

140392

10285 F 675

INSTITUUT VOOR ZEEWETENSCHAPPELIJK ONDERZOEK
8420 Koninklijke baan KLEMSKERKE

IONIC REGULATION IN *CRANGON VULGARIS* (FABR.) (CRUSTACEA, NATANTIA) FROM BRACKISH WATER

LARS HAGERMAN

Marine Biological Laboratory, DK-3000 Helsingør, Denmark

ABSTRACT

The hyper-hypoosmotic (isosmotic at 23 ‰) shrimp *Crangon vulgaris* was found to maintain Ca^{2+} and K^{+} more concentrated than the medium in 5-25 ‰, Na^{+} and Cl^{-} more concentrated in the range 5-30(23) ‰ and less concentrated in higher salinities. Mg^{2+} was held at a very low level throughout the salinity range. The importance of various mechanisms (low permeability, active ion transport and hypo-osmotic urine) in assisting this efficient ion regulation is discussed.

INTRODUCTION

The sand-living shrimp *Crangon vulgaris* (Fabr.) is most abundant in shallow brackish water, although its salinity distribution lies within 5-35 ‰ (see a. o. Muus, 1967). *Crangon vulgaris* is a homoiosmotic species, hyperosmotic in salinities below 21-26 ‰ and hypo-osmotic in higher salinities (Broekema, 1941; Flügel, 1960; Weber & Spaargaren, 1970; Spaargaren, 1971; Hagerman, 1971). Both in shallow water in summertime and during its migration to and from deeper water in autumn and spring the species is subject to sometimes rather violently fluctuating salinities which exert great demands upon the tolerance of the organism.

In this paper the ionic regulation of adult intermoult *C. vulgaris* adapted to different salinities are studied. The work is part of an investigation on the eco-physiological complex of *Crangon vulgaris* from the Øresund (Hagerman, 1970a, b; 1971; 1973).

MATERIAL AND METHODS

Specimens of *Crangon vulgaris* were collected in shallow water (0-4 m) in the Øresund, north of Helsingør (salinity 10-15 ‰). The animals were placed singly in small containers (diam. 12 cm) and left to acclimatize to the experimental salinities (5-35 ‰) for at least three days and not more than seven days. Only specimens in the intermoult stage C-D, were considered suitable for further treatment. All measurements were made on animals acclimatized to $+10^{\circ}\text{C}$. The animals were not fed during the acclimatization period.

Before sampling the animals were gently dried on soft filter paper and placed on a special surgery-table where they were held in place by a small plate pressing gently on the carapace. Hæmolymph was sucked from the heart by means of a glass-syringe, and placed under liquid paraffin. It was usually possible to get 20-35 μ l hæmolymph from a *Crangon* 40-50 mm long. The hæmolymph was divided into subsamples by means of 5 or 10 μ l Drummond microcaps and diluted with distilled water.

Sodium, potassium, calcium and magnesium was measured on a Unicam SP 90 Series 2 atomic absorption spectrophotometer. The hæmolymph samples were diluted $1000 \times$ (Na^+), $100\text{-}200 \times$ (K^+), $51 \times$ (Ca^{2+}) and $510 \times$ (Mg^{2+}). Calcium was measured after the addition of lanthanumchloride. The results were recorded on a Goerz Servogor RE 511 recorder. Chloride was measured on 10 μ l samples with an Aminco-Buchler-Cotlove direct reading chloridometer.

The investigation was partly supported by a grant from the Danish Science Research Council. Dr. Donald McLusky, University of Stirling, Scotland, kindly read and criticized the manuscript.

RESULTS

The regulation of sodium in salinities between 5-35‰ has been discussed in an earlier paper (Hagerman, 1971). The regulation of the major anion, chloride, is shown in Fig. 1. It has long been stated that chloride passively follows the sodium fluxes. When the chloride and the sodium curves are compared it is also evident that they follow the same pattern, i.e. a higher concentration than the outer medium below ca. 425 mEq Na^+ and 340 mEq Cl^- , corresponding to a salinity of 30 and 23‰ respectively. This discrepancy of 7‰, a higher concentration of Na^+ when *Crangon* is isosmotic with respect to chloride, points to changes in the ratio of sodium and chloride fluxes, a better and thus less passive transport of chloride the closer to the isosmotic salinity in which the animal lives. The level at which hæmolymph chloride is the same as the surrounding medium is the same as when the hæmolymph is isosmotic with regard to the total osmoconcentration (Hagerman, 1971). In salinities above 23‰ chloride is held at a concentration below the outer medium, the difference between medium and chloride being appr. 50 mEq. at 30‰.

The regulation of magnesium in the salinity range 5-35‰ is shown in Fig. 2. Throughout the entire salinity range magnesium is held at a level far below the outer medium. The curve is slightly S-shaped, a rather steep increase in magnesium ion concentration between 5-10‰, a very efficient and constant regulation between 10-25‰ (i.e. within the normal salinity range in northern Øresund), and then an increase in the hæmolymph magnesium concentration in 25-35‰, but still very far below the outer medium. Compared to many other

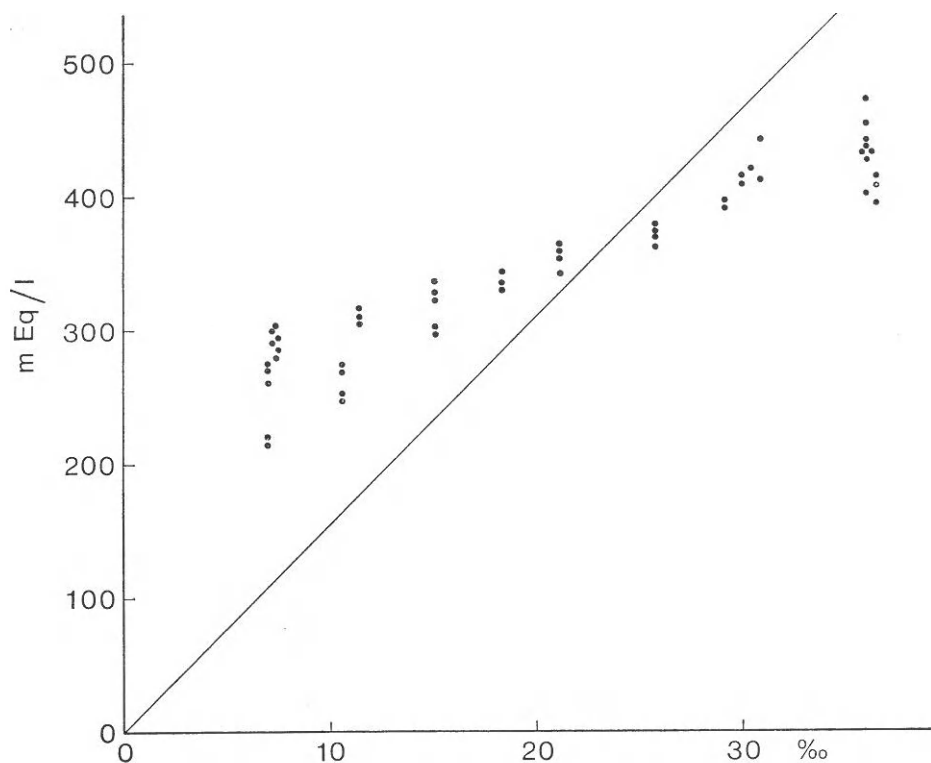


FIG. 1. The concentration of chloride ions in the blood of *Crangon vulgaris*.

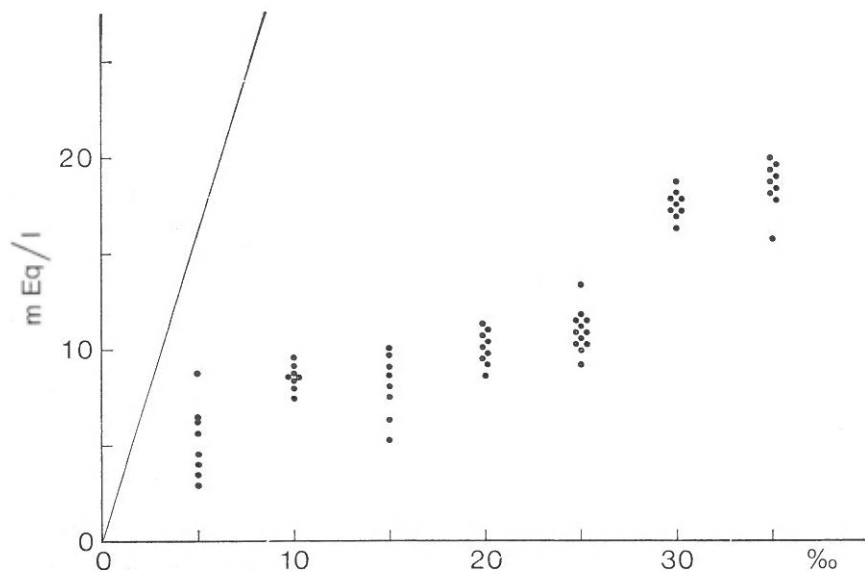


FIG. 2. The concentration of magnesium ions in the blood of *Crangon vulgaris*.

marine crustaceans *Crangon* is very efficient in keeping the magnesium ion concentration in the h  molymp low (see Nicol (1967) for details on other species).

It has long been recognized that a low magnesium ion concentration in crustaceans is associated with high activity. Thus, since *Crangon* is an animal capable of very intense outbursts of activity (Hagerman, 1970b) it must have a low magnesium ion concentration in the blood.

The analysis of potassium ions in different salinities showed a concentration in the blood higher than the surrounding medium. (Fig. 3). The potassium ion concentration showed a very great variability, 2.5 mEq within the same salinity. This could be due to technical reasons (all animals examined were of stage C-D₁) or it could be due to residues of breakdowned cells as the rather high cellular potassium concentration can elevate the blood concentration in some animals when whole blood is analyzed (Potts & Parry, 1964). A still greater variability was found by McLusky (1968) for *Corophium volutator*. The concentration of potassium in *Crangon* is slowly approaching the outer medium in higher salinities, above 9 mEq/l (i.e. above 30‰) no difference between blood and sea water was found. This pattern of potassium regulation in lower salinities and the relative ionic content is similar to that noted for other brackish water crustaceans (Nicol, 1967). The obviously decreasing ability to maintain the great difference between blood and medium must be correlated to the general decrease in total hyperconcentration and in many cases a hypoconcentration. That the potassium concentration in the blood in higher salinities could be most variable, also within the same species, is well known (Adelung, 1971).

Calcium (Fig. 4) was consistently held more concentrated than the medium, with differences of 2 to 7 mEq for minimum values within the salinity range. The difference between inner and outer medium is greater the lower the salinity. The large difference between the highest and lowest concentration in a specific salinity is due to the variation in stored calcium for ecdysis in moult stages C-D₁.

The blood analyses are summarized in Tab. 1. The pattern of regulation for *C. vulgaris* in the hyperosmotic range; an increase of sodium, potassium, calcium and chloride relative to the medium and a strong decrease in magnesium ion concentration, and also the relative ionic content, is similar to that noted for other crustaceans (Nicol, 1967; Lockwood, 1968). In the hypo-osmotic range *C. vulgaris* showed a decrease of sodium and chloride relative to the medium, while calcium was still higher and potassium almost isosmotic to the medium. Magnesium was still very far below medium concentration. This pattern of regulation is not so well known for marine and brackish water animals, partly because rather few hypo-osmotic species has been investigated with regard to ionic regulation (Potts & Parry, 1964; Parry, 1954; Dehnel & Carefoot, 1965). With respect to sodium, calcium, magnesium and chloride similar results were found for *Leander (Palaemon) serratus* by Parry (op.cit.) while potassium showed a considerably lower blood concentration in *Leander* than in *Crangon*.

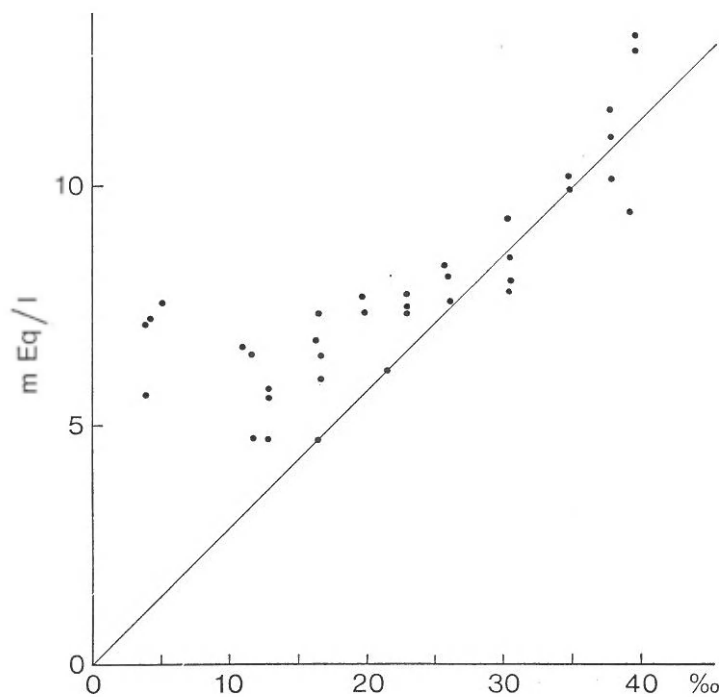


FIG. 3. The concentration of potassium ions in the blood of *Crangon vulgaris*.

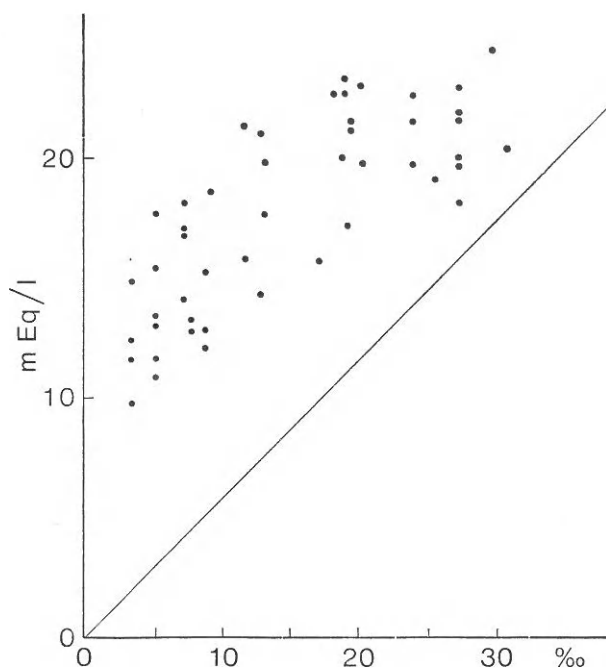


FIG. 4. The concentration of calcium ions in the blood of *Crangon vulgaris*.

TABLE 1. Analysis of inorganics in the blood of *Crangon vulgaris* in moult stage C-D₁.

Salinity of medium ‰	$\Delta^{\circ}\text{C}^*$	NaCl mM ***	Cations (mEq/l)				Total cations	Discr.	Anion (mEq/l) Cl	Total anions (+40 mEq/l for SO ₄ **)
			Na ⁺	K ⁺	Ca ⁺⁺	Mg ⁺⁺				
5	0,9	257,4	205	6,6	14,7	4,5	230,8	26,6	245	285
10	1,2	343,2	310	6,0	16	8,3	340,3	2,9	268	308
15	1,4	400,4	320	6,3	18	9,0	353,3	47,1	312	352
20	1,4	400,4	348	7,1	20	10,1	385,2	15,2	352	392
25	1,5	429,0	387	7,9	20,5	10,6	426,0	3,0	360	400
30	1,6	457,6	390	8,0	21	17,5	436,0	21,6	405	445
35	1,9	543,4	410	—	23	18,1	461,0	82,4	421	461

* According to Hagerman (1971)

** According to Nicol (1967)

*** mM = $\Delta \times 286$

DISCUSSION

The investigations on the osmotic and ionic balance in *Crangon vulgaris* has indicated that the intermoult stage is very well fitted for life under changing or low salinity conditions, i.e. in estuaries, in tidal areas and in permanently brackish waters. Within the hyperosmotic range the ions accounts for the majority of the osmotic pressure, but a small discrepancy was found at high salinities (see Table 1). This discrepancy may be suggested to be due to nonelectrolytes such as amino-acids. For hyperosmotic crustaceans it is a general statement that the concentration of amino-acids increase as salinity decrease (Lockwood, 1968).

To keep the haemolymph at concentrations deviating from the medium requires mechanisms with which the animal can protect itself from the negative influence of the medium; i.e. a low surface permeability, an ability to transport ions against a concentration gradient, and in some cases an ability to produce an urine with a composition other than the haemolymph. *Crangon vulgaris* has a very low surface permeability. When the silver-staining technique of McLusky (1968) was used the only stained areas for intermoult animals throughout the year were the gills, the excretory organs and the flagellum of the second antenna (probably a center for chemoreceptory stimulus). Thus in this respect *C. vulgaris* is very well suited for a life in an estuarine habitat. The sensitive phases with regard to permeability are the ecdyses, as it is shown that the permeability of the cuticle changes during and immediately after the ecdysis (Hagerman, 1973).

According to Spaargaren (1971) *C. vulgaris* (*C. crangon*) produces urine isosmotic to the blood. This is an obvious disadvantage for such a good osmoregulator as *C. vulgaris* and in contrast to some other species living in similar biotopes: *Gammarus duebeni* (Lockwood, 1961), *Corophium volutator* (McLusky,

1968) and *Nereis diversicolor* (Smith, 1970). A few experiments on *C. vulgaris* from the Øresund indicates that here the urine in fact is hypo-osmotic to the blood. This will be further investigated.

A discrepancy between the total osmotic concentration of the blood and the ionic concentration is found at all salinities with the largest difference at the highest salinity, 35‰. The difference, i.e. the amount of non-electrolytes, shows the same variations versus the salinity of the medium as shown by Spaargaren (1971) for the same species. The non-electrolyte fraction seems to vary between almost 0 to 15-20 % of the total osmolarity, dependent on the salinity, and according to Spaargaren (op.cit.) it is also dependent on the temperature.

REFERENCES

- ADELUNG, D. 1971. Untersuchungen zur Häutungsphysiologie der dekapoden Krebse am Beispiel der Strandkrabbe *Carcinus maenas*. Helgoländer wiss. Meeresunters., **22**: 66-119.
- BROEKEMA, M.M. 1941. Seasonal movements and the osmotic behaviour of the shrimp, *Crangon crangon* L. Archs néerl. Zool., **6**: 1-100.
- DEHNEL, P.A. & CAREFOOT, T.H. 1965. Ion regulation in two species of intertidal crabs. Comp. Biochem. Physiol., **15**: 377-397.
- FLÜGEL, H. 1960. Über den Einfluss der Temperatur auf die osmotische Resistenz und die Osmoregulation der dekapoden Garnele *Crangon crangon* L. Kieler Meeresforsch., **16**: 186-200.
- HAGERMAN, L. 1970a. The oxygen consumption of *Crangon vulgaris* (Fabricius) (Crustacea, Natantia) in relation to salinity. Ophelia, **7**: 283-292.
- 1970b. Locomotory activity patterns of *Crangon vulgaris* (Fabricius) (Crustacea, Natantia). Ibid., **8**: 255-266.
- 1971. Osmoregulation and sodium balance in *Crangon vulgaris* (Fabricius) (Crustacea, Natantia) in varying salinities. Ibid., **9**: 21-30.
- 1973. Ionic regulation in relation to the moult cycle of *Crangon vulgaris* (Fabricius) (Crustacea, Natantia) from brackish water. Ibid., **12**: 141-149.
- LOCKWOOD, A.P.M. 1961. The urine of *Gammarus duebeni* and *G. pulex*. J. exp. Biol., **38**: 647-658.
- 1968. Aspects of the physiology of Crustacea. Oliver & Boyd, Edinburgh, 328 pp.
- McLUSKY, D.S. 1968. Aspects of osmotic and ionic regulations in *Corophium volutator* (Amphipoda). J.mar.biol.Ass. U.K., **48**: 769-781.
- MUUS, B.J. 1967. The fauna of Danish estuaries and lagoons. Meddr Danm. Fisk.- og Havunders., N.S., **5**: 1-316.
- NICOL, J.A. 1967. The biology of marine animals. Pitman, London, 699 pp.
- PARRY, G. 1954. Ionic regulation in the palaemonid prawn *Palaemon* (= *Leander*) *serratus*. J.exp.Biol., **31**: 601-613.
- POTT, W.T.W. & PARRY, G. 1964. Osmotic and ionic regulation in animals. Pergamon Press, Oxford, 423 pp.
- SMITH, R.I. 1970. Hypo-osmotic urine in *Nereis diversicolor*. J.exp.Biol., **53**: 101-108.
- SPAARGAREN, D.H. 1971. Aspects of the osmotic regulation in the shrimps *Crangon crangon* and *Crangon allmanni*. Neth.J.Sea Res., **5**: 275-333.
- WEBER, R.E. & SPAARGAREN, D.H. 1970. On the influence of temperature on the osmoregulation of *Crangon crangon* and its significance under estuarine conditions. Ibid., **5**: 108-120.