ASPECTS OF THE OSMOTIC REGULATION IN THE SHRIMPS CRANGON CRANGON AND CRANGON ALLMANNI

D. H. SPAARGAREN

STELLINGEN

I

De problematiek die neergelegd is in

Man kann nicht wissen
was Fische pissen
Unter 's Wasser sieht man es nicht
und über 's Wasser tun sie es nicht

kan experimenteel worden benaderd.

Volkswijsheid.

II

De met osmoregulatie samenhangende seizoensmigratie, zoals deze gevonden werd voor de gewone garnaal en de koningsgarnaal, maakt een studie van de osmoregulatiemechanismen van verwante soorten, die niet deze mogelijkheid tot habitatkeuze hebben, gewenst.

III

Een opzwellen van het spijsverteringskanaal van adulte Ephemeroptera kan een rol spelen bij het uitstoten van de geslachtsproducten; de hypothese dat het spijsverteringskanaal een aerostatische functie heeft is niet aannemelijk.

A. D. Imms, 1957. A general textbook of entomology: 284–293.

IV

Op goede gronden rekent Edney de isopode *Philoscia muscorum* tot de weinig aan het landleven aangepaste soorten. Op betere gronden moet deze soort echter tot de best aan het land geadapteerde Isopoda worden gerekend.

E. B. Edney, 1954. Biol. Rev. **29**: 185–220.

V

De nachtelijke excursies van diverse soorten pissebedden dienen niet voor het verliezen van water dat gedurende hun verblijf in een vochtige schuilplaats is opgenomen. Waarschijnlijk zijn het foerageertochten, waarvan de duur samen kan hangen met de luchtvochtigheid.

P. J. den Boer, 1961. Proefschrift, Leiden.

VI

De experimenten van NISHIMURA geven onvoldoende grond voor de functie die hij toeschrijft aan cytochroom b in de fotosynthesketen.

M. NISHIMURA, 1968. Biochim. Biophys. Acta **153**: 838–847.

VII

De conclusie van WEBSTER dat *Hymenolepis diminuta* een osmoconformer is lijkt zeer aannemelijk, maar volgt niet uit de experimenten.

L. A. WEBSTER, 1970. Comp. Biochem. Physiol. **37**: 271–273.

VIII

De invloed van de temperatuur op de zuurstofbinding door verschillende bloedpigmenten ligt niet in dezelfde orde van grootte, zoals KLOTZ & KLOTZ vanuit een thermodynamische beschouwing suggereren, maar kan een adaptieve variatie vertonen.

I. M. KLOTZ & T. A. KLOTZ, 1955. Science **121**: 477–480.

IX

Voor de bepaling van de hoeveelheid extra-cellulaire vloeistof in aquatische evertebraten kan men in plaats van een fysiologisch inerte stof, als het algemeen toegepaste inuline, beter gebruik maken van een fysiologisch belangrijke stof waarvan de concentratie op cellulair niveau sterk gereguleerd wordt.

X

Om praktische redenen is de combinatie van Cetaceeën onderzoek en een dolfijnenshow erg gunstig. De kosten van dit onderzoek horen hierbij echter niet op de bezoekers van de show te rusten.

XI

Reactiesnelheid en concentratievermogen zijn in het verkeer belangrijker criteria dan het alcoholpercentage in het bloed; bovendien zijn deze grootheden eenvoudiger te bepalen.

D. H. Spaargaren

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ACADEMISCH PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE WISKUNDE EN NATUURWETENSCHAPPEN
AAN DE UNIVERSITEIT VAN AMSTERDAM OP GEZAG
VAN DE RECTOR MAGNIFICUS MR. A. D. BELINFANTE,
HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID,
IN HET OPENBAAR TE VERDEDIGEN
IN DE AULA DER UNIVERSITEIT
(TIJDELIJK IN DE LUTHERSE KERK,
INGANG SINGEL 411, HOEK SPUI) OP
WOENSDAG 21 APRIL 1971 DES NAMIDDAGS TE 3 UUR PRECIES

DOOR

DIRK HERMANNUS SPAARGAREN
geboren te Eindhoven

E. J. BRILL LEIDEN 1971

Promotor: Prof. Dr. A. Punt
Co-Referent: Dr. R. E. Weber

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Aan mijn ouders
Aan mijn vrouw

ASPECTS OF THE OSMOTIC REGULATION IN THE SHRIMPS CRANGON CRANGON AND CRANGON ALLMANNI

by

D. H. SPAARGAREN

(*Netherlands Institute for Sea Research, Texel, The Netherlands*)

CONTENTS

I. INTRODUCTION

Two species of shrimps are very common in the North Sea: *Crangon crangon* and *Crangon allmanni*. Their distribution, however, is different. The greater part of *C. crangon* migrates to coastal and inland waters, such as the Wadden Sea, in spring, and back again towards the North Sea in early winter, while *C. allmanni* remains in deeper waters all the year round, though it may move slightly nearer to the coast in winter—a direction opposite to that of *C. crangon* (VERWEY, 1957).

BROEKEMA (1942) found a correlation between migration and regulation of the electrolyte concentration in the blood of *C. crangon*. From her data VERWEY (1957) derived the hypothesis that the species migrates towards salinities where it can keep the difference between its internal osmotic pressure—dependent on temperature—and that of the surrounding water at a constant value.

This led to three questions which form the theme of the underlying research: (*1*) in which way do the environmental factors salinity and temperature influence the internal osmotic concentration of *C. crangon*, (*2*) how does the internal regulation of the related, but non-migratory species vary at various salinities and temperatures, and (*3*) which mechanisms play a part in the osmotic regulation.

Water and soluble matter may enter or leave an animal body in various ways. In crustaceans the exchange between the animal and its environment is limited by the presence of a relatively impermeable exoskeleton. Various regulation processes may affect other exchange possibilities, *e.g.* by the kidneys, the gills or the gut. Non-electrolytes, such as amino acids, glucose, lipids, of which the excreted amount is negligible, may also influence the internal concentration. The internal concentration of crustaceans may thus be differently affected under varying circumstances. In some cases it remains isosmotic with the medium at various salinities, or it maintains a constant difference: "conformity". In other species the internal concentration may remain

more or less constant though salinity of the medium varies: "regulation" (Lockwood, 1962; Potts & Parry, 1964).

In the present research the internal osmotic concentration as well as the concentration of electrolytes and non-electrolytes, at various temperatures and salinities, were determined in both species of shrimps. In addition the transport of the dissolved substances under varying circumstances was investigated. This included the transport of water and electrolytes via the urine and the extra-renal transport of electrolytes from the animal to its environment, and the transport of water, electrolytes and non-electrolytes between tissues and blood.

Acknowledgements.—To Professor Dr. A. Punt (Laboratory of Animal Physiology, Amsterdam University) I want to express my sincere thanks for his stimulating interest and valuable advice during the course of this research. I am also very grateful to Dr. R. E. Weber (Netherlands Institute for Sea Research, Texel) for many instructive discussions and help. Further I am indebted to Dr. J. Verwey (former director of our Institute) for his advices and his interest in the subject.

I enjoyed the cooperation of many colleagues in our laboratory of whom I mention especially Drs. J. W. de Blok, who adviced me on the composition of the manuscript, Mr. H. Hobbelink, who prepared the diagrams, Mr. E. Pauptit and Mr. G. W. de Kraay, who were always willing to help with the experiments. Mr. J. J. A. van Weereld helped to overcome technical complications and Miss S. M. van der Baan translated the manuscript into English. Their kind cooperation is warmly acknowledged.

II. MATERIAL

The research was carried out with two shrimp species: *Crangon crangon* (L.) and *Crangon allmanni* Kinahan. *C. crangon* was caught off the island of Texel all year round, in water of which salinities varied between 26‰ and 33‰. In summer this species is common in the Wadden Sea and in coastal waters of the Southern Bight. In winter the animals migrate to deeper water of a higher salinity (Havinga, 1930; Broekema, 1942; Lloyd & Yonge, 1947). *C. allmanni* was collected in the spring of 1969 and 1970, in the North Sea between Texel and Heligoland. The species is common in deeper water, but late in winter it is also found in waters nearer to the coast.

C. crangon is euryhaline as well as eurytherm, but it can stand lower salinities better when the temperature is high, and higher salinities when the temperature is low (Broekema, 1942; see also Haefner, 1969). On the other hand *C. allmanni* is found in a region where the

range of variation in salinity is narrow (32 to 34‰ S), and where temperatures are relatively low (4 to 12 °C).

Male and non-ovigerous female specimens were used in the experiments.

Adaptation to various salinities between 5‰ and 40‰ and to three temperatures (about 5 °C, 15 °C and 21 °C) was carried out in small glass aquaria (30 × 22 × 23 cm) provided with a bottom filter of shell grit covered with sand. Circulation as well as gaseous equilibrium and microbial cleaning of the aquarium water was obtained by placing a diffuser block in the grit and covering it with an inverted funnel. Lowered salinities were obtained by mixing seawater with tapwater. The local tapwater did not contain metal ions in harmful concentrations. To obtain higher salinities than that of the available seawater, salt from evaporated seawater was added (Dr. Ritters Südsalz). This added "Südsalz" contains relatively less calcium, since during evaporation part of this calcium is precipitated irreversibly. Calcium affects the permeability of animal membranes (PANTIN, 1931a, 1931b; KITCHENER, 1957), however, it will be supplemented from the shell grit covering the bottom.

Adaptation of the animals to higher or lower salinities was performed in daily steps of 5‰ S at the most. Twice a week the animals were fed with *Tubifex* or chopped fish. In the case of *C. crangon* mortality was low, except at the highest temperature (21 °C) and, for all temperatures studied, at the lowest salinity (5‰ S). In *C. allmanni* mortality was high at 21 °C; at this temperature salinities below about 25‰ were lethal (page 282).

III. OSMOTIC CONCENTRATION OF THE BLOOD

1. INTRODUCTION

Several authors (BROEKEMA, 1942; FLÜGEL, 1960, 1963; GRIMM, 1969) found that *C. crangon* possesses osmoregulatory powers. At low salinities the blood was hyperosmotic to the medium, at high salinities it was hypo-osmotic. The diagrams representing the changes in the osmotic concentration of the blood (Δ_i) as a function of that of the medium (Δ_e) are, however, rather divergent. In order to check whether those differences were due to differences in the animals used or to the different measuring techniques this relation was determined anew (WEBER & SPAARGAREN, 1970). It appeared that the differences could be seen as physiological adaptations to the temperature and salinity conditions of the areas in which the animals were collected. Compara-

tive research indicated that an influence of temperature on osmotic concentration, similar to that found in *C. crangon*, was also found in experiments with other crustaceans, where it could be explained as a similar adaptation. An extensive discussion of the possible ecological implications of the Δ_i/Δ_e regulation pattern and the influence of temperature on this is to be found in the above paper.

In *C. allmanni* GRIMM (1969) found a high degree of "conformity" at 10 °C, the blood concentration being practically isosmotic to the medium at all salinities. Supplementary observations were needed to determine whether this non-estuarine species does also show an influence of temperature on osmotic blood concentration and whether our animals and those used by GRIMM—obtained from the Marine Station, Millport, Isle of Cumbrae—represent physiologically different strains.

When measuring the osmotic concentration of blood it is necessary to know whether adaptation to a given salinity has been complete. In experiments with *C. crangon* in which the animals were transferred from 34‰ S to 24‰ S at a temperature of 10°C, GRIMM (1969: 14,15) found that the osmotic, chloride and sodium concentrations of blood and urine decreased at a very slow rate. This does not agree with other observations. In similar experiments with *C. crangon* BROEKEMA (1942) found a rapid decrease in blood conductivity during the first 5 hours, followed by a slow decrease in blood conductivity over the next 35 hours. In *Carcinus maenas* MARGARIA (1931) and SHAW (1961a) also found a rapid decrease of respectively osmotic concentration and sodium concentration in the blood. GROSS (1957) found a rapid osmotic adaptation even in semi-terrestrial and terrestrial decapods. For this reason observations on the rate of salinity adaptation in the animals to be used in the experiments were carried out before starting the determinations on blood osmotic concentrations.

2. METHODS

As a measure for the osmotic concentration the freezing point depression of the blood was determined with the aid of a Knauer semi-micro osmometer. To this end blood was removed from the pericardial cavity by penetrating the membrane between carapace and first abdominal segment with a finely drawn out calibrated glass capillary. The amount of about 20 µl thus obtained, diluted with 50 µl of distilled water, permitted the determination of the osmotic concentration of the blood of individual animals.

3. RESULTS AND DISCUSSION

a. Changes in the blood concentration

When measuring the changes in blood concentration after transferring *C. crangon* from 30‰ S to 15‰ S it appeared that the rate of adaptation was strongly dependent on temperature. The "half-time" values for accomodation of Δ_i were 10.2, 2.4 and 1.2 hours at temperatures of 4 to 6 °C, 15 °C and 21 °C, respectively.

A closer examination of GRIMM's (1969: 13, Fig. 2) observations revealed that the blood concentrations before the animals were transferred to lower salinity were too low to represent complete adaptation to a salinity of 34‰ S. Therefore it seems plausible that the contradiction between GRIMM's results and those obtained here and elsewhere may be explained by assuming that GRIMM's animals had not been adapted to 34‰ S, but to a lower salinity.

Measurements concerning the rate of change in blood concentration under the influence of temperature have not been described previously. Some indications have been found (page 317) that this change proceeds at a much slower rate than salinity adaptation, but that it is completed within 5 days. Measurements of the osmotic values of the blood of animals adapted for longer periods to a given salinity did not differ significantly from those of animals adapted for 5 days.

The change in blood concentration after a change in salinity of the medium is an important factor in the ecology of a species (GROSS, 1957; KINNE, 1964b, 1964c). Generally, marine species show a quicker adaptation rate than estuarine species. Among other factors permeability plays a role (pages 299 and 313). In accordance with this general tendency GRIMM (1969: 11–16) found a rapid change in the blood concentration of *C. allmanni* when the salinity of the medium decreased: a practically complete adaptation had been reached within 4 hours.

The above data led us to assume that in all further experiments an adaptation period of at least 5 days would be sufficient for complete adaptation, both for *C. crangon* and for *C. allmanni*.

b. Osmotic concentration in the blood of *C. crangon*

At various temperatures the relation between osmotic concentration in the haemolymph and salinity in the medium shows practically the same relation (Fig. 1). Below about 15‰ S and above about 30‰ S there is a considerable "conformity". Between those values the blood concentration exhibits a certain amount of "regulation". The salinity at which maximal regulation occurs (where the slope of the curve is

minimal) decreases, however, with higher temperatures. The figures for the blood concentration and the isosmotic point do also show an inverse relation with the temperature.

These results agree very well with data on shrimps of similar origin which BROEKEMA (1942) obtained from conductivity measurements. There are obvious differences, however, with the relations FLÜGEL (1963) and GRIMM (1969) determined for this species with animals collected from the Baltic and from Clackmannanshire, Scotland, respectively (WEBER & SPAARGAREN, 1970).

The shifting of the regulation range to lower salinities at higher temperatures may be correlated with the more brackish water of the coast and the Wadden Sea in summer (BROEKEMA, 1942). Furthermore it appeared that the combination of high temperatures and high salinities of the medium and, on the other hand, of low temperatures together with low salinities, resulted in maximal mortality (*cf.* HAEFNER, 1969). This may be correlated with the fact that under those circumstances the blood concentration differs maximally from the value at which Δ_i is best regulated.

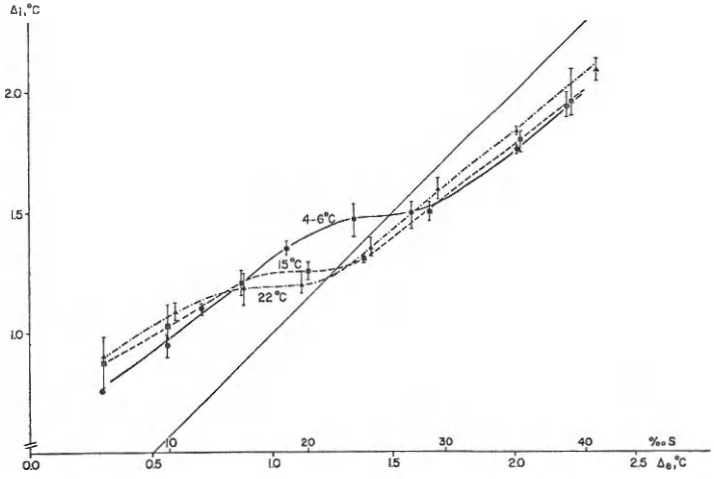


Fig. 1. Osmotic concentration of the blood of *C. crangon* as a function of osmotic concentration of the medium at 3 temperatures. ● 4–6 °C, ■ 15 °C, ▲ 21 °C; the vertical lines show the range of values found.

c. Osmotic concentration in the blood of *C. allmanni*

In *C. allmanni* the relation between osmotic concentration in the blood and salinity of the medium shows a very high degree of "conformity". At all salinities the blood concentration is practically isosmotic to the

medium. At low salinities, however, a very slight hypertonicity is found, at higher salinities a slight hypotonicity, which is most obvious at 21 °C.

At the temperatures at which the species is normally found there is no temperature effect. The blood concentrations determined at 15 °C do not differ significantly from the values measured at 5 to 7 °C.

In this species no difference in osmotic response is found in comparison with the animals used by GRIMM. The high degree of conformity is in accordance with what is to be expected for a stenohaline, marine species (LOCKWOOD, 1963).

At 21 °C *C. allmanni* cannot stand salinities below 25‰. At 5 to 7 °C salinities as low as about 10‰ can still be tolerated. The observations on mortality yield the reverse picture of those obtained with *C. crangon*, where lower salinities are better tolerated at higher temperatures. This agrees with the difference in distribution: in winter *C. allmanni* may be also found nearer to the coast, while on the other hand *C. crangon* migrates to deeper water.

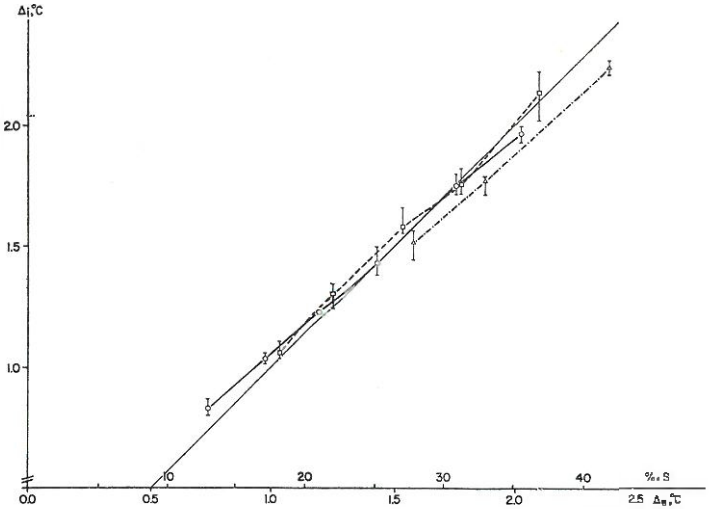


Fig. 2. Osmotic concentration of the blood of *C. allmanni* as a function of osmotic concentration of the medium at 3 temperatures. ○ 5–7 °C, □ 15 °C, △ 21 °C; the vertical lines show the range of values found.

IV. WATER CONTENT

1. INTRODUCTION

Whenever the internal concentration of an animal is hypertonic to the surrounding medium, water will enter the body by osmosis, conversely

an animal will loose water in a hypertonic medium. Thus changes in the internal amount of water may occur which influence osmotic concentration of the haemolymph. On the other hand crustaceans possess a tough exoskeleton, capable of limiting water uptake in a hypotonic medium by exerting turgor pressure. There is also the possibility of an active regulation of water content by *e.g.* drinking and urine excretion.

The above considerations led to the investigation whether any effective regulation of the amount of water occurred under various circumstances. To this end the water content was determined in specimens of *C. crangon*, adapted to various salinities and temperatures.

At the same time, the effect of a change in total water content on osmotic concentration of the blood could be studied.

In varying external conditions the blood of *C. allmanni* remains practically isosmotic to the medium, probably merely by the uptake and loss of salts. For this reason changes in the water content are less likely to occur in this species.

2. RESULTS AND INTERPRETATION

The values obtained for the water content of animals adapted to the same temperature and salinity were widely divergent (Fig. 3). No correlation with the size of the animal could be found. Probably the divergencies are the outcome of the inaccuracy in the determinations of the wet weights. Besides, the amount of blood that can be drawn from various shrimps may differ to such an extent that they seem to indicate the existence of a considerable individual variation, perhaps connected with the moulting cycle (ROBERTSON, 1960).

The difference in mean water contents of animals adapted to low (about 10‰) and to high salinities (about 40‰) is, however, significant: $p < 0.01$, Student's test with Bessel correction. BROEKEMA (1942: 92–95) found no change in weight in *C. crangon* when transferred to lower or higher salinities, which might point to a very efficient water regulation. Our data are also in agreement with an efficient water regulation, though in extreme conditions some change in water content may be found.

The salinities at which maximal water regulation takes place are about the same as those at which Δ_i is regulated maximally. The curves in Fig. 3 (lower diagram) represent the most likely relations between water content and salinity at 3 temperatures. When considering the water content we find that at higher temperatures the salinity traject at which maximal regulation occurs shifts to lower salinities.

There exists an inverse relation between water content and salinity.

The relation between blood concentration and salinity, however, is direct. The effect of temperature on water content and blood concentration in the regulation range is also the reverse. This suggests that the

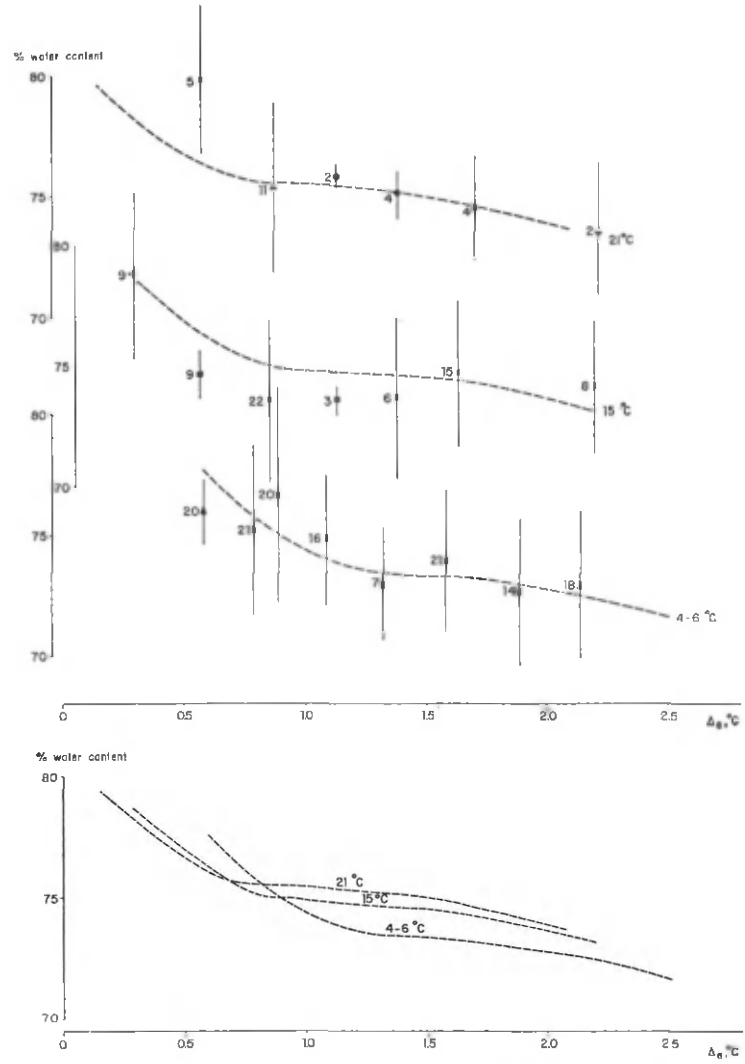


Fig.3. Water content of *C. crangon* at various salinities of the medium and at 3 temperatures. The symbols indicate average values, the vertical lines the standard deviations. The figures show the number of measurements. In these and all following diagrams the symbols give the values as determined; the broken lines the most likely interpretation of the relation. In the lower diagram the curves of the upper diagrams are shown together for better comparison of their mutual positions.

changes in Δ_i under influence of salinity and temperature may be brought about to a certain extent by changes in the water content. The effect of changes in water content on blood concentration may be calculated by a simple formula.

The molecules and ions in an animal may be divided into three groups: (*1*) water molecules (N moles), (*2*) molecules and ions dissolved in water (n moles) and (*3*) other molecules and ions. The water content (W) of the animal—the weight of the water molecules as a percentage of the total weight—may be represented by:

$$W = \frac{N \cdot M_{H_2O}}{N \cdot M_{H_2O} + n \cdot a + b} \times 100$$

where M_{H_2O} denotes the molecular weight of water; a, the weighted mean of the molecular weights of the dissolved substances; and b the weight of the other molecules and ions.

The freezing point of a solution may be represented by $\Delta = c \cdot n/N$ (c being a constant; GLASSTONE & LEWIS, 1965: 241). Since probably no osmotic differentiation can exist within the body (page 319; FLORKIN & SCHOFFENIELS, 1969) we may also say: $\Delta_i = c \cdot n/N$. By substituting N in the formula for water content and introducing k for the constant $\frac{n \cdot a + b}{c \cdot n \cdot M_{H_2O}}$ we arrive at:

$$W = \frac{100}{k\Delta_i + 1}$$

This formula served to calculate the changes in water content under the influence of salinity and temperature respectively, and their effects on internal concentration.

The first calculation concerned the water contents necessary to explain the range of blood concentration as measured in various salinities, assuming that no changes occurred in the amount of dissolved substances. Here the factor k could be calculated by inserting the values for Δ_i and W, as measured in the isosmotic point (at 4 to 6 °C as 1.48 °C and 73.3% respectively).

When the experimentally determined water contents are compared with the calculated results (Fig. 4) it appears that generally the changes in water content in *C. crangon* are too small to cause the differences in Δ_i. In a hypertonic medium the increase in blood concentration will even be mainly brought about by an increase in dissolved substances. In a hypotonic medium, however, the decrease in internal concentration with a lowered salinity is only partly caused by a decrease of dissolved substances. Here the increase in water content is a main factor in the decrease of the blood concentration.

In a second calculation the values for Δ_i in the relation between blood concentration and salinity were corrected for differences in water contents. It appears that the curves for different temperatures (not shown here)—especially at lower salinities—approach, but do not

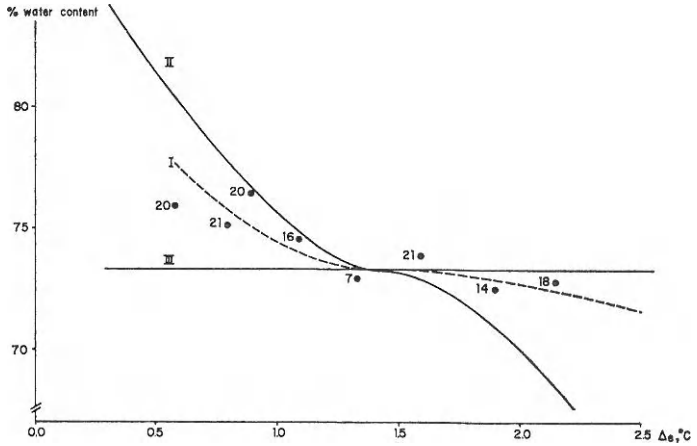


Fig.4. The relation between water content of *C. crangon* and salinity as derived theoretically and as determined. I, measured values; II, water contents calculated if no net transport of dissolved substances should occur when passing from one salinity to another; III, water contents if no net transport of water should occur. Temperature 4–6 °C. The figures next to the symbols show the number of measurements.

overlap each other. The conclusion is that the effect of temperature on internal concentration is not wholly due to the influence of temperature on water content. When the temperature changes there must be some change in the amount of dissolved substances. Further indications to this effect are discussed in Chapter VII.

V. WATER REGULATION

1. INTRODUCTION

In the range of salinity where regulation of internal concentration is maximal, the difference between Δ_i and Δ_e is variable. A greater difference does not result in a net uptake or loss of water. Since at the above mentioned salinities the water content is practically constant, while it varies at extreme salinities, where the difference between Δ_i and Δ_e is less variable, it is clear that water regulation does indeed occur.

In a hypotonic medium, regulation means that the water entering the body has to be removed. It appears that in crustaceans the ability to excrete water with urine hypotonic to blood, is confined to a few fresh water species, such as *Procambarus clarkii* (LIENEMAN, 1938), *Astacus fluviatilis* (BRYAN, 1960a), *Gammarus duebeni*, *G. pulex* (LOCKWOOD, 1961), and *Orconectes virilis* (RIEGEL, 1961). Other crustaceans from fresh and brackish water produce urine which is practically isosmotic to the blood, *e.g. Potamon edule* (DUVAL, 1925), *Eriocheir sinensis* (SCHOLLES, 1933), *Carcinus maenas* (NAGEL, 1934), *Palaemonetes varians* (PANNIKAR, 1941), and *Palaemonetes antennarius* (PARRY, 1957).

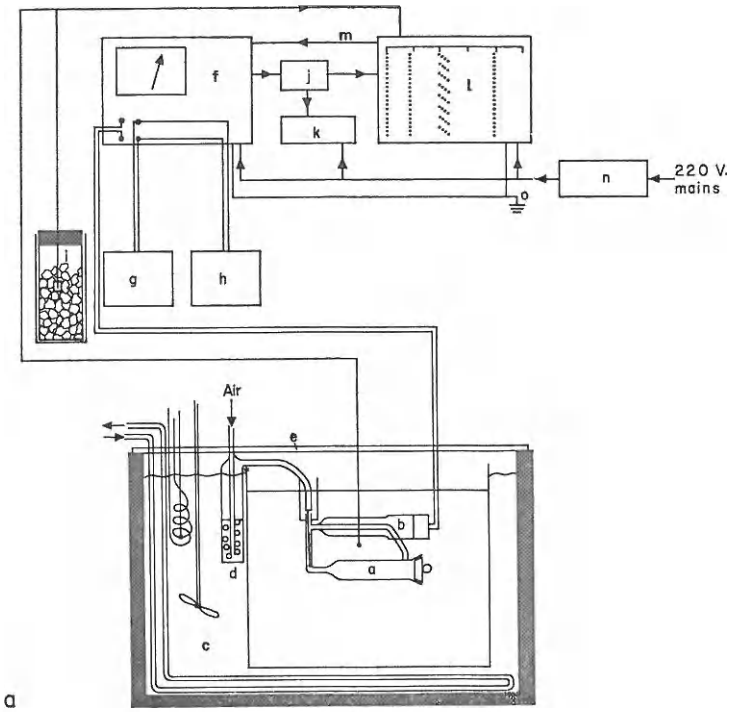
Besides the production of urine there is the possibility of an active extra-renal water excretion. A suggestion to this effect was obtained with *Potamon edule*, where the relatively low urine production might be the result of an extremely low permeability or an extra-renal water excretion (POTTS & PARRY, 1964: 174).

With water regulation in a hypertonic medium water must be actively taken up from the medium. In various crustaceans both oral and anal intake of seawater has been demonstrated (FOX, 1953; DALL, 1966). Absorption of water and salt may take place in the gut. Salt excretion may be effected by the gills, *e.g.* in *Artemia salina* (CROGHAN, 1958a, 1958b, 1958c) and *Ocypode albicans* (GREEN, HARSCH, BARR & PROSSER, 1959; FLEMISTER, 1959) or, as could be demonstrated for the latter species, by production of hypertonic urine (FLEMISTER & FLEMISTER, 1951).

In most aquatic crustaceans the urine is isosmotic to the blood, even in a hypertonic medium. This is the case with *Pachygrapsus grassipes* (JONES, 1941; PROSSER, GREEN & CHOW, 1955), *Palaemon serratus* (PARRY, 1954), and *Palaemonetes varians* (PARRY, 1955). In a hypertonic medium the production of urine isosmotic to the blood means an extra loss of water.

Apart from these possibilities of uptake of seawater coupled with extra-renal and renal excretion of the salts, there are also some indications for the occurrence of active water absorption. This could take place by means of the gut, *e.g.* in *Uca pugnax* (GREEN *et al.*, 1959) and *Metapenaeus bennettae* (DALL, 1966), the gills (GREEN *et al.*, 1959) or the pericardial sacs (BLISS, 1963), which also occur in some aquatic crustaceans, *e.g.* in *Gecarcinus lateralis* (BLISS, WANG & MARTINEZ, 1966).

As to the water regulation of *C. crangon*, both in a hypotonic and in a hypertonic medium, production and concentration of urine may play a role, apart from possible active extra-renal water excretion or an active absorption of water. GRIMM (1969) already found that the concentration of the urine of *C. crangon*, just as with *C. allmanni* and many



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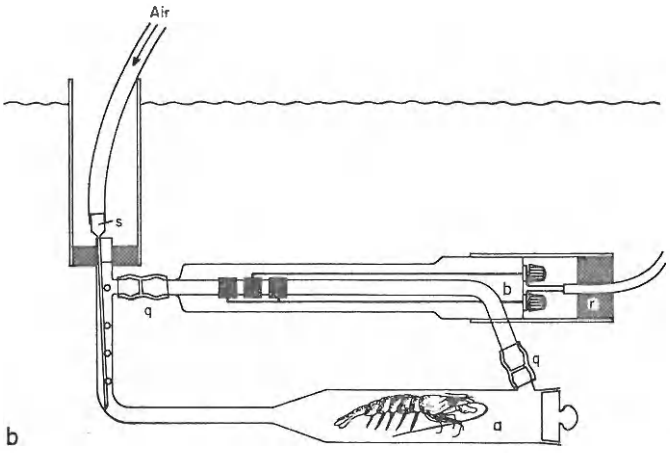

b

other crustaceans, was isosmotic to the blood. In the present research the effect of the amount of the urine at various salinities and temperatures is examined more closely.

2. METHODS

For the determination of urine production in crustaceans various techniques have been described: measuring changes in weight after blocking the nephropores (see a.o. HERMANN, 1931; SCHOLLES, 1933; PARRY, 1955; BRYAN, 1960; KAMEMOTO, KATO & TUCKER, 1966), periodically removing the bladder contents by suction (LIENEMAN, 1938; MALUF, 1941b; RIEGEL, 1961), catheterizing the nephropores (PARRY, 1955; KAMEMOTO & ONO, 1968), and injection of dyes (PARRY, 1955). All these methods had a limited exactness and entailed a considerable amount of handling of the animals.

The method used in this study is based on the principle that by intake and excretion of water and electrolytes an animal affects the composition of the surrounding medium. In a closed volume of liquid these changes could be measured by means of a sensitive continuous recording of the electric conductance.

Fig. 5 gives a scheme of the experimental set-up. The animal is put in a measuring cell (a). This cell consists of a glass tube which by means of two glass-to-glass connections forms a circuit with a micro-conductivity cell. Moist air, brought in through a long injection needle (s), circulates and aerates the water filling the measuring cell. The total amount of water circulating past the animal varies between 11 and 12 ml, *i.e.* 10 to 30 times the volume of the animal. The conductivity electrode (b) is connected to a direct-reading conductivity measuring bridge (f) where resistance and capacitance are measured against a reference resistance (g) and reference capacitance (h). By way of an attenuator and zero depression apparatus the difference in conductivity is registered by a multi-point recorder (l).

Fig.5. Set-up for measuring urine production; a, general scheme; b, detail of measuring cell. Indicated are: a, chamber for the animal; b, conductivity measuring cell (PHILIPS, type PW 9513); c, thermostated water bath; d, gas wash bottle with distilled water for saturating the air for aeration of the measuring cell with water vapour; e, temperature insulating cover plate; f, conductivity meter (PHILIPS, type PR 9501/01); g, resistance box, 10Ω to 21 KΩ; h, decade capacitor, 0 to 20 nF; i, temperature reference: vacuum jar with ice cubes; j, attenuator and zero depression apparatus; k, digital read-out; l, multipoint recorder (PHILIPS, type PR 3500); m, switch for changing over between conductivity and differential conductivity measurement; n, voltage stabilizer; q, glass-to-glass connection; r, waterproof housing for electrode connections; s, injection needle.

Sensitivity to temperature.—The conductivity of seawater is strongly dependent on temperature (temperature coefficient of seawater: 2–3% per °C, BRUNS, 1962). The sensitivity of the instrument was chosen at 2% conductivity difference for full scale deflection of the recorder. This entailed the maintenance of a very stable temperature, obtained by keeping the measuring cell in a water tank which was kept in a thermostated water bath in which fluctuations in temperature did not exceed 0.01 °C.

Testing.—The changes in conductivity, measured when an animal was present, may be expressed in terms of μmol NaCl equivalents, although an increase in conductivity will not be caused by the loss of NaCl only.

Changes in conductivity of the medium may be caused by exchange diffusion. Water absorption or water excretion will also–though to a small extent–affect the conductivity. If the change in conductivity proceeds gradually the influence of the above mentioned processes in the measurements cannot be separated. In the quick process of urine discharge, however, the effect of the slow processes can be disregarded. The scale could be calibrated by theoretical calculation. The values thus found agree with those measured when NaCl solutions of known concentrations were added.

Deduction of the calibration.—Assume an urinating animal adds x ml urine with a concentration equalling y μmol NaCl·ml^{-1}, to b ml of the medium with a salinity of S_0‰ and a temperature of t °C. The concentration of the medium with a conductivity $\varkappa^{t}_{0}$ may also be expressed as C_0 μmol NaCl·ml^{-1} (WOLF, 1966: table 5a). The change in concentration of b ml, C_0 μmol NaCl·ml^{-1} after addition of x ml, y μmol NaCl·ml^{-1} may then be represented as:

$$dC = \frac{b \cdot C_0 + x \cdot y}{b + x} - C_0$$

or

$$dC = \frac{y - C_0}{\frac{b}{x} + 1} \qquad (1)$$

Within a limited range of concentration, conductivity and concentration may be considered as directly proportional:

$$d\,\varkappa^{t} = K_{t,C} \cdot dC \qquad (2)$$

In this formula the factor $K_{t,C}$ determines the changes in conductivity occurring at a given change in concentration. In the experiments it

varied between 0.74 and 0.99. Usually the sensitivity was adjusted in such a way that 2% change in conductivity resulted in full scale deflection (scale deflection, $\Delta U = 250$ mm) of the recorder:

$$\Delta U = \frac{250 \cdot dx^t}{0.02 \varkappa \, {}^t_0} \tag{3}$$

Substitution of (1) and (2) in (3) gives:

$$\Delta U = 250 \cdot K_{t,C} \cdot \frac{\frac{y - C_0}{\frac{b}{x} + 1}}{0.02 \varkappa \, {}^t_0} \tag{4}$$

In this formula ΔU is the deflection in mm; $K_{t,C}$, the factor for sensitivity of the meter to changes in concentration in $m \cdot \Omega^{-1} \cdot cm^{-1}$ per μmol NaCl $\cdot$ ml^{-1}; y, urine concentration in μmol NaCl $\cdot$ ml^{-1}; C_0, initial concentration of the medium in μmol NaCl $\cdot$ ml^{-1}; b, volume of the medium in ml; x, urine volume in ml; $\varkappa \, {}^t_0$, conductivity of the measuring liquid at t °C at zero time in $m \cdot \Omega^{-1} \cdot cm^{-1}$ = reference conductivity.

With the aid of this formula the changes in conductivity at various salinities and temperatures could be converted into volume and concentration of the urine (pages 296 and 303).

3. RESULTS AND INTERPRETATION

A. WATER REGULATION IN C. CRANGON

a. General remarks

The production of urine by *C. crangon* was measured with animals adapted to various salinties and temperatures. During the measurements the animals were kept in water from the aquarium in which they had been kept for considerable time. Additional experiments have been carried out in which the animals studied were suddenly transferred to a lower salinity.

In a hypotonic medium the conductivity registration is serrated (Fig. 6a). The periodical quick increase in conductivity of the medium is caused by urine discharge. The increase in conductivity occurs in less than one minute. In this time none or only a few points are plotted on the registration. The increase in conductivity is followed by a slow decrease, due to an active uptake of electrolytes from the medium.

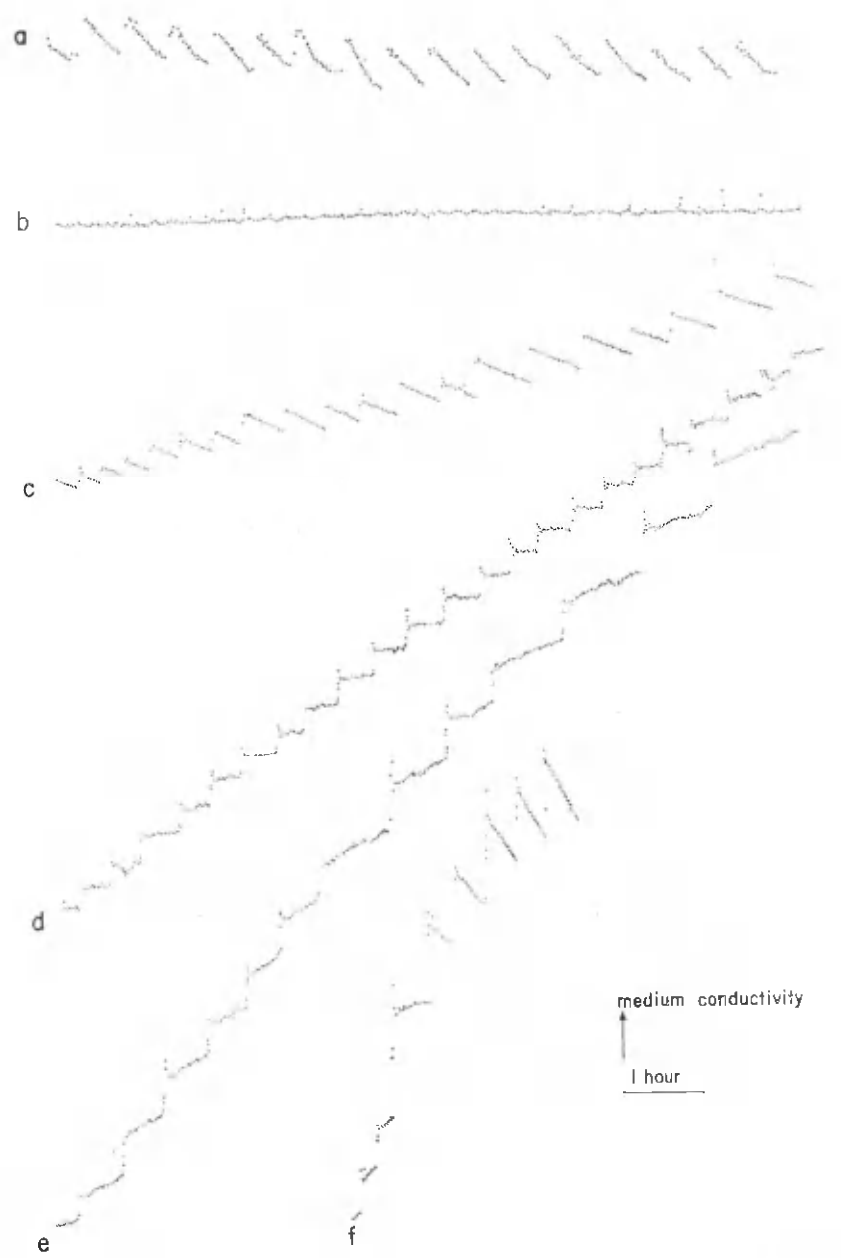

Fig.6. Examples of conductivity changes registered for *C. crangon* (on a different scale for the individual registrations); a, conductivity changes in a hypo-osmotic medium, salinity of medium (S_m) the same as to which the animal had been adapted ($S_m = S_a = 7‰$ S); b, conductivity in a hyperosmotic medium ($S_m = S_a = 36.3‰$); c, conductivity changes after transfer to lower salinity in a hypo-osmotic medium ($S_a = 12.1‰$, $S_m = 7.3‰$); d, as c ($S_a = 20.7‰$, $S_m = 11.1‰$); e, as c ($S_a = 20.7‰$, $S_m = 11.1‰$); f, as c ($S_a = 12.1‰$, $S_m = 7.3‰$). Temperature 21 °C.

These characteristics are not found in a hypertonic medium (Fig. 6b), where over a long time no changes in conductivity of the medium can be recorded. This means that either the production of urine is now much reduced or that the conductivity of urine and medium is the same, whereby addition of urine to the medium cannot be detected. If the urine in a hypertonic medium has the same conductivity as the medium then it would be hypertonic to the blood. This would be an advantage for the water regulation at the higher salinities, but the measurements are not conclusive in this respect.

When an animal is transferred suddenly to a lower salinity the medium will show a net increase of electrolyte, lost from the animal. This loss may be partly compensated by an active uptake (Fig. 6c). Sometimes the extra-renal loss compensates the active uptake: electrolyte loss occurs purely via the urine (Fig. 6d). In addition to renal loss a net extra-renal loss of electrolytes may occur (Fig. 6e). The changes in urine concentration, frequency of urine discharge and net loss in a given period of time correspond to changes in internal concentration. After some time active uptake may surpass extra-renal loss (Fig. 6f), probably by a decrease in the latter, related to a decrease in the internal concentration after some time of net electrolyte loss.

When an animal had died a gradual increase or decrease of conductivity in the medium was found in respectively a hypotonic and a hypertonic medium. This may be explained in terms of a gradual equalizing of the internal and medium concentrations. With the discharge of salt from a dead animal in a hypotonic medium there appeared to be an obvious break in the conductivity curve with the time. This suggests that the electrolytes in the body are confined to places where exchange with the medium proceeds at different rates. In this research this phenomenon has not been investigated any further.

b. Frequency of urination

The measurements give a very good impression of the frequency of urine discharge. During the experiment the animals remain undisturbed in the cell. Since the cell has to be temperature equilibrated, observations during the first hour are left out of consideration. Most observations lasted for more than 48 hours. In two cases the frequency of urination of an animal could be checked over a whole week. Even in these long periods—in which the animal did not get any food—no changes in the rhythm of urination could be found.

Though generally crustaceans are said to be very sensitive to handling the effect here was minimal. However, it was possible to disturb the regular rhythm by tapping on the cell. In that case a

significant correlation could be found between the period between successive urine discharges—induced by tapping on the cell—and the volume of the urine (Fig. 7).

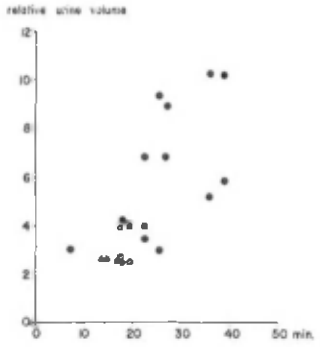

Fig.7. Urine volume of *C. crangon* after at different times experimentally induced discharges. Plotted are the relative urine volumes as a function of time between two successive urine discharges.

A gradual change of urination frequency was found in a few experiments in which the animals were transferred to a lower salinity. Generally a higher initial frequency was found which decreased gradually (Fig. 6e). In some cases, however, an increase in frequency was found with animals which became moribund as a result of the quick decrease in salinity. More extensive measurements on the response to changes in salinity have not been carried out.

No significant individual differences in urination frequency have been found between animals adapted to the same temperature and salinity, not even when they were of greatly differing sizes.

The frequency of urination is dependent on temperature and salinity (Fig. 8).

At low salinities the frequency of urination reaches a certain maximum, at higher salinities there is a decrease up to the isosmotic point. At 21 °C a slight decrease is found with the lowest salinities. PARRY (1955), in her research on *Palaemonetes varians* in hypotonic salinities, found a similar decrease of urine production towards the isosmotic point. A maximum in the lower salinities is not found here. SHAW (1961a) found the same for *Carcinus maenas*.

In the range of salinities at which maximal regulation of the internal concentration and water content is found, the frequency of urine discharge increases with decreasing salinity. This relation exists at 5 °C as well as at 21 °C: the shift of the regulation range in which

Δ_i and water content are regulated (Figs 1 and 3) is correlated with a shift of the salinities at which the rhythm of urine discharge changes.

At lower salinities, where conformity of Δ_i and increase of water content were found, the frequency of urine discharge is practically constant, and at its maximal value. That at lower salinities no maximal

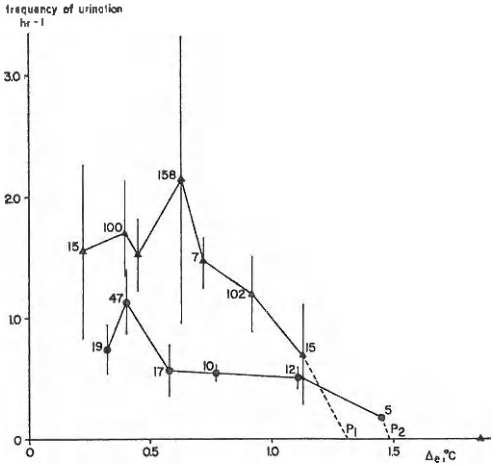

Fig.8. Urination frequency of *C. crangon* at various salinities and two temperatures. Represented are average values and standard deviations. The figures next to the symbols indicate the number of observations. P_1 and P_2 represent the isosmotic points at (●) 5 °C and (▲) 21 °C.

urine production was found with *Palaemonetes varians* (PARRY, 1955) and *Carcinus maenas* (SHAW, 1961a) may be due to the fact that with the salinities used in the experiments there is hardly any conformity between blood concentration and salinity of the medium. This is apparent, when the above data on urine production are compared with the relation Δ_i/Δ_e as determined for *P. varians* by PANNIKAR (1941) and for *C. maenas* by various authors (NAGEL, 1934; BETHE, VON HOLST & HUFF, 1935; HUFF, 1936; WEBB, 1940; SHAW, 1961a). The attainment of a maximal urinating frequency or a decrease of this frequency at low salinities may limit the renal loss of electrolytes (page 303).

In hypertonic salinities no sudden changes in conductivity of the medium were found. If under these circumstances the urine is also isosmotic to the blood this indicates that no urine was produced here. There are slight indications, however, that under these conditions the urine had a somewhat higher conductivity than blood (page 304). Since at high salinities the internal concentration does not differ greatly with the concentration of the medium it might be possible

that in such circumstances the discharge of urine is not detectable by our method. From the increase in weight of animals (*P. varians*) with blocked nephropores, PARRY (1955) concludes to a slight urine production in hypertonic salinities. On the other hand this conclusion is not supported by observations on the size and the contractions of the "epigastric" sac. The production of urine in a hypertonic medium remains an unsolved problem. Excretion of isosmotic urine at high salinities is inconsistent with water regulation, but it may function in ionic regulation.

c. Average volume of the excreted urine

If the concentration of the urine is known, the amount may be derived from the change in conductivity associated with the production of urine (page 291). Assuming that in *C. crangon*—just as in most marine crustaceans—the urine is isosmotic to the blood, the average volume of discharged urine in animals of various sizes was calculated. It appeared that a linear relation exists between the size of the animal and the average volume of the urine excreted at a time: $V_u = 9.86\ w$ (V_u = urine volume in µl; w = weight of the animal in grams). The volume of the urine discharged per time is approximately 1.07% of the body volume. This average volume of discharged urine is probably lower than the maximal volume of the bladders, since it may happen that discharge takes place before the bladders are completely filled (page 294), and also since there might be some variation in the amount of discharge. The variation is, however, only slight in undisturbed animals (Fig. 6a). In the two *Crangon* species (size not specified) GRIMM (1969) found for the volume of the bladders values ranging from 0.4 to 15µl. These values are of the same order as might be derived from the above relation.

d. Renal water loss

The water loss via the urine was determined by the frequency of urine discharges and the volume of the discharges. Table I gives the values obtained for urine production of *C. crangon* after adaptation to various salinities at 5 °C and 21 °C.

A direct comparison with the values found by various authors (page 289) for urine production in various organisms is handicapped by the differences in osmotic concentration between blood and medium, in temperature and in surface to volume ratio in the various animals.

From the values in our measurements for frequency of urine discharge, blood concentration and water content at various temperatures

TABLE I

Urine discharge and renal electrolyte loss of *C. crangon* at two temperatures and various salinities.

Temperature °C	*Salinity* ‰	*Urine discharge* $\mu l \cdot g^{-1} \cdot hr^{-1}$	*Renal electrolyte loss* $\mu mol\ NaCl \cdot g^{-1} \cdot hr^{-1}$
5	5.7	7.3	1.61
	7.0	11.2	2.67
	10.1	5.6	1.46
	13.5	5.3	1.70
	19.6	5.1	1.99
	25.5	1.7	0.71
21	3.8	15.3	3.67
	7.0	16.8	4.55
	7.9	15.0	4.12
	11.1	21.1	6.55
	12.7	14.4	4.63
	16.1	11.8	3.94
	19.9	6.8	2.31

and salinities the following relations were already apparent: (*1*) in the salinity range in which Δ_i and water content are regulated maximally the frequency of urine discharges, and thus of the urine production, increases in lowered salinity, (*2*) at lower salinities Δ_i is approaching conformity and the water content increases, while urine production is kept constantly at a maximal rate. These correlations have been found at a temperature of 5 °C as well as of 21 °C. It suggests that in the range of maximal regulation the water which enters at an increasing rate as salinity in the medium decreases, can be excreted by a proportionally increasing production of urine. As long as the frequency of urination can keep up with the lowering salinity the water content remains constant. At lower salinities, excretion no longer suffices and the water content increases. In hyposmotic salinities this increase in water content plays an important role in the decrease of blood concentration (page 285).

With a passive water influx by osmosis the amount of water entering the body—permeability being equal—is proportional to the difference between osmotic pressure in the animal (π_i) and that of seawater (π_e). For the relation between osmotic pressure in seawater and chlorinity MIYAKE (1939) gives the empirical formula: $\pi_t = 1.240\ Cl + 0.00454\ Cl\ t$ (π_t in atm, Cl in ‰, t in °C). Chlorinity may be derived from salinity by the formula: $S = 0.030 + 1.8050\ Cl$. Between Δ and Cl MIYAKE gives the relation $\Delta = 0.102710\ Cl$.

In Fig. 9 urine production has been plotted against $\pi_i - \pi_e$. It appears that, as the difference between Δ_i and Δ_e gets bigger—at lower salinities than at which Δ_i and water content are regulated—the values for urine production have not increased proportionally. There are three possible causes: (*1*) decrease of permeability for water at lower salinities, (*2*) increase of internal hydrostatic pressure and (*3*) a more than proportionally increasing extra-renal water excretion.

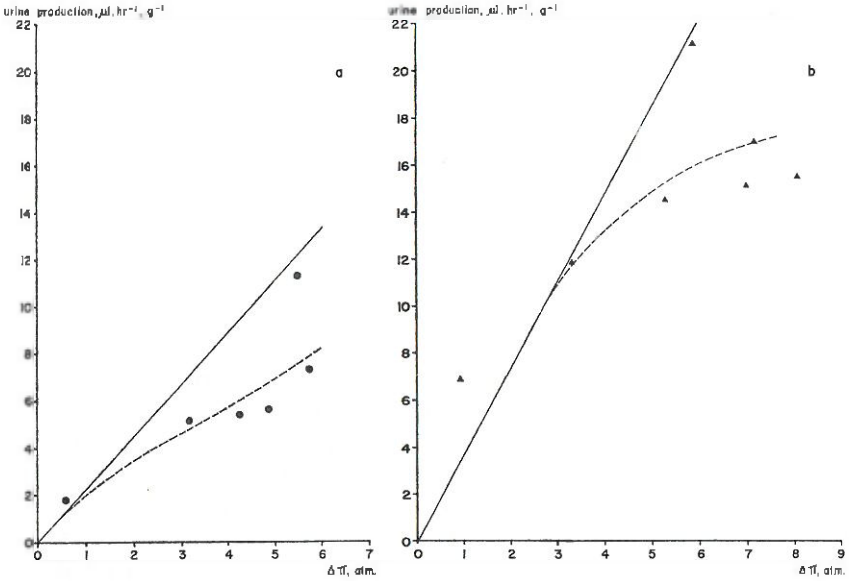

Fig.9. Urine production of *C. crangon* as a function of the difference in osmotic pressure between blood and medium. The drawn line gives the hypothetical increase in urine production if no increase in water content should occur. a, at 5 °C (●); b, at 21 °C (▲).

At low salinities the water content of *C. crangon* increases. As there is a hard exoskeleton the volume of the animal can only increase to a limited extent. The intake of water will result in a certain internal pressure. The amount of turgor may be assumed to depend on the increase of water content over that at the isosmotic point, or:

$$\pi_h = c\,\Delta\,W \qquad (1).$$

In this formula π_h is the internal hydrostatic pressure in atm, c is a proportionality constant in atm·‰$^{-1}$. For a simple osmotic process the relation between water influx, permeability and difference in osmotic pressure may be written as:

$$i = p\,(\pi_i - \pi_h - \pi_e) \qquad (2),$$

in which i is the net influx in $\mu l \cdot g^{-1} \cdot hr^{-1}$, p is permeability in $\mu l \cdot atm^{-1} \cdot g^{-1} \cdot hr^{-1}$. Substitution of (1) in (2) and dividing by $\Delta\pi = \pi_i - \pi_e$ gives:

$$\frac{i}{\Delta\pi} = p - c \cdot p \frac{\Delta W}{\Delta\pi} \qquad (3).$$

Both at 5 °C and at 21 °C a linear relation seems to exist between the quotient of urine production and $\Delta\pi$ and the quotient $\frac{\Delta W}{\Delta\pi}$ (Fig. 10). This means that for a given temperature the values of c and p are constant. At 5 °C these values are lower than at 21 °C: $c_5 = 0.33$ atm·%$^{-1}$, $c_{21} = 1.19$ atm·%$^{-1}$, and $p_5 = 2.20$ $\mu l \cdot atm^{-1} \cdot g^{-1} \cdot hr^{-1}$ and $p_{21} = 3.88$ $\mu l \cdot atm^{-1} \cdot g^{-1} \cdot hr^{-1}$.

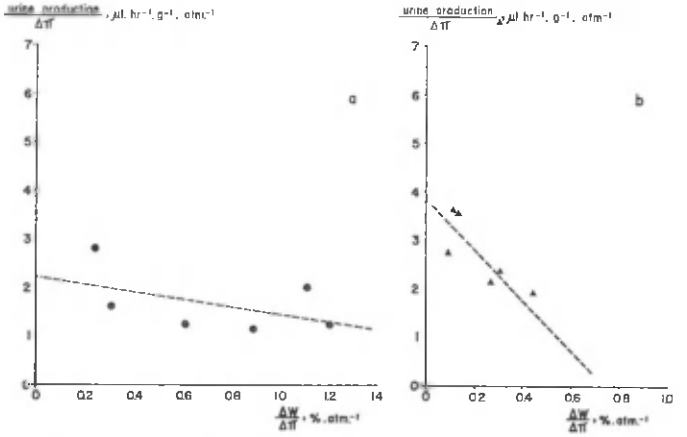

Fig. 10. The relation between urine production and increase in water content in *C. crangon*, corrected for differences in osmotic pressure between blood and medium. a, at 5 °C (●); b, at 21 °C (▲).

Thus the permeability does not depend on the salinity of the medium, and therefore it cannot be the cause of the relatively low urine production at low salinities. It appeared from experiments in which the uptake of tritiated water was determined that the brackish water crustaceans *Carcinus maenas* and *Palaemonetes varians* do not either show any changes in water permeability at different salinities of the medium (Rudy, 1967).

In only a few cases the urine production was determined at different temperatures. Hevesy, Høfer & Krogh (1935) found a marked

increase in urine production at higher temperatures in the frog, *Rana temporaria*. For *Petromyzon* WIKGREN (1953) also found a substantial increase. The temperature effect may be expressed in the Q_{10} value. If V_1 and V_2 are the rates at which the process goes on at temperatures t_1 and t_2 °C respectively, then Q_{10} may be derived from:

$$\log Q_{10} = \frac{10}{t_1 - t_2} (\log V_1 - \log V_2)$$

The observations of HEVESY, HØFER & KROGH and WIKGREN yielded a Q_{10} value of about 2. SHAW's (1955a) data on the permeability for heavy water of *Sialis lutaria* show a Q_{10} value of about 3. From the values for water permeability obtained here at two temperatures, it follows that $Q_{10} = 1.36$ which is lower than the values published by the other authors. Processes entailing catalization by enzymes usually have Q_{10} values of about 2 to 3, physical processes, such as diffusion and viscosity, have a Q_{10} value not much in excess of 1. The above value found for Q_{10} suggests that the water influx is brought about by a simple physical process. This suggestion is supported by the fact that urine production may be described by the formula (3) derived for osmotic processes (Fig. 10).

The probable linearity in the relation of Fig. 10 suggests that urine production is proportional to water influx. From the data obtained no conclusions can be derived on the existence of a possible net extrarenal water excretion. Active water transport, however, is an unusual phenomenon in crustaceans (*cf.* POTTS & PARRY, 1964: 40). As a rule it is assumed that urine production equals net water influx.

The internal hydrostatic pressure which in this case causes a reduction of water influx, may also result in a higher urine production (BEADLE, 1937). The increase in water content and internal hydrostatic pressure which does not result in a higher frequency of urination points to an active regulation of urine production.

The urine of *C. crangon* is practically isosmotic to the blood. This means that with urine production in a hypo-osmotic medium electrolytes are lost. This loss of ions is probably selective. Analyses of seperate ions in the urine of marine decapods usually indicate that concentrations of monovalent ions are relatively similar to their concentration in the blood. Divalent ions—calcium, magnesium and sulphate—are usually selectively excreted or absorbed in the antennal gland (see a.o. PANTIN, *1931c*; ROBERTSON, *1939*; RIEGEL & LOCKWOOD, 1961). The monovalent ions lost with the urine will have to be actively regained. This ion loss would make production of urine in a hypotonic medium a disadvantage to the animal. Here, however, the urine is mainly of importance for regulation of the water content. At

salinities where Δ_i and water content show maximal regulation the renal and extra-renal loss of electrolytes may be regained by active uptake.

At lower salinities no further increase in urine production is found; a small decrease even occurs at 21 °C. This is of consequence for the restriction of renal and extra-renal electrolyte loss under these circumstances.

As at lower salinities there is no longer an increase in urine production the water content increases. Then the osmotic water influx is reduced by the hydrostatic pressure due to the increased water content. In this way the renal electrolyte loss is limited.

The increase in water content is an important factor in the decrease of osmotic concentration in the blood. The effects of the decrease of blood concentration are threefold: (*1*) as Δ_i gets lower the difference between Δ_i and Δ_e at lower salinities will not longer increase which again restricts water influx and thereby electrolyte loss by way of the urine, (*2*) the concentration of the urine gets lower, and (*3*) as Δ_i gets lower the extra-renal loss of electrolytes will also be restricted (Chapter VI).

It is clear, therefore, that regulation of urine production is of primary importance to osmoregulation. On the one hand, urine production has to be adjusted to osmotic influx of water, so that no dilution of body fluids is caused by the intake of water; on the other hand, urine production has to be reduced at lower salinties, when the active uptake of electrolytes can no longer compensate renal and extra-renal losses.

It is likely that the production of urine is governed by hormones. Urine production increases when the eyestalks are ligatured in *Eriocheir sinensis* (DE LEERSNYDER, 1967), *Procambarus clarkii* (KAMEMOTO & ONO, 1968) and *Metapograpsus mesor* (KATO & KAMEMOTO, 1969). Here, however, the results are interpreted as the consequence of an increased water influx. THOMPSON (1967) mentions an increase in permeability for tritiated water after removal of the eyestalks of the freshwater crab *Pseudothelpusa jouyi*. MANTEL (1967) suggested a neuroendocrine control on water transport in the foregut of *Gecarcinus lateralis*. The present data, like those of RUDY (1967), however, do not point to any differences in permeability for water in various salinities. For *C. crangon* a regulation by way of the antennal gland has to be assumed.

B. WATER REGULATION IN C. ALLMANNI

Contrary to *C. crangon*, *C. allmanni* does not possess an effective osmoregulation mechanism, but still it is to be expected that in this species the

water content is more constant than in *C. crangon*. Determinations by GRIMM (1969) gave results that did not differ significantly with various salinities (75.3 ± 0.5% in 110% seawater, 74.9 ± 0.7% in 100% seawater, 76.5 ± 0.3% in 80% seawater; temperature 10 °C). These values are of the same order as the values at which *C. crangon* regulates its water content. It is regretted that more extreme conditions have not been tested. Probably the stability is related with the higher permeability for electrolytes (page 313) which entails that no difference between internal and medium concentration can be maintained.

When *C. allmanni* was subjected to the same experiments as *C. crangon* (Chapter V-3-A), in all the 65 hours that a measurement lasted the typical changes in conductivity of the medium were never found, not even when the sensitivity of the apparatus had been increased 20 times. Different medium salinities were tested. In *C. allmanni* the urine is also isosmotic to the blood and, since the blood is practically isosmotic to the medium, excretion of urine will influence the conductivity of the medium far less. Still it does not seem plausible to attribute the absence of conductivity leaps to the possibility of the urine and medium being exactly similar in conductivity. Since the urine is expected to play a role in ionic regulation small differences were expected. Besides, as there is practically no difference between internal concentration and concentration in the medium, the frequency of urination might very well be extremely low.

That *C. allmanni* does in effect produce urine was apparent when it was suddenly transferred to a lower salinity. In a number of experiments 1 ml of measuring liquid was sucked up (by putting an injection needle through the vent tube) and replaced by distilled water, while measuring was in progress. After a sharp drop in the conductivity due to the mixing with distilled water a slow continuous increase set in which lasted for about one hour. During this period of slowly increasing conductivity, in three cases one single sudden increase in conductivity was found, which could be attributed to the production of urine. From the observation that one or in most cases no conductivity leap was found after lowering the salinity of the medium it may be concluded that in *C. allmanni* the adaptation of the blood concentration to changes in salinity of the medium proceeds very rapidly. This suggests a high permeability (*cf.* page 280).

VI. ELECTROLYTE BALANCE

In a hypo-osmotic medium *C. crangon* loses a certain amount of electrolytes via the periodical excretion of urine. In addition considerable amounts of electrolytes are lost by diffusion through the body's surface,

especially through the respiratory epithelium. In a hyperosmotic medium the loss of electrolytes will partly take place by a continuously active secretion.

In animals adapted to a given salinity theser enal and extra-renal losses will be compensated by a passive uptake (diffusion) and, when the medium is hypo-osmotic, by an active uptake of electrolytes. In *C. allmanni* the renal loss of electrolytes is probably only slight, just as the active intake and secretion of electrolytes.

In this chapter the various means of uptake as well as loss of electrolytes are dealt with for each species, together with their relation to temperature and salinity of the medium.

A. RENAL ELECTROLYTE LOSS

The urine of marine crustaceans seems to be formed in the antennal gland as a protein-free ultrafiltrate of the blood (PICKEN, 1936; RIEGEL & KIRSCHNER, 1960; WEBER, 1971; *cf.* MALUF, 1941a, 1941b, 1941c). Both *C.crangon* and *C. allmanni* yielded the same values in blood and urine for the freezing point depression, the Na- and the Cl concentrations (GRIMM, 1969). Though urine may probably play a part in electrolyte regulation (page 300) Na and Cl are not regulated.

The results of the present research confirmed that in *C. crangon* the urine remains practically isosmotic to the blood at various salinities and temperatures. The electrolyte concentration of the urine could be derived by substituting the mean change in conductivity of the medium and the estimate of the volume of the excreted urine in formula (4) of page 291. These calculations were derived from a number of observations of animals adapted to various circumstances, which were independent of the observations used for the calculation of the average volume of the excreted urine. No clear differences between urine electrolyte concentration (Δ_u) and osmotic concentration of the blood were found (Fig. 11). Therefore the effect of temperature on internal osmotic concentration (Fig. 1) cannot be due to an influence of temperature on the osmolarity of the urine. At higher temperatures, however, a slight tendency was found for the urine concentrations to be slightly lower at lower blood concentrations. The lowest value of urine concentration, however, is still higher than the concentration of the medium. It is possible that this difference, though only slight, still helps to reduce renal loss of electrolytes. Though the production of urine and its effect on water regulation are essential to osmoregulation and may play a part in regulation of mainly divalent ions, the discharge of urine is still disadvantageous to the electrolyte balance.

From urine production and urine concentration at various salinities

and two temperatures the renal electrolyte loss in *C. crangon* under various conditions could be estimated (Table I, page 297).

On the average the values for renal electrolyte loss are higher than similar values with freshwater crustaceans, a.o. *Eriocheir sinensis*: 0.42 μM $Cl \cdot g^{-1} \cdot hr^{-1}$ (Scholles, 1933); *Astacus fluviatilis*: 0.021 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (Bryan, 1960a); *Potamon niloticus*: 0.005 to 0.06 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (Shaw, 1959). Only for *Palaemonetes antennarius* values of the same order have been found: 4.40 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (Parry, 1957, 1961).

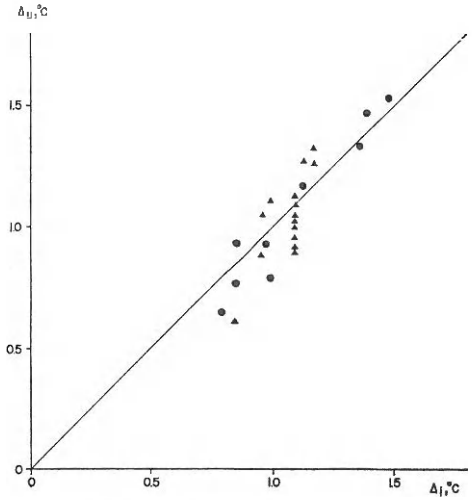

Fig. 11. Osmotic concentration of urine of *C. crangon* at various blood concentration and two temperatures; ● 4–6 °C, ▲ 21 °C.

B. EXTRA-RENAL ELECTROLYTE LOSS

1. INTRODUCTION

Besides renal loss electrolytes may leave the body by diffusion and by active extra-renal excretion. As a rule the extra-renal loss is considerably greater than the renal loss.

Most data on extra-renal loss of electrolytes have been obtained with brackish- and fresh water species which have been placed, with blocked or open nephropores, in distilled water. After some time the Na, K or Cl concentration in this water was determined. Radio isotopes have been used in some cases. By the latter method the efflux could also be determined in seawater of various salinities.

The values for extra-renal electrolyte loss do also show some relation to the environment. They are generally lower in freshwater animals, higher in brackish water and marine species. For instance *Astacus fluviatilis*: 0.37 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (BRYAN, 1960a), *Eriocheir sinensis*: 1.7 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (SHAW, 1961b), *Carcinus maenas*: 18 μM $Na \cdot g^{-1} \cdot hr^{-1}$ (SHAW, 1961a), *C. crangon*: 12.8 to 49.5 μM $Na \cdot g^{-1} \cdot hr^{-1}$ in 10 to 110% sea water and *C. allmanni*: 46.2 to 54.8 μM $Na \cdot g^{-1} \cdot hr^{-1}$ in 80 to 110% sea water (GRIMM, 1969).

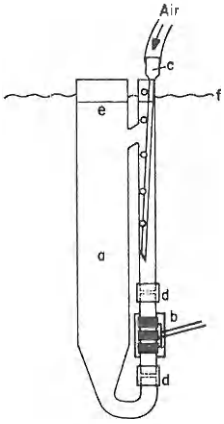

Fig.12. Diagram of the measuring cell used for determination of extra-renal electrolyte loss in a solution of manitol in distilled water: a, chamber for the animal; b, conductivity electrode; c, injection needle; d, glass-to-glass connection; e, level of measuring liquid; f, water level in the thermostat bath.

The variation found for animals from the same environment may be due to differences in permeability, blood concentration, temperature and surface to volume ratio in the different species.

The aim of the experiments described in the following pages was to trace the influence of temperature and blood concentration on extra-renal efflux in the two *Crangon* species.

2. METHOD

The gross extra-renal electrolyte loss was determined by continuously measuring electrical conductivity in a manitol solution to which an animal had been transferred. The solution of the non-electrolyte manitol was always isosmotic to the seawater to which the animal had been adapted (THUET, MOTAIS & MAETZ, 1968).

In the solution of manitol in distilled water the animal will lose electrolytes. Uptake of electrolytes from this medium is negligible;

even after the animal had been in the medium for half an hour the electrolyte concentration remained extremely low. At low concentrations the sensitivity of the conductivity meter for changes in electrolyte concentration was high enough to determine the electrolyte loss of an animal by measuring the absolute conductivity of the medium. In this case the influence of changes in temperature is much less, so that measurements could be carried out in an open cell (Fig. 12), placed directly in a thermostated water bath. Temperature equilibrium is

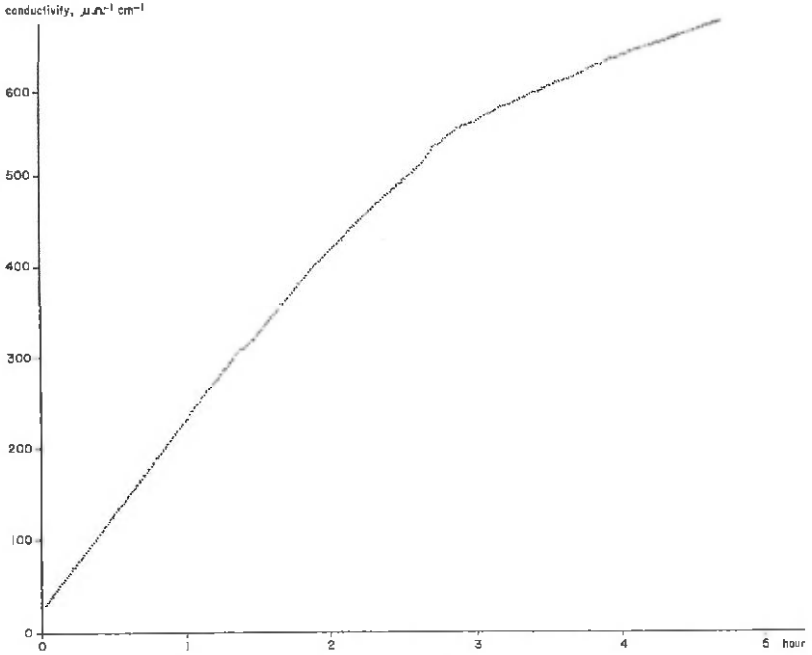

Fig. 13. Example of a long-term registration of the conductivity of a manitol solution to which a specimen of *C. crangon* is transferred. In this case the increase is only due to extra-renal loss of electrolytes. The animal was adapted to 31.7‰ S. Temperature 21 °C.

reached quickly—but less accurately than in the set-up of Fig. 5—which favoured the determination of electrolyte loss soon after the animal had been transferred to the manitol solution. After the animal had been rinsed in the measuring liquid and wiped off, it could be transferred, without any further handling, to a known volume of manitol solution kept ready in the measuring cell.

Immediately after transfer the animal starts to lose electrolytes at a constant rate (Fig. 13). After some time, dependent on the size of the

animal, a decrease of electrolyte efflux is observed. Most measurements lasted 20 to 30 minutes, a period in which no significant decrease in the amount of efflux occurred. The animal was then weighed. To find the gross efflux a line was drawn representing the relation between increasing conductivity of the measuring liquid and time, and its slope was determined between t = 5 min and t = 15 min. Urine discharge, which seldom occurred during this short period, produces sudden displacements of the curve, but does not alter the slope (Fig. 14). This method made it possible to distinguish between extra-renal and renal loss without having to resort to blocking the nephropores.

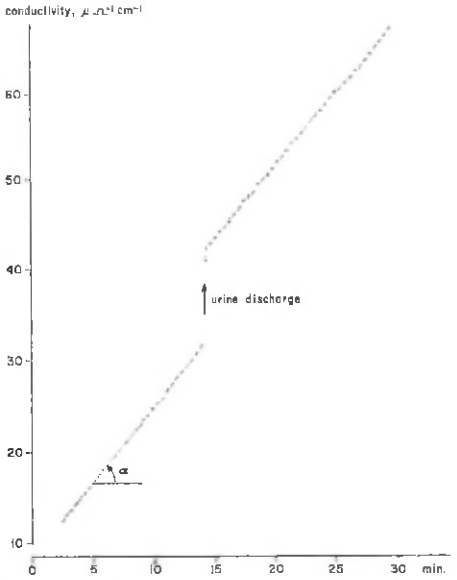

Fig.14. Example of a registration of the conductivity of a manitol solution to which a specimen of *C. crangon* is transferred. Both a gradual increase due to extra-renal loss of electrolytes and a sudden increase due to urine production can be seen. The animal was adapted to 11.0‰ S. Animal weight 0.450 g. Temperature 21 °C.

The electrolyte efflux could be derived from the formula:

$$\Phi_{eff} = \frac{1}{w} \cdot V \cdot S \cdot \frac{\Delta U}{\Delta T}$$

in which Φ_{eff} is the gross extra-renal electrolyte efflux in μM NaCl $\cdot g^{-1} \cdot hr^{-1}$, w the weight of the animal in grams, V the volume of the measuring liquid in ml (in these experiments 12.48 ml), S the calibration in μM NaCl $\cdot ml^{-1} \cdot sd^{-1}$ (fixed at 8.86×10^{-3} μM NaCl $\cdot ml^{-1} \cdot sd^{-1}$

at 6 °C, at 6.19×10^{-3} μM NaCl·ml^{-1}·sd^{-1} at 21 °C), ΔU the deflection of the meter in scale divisions (sd), ΔT the time in hours.

3. RESULTS AND INTERPRETATION

In animals adapted to various salinities the gross extra-renal efflux was determined, for *C. crangon* at 6 °C and 21 °C, for *C. allmanni* only at 6 °C. The results with *C. crangon* are plotted against concentration of the electrolytes in the blood (Fig. 15).

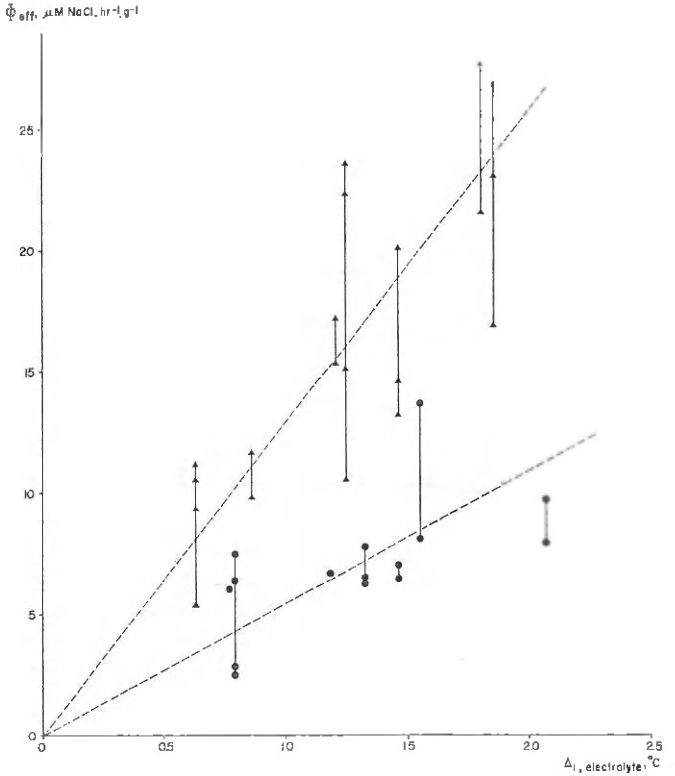

Fig. 15. Efflux of electrolytes in *C. crangon* as a function of electrolyte concentration in the blood. The electrolyte concentration of the blood is expressed as the freezing-point depression of seawater with the same conductivity. ● 6 °C, ▲ 21 °C.

It appears that the individual variation in extra-renal efflux is rather great. Animals of various sizes have been used for the experiments. The gross efflux per gram was calculated. Lockwood (1962) uses the term "specific loss" which he defines as loss·hr^{-1}·g$^{-2/3}$. To

find out whether the electrolyte loss was less variable if related to the surface of the animal the Φ_{eff} values, corrected for differences in blood concentration, were correlated with weight and (weight)$^{-2/3}$. The correlation coefficients at 6 °C and 21 °C were in correlation with weight 0.804 and 0.780 respectively and in correlation with (weight)$^{-2/3}$ 0.782 and 0.766. Though the differences are small the correlation with weight fits better—for both temperatures—than with surface.

With *Eriocheir sinensis* a similar great variation in gross efflux has been found; per crab 450 to 24 μM Na·hr^{-1} (Koch & Evans, 1956).

The gross efflux increases with concentration of electrolytes in the blood (*cf.* Bryan, 1960c).

In a hypertonic medium part of the gross electrolyte efflux takes place actively. Using the method described here this active excretion cannot be distinguished from the passive extra-renal loss. In animals adapted to hypertonic media on the average a higher quotient $\Phi_{eff}/\Delta_{i,\ electr}$ was found, but the differences may not be significant. This may be due to the great individual variation, but it is also conceivable that in the manitol solution which is poor in electrolytes, the active efflux stops quickly. The data obtained cannot give an answer to this question.

At 6 °C and 21 °C the average value of the above mentioned quotient $\Phi_{eff}/\Delta_{i,\ electr}$ was 4.97 μM NaCl·g^{-1}·hr^{-1}·°C^{-1} (N = 15) and 12.96 μM NaCl·g^{-1}·hr^{-1}·°C^{-1} (N = 20) respectively. Grimm (1969) measured the extra-renal loss of Na with the aid of isotope techniques and also found a positive correlation with blood concentration. From this the average value for Φ_{eff}/Δ_i can be calculated: 23.1 μM Na·g^{-1}·hr^{-1}·°C^{-1} (temperature 10 °C).

The value for extra-renal efflux derived from Grimm's measurements is considerably higher than the value found here. Similar differences were found with *C. allmanni*: $\Phi_{eff}/\Delta_{i,\ electr}$ = 9.38 μM NaCl·g^{-1}·hr^{-1}·°C^{-1} (own observations, temperature 6 °C), Φ_{eff}/Δ_i = 26.7 μM Na·g^{-1}·hr^{-1}·°C^{-1} (Grimm, temperature 10 °C).

It is difficult to compare these figures, since Grimm's values concern the Na efflux and ours the total electrolyte efflux in NaCl equivalents. 1 μM NaCl equivalent contains less than 1 μM Na. This strengthens the conclusion that Grimm's values are higher for both species. So does the fact that in his case the efflux is related to blood osmolarity and not to the osmotic value of the electrolytes in the blood as in this study.

The differences between the values for extra-renal efflux as found here and those given by Grimm might be due to a different origin of the experimental animals (*cf.* Weber & Spaargaren, 1970). Grimm's animals came from an environment where the average salinity was

higher. This is usually associated with a higher permeability (page 313). Since, however, for *C. allmanni* no difference in osmoregulation was found between the animals used for both the experiments, despite differences in gross efflux, it seems less plausible to attribute the differences found for *C. crangon* to differences in origin.

For *Astacus fluviatilis* indications have been found that a loss of ions to the medium occurs when Na was actively absorbed. When the Na pump stops, as is the case in distilled water, this leaking ceases and the extra-renal efflux is reduced (BRYAN, 1960b). In *C. allmanni* the Na concentration in the haemolymph is higher than in the medium (GRIMM, 1969: 18), just as in *C. crangon* in hypotonic media. Therefore both species must have some mechanism for active uptake of ions. The mechanism described by BRYAN for *Astacus fluviatilis* may also explain the lower efflux values in the manitol solutions. On the other hand, the differences may as well point to a considerable exchange diffusion.

The influence of temperature on the gross extra-renal efflux in *C. crangon* has a Q_{10} of 1.82. Little is known about the temperature influence on extra-renal efflux in other species. Only in *Asellus aquaticus* it was found that the extra-renal electrolyte loss was independent of temperature (LOCKWOOD, 1960).

4. DISCUSSION

a. The relation between renal and extra-renal electrolyte loss

In *C. crangon*, just as in many crustaceans, the gross extra-renal efflux surpasses the renal electrolyte loss (*cf.* SHAW, 1961a; POTTS & PARRY, 1964b). In a hyperosmotic medium the renal loss is probably very small, at lower salinities the electrolyte loss via the urine increases to about 30% of the total electrolyte loss. This agrees with the value of 24% calculated by SHAW (1961b) from his determinations on Na loss, and SCHWABE's (1933) estimate of urine production in *Eriocheir sinensis* (*cf.* KROGH, 1939). If in the manitol solution a reduction of efflux has taken place, the actual contribution of the renal electrolyte loss to the total electrolyte loss will be lower.

As *C. allmanni* probably has a very low urine production, and, compared with *C. crangon*, a higher extra-renal efflux, its renal electrolyte loss will amount to an even smaller part of the total efflux.

b. Active uptake of electrolytes

The sum of renal and extra-renal efflux gives the total gross efflux (Fig. 16). In a state of equilibrium the total efflux will be compensated by an equal influx. The latter will be partly passive by diffusion, but

will at least be partly active in a hypo-osmotic medium. Since passive extra-renal efflux seemed to be proportional to electrolyte concentration in the blood, it may also be assumed that passive influx is proportional to salinity (*cf.* SHAW, 1961a). At those values of salinity where conductivity of blood and seawater are equal, the gross influx will be equal to the gross efflux, since in this case active uptake and renal loss are negligible. The difference between total efflux and passive influx gives an indication of the active uptake of electrolytes.

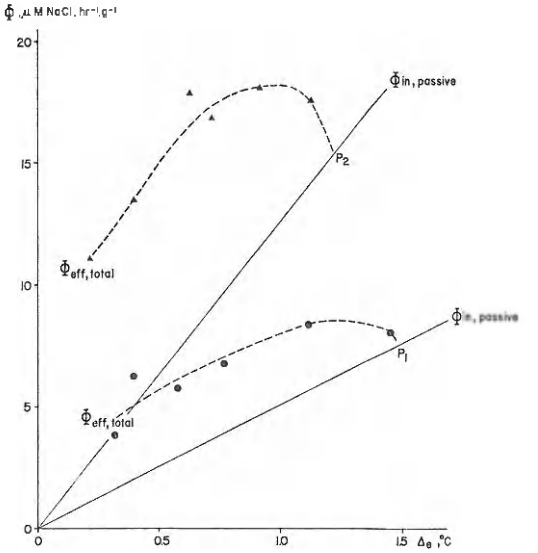

Fig.16. Total electrolyte efflux, and hypothetical passive electrolyte influx in *C. crangon* at various osmolarities of the medium. P_1 and P_2 are the isosmotic salinities at 6 °C (●) and 21 °C (▲) respectively.

From the point of iso-conductivity the active influx increases towards lower salinities to reach a maximum at low salinities. The salinity at which the active uptake no longer increases corresponds with the lower boundary of the regulation range of Δ_i. This relation is found at 6 °C as well as at 21 °C. From these data the maximal active uptake can be estimated at 3.1 μM $NaCl \cdot g^{-1} \cdot hr^{-1}$ and 8.5 μM $NaCl \cdot g^{-1} \cdot hr^{-1}$ respectively. If the values for extra-renal efflux are too low, due to a reduction of efflux in the manitol solution, these figures will be too high.

For *Carcinus maenas* a maximal active influx of 8.2 μM $Na \cdot g^{-1} \cdot hr^{-1}$ has been found (SHAW, 1961a, temperature not specified). As far as

could be traced, no data are known for other brackish water or marine species.

The Q_{10} value of the active uptake is 1.88. This value is very similar to the temperature influence on gross extra-renal efflux ($Q_{10} = 1.82$). As at higher temperatures the active uptake may increase practically proportionally to the gross electrolyte loss, the internal electrolyte concentration may remain practically the same as temperature changes. The difference between the Q_{10} values for active uptake and gross extra-renal electrolyte efflux may account for the relatively small increase in renal electrolyte loss at higher temperatures.

c. Localization of active uptake

Some indications as to the site where active uptake occurs could be obtained by experiments with animals in which the gill cavities had been shut off. In the measuring cell of the urine production set-up (Fig. 5) an animal was placed of which both gill cavities had been shut off by quickly injecting vaseline by means of a syringe. In hypo-osmotic seawater this yielded (*1*) a reduction of the urine discharge and (*2*) a continuation of active electrolyte uptake for some time before the animal died. The failure of urine production may be an indication that perhaps part of the water enters by way of the gills. It is more obvious, however, that with the gill cavities blocked the active uptake of electrolytes still goes on for some time. This indicates that active uptake of electrolytes may take place at some other site than the gills. In *Eriocheir sinensis*, however, Koch, Evans & Schicks (1954) proved active uptake in isolated gills. Moreover Nagel (1934), using normal specimens of *Carcinus maenas* and specimens in which the gut had been blocked found no difference in the results. Here too, active uptake must take place at the outer surface. In the same species Webb (1940) and Shaw (1961a) demonstrated salt absorption through the gills. The present results seem remarkable when compared to those observations. Only Croghan (1958a), working with *Artemia salina* found some indications for salt uptake in the gut. In this research experiments on animals in which the gullet had been shut off by vaseline yielded no further results, since the animals either swallowed the vaseline or regurgitated it.

d. Active excretion of electrolytes

In *C. crangon* active excretion of salts takes place in a hyperosmotic medium. It appears already from the curves relating Δ_i to Δ_e that the facilities for active efflux are less well developed than the facilities for active uptake in hypo-osmotic media. Assuming that no renal loss of salts occurs from *C. crangon* in a hyperosmotic medium, the maximal

active efflux may be estimated at about 2.19 μM NaCl·g^{-1}·hr^{-1} at 6 °C and 5.71 μM NaCl·g^{-1}·hr^{-1} at 21 °C. The influence of temperature on active salt excretion is practically equal to that on active uptake; Q_{10} being 1.89 and 1.88 respectively. It is interesting to consider the possibility that active uptake and active secretion may be governed by the same mechanism. For active salt excretion several indications have been found for localization in the gut (GREEN, HARSCH, BARR & PROSSER, 1959; GIFFORD, 1962; DALL, 1966). Some data point to active excretion by way of the gills (CROGHAN, 1958b; FLEMISTER, 1959).

e. Permeability for electrolytes

The gross extra-renal efflux yields some information on permeability for electrolytes. For *C. crangon*, starting from the quotient Φ_{eff}/Δ_i the permeability at a concentration difference of 1 mol could be derived: 17.8 μM NaCl·g^{-1}·hr^{-1}·mol^{-1} at 6 °C and 45.5 μM NaCl·g^{-1}·hr^{-1}·mol^{-1} at 21 °C. For *C. allmanni* this value was 32.6 μM NaCl·g^{-1}·hr^{-1}·mol^{-1} at 6 °C.

Permeability greatly influences the energy needed for maintaining a difference between blood and medium concentrations. As a rule the permeability for electrolytes increases from freshwater species to brackish water and marine species (NAGEL, 1934; GROSS, 1957). This tendency is also apparent in the difference in permeability between *C. crangon* and *C. allmanni*.

The lower permeability of fresh- and brackish water species is again reflected in a slower rate of extra-renal loss in a solution poor in electrolytes. Both with *C. crangon* and *C. allmanni* a decrease of gross efflux was measured within some hours. With *Eriocheir sinensis* KROGH (1939) found on the first day an efflux of 1400 to 1800 μM NaCl·g^{-1}·hr^{-1}, on the second day it was still 750 μM·g^{-1}·hr^{-1}. BERGER (1931) had found a slightly quicker decrease with the same species: 1400 μM Cl·g^{-1}·hr^{-1} on the first day, 280 μM·g^{-1}·hr^{-1} on the second day, and 100 μM·g^{-1}·hr^{-1} after a week. Similar slow decreases of efflux have been found in freshwater species (a.o. *Gammarus pulex*, LOCKWOOD, 1961).

Measurements of extra-renal efflux in a manitol solution, using animals with blocked gills, proved that the greater part of electrolyte efflux takes place by way of the gills. Blocking of the gill cavities resulted in a reduction of electrolyte efflux by about 52% ($N = 7$, $\sigma = 17{,}7$).

A Q_{10} of 1.82 for electrolyte efflux (page 310) and 1.36 for water transport (page 300) indicate that, to a certain extent, permeability for electrolytes and for water are independent of each other (*cf. Potamon niloticus*, SHAW, 1959c).

VII. NON-ELECTROLYTES IN THE BLOOD

1. INTRODUCTION

Non-electrolytes also contribute to the osmotic pressure in the haemolymph. Their contribution will be considerably smaller than that of the various ions in the blood, since the former molecules are less numerous.

Some idea about the contribution of the non-electrolytes could be gained by comparing freezing-point determinations and conductivity determinations of the blood. The value of the blood conductivity was compared with conductivity values of seawater to find out which seawater concentration yielded an identical figure (BRUNS, 1962). The freezing-point depression of seawater can be derived from the empirical formula of MIYAKE (1939). The difference between the freezing-point depression as measured and that of the electrolytes obtained by the above procedure ($\Delta_i - \Delta_{i,\ electr}$) is an indication for the concentration of non-electrolytes.

Blood samples were obtained from specimens of *C. allmanni*, adapted to various salinities and temperatures, and freezing-point as well as conductivity of the same sample were determined. To this end 20 µl of blood was diluted with 1 ml distilled water. Of this mixture the conductivity at 20 °C was measured. The conductivity of the blood of *C. crangon* was determined by BROEKEMA (1942). Her specimens came from the same area as the species used in the present freezing-point determinations. The concentration of free amino-acids in the blood of *C. crangon* was also measured directly in our institute by R. E. WEBER. The results will be published later.

2. RESULTS AND INTERPRETATION

The differences between Δ_i and $\Delta_{i,\ electr}$ for *C.crangon* have been plotted against medium osmolarity (Fig. 17). It appears that at various concentrations of the medium the share of non-electrolytes does not remain constant. At 4 to 6 °C and freezing-point depressions of the medium between 1.1 and 2.0 °C non-electrolyte concentrations are lower, with a minimum at $\Delta_e = 1.7$ °C. At 21 to 22 °C a similar drop is found at lower salinities: at $\Delta_e = 1.1$ °C there is practically no non-electrolyte left in the haemolymph, at medium osmolarities higher than 1.5 °C the change in concentration of the non-electrolytes is relatively smaller. The maximum concentration of non-electrolytes is of the same order at both temperatures.

At low salinities *C. allmanni* exhibits relatively small variations in concentration of non-electrolytes in the haemolymph (Fig. 18). The

amount is similar to the maximal concentration of non-electrolytes in *C. crangon*. At higher temperatures (21 °C) less non-electrolytes are found in the blood. This corresponds to high mortality observed at this temperature.

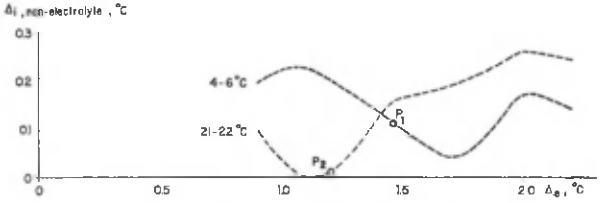

Fig.17. Osmotic concentration of non-electrolytes in the blood of *C. crangon* as a function of osmotic concentration of the medium. P_1 and P_2 are the isosmotic salinities at 4 °C and 21 °C.

a. The significance of variation in non-electrolyte concentration in the blood of *C. allmanni*.

At lower salinities electrolyte concentration in the blood of *C. allmanni* is practically equal to that in the medium. Here the low hypertonic value of the blood is due to the contribution of non-electrolytes. At higher salinities non-electrolytes disappear from the blood. Here the blood is isotonic or slightly hypotonic since on the one hand the con-

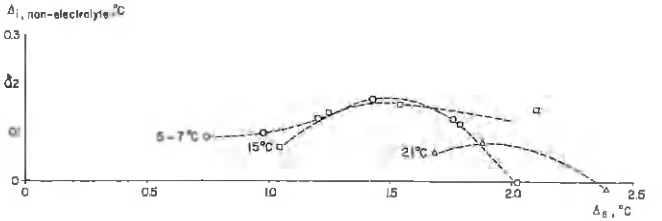

Fig.18. Osmotic concentration of non-electrolytes in the blood of *C. allmanni* as a function of osmotic concentration of the medium. ○ 5–7 °C, □ 15 °C, △ 21 °C.

centration of non-electrolytes had decreased and on the other hand there is a small active excretion of electrolytes (especially at the high temperature of 21 °C). These data suggest that the variation in non-electrolyte concentration in *C. allmanni* is connected with a slight osmoregulation. For a species with a relative high permeability for electrolytes, such as *C. allmanni*, this form of osmotic regulation may perhaps save more energy than maintaining the same osmotic difference between blood and medium by means of electrolytes.

b. The effect of temperature on osmotic concentration of the blood of *C. crangon*

The curves indicating the relation between Δ_i and Δ_e in *C. crangon* at various temperatures (Fig. 1) do not quite overlap. The effect of temperature may be indicated by the difference $\Delta_i^{21°} - \Delta_i^{5°}$ (Fig. 19, drawn line). In the salinity range between about 14 and 28‰ (Δ_e about 0.8 to 1.6 °C) Δ_i decreases when the temperature increases. Outside these boundaries a positive correlation between temperature and Δ_i is found.

The effect of temperature on the concentration of electrolytes and non-electrolytes is represented in the same way (Fig. 19). It appears that the effect of temperature on internal osmotic concentration is strongly correlated with the same effect of non-electrolyte concentration. The change in total osmotic concentration at rising temperatures is also correlated with a change in the electrolyte concentration, but to a much smaller extent.

The shift of the regulation range towards lower salinities and lower Δ_i values when the temperature rises may thus mainly result from the changes occurring in the concentration of non-electrolytes. The ecological consequences as to seasonal migration have already been indicated (page 281).

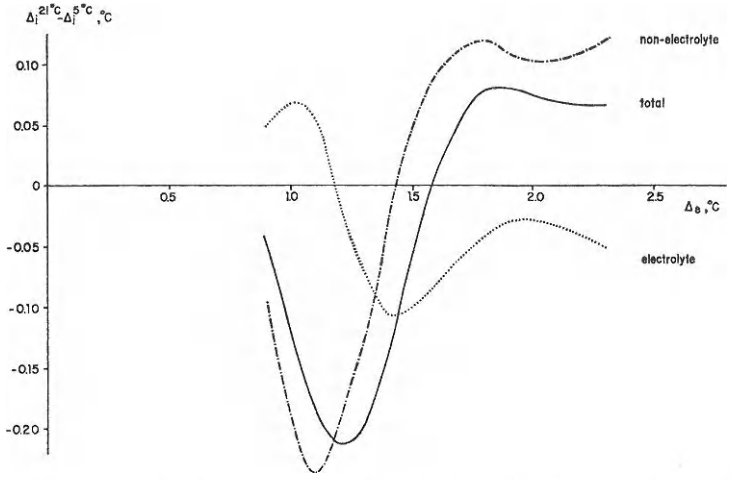

Fig. 19. Effect of a rise in temperature from 5 °C to 21 °C on total osmotic concentration, electrolyte concentration and non-electrolyte concentration in the blood of *C. crangon* as a function of the osmolarity of the medium.

c. Adaptation to temperature in *C. crangon*

Supplementary indications for the effect of non-electrolytes on the

change in Δ_i under the influence of temperature have been obtained by measuring the rate of temperature adaptation. About 50 specimens were adapted to 21‰ S at 4 °C. With this salinity the influence of temperature on blood concentration is relatively great (Fig. 1). The blood of a number of these animals was collected and freezing-point as well as conductivity was measured. The temperature of the aquarium was then increased to 21 °C during a period of 1 hour. In the next seven hours blood samples were taken from 20 specimens, and Δ_i and conductivity were determined. It appeared that during this period no significant decrease in blood concentration occurred. Neither was the expected decrease found in 8 measurements, carried out after 24½ hours.

Contrary to the swift decrease in Δ_i at a change of medium salinity (page 280) the adaptation to temperature takes a longer time. In nature too these changes will proceed very gradually. With the changes in salinity it is the other way round: especially in summer, when the animals are found in coastal waters or in the Wadden Sea, considerable and sudden alterations in salinity may occur. The quick adaptation to these conditions is connected with the relatively high fluxes of electrolytes. The slow development of temperature adaptation suggests that it is not brought about by electrolyte transport, but by a change in concentration of non-electrolytes, which apparently can only proceed at a slow rate.

d. Conclusion

For the contribution of the concentration of Na and Cl to the osmotic concentration GRIMM (1969: 20) found values, both with *C. crangon* and *C. allmanni* which varied between 88 and 92% of the total osmotic concentration. His method of derivation does not exclude the possibility that these values are somewhat on the high side. Though GRIMM suggests that a constant contribution of non-electrolytes under changing conditions is a very common phenomenon in the body fluids of many species of animals, the present results point to another conclusion. It appeared that the non-electrolyte concentration might vary between approximately 0 and 20% of the total osmolarity, dependent on temperature and salinity. This variation is connected with a form of osmotic regulation in *C. allmanni*. In *C. crangon* the adaptive shifting of the regulation range, dependent on temperature, may mainly result from this variation in non-electrolytes.

VIII. CELLULAR REGULATION

1. INTRODUCTION

Metabolic processes are dependent on certain optimal concentrations of various substances in the cells. GRIMM (1969) determined Na concentrations in solutions, obtained by dissolving complete specimens of *C. crangon* and *C. allmanni*, which had been adapted to various salinities, in nitric acid. The results suggested different Na concentrations for tissues and blood, the differences being greatest for *C. allmanni*. This implied that there existed a regulation of certain substances at cell level, besides the regulation of blood concentration.

It seemed desirable to supplement these data with observations on the animals of the present populations. GRIMM's animals showed a slightly different osmoregulation and moreover the above observations had been carried out at only one temperature and at five salinities, of which only two in the regulation range.

In both species it is possible to isolate muscle tissue from the abdomen. After incineration or homogenisation the concentration of various components may be determined. A disadvantage is that an unknown quantity of blood remains in the tissue. For this reason similarly whole specimens were used, which made it possible to take the quantity and concentration of the blood into account, and also to give a rough picture of the average amount of dissolved substances in shrimp tissues.

Animals adapted to eight different salinities, both at 4 °C and at 21 °C were homogenized in a Potter tube. To this homogenate was added about 8 ml of distilled water and of the liquid thus obtained the freezing-point, conductivity and chloride concentration were determined.

The water present in an animal may be considered to occupy various compartments, such as blood, urine, intracellular water, of which each volume has a certain concentration of dissolved substances. Previous measurements (Chapter III) gave the water content of *C. crangon* in relation to temperature and salinity. For the water content of *C. allmanni* the average of the values obtained by GRIMM were taken. From the figures for freezing-point depression, conductivity, and chloride concentration, obtained in the diluted homogenate, the average concentrations of the water present in the animals were calculated. In order to ensure a better comparison between osmotic concentration, electrolyte concentration and chloride concentration the values have all been expressed as freezing-point depressions in degrees centigrade.

2. RESULTS AND INTERPRETATION

a. Tissue osmotic concentration

The average concentration of the body fluids (Δ_h, freezing-point determined in homogenized animals) is higher than that of the haemolymph (Fig. 20). In the salinity range where blood concentration of *C. crangon* is strongly regulated, and at hypertonic seawater concentrations, Δ_h follows a practically parallel course to that of Δ_i. At 4 °C the difference between the two is practically the same as at 21 °C. Analogous to the shift in the osmoregulation range for increase in temperature towards lower salinities and lower Δ_i values, the range of Δ_h regulation shifts when the two temperatures are compared in a plot of Δ_h against Δ_e. At lower salinities, however, the difference between Δ_i and Δ_h is less at 4 °C, and at higher salinities it is somewhat greater at 21 °C. These changes correspond with conditions unfavourable for *C. crangon*.

For *C. allmanni* a similar, constant, but smaller difference was found between Δ_h and Δ_i (Fig. 20c).

If the body fluids are divided in a compartment, V_1 with an osmolarity of Δ_i, comprising blood, urine, *etc.*, and a compartment V_2 with a different osmolarity Δ_2 then $(V_1 + V_2)\Delta_h = V_1\Delta_i + V_2\Delta_2$ or:

$$\Delta_h = \frac{V_1}{V_1 + V_2} \cdot \Delta_i + \frac{V_2}{V_1 + V_2} \cdot \Delta_2 \qquad (1).$$

Since for a large range of salinities the difference between Δ_i and Δ_h is constant, correlation of the two (Fig. 20) in this traject will give a straight line with a slope $V_1/(V_1 + V_2) = 1$. This means that practically all the body fluid, and therefore probably also the intracellular fluid, is isosmotic to the blood. In addition, there will be a number of osmotic substances, associated with only a very small amount of water, or occurring in undissolved state in the body. This quantity may be estimated for *C. crangon* and for *C. allmanni* at 86 μM NaCl equiv · g^{-1} and 64 μM NaCl equiv · g^{-1} respectively. Under unfavourable circumstances these quantities are subject to changes.

Because of the large surface of the cells and the relatively high permeability of the cell membranes, concerned with the intensive transport of matter between cells and blood, FLORKIN & SCHOFFENIELS (1969) assumed that no differences can exist between osmotic concentration in cells and in blood. SHAW (1958b) also found for *Carcinus maenas* in undiluted seawater and in 40% seawater that in isolated muscle tissue the freezing-point depression was higher than in blood. He also expects that the intracellular fluid will be isosmotic to the blood. He ascribes the higher osmotic value to autolysis of organic

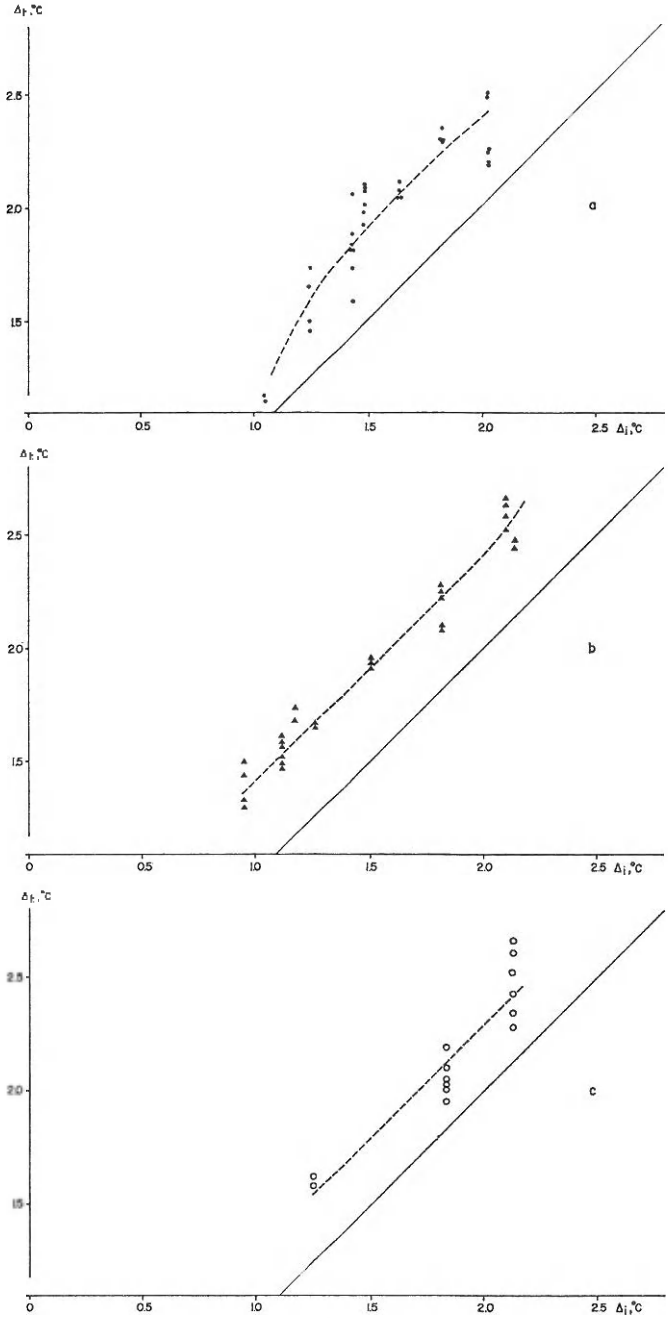

Fig.20. The relation between osmotic concentration of shrimp homogenates to that of the blood. a (●), *C. crangon* at 4 °C; b (▲), *C. crangon* at 21 °C; c (○), *C. allmanni* at 4 °C.

components, such as adenosine-tri-phosphate. The above relation between $\Delta_i - \Delta_h$ and salinity, however, suggests that there may be an amount of osmotic substances stored in the tissues. Under favourable conditions of higher salinities together with high temperatures a further storage occurs, and of low salinities together with low temperatures part of the storage is mobilized (both conditions are unfavourable for *C. crangon*).

b. Tissue electrolyte concentration

The average electrolyte concentration in the body ($\Delta_{h,\ electr}$) is plotted against electrolyte concentration in the blood (Fig. 21). Though from the above data it seemed likely that the cell fluid would be isosmotic to the blood, it appears that the electrolyte concentrations in cell and blood may differ (*cf.* GRIMM, 1969). The course of $\Delta_{h,\ electr}$ even indicates that at fairly high variations of electrolyte concentrations in the blood, occurring under the influence of an even greater variation in salinity of the medium, the electrolyte concentration in the cells must be constant to a high degree.

Even in *C. allmanni*, which only in hyperosmotic salinities shows some slight regulation of electrolytes in the blood, the electrolyte concentration in the cell appears to be regulated.

In other species of crustaceans an ion regulation at cell level has also been found. SHAW (1958b) found in muscle tissue from *Carcinus maenas*, with decreasing salinity in the medium, a decrease of Na, K and Cl concentration which was very small compared to the decrease of these substances in the blood. ROBERTSON (1961) found an active K regulation in muscle tissue from *Nephrops norvegicus*. In some cases it could be proved that the ion concentrations in the cells were—as expected—in a Donnan equilibrium with the dissolved organic substances, such as K and Cl concentrations in nerve tissue of *Loligo* sp. (HODGKIN, 1956), in *Carcinus maenas* (LEWIS, 1952) and in *C. maenas* muscle tissue (SHAW, 1958a).

If $\Delta_{h,\ electr}$ is equal to $\Delta_{i,\ electr}$, the electrolyte concentration in the cells is the same as the electrolyte concentration in the blood. Analogous to (1)

$$(V_1 + V_2)\Delta_{h,\ electr} = V_1\Delta_{i,\ electr} + V_2\Delta_2$$

and when $\Delta_{h,\ electr} = \Delta_{i,\ electr}$ then:

$$(V_1 + V_2)\Delta_{h,\ electr} = V_1\Delta_{h,\ electr} + V_2\Delta_2$$

and consequently $\Delta_2 = \Delta_{h,\ electr} = \Delta_{i,\ electr}$.

This condition is fulfilled in the experiments with *C. crangon* at 4 °C and at 21 °C at electrolyte concentrations of about 364 and 336

mM NaCl equiv · l^{-1}, respectively. With *C. allmanni* this concentration is about 316 mM NaCl equiv · l^{-1}.

Probably equal electrolyte concentrations for intracellular fluid and haemolymph represent optimal concentrations for metabolism. In *C. crangon* these concentrations are practically reached in the iso-conductivity points. Since the differences between the obtained values

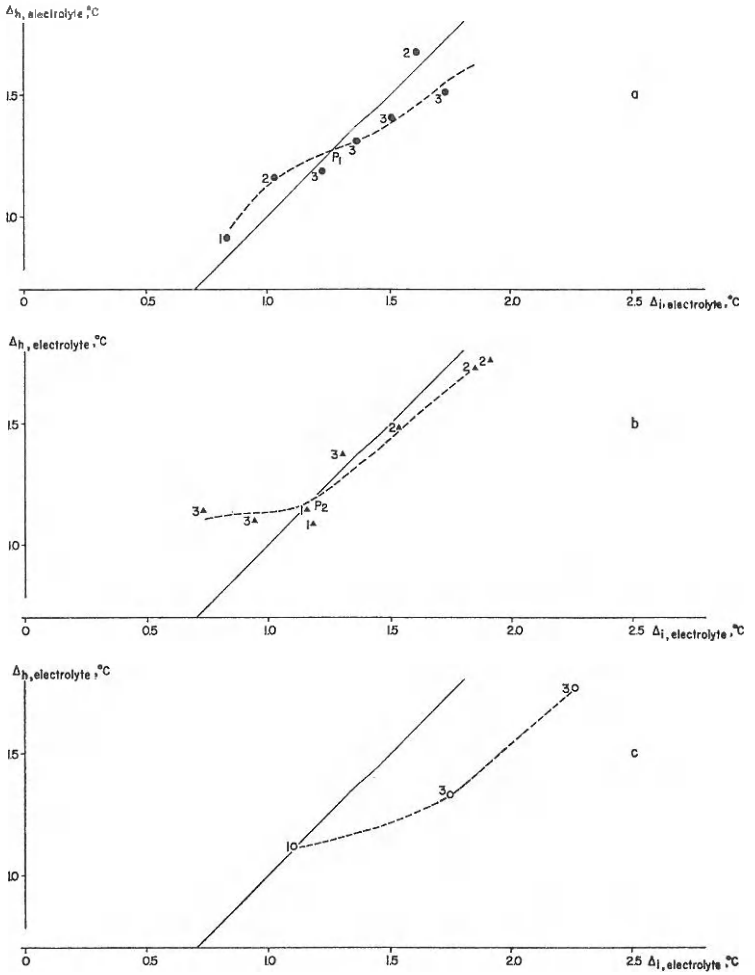

Fig.21. The relation between osmotic concentration of electrolytes in homogenates to that of electrolytes in the blood. a (●), *C. crangon* at 4 °C; b (▲), *C. crangon* at 21 °C; c (○), *C. allmanni* at 4 °C. At P_1 and P_2 the blood of *C. crangon* had the same conductivity as the medium at 4 °C and 21 °C respectively. The figures next to the symbols are the numbers of observations.

are small and probably not significant it might well be possible that the optimal electrolyte concentration is the same for the two species, and that this concentration is not dependent on temperature.

Chloride concentration in the body fluid.—Both *C. crangon* and *C. allmanni* show an obvious regulation in the average chloride concentration in body fluids at various salinities (Fig. 22). The chloride concentration in the body fluid of *C. crangon* is hardly influenced by temperature (Chapter V). Practically over the entire salinity range the chloride content of *C. allmanni* is equal to that of *C. crangon*. Only in diluted seawater the values of the former are somewhat lower. This corresponds with a decrease in ion concentration in the blood of this species when the salinity of the medium decreases. GRIMM (1969: 29,47) found some

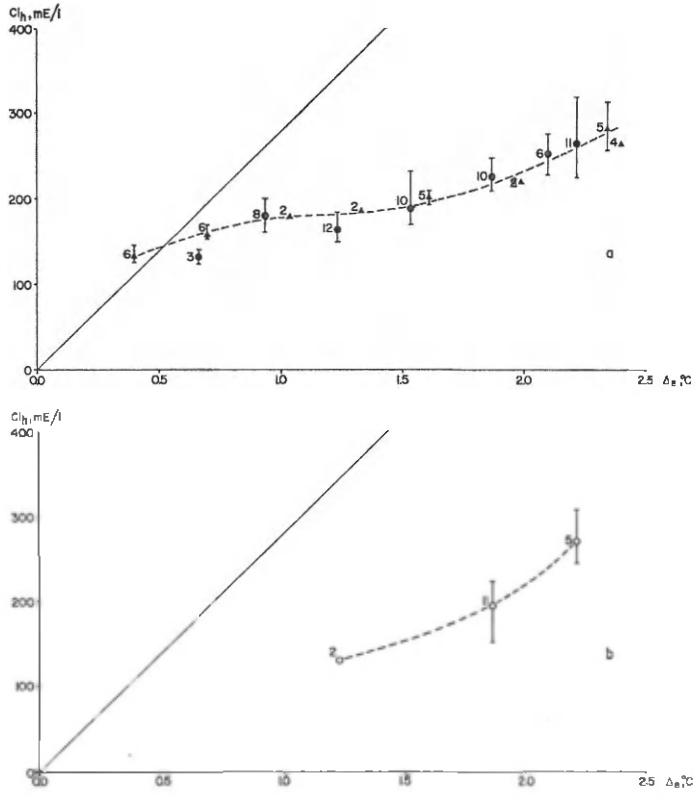

Fig.22. Chloride concentration in the homogenate of a shrimp as a function of the osmolarity of the medium. a, *C. crangon* at 4 °C (●), and at 21 °C (▲); b, *C. allmanni* at 4 °C (○). Represented are averages and extreme values. Figures next to the symbols indicate the numbers of observations.

indications that in *C. crangon* electrolyte regulation is attained by active uptake of chloride at low salinities of the medium, Na following passively.

The part played by chloride in the electrolyte concentration of body fluids is smaller than that of chlorides in the electrolyte concentration of seawater. In Fig. 23 the chloride concentration of the body fluids has been plotted against the electrolyte concentration. Of the total electrolyte concentration the percentage of chlorides varies with the average electrolyte concentration in the animal and thereby with the salinity of the medium. At high salinities relatively more chlorides are present, while the amount of other electrolytes remains more or less constant at various salinities. This amount does not depend on temperature and is also the same for both species of shrimps. Probably those other ions of which the concentration is kept more constant under varying circumstances will play a more essential part in cell metabolism.

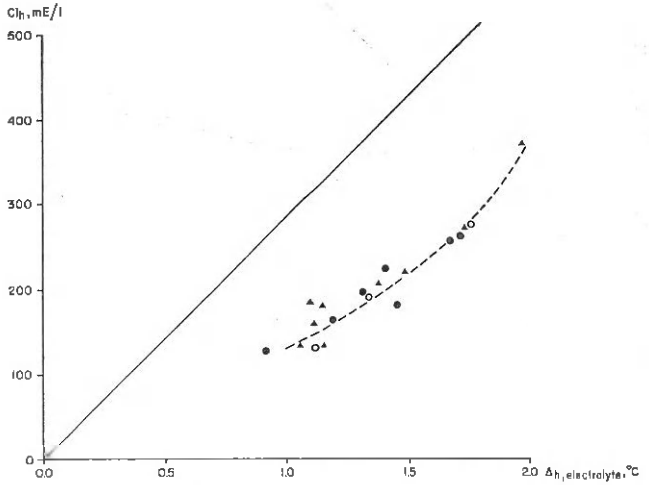

Fig. 23. Relation between chloride concentration and total electrolyte concentration in shrimp homogenates (● *C. crangon* at 4 °C; ▲ *C. crangon* at 21 °C; ○ *C. allmanni* at 4 °C) and in seawater (drawn line).

c. Tissue non-electrolyte concentration

In both species the average non-electrolyte concentration ($\Delta_{h,\,non\text{-}electr}$) of the body fluids increases with salinity of the medium (Fig. 24). It appears that this increase with salinity is greater than that of the average electrolyte concentration, so that at higher salinities the part of non-electrolytes has increased to about one third of the total average osmotic concentration.

Compared to the concentration of the non-electrolytes in the blood a remarkable difference can be seen (Fig. 24, I and II). This enables us to decide whether at a given change in salinity of the medium the non-electrolyte concentration in the tissues gets higher or lower. For, if a decrease in average non-electrolyte concentration of the body fluids is found, while at the same time the non-electrolyte concentration of the blood increases, it means that a greater decrease of non-electrolyte concentration must have taken place in the tissues.

At a decrease of salinity in a hypo-osmotic medium (Fig. 24a and b, *C. crangon*, at 4 °C and 21 °C to the left of P_1 and P_2 respectively) non-electrolytes disappear from the tissues, while non-electrolyte concentration increases in the blood. This may indicate a transport of non-electrolytes from the cells to the blood by which the osmotic concentration in the cells can be adapted to the lowered osmotic concentration of the blood. In this way great modifications of electrolyte concentrations in the cell are avoided. Furthermore the decrease in osmotic concentration in the blood is slightly counteracted by the increase of non-electrolyte concentration.

Generally the concentration of non-electrolytes is considerably higher in the cells than in the blood. Under unfavourable circumstances, however, such as low temperatures and low salinities, the non-electrolyte concentration in tissues may decrease considerably and even become equal to or slightly lower than the non-electrolyte concentration in the blood (Fig. 24a). Besides this possibility of migration of non-electrolytes from cells to the blood there is obviously also a loss of non-electrolytes, as appears from the decrease of the average non-electrolyte concentration of the body fluids at lower salinities.

In the range of salinities at which the blood concentration is kept at a practically constant level, the concentration of non-electrolytes in the blood is relatively small and the concentration in the cells will probably remain constant. When salinity increases, the average concentration of non-electrolytes in the body fluids increases too. They are probably produced in the tissues, whereby the osmotic concentration of the cells is able to adapt itself to the increase in osmotic concentration of the blood; the electrolyte concentration in the cells remaining more or less constant. Part of the non-electrolytes will pass to the blood and cause an increase of non-electrolyte concentration, up to a certain maximum. Under unfavourable circumstances of high temperature and high salinity (Fig. 24b) the production of non-electrolytes is limited, resulting in a maximal non-electrolyte concentration in the body.

In *C. allmanni* similar relations were found. At lower salinities non-electrolytes pass from cell to blood when concentration in the medium

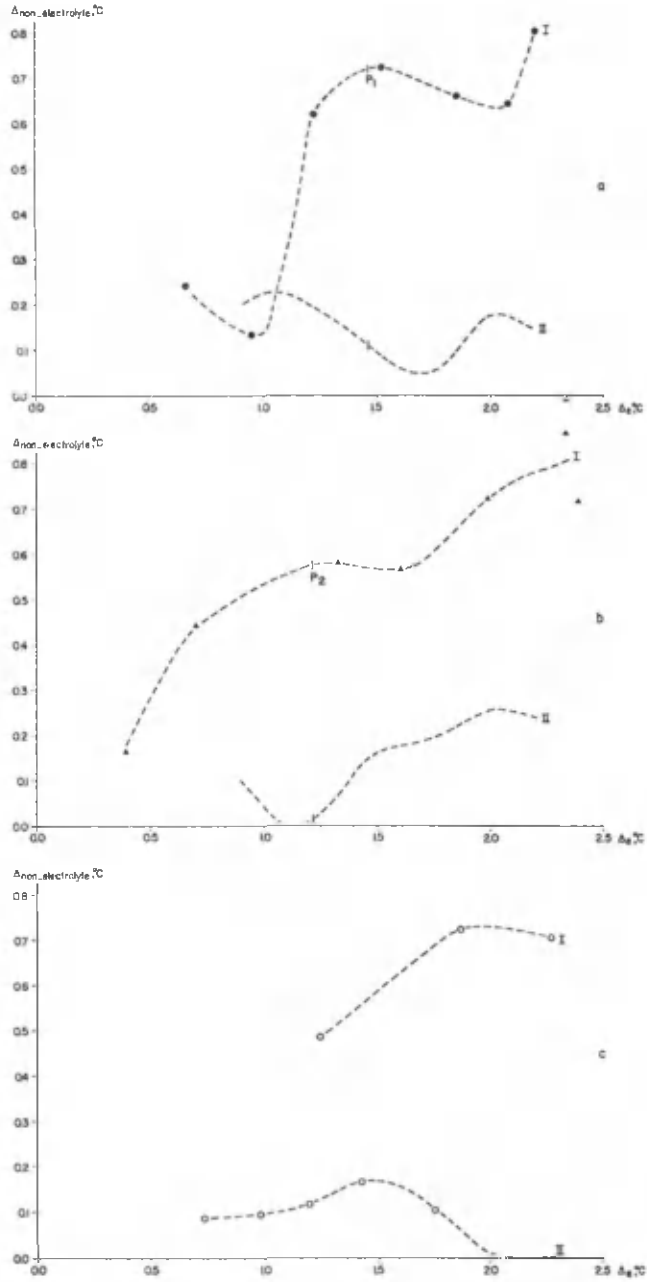

Fig.24. Osmotic concentration of non-electrolytes in homogenates (I) and in blood (II) at various osmolarities of the medium. a (●), *C. crangon* at 4 °C; b (▲), *C. crangon* at 21 °C; c (○), *C. allmanni* at 4 °C. At P_1 and P_2 the blood is isosmotic to the medium at 4 °C and 21 °C respectively.

—and therefore in the blood—gets lower. This means an osmotic adaptation in which electrolyte concentration in the cells remains practically constant. At higher salinities the non-electrolyte concentration in the cells does not increase any longer with the increase of concentration in the blood. The osmotic adaptation of cells and blood then corresponds to an increase of electrolyte concentration in the cells (Fig. 21c).

In other crustaceans similar indications for a variation in concentration of organic components in tissues have been found, thus effecting the isotonicity of blood and intracellular liquid. SHAW (1958b) found that when the salinity of the medium, in which *Carcinus maenas* was kept, was lowered, the salinity of the muscle fibres decreased, together with a considerable reduction in the concentration of trimethylamineoxyde and betaine. However, the decrease in Na, K and Cl concentration was much smaller. Moreover DUCHATEAU, FLORKIN & JEUNIAUX (1959) found that in this species the concentrations of alanine, arginine and asparagine did not change when salinity decreased, but that considerable reductions occurred in the concentrations of glutamic acid, glycine and proline. Another example is given in the work of VELANKAR & GOVINDAN (1960), who found that in *Penaeus* and *Metapenaeus* spp. the trimethylamineoxyde concentration decreased greatly with decreasing salinities.

In the two species used in the experiments, the osmotic adaptation of the cell liquid to the blood concentration under various circumstances was mainly brought about by the changes in non-electrolyte concentrations. In this way the electrolyte concentrations in the cells could remain more or less constant. Probably of these electrolytes mainly ions other than chloride are essential. When the blood concentration is lowered, non-electrolyte passes from the cells to the blood, so that the contribution to the blood osmotic concentration by non-electrolytes increases. At lower temperatures and salinities the cellular non-electrolyte concentration in *C. crangon* will decrease to a certain minimum value, roughly equal to the non-electrolyte concentration in the blood. A further lowering of salinity of the medium may result in a lethal decrease in cellular electrolyte concentration. At higher temperatures and salinities an increase of non-electrolyte concentration takes place in the cells, which in *C. crangon* may reach a certain maximum at high temperatures. An increase of salinity in the medium may now result in an increase of electrolyte concentration in the cells which may also become lethal.

In this way the osmotic adaptation from the cells to the blood by means of non-electrolytes and the resulting maintainance of cellular electrolyte concentration may be correlated to mortality in *C. crangon*

under various conditions. In natural conditions the unfavourable combinations of temperature and salinity are avoided by the seasonal migration.

The cellular regulation in *C. crangon* and *C. allmanni* are similar in many respects. In the course of evolution a species with a similar cellular regulation as *C. allmanni* may have developed a form with a lower permeability to water and electrolytes. Only then a more effective osmotic regulation of the blood could develop. The double regulation in *C. crangon*, of which it seems plausible that it requires relatively less energy, enabled the latter species to endure greater variations in salinity, and thereby to extend the area in which life conditions are favourable.

IX. SUMMARY

On two species of shrimps, *Crangon crangon* and *Crangon allmanni*, data were obtained on internal osmotic concentration after adaptation to various salinities and temperatures. The exchange between the environment and two components of osmotic concentration, *viz*: water and electrolytes, was investigated. Osmotic concentration, electrolyte concentration and non-electrolyte concentration were related to tissues and blood separately. The differences obtained for the two species can be related to their habitats.

Between about 15‰ and 30‰ S, *C. crangon* is able to keep its internal osmotic concentration at a more or less constant level. The salinity at which maximal regulation occurs, the isosmotic point and the value at which the blood concentration is regulated are inversely related to temperature. This may be correlated with data on mortality and seasonal migration of the species. With *C. allmanni* the osmolarity of the blood is only slightly different from that of the medium, under various circumstances.

The water content of *C. crangon* is inversely related to blood osmolarity. Frequency of urination and volume of urine discharges could be determined under various conditions. At circumstances where water content and blood concentration are regulated, urine production increases with decreasing salinity. At lower salinities urine production reaches a maximum, water content increases and blood concentration shows conformity. This reduction of urine flow, which is probably controlled by hormones, may effect the reduction of renal and extrarenal electrolyte loss. Water influx seems to be a passive process, which

at low salinities is limited by internal hydrostatic pressure, increasing with water content. In a hypertonic medium the urine production of *C. crangon* is probably very low. With *C. allmanni* urine production is probably always very low.

The urine of *C. crangon*—and probably also of *C. allmanni*—is practically isosmotic to the blood under various conditions. The extra-renal loss is positively correlated with electrolyte concentration of the blood for both species. Extra-renal loss exceeds renal loss which is at the utmost about 30% of the total efflux. It appeared that the active uptake was not localized in the gills. In *C. crangon* the maximal active uptake at 6 °C and 21 °C may be estimated at 3.1 and 8.5 μM $NaCl \cdot g^{-1} \cdot hr^{-1}$ respectively. The Q_{10} value for temperature influence on active uptake (1.88) is of the same order as for temperature influence on gross efflux (1.82). The effect of temperature on active secretion in hypertonic salinities is practically the same (1.89). There are several indications that in *C. crangon* permeability, both to water and electrolytes, is lower than in *C. allmanni*. Permeability is not influenced by salinity, but increases with temperature.

The concentrations of non-electrolytes in the blood of both shrimps show the same variation of 0 to 20% of the total osmotic concentration. At salinities at which the blood concentration of *C. crangon* is regulated, the non-electrolyte concentration is lower. The effect of temperature on blood osmolarity of this species is mainly attained by this variation of non-electrolyte concentration in the blood. The slight hypertonic values of the blood of *C. allmanni* at lower salinities are caused by non-electrolytes. The changes in non-electrolyte concentration occur at a slower rate than the changes in electrolyte concentration in the blood.

There are indications that in both species of shrimps the blood and the intracellular fluid are isosmotic. The electrolyte concentration in the cells is practically independent of temperature and kept at almost the same constant value in both species. At low salinities the osmotic adaptation of intracellular fluid to blood osmolarity takes place by non-electrolytes passing from the cells to the blood; at high salinities the intracellular concentration of non-electrolytes increases. At the combination of low temperatures and low salinities the intracellular concentration of non-electrolytes reaches a minimum, at high temperatures and high salinities a maximum in *C. crangon*. In both cases the osmotic concentrations of the cells cannot remain isosmotic to the blood without a change in electrolyte concentration in the cells. These conditions, which correspond with higher mortality are in natural circumstances avoided by migration.

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SAMENVATTING

Van twee soorten garnalen, *Crangon crangon* en *Crangon allmanni* werden gegevens verkregen over de inwendige osmotische concentratie na adaptatie aan verschillende saliniteiten en temperaturen. Van twee componenten van de osmotische concentratie, water en elektrolyten, werd de wisselwerking met het milieu onder verschillende omstandigheden nagegaan. Voor de osmotische concentratie, de elektrolyt concentratie en de niet-elektrolyt concentratie werd onderscheid gemaakt tussen de weefsels en het bloed. De verschillen die hierbij tussen beide soorten optreden kunnen in verband worden gebracht met verschillen in hun verspreiding.

Tussen omstreeks 15‰ S en 30‰ S is *C. crangon* in staat zijn inwendige osmotische concentratie min of meer konstant te houden. De saliniteit waarbij maximale regulatie optreedt, het isosmotisch punt en de waarde waarop de bloedconcentratie gereguleerd wordt, vertonen een omgekeerde relatie met de temperatuur. Dit kan gecorreleerd worden met sterftegegevens en de seizoensmigratie van deze soort. De osmolariteit van het bloed van *C. allmanni* wijkt onder verschillende omstandigheden slechts weinig af van die van het medium.

Het watergehalte van *C. crangon* vertoont een omgekeerde relatie met de osmolariteit van het bloed. De frequentie van de urinelozingen en het volume van de geproduceerde urine konden bij *C. crangon* onder verschillende omstandigheden worden vastgesteld. Bij de saliniteiten waarin het watergehalte—en de concentratie van het bloed—gereguleerd worden neemt de urineproduktie toe bij afname van de saliniteit. Bij lagere saliniteiten bereikt de urineproduktie een maximum, stijgt het watergehalte en vertoont de concentratie van het bloed conformiteit. Deze beperking van de urineproduktie, die waarschijnlijk hormonaal gereguleerd wordt, kan van belang zijn voor een reduktie van het renale en extra-renale elektrolytverlies. De wateropname geschiedt waarschijnlijk passief en wordt bij lagere saliniteiten beperkt door een inwendige turgor die samengaat met de toename van het watergehalte. In een hyperosmotisch milieu is de urineproduktie van *C. crangon* waarschijnlijk zeer gering. Bij *C. allmanni* is de urineproduktie waarschijnlijk altijd zeer gering.

De urine van *C. crangon* en *C. allmanni* is onder verschillende omstandigheden vrijwel isosmotisch met het bloed. Het extra-renale elektrolytverlies vertoont voor beide soorten een positieve correlatie met de elektrolytconcentratie in het bloed. De waarden hiervoor zijn aanzienlijk hoger dan het renale elektrolytverlies dat maximaal omstreeks 30% van de totale afgifte kan bedragen. De aktieve opname blijkt niet in de kieuwen gelokaliseerd te zijn. Bij *C. crangon* kan de

maximale aktieve opname bij 6 °C en 21 °C worden geschat op respectievelijk 3,1 en 8,5 μM $NaCl \cdot g^{-1} \cdot hr^{-1}$. De Q_{10} waarde van de temperatuurinvloed op de aktieve opname (1,88) is nagenoeg gelijk aan de Q_{10} van de temperatuurinvloed op de bruto afgifte (1,82). Het temperatuureffekt op de aktieve afgifte in hypertonische saliniteiten ligt in de zelfde orde van grootte ($Q_{10} = 1{,}89$). Uit velerlei aanwijzingen blijkt dat de permeabiliteit van *C. crangon* zowel voor water als voor elektrolyten kleiner is dan bij *C. allmanni* .De permeabiliteit voor water en voor elektrolyten lijken in zekere mate onafhankelijk van elkaar. De permeabiliteit wordt niet beinvloed door de saliniteit, maar neemt wel toe met de temperatuur.

De concentraties van de niet-elektrolyten in het bloed van beide soorten garnalen vertonen dezelfde variatie van 0 tot 20% van de totale osmotische concentratie. Bij de saliniteiten waarin de bloedconcentratie van *C. crangon* gereguleerd wordt, treedt een daling op van de niet-elektrolyten concentratie in het bloed. Het temperatuureffekt op de osmolariteit van het bloed van deze soort komt voor een groot deel tot stand door deze variatie in concentratie van de niet-elektrolyten in het bloed. De geringe hypertonie bij lage saliniteiten van het bloed van *C. allmanni* wordt veroorzaakt door de niet-elektrolyten. De veranderingen in de concentratie van niet-elektrolyten komen langzamer tot stand dan de veranderingen van de elektrolyt concentraties in het bloed.

Er werden aanwijzingen gevonden dat bij beide soorten garnalen de intracellulaire vloeistof isosmotisch is met het bloed. De elektrolyt concentratie in de cellen wordt zowel bij *C. crangon* als bij *C. allmanni* bij verschillende saliniteiten op een konstante waarde gereguleerd. Deze waarde is slechts in geringe mate afhankelijk van de temperatuur en verschilt voor de beide soorten weinig. De osmotische adaptatie van de intra-cellulaire vloeistof aan de osmolariteit van het bloed vindt plaats bij lage saliniteiten door migratie van niet-elektrolyten uit de cellen naar het bloed; bij hoge saliniteiten stijgt de intra-cellulaire concentratie aan niet-elektrolyten. Bij kombinatie van lage temperatuur en lage saliniteit bereikt bij *C. crangon* de intra-cellulaire concentratie van niet-elektrolyten een minimale waarde, bij hoge temperatuur en hoge saliniteit een maximale waarde. In beide omstandigheden kan de osmotische concentratie binnen de cellen niet meer gelijk blijven aan de osmolariteit van het bloed zonder verandering van de elektrolytconcentratie in de cellen. Deze omstandigheden, die corresponderen met een hogere sterfte, worden in de natuur door migratie vermeden.