

Osmotic and Ionic Regulation in the Ghost Crab *Ocypode saratan* in Hypoionic and Hyperionic Media and Following Desiccation in Air

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ABSTRACT. 1. Sodium, potassium and magnesium ion concentrations in the blood of *Ocypode* were hypoionic to normal and concentrated sea water.

2. Over the entire salinity range tested calcium ion concentration was found to remain fairly constant and hyperionic to all media.

3. The changes in haemolymph sodium concentration reflect the changes in total osmotic concentration.

4. Death from desiccation related directly to weight loss, indicating that water loss probably as circulating haemolymph volume was the critical factor, rather than ionic concentration.

Introduction

The decapod Crustacea include groups such as the brachyuran and anomuran crabs, which present a full spectrum of intermediaries between aquatic and terrestrial forms. The subject of terrestriality in crustaceans was reviewed by Edney^[1]. Crabs showing various degrees of terrestriality typically maintain the osmotic concentration of their blood below that of the sea i.e. they hyporegulate^[2,3]. Ionic and water regulation has been examined in detail in the semi-terrestrial crab *Pachygrapsus crassipes*,^[3-7] and in several species of *Uca*^[8]. Land crabs are most often found close to a source of water which they enter at regular intervals to excrete waste products such as CO₂ and ammonia and to ionoregulate^[9,10]. This source of water may be saline or relatively fresh, presenting a variable osmotic and ionic problem to the crab. The

role of behavior in the regulation of water balance in land crabs was discussed by Gross and Holland^[11] and Gross^[12].

A few crustaceans that regulate hyperosmotically also regulate hyposmotically^[13]. Many crabs that spend time on land are capable of hyposmotic regulation. The fiddler crab *Uca* spends long periods on sand and mud flats; it is a good hyposmotic regulator, its blood concentration increases less rapidly during prolonged exposure to air than that of rock crabs such as *Hemigrapsus*^[14].

The ghost crab *Ocypode saratan* is the most conspicuous member of sandy beach communities in the Red Sea. Its environment is bordered by desert and environmental air temperatures may exceed 40°C in summer and drop below 15°C during winter nights. Relative humidities are also greatly variable, from below 5% to 100%^[15]. Spaargaren^[16] has studied osmotic regulation in *Ocypode saratan*, it maintains a very constant internal concentration when exposed to a wide range of salinities.

The present work was undertaken in order to study the regulation of blood cations and total blood osmotic concentration in *Ocypode saratan* adapted to normal, concentrated and diluted sea water. Also the effect of desiccation on blood osmolarity was examined and discussed.

Material and Methods

Specimens of the ghost crab *Ocypode saratan* of either sex and weighing between (20-60 g); were collected from local beaches close to the Faculty of Marine Sciences, Jeddah, Saudi Arabia. The crabs were maintained for one-two weeks in tanks containing damp sand, with access to sea water at 22°C. The water was changed daily and the animals were fed with chopped heart and liver. The sea water was obtained from the same site of collection. Experimental crabs were starved for three days before and during the experiments. Crabs were exposed to a variety of salinities between 25% and 175% with 100% S.W. = 34‰ as measured by titration with silver nitrate^[17]. The water level in the tanks was adjusted to enable the crabs to raise the front of their bodies sufficiently to bring the exhalent openings of the branchial chambers, which are situated above the mouthparts, just clear of the water surface. Specimens were exposed to test salinities for 24 h, after which time blood samples were obtained. In some experiments, crabs were left for four days at test salinities to ensure that 24 h period was sufficient to obtain a steady state of ionic composition in the haemolymph. The osmolarity of the blood of crabs was determined by measuring blood cations concentrations and total osmotic concentration. For cations concentrations, blood was sampled from crabs by inserting a glass disposable micropipette (50 µl) into the basal joint of one of the walking legs. The area was first carefully dried. The micropipette was then transferred to a 50 ml measuring flask, broken up and the contents were diluted by adding deionized water. Cations concentrations were determined using a flame atomic absorption spectrophotometry (A-A-670 Shimatz Corporation).

For the determination of total osmotic concentration, a jonotec osmometer (030D) was used. The same technique was used in withdrawing the blood, but samples were collected in (50 μ l) epidural tubes.

The crabs were then taken from water of various salinities and placed in air in open tanks. The relative humidity and air temperature were 47 and 22°C respectively. Before death from desiccation the crabs were weighed and a second blood sample was taken for the determination of blood total osmotic concentration. The approach of death was manifested by the crabs decreasing activity.

Unless otherwise described, measured variables are given in the text as mean values \pm S.E. of mean, followed by the number of observations (n) in parentheses. Students t-test was used to test the significance of any difference between mean values and the term "significant" in the present report refers to 95% confidence level ($P < 0.05$).

Results

1. Haemolymph cation concentration

Blood cations concentrations as a function of salinity are shown in Fig. 1. Haemolymph magnesium concentration remained constant over a wide range of salinities Fig. 1,a. Only in diluted sea water (25% S.W.) did the haemolymph magnesium decrease significantly ($P < 0.01$) but remained hyperionic to the medium. Haemolymph potassium concentration remained unchanged over the entire salinity range with the exception of very concentrated sea water (175% S.W.) in which the haemolymph potassium concentration increased significantly ($P < 0.01$) by 5 m equiv.l⁻¹.

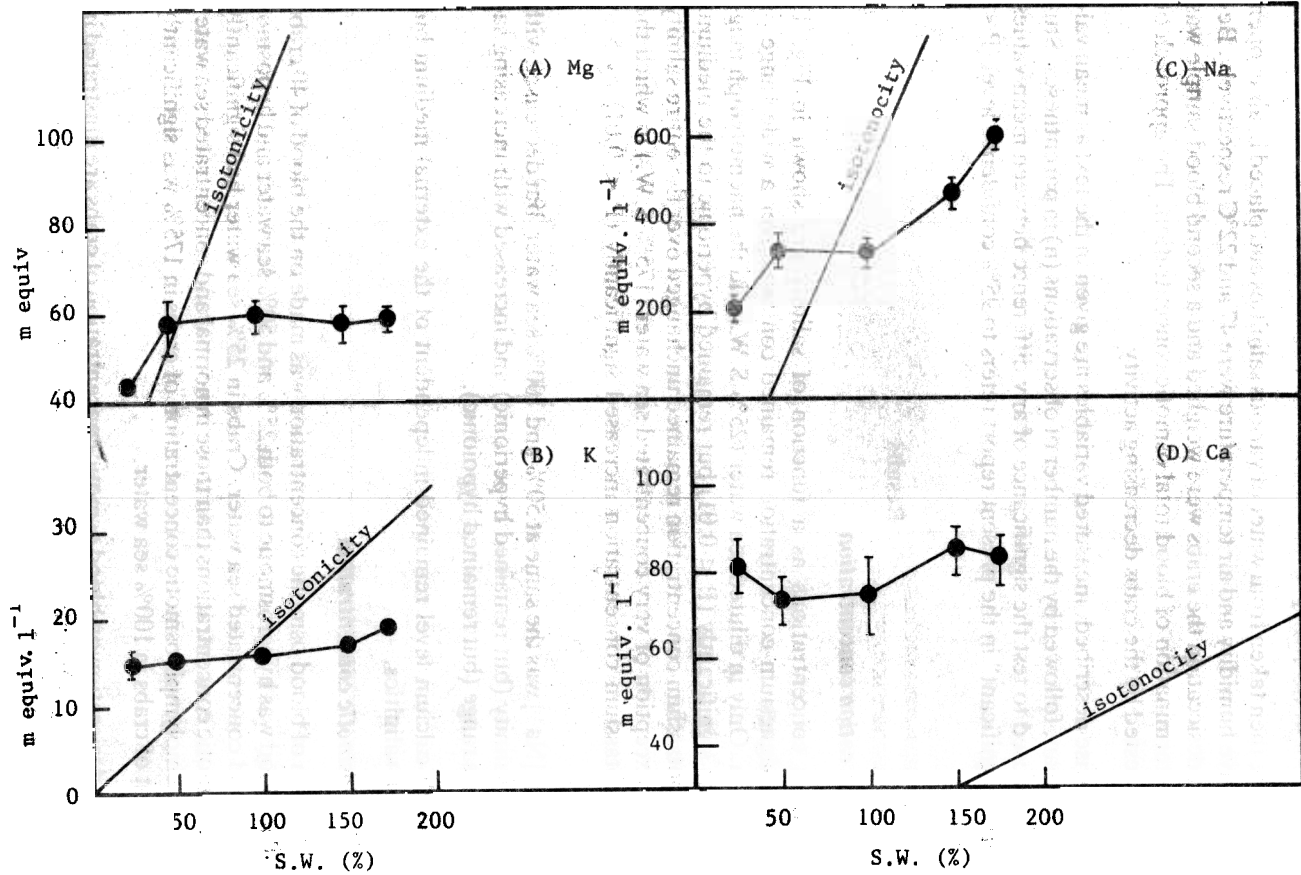
Haemolymph [Na⁺] was the same at 50% and 100% sea water, but decreased with decreasing the salinity (but remained hyperionic) and increased with increasing salinity outside this range (but remained hypoionic).

Haemolymph calcium level remained independent of the external medium but hyperionic to all salinities.

2. Total blood osmotic concentration

Determination of blood osmotic concentration was made on the blood of 40 crabs (Fig. 2). The blood was hyperosmotic to both 25% and 50% sea water and hyposmotic to normal and concentrated sea water. Crabs in 25% sea water had significantly lower blood osmotic concentrations than those in normal and concentrated sea water ($P < 0.05$). Haemolymph osmotic concentration of crabs in 175% was significantly higher than that of crabs in 100% sea water.

The effect of desiccation on blood osmotic concentration of crabs are illustrated in Fig. 3. Blood osmotic concentration at death varied in relation to the salinity from which the crabs were taken prior to desiccation.



10. *Ocyropsis saratan*. The relationship between concentration in the haemolymph (m equiv. l^{-1}) and the external sea water concentration (% sea water) for A, Magnesium; B, potassium; C, sodium and D, calcium.

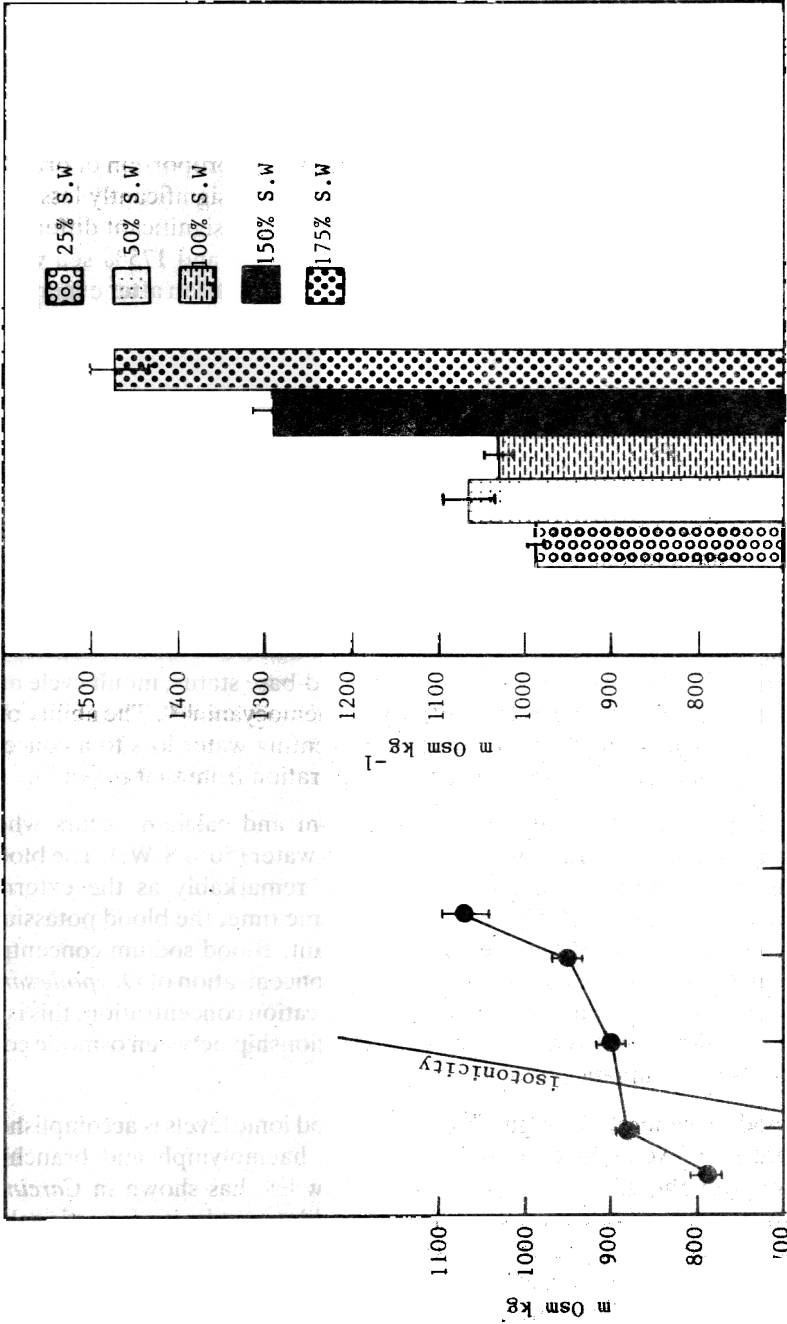


FIG. 3. *O. saratan*. The osmotic concentration (m Osm. kg⁻¹) of haemolymph, after death by desiccation, of crabs removed from water at various salinities (% sea water).

O. saratan. The relationship between the total osmolarity of the haemolymph (m Osm. kg⁻¹) and the external concentration of sea water (% sea water).

3. Changes in body mass during desiccation

Crabs weighed immediately on withdrawal from water of various salinities, exposed in dry air until they seemed to have died from desiccation and then reweighed showed very similar patterns of weight loss. Crabs removed from 25% sea water showed a mean weight loss of $12.2 \pm 0.8\%$, from 50% sea water a loss of $12.9 \pm 1.0\%$; from 100% sea water a loss of $10.2 \pm 0.4\%$ and crabs from 175% sea water lost $14.0 \pm 0.8\%$ of their original, hydrated weight. Although the proportion of original body weight lost by crabs removed from 100% sea water was significantly less than that lost by crabs from 50% at death ($P < 0.05$), there was no significant difference between the losses experienced by crabs from water of 25% and 175% sea water salinities and these data indicate that *Ocypode* dies from desiccation after experiencing water loss equivalent to 10-14% of hydrated mass irrespective of the salinity to which it is previously exposed and adapted.

Discussion

The present investigation revealed that *Ocypode saratan* effectively hyporegulates in normal and in concentrated sea water, and hyperregulates in dilute media. When *Ocypode* is adapted to normal and concentrated sea water, the blood concentrations of sodium, potassium and magnesium are kept somewhat below their corresponding levels in the external medium. The calcium concentration of blood appears to be higher than that of the medium and remained fairly constant over the entire salinity range. This may indicate that a proportion of calcium ion in crab blood is found in an indiffusible form^[18-21]. Also calcium levels relate to acid-base status, moult cycle and the specific effect of $[Ca^{2+}]$ on the oxygen affinity of haemocyanin^[22]. The ability of a land crab to hyporegulate might be of value in preventing water loss to a concentrated fluid in the gill chamber, itself caused by evaporation from that region^[3].

Hyperionic regulation of blood sodium, potassium and calcium occurs when *Ocypode* is adapted to concentrations below 100% sea water (50% S.W.). The blood levels of sodium and magnesium tend to decrease remarkably as the external medium becomes more diluted (25% S.W.); at the same time, the blood potassium and calcium concentrations are kept relatively constant. Blood sodium concentrations, reflect completely the changes in total osmotic concentration of *Ocypode saratan* blood. As it represents about 75% of haemolymph cation concentration, this is to be expected and it agrees with the observed close relationship between osmotic concentration and $[Na^+]$ in aquatic crabs^[23].

In the decapods in general, the regulation of the blood ionic levels is accomplished by active uptake or excretion of ions between the haemolymph and branchial chamber water over the gills^[18,24-27]. Moreover, Shaw^[28], has shown in *Carcinus maenas* that the rate of active uptake of sodium from dilute media is proportional to the difference between the concentrations of sodium in blood and medium. Under steady-state conditions, a given concentration gradient between blood and medium, in the absence of a significant potential difference, can be maintained only if the rate

of ion uptake fully compensates for the losses in the opposite direction. Assuming that the permeability of the surfaces involved in ionic exchange between blood and medium remains constant, regardless of the external concentration, the passive ion loss will always be proportional to the gradient itself, and it follows that the rate of uptake must also be proportional to this concentration difference, if balance is to be maintained^[29].

The participation of the antennary glands of the crustaceans in ionic regulation is that of removing magnesium and sulphate from the blood and excreting these ions in the urine, together with some other unwanted metabolites and excess water^[5,12,27,30-35]. Nonetheless, these organs may contribute to some extent to the regulation of the blood^[32,33,36]. Active regulation of the ionic concentration of urine during desiccation was described in the crayfish with $[Na^+]$, $[Mg^{2+}]$ and $[Cl^-]$ all increasing^[37] although in this species urine flow ceased after 24 h in air. The possible role of the antennary glands in osmotic and ion regulation in *Ocypode saratan* is the subject of current experimental work.

In desiccation experiments, the osmotic pressures (F_p) of the various individuals prior to death ranged from 1.83-3.13 \pm C. The individuals with the highest blood osmotic pressure at death were those which came from the most concentrated sea water and which therefore had the highest initial blood osmotic pressure, see Fig. 3.

The percentage increase in blood osmotic pressure, at death in air for crabs taken out of 25%, 100% and 150% sea water, were 20%, 12% and 29%, respectively. This will be as a result of water loss by evaporation and/or increased activity of the antennary glands.

Although crabs died at an internal osmotic pressure as low as 1.83 during desiccation for crabs taken out of 25% sea water, they were able, however, to tolerate and survive for many days a blood osmotic pressure of 2.48 in concentrated sea water. It seems that death from desiccation relates to water loss in air as all crabs died after losing 10-14% of body mass. Assuming a haemolymph volume of 20-30%^[38] and that the losses represented loss of circulating haemolymph as described in the crayfish^[38], this indicates that crabs died after haemolymph volume was reduced to 50% of the hydrated value, resulting in circulatory failure. It was difficult to sample haemolymph from desiccated crabs indicating that the volume was reduced. The branchial chamber water may also be used in the regulation of the blood acid-base balance and its evaporation from that region may lead to an accumulating respiratory and metabolic acidosis in the blood, (personal communication with E.W. Taylor). The role of the gills of *Ocypode saratan* in ionic and acid-base balance regulation should be further investigated under normal and desiccating conditions.

The adaptive value of ionic regulation by branchial ion exchange for the land habit in crabs remains in question, as it is difficult to assign it a specific function when they are not immersed in water^[39]. On the other hand, amphibious and terrestrial crabs are commonly found in regions of fluctuating salinities (e.g. estuaries or pools flooded by tides or heavy rainfall), and it may well be that selective pressures which encour-

aged the land habit were included with those favouring hypo- and hyperosmotic regulation^[40]. Under normal circumstances, however, the burrowing mode of *Ocypode saratan* enables it to avoid the dangers of desiccation. The temperature within the burrow is fairly constant, avoiding environmental extremes, and the relative humidity is high^[15].

References

- [1] Edney, E.B., Terrestrial adaptations. In: Waterman, T.H. (ed.) *The physiology of Crustacea*, Vol. 1, New York: Academic Press, pp. 367-393 (1960).
- [2] Pearse, A.S., *Freezing points of blood of certain Littoral and estuarine animals*, Carnegie Inst. of Washington Papers from Tortugas Laboratory, **28**: 93-102 (1934).
- [3] Jones, L.I., Osmotic regulation in several crabs of the Pacific Coast of North America, *J. Cell. Comp. Physiol* **18**: 79-91 (1941).
- [4] Prosser, C.L., Green, J.W. and Chow, T., Ionic and osmotic concentrations in blood and urine of *Pachygrapsus crassipies* acclimated to different salinities, *Biol. Bull.* **109**: 99-107 (1955).
- [5] Gross, W.J., An analysis of the response to osmotic stress in selected decapod crustaceans, *Biol. Bull.* **112**: 43-62 (1957).
- [6] Gross, W.J., Potassium and sodium regulation in an intertidal crab, *Biol. Bull.* **114**: 334-347 (1958).
- [7] ———, The effect of osmotic stress on the ionic exchange of a shore crab, *Biol. Bull.* **116**: 248-257 (1959).
- [8] Wright, D.A., Zanders, I.P. and Pait, A., Ionic regulation in three species of *Uca*: A comparative study. *Comp. Biochem. Physiol.* **78A** (1): 175-179 (1984).
- [9] Wood, C.M. and Randall, D.J., Oxygen and carbon dioxide exchange during exercise in the land crab (*Cardisoma carnifex*). *J. exp. Zool.* **218**: 7-22 (1981).
- [10] Innes, A.J. and Taylor, E.W., The evolution of air breathing in crustaceans: a functional analysis of branchial, cutaneous and pulmonary gas exchange, *Comp. Biochem. Physiol.* **85A**: 621-637 (1986).
- [11] Gross, W.J. and Holland, P.V., Water and ionic regulation in a terrestrial hermit crab, *Physiol. Zool.* **33**: 21-28 (1960).
- [12] Gross, W.J., Trends in water and salt regulation among aquatic and amphibious crabs, *Biol. Bull.* **127**: 447-466 (1964).
- [13] Gross, W.J., Lasiewski, R.W., Dennis, M. and Rudy, J.R., Salt and water balance in selected crabs of Madagascar, *Comp. Biochem. Physiol.* **17**: 641-660 (1966).
- [14] Prosser, C.L., *Comparative animal physiology volume I*, third edition (ed. Ladd Prosser, C.) W.B. Saunders Company, Philadelphia-London-Toronto (1973).
- [15] Eshky, A.A., *Aspects of the ecology, behaviour and physiology of the ghost crab Ocypode seration* (Ph.D.) Thesis, Univ. Marine Biological Station, Millpool and Dept. of Zool. Glasgow Univ. (1985).
- [16] Spargareen, G.L., On the water and salt economy of some decapod crustaceans from the Gulf of Aqaba (Red Sea), *Neth. J. Sea. Res.* **11**: 99-106 (1955).
- [17] Harvey, H.W., *The chemistry and fertility of sea waters*. Cambridge University, 1-224 (1955).
- [18] Webb, D.A., Ionic regulation in *Carcinus maenas*, *Proc. R. Soc.* **129**: (Ser. B) 197-136 (1940).
- [19] Robertson, J.D., Some features of the calcium metabolism of the shore crab (*Carcinus maenas* Pennant). *Proc. R. Soc.* (Ser. B.) **124**: 162-182 (1937).
- [20] Zanders, I.P., *Dynamics of ionic balance and its control in Carcinus maenas (L.)*, Ph.D. Thesis, University of Newcastle-upon-Tyne. (1975).
- [21] Greenaway, P., The regulation of haemolymph calcium concentration of the crab *Carcinus maenas* (L.). *J. exp. Biol.* **64**: 149-157 (1976).
- [22] Morris, S., Tyler-Jones, R. and Taylor, E.W., The regulation of haemocyanin oxygen affinity during emersion of the crayfish *Austrapotamobius pallipes*, *J. exp. Biol.* **121**: 315-326 (1986).
- [23] Taylor, E.W., Butler, P.J. and Al-Wassia, A., The effect of a decrease in salinity on respiration, osmoregulation and activity in the shore crab, *Carcinus maenas* (L.), *J. Comp. Physiol.* **119**: 155-170 (1977).

- [24] Krogh, A., *Osmotic Regulation in Aquatic Animals*, C.U.P. Cambridge, 242 p. (1939).
- [25] Flemister, L.J., Salt and water anatomy, constancy and regulation in related crabs from marine and terrestrial habitats, *Biol. Bull.* **115**: 180-200 (1958).
- [26] Robertson, J.D., Ionic and osmotic regulation, In: Waterman, T.H. (ed.) *The Physiology of Crustacea*, Vol. 1, Academic Press, New York, pp. 317-339 (1960).
- [27] Smith, D.S. and Linton, J.R., Potentiometric evidence for the active transport of sodium and chloride across the excised gill of *Callinectes sapidus*, *Comp. Biochem. Physiol.* **38**: 367-378 (1971).
- [28] Shaw, J., Studies on the ionic regulation of *Carcinus maenas* (L.) I. Sodium balance, *J. Exp. Biol.* **38**: 135-152 (1961a).
- [29] Zanders, I.P., Ionic regulation in the mangrove crab *Goniopsis cruentata*, *Comp. Biochem. Physiol.* **60**: 293-302 (1977).
- [30] Gross, W.J., Osmotic tolerance and regulation in crabs from a hypersaline lagoon, *Biol. Bull.* **121**: 290-301 (1961).
- [31] Green, J.W., Harsch, M., Barr, L. and Prosser, C.L., The regulation of water and salt by the fiddler crabs, *Uca Pugnax* and *Uca pugnator*, *Biol. Bull.* **116**: 76-87 (1959).
- [32] Riegel, J.A. and Lockwood, A.P.M., The role of the antennal gland in the osmotic and ionic regulation of *Carcinus maenas*, *J. Exp. Biol.* **38**: 491-499 (1961).
- [33] Lockwood, A.P.M., The osmoregulation of Crustacea, *Biol. Rev.* **37**: 257-305 (1962).
- [34] Gross, W.J. and Capen, R.L., Some functions of the urinary bladder in a crab, *Biol. Bull.* **131**: 272-291 (1966).
- [35] Lockwood, A.P.M. and Riegel, J.A., The excretion of magnesium by *Carcinus maenas*, *J. Exp. Biol.* **51**: 575-590 (1969).
- [36] Dehnel, P.A. and Carefoot, T.H., Ion regulation in two species of intertidal crabs, *Comp. Biochem. Physiol.* **15**: 377-397 (1965).
- [37] Tyler-Jones, R. and Taylor, E.W., Urine flow and the role of the antennal glands in water balance during aerial exposure in the crayfish *Austropotamobius pallipes* (Lereboullet), *J. Comp. Physiol.* **B156**: 529-535 (1986).
- [38] Taylor, E.W., Tyler-Jones, R. and Wheatly, M.G., The effect of aerial exposure on the distribution of body water and ions in the freshwater crayfish *Austropotamobius pallipes* (Lereboullet), *J. Exp. Biol.* **128**: 307-322 (1987).
- [39] Gross, W.J., Aspects of osmotic regulation in crabs showing the terrestrial habit, *Am. Nat.* **89**: 205-222 (1955).
- [40] ———, Water balance in anomuran land crabs on a dry atoll, *Biol. Bull.* **126**: 54-68 (1964).

التنظيم الأسموزي والأيوني في السرطان أوسيبود سرطان في تراكيز مختلفة من الملوحة وأثناء الجفاف

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المستخلص . أجريت هذه الدراسة للتعرف على مدى مقدرة السرطان (أوسيبود سرطان) على التنظيم الأيوني والأسموزي وذلك في أوساط ملحية مختلفة التراكيز ، بالإضافة إلى محاولة إلقاء بعض الضوء على سبب موت هذا الكائن عند تعرضه للجفاف . وقد تم التوصل إلى ما يلي :

- ١ - تركيز أيوني البوتاسيوم والمغنسيوم في الدم هو أقل من تركيزهما في ماء البحر الطبيعي (١٠٠٪) وماء البحر المركز (١٥٠٪ ، ١٧٥٪) .
- ٢ - تركيز أيون الكالسيوم ثابت وأعلى في دم السرطانات من تركيزه في الوسط الخارجي وذلك في جميع التراكيز الملحية المستخدمة .
- ٣ - تركيز أيون الصوديوم في الدم يسلك سلوكا مشابها للضغط الأسموزي الكلي للدم وذلك في جميع الأوساط الملحية المستخدمة .
- ٤ - إن موت السرطان من الجفاف يبدو أنه يتعلق مباشرة بكمية الماء التي يفقدها جسمه ، إذ إن كل السرطانات تموت عند فقدانها من ١٠-١٤٪ من وزنها الرطب . وبالرجوع إلى أبحاث الآخرين ، وجد أن حجم الهيموليمف يمثل من ٢٠-٣٠٪ من وزن الجسم ، أي إن مقدار ما يفقده السرطان عند الموت من الماء نتيجة للجفاف يعادل ٥٠٪ من حجم الهيموليمف . وحيث إن السرطانات أظهرت مقدرة فائقة على تحمل الملوحة العالية وما يصاحب ذلك من ارتفاع في ضغط دمها الأسموزي ، فإن سبب موتها من الجفاف يرجع إلى عطل في جهازها الدوري وليس إلى فشل أسموزي .