in *Sesarma huzardi* (17.71 ± 1.16 g/dL) than levels recorded in C*ardiosoma armatum* (17.71 ± 1.16 g/dL) and *Goniopsis pelii* (15.06 ± 1.17g/dL).

However, there was no significant difference within the group.

Table 3. Serum biochemical profile of Bracyuran Crabs *Cardisoma armatum*, *Sesarma huzardi* and *Goniopsis pelii*

Parameters	*Cardiosoma Armatum*	*Sesarma huzardi*	*Goniopsis Pelii*
Aspartate Aminotransferase (AST) IU	*50.00±1.81	32.26 ±2.19	51.08±4.86
Alanine Aminotransferase (ALT) IU	38.05±0.86	50.00±2.88	54.00±5.34
Alanine phosphatase (ALP) IU	52.34±2.54	28.82±1.67	29.64±1.58
Glucose (GLU) mg/100mL	42.55±3.11	50.58±3.55	36.54±1.70
Cholesterol (CHO) mg/dl	14.74±5.40	26.54±0.72	13.56±2.95
Lactate Dehydrogenase (LDH) U/I	271.70±9.40	121.69±14.87	120.61±21.14
Protein (PRO) g/l	17.71±1.16	17.71±1.16	15.06±1.17

*Mean ± SE

DISCUSSION

Over the years, condition factor of species has been used to describe the well-being, corpulence and fatness of the organism (Etim and Taege 1993). Size-frequency measurement is one of the most widely used methods for growth pattern assessment in the wild (Olalekan *et al.*, 2015). The result of biometric indices in this study indicates that, *Sesarma huzardi* was the smallest in terms of size when compared to other crab species examined. Lawal-Are and Nwankwo (2011) previously investigated the size and growth pattern of the hairy mangrove Crab, *Sesarma huzardi* in the Lagos Lagoon mangrove area. From their study, carapace length of examined crabs (1.5 cm to 4.7cm) is slightly higher than the values recorded in present study for the three crab species under consideration. Contrary to this, the maximum carapace length reported by Akin-oriola *et al.* (2005) for *Cardiosoma armatum* (6.0 cm) and *Callinectes pallidus* (7.0 cm), *Cardiosoma* sp from Wouri River Estuary of Douala (Ngo-Massou *et al.*, 2014) as well as the levels reported by Olalekan *et al.* (2015) for the Lagos Lagoon was relatively higher. The maximum size attainable by any fish or shellfish species has been shown to be location specific (King, 1996) as well as incessant factors such as pollution, high fishing pressure and environmental degradation (Abowei and Hart2009).

Haemocytes play a central role in the immune defenses of crustaceans (Söderhäll and Cerenius, 1992; Zhang *et al.*, 2006). Consequently, total and differential hemocyte counts provide a useful way of assessing the physiological state of an animal (Battison *et al.*, 2003). Although there was a considerable variation in the total haemocytes count (THC) recorded for the three crabs species examined in the present study, recorded mean THC for all examined crabs is in accordance with the range reported by Celi *et al.* (2013) and Filiciotto *et al.* (2014) for other crustaceans. Notably, hyalinocytes and semigranulocytes were more represented in the circulating hemolymph of *Cardiosoma armatum* and *Sesarma huzardi than Goniopsis pelii*. Such variations in blood parameters have been attributed to responses to a changed physiological and energetic requirement by most researchers (Kutz *et al.*, 2004, Ochang *et al.*, 2007; Adeogun, 2011). Such variation is also considered to be an early warning sign of stress before the onset of a population decline (Adeogun *et al.*, 2015). Furthermore, the bilateral movement of haemocytes from tissue to haemolymph due to the presence of pathogens can result in an increase in THC according to the reports of Allam *et al.* (2000) and Comesana *et al.* (2012) as cited by Adeogun *et al.* (2015). On the other hand, Amachree *et al.* (2013) attributed such condition to an exposure to contaminants.

The glucose levels of all three species of crabs studied with values ranging from 26.4- 66mg/100mL for *Sersema huzardi*, 27.34 to 45.3 mg/100mL for *Goniopsis pelii* and 24.51 to 53.73 mg/100mL for *Cardiosoma armatum* in our study falls within the range (44.23 - 52.03 mg/100mL) reported for *C. amnicola* from Epe and Lagos Lagoon by Adeogun *et al.* (2015). Aminotransaminases and phosphatases are important diagnostic tools for evaluating tissue damage in organisms (Vijayavel *et al.*, 2006; Adeogun *et al.*, 2012). As such, liver transaminase enzymes (alanine and aspartate transaminase) are frequently used as biomarkers of water contaminants (Kim *et al.,* 2008) and in the diagnosis of damage or metabolic alterations caused by pollutants in fish (De La Torre *et al.,* 2005; Teles *et al.,* 2003). According to Philip and Rajasree, (1996), alteration in the biochemical stress biomarker, ALT allows the identification of damage in several tissues and organs such as liver. De La

The result of the mean values of ALT, AST and ALP in crabs from the present study was within the mean values (42.93±5.92; 54.2±7.76; 42.17±7.99 IU) reported earlier by Adeogun *et al.,* (2015) for *Callinectes amnicola* from some locations on the Lagos Lagoon. There was however a slight variation from the mean values of ALT, AST and ALP (37.37±8.24; 48.43±10.90; 34.93±8.52IU) reported for the blue crab from Epe Lagoon (Adeogun *et al.,* (2015). The higher activities of ALT, AST and ALP in Lagos Lagoon crabs suggest possible incidence of liver tissue damage in the examined crabs. Significantly ($p < 0.05$) higher levels of ALP were also observed in *Cardiosoma armatum*. The relatively higher values of ALT, AST and ALP obtained for *Cardiosoma armatum* might suggest possible incidence of liver tissue damage. Similarly, Torre *et al.* (2005) reported increases in the levels of hepatic transaminases, ALT and AST as biomarkers of polluted waters in the fish *Cnesterodon decemmaculatus*. The high level of alkaline phosphatase (ALP) might depict a blockage in the bile system of the organism (Basten, 2010).

An assessment of protein content in different tissues can also be used as a diagnostic tool for determining the physiological status of an organism (Prasath and Arivoli, 2008). The result of the present investigation shows no significant variation in the total protein content in the examined crab species. However, obtained levels were significantly higher than previously reported values for crabs from Epe and Lagos Lagoon by Adeogun *et al.* (2015). Adeogun *et al.* (2015) attributed observed lower concentration of total serum protein in Lagos Lagoon crabs to increased breakdown of serum peptidic material and modulation of their involvement in various biological processes from environmental stress.

CONCLUSION

The present study has shown that haematological and biochemical characteristics of organisms inhabiting highly impacted ecosystems most often may be modulated in response to prevailing environmental conditions. The hemolymph characteristic of ecologically important sentinel organisms like the mangrove crabs could therefore be useful for health status assessments and evaluation of mangrove habitat quality. Significant variations were observed in blood parameters of the three brachyuran crabs studied. However, the higher activities of ALT, AST and ALP in the crabs suggest possible incidence of liver tissue damage due to contaminant presence and environmental quality. This calls for stringent environmental regulations for the protection of socioeconomically important biota and other ecological receptors.

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