www.ajbrui.net

Afr. J. Biomed. Res. Vol.18 (September, 2015); 233 - 247

Full Length Research Paper

Haematological and Serum Biochemical Profile of the Blue Crab, *Callinectes amnicola* from two Tropical Lagoon Ecosystems

Adeogun A.O*[1], Salami O.A[2] Chukwuka A.V[2] and Alaka O.O[3]

[1]*Hydrobiology and Fisheries Research Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria*
[2] *Ecology and Environmental Biology Unit, Department of Zoology, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Veterinary Pathology, University of Ibadan, Ibadan, Nigeria*

ABSTRACT

Haematology and serum biochemistry of the crab, *Callinectes amnicola* from Epe and Lagos Lagoon in southwest Nigeria were investigated from March –August, 2013. Haemocyte samples were analyzed for haematological and biochemical parameters. The Total Haemocyte count (THC) of *C. amnicola* from Epe and Lagos Lagoon were 3.08±0.52 and 3.19±0.48 x10^7/mL respectively and three types of haemocyte subpopulation were identified viz: hyalinocytes, semigranulocytes and granulocytes. The haemocytic subpopulation of crabs from both sites were dominated by hyalinocytes (71.36±6.42; 64.80±2.54%), granulocytes (19.80±6.45; 26.97±3.85%) and semigranulocytes (8.66±0.86; 8.09±3.44%) and crabs from Lagos Lagoon had a higher granulocyte (26.97%) subpopulation than crabs from Epe Lagoon (19.80%). Hyalinocytes had the smallest cell size (8.95±1.62µm); semigranulocytes were intermediate (13.49±2.37µm) while granulocytes were the largest of all cell types (19.37±2.76µm). Principal component (PCA) biplots showed that dissolved oxygen (R^2=-0.73) and pH (R^2=-0.83) were positively correlated with hyalinocytes (R^2=- 0.88) for Epe Lagoon crabs while granulocytes and THC (R^2=0.47) showed a negative correlation with pH (R^2=-0.80) and DO (R^2=-0.76) for Lagos Lagoon crabs. Albumin (R^2=-0.75) and total protein (R^2=-0.57) showed positive correlation with pH (R^2=-0.84) and DO (R^2=-0.69) for Epe Lagoon crabs while creatinine (R^2=0.68), potassium (R^2=0.93) and sodium (R^2=0.69) showed positive correlation with globulin (R^2=0.81) and negative correlation with pH (R^2=-0.84) and DO (R^2=-0.69) Although the baseline values for *C. amnicola* were provided for these aquatic ecosystems, values show a great dependence on habitat quality and is an indication of organism response to varying habitat conditions.

Key words: haemocytes**,** haematology**,** serum biochemistry, *Callinectes amnicola*, Epe and Lagos Lagoon

INTRODUCTION

Shellfish, a broad description for aquatic invertebrate animals possessing a hard outer covering includes the molluscs i.e. oysters and clams, the crustaceans i.e. shrimps, prawns, lobsters and crabs (Darren *et al*., 2008). Crabs are found throughout the tropical and subtropical regions of the world where they live in a wide range of water bodies, from fast flowing rivers to swamps, as well as in tree holes or caves. The blue crab, *Callinectes* species is an ecologically and economically relevant crustacean with a biogeographic range that includes

*Corresponding author:
E-mail: ainaadeogun@yahoo.com
Tel: +2348023342826

Date Received: December, 2014
Date Accepted:, June, 2015

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

West Africa and tropical South America (Chindah *et al*., 2000; Lawal-Are, 2010; Castejón and Guerao, 2013). Some species provide important food sources for various vertebrates (Darren *et al*., 2008) and are among the most popular types of seafood with the flesh having a sweet delicate flavour and is considered to be a healthy diet choice rich in antioxidants (Oksuz, *et al*., 2009).

Crustaceans are an invertebrate group with the most diverse morphotypes and occur in both freshwater and marine systems (Ruppert and Barnes, 1994). This group like other arthropods have an open vascular system with freely circulating haemocytes in the haemolymph and haemocytes originate from a specialized haematopoietic tissue situated on the dorsal and dorsolateral sides of the stomach surrounded by connective tissue (Matozzo and Marin, 2010). The circulating haemocytes of crustaeceans perform important roles in the host immune response by stimulating the defense system using different mechanisms against various infectious organisms (Söderhäll and Cerenius, 1992). Aside from controlling the invasion of foreign organisms, haemocytes participate in recognition, phagocytosis, melanization, cytotoxicity, modulation, encapsulation and communication between cells (Söderhäll and Cerenius, 1992). They differ from species to species and the magnitude of interspecific variation in the haemocyte population of crustaceans appears to be very wide, ranging from 286 cells/mm^3 (*Astacus fluviatilis*) to 128,000 cells/mm^8, (*Callinectes sapidus)* (Hardy, 1892; Sawyer *et al*., 1970).

Haematological profiles are often applied as an index of physiological condition of various organisms and thus provide information about the health status of local populations (Hardy and Depledge, 1999; Petri *et al*., 2006). Haemolymph profiles are critical to the survival and adaptive capacity of crustaceans (Clare and Lumb, 1994; Destoumieux *et al*., 1997) and details of crustacean haematology including the function of each cell type have been described by a number of authors (Ratcliffe *et al*., 1985; Bachere *et al*., 1995; Roch, 1999; Yildiz and Atar, 2002; Jiravanichpaisal *et al*., 2006). Yildiz and Atar (2002) reported three types of haemocytes i.e. hyalinocytes, semigranulocytes and granulocytes in the freshwater crab, *Potamon fluvialilis* and observed their relative occurrence as 15.00%, 54.25% and 30.75% respectively. Reports on another freshwater crab *Sartoriana spinigera* also showed similar ratios for hyalinocytes (19%), semigranulocytes (43%), granulocytes (30%) and adipohaemocytes (8%) (Nayan *et al*., 2010).

Studies of blood chemistry involve measurement of the chemical components in serum or plasma - the non-cellular part of blood and this includes a wide range of electrolytes, enzymes and hormones (Petri *et al*., 2006). Serum and plasma from aquatic organisms are characterized by a number of proteins (trypsin, lysozyme, antibodies, C-protein, complement factors and other lytic factors) which play critical roles as antimicrobial agents, and are the first line of defense (Dalmo *et al*., 1997; Jones, 2001), primary barrier against invasion (Dalmo *et al*., 1997; Jones, 2001) and contain the proliferation of pathogens, including parasites (Jones, 2001). The biochemical aspects of haemolymph which is also vital for understanding tissue injury and adaptive responses of crustaceans to its environment have also been documented (Wu *et al*., 2002; Song *et al*., 2003; Yoganandhan *et al*., 2003; Battison, 2006; MohanKumar and Ramasamy, 2006). Studies have also shown that the physiological profile of crustaceans may differ depending on the selective pressure to tolerate or cope with certain environmental factors (Klerks and Weis, 1987; Rainbow *et al*, 1999)
Members of Members of the *Callinectes* species are notable for their ability to survive in salinities ranging from fresh to hypersaline water. They have high water and salt permeabilities (Cameron, 1978) characteristic of a marine species, but will achieve complete moulting and higher specific growth rate at salinities between 5-20 ppm (Lawal-Are and Kusemiju, 2010). Some authors have documented aspects of the biology of *C. amnicola* in Nigeria ranging from ecology (Lawal-Are and Kusemiju, 2010), population characteristics (Abowei and George, 2010; Udoh and Nlewadim, 2011), growth and fecundity (Lawal-Are, 2010; Lawson and Oloko, 2013), mating behavior and recruitment (Lawal-Are *et al*., 2010) and parasite fauna (Ekanem *et al*., 2013). Clare and Lumb (1994) while describing the morphological aspects of haemocytes in this species reported the presence of pseudopodia processes in small granulocytes and attributed this feature to their role in cellular defense via phagocytosis. They also reported that hyaline cells and not the granulocytes were responsible for initiating clotting in *Callinectes* species. Omari *et al*., (1989) also reported the presence of hyaline, semigranulocytes and granulocytes in the haemolymph of the ridgeback prawn, *Sicyonia ingentis* and stated that hyaline cells could be distinguished from granulocytes primarily on the basis of size; being approximately half of granulocyte size and their nucleo-cytoplasmic ratio is high with a corresponding low granule number.

To understand the innate immune mechanism of the haemocyte of shellfishes clearly, the structure and function of different haemocyte types need to be studied. Studies conducted on several invertebrate species had focused on the separation of haemocyte population and

determination of their roles, but until now, little or no known published information is available on the cell morphology and haematic indices of *Callinectes amnicola* (Malacostraca: Portunidae; De Rochebourne, 1883) in Nigeria. This study to our knowledge is the first study aimed at examining the haemocyte population, haematological and biochemical profiles of *C. amnicola* in Nigeria.

MATERIALS AND METHODS

Study sites: Epe and Lagos Lagoons

Epe Lagoon lies between longitude 5°30' - 5^0 40'E and latitude 3°50' - 4° 10'N and has a surface area of about 225km^2 and a maximum depth of 6m. The lagoon is sandwiched between the Lagos and Lekki Lagoons. However, a large area of the lagoon is relatively shallow with a minimum depth of 1m and the vegetation surrounding the lagoon is of the mangrove swampy type (Balogun, 1987). The lagoon opens into the Gulf of Guinea via Lagos harbor (Fig. 1).

Lagos Lagoon (Ikorodu) is located between longitude 3^0 23 – 3^0 40E and latitude 6^0 22' – 6^0 38N which according to Oyebande, *et al*., (2003) is referred to as the Lower Ogun River Basin Wetlands of Lagos Lagoon and is a major tidal wetland area whose hydrology is driven by tides. This wetland is fed by Ogun and Majidun Rivers. However, Ogun River remains the major source of freshwater to Lagos Lagoon (Ikorodu).

Collection of samples

Bimonthly samples of the adult blue crab *Callinectes amnicola* (n=144 per site) were collected from Epe Lagoon and Lagos Lagoon between March and August, 2013 from three sampling locations in each study site using a funnel trap. The funnel trap was designed using the EPA level 3 protocol for sampling invertebrates (USEPA, 2012) , had a two non-return end and a total length and opening aperture of 1.0 x 0.3 m respectively and was placed in sampling sites for 12 hrs for crab collection. Crabs were transported to the laboratory,acclimatized for 48hrs and fed with sampling location mud rich organic matter until further analysis..

Collection of haemolymph

Crabs were anaesthetized on ice for 10 mins and haemolymph was 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. 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 5mL lithium heparin bottle kept in an ice chest and haemolymph of crabs (n=10 per month and site) were analyzed immediately for haemocyte morphology, haematological and biochemical indices of *C. amnicola*. The total haemolymph obtained from individual crabs were divided into three aliquots for haemocyte count and morphology, haemocyte sedimentation rate and serum biochemistry

Haemolymph analysis

Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC): Total haemocyte count (THC) and differential count (DHC) of haemocyte population were determined immediately after sampling using an improved Neubaeur heamocytometer according to methods described by Blaxhall and Daisley (1973).

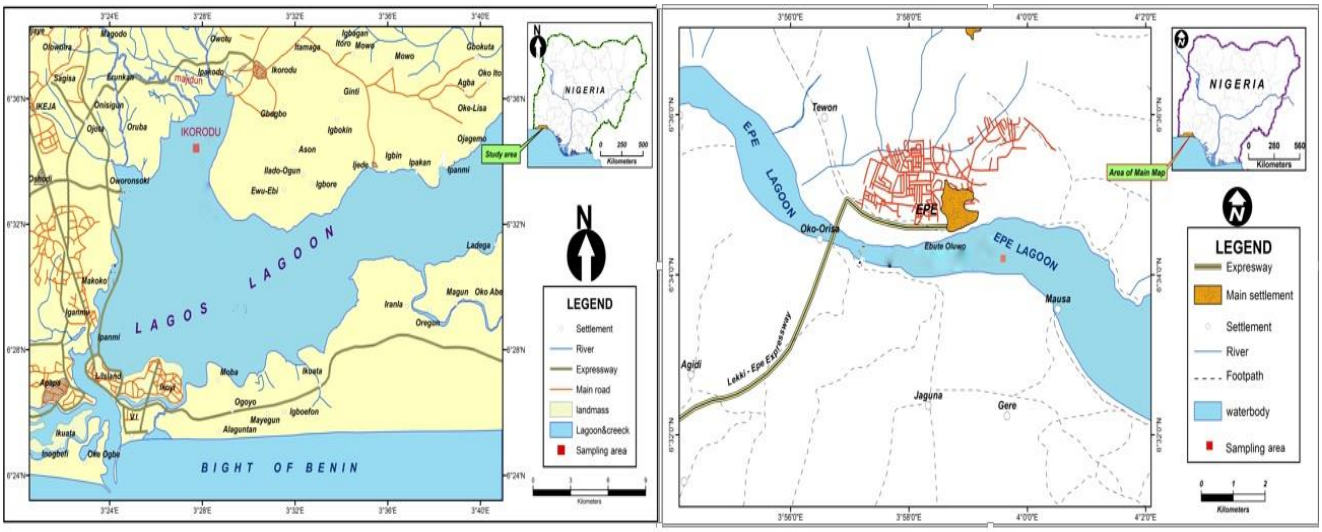

Figure 1:
Map showing Epe Lagoon and Lagos Lagoon.

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.

Haemocyte sedimentation rate (HSR): The micro-wintrobe method was used to determine haematocyte sedimentation rate as described by Blaxhall and Daisley (1973). Briefly, a microhaematocrit tube was placed at an angle in heparinized collection tubes and allowed to flow by gravity into the tube. One end of the tube was sealed with plasticine and the tube was kept in a vertical position for 1hr at room temperature. Readings for HSR were determined with a millimeter graph paper mounted on a card and measurements made from the top of the column of settled haemocytes to the surface of the serum were reported in mm/h (Westergreen, 1957).

Haemocyte morphology: Haemocyte identification from freshly collected haemolymph samples were based on cell size, shape and granularity (Bachau, 1981) using a combination of wet mount and permanent staining. One drop of haemolymph was introduced onto a slide and thin smears were viewed immediately with a 100x light microscope for wet mount while for permanent staining, three drops of haemolymph were placed on individual slides, smeared and fixed with absolute methanol for 3 mins with three types of stains: Geimsa, Wright and Maygrunwald/Wright applied for 10 mins to determine the best stain for crab haemocyte morphology. The stains were rinsed with distilled water, slides viewed under the microscope for the different cell morphotypes and Geimsa stain gave the best result for haemocyte morphology in *C. amnicola*.

Biochemical analysis: Haemolymph samples were centrifuged for 10 mins at 5000 g with a Hawskley micro-haematocrit centrifuge and the serum derived was stored at -20^{o}C for further analysis. 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). Total protein was determined by the Buiret method, albumin levels were determined with a Boehringer Mannheim's albumin reagent and globulin was determined by subtracting the concentration of albumin from the total protein (Coles, 1986).

Physicochemical parameters: Physicochemical parameters (pH, Conductivity, Salinity, Temperature, Total Dissolved Solids (TDS), Ion and Dissolved oxygen (DO)) were measured *in situ* with a Consort CT-C933T Electrochemisrty meter (TOPAC INSTRUMENTS).

Data analysis: Data were subjected to Independent samples t-test for significant difference in means of haematological and serum biochemical parameters between sampling locations. Differences in means were considered significant when $p<0.05$. Principal Component Analysis (PCA) was used to elucidate patterns of variation in physicochemical parameters and haematological and serum biochemical parameters of *C. amnicola* and were reported as significant at $p<0.05$. All data were analyzed using Statistica version 8 (Statsoft Inc. USA).

RESULTS

Biometric indices of *C. amnicola* from Epe and Lagos Lagoons

Mean values of body weight for the blue crab *Callinectes amnicola* are given in Table 1. Crabs from Epe Lagoon were about 1.5 times heavier (36.45±15.19 g) than those from Lagos Lagoon (23.25±6.50 g).

Table 1:
Body weight and Haematic Indices of *Callinectes amnicola* from Epe and Lagos Lagoons

Study Sites (Lagoons)	Body Weight (g)	THC	HSR (mm/hr)	Hyalinocyte	Semi granulocyte	Granulocyte
Epe	36.45±15.19	3.08±0.52	4.56±1.50	71.36±6.42*	8.66±0.86	19.80±6.45
Lagos	23.25±6.50	3.19±0.48	4.78±2.08	64.80±2.54	8.09±3.44	26.97±3.85*

***= variables with significant differences across sites**

Table 2:
Haemocyte Morphotypes of *Callinectes amnicola* from Epe and Lagos Lagoons

Cell type	Cell Diameter (µm)	Nucleus (µm)	N/C ratio
Hyalinocyte	8.95±1.62	7.08±0.08	0.79±0.03
Semi granulocyte	13.49±2.37	8.62±0.71	0.64±0.05
Hyalinocyte	19.37±2.76	12.78±0.95	0.66±0.03

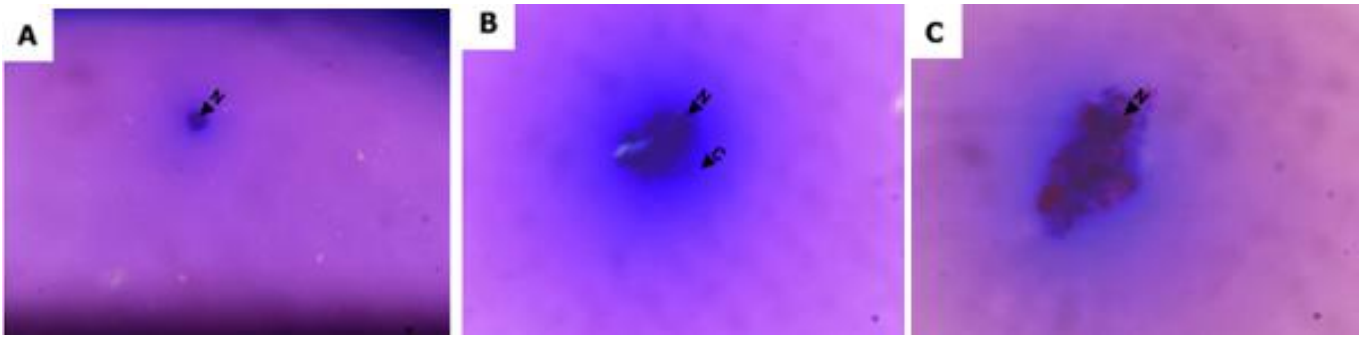

Figure 2:
Haemocyte morphotypes of *Callinectes amnicola* from Epe and Lagos Lagoons. A: Hyalinocyte. B: Semi granulocyte. C: Granulocyte.

Haematological Variables

Total Haemocyte count (THC), and Hyaemocyte sub-population of *Callinectes amnicola* from Epe and Lagos Lagoons

The total haemocyte count (THC) in the circulating haemocytes of *C. amnicola* (n=60 per sampling location) were similar for both Epe (3.08±0.52 x 10^7cells /mL) and Lagos Lagoon (3.19±0.48 x 10^7 cells/mL). Hyalinocyte sub-population was highest in the circulating haemocytes of crabs from Epe and Lagos Lagoon respectively with a higher population from Epe Lagoon (71.36±6.42%) compared to Lagos Lagoon (64.80±2.54%). The semigranulocyte sub- population of *C. amnicola* were lowest in the circulating haemocyte for both sampling locations and similar for the Epe population (8.66±0.86%) compared to the Lagos Lagoon population (8.09±3.44%). Granulocyte population in circulating haemocytes were intermediate between hyalinocytes and semigranulocytes and a higher population was recorded in *C. amnicola* from Lagos Lagoon (26.97±3.85%) compared with Epe Lagoon (19.80±6.45%). Haematological variables showed that, THC and semigranulocyte population did not differ considerably between crabs from Epe and Lagos Lagoons. Significant differences were however recorded for hyalinocytes and granulocytes with Epe crabs having a higher sub-population of hyalinocytes (71.36±6.42%) than Lagos Lagoon crabs (64.80±2.54). A significantly higher sub-population of granulocytes were also recorded in circulation for Lagos Lagoon crabs (26.97±3.85) compared to Epe Lagoon crabs (19.80±6.45) (Table 1).

Haemocytes of *C. amnicola* was classified into three morphotypes based on cell size and shape of granules, position of nucleus and nucleo: cytoplasmic ratio. These were hyalinocytes, semigranulocytes and granulocytes (Table 2, Fig. 2). Hyalinocytes had the smallest size (8.95±1.62µm) of the three morphotypes recorded in *C. amnicola* and were circular or ellipsoidal in shape and characterized by a small centrally located agranular nucleus (7.08±0.08µm) (Fig. 2A). Both nucleus and cytoplasm stained blue with Geimsa stain (basophilic) with a nucleocytoplasmic ratio of 0.79±0.03µm. Semigranulocytes had two nuclei morphotypes ranging from circular to ovoid cells (13.49±2.37µm) and the nucleus were centrally located with numerous small and a few large granules (8.62±0.71µm) (Fig. 2B). Nucleus and cytoplasm were also basophilic staining blue with Geimsa stain and the nucleo cytoplasmic ratios were 0.64±0.05µm. Granulocytes were the haemocytes with the largest size (19.37±2.76µm) of all the circulating haemocyte sub-population of *C. amnicola* from Epe and Lagos Lagoon. The nucluei were ellipsoidal (12.78±0.95µm) with densely populated large blue coloured granules (Fig. 2C). Both cytoplasm and nucleus were basophilic and cells were ellipsoidal in shape with a nucleo:cytoplasmic ratio of 0.66±0.03µm.

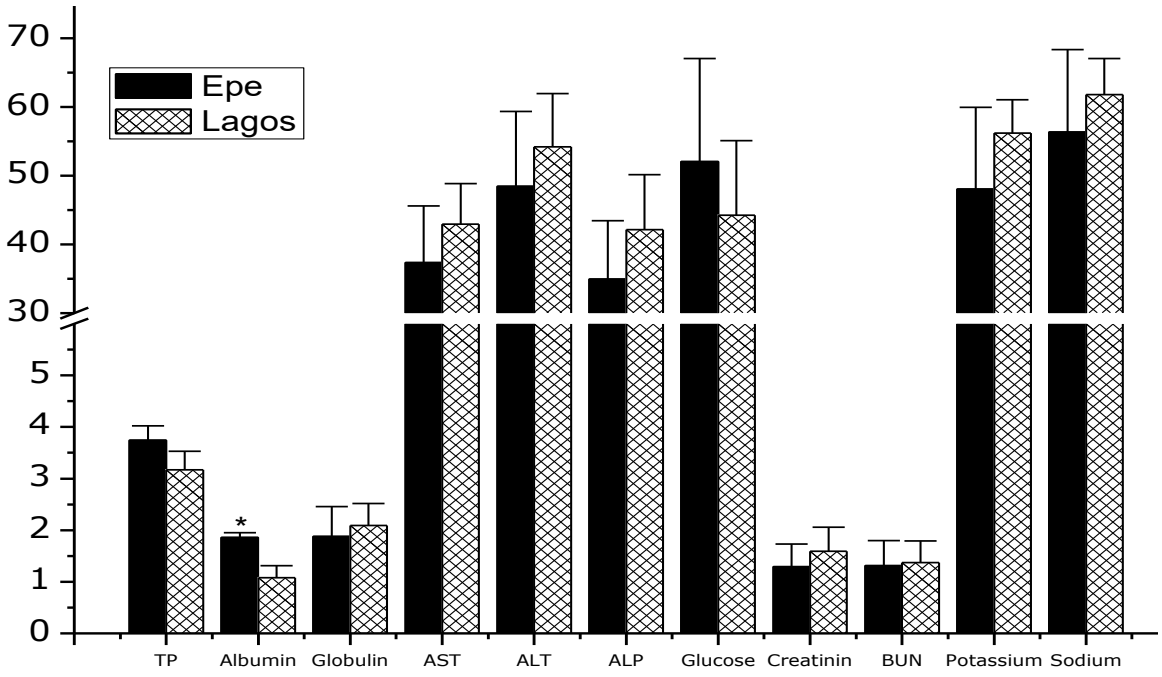

Figure 3:
Serum biochemical profile of *Callinectes amnicola* from Epe and Lagos Lagoons.

Serum Biochemistry of *Callinectes amnicola* from Epe and Lagos Lagoons

Total protein was higher in Epe crabs (3.74±0.28 g/dL) than those from Lagos Lagoon (3.17±0.36g/dL). Serum albumin concentrations were significantly higher in Epe crabs (1.86±0.09 g/dL) compared with Lagos Lagoon (1.08± 0.23 g/dL) crabs. Globulin levels in Epe crabs (1.88±0.58 g/dL) were also lower than those from Lagos Lagoon (2.09±0.43 g/dL). Transaminases AST, ALT and the phosphatase ALP also showed lower activities in Epe crabs (37.37±8.24; 48.43±10.90; 34.93±8.52IU) compared to Lagos Lagoon crabs (42.93±5.92; 54.2±7.76; 42.17±7.99 IU) respectively. Glucose levels were significantly higher in Epe crabs (52.01±15.03 mg/100mL) compared to Lagos Lagoon crabs (44.23±10.85 mg/100mL). Electrolytes indicative of kidney function i.e. creatinine, potassium and sodium in Epe crabs (1.29±0.44 mg/dL; 48.07±11.86 mmol/L; 56.33±12.04 mmol/L) also showed lower values than recorded for Lagos Lagoon crabs (1.59±0.47 mg/dL; 56.2±4.83 mmol/L; 61.8±5.26 mmol/L) respectively. Blood urea nitrogen (BUN) was lower in Epe crabs (1.31± 0.49 mmol/L) but not significantly different from values in Lagos Lagoon crabs (1.37±0.42mmol/L) (Fig. 3).

Principal Component Analysis (PCA): Correlation between biological and environmental variables

Haematology

Interactions between physicochemical parameters of surface water from sampling sites and haematological variables of crabs from each site are given in Figure 4 and Table 4 with PC 1 accounting for the highest variance (36.35%) for this interaction. The orientation of variables in the ordination plot showed that dissolved oxygen (R^2= -0.73) and pH (R^2= -0.80) had positive correlations with hyalinocytes (R^2=-0.88), in crabs from Epe Lagoon. This implies that higher dissolved oxygen (DO) content in water and slightly alkaline pH allowed for a corresponding proliferation of hyalinocytes in the haemolymph of crabs in Epe Lagoon. In blue crabs from Lagos Lagoon, granulocyte and THC (R^2= 0.47) in haemolymph showed a negative correlation with pH (R^2= -0.80) and dissolved oxygen (R^2= -0.76). This implies that lower DO content of water i.e. hypoxic conditions and lower pH (deviations from alkalinity) resulted in higher concentrations of granulocytes and THC in crab haemolyph in Lagos Lagoon. The higher THC (R^2= 0.47) and granulocyte (R^2= 0.62) count also showed a positive correlation with salinity (R^2= 0.73) and concentrations of ions (R^2= 0.52) in Lagos Lagoon *C. amnicola* population.

PC 2 accounted for 18.24% of the total variance between the interactions in ordination plot and indicated that Electrical conductivity (EC) (R^2= 0.86) and TDS (R^2= 0.87) showed the strongest positive correlation with HSR (R^2= 0.63) and negative correlations with THC (R^2= -0.70) implying that increased conductivity and TDS resulted in a reduction in total haemocyte count and increase in haemocyte sedimentation rate (HSR) in Lagos Lagoon crabs.

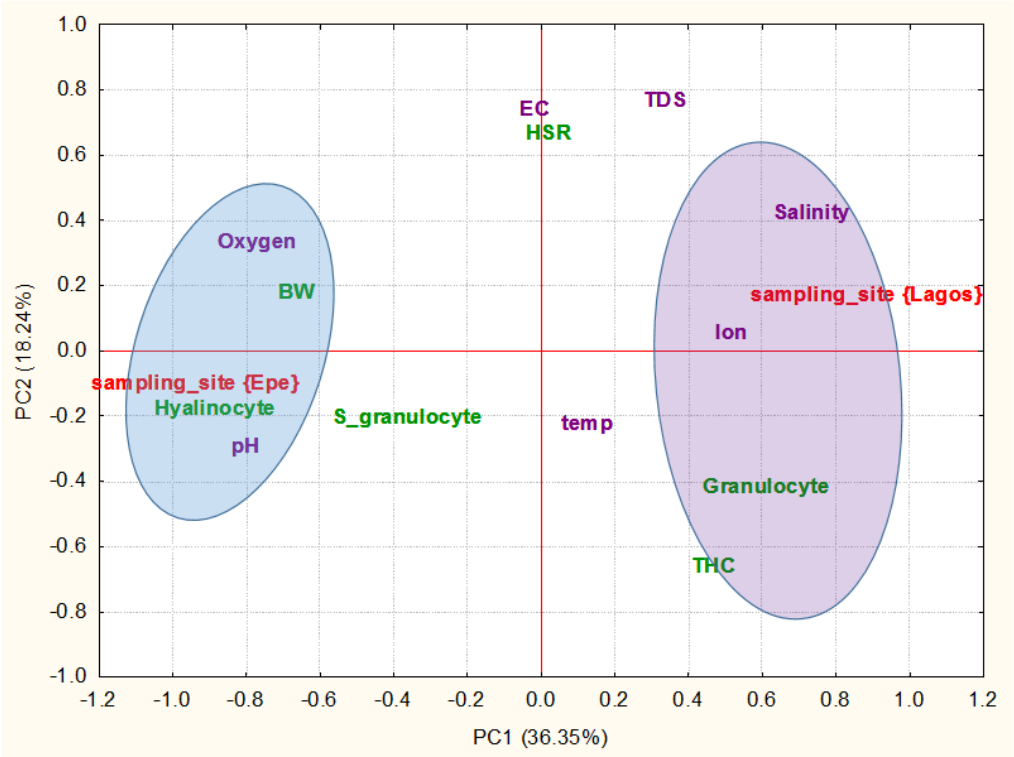

Figure 4:
Principal component biplot of haematological and environmental variables of *Callinectes amnicola* from Epe and Lagos Lagoons

Table 4:
Principal component scores for body weight, haematological and environmental variables of *Callinectes amnicola* from Epe and Lagos Lagoons

	PC 1	PC 2	PC 3
pH	-0.80	-0.33	-0.38
Ion	0.52	0.02	0.26
EC	-0.02	0.67	-0.14
TDS	0.33	0.67	-0.02
Salinity	0.73	0.39	0.05
Oxygen	-0.76	0.26	0.44
Temp	0.13	-0.26	0.80
BW	-0.66	0.14	0.65
THC	0.47	-0.70	-0.20
HSR	0.02	0.63	-0.44
Hyalinocyte	-0.88	-0.21	-0.11
Granulocyte	0.62	-0.45	-0.11
S_granulocyte	-0.36	-0.24	-0.55
sampling_site {Lagos}	0.94	0.03	0.20
sampling_site {Epe}	-0.94	-0.03	-0.20
% Variance	**36.35**	**18.24**	**13.97**
Cumulative	**36.35**	**54.59**	**68.56**

Serum biochemistry

Principal Component biplot showed that PC 1 accounted for 37.55% in total variations for the relationship between biochemical indices and environmental variables while PC 2 accounted for a variance of 16.93% (Fig. 5, Table 5). Orientation of variables in ordination space showed that albumin (R^2=-0.75) and total protein (R^2=-0.57) levels in serum samples of blue crabs at Epe Lagoon were positively correlated with pH (R^2= -0.84) and dissolved oxygen (R^2= -0.69). On the other hand creatinine (R^2= 0.68), potassium (R^2= 0.93), sodium (R^2= 0.69) were positively correlated with globulin (R^2= 0.81) in serum samples of blue crabs from Lagos Lagoon. These variables i.e. creatinine, potassium, sodium and globulin also showed a strong positive correlation with TDS (R^2= 0.56) and salinity (R^2= 0.75) and a negative correlation with pH (R^2=-0.84) and dissolved oxygen (R^2=-0.69) for Lagos Lagoon crabs

DISCUSSION

Haematological and biochemical changes in crustaceans are often modulated in response to environmental factors to bring about homeostatic control within the organism and as a result are used as diagnostic tools for assessing the health of wild populations (Macpherson, 2002; Giro´ n-Pe´ rez *et al*., 2008; Velisek *et al*., 2009).

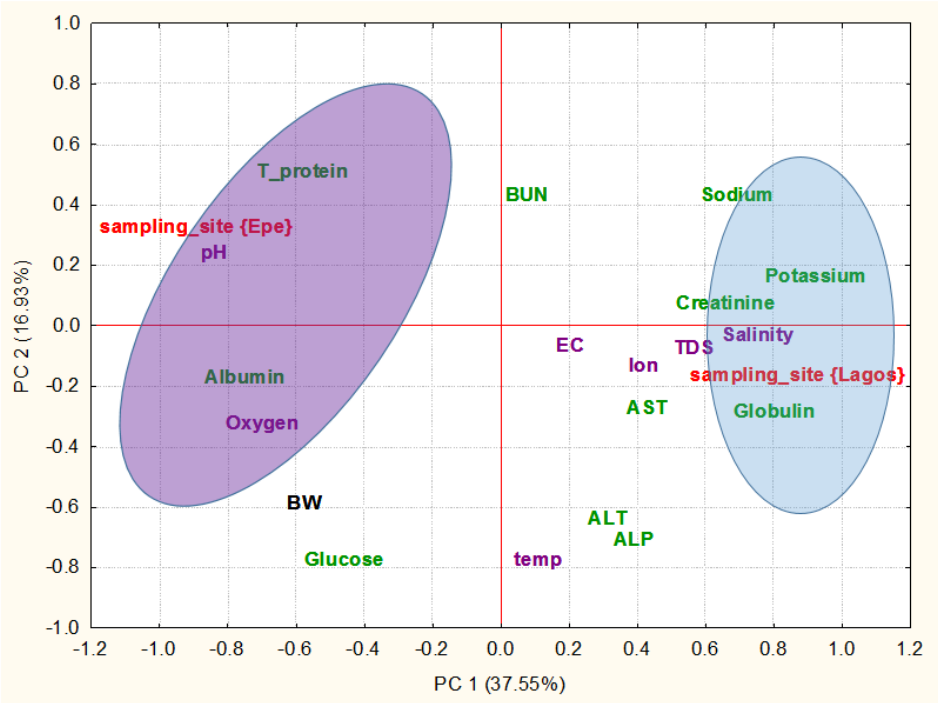

Figure 5:
Principal component biplot of body weight, bicochemical and environmental variables of *Callinectes amnicola* from Epe and Lagos Lagoon

Table 5:
Principal component scores for body weight, biochemical and environmental variables of *Callinectes amnicola* from Epe and Lagos Lagoons

	PC 1	PC 2	PC 3
pH	-0.84	0.21	0.00
Ion	0.42	-0.17	-0.06
EC	0.20	-0.16	0.75
TDS	0.56	-0.11	0.56
Salinity	0.75	-0.07	0.20
Oxygen	-0.69	-0.40	0.31
Temp	0.11	-0.81	-0.19
BW	-0.58	-0.62	0.12
T_protein	-0.57	0.47	0.00
Albumin	-0.75	-0.21	0.41
Globulin	0.81	-0.22	0.27
AST	0.43	-0.31	-0.26
ALT	0.31	-0.67	-0.08
ALP	0.39	-0.75	-0.29
BUN	0.08	0.40	-0.56
Glucose	-0.45	-0.81	-0.14
Creatinine	0.68	0.00	0.61
Potassium	0.93	0.13	0.12
Sodium	0.69	0.40	-0.13
sampling_site {Lagos}	0.95	-0.18	-0.16
sampling_site {Epe}	-0.95	0.18	0.16
% Variance	**37.55**	**16.93**	**13.51**
Cumulative	**37.55**	**54.48**	**67.99**

Variations in blood parameters have been attributed to responses to a changed physiological and energetic requirements (Kutz *et al*. 2004, Ochang *et al*., 2007; Adeogun, 2011) and may be an early warning measure of stress before population declines are observed. The significantly higher level of granulocytes in crabs from Lagos Lagoon may be a stress response to unfavorable environmental conditions. Granulocytes have been reported to play a significant role in the crustacean defense system because of their antibacterial activity and function in secreting extracellular matrix proteins that stops the action of invading organisms, when the host is attacked by either extremelylarge particles or numerous tiny particles (Soderhall and Cerenius, 1992 Chisholm and Smith, (1995). There are similar reports for crayfish (Johansson *et al*., 1995), *Penaeus monodon* (Sritunyaluksana *et al*., 2001) and *Litopenaeus vannamei* (Liu *et al*., 2004). The increased THC in crabs from Lagos Lagoon appeared to be associated with lower dissolved oxygen in water. Reports have indicated that the increase in number of circulating haemocytes under hypoxic condition is a compensatory response to maintain oxygen tissue perfusion in crabs (Sussarellu *et al*., 2012). Other reports have shown that the bilateral movement of haemocytes from tissue to haemolymph can result in an increase in THC and could be attributed to the presence of pathogens (Allam *et al*., 2000; Comesana *et al*., 2012) or exposure to contaminants (Amachree *et al*., 2013). This may also explain why the increased THC in blue crabs from Lagos Lagoon was

positively correlated with higher concentrations of ions in surface water. Haemocytes are activated by microorganisms (Vargas-Albores, 1995; Vargas-Albores, *et al*., 1997) and are involved in the elimination of foreign particles (Hose and Martin, 1989; Bachère *et al*., 1995). A number of field studies on aquatic species have linked poor water quality particularly reduced levels of dissolved oxygen with increased occurrence of infection (Hargis *et al*., 1989; Landsberg *et al*., 1998). It has also been documented that periods of hypoxia may lead to physiological stress, for example, Albert and Ellington (1985); Vermeer, (1987) Schmitt and Uglow, (1997); Taylor and Waldron, (1997); Wileman *et al*., (1999), and Paterson and Spanoghe (1997) provided useful insights on various haemolymph components as indicators of stress in decapod crustaceans. A number of studies have also attributed increased susceptibility of crustaceans to bacterial pathogens during hypoxia to the reduced ability of the host to clear the pathogen from its tissues (Burnett and Stickle, 2001; Burnett and Burnett, 2005). Acute exposures are emphasized as a factor in the haemocyte profile of crabs because chronic hypoxia may ultimately result in reduced immunological characteristics of serum. Noga *et al* .,(1994) reported that the blue crab, *Callinectes sapidus* under chronic hypoxia showed reduced serum bacteriostatic activity compared to populations exposed to intermittent acute hypoxia.

The higher HSR in *C. amnicola* from Lagos Lagoon can be related to inflammatory reactions in tissues leading to faster cell aggreagation or an increase in the percolation of cells. Jain (1986) and Adeogun (2011) linked such responses to a basic protective response to tissue damage and is a common adaptive response to maintain tissue intergrity and provide a functional blood supply in all organisms.

The three haemocyte morphotypes reported in this study is consistent with reports on the circulating haemocyte sub-population of *Callinectes sapidus* (Bodammer, 1987; Yildiz and Atar, 2002, Li and Shields, 2007) and *Carcinus aestuarii* (Matozzo and Marin, 2010) by some authors. The highest population for hyalinocytes of *C. amnicola* from Epe and Lagos Lagoon is consistent with the reports of Bachau (1973) in the crab *Eriocheir sinesis* where hyalinocytes were 5-8 times more abundant than granulocytes and (Hose *et al*., 1990) reported that 56% of the circulating haemocytes in the lobster, *Panulirus interruptus* were hyalinocytes . Li and Shields (2007) reported a hyalinocyte population of 48% from *C. sapidus* from the Chesapeake Bay, USA and although the percentage of haemocytes reported were lower than the 71% recorded in this study; high variability is generally reported by authors for varying number of crustaecean species. Some other reports have proposed much higher morphotype sub-populations for some decapod crusteaceans (Hose and Martin, 1989; Omari *et al*., 1989; Manjula *et al*., 1997). Matozzo and Martin (2010) were of the opinion that the morphological characteristics of *C. aestuarii* (central nucleus, high nucleo: cytoplasmic ratio and lack of granules) which is similar to *C. amnicola* in this study suggest a close resemblance to undifferentiated stem cells.

The significantly higher weight of crabs from Epe compared to those from Lagos Lagoon may be an indication of the better physiological condition of Epe crabs attributable to less polluted environment and probable increased foraging opportunities.

The total serum protein in Epe and Lagos Lagoon crabs (3.17 -3.74 g/dL) were within close range of values reported by Pauley *et al*., (1976) in blue crabs (3.30 g/dL) and Stewart *et al*., (1967) in lobsters. The lower concentration of total serum protein in Lagos Lagoon crabs may be attributed to increased breakdown of serum peptidic material and modulation of their involvement in various biological processes due to environmental stress. Prolonged exposure to stress has been implicated in increased metabolism of amino acid carbon skeleton (Huggins and Munday, 1968). In addition haemolymph proteins have been reported as evidence of energy reserve in invertebrates, as such lower concentrations may depict lower food availability in a population (Smith and Dall, 1982; Rosas *et al*., 2012) while higher serum protein concentrations have been associated with higher live wet weight and diet quality (Stewart *et al*, 1967; Hagerman, 1983).

The mean albumin levels 1.08- 1.86 g/dL in *C. amnicola* from Epe and Lagos Lagoons respectively were significantly lower than values i.e. 2.58g/dL reported for lobsters under favourable environmental conditions in the temperate region (Dove *et al*., 2005). This significant difference may be attributed to differences in climate because crustaceans under experimental conditions of higher temperature have been documented to have lower albumin concentrations in serum (Dove *et al*., 2005). The higher concentration of globulin in the serum of Lagos Lagoon crabs may be indicative of increased immune responses.

The glucose levels of *C. amnicola* for both Epe and Lagos Lagoon (44.23- 52.03mg/100mL) in this study were higher compared to values reported for glucose levels in the serum of non-parasitized blue crab *C. sapidus* (37.30 ± 28.9mg/100mL) (Pauley *et al*., 1975). Creatinine levels reported for crabs from both sites were significantly lower than ranges (2.0mg/dL) recorded for lobsters in the wild. The higher levels of potassium and

sodium in blue crabs from Lagos compared to Epe Lagoon may be attributed to isotonic conformation of body fluid to the higher salinity of surface waters in Lagos Lagoon. Amado *et al*., (2006) reported increased haemolymph concentrations of Na^+, K^+, Cl^-, and osmolality upon exposure of the freshwater crab *D. pagei* to increased salinity. Exposure to pollutants in the environment have also been implicated in disruption of normal osmoregulation potential of crustacean cells. Aquatic organisms especially freshwater fish and crustaceans have been documented to be vulnerable to nitrite poisoning (Boyd, 1982; Boyd and Tucker, 1998) and exposure to nitrite has been reported to stimulate a net loss of potassium ions from muscle tissues resulting in serum hyperkalemia and increased excretion of potassium (Gupta, 2012).

The higher activites of ALT, AST and ALP in Lagos Lagoon crabs compared to Epe Lagoon crabs also suggest possible incidence of liver tissue damage in blue crabs from Lagos Lagoon. This is because transaminases and phosphatases are important diagnostic tools for evaluating tissue damage in organisms (Vijayavel *et al*., 2006; Adeogun *et al*., 2012). Hypoxia or tissue damage may result in enzyme leakage into extracellular fluid, is indicative of cellular damage (Adeogun *et al*., 2012) and may be related to environmental pollution. Such responses have been attributed to the fact that transaminases are involved in the maintenance of a balanced pool of free amino acids with ALT catalyzing the transfer of amino group from alanine to α-ketoglutarate to form glutarate and pyruvate while AST on the other hand catalyses the transfer of an amino group from α-ketoglutarate to glutarate and oxaloacetate (Moss *et al*., 1986). Increased ALT and AST activites in *C. amnicola* from Lagos compared to Epe Lagoon may be a direct consequence of stress induced protein metabolism in the tissue of blue crabs. The higher activity of ALP may be due to the fact that being a brush border enzyme; ALP is involved in splitting of various phosphorous esters at an alkaline pH and is involved in protein and enzyme synthesis, growth and differentiation and transport to phosphorylated intermediates across cell membranes (Zhou *et al*., 2000). As such, an increase in ALP activity is also indicative of variations in protein metabolism in tissue of *C. amnicola* in Lagos compared to Epe Lagoon.

Most environmental factors have been reported to affect serum protein concentration in crustaceans by influencing appetite and feeding behaviour rather than having a direct effect on serum protein itself (Scott and Solman, 2004)

From PC1, the positive correlation between hyalinocytes, body weight and semi-granulocytes with dissolved oxygen in surface water of the Epe Lagoon environment suggest that oxygenated conditions support better growth and lower incidence of infections via opportunistic microbes. As such the negative correlation between THC and granulocytes with dissolved oxygen in surface water of Lagos Lagoon implies that hypoxic conditions allow for increased incidences of infection in resident blue crabs. The negative correlation between granulocytes and pH indicates that a lower pH of surface water may lead to increased incidence of infection in resident crabs. In essence deviation from alkaline pH of freshwater will result in deteriorated physiological conditions. Alkaline pH of aquatic ecosystems has been documented to be more favourable for the proliferation of crab species. In decapods, pH influences the metabolism, physiology and maturation process (Muthu and Laxminarayana, 1977) while a dysfunction in electrolyte and acid–base regulation have been reported for fish under low pH conditions (McDonald and Wood, 1981; Wright and Wood, 1985; Wilkie and Wood, 1991).

For *C. amnicola* from Lagos Lagoon, PCA also showed that hyalinocytes were negatively correlated with granulocytes and this may just be a reflection of a challenged immune system under pollution pressure because lower hyalinocyte levels may easily characterize crustaceans with higher infection burden. Bachere, (2000) described hyalinocytes as being small in size, having few granules and chiefly involved in phagocytosis. Other reports also showed that the smallest and least numerous haemocytes are the hyalinocytes (Söderhäll and Cerenius, 1992) as such intense or chronic pollution are likely to result in a reduction in hyalinocyte number and an increased mobilization of granulocytes for more specific immune responses. Early reports (Cornick and Stewart, 1973) portrayed hyalinocytes as a rudimentary antibody or recognition system in crustaceans also confirming their role in non-specific immune responses and the likelihood of their decrease under chronic pollution/infection.

From PC 2 the negative relationship between EC and THC may be a reflection of the toxic effects of contaminants on haematological variables in Lagos Lagoon blue crabs. Conductivity is a measure of the ability of water to pass an electrical current and is affected by the presence of inorganic dissolved solids such as chloride, nitrate, sulfate, and phosphate anions (ions that carry a negative charge) or sodium, magnesium, calcium, iron, and aluminum cations (ions that carry a positive charge) (WHO, 1996). Chandanshive *et al*., (2012) reported a reduction in erythrocyte count and increased hematocrit in fishes

exposed to heavy metals, suggesting that heavy metals alter the membrane integrity of blood cells, causing defective osmoregulation and ultimate hemolysis of cells. Other reports have shown that exposure to heavy metals can cause severe constriction of the blood spaces which reduce blood flow and make oxygen transport less efficient (Oronsaye and Brafield 1984; Oronsaye, 1997). Although metals were not assayed for in this study, reports of higher heavy metal contamination of Lagos Lagoon have been well documented (Ajao, 1996; Don Pedro *et al*., 2004). Also from PC 2, the positive correlation between electrical conductivity (EC) and HSR for blue crabs from Lagos Lagoon suggests that increased exposure and uptake of metals could have resulted in increased HSR values. Increased sedimentation rate of blood cells have been attributed to non-specific incidences of inflammation or autoimmune responses (Bedell and Bush, 1985; Sox and Liang, 1986) and a number of reports have documented that heavy metal toxicity may elicit inflammation in living cells (Xu *et al*., 1999; Figueroa *et al*., 2001; Valko *et al*., 2005). Moody *et al*., (2013) also reported increased sedimentation rate of red cells for fishes living in a metal polluted river.

The relationship between serum concentrations of potassium and salinity of a water body highlights the osmoregulatory responses of the crustacean cell in its environment. The significantly higher concentration of potassium ion in serum of Lagos Lagoon crabs on the other hand may be a reflection of environmental stress or probable parasite burden. Increased sodium and chloride levels have been reported as part of the biochemical changes that characterize parasitized aquatic species (Tavares-Dias *et al*., 2007). Sodium and potassium are electrolytes critical for the homeostatic state of the internal environment of crustaceans and regulated sodium uptake and retention is a measure to control haemocyte pressure and volume in shellfishes. A damaged epidermis and increased permeability of integument has also been implicated in the increased diffusion of ions from water into fish body (Zahran and Risha, 2013). The positive correlation between globulin, potassium and sodium in Lagos Lagoon crabs emphasize a possible relationship between higher potassium and sodium concentrations with incidence of pollution in the Lagos Lagoon crab environment.

In conclusion, three haemocyte sub-population morphotypes: hyalinocytes, semigranulocytes and granulocytes were identified in *Callinectes amnicola* from Epe and Lagos Lagoons with hyalinocytes having the highest population of circulating heamocytes for both lagoons. Haematological and biochemical indices in haemolymph depict the blue crab population from Lagos Lagoon as stressed and attributes its lower physiological condition compared to blue crab population at Epe Lagoon to sub-optimal environmental parameters. This study to the best of our knowledge represents the first published profile for serum haematology and serum biochemistry of *C. amnicola* in Epe and Lagos Lagoons and such species-specific or taxon-specific baseline values provide a reference point for comparing responses of members of this taxon to differential habitat quality.

REFERENCES

Abowei J.F.N. and George A.D.I. (2010): The morphology, abundance, size and sex distribution of *Callinectes amnicola* (De Rochebrune, 1883) from Okpoka Creek, Niger Delta, Nigeria. Current. Research Journal of Biological Sciences 2(1): 27-34.

Adeogun, A. O. (2011): Haematological profiles of the African Clariid Catfish (*Clarias gariepinus*) exposed to sublethal concentrations of the methanolic extracts of *Raphia hookeri*. Tropical Veterinarian 29(4):27-43

Adeogun, A. O., Onocha P. A. ,Oladoyinbo S. O. (2012): Variations In Plasma Biochemical Parameters of *Clarias gariepinus* Exposed To Sub-Lethal Concentrations of the Methanolic Extracts of *Raphia hookeri*. Tropical Veterinarian 30(1): 17-31.

Ajao E.A. (1996): Review of the State of Pollution of the Lagos Lagoon. NIOMR Tech. paper No 106.

Albert, J.L., Ellington, W.R., (1985): Patterns of energy metabolism in the stone crab, Menippe mercenaria, during severe hypoxia and subsequent recovery. J. Exp. Zool. 234, 175–183

Alberto B.B. (2006): Veterinary Clinics of North America: Ectoparasite Control. Food Ani. Pract. 22(2):463-474.

Allam B, Paillard C, Howard A, Le Pennec M (2000): Isolation of the pathogen Vibrio tapetis and defense parameters in brown ring diseased Manila clams *Ruditapes philippinarum* cultivated in England. Dis Aquat Org 41:105–113

Amachree, D., Moody, A.J., Handy, R.D., (2013): Comparison of intermittent and continuous exposures to cadmium in the blue mussel, *Mytilus edulus*: accumulation and sub-lethal physiological effects. Ecotoxicol. Environ. Saf. 95, 19e 26.

Amado E.M., Freire C.A., Souza M.M. (2006): Osmoregulation and tissue water regulation in the freshwater red crab Dilocarcinus pagei (Crustacea, Decapoda), and the effect of waterborne inorganic lead. Aquatic Toxicol 79:1–8.

Bachère, E., Mialhe, E. and Rodriguez, J. (1995): Identification of defense effectors in the haemolymph of crustaeceans with particular reference to the shrimp, *Penaeus japonicus* (Bate): prospects and applications. Fish Shellfish Immunol. 5:597-612

Bachère, E. (2000): Shrimp immunity and disease control. Aquaculture 191 (1-3): 3-11

Bachau A.G. (1981): Crustaeceans, In: Ratcliffe N.A., Rowley A.F. (eds). Invertebrate Blood Cells, Vol 2, London: Academic Press. pp 385-420

Balogun J.K. (1987): Seasonal fluctuations of salinity and fish occurrence in and around Epe Lagoon, Nigeria. Afr. J. Ecol., 25(1): 55 – 61.

Battison, A., (2006). Tissue distribution and hemolymph activity of six enzymes in the American lobster (*Homarus americanus*): potential markers of tissue injury. J. Shellfish Res. 25, 553–560

Bedell S. E. and Bush B. T. (1985): Erythrocyte sedimentation rate from folklore to facts Am. J. Med. 78 1001-9

Blaxhall P.C., Daisley K.W. (1973): Routine haematological methods for use with fish blood. Journal of Fish Biology 5, 771-781.**Bodammer, J.J. (1978)**: Cytological observations on the blood and haemopoietic tissue in the blue crab, *Callinectes sapidus*. Cell Tissue Res. 187:79-96

Boyd, C. E.(1982): Hydrology of small experimental fish ponds at Auburn, Alabama. Trans. Amer. Fish. Soc., 111: 638–644

Boyd, C. E., C. S. Tucker (1998): Pond Aquaculture Water Quality Management. Boston: Kluwer Academic Publishers (1998).

Burnett, K. G., L. E. Burnett. (2005):. The impacts of hypoxia on disease resistance in crustaceans. In: (Eds. Cipriano, R.C., Shchelkunov, I.S., and Faisal M.) Health and Diseases of Aquatic Organisms: Bilateral Perspectives. Proceedings of the Second Bilateral Conference between Russia and the United States (21 - 28 September 2003, Shepherdstown, West Virginia. Michigan State University, East Lansing, MI. (363 pp., ISBN 0-9765653-0-7)

Burnett, L. E., W. B. Stickle. (2001) Physiological responses to hypoxia, Pages 101-114 in Nancy N. Rabalais and R. Eugene Turner (eds.), Coastal Hypoxia: Consequences for Living Resources and Ecosystems. Coastal and Estuarine Studies 58, American Geophysical Union, Washington, D.C.

Cameron, J.N. (1978): Effects of hypercapnia on blood acid-base status, NaCl fluxes and trans-gill potential in freshwater blue crabs, *Callinectes sapidus*. J. Comp. Physiol. 123:137-141

Castejón, D., Guerao, G., (2013): A new record of the American blue crab. *Callinectes sapidus* Rathbun, 1896 (Decapoda: Brachyura: Portunidae), from the Mediterranean coast of Iberian Peninsula. BioInvasions Record, 2 (2), 141-143.

Chandanshive S.S. P.P.Sarwade, Atul Humbe A. D. Mohekar (2012): Effect of heavy metal model mixture on haematological parameters of *Labeo rohita* from Gharni Dam Nalegaon, Latur. International Multidisciplinary Research Journal 2012, 2(4):10-12 ISSN: 2231-6302 Available Online: http://irjs.info/

Chindah A.C., Tawari C.C., Ifechukwude K.A. (2000): The food and feeding habits of the swimming crab, *Callinectes amnicola* (Portunidae) of the New Calabar River, Nigeria. J Appl Sci Environ Manage 4: 51-57

Chisolm, L.A., Smith, C.L. (1995): The sea urchin complement homologue, SpC3, functions as an opsonin. J Exp Biol 207: 2147–2155.

Clare, A. S. Lumb, G. (1994): Identification of haemocytes and their role in clotting in the blue crab *Callinectes sapidus*. Mar. Biol. 118(4): 601-610.

Coles, E.H. (1986): Veterinary Clinical Pathology. W.B. Saunders, Philadelphia, PA, USA, pp.1-42.

Comesaña, P., Casas, S.M., Cao, A., Abollo, E., Arzul, I., Morga, B., Villalba, A., (2012): Comparison of haemocytic parameters among flat oyster *Ostrea edulis* stocks with different susceptibility to bonamiosis and the Pacific oyster *Crassostrea gigas*. J. Invertebr. Pathol. 109, 274–286.

Cornick, J.W., Stewart, J.E., (1973): Partial characterization of a natural agglutinin in the hemolymph of the lobster, *Homarus americanus*. J. Invertebr. Pathol. 21, 255–262.

Dalmo, R. A, K. Igebregstsen, J. Bogwald (1997): Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). J. Fish Dis. 20, 241-273.

: Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. .Hydrobiologia. 595 (1): 275–286

Destoumieux, D., Bulet, P., Loew, D., VanDorsselaer, A., Rodriguez, J. Bachere, E. (1997): Penaeidins, a new family of antimicrobial peptides isolated from the shrimp *Penaeus vannmei*. J. Biol. Chem. 272: 28398-28406

Don-Pedro K.N., Oyewo E.O., Otitoloju A.A. (2004): Trend of heavy metals concentrations in Lagos Lagoon ecosystem, Nigeria. West Afr. J. Appl. Ecol., 5: 103–114.

Dove, A.D.M., Allam, B., Powers, J.J., Sokolowski, M.S. (2005): A prolonged thermal stress experiment on the American lobster. J. Shellfish Res. 24: 761– 766.

Ekanem A.P., Victor O. Eyo, Imaobong E. Ekpo, Blessing O. Bassey (2013): Parasites of Blue Crab (*Callinectes amnicola*) in the Cross River Estuary, Nigeria. International Journal of Fisheries and Aquatic Studies 2013; 1 (1): 18-21

Figueroa, Y.G.; Blank, V.; Beckman, B.S.; Alam, J. J.(2001): Biol. Chem. 276, 27018-27025**George G.B. Parker K. (2003):** Understanding the complete blood count with differential. J. Perianesth Nurs. 19:96-114.

Giro´ n-Pe´ rez, M.I., Montes-Lo´ pez, M., Gracı´a-Ramı´rez, L.A., Romero-Ban˜uelos, C.A., Robledo-Marenco, M.L., (2008): Effect of sub-lethal concentrations of endosulfan on phagocytic and hematological parameters in Nile tilapia (*Oreochromis niloticus*). Bull. Environ. Contam. Toxicol. 80, 266–269.

Gupta R.C. (2012): Veterinary Toxicology, Second Edition: Basic and Clinical Principles. ISBN-13: 978-0123859266. ISBN-10: 0123859263.

Hagerman, L. (1983): Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. Mar. Biol. 77:11–17

Handy R.D., Depledge, M.H. (1999): Physiological responses: their measurement and use as environmental biomarkers in ecotoxicology. Ecotoxicology 8,329-349.

Hardy, W. B., (1892): The blood corpuscles of the crustacea, together with a suggestion as to the origin of the crustacean fibrin-ferment. J. Physiol., 13: 165-190.

Hargis, W. J. Jr.. D. E. Zwerner, D. A. Thoney, K. L. Kelly, J. E. Warin-ner III.(1989): Neoplasms in mummichogs from the Elizabeth River. Virginia. / Aquat. Anim. Health 1 :1 65-1 72.

Hose, J.E., Martin, G.G. (1989): Defense functions of granulocytes in the ridgeback prawn, *Sicyonia ingentis.* Journal of Invertebrate Pathology 53(3):335-346

Hose, J.E., Martin, G.G., Gerard, A.S. (1990): A decapod hemocyte classification scheme intergrating morphology, cytochemistry and function. Biol. Bull. 178:33-45

Huggins, A.K., K.A. Munday (1968): Crustacean metabolism. Adv. Comp. Physiol. Biochem., 3t271-378.

Jain N C. (1986): Schalms Veterinary Haematology, 4th Edn.Lea and Febiger, 600 Washington square, Philadelphia, PA 19106-4198, USA.pp 30-34.

Jiravanichpaisal, P., Söderhäll, K. and Söderhäll, I. (2006): Characterization of white spot syndrome virus replication in in vitro-cultured haematopoietic stem cells of freshwater crayfish, *Pacifastacus leniusculus*. J. Gen. Virol. 87:847-854

Johansson, M.W., Lind, M.I., Holmbald, T., Thornqvist, P.O. and Soderhall, K. (1995): Peroxinectin, a novel cell adhesion protein from crayfish blood. Biochem. Biophys. Res. Comm. 216: 1079–1087.

Johnson, P.T., (1980): Histology of the Blue Crab, *Callinectes sapidus*. Praeger Publishers, New York, USA

Jones, S. R. M. (2001): The occurrence and mechanisms of innate immunity against parasites in fish. Dev. Comp. Immunol. 25, 841-852.

Klerks P.L., Weis J.S. (1987): Genetic adaptation to heavy metals in aquatic organisms: a review. Environ Pollut 45:173–205

Kutz S.J., Hoberg E.P., Nagy J., Polley L., Elkin B. (2004): Emerging parasitic infections in arctic ungulates. Integr. Comp. Biol. 44:109–118

Landsberg, J. H., B. A. Blakesley, R. O. Reese, G. McRae, P. R. Forstchen. (1998): Parasites offish as indicators of environmental stress. Environm. Monitor. Assess. 51:211-232.

.**Lawal-Are A.O. (2010)**: Reproductive Biology of the Blue Crab, *Callinectes amnicola* (De Rocheburne) in the Lagos Lagoon, Nigeria. Turkish Journal of Fisheries and Aquatic Sciences 10: 1-7.

Lawal-Are A.O., Kusemiju K (2010): Effect of salinity on survival and growth of blue crab, *Callinectes amnicola* from Lagos Lagoon, Nigeria. J Environ Biol 31: 461-464

Lawson, E. O., Oloko, R.T. (2013): Growth patterns, Sex ratios and Fecundity estimates in Blue Crab (*Callinectes amnicola*) from Yewa River, Southwest Nigeria.

Li, C., J.D. Shields (2007): Characterization and Primary Culture of Hemocytes from the Blue Crab, *Callinectes sapidus*. In Cai, S.L. (ed). 5th World Chinese Symposium for Crustaecean Aquaculture. Ocean Press, Beijing, China. Transactions of the Chinese Crustaecean Society 5: 25-35

Li C, Shields J.D., Ratzlaff R.E., Butler M.J. IV (2008): Pathology and hematology of the Caribbean spiny lobster experimentally infected with *Panulirus argus* virus 1 (PaV1). Virus Res 132:104–113.

Liu, C.H., Cheng, W., Kuo, C.M. and Chen, J.C. (2004): Molecular cloning and characterization of a cell adhesion molecule, Peroxinectin from the white shrimp *Litopenaeus vannamei*. Fish Shellfish Immunol. 17: 13–26.

Macpherson E (2002): Large-scale species-richness gradients in the Atlantic Ocean. Proc Biol Sci 269: 1715-1720.

Manjula, P.L., Rahman, K. Abraham, T.J. (1997): Haemocyte classification and differential counts in the Indian spiny lobster. *Panulirus homarus* (Linnaeus). J. Aquacult. In the Tropics 12(2): 113-121

Matozzo V. Martin, M. G. (2010): First cytochemical study of haemocytes from the crab Carcinus aestuarii (Crustaecean:Decapoda). European Journal of Histochemistry 54(1): 44-49

Mc Donald, D. G., Wood, C. M. (1981): Branchial and renal acid and ion fluxes in the rainbow trout, Salmo gairdneri, at low environmental pH. J. Exp. Biol. 93, 101–118.

Mohankumar, K., Ramasamy, P., (2006). Activities of membrane bound phosphates,transaminases and mitochondrial enzymes in white spot syndrome virus infected tissues of *Fenneropenaeus indicus*. Virus. Res. 118, 130–135.

Moody F. O., Akinwande A. A., Adewunmi A.A. (2013): Bioaccumulation of some heavy metals and the haematological indices in some selected freshwater fishes in river Manyara, Nigeria International Journal of AgriScience 3(8): 602-607

Moss D.W., Henderson A.R., Kochmar J.F. (1986): *Enzymes: principle of diagnostic enzymology and the aminotransferases.* In: Tietz N.W. (ed). Textbook of clinical chemistry. Saunders, Philadelphia, pp663-678.

Mukut S., Ran S., Xiu L.G., Brian L.F., Ellen T.M., Virginia J.S. , Eric P.C., John E.M. (2001). Early detection of radiation-induced glomerular injury by albumin permeability assay. Bioone.155 (3):474-480.

Muthu M.S., Laxminarayana A. (1977): Induced maturation and spawning of Indian penaeid prawns. Indian Journal of Fisheries 24.

Nayan, P., R.N. Prasad, S. Paul S. Besra (2010): Ecology and Haematology of Freshwater Crab, *Sartoriana spinigera*, Wood Mason (1871), with Special Reference to Haemocytes Classification and its Differential Count. The Bioscan 2:349-356

Noga, E.J., Engel, D.P., Arrol, T.W., Mckenna, S. Davidian, M. (1994): Low serum antibacterial activity coincides with increased prevalence of shell disease in blue crabs *Callinectes sapidus*. Dis. Aquat. Org. 19:121-128

Ochang S.N, Fagbenro O.A., Adebayo O.T. (2007): Growth performance, Body composition, haematology and Product quality of the African Catfish (*Clarias gariepinus*) fed diets with Palm oil.

Oksuz, A., A. Ozilmaz, M. Aktas, G. Gercek, J. Motte (2009): A Comparative Study on Proximate, Mineral and Fatty Acid Compositions of Deep Seawater Rose Shrimp (*Parapenaeus longirostris*) and Red Shrimp (*Plesionika martia*). J. Anim. Vet. Adv., 8 (1): 183-189

Omari, S. A., Martin, G. G. Hose. J. E. (1989): Mophology of haemocyte lysis and clotting in the ridgeback prawn, *Sicyonia ingentis*. Cell Tissue Res. 255: 117-123.

Oronsaye, J. A. O. (1997): Ultrastructural changes in the gills of the stickleback, *Gasterosteus aculeatus* (L.) exposed to dissolved cadmium in hard and soft water. Journal of Aquatic Sciences, 12: 59 – 66.

Oronsaye, J. A. O., Brafield, A. E. (1984): The effects of dissolved cadmium on the chloride cells of the gills of the stickleback, Gasterosteus acculeatus (L.) Journal of Fish Biology, 26: 253 – 258.

Oyebande, L., Obot, E. O., Bdiliya, H. H. (2003): *An inventory of wetlands in Nigeria*. Report prepared for World Conservation Union–IUCN, West African Regional Office, Ouagadougou, Burkina Faso.

Paterson, B.D., Spanoghe, P.T.,(1997): Stress indicators in marine decapod crustaceans, with particular reference to the grading of western rock lobsters *Panulirus cygnus* during commercial handling. Mar. Freshwater Res. 48, 829–834.

Pauley G.B, Martin W. Newman, E. Gould (1975): Serum Changes in the Blue Crab, *Callinectes sapidus*, associated with *Paramoeba perniciosa*, the causative agent of Gray Crab Disease. MFR Paper 1145. Marine Fisheries Review, 37, 5-6.

Petri D., Glover C.N., Ylving S., Kolas K., Fremmersvik G., Waagbo R. Berntssen M.H.G., (2006): Sensitivity of Atlantic salmon (*Salmo salar*) to dietary endosulfan as assessed by haematology, blood biochemistry, and growth parameters. Aquatic Toxicology, 80, 207-216.

Rainbow P.S., Amiard-Triquet C., Amiard J.C., Smith B.D., Best S.L., Nassiri Y., Langston W.J. (1999): Trace metal uptake rates in crustaceans (amphipods and crabs) from coastal sites in NW Europe differentially enriched with trace metals. Mar Ecol Prog Ser 183:189–203

Ratcliffe, N. A., Rowley, A. F., Fitzgerald, S.W. Rhodes, C.P. (1985): Invertebrate immunity: basic concepts and recent advances. Int. Rev. Cytol. 97, 183-350

Rej R (1989): Aminotransferase in Disease, Clin. Lab. Med, 9(4):667-87.

Roch, P., (1999): Defense mechanisms and disease prevention in farmed marine invertebrates. Aquaculture 172, 125–145.

Rosa R.D., J. de Lorgeril, P. Tailliez, R. Bruno, D. Piquemal, E. Bachère (2012): A hemocyte gene expression signature correlated with predictive capacity of oysters to survive Vibrio infections BMC Genomics, 13 p. 252

Ruppert, E.E., Barnes, R.D. (1994): Invertebrate Zoology. Saunders College Publishing and Harcourt Brace Jovanovich. Orlando, Florida. 1056p.

Sawyer T.K, R.Cox, M.Higginbottom (1970): Hemocyte values in healthy blues crabs, *Callinectes sapidus* and crabs infected with the amoeba, *Paramoeba perniciosa.* J. Invertebr. Pathol. 15:440-446

Schmitt, A.S.C., Uglow, R.F., (1997): Haemolymph constituent levels and ammonia efflux rates of *Nephrops norvegicus* during emersion. Mar. Biol. 127, 403–410.

Scott, G.R.,, Sloman, K.A., (2004): The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology 68, 369-392.

Shields, J.D., Behringer, D.C., (2004): A pathogenic herpes-like virus in the Caribbean spiny lobster, *Panulirus argus*, from the Florida Key. Dis. Aquat. Org. 59, 109–118.

Smith, D. M., W. Dall. (1982): Blood protein, blood volume and extracellular space relationships in two *Penaeus* spp. (Decapoda: Crustacea). Journal of Experimental Marine Biology and Ecology 63:1-15.**Söderhäll, K. Cerenius L. (1992):** Crustaecean Immunity. Annual Rev. Fish Dis. 2, 3-23

Song, Y.L., Yu, C.I., Lien, T.W., Huang, C.C., Lin, M.N., (2003): Hemolymph parameters of Pacific white shrimp (*Litopenaeus vannamei*) infected with Taura syndrome virus. Fish Shellfish Immunol. 14, 317–331.

Sox H. C. Jr, Liang M. H. (1986): The erythrocyte sedimentation rate, guideline for rational use Ann. Int. Med. 104 515-23

Stewart, J. E., J. W. Cornick, J. R. Dingle. (1967): An electronic method for counting lobster (*Homams americanus* :Milne Edwards) hemocytes and the influence of diet on hemocyte numbers and hemolymph proteins. Can. J. Zool. 45:291-304.

Sritunyalucksana, K. Wongsuebsantati, K., Johansson, M.W. and Söderhäll, K. (2001): Peroxinectin, a cell adhesive protein associated with the proPO system for the black tiger shrimp, *Penaeus monodon*. Dev. Comp. Immunol. 25:353-363

Sussarellu, R., Fabioux, C., Camacho Sanchez, M., Le Goïc, N., Lambert, C., Soudant, P., Moraga, D., (2012): Molecular and cellular response to short-term oxygen variations in the Pacific oyster *Crassostrea gigas*. J. Exp. Mar. Biol. Ecol. 412, 87e95.

Tavares-Dias M, Ruas de Moraes F, Onaka E.M., Bonadio Rezende P.C. (2007): Changes in blood parameters of hybrid tambacu fish parasitized by *Dolops carvalhoi* (Crustacea, Branchiura), a fish louse. Veterinarski Arhiv 77:355-363

Taylor, E. H.(1989): *Clinical Chemistry*. New York: John Wiley and Sons. pp. 4, 58–62.

Taylor, H., Waldron, F.M., (1997): Respiratory responses to air-exposure in the southern rock lobster, *Jasus edwardsii*, Hutton Decapoda: Palinuridae . Mar. Freshwater Res. 48, 889–897.

Tripathi N.K., Latimer K.S., Gregory C.R., Ritchie B.W., Wooley R.E., Walker R.L. (2005) Development and evaluation of an experimental model of *Cutaneous columnaris* disease in Koi *Cyprinus carpio*. J Vet Diagn Investig 17(1):45-54

Udoh J. P., Nlewadim A. A., (2011): Population characteristics of the swimming crab *Callinectes amnicola* De Rocheburne, 1883 (Crustacea, Brachyura, Portunidae) in the Qua Iboe River estuary, Nigeria. AACL Bioflux 4(3):412-422.

United States Environmental Protection Agency USEPA (2012): Water: Monitoring and Assessment; Sampling Methods and Analysis: Macroinvertebrates and Amphibians.

Valko M, Morris H, Cronin M.T.D. (2005): Metals, toxicity and oxidative stress. Curr. Med. Chem. 12:1161–1208.

Vargas-Albores, F., (1995): The defense system of brown shrimp *_Penaeus californiensis*.: humoral recognition and cellular responses. J. Mar. Biotechnol. 3, 153–156.

Vargas-Albores, F., Jime´nez-Vega, F., Yepiz-Plascencia, G., (1997): Purification and comparison of b-1,3-glucan binding protein from white shrimp *_Penaeus Íannamei*.. Comp. Biochem. Physiol. 116B, 453–458.

Velisek, J., Svobodova, Z., Piackova, V., Sudova, E.,(2009). Effects of acute exposure to metribuzin on some hematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). Bull. Environ. Contam. Toxicol. 82, 492–495.

Vermeer, G.K., (1987): Effects of air exposure on desiccation rate, haemolymph chemistry, and escape behavior of the spiny lobster, *Panulirus argus*. Fish. Bull. 85, 45–52.

Vijayavel, K. C. Anbuselvam, M.P. Balasubramanian, V. Deepak Samuel, S. Gopalakrishnan (2006): Assessment of biochemical and enzyme acvtivites in the estuarine crab *Scylla tranquebarica* from naphthalene contaminated habitants. Ecotoxicology 15:469-476

Westergren, A. (1957): "Diagnostic tests: the erythrocyte sedimentation rate range and limitations of the technique". Triangle 3 (1): 20–5.

World Health Organization, WHO (1996): Guidelines for Drinking-Water Quality, second ed.Health Criteria and Other Supporting Information, Vol. 2 WHO, Geneva, Switzerland.

Wileman, D.A., Sangster, G.I., Breen, M., Ulmestrand, M., Soldal, A.V., Harris, R.R., (1999): Roundfish and Nephrops survival after escape from commercial fishing gear. Final report to the EC FAIR-CT95-0753 .

Wilkie, M. P. Wood, C. M. (1991): Nitrogenous waste excretion, acid–base regulation and ionoregulation in rainbow trout (*Oncorhynchus mykiss*) exposed to extremely alkaline water. Physiol. Zool. 64, 1069–1086.

Wood, C.M., Simons, B.P., Mount, D.R. Bergman, H.L. (1988): Physiological evidence of acclimation to acid/aluminium stress in adult brook trout *(Salvelirzus fontinal). 2.* Blood parameters by cannulation. Can. J. Fish. Aquat. Sci. 45, 1587-1596.

Wright, P. A.,Wood, C. M. (1985): An analysis of branchial ammonia excretion in the freshwater rainbow trout: effects of environmental pH change and sodium uptake blockade. J. exp. Biol. 114, 329–353

Wu, J.L., Nishioka, T., Mori, K., Nishizawa, T., Muroga, K., (2002): A timecourse study on the resistance of *Penaeus japonicus* induced by artificial infection with white spot syndrome virus. Fish Shellfish Immunol. 13,391–403.

Xu, G.; Zhou, G.; Jin, T.; Zhou, T.; Hammarstrom, S.; Bergh, A.; Nordberg, G. (1999): Biometals 12, 131–139.

Yildiz, H.Y., Atar, H.H. (2002): Haemocytes classification and differential counts in the freshwater crab, *Potamon fluviatilis* Turk. J. Vet. Anim. Sci. 26: 403-406

Yoganandhan, K., Thirupathi, S., Sahul Hameed, A.S.,(2003): Biochemical, physiological and hematological changes in white spot syndrome virus-infected shrimp, *Penaeus indicus*. Aquaculture 221, 1–11.

Zhou, X.W., Chen,Q.X., Chen, Z.,He Z.Q., Zhou H.M. (2000): Effects of oxodiperoxovanadate (V) complexes on the activity of green crab (Scylla serrata) alkaline phosphatase. Biochemistry 65(12); 1424-1428.

.