PHYSIOLOGICAL RESPONSES TO HYPER-SALINE WATERS IN NECORA PUBER (VELVET CRAB)

Diaa B. Al-Azhary[(1)], Nagy S. Tawfek[(1)], Noha M. Meligi[(1,2)] and Michael Elliott [(2)]

1. Zoology Department, Faculty of Science, Minia University 61519, Minia, Egypt
2. Institute of Estuarine & Coastal Studies (IECS), University of Hull, Hull, HU6 7RX, United Kingdom

Background: Brine (hypersaline solution) is increasingly discharged into coastal areas as the result of solute mining and desalination schemes. Therefore, more information on physiological and biochemical responses of marine organisms to those solutions is needed to clarify their tolerance to salinity changes. However, this information is lacking, so the goal of the present study was to assess how velvet crab responds physiologically to such solutions. **Methods:** Adult crabs were placed in large, plastic tanks filled with 35 psu water, 10 crabs were removed for blood collection. At the completion of sampling, the salinity was raised to 40 psu and held at 40 psu for 4 days and 10 more crabs were sampled. This basic procedure was repeated at 50, 55 and 60 psu and there were a control group at salinity 35 for each experimental group. **Results:** At higher salinities (55 and 60 psu), the levels of haemolymph total proteins, glucose, lactate and total ammonia increased significantly with respect to the control groups. Moreover, at higher salinities, haemolymph ion levels rose significantly. In contrary, significant decreases of haemolymph *p*H and haemocyanin were observed at higher salinities. **Conclusion:** Salinity stress induced physiological and biochemical changes in the haemolymph of velvet crabs, therefore, this study gives attention to the importance of finding ways for determine the optimal dilution of hypersaline brine discharges into water to minimize any impact on its communities.

Keywords: Hypersalinity, Salinity, Necora puber, Biochemical responses

INTRODUCTION

Salinity and its potential variations are among the main factors influencing reproduction, dispersal and recruitment of organisms in marine, coastal and estuarine habitats.[1] Hypersaline solutions (i.e., those which have considerably greater salinity than that of ambient seawater) are now discharged into coastal areas from several types of industrial process. In particular, due to the increasing demand for fresh water, the number of desalination plants and the discharge of brine have increased in recent years. As another source, solute mining, such as the creation of gas storage cavities in salt strata adjacent to coasts, also produces hypersaline effluent.[2] This is a major source of brine effluent and a number of underground storage facilities have been proposed or are being constructed around the British coast (e.g., Aldbrough on the Holderness coast and Preesall Saltfield in Lancashire). Excavation is carried out by dissolution of the underlying salt deposits through the injection of seawater, resulting in the production of a hyper-saline effluent which is then discharged into the adjacent coastal waters.[2] This effluent can have a salinity of 280, i.e., eight times ambient sea water.

Salinity critically controls the distribution, reproduction, dispersal and recruitment of marine and estuarine organisms and brine discharges therefore have the potential to adversely affect marine communities.[2] These impacts could be through direct mortality, reduced reproductive success, individual growth, population growth and increased susceptibility to other pollutants; or indirect whereby an impact on prey species could affect higher trophic levels and impact on community structure and ecosystem functioning.[3] Ultimately, this could lead to negative impacts on the conservation status of marine and estuarine areas and on the status of commercially important species. Vernberg and Vernberg[4] reported that salinity may affect the duration of larval development, larvae of the stenohaline crab Hepatus epheliticus develop at a slower rate in salinity, 25% compared to that in salinties 30 and 35% and the duration of complete development of Callinectes sapidus is longer at the higher salinity 32%, but the megalops develops more slowly at lower than higher salinities. Moreover, Homarus americaus larvae develop slowly in salinities both above and below 25%.

Historically studies of salinity tolerance have focussed on the effects of low and fluctuating salinity, within the normal range of ambient seawater, on various marine and estuarine organisms or the effects of hypersalinity on organisms already adapted to such conditions.[5] Until recently, very few studies have addressed the effects of hypersalinity on marine organisms which are not usually exposed to abnormally high salinity. Therefore, the present study aims to investigate the physiological changes in velvet crabs exposed to hypersaline solutions.

MATERIALS AND METHODS

Adult specimens of Necora puber (>6 cm carapace width) were purchased from a local merchant in Bridlington, England. Crabs were held in Hull in plastic tanks supplied with re-circulating, aerated seawater (salinity of 35±1, 7 °C ±1), and a 12:12 h light:dark photoperiod to promote natural behaviour and reduce stress. A biological filter (to keep ammonia levels low) was constructed for each re-circulation system and consisted of layers of charcoal and

absorbent pellets. Following collection, animals were held for at least a week prior to experiments. This time was allowed to ensure temperature acclimation and recovery from stresses caused by transportation and handling. Salinity was monitored regularly with a refractometer and adjusted as needed.

Experimental series and protocol

Adult crabs were placed in large, plastic tanks filled with 35 psu (practical salinity unit) water (the same salinity as the water in which they were being held for at least a week) and allowed to recover for 4 days. To start the test, 10 surviving crabs were removed for blood collection. At the completion of sampling, the salinity was raised by adding ocean salts to 40 psu. Crabs were held at 40 psu for 4 days (96 hrs) and 10 more crabs were sampled. This basic procedure was repeated at 50, 55 and 60 psu and there were a control group at salinity 35 for each experimental group.

Collection and Storage of Samples

Haemolymph samples were obtained by either capillary puncture using a glass pipette or a hypodermic needle (21 gauge) through the arthrodial membrane at the base of the 4^{th} or 5^{th} pereiopod (pre-branchial haemolymph). Prior to puncturing, the sampling area was thoroughly dried with absorbent paper. Volumes of 0.5–1.0 ml of haemolymph were obtained from each individual. The time taken for haemolymph collection was kept between 20–30 sec in order to minimize stress to the animals. This time restriction was important in experiments involving analysis of glucose to avoid a hyperglycemic response to stress.[6] The haemolymph was then subdivided into aliquots depending on the subsequent analysis. Analysis of glucose and lactate required deproteinisation which was necessary to prevent changes to the haemolymph following sampling and to prevent the interference of haemocyanin at diagnostic wavelengths. Haemolymph samples (200 µl) were diluted 1:1 (v:v) with ice cold perchloric acid (6%), then centrifuged at 8,000 rpm for 5 min. Haemplymph glucose and lactate were determined using colourimetric glucose oxidase kits (Sigma, Cat. 510) and a colourimetric kit from Trinity Biotech (Cat. 735) respectively.

Analysis of total ammonia required haemolymph dilution before analysis. Haemolymph samples (500 µl) were diluted with 500 µl of distilled water and centrifuged (8,000 rpm, 5 min) to prevent coagulation. A further dilution of between 4 and 10 times was necessary for total ammonia (TA) analysis, prior to analysis. Taking multiple haemolymph samples from individuals was avoided where possible. The samples were stored frozen in airtight containers until used. Total ammonia (TA) assays of haemolymph was processed using the flow injection gas diffusion technique (FIGD).[7] Haemolymph protein concentrations were determined using a hand-held clinical refractometer (ATAGO, Cat. No. 2734). Measurements of Haemolymph *p*H were made immediately using a Jencon PHM2 *p*H meter and a Whatman protein-resistant microelectrode. Haemolymph haemocyanin concentrations were determined according to Nickerson and Van Hold [8]. Inductively coupled plasma-optical emission spectrometry (ICP-OES) was used to determine the ionic composition of the haemolymph.

Statistical analyses

The data are expressed as Means±Standard Error. The number of animals measured (n) is given in parenthesis. Data were tested for normality and equality of variances, prior to testing for one way ANOVA. If significant differences were detected, the post hoc tests, Turkey (equal number of observations in each group) or Scheffé's test (unequal number of observations in each group) were used to identify where the differences were. However, the data of unequal variance were compared with the non-parametric Kruskal Wallis or Mann-Whitney U. Significant differences were made at $p<0.05$ level. Data analyses and presentation of graphs were performed using SPSS (Version 15.0), Sigma Plot (Version 8.0) and Microsoft Excel (XP).

RESULTS

Exposure to hyper-saline solutions at 55 and above caused significant decrease in the *p*H values ($p<0.05$) compared with the control groups (Table-1, Figure-1). Haemolymph total protein values were also affected at salinities 55 and 60 psu which significantly ($p<0.05$) increased against control groups (Table-1, Figure-2). Exposure to elevated salinity also led to significant ($p<0.05$) increases in the haemolymph glucose and lactate levels at salinities 55 and 60 psu (Table-1, Figures-3, & 4). Haemolymph haemocyanin concentrations were not significantly different to the controls until salinity reached 55 then there were significant decrease (Turkey test, $p<0.05$) with respect to controls (Table-1, inset in Figure-5). Haemolymph total ammonia concentrations in Necora puber increased progressively during the experiment. Significant increase (Turkey test, $p<0.05$) occurred in the 55 and 60 psu groups compared to the control groups (Table-1, Figure-5).

The concentration of the major ions in the haemolymph samples are given in Table-2. The mean sodium concentrations followed the same trend as hyper saline waters concentrations, increasing over time following salinity change. The mean sodium concentrations at salinity 45 and higher were significantly increased (Turkey test, $p<0.05$) compared with the control groups (Figure-6). Both potassium and magnesium concentrations (Figure-7) increased directly with salinity increased to reach values that were significantly higher (Turkey test, $p<0.05$) than the control groups. The mean calcium concentrations at salinities 50, 55 and 50 had increased significantly (Turkey test, $p<0.05$) compared with the control groups (Figure-7). Finally, copper concentrations during the experiment did not attain significance in any on the groups ($p=1.00$) (Figure-7).

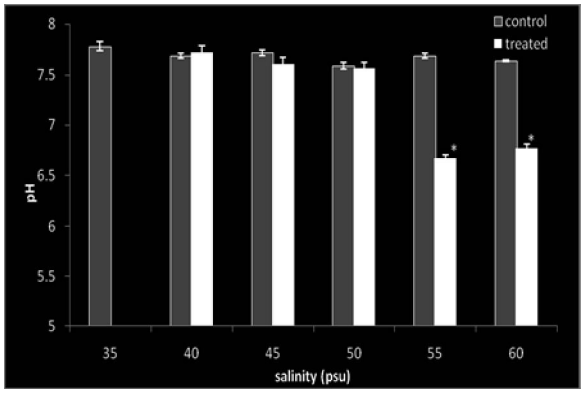

Figure-1: The effect of salinity on haemolymph *p*H. (Means±SE) (n=10). Asterisks indicate significant differences from values at control group.

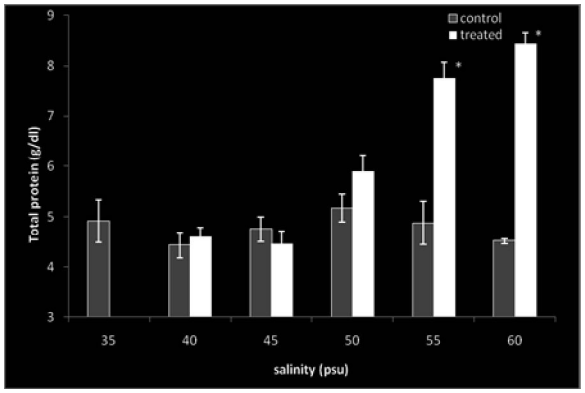

Figure-2: The effect of salinity on haemolymph total protein levels. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups.

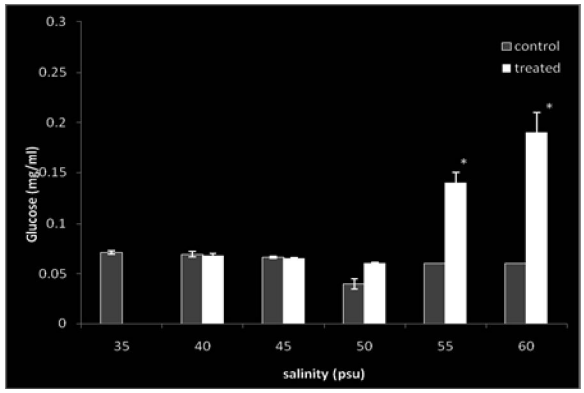

Figure-3: The effect of salinity on haemolymph glucose levels. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups.

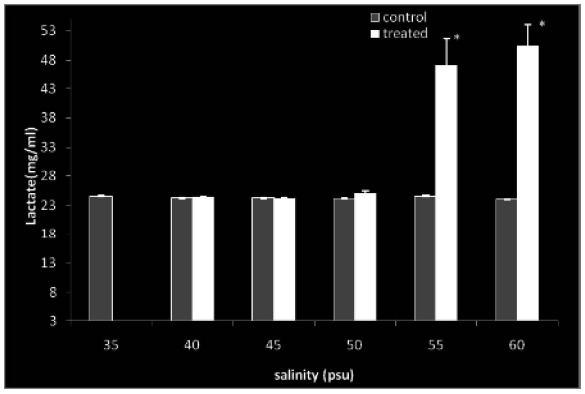

Figure-4: The effect of salinity on haemolymph lactate levels. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups.

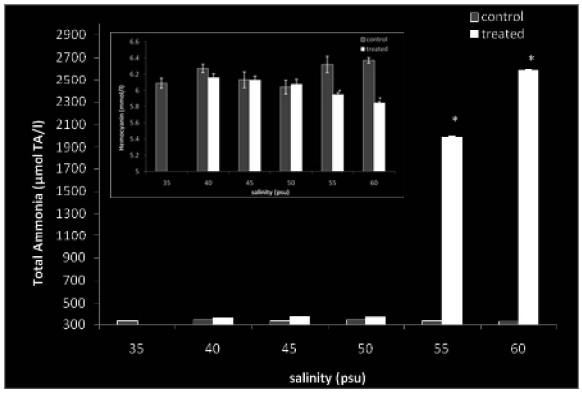

Figure-5: The effect of salinity on haemolymph total ammonia levels. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups. The inset Fig. represents the effect of salinity on haemolymph haemocyanin levels.

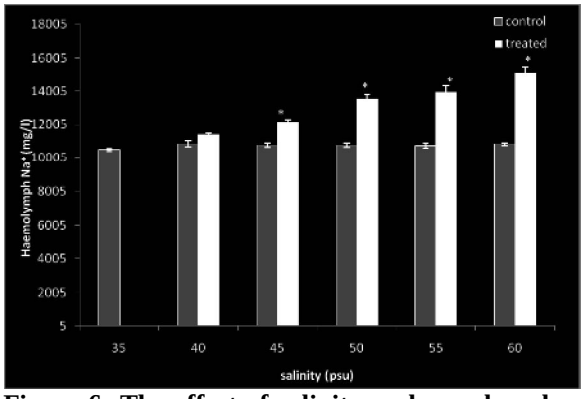

Figure-6: The effect of salinity on haemolymph Na^+ concentrations. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups.

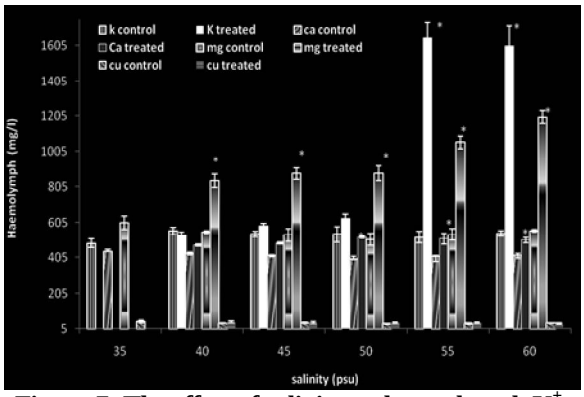

Figure-7: The effect of salinity on haemolymph K^+, Ca^{2+}, Mg^{2+} and Cu^{2+} concentrations. (Means±SE) (n=10). Asterisks indicate significant differences from values at control groups.

DISCUSSION

As shown from the results of the present study, salinity increase induced different biochemical changes in the haemolymph of velvet crabs exposed to higher salinities. Haemolymph lactate and glucose concentrations showed significant increases at salinity 50 and above. It is well known that, the exposure to elevated salinity led to a significant increase in the rate of oxygen consumption, for example mollies at 60 psu consumed oxygen at a rate over 40% higher than those at 35 psu and when salinity was raised to 80 psu rate of oxygen consumption climbed to a level about 130% higher than at 35 psu.[9]

Table-1: Effect of salinity on different biochemical parameters in haemolymph of Necora puber. (Mean±SE) (n=10)

Salinity Concentrations (psu)	Mean Haemolymph *p*H	Mean Total Protein (g/dl)	Mean Glucose (mg/ml)	Mean Lactate (mg/dl)	Mean Haemocyanin (mmol/l)	Mean Total Ammonia (µmol TA/L)
Control	7.78± 0.04	4.92± 0.42	0.07± 0.001	24.55± 0.14	6.09± 0.06	337.39± 39.12
Control	7.72±0.06	4.44± 0.26	0.069± 0.002	24.23± 0.13	6.27± 0.05	344.00±60.54
40/treated	7.69± 0.02	4.61± 0.18	0.07± 0.002	24.37± 0.14	6.16± 0.05	364.31±36.64
Control	7.72± 0.03	4.46± 0.27	0.065± 0.01	24.24± 0.13	6.13± 0.09	338.00± 73.31
45/treated	7.61± 0.06	4.76± 0.25	0.06± 0.001	24.21± 0.12	6.13± 0.05	376.04± 49.52
Control	7.59± 0.03	5.18± 0.28	0.04± 0.005	24.11± 0.11	6.04± 0.08	343.47± 55.82
50/treated	7.57± 0.06	5.90± 0.32	0.06± 0.001	25.02± 0.41	6.08± 0.06	370.48± 46.79
Control	7.69± 0.03	4.88± 0.43	0.060± 0.00	24.53± 13	6.32± 0.09	337.00± 67.63
55/treated	6.67± 0.04	7.76± 0.32	0.14±0.01	47.08± 4.57	5.95± 0.02	1993.15± 393.17
Control	7.64± 0.01	4.52± 0.05	0.060± 0.00	24.05± 0.06	6.37± 0.03	333.27± 62.04
60/treated	6.77± 0.04	8.44± 0.21	0.19± 0.02	50.46± 3.54	5.85± 0.01	2586.82± 298.70

Table-2: The effect of salinity on haemolymph ionic compositions levels in Necora puber. (Mean±SE) (n=10)

Salinity Con. (psu)	Mean Na^+ (mg/l)	Mean K^+ (mg/l)	Mean Ca^{2+} (mg/l)	Mean Mg^{2+} (mg/l)	Mean Cu^{2+} (mg/l)
Control	10482.32±80.64	478.55±23.72	441.27±11.28	603.29±38.26	45.97±7.39
Control	10862.13±171.59	556.42±20.87	429.22±8.26	550.08±9.14	35.99±3.32
40/treated	11422.95±85.77	532.64±16.37	476.53±6.85	838.58±37.88	42.58±5.62
Control	10769.08±142.72	538.88±16.24	417.35±4.50	533.36±34.81	38.42±4.74
45/treated	12137.14±157.72	586.29±14.98	487.91±6.23	878.01±30.69	39.66±5.52
Control	10763.08±142.72	537.91±42.84	399.80±13.92	509.36±34.81	33.19±1.38
50/treated	13548.93±221.36	626.05±25.80	522.89±5.28	880.44±41.07	38.11±4.86
Control	10749.75±138.44	521.95±33.31	412.40±5.07	536.45±32.72	34.34±3.19
55/treated	13962.42±373.93	1647.27±86.81	513.38±30.60	1057.74±35.94	37.65±3.87
Control	10837.10±82.99	544.62±14.94	418.40±8.97	559.21±7.37	36.69±0.76
60/treated	15121.67±341.89	1602.26±110.65	506.79±15.39	1199.54±37.03	33.91±3.87

Chen and Fang[10] also showed that there was a respiratory depression in Metapenaeusensis after a salinity change and Clark[11] observed a decrease in the respiration rate of shrimp after a salinity shock. Therefore, the increase in the rate of oxygen consumption and severe tissue oxygen deprivation (in the previous studies) served to lead to lactic acidosis which is characterized by weakness and stupor. A haemolymph acidosis (revealed by an accumulation of lactate accompanied by a hyperglycaemia) indicates a switch to anaerobic metabolism, and has been reported for a range of marine crustaceans following periods of exercise or emersion.[12] It is well known that when the oxygen supply is fully utilized, additional energy is released by metabolizing glycogen to pyruvate and lactate. So, anaerobic metabolism consumes lots of glycogen and produces lots of lactate. Thus, two of the main features of anaerobic metabolism are glycogen depletion and lactic acidosis. This is consistent with results of Lorenzon *et al*[13] who reported that hyperglycaemia is induced by a release of crustacean hyperglycaemic hormone in response to stress leading to an increased glycogen utilisation, and has been reported for a number of crustaceans.

According to Storey and Storey when ambient oxygen falls below a critical level, ATP production may be replaced by a strong metabolic depression.[14] This will ration the use of carbohydrates and maximise survival

time. However, reduced activity and metabolic rate will also depress some glucose utilisation which may induce hyperglycaemia as suggested by Schmitt and Santos.[15] So, the combination of excess glucose production and low glucose utilization in the body raises levels of the haemolymph glucose in the present study.

Protein concentration in haemolymph has been recognised as an important health indicator for crustaceans. In this study the haemolymph total protein values were increased significantly at salinities 55 and 60 psu which is possibly a reflection of the high dehydration of the tissue of velvet crabs. This is supported by the findings of Hoeger and Abe,[16] who found that the total protein content of the body wall of Nereis japonica following adaptation to higher salinity was very high indicating dehydration of the tissue.

There was a decrease in *p*H values with increasing salinity and this was accompanied by increasing lactate levels (metabolic acidosis). Acidosis was probably also related to respiratory difficulties which induces CO_2 accumulation (respiratory acidosis). Investigation of Macrobrachium olfersii, Callinectes sapidus and Chasmognathus granulate[17] showed an activity decrease in gills following adaptation to increased salinities. Such events may cause also CO_2 accumulation which, with increased lactate levels, may result in a blood acidosis. As a result of decreasing *p*H values, poor health, morbidity and mortality have been reported to be well correlated to low *p*H values in crustaceans.[18]

In this study, ammonia was found to accumulate in the haemolymph of Necora puber. This accumulation was significantly higher at salinities 55 and 60 psu. Ammonia is the primary nitrogenous waste in aquatic crustaceans, and is excreted continuously via the gills. Ammonia excretion is a continuous process, and its rapid elimination into the environmental sea water protects all the body compartments from the toxic effects of high ammonia levels.[19] Ammonia is generally accepted as being toxic to crustaceans but few studies have been made on the mechanisms and levels of its toxicity for any crustacean species. Sub lethal concentrations of haemolymph TA have been reported to affect growth, oxygen consumption, Na-K-ATPase activities, and osmoregulation in the shrimp, Penaeus monodon which was weakened and eventually died when 1.4-1.5 mmol accumulated in the haemolymph.[20] It has been suggested in a previous study that the activity of gills decreases following exposure to increased salinities and can no longer function as efficiently.[21] As the result, the ability to excrete ammonia is much reduced and accumulation of ammonia in the haemolymph as observed in the present work may occur.

This work indicated a decrease in haemocyanin levels at higher salinity concentrations. It is known that haemocyanins are large multi-subunit copper proteins composed of different subunit types and found freely dissolved in the haemolymph of arthropods and molluscs.[22] Their primary biological function is the transport of oxygen. The haemocyanin concentration in haemolymph has been described to vary under the influence of a range of factors such as gender, food availability, and other environmental factors such as ambient ammonia and dissolved oxygen. Bernasconi[23] found that a decline of *p*H value of haemolymph on haemocyanin can, result in a reduction of the total amount of oxygen delivered to the tissue, as full saturation at the gills cannot occur when blood is too acidic and may cause a decrease in haemocyanin concentration at higher concentrations.

In general at higher salinity ion levels rose more suggesting that the ability to absorb water and excrete salts would become increasingly problematic and internal ion disturbances. Other species exhibit similar patters when exposed to hyper-salinities. For example, Oreochromis mossambicus displays a very similar pattern in plasma ions levels during exposure to hyper-salinities.[24]

The results of the present investigation indicated increase of Na^+ and K^+ levels at higher salinities. It is known that the Na-K-ATPase activity in euryhaline decapods, was impacted by saline media where there is a significant decrease of the Na-K-ATPase activity during acclimation to high salinity.[17] It has been suggested that branchial Na-K-ATPase is of central importance in ion regulation by aquatic vertebrates and invertebrates which Located in the basolateral membranes of the epithelial cells of gills, and is responsible for the transport of Na^+ with K^+ from the cell into the haemolymph[25] which suggesting a disturbance in these ions might occur.

The concentrations of Mg^{2+} increased significantly from salinity 40 and higher. Walters and Uglow[26] found a negative correlation between haemolymph Mg concentrations and the apparent level of activity of different decapods species. Thus slow moving species (e.g., Lithodes maja and Maja squinado) have relatively high magnesium concentrations in their haemolymph than more active species (e.g., Nephrops norvegicus and Carcinus maenas) have much lower magnesium concentrations. Similarly, Frederich *et al*[27] demonstrated that there was a significant correlation between the scope for activity and decreasing magnesium concentrations. The results here are consistent with these findings where there was a decrease in activity and low metabolic rate with the exposure time.

The elevated Ca^{2+} in this study at higher salinities suggested that the respiratory and metabolic acidosis are compensated by the mobilisation of an internal source of buffer base ($CaCO_3$ from the exoskeleton) which elevates haemolymph Ca^{2+} levels as described in Carcinus maenas and Austropotamoobius

pallipes.[28] It is also probable that the animals in this study became dehydrated (water loss) resulting in elevated haemolymph ion concentrations including calcium.

Haemolymph copper concentration shows broad variations in relation to salinity stress, hypoxia and starvation.[29] The copper concentrations in this study did not change significantly throughout the experimental procedure. This result is in accordance with the finding that copper content in decapods crustaceans is regulated within a narrow range.[30]

CONCLUSION

Salinity stress induced metabolic abnormalities and changes in the internal environment of velvet crab, suggesting a probable reduction in respiration, growth and survival; the processes which control reproduction and distribution of this commercial species. The study, therefore, gives attention to the importance of finding ways for determine the optimal dilution of hypersaline brine discharges into water to minimize any impact on its communities.

REFERENCES

1. Anger K. Salinity as a key parameter in the larval biology of decapod crustaceans. Inv. Repr. Dev 2003;43:29–45.
2. Bérest P, Bergues J, Brouard B, Durup JG, Guerber B. A salt caven abandonment test. Int. J. Rock Mech. Min. Sci 2001;38:357–68.
3. Fleeger JW, Carman KR, Nisbet RM. Indirect effects of contaminants in aquatic ecosystems. Sci. Total Environ 2003;317:207–33.
4. Vernberg FJ, Vernberg WB. The Biology of Crustacea. Vol 7. Behavior and Ecology. London, 1983; p. 246.
5. Anger K. Salinity tolerance of the larvae and first juveniles of a semiterrestrial grapsid crab, Armases miersii (Rathbun). J. Exp. Mar. Biol. Ecol 1996;202(2):205–23.
6. Kleinholz LH, Little BC. Studies in the regulation of blood suger concentration in crustaceans. I. Normal values and experimental hyperglycaemia in Libinia emerginata. Biol. Bull 1949;96:218–27.
7. Hunter DA, Uglow RF. Moult stage-dependent variability of haemolymph ammonia and total protein in Crangon crangon (L.) (Crustacea, Decapoda). Ophelia 1993;37:41–50.
8. Nickerson KW, Van Hold KE. A comparison of molluscan and arthropod haemocyanin. I. Circular dischroism and absorption spectra. Com. Biochem. Physiol 1971;39:855–872.
9. Gonzalez RJ, Cooper J, Head D. Physiological responses to hyper-saline waters in sailfin mollies (Poecilia latipinna). Com. Biochem. Physiol 2005;142:397–403.
10. Chen LM, Fang LS. Metabolic and osmotic responses of Metapenaeus ensis (De Haan) subjected to sudden salinity change. In: Maclean, J.L.; Dizon, L.B. and Hosillos, L.V. editors. The First Asian Fisheries Forum. Manila: Asian Fisheries Society, 1986;P. 629–32.
11. Clark JV. Physiological responses of adult Penaeus semisulcatus (De Haan) to changes in salinity. Com. Biochem. Physiol 1992;101:117–9.
12. Schmitt ASC, Uglow RF. Haemolymph constituent levels and ammoni efflux rates of Nephrops norvegicus durind emersion. Mar. Biol 1997;127:403–10.
13. Lorenzon S, Giulianini PG, Ferrero EA. Lipopolysaccharide-induced hyperglycaemia is mediated by CHH release in crustaceans. Gen. Comp. Endocrinol 1997;108:395–405.
14. Storey KB, Storey JB. Metabolical rate depression and biochemical adapatation in anaerobiosis hibernation and estivation. Q. Rev. Biol 1990;65(2):145–74.
15. Schmitt ASC, Santos EA. Lipid and carbohydrates metabolism of the intertidal crab Chasmagnathus granulate (Dana, 1851) (Crustacea: Decapod) during emersion. Com. Biochem. Physiol 1993;106(2):329–36.
16. Hoeger U, Abe H. B-Alanine and other free amino acids during salinity adaptation of the polychaete Nereis japonica. Comp. Biochem. Physiol 2004;137:161–71.
17. Castilho PC, Martins IA, Bianchini A. Gill Na^+-K^+-ATPase and osmoregulation in the estuarine crab, Chasmagnathus granulate Dana, 1851 (Decapoda, Grapsidae). J. Exp. Mar. Biol. Ecol 2001;256:215–27.
18. Paterson BD. Respiration rate of the Kurma prawn, Paenaeus japonicas Bate, is not increased by handling at low temperature (12 C^+). Aquaculture 1993;114:229–35.
19. Durand F, Chausson F, Regnault M. Increases in tissue free amino acid levels in response to prolonged emersion in marine crabs: an ammonia-detoxifying process efficient in the intertidal Carcinus maenas but not in the subtidal Necora puber. J. Exp. Biol 1999;202:2191–202.
20. Chen JC, Kou YZ. Accumulation of ammonia-N excretion of Penaeus monodon exposed to ambient ammonia. Aquaculture 1993;109:177–85.
21. Pan LQ, Luan ZH, Jin CX. Effects of Na^+/ K^+ and Mg^{2+}/ Ca^{2+} ratios in saline groundwaters on Na^+-K^+-ATPase activity, survival and growth of Marsupenaeus japonicas postlarvae. Aquaculture 2006;261:1396–402.
22. Terwilliger NB. Functional adaptations of oxygen-transport proteins. J. Exp. Biol 1998;201:1085–98.
23. Bernasconi CJ. Physiological, biochemical and immunological responses of Nephrops norvegicus to current and novel post harvest treatment. PhD thesis, Hull University, 2006;p.23.
24. Sardella BA, Matey V, Cooper J, Gonzalez RJ , Brauner CJ. Physiological, biochemical and morphological indicators of osmoregulatory stress in California Mozambique tilapia (Oreochromis mossambicus x O. Urolepis hornorum) exposed to hypersaline waters. J. Exp. Biol 2004;207:1399–413.
25. Towle DW. Membrane-bound ATPase in arthropod ion-transporting tissues. Am. Zool 1984;41(4):770–80.
26. Walters NJ, Uglow RF. Haemolymph magnesium and relative heart activity of some species of marine decapod crustaceans. J. Exp. Mar. Biol 1981;55:255–65.
27. Frederich M, DeWachter B, Sartoris FJ, Pörtner HO. Cold tolerance and the regulation of cardiac performance and haemolymph distribution in Maja squinado (Crustacea: Decapoda). Physiol. Biochem. Zool 2000;73(4):406–15.
28. TaylorEW, Wheatly MG. The effect of long-term aerial exposure on heart rate, ventilation, respiratiory gas exchange and acid-base status in the crayfish Austropotamobius pallipes. J. Exp. Biol 1981;92:109–24.
29. Depledge MH, Bjerregaard P. Haemolymph protein composition and copper levels in decapod crustaceans. Mar. Environ. Res 1989;27:115–26.
30. Devescovi M, Lucu C. Growth of tissues related to haemolymph copper throughout the moult cycle of the lobster Homarus Gammarus. Mar. Ecol. Prog. Ser 2003;247:165–72.

Address for Correspondence:

Noha. M. Meligi, Department of Zoology, Faculty of Science, Minia University 61519, Minia, Egypt. Tel: +2-068-2359738, +2-011-7456335

E-mail: Nohameligi@yahoo.co.uk