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OSMOTIC AND IONIC REGULATION IN *Chasmagnathus granulata* DANA, 1851 (DECAPODA, GRAPSIDAE) DURING HYPOSMOTIC STRESS

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ABSTRACT

The dynamics of osmotic and ionic regulation, as well as possible seasonal changes, were investigated in the estuarine crab *Chasmagnathus granulata*. Osmotic and ionic concentrations (Na^+, K^+, Ca^{++} and Cl^-) of the hemolymph were determined, both during summer and winter, in animals exposed to 0, 10 and 20 ‰ salinity, at the beginning of the experiments (T 0), and 24, 72, 168 and 360 h after salinity changes. The results show that this species is capable of both short and long term hyperregulation, but there may be a necessity of long acclimation and/or acclimatization periods. The data indicate a greater capability of hyperosmotic regulation in summer, possibly related to seasonal salinity changes in the habitat. Significant alterations of the measured parameters were observed during salinity stress, suggesting that intracellular isosmotic regulation must be considered as part of the mechanisms involved in the osmotic and ionic regulation of this species.

Keywords: Osmoregulation, ionoregulation, salinity, crab, hyposmotic stress

INTRODUCTION

Crustaceans live in aquatic, semi-terrestrial and terrestrial environments. This requires special biochemical, physiological, morphological and/or behavioral adaptations to deal, among other things, with the movements of water and ions between the animals and their surroundings. The most commonly studied variables related to these osmotic and ionic adaptations in crustaceans are: the salinity at which the internal medium is isosmotic with the external medium, the degree of osmotic and ionic regulation, and the tolerated salinity range (see Mantel and Farmer, 1983 for review).

The mechanisms that interact to maintain the chemical homeostasis of the tissues can occur at two distinct levels: (1) anisosmotic regulation of the extracellular fluid, which comprises the mechanisms involved in the maintenance of the hemolymph osmotic concentration more or less independent of the osmotic concentration of the external medium and (2) isosmotic intracellular regulation, comprising the mechanisms involved on the maintenance of an osmotic equilibrium between the intra and extracellular

fluids. Both forms of regulation act as to avoid, or at least reduce, the fluxes of water and ions at the cellular level (Gilles and Pequeux, 1983).

The osmo and ionoregulatory capacities of hyper-regulating species (those that face a diffusive gain of water and loss of ions) has been attributed to permeability decreases, to both salt and water, that occur mainly in the gills, as well as to modifications in the rate of urine production and activation of mechanisms related to active uptake of salts occurring in specialized boundary epithelia, specially of the gills, gut and excretory organs (Lockwood, 1977; Kirschner, 1979; Pequeux *et al.*, 1988; Lucu, 1990).

Among the parameters that interfere with osmotic and ionic regulation in crustaceans are those related to seasonal changes. Species such as *Callinectes sapidus* (Mantel, 1967), *Hemigrapsus nudus* and *H. oregonensis* (Dehnel and Carefoot, 1965) show variations in their osmorregulatory capabilities between summer and winter. Although Kamemoto and Tullis (1972) have observed seasonal influences on the neuroendocrine control of hydromineral balance, the mechanisms involved in such modification of the osmoregulatory capacity are poorly understood.

Chasmagnathus granulata, an estuarine crab distributed along the coast of southern Brazil, Uruguay and Argentina (Boschi, 1964; Botto and Irigoyen, 1979) has been object of a variety of physiological studies (Santos and Colares, 1986; Santos *et al.*, 1987; Santos *et al.*, 1988; Schmitt and Santos, 1993a and b; among others). Although it has been characterized as an hyper/hyporegulating crab by Mañe-Garzon *et al.* (1974) and Gnazzo *et al.* (1978), nothing is known about the dynamics of the processes involved in its osmotic and ionic regulation, as well as possible modifications related to seasonal changes. The study of such aspects constitutes the main objective of the present investigation.

MATERIAL AND METHODS

The crabs, adult males of *Chasmagnathus granulata* on stage C or early D of the intermolt cycle (Drach and Tchernigovtzeff, 1967), were collected on salt marshes around the city of Rio Grande (southern Brazil), during winter (June-August) and summer (December-Frebruary), being immediately transported to the laboratory. The animals had a mean wet weight ($\pm$ SD) of 7.8 $\pm$ 1.9 g in the winter and 9.3 $\pm$ 2.4 g in the summer.

They were acclimated to laboratory conditions for 30 days in tanks with a salinity of 20 ‰ and a temperature of 20-24°C. During this period the animals were regularly fed ground beef.

After acclimation the crabs were separated in groups (see results for number of animals per group) and submitted to the experimental salinities (0, 10 and 20 ‰) during 15 days. The animals transferred to 0 ‰ in winter presented high mortality (see results), making it necessary to repeat such experiment. The temperature during the experimental period was also kept between 20-24°C.

All animals were starved since 24 h prior to the transfer to the experimental salinities.

Water for these experiments was obtained by appropriate dilution of local sea water. Salinity was determined by hand refractometry (American Optical Inc.).

Hemolymph samples were obtained immediately before the transfer of the crabs to the experimental salinities (T 0) and after 24, 72, 168 and 360 h. Water samples from the tanks were also taken at these same time intervals. Hemolymph was sampled in animals maintained at 20 ‰ with the objective of verifying the possible interference of stress (possibly caused by handling) and starvation on the osmotic and ionic regulation.

The hemolymph was drawn from the blood sinus at the base of the fourth or fifth pair of pereiopods with a siliconized syringe, centrifuged for about 15 min at 3000 rpm, and the supernatant kept frozen at -20°C. Water samples were similarly stored.

Cation (Na^+, K^+ and Ca^{++}) concentrations were obtained by flame photometry (Procyon, model SP-45). Chloride was measured by microtitration (Beckman/Spincco, model 150) with mercury nitrate in the presence of nitric acid and s-diphenylcarbazone. All results are given as mEq/l.

In the summer experiments the osmotic concentrations of both, hemolymph and water, were determined with a semi-micro osmometer (Knauer, model ML). At three specific moments hemolymph of two animals had to be pooled in order to obtain the minimum volume necessary for such analysis, 24 h after the salinity changes (0 and 10 ‰) and at T 0 (20 ‰).

In the winter, due to the fact that only very small hemolymph volumes were obtained, osmolality was determined by means of a micro-osmometer as described by Salomão (1980). Results are given as mOsm/kg H_2O.

The results were submitted to one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. Data were, when necessary, submitted to mathematical transformations so as to conform with the requisites of such analysis (normality and homocedatiscity) (Sokal and Rolph, 1981). If such requisites were, nevertheless, not obtained the results were submitted to the non-parametric test of Kruskal-Wallis. In all cases differences were considered to be significant, and referred as such in the text, when $P< 0.05$.

RESULTS

The data obtained for the osmotic and ionic concentrations of water at different salinities is presented in Table 1. There seems to be some differences, although relatively small, regarding the real salinity of the water utilized in the experiments and that obtained by hand refractometry (see for example the osmotic concentrations of the water considered as 0 ‰). Throughout the text, for easiness of understanding, the values obtained by hand refractometry will be utilized.

Table 1. Ionic (mEq/l) and osmotic (mOsm/kg H2O) concentrations of the water employed in the different experiments (N=20) and of the hemolymph of *Chasmagnathus granulata* (N=9-18), expressed as mean+SEM, after 360 h of exposition to the indicated salinities during winter and summer. P = probability according to variance analysis.

Salinity	Season	Na^+	K^+	Ca^{++}	Cl^-	Osmol.
	Water	0.0±0.0	0.0±0.0	0.0±0.0	5.5±0.9	36±2
0‰	Winter	394±14	4.3±0.2	7.2±0.3	268±7	559±14
	Summer	381±8	8.1±0.2	12.5±0.3	338±6	645±20
	P	0.48	<0.001	<0.001	<0.001	<0.002
	Water	166±3	2.3±0.2	4.9±0.2	163±8	314±17
10‰	Winter	438±7	6.3±0.3	11.8±0.4	381±8	743±30
	Summer	390±7	9.2±0.2	13.2±0.4	330±11	684±22
	P	<0.001	<0.001	0.024	<0.001	0.14
	Water	346±6	6.1±0.1	10.3±0.3	304±7	679±10
20‰	Winter	531±10	8.4±0.4	13.0±0.3	367±12	749±25
	Summer	441±3	9.7±0.4	14.7±0.5	380±14	816±8
	P	<0.001	0.032	0.004	0.51	0.022

1. Winter Experiments

The animals exposed to 0 ‰ had, in the first experiment, a mortality of 62 %. For this reason such experiment was repeated and, in this new group, mortality was only 3.8 % The reasons for such difference are not known. Nevertheless, previous statistical analysis did not detect any significant difference between the behavior of the osmotic and ionic concentrations of the surviving animals in these two experimental groups. For this reason such results were grouped in all subsequent analysis. No mortality was observed in the other experimental groups.

The changes observed in the Na^+ concentrations after the salinity changes are shown in Fig. 1A. As expected, they were more pronounced after the exposition to 0 ‰ than to 10 ‰. At the former salinity there was a fast and significant decrease in hemolymph Na^+ followed by a more or less stable period up to 168 h after the salinity change. Following this period a partial recovery was observed. At 10 ‰ such changes were relatively small, although significant. A complete recovery occurred in this case. At the end of the experimental period (360 h) the Na^+ concentration in the hemolymph of animals exposed to 0 and 10 ‰ was not significantly different from that of T 0 but, nevertheless, significantly lower than that of the crabs maintained at 20 ‰. This

was due mainly to a significant increase in the hemolymph Na^+ in this later group between 168 and 360 h, for which we have no explanation at present, but believe to be a consequence of starvation.

The overall changes observed in the hemolymph K^+ concentrations are similar to those of Na^+ (Fig. 2A). The main differences are related to the fact that there was a transitory fall in the concentration of this ion after 24 h in the animals maintained in 20 ‰. Also, the statistical analysis indicates a significant difference in the K^+ levels at the end of the experimental period between all three salinities.

Regarding Ca^{++}, the results are presented in Fig. 3A. A significant change in Ca^{++} concentration was observed in all three salinities after 24 h. Nevertheless, a complete recovery occurred at 20 and 10 ‰ between 72 and 168 h. At 0 ‰ the Ca^{++} levels decreased even further until the end of the experiment.

The changes in Cl^- concentration are similar to those described for Ca^{++} (Fig. 4A).

As expected, the changes in osmolality reflect the changes observed in the individual ions. At 0 ‰ a pronounced and significant change was detected after 24 h followed by a small and incomplete recovery. Osmotic concentration remained nearly constant, and not significantly different after 360 h, at 10 and 20 ‰.

2. Summer Experiments

Mortality in the summer experiments was also considerably low. After the salinity change only the animals maintained in 0 ‰ presented some mortality (8.3 %).

The Na^+ concentration in the hemolymph after the salinity changes during the summer is shown in Fig. 1B. No significant changes were observed along exposition time in animals kept in either 10 or 20 ‰. In 0 ‰ a fast and significant decrease was observed after 24 h, which was immediately followed by a partial recovery at 72 h. After this period the Na^+ concentration remained relatively stable.

After the salinity changes, the K^+ concentration decreased significantly at both 0 and 10 ‰ (Fig. 2B). The changes were, as expected, more pronounced in the lower salinity. The recovery was also slower, and only partial, at 0 ‰.

No significant changes in hemolymph Ca^{++} levels were detected in either 10 or 20 ‰ (Fig. 3B). At 0 ‰ a slow decline was observed until 72 h, which was followed by a recovery period. After 168 h this concentration was not significantly different from that observed at the beginning of the experiment.

Concerning the hemolymph Cl^- (Fig. 4B), no significant change was detected at 20 ‰. At 10 ‰ the Cl^- concentration started to fall after 24 h, but was almost completely recovered by the end of the experiment. The statistical analysis does not indicate significant differences in these levels between T 0 and 360 h at this salinity. The animals kept in 0 ‰ had a fast and significant decrease of their hemolymph Cl^-, which was not recovered up to the end of the experiment.

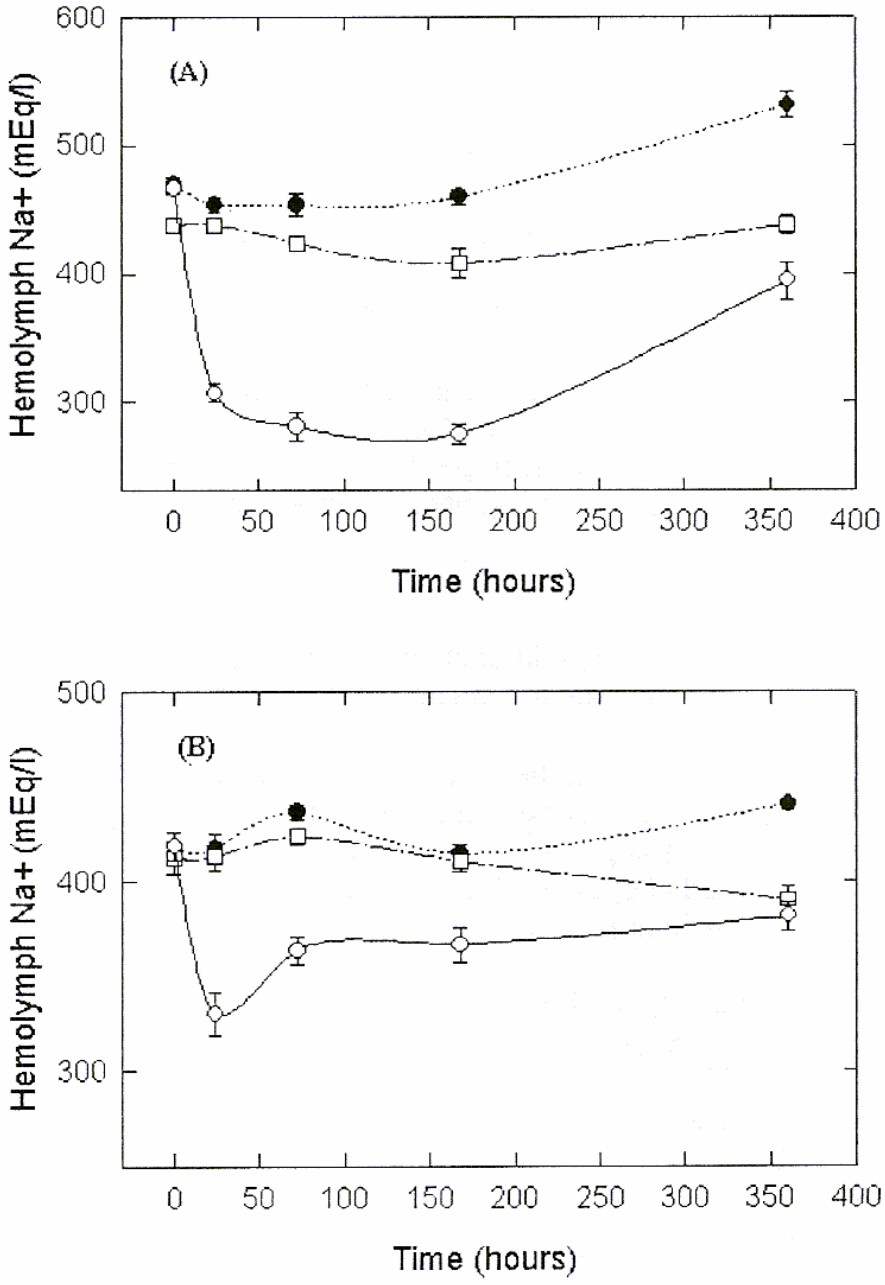

Figure 1. Effect of salinity changes on hemolymph Na^+ concentration (mEq/l) of *Chasmagnathus granulata*, as a function of time, during winter (A) and summer (B). Data expressed as mean±SEM (N=10-19). Closed circles=20‰, squares=10‰, opened circles=0‰.

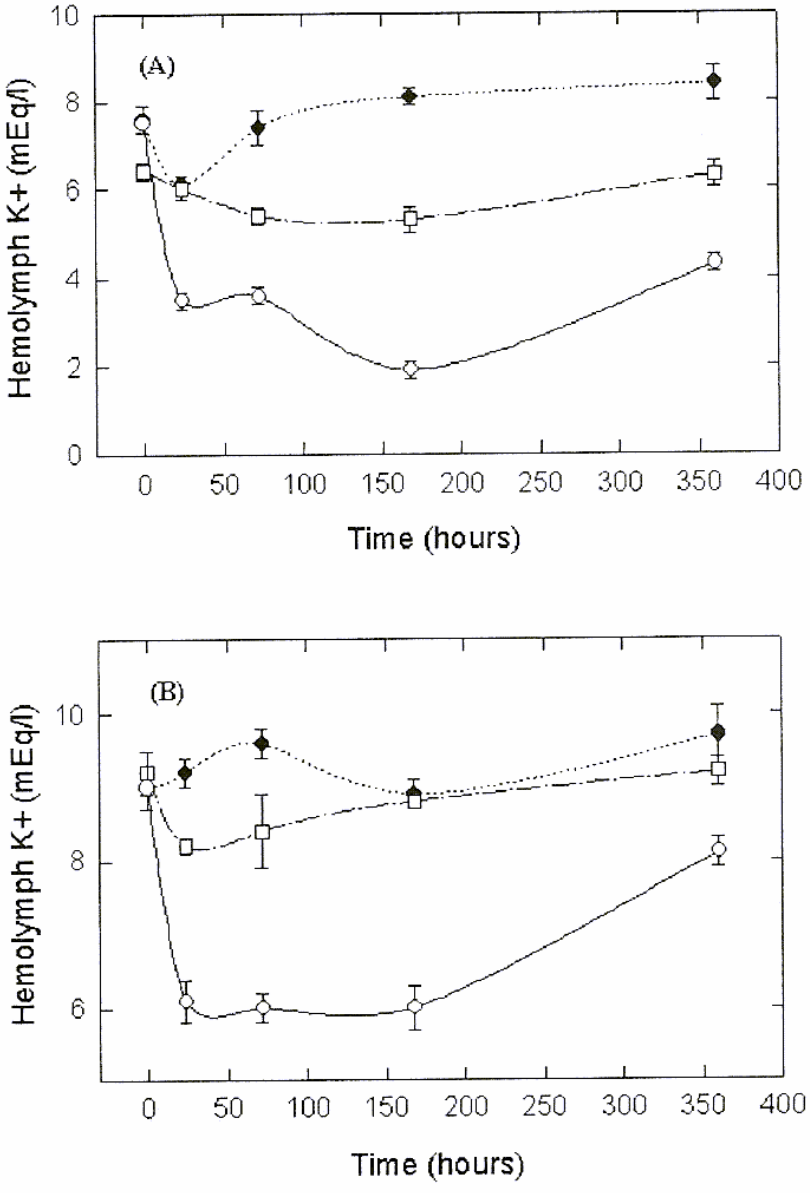

Figure 2. Effect of salinity changes on hemolymph K^+ concentration (mEq/l) of *Chasmagnathus granulata*, as a function of time, during winter (A) and summer (B). Data expressed as mean±SEM (N=10-18). Closed circles=20‰, squares=10‰, opened circles=0‰.

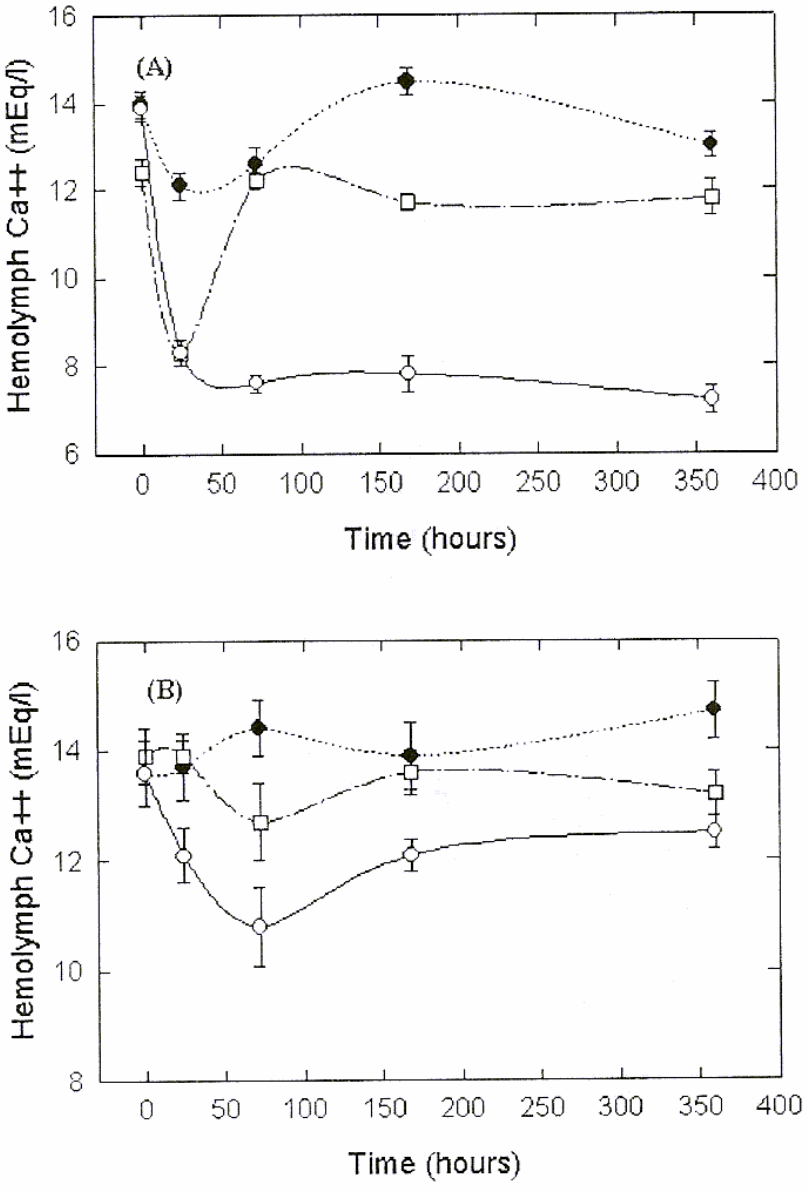

Figure 3. Effect of salinity changes on hemolymph Ca^{++} concentration (mEq/l) of *Chasmagnathus granulata*, as a function of time, during winter (A) and summer (B). Data expressed as mean±SEM (N=7-18). Closed circles=20‰, squares=10‰, opened circles=0‰.

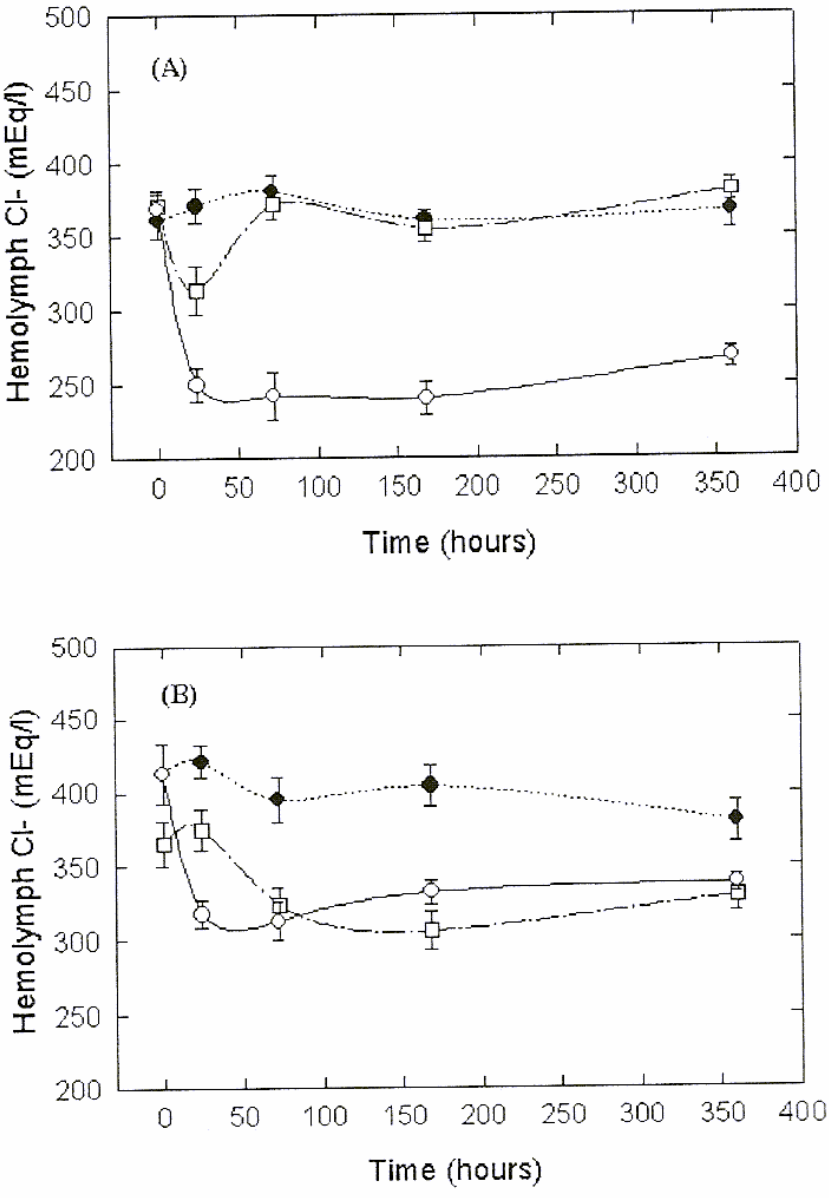

Figure 4. Effect of salinity changes on hemolymph Cl^- concentration (mEq/l) of *Chasmagnathus granulata*, as a function of time, during winter (A) and summer (B). Data expressed as mean±SEM (N=8-19). Closed circles=20‰, squares=10‰, opened circles=0‰.

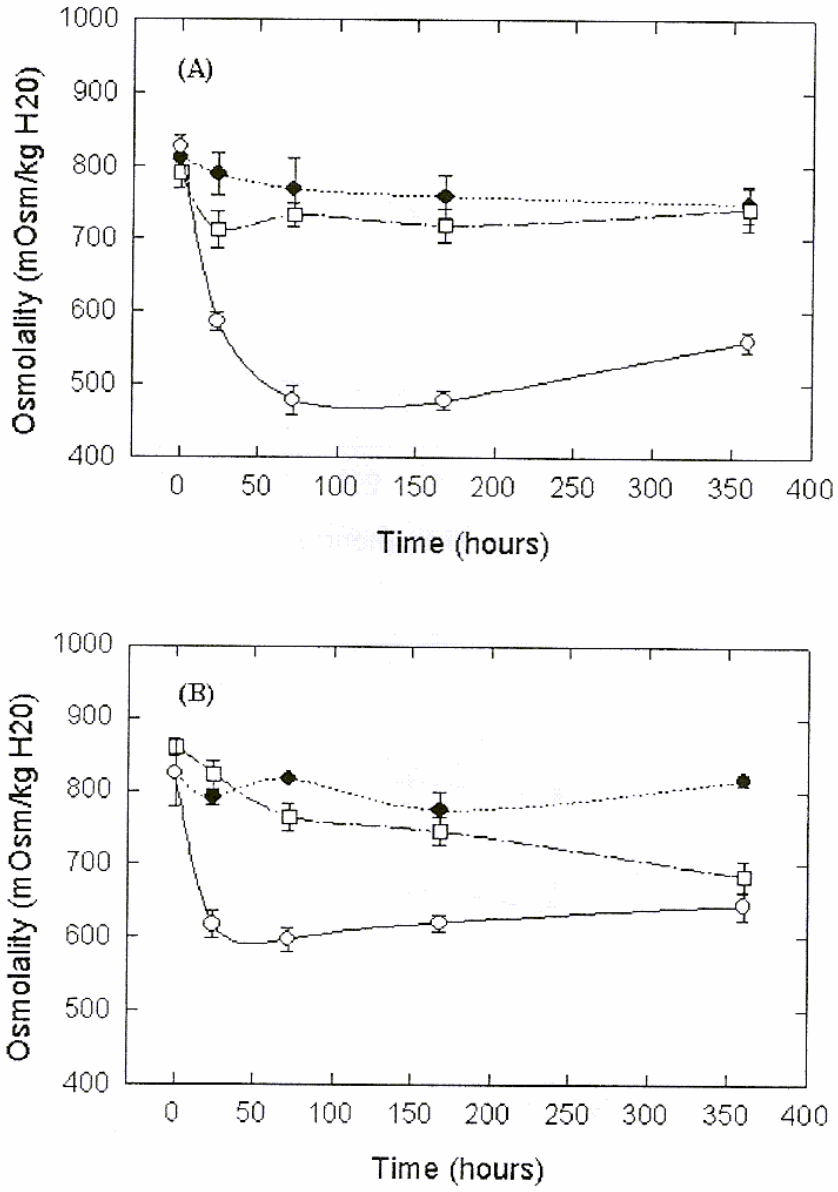

Figure 5. Effect of salinity changes on hemolymph osmotic concentration (mOsm/kg H_2O) of *Chasmagnathus granulata*, as a function of time, during winter (A) and summer (B). Data expressed as mean±SEM (N=6-19). Closed circles=20‰, squares=10‰, opened circles=0‰.

The osmolality (Fig. 5B) was almost constant in animals kept at 20 ‰. In 10 ‰ an almost linear decrease in osmolality could be observed until the end of the experiment. Nevertheless, such decrease was relatively fast (already after 24 h) in the animals transferred to 0 ‰. By the end of the experiment hemolymph osmotic concentration was similar in the animals maintained at 0 and 10 ‰, but significantly lower than that of the crabs kept in 20 ‰.

3. Ionic and Osmotic Concentrations in Summer and Winter

The comparison of the ionic and osmotic concentrations at the beginning of the experiments between summer and winter animals shows that Na^+ and K^+ concentrations in the hemolymph are respectively lower and higher in the summer. The other analyzed parameters (Cl^-, Ca^{++} and osmotic concentration) did not show a clear trend. In some groups they were significantly higher in the summer but, in others, no significant difference could be detected.

After 360 h hemolymph Na^+ concentrations also tend to be lower in summer than in winter, the opposite being observed with K^+, Ca^{++} and Cl^-, except for this later ion at 10 ‰ (Table 1). At the end of the experimental period hemolymph osmolality is also generally lower in winter than in summer animals, also with the exception of those kept at 10 ‰, in which the values are not significantly different.

DISCUSSION AND CONCLUSIONS

The analysis of the alterations in osmotic and ionic concentrations of the hemolymph of *C. granulata* after the hyposmotic shock shows that this species is capable of both short and long term hyperregulation in a salinity range of 0 to 20 ‰. Both types of regulation can be very important for the survival of these animals, in whose habitat the salinity can present sudden and large fluctuations, which can be maintained for extended periods of time (days or even weeks) (D'Incao *et al.*, 1992).

The results obtained clearly show that the osmo and ionic concentrations of the hemolymph can change even after 168 h after the osmotic shock and, in some cases, they seem to have not stabilized up to 360 h. Such results point towards the necessity of long acclimation and /or acclimatization periods, which should be taken into account also during other physiological studies with this species.

The maintenance of osmotic and ionic concentrations of the hemolymph more or less independent of the medium, as it seems to occur in *C. granulata*, depends mostly on two factors: (a) apparent permeability to water (APW) and/or ions and (b) active ion transport.

The diffusive flux of water between the animal and the medium, which occurs mostly at the gill level, can be regulated by changes of the APW of the

integument (Lockwood, 1977; Kirschner, 1979; Mantel and Farmer, 1983; Lucu, 1990). It is possible that *C. granulata* suffered a decrease in its branchial APW, since it seems to be a typical phenomenon occurring in osmoregulators exposed to diluted media. Also, the concomitant fall in the hemolymph concentration of all ions, as well as of the osmolality, after the transfer to 0 ‰, suggests that a more general mechanism, such as a change in APW, may occur.

It is also possible that specific modifications of the gill permeability to ions occurred. According to Pequeux and Gilles (1988), a low permeability to Na^+, combined with a decrease in APW, seems to be indispensable to the adaptation of osmoregulators to low salinities. Nevertheless, such generalizations do not seem to be possible in relation to Ca^{++}, K^+ and, Na^+, although changes of this type have been observed for Cl^- in other crustaceans (Gocha *et al.*, 1987; Bayliss and Harris, 1988).

The results obtained for the Na^+ regulation in *C. granulata* admit both short and long term adaptative strategies for the activation of Na^+/K^+ ATPase. The constancy of the Na^+ concentration during the first 72 h after the hyposmotic shock at 10 ‰ could be related to a short term activation of pre-existing enzymes. The same activation probably also occurs at 0 ‰, but in this case it may not have been sufficient to counterbalance the efflux of Na^+, since the concentration gradient was much greater. The tendency for recovery of the initial hemolymph Na^+ after 168 h at 0 ‰ could be considered as an evidence for a long term strategy involving synthesis of Na^+/K^+ ATPase.

The changes in hemolymph concentration of Cl^-, K^+ and Ca^{++} indicate that these ions are actively absorbed from the medium. Notwithstanding, the discussion of the mechanisms involved in their regulation is difficult, since studies on active transport of such ions are scarce and a decrease of gill permeability for them during hyperregulation does not seem to be the rule (Gilles and Pequeux, 1983; Gocha *et al.*, 1987; Bianchini *et al.*, 1988; Proverbio *et al.*, 1990).

The comparison of the results obtained for *C. granulata* in the winter and summer experiments indicate a greater capability of hyperosmotic regulation at 0 ‰ in the summer. The osmotic and ionic concentrations of the hemolymph showed smaller alterations, and/or the recovery to the initial levels was faster during this season (except for Cl^-). It is possible that this difference between summer and winter is somehow related to the seasonal salinity changes in the habitat. The mean salinities in the field are about 11 and 26 ‰ in the winter and summer respectively (D'Incao *et al.*, 1992). Thus, it seems that animals exposed for longer periods to higher salinities are better hyperregulators. Mañe-Garzon *et al.* (1974) obtained similar results with *C. granulata* collected at two localities of the river La Plata (Montevideo/Uruguay). One of these localities had a salinity around 5 ‰, and the other around 19 ‰. After acclimation of the animals in a salinity of 6 ‰ for 5 days, the animals provenient from 19 ‰ maintained higher hemolymph osmotic and ionic concentrations.

If one considers that the differences observed in regulatory capabilities between winter and summer animals are caused by seasonal salinity variations

of the habitat, it must be assumed that the acclimation for 30 days was not sufficient to eliminate such differences. Similar observations have also been reported by Dehnel and Carefoot (1965) for *Hemigrapsus nudus*.

It is known that prolonged exposition of crustaceans to determined salinity and temperature combinations can lead to changes in the adaptative processes of membrane bound enzymes. According to Somero and Hochachka (1971) such changes can be either qualitative or quantitative. Among these, the most widely recognized is that related to changes of the lipid composition of the membrane, which can lead to modifications of the properties of the transport systems. Both, the proportion of saturated and unsaturated fatty acids of specific phospholipids, as well as the type of phospholipid itself can change, and thus lead to different membrane fluidity. Such adaptative phenomenon was called by Chapelle *et al.* (1982) as viscotropic regulation.

Some crustaceans show a characteristic annual cycle in the proportion of unsaturated/satured fatty acids in the membrane phospholipids. Such relation is increased particularly in species that are active during the winter (see Chapelle, 1986 and Pruitt, 1990 for reviews). Also, the relation phosphatidylethanolamine/phosphatidylcholine changes in response to temperature and salinity changes, being augmented in low salinities and temperatures (Chapelle *et al.*, 1977; Chapelle, 1978; Brichon *et al.*, 1980; Chapelle *et al.*, 1982). Such phospholipids, as well as phosphatidylserine, have a modulating effect on the activity of $Na^+/K^=$ ATPase of the posterior gills of *Eriocheir sinensis* (Chapelle and Zwingelstein, 1984; Chapelle *et al.*, 1982; Chapelle, 1986, Gibbs and Somero, 1990). On the other hand, if increased fluidity is related to increases in electrogenic pump activity, it also seems to lead to increased permeability to water (Holtzman and Novikoff, 1985).

Considering the reported differences in temperature and salinity to which *C. granulata* is exposed in the field during winter and summer, as well as considering that the acclimation period did not seem to be enough to completely eliminate such differences, the results here presented support the idea of a viscotropic adaptation of the membranes, as suggested by Miranda (1994). Thus, winter acclimated crabs would have, for the same temperature-salinity conditions, higher membrane fluidity and as consequence, higher water permeability and increased Na^+/K^+ ATPase activity. Such idea is supported by the fact that winter animals have lower K^+, Ca^{++}, Cl^- and osmotic concentrations after 360 h at 0 ‰ when compared to summer animals. Another important fact is that the minimum values reached after transferring animals from 20 to 0 ‰ were always observed in winter animals, what is also an evidence of higher water permeability, maybe related to the viscotropic changes of the membranes. On the other hand, if such hypothesis is true, the hemolymph Na^+ concentration should have followed the same pattern. Nevertheless, the increased membrane fluidity of winter animals, as already mentioned, may have been related to higher activity of the Na^+/K^+ ATPase of the basolateral membranes of the gills, what may lead to hemolymph Na^+ accumulation.

The K^+ concentration of the hemolymph of winter animals suffered a considerable decrease, reaching values as low as 1.9 mEq/l. The lower K^+

concentrations in the literature are, to our knowledge, 3.8 mEq/l for *P. leniusculus* submitted to freshwater for 21 days (Henry and Wheatly, 1988) and 3.1 mEq/l for *Astacus fluviatilis*, a freshwater decapod (Shaw, 1959). It is surprising that *C. granulata* can survive such low hemolymph K^+ concentrations.

In spite of the seasonal differences in the osmoregulatory dynamic, it seems that the osmotic concentration of the hemolymph tends to be maintained at a "common level" after the hyposmotic stress, both in winter and summer. It can be observed that, in the summer, the osmotic concentration of the hemolymph of animals submitted to 10 ‰ presented a slow fall until the end of the experiment, reaching the same level of the hemolymph concentration of animals submitted to 0 ‰. This later group showed a small but significant recovery of the osmotic concentration. It seems that *C. granulata* use an evasive strategy at 10 ‰, decreasing its osmotic concentration.

Although *C. granulata* was capable of hyperregulating the osmotic and ionic concentrations of the hemolymph, significant alterations were observed during the hyposmotic stress, suggesting that intracellular isosmotic regulation must be considered as part of the mechanisms involved in the osmotic and ionic adaptation in these animals.

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REFERENCES

BAYLISS, D. & R. R. HARRIS. 1988. Chloride ion regulation in the freshwater amphipod *Corophium curvispinun* and acclimatory effects of external Cl^-. J. Comp. Physiol., 158B: 81-90.

BIANCHINI, A., A. PEQUEUX & R. GILLES. 1988. Effects of TPA and DPC on the transepithelial potential difference of isolated perfused gills of the freshwater acclimated crab *Eriocheir sinensis*. Comp. Biochem. Physiol., 37: 85-91.

BOSCHI, E. E. 1964. Los crustáceos decápodos brachyura del litoral Bonaerense (Republica Argentina). Bol. Inst. Mar. (Mar del Plata), 6: 1-76.

BOTTO, J. L. & H. R. IRIGOYEN. 1979. Bioecologia de la comunidad del cangrejal. I-Contribucion al conocimiento biológico del cangrejo de estuário, *Chasmagnathus granulata* Dana (Crustacea, Decapoda, Grapsidae) en la desembocadura del rio Salado, Provincia de Buenos Aires. Semin. Biol. Bentonica y Sedimentacion de la Pataf. Cont. Del Atlantico Sur, Unesco, Montevideo, p. 161-169.

BRICHON, G., S. CHAPELLE & G. ZWINGLESTEIN. 1980. Phospholipids composition and metabolism in the hemolymph of *Carcinus maenas* (Crustacea, Decapoda). Effect of temperature. Comp. Biochem. Physiol., 67B: 647-652.

CHAPELLE, S. 1978. The influence of acclimation temperature on teh fatty acid composition of an aquatic crustacean (*Carcinus maenas*). J. Exp. Zool., 204: 337-346.

CHAPELLE, S. 1986. Aspects of phospholipid metabolism in crustaceans as related to changes in environmental temperatures and salinities. Comp. Biochem. Physiol., 84B: 423-439.

CHAPELLE, S., R. MEISTER, G. BRICHON & G. ZWINGELSTEIN. 1977. Influence of temperature on the phospholipid metabolis of various tissues from the crab *Carcinus maenas*. Comp. Biochem. Physiol., 58B: 413-417.

CHAPELLE, S., J. M. CHANTRINE & A. PEQUEUX. 1982. Gill phospholipids of mitochondria in euryhaline crustaceans as related to environmental changes. Bloch. System. Ecol., 10: 65-70.

CHAPELLE, S. & G. ZWINGELSTEIN. 1984. Phospholipids and metabolism of crustacean gills as related to changes in environmental salinities. Relationship between Na^+/K^+ ATPase activity and phospholipids. Comp. Biochem. Physiol., 78B: 363-372.

DEHNEL, P. A. & T. H. CAREFOOT. 1965. Ion regulation in two species of intertidal crabs. Comp. Biochem. Physiol., 15: 377-397.

D'INCAO, F., M. L. RUFINO, K. GRUBEL & A. L. BRAGA. 1992. Responses of *Chasmagnathus granulata* (Dana, 1851) (Decapoda, Grapsidae) to salt marsh environmental variations. J. Expl. Mar. Biol. Ecol., 161: 179-188.

DRACH, P. & C. THECRENIGOVTZEFF. 1967. Sur la methode de determination des stades d'intermue et son application generale aux crustaces. Vie Milieu, 18: 595-607.

GIBBS, A. & G. N. SOMERO. 1990. Pressure adaptation of teleost gill Na^+/K^+-adenosine triphosphatase: role of the lipid and protein moieties. J. Comp. Physiol., 160B: 431-439.

GILLES R. & A. PEQUEUX. 1983. Interactions of chemical and osmotic regulation with the environment. *In*: Vernberg, F. J. & W. B. Vernberg (Eds.) *The Biology of Crustacea. Environmental Adaptations*. Vol. 8, Academic Press Inc., New York, p. 109-177.

GNAZZO, G., F. MAÑE-GARZON & J. CEYMONIE. 1978. Estudios sobre la biologia del cangrejo de estuário *Chasmagnathus granulata* Dana, 1851. IV. Ionograma normal em poblaciones de diferentes salinidad. Rev. Biol. del Uruguay, 6: 5-17.

GOTCHA, N., A. PEQUEUX, S. WANSON & R. GILLES. 1987. Cl^- fluxes across isolated gills of the chinese crab *Eriocheir sinensis* (M. Edw. acclimated to fresh water. Comp. Biochem. Physiol., 88A: 581-584.

HENRY, R. P. & M. G. WHEATLY. 1988. Dynamics of salinity adaptations in the euryhaline crayfish *Pacifastacus leniusculus*. Physiol. Zool., 61: 260-271.

HOLTZMAN, E. & A. B. NOVIKOFF. 1985. Células e Estrutura Celular. Ed. Guanabara, Rio de Janeiro, 630 pp.

KAMEMOTO F. I. & R. E. TULLIS. 1972 Hydromineral regulation in decapod crustaceans. Gen. Comp. Endocrinol., Suppl., 3: 299-307.

KIRSCHNER, L. B. 1979. Control mechanisms in crustaceans and fishes. In: Gilles, R. (Ed.) Mechanisms of Osmoregulation in Animals: Maintenance of Cell Volume. John Wiley & Sons, Chichester, p. 157-222.

LOCKWOOD, A. P. M. 1977. Transport and osmorregulation in crustacea. In: Gupta, B. L.; R. B. Moreton; J. L. Oshman & B. J. Wall (Eds.) *Transport of ions and water in animals*. Academic Press Inc., London, p. 671-705.

LUCU, C. 1990 Ionic regulatory mechanism in crustacean gill epithelia. Comp. Biochem. Physiol., 97A: 297-306.

MAÑE-GARZON, F., E. DEI-CAS, B. HOLLMAN-SPECTOR & J. LEYMONIE. 1974. Estudios sobre la biologia del cangrejo de estuário *Chasmagnathus granulata* Dana, 1851. I. Osmorregulacion frente a cambios de salinidad. Physis, 33A: 163-171.

MANTEL, L. H. 1967. Asymmetry potentials, metabolism and sodium fluxes in gills of blue crab *Callinectes sapidus.* Comp. Biochem. Physiol. 20: 743-753.

MANTEL, L. H. & L. L. FARMER. 1983. Osmotic and ionic regulation. *In*: Mantel, L. H. (Ed.) The Biology of Crustacea. Internal Anatomy and Physiological Regulation. Vol. 5, Academic Press Inc., New York, p. 53-161.

MIRANDA, R. B. 1994. Efeitos da temperatura e da salinidade sobre a tolerância e a ionorregulação de *Chasmagnathus granulata* Dana, 1851. Fundação Universidade do Rio Grande, Rio Grande/RS, Tese de Mestrado (Master Thesis), 176 pp.

PEQUEUX, A.& R. GILLES. 1988. The transepithelial potential difference of crab isolated perfused gills of the chinese crab *Eriocheir sinensis* Acclimated to fresh water. Comp. Biochem. Physiol., 89A: 163-172.

PEQUEUX A., R. GILLES & W. S. MARSHALL. 1988. NaCl transport in gills and related structures. *In*: Greger,R. (Ed.) Advances in Comparative Environmental Physiology. Vol. 1, Springer-Verlag, Berlin, p. 2-63.

PROVERBIO, T., I. P. ZANDERS, R. MARIN, J. M. RODRIGUEZ & F. PROVERBIO. 1990. Effects of sodium and/or potassium on the magnesium-dependent ATPase activities in shrimp (*Macrobrachium amazonicum*) gill homogenates. Comp. Biochem. Physiol., 97B: 383-390.

PRUITT, N. L. 1990. Adaptations to temperature in the cellular membranes of crustacea: membrane structure and metabolism. J. Thermal Biol., 15: 1-8.

SANTOS, E. A., B. BALDISSEROTO, A. BIANCHINI, E. P. COLARES, L. E. M. NERY & G. C. MANZONI. 1987. Respiratory mechanisms and metabolic adaptations of an intertidal crab, *Chasmagnathus granulata* Dana, 1851. Comp. Biochem. Physiol., 88A:21-25.

SANTOS, E. A. & E. P. COLARES. 1986 Blood glucose regulation in an intertidal crab *Chasmagnathus granulata* Dana, 1851. Comp. Biochem. Physiol., 83A: 673-675.

SANTOS, E. A., L. E. M. NERY & G. C. MANZONI. 1988 Action of the crustacean hyperglycemic hormone of *Chasmagnathus granulata* Dana, 1851 (Decapoda, Grapsidae). Comp. Biochem. Physiol., 89A: 329-332.

SCHMITT, A. S. C. & E. A. SANTOS. 1993a. Lipid and carbohydrate metabolism of the intertidal crab *Chasmagnathus granulata* Dana, 1851 (Crustacea:Decapoda) during emersion. Comp. Biochem. Physiol., 106A: 329-336.

SCHMITT, A. S. C. & E. A. SANTOS. 1993b. Behaviour and haemolymphatic ionic composition of the intertidal crab *Chasmagnathus granulata* Dana, 1851 (Crustacea: Decapoda) during emersion. Comp. Biochem. Physiol., 106A: 337-342.

SHAW, J. 1959 Salt and water balance in the east African freshwater crab *Potamon niloticus* (M. Edw.). J. Exp. Biol., 36: 157-176.

SOMERO, G. N. & P. W. HOCHACHKA. 1971. Biochemical adaptation to the environment. Am. Zool., 11:159-167.