ASPECTS OF OSMOREGULATION IN THE COMMON PRAWN, *PALAEMON SERRATUS* (PENNANT, 1777)

by

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ABSTRACT

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ASPECTS OF OSMOREGULATION IN THE COMMON PRAWN,

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The common prawn, Palaemon serratus (Pennant) regulates the sodium in its haemolymph hypertonically in a sea water concentration below 70%, whereas in a medium concentration above 70% SW the animal regulates its sodium concentration in the haemolymph hypotonically. In normal sea water, there is an inverse correlation between the haemolymph sodium concentration and temperature. In 50% SW the haemolymph sodium concentration increases irregularly with increased temperature. In 125% SW, within a temperature range of 5^{o} to 15^{o}C, the sodium concentration in the haemolymph of the prawn decreases with increasing temperature, but it increases with increasing temperature when the prawn is exposed to temperatures ranging from 15^{o} to 25^{o}C. Within the range of salinity between 50% to 125% SW, the prawn regulates its water content very efficiently especially in a temperature range from 5^{o} to 25^{o}C.

Reverse peristalsis in the hind gut and water drinking by prawns exposed to different osmotic stresses were observed. The animal takes in the water via the mouth and the anus. In normal sea water the drinking rate via both routes, was about 0.69 $\pm$ 0.13% body wt/hr. The drinking was continuous and decreased irregularly

with decreased salinity varying from about 0.42 ± 0.08% body wt/hr to 0.26 ± 0.07% body wt/hr in 70% and 50% SW respectively. Temperature also affects the drinking rate, a reduction of temperature from 15° to 5°C results in a decrease in the drinking rate by about a half.

The haemolymph volume of prawns acclimated to a salinity range of 10% to 100% SW lies between 19.44 ± 2.28% and 21.52 ± 4.76% body wt. The systems responsible for control of the haemolymph volume are the antennal glands and the sodium transport mechanisms. The former is responsible for eliminating or retaining water whereas the latter is involved in maintaining the constancy of osmotic and ionic levels of the haemolymph. The bladder volume of prawns averaged about 2.61% body weight though with considerable variability.

In 100% SW the prawn produced urine at a rate of 0.44% body wt/hr whilst the rate in 50% SW was 0.93% body wt/hr. In an isotonic medium, 70% SW, urine production rate was 0.34% body wt/hr. The prawn increases its urine flow as the salinity decreases. At a salinity near the lower limit of its tolerance, 10% SW, the animal increases its urine flow to about twice the rate in 50% SW. When temperature is lowered the rate of urine flow decreases, dropping from 0.44% body wt/hr at 15°C to 0.19% body wt/hr at 5°C.

The U/H ratios for Cr-51 EDTA of prawns kept in 100%, 70%, 50%, and 10% SW are 2.34, 2.16, 1.77, and 1.22 respectively, indicating maximum reabsorption of fluid is taking place in normal sea water. The rate of water reabsorption from the urinary bladder decreases as the salinity of the medium declines.

Under steady state conditions, the osmoregulatory process in 100% SW involved a continuous swallowing of medium with absorption of univalent ions and water from the gut epithelium; the excess ions were then excreted through the gills by active extrusion pumps to give a net uptake of water to balance the passive osmotic loss. The antennal glands serve to excrete divalent ions.

A sodium-potassium exchange pump involved in sodium extrusion has been demonstrated in the gills. The pump is located on the external membrane, and the transport system is inhibited when ouabain is placed in the bathing medium. The inhibitory action of this glycoside can be antagonised by adding excess K^+ to the external medium.

In dilute sea water, the prawn swallows very little medium, ions are actively taken up through the gills, and the osmotic inflow of water is balanced by the production of large quantities of isotonic urine.

The possible hormonal control of salts and water balance in the prawn was studied by bilateral eyestalk ablation experiments. In 100% SW the haemolymph sodium concentration and the rate of urine flow of animals with ligatured eyestalks are similar to that of the controls, but there are significant differences in the rate of water drinking, the haemolymph volume, and the rate of water reabsorption in the bladder. Eyestalk ablation elevates the U/H ratio for Cr-51 EDTA of the prawn from 2.34 to 2.94 which indicates a higher rate of water reabsorption from the bladder than the normal prawn, suggesting that the water reabsorption from the urinary bladder may be controlled by an eyestalk factor.

When prawns are subjected to a sudden change from 100% to 50% SW the haemolymph sodium concentration of animals with ligatured eyestalks and normal animals, reached a new steady state within 6 hours after the transfer whilst animals with their eyestalk ligatured maintained a sodium steady state higher than the normal animals. In 50% SW, eyestalk ablation caused no change in urine flow or haemolymph volume, but it raises the concentration of sodium in the haemolymph, and also the rate of sodium efflux. By using a basic dye, thionine, evidence is produced which suggested the possibility that the sodium pump at the gills is controlled by eyestalk factors.

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TABLE OF CONTENTS

I. GENERAL INTRODUCTION

Prawns and shrimps of commercial importance are placed in the order Decapoda and consists of two tribes, Penaeidea, and Caridea. The two families under the first tribe are Penaeidae and the Sergestidae. The species which are of interest in culture are those belonging to the genera Penaeus, Metapenaeus, and related forms.

Under the tribe Caridea, there are several prawns and shrimps which are also suitable for pond culture. Among them are the common prawn, Palaemon serratus (Pennant), and Macrobrachium rosenbergii (de Man). In the United Kingdom the former has received considerable attention within the past decade owing to the increased commercial importance of the species and its possible use in pond culture.

Experiments designed to investigate the possibility of cultivating prawns on a commercial scale in the United Kingdom were initiated in 1964 at the Shellfish Culture Unit of the Ministry of Agriculture, Fisheries and Food, Conway, North Wales. The development of rearing techniques for the common prawn has been reported by Reeve (1969), Forster (1970), and Wickins (1972). The main conclusions from these works are that from a biological standpoint the mass culture of the common prawn is technically feasible but is unlikely to be economically successful. As a result of the work, it is believed that, if the prawns are to be reared successfully on a commercial basis in the United Kingdom, intensive methods of culture must be developed. Before such system can be operated successfully more knowledge of the biology and the

physiology of the prawn is needed. For the latter, more extensive information on the process of adaptation in the cultured species would be valuable, especially knowledge concerned with the osmoregulatory mechanisms which enable the animals to cope with the osmotic problem posed by external environments. It is the purpose of this thesis to study the osmoregulatory mechanisms of the common prawn, P. serratus (Pennant) in some detail.

II. HISTORICAL BACKGROUND

1. Natural History.

The common prawn, Palaemon serratus (Pennant, 1777), was formerly known as Leander serratus Sharp, 1893 (see Holthuis, 1950 for history of synonyms). Keys for the purpose of identification of the species are provided by Holthuis (1950), and also Kemp (1910).

The prawn is common in the rock pools and sub-littoral region around the coasts of the British Isles up to a line extending from the Clyde to the Wash, and including Ireland. Its geographical range extends from Britain to the Mediterranean (Kemp, 1910) and it is also common around the shores of France, Italy, Greece, and Algeria (Gurney, 1923).

The life history and ecology of this species were the subjects of papers by Gurney (1923), Forster (1951), and Cole (1958), for the populations around the coasts of southern England and North Wales. Studies of the development of the larvae have been made by Gurney (1924) on specimens from the plankton, and by Sollaud (1923) from laboratory reared animals.

2. Previous works on osmoregulation in the Caridea, Crustacea.

The osmoregulatory capacities of decapod crustaceans have been relatively well studied in the group as a whole. Within the past few decades several works exclusively or partially concerned with osmoregulation of Crustacea have appeared. Among them are Krogh (1939), Beadle (1943, 1957), Robertson (1957, 1960), Prosser and Brown (1961), Lockwood (1962, 1968), Potts and Parry (1964a).

However, there is little relatively evidence available with regard to osmoregulation in the Caridea. An outline of relevant literature is reviewed chronologically below.

The foundations of knowledge of the osmoregulatory mechanisms operating in caridean shrimp and prawns were laid down some thirty years ago by Panikkar (1941), and Broekema (1942). Panikkar (1941) investigated the osmotic behaviour of haemolymph and urine in the palaemonid prawns, Leander serratus, L. squilla, and Palaemonetes varians by means of vapour-pressure measurements. He established the hypotonicity of the haemolymph and urine of the three palaemonid prawns living in normal sea water. He also demonstrated their hypertonicity to the medium in brackish water at the lower end of the tolerance range. Urine is isotonic with the haemolymph at all sea water dilutions.

At Den Helder, Broekema (1942) made a careful analysis of the distribution and physiology of shrimp, Crangon crangon (Linnaeus), she found that young C. crangon in summer tolerate changes in salinities better than adults. Adults seek a higher salinity in winter and a lower salinity in summer. The young, after a period of estuarine existence, migrate to the sea in the fall and return to the estuary as adults the following spring. She also found that with continued dilution of the external medium within limits, but under conditions of constant salinity, the concentration of the haemolymph increased with a fall in temperature.

Since 1950, more attention has been given to the problems of osmotic and ionic behaviour of shrimps and prawns. Parry (1954, 1955, 1957, and 1961) studied aspects of osmoregulation in P. serratus, and Palaemonetes antennarius, and also made comparative

observation on P. varians and Palaemon longirostris. She confirmed the finding of Panikkar (1941) on P. varians.

Williams (1960) has studied the effect of temperature on osmotic regulation in two shrimps, Penaeus duorarum, and P. aztecus. Both penaeid shrimps regulated hyperosmotically in sea water of salinity below 30‰, and hypoosmotically in salinity above 30‰. As the temperature was lowered from 28° to 18°C and 18° to 8°C the haemolymph concentration of both species of shrimp tended to approach isotonicity at all experimental salinities.

Flügel (1960, 1963), on the basis of cryoscopic and conductivity experiments, showed that osmotic resistance of C. crangon to low and high salinities (1 to 5‰) is higher at 5°C than at 15° and 20°C. The efficiency of osmoregulation of the shrimp is correlated with the fact that in the northern Baltic sea C. crangon is capable of survival in low salinity water at temperatures near freezing point.

Bryan and Ward (1962) have made a comparative study on the accumulation and loss of Cs-137 in relation to K metabolism in P. serratus, and P. varians. They observed that inulin U/H ratios of P. serratus ranged from 1.5 to 2.0 suggesting that water reabsorption occurs in the prawn when it is kept in normal sea water.

Dobkin and Manning (1964) have studied the osmoregulation of two species of American caridean shrimp, Palaemonetes paludosus and P. intermedius which are comparable to the European species, P. antennarius and P. varians respectively.

Recently, interest has been centred on the control of ionic balance, Potts and Parry (1964b) showed that in full strength sea water sodium ions are actively removed by P. varians extrarenally. In 2% SW chloride ions are actively absorbed. In salinities below 2% SW uptake of ions declined and animals can no longer maintain equilibrium.

Rudy (1967), using tritiated water, found adaptive differences in water permeability among several different species of marine, brackish water, and fresh water crustaceans. He stated, on the basis of his data, that P. varians could not significantly alter its integumental water permeability.

More recent works on osmoregulatory studies of caridean shrimps have been performed. Among them are Born, (1968) on Palaemon macrodactylus, and Syncaris pacifica; Denne (1968) on Macrobrachium australiense, and M. equidens; Grimm (1969) on Crangon vulgaris, and C. allmanni; Haefner (1969) on C. septemspinosa.

Hagerman (1971) studied osmoregulation and sodium balance in C. vulgaris in varying salinities. He found that the shrimps were hyperosmotic in salinities below 25‰, and hypoosmotic above this salinity. Spaargaren (1971) worked on P. serratus and Lysmata seticaudata, he found that osmotic concentration of haemolymph and of muscles (as measured on homogenates) of P. serratus are strongly influenced by temperature while L. seticaudata is independent of temperature. Spaargaren and Kraay (1973) found that the shrimp, C. crangon regulates the chloride concentration in its haemolymph at values above 395 mE/l. The regulation depends on the temperature and is more effective at 21°C than at 5°C. The chloride

concentration in the cells is practically independent of temperature and salinity.

From the foregoing data, it appears that there is a noticeable gap in the literature as to information on the response of brackish and marine species of prawns to a large range of temperature and salinity. Although there are some observations available on the influence of temperature and salinity on osmotic concentration systematic experimental tests are still needed.

In the condition of artificial prawn culture, the animals have to live in shallow water ponds of varying salinities and temperatures which are subjected to change from time to time. In order to survive in such unstable environments the animals need the means to regulate their intra and extra-cellular fluids with respect to water and salts in order to maintain their tissues in a proper functioning condition in environments which may be both unfavourable and fluctuating. The over all regulatory process in these animals is the sum of those concerned with osmotic and ionic regulation. These two processes are linked together, but they are independent in physiological mechanisms. The physio-chemical mechanism involved, especially in water permeability and active ion transport of prawns, are not well understood. The relationship between temperature, salinity, and their combined effects on such regulatory processes has not yet been fully explained and is still the subject of much debate.

Knowledge of the physiology of the cultured species is very important, since it can be applied in prawn farming techniques to the selection of environments to obtain a higher yield and lower natural mortality. It can not be said that the existing knowledge

of the subject is comprehensive enough for immediate application and further work is needed. In view of this, it was hoped that a fuller knowledge of the physiology of prawns, and a critical evaluation of the influence of environmental conditions might help in the practical application in prawn culture.

However, information obtained from these studies is not only important for prawn culture, but it is useful to determine the suitability of environmental conditions for rearing this species in the laboratory. Although the results obtained apply specifically to this species many of the conclusions may be applied generally to other species of prawns which may be considered for their potential as cultivated species.

3. Present work.

The prawn, P. serratus has been chosen for the present studies, since it is

(1) the largest species available in Britain,

(2) is of commercial value,

(3) adapts well to laboratory conditions,

(4) is readily available, and

(5) is a species potentially feasible for pond culture.

This thesis is an account of experiments undertaken to test the physiological response of the prawn in terms of

(1) the capacity of the prawn to regulate its sodium concentration in media of varying salinities and temperatures,

(2) to determine the effects of temperatures on the ability of the prawn to regulate osmotically when exposed to various concentration of sea water,

(3) to determine the mechanisms which permit the prawn to adapt to water of different salinities and temperatures, and

(4) to determine the effects of eyestalk removal on the water and sodium balance.

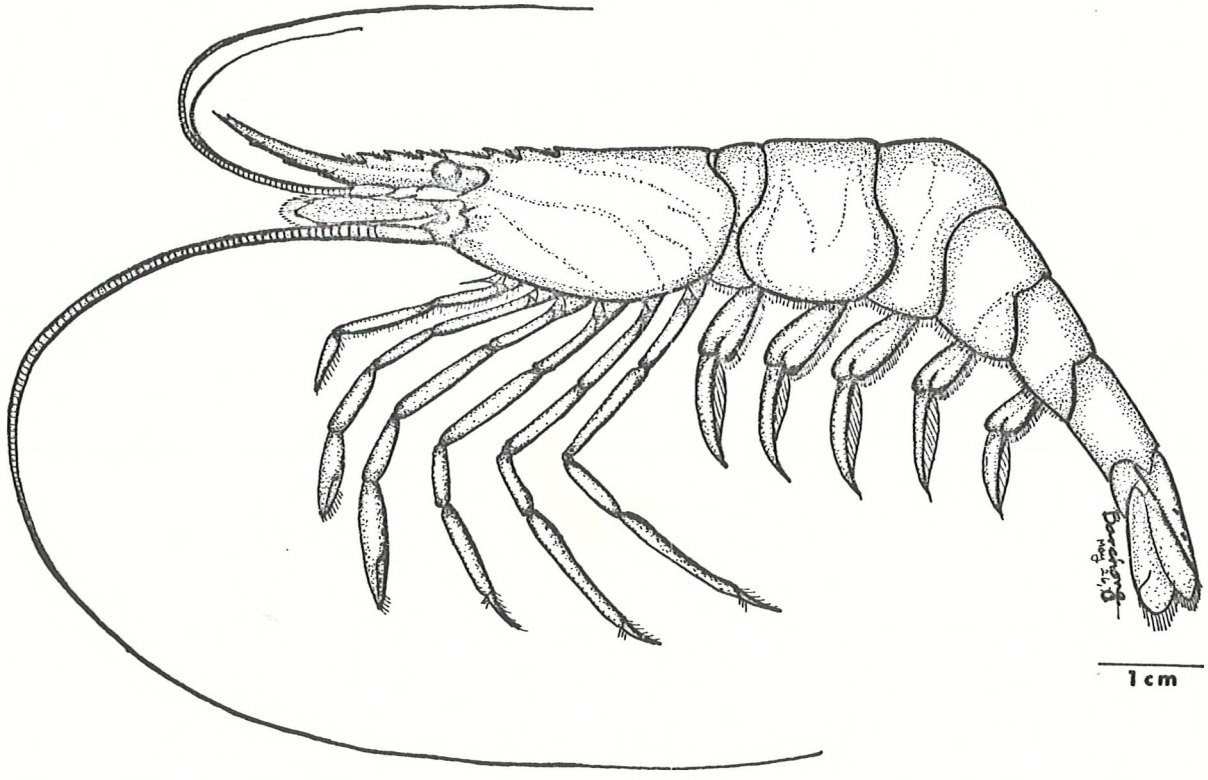

Figure 2.0 The common prawn Palaemon serratus (Pennant, 1777)

III. MATERIALS AND METHODS

1. Collection and handling of experimental animals.

Prawns used in the present work were collected by trawling from Calshot, Warsash, and Solent areas during the winter months whereas during the summer most prawns were obtained from Kemp's boat yard by potting, from Weymouth by scoop net and from Studland Bay, Dorset, by hand net. Animals were transported directly to the laboratory and introduced into holding tanks provided with continuously flowing unfiltered sea water (34‰). The size of specimens varied from 0.15 to 8.5g, but, whenever possible, comparative experiments were done using animals of similar size. Animals were identified following Kemp (1910), and Holthuis (1950). The animals were used indiscriminately as collected but excluding berried females and those which were damaged or moribund. Molt stages were determined by the method of Drach and Tchernigovtzeff (1967). Except where stated otherwise only intermolt prawns (State C) were used in order to reduce molting as a factor in the experiments. Animals in all cases were kept in the laboratory for three days before each experiment began.

During the experiments the animals were not fed. The prawns were acclimated in 13-litre plexiglass aquaria of 30x20x20 cm which were placed inside a water-bath of approximately 340 litre capacity, the remaining space of which was filled with tap water. The aquarium was somewhat higher than the water-bath, and surrounded with tap water for about 3/4 of the height. The experimental container was covered with a plastic mesh in order to prevent the prawns jumping out of the vessel. During the acclimation period,

and the course of the experiment the water was renewed every two days in order to minimize the changing concentration of the experimental medium.

2. Removal of eyestalks.

The following treatment was used to prepare for removal of eyestalks: each prawn was placed on an operating board and one eyestalk was removed. The second eyestalk was removed 48 hours later. In some experiments the eyestalk ablation was also accomplished by firmly ligaturing the bases of the eyestalks with cotton thread. This results in the isolation of eyestalks without the loss of haemolymph which usually occurs after surgical ablation. The ligatured animals displayed effects similar to those in which eyestalks were removed. After operation the animals were immediately returned to well aerated 100% SW, and kept for three days for recovery before further experimental use.

3. Experimental media.

In order to be able to alter the concentration, or completely remove one major ion from the experimental solution, whilst maintaining the composition of the other ions constant, artificial salines were prepared.

Artificial sea water was made to give a final concentration of 470mM/l Na^+, 548 mM/l Cl^-, 10 mM/l K^+, 10 mM/l Ca^{++}, 54 mM/l Mg^{++}, 2 mM/l HCO_3^-, and 28 mM/l SO_4^{--} (Hale, 1957). Potassium-free artificial sea water was made to give a final concentration of 470 mM/l Na^+, 538 mM/l Cl^-, 10 mM/l Ca^{++}, 54 mM/l Mg^{++}, 2 mM/l HCO_3^-, and 28 mM/l SO_4^{--}.

4. Temperature.

The experimental temperatures were chosen in relation to the average seasonal sea temperature. For example, the seasonal range at Calshot was from about 4° to 20°C. At Marchwood the minimum temperature recorded was 0°C and the maximum was 22°C (Raymont, 1972, Figure 3.0). Using this temperature as a guide, the lowest temperature chosen for experiments was 5°C and the highest temperature was 25°C. The water temperature was kept constant by individual thermoregulating water-baths in which the temperatures were maintained within $\pm$ 1°C.

5. Salinity.

For convenience the terms "normal sea water" or "100% SW" used in this work refer to Southampton sea water of 34-35 parts per thousand salinity, and the other salinities are referred to as percentage of this standard. Sea water was brought to the laboratory from the Solent area. A medium of salinity greater than Southampton water was prepared by means of evaporation at a temperature of 50°C, and room pressure until the salinity had risen to about 55 parts per thousand. From this supply of concentrated sea water, the various salinities above 100% SW were obtained by dilution with deionized glass distilled water. Salinities less than that of sea water were made by mixing Southampton sea water with deionized distilled water. The salinity was then determined by means of conductivity measurements.

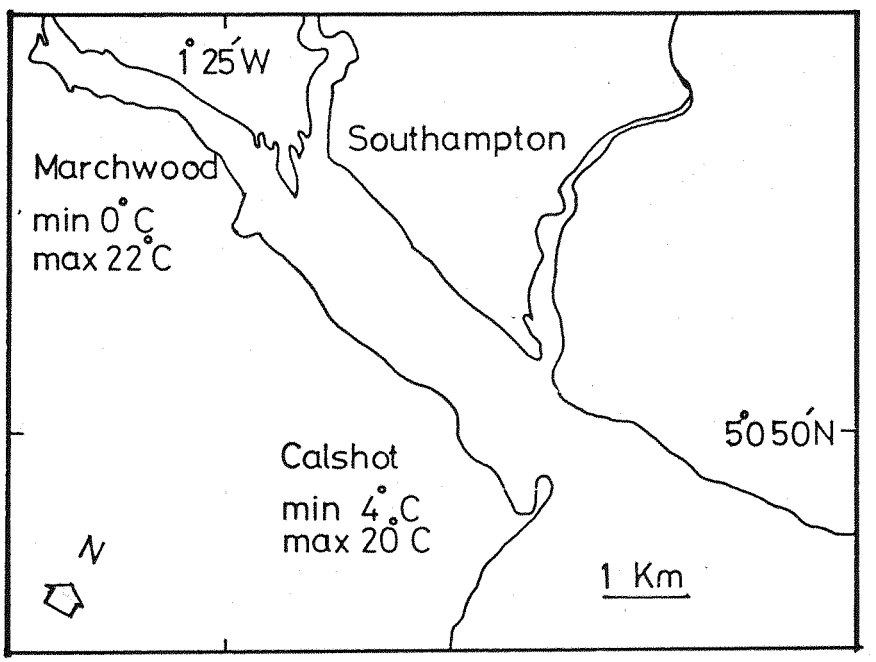

Figure 3.0 Map showing the annual temperature range in Southampton area.

6. Haemolymph sampling.

Haemolymph samples were collected by means of a micropipette. The pipette was made from 2 mm internal diameter thick walled pyrex tubing which had been drawn out to a fine tapering point. Before use, it was cleaned throughly in chromic acid and the tip coated on the outside with a layer of silicone (Repelcote). At the other end of the pipette was attached a piece of rubber tubing which served to facilitate emptying or filling by mouth. The haemolymph samples were used without removal of corpuscles, since Parry (1954) working with P. serratus found that the removal of corpuscles by centrifugation was impractical with such small sample since there was a risk that manipulation would lead to increase of errors in analysis.

Samples of haemolymph were taken from individual prawns by introducing the small pipette into the pericardial cavity at the point where it is easily seen from outside under the median frontal part of the carapace. The normal vigorous struggling of the animal was inhibited if the prawn was held with extended fingers firmly surrounding the anterior of the abdomen. Filling of the pipette usually occurred by capillarity as soon as the heart was pierced. Prior to making the puncture, the postero-dorsal border of the carapace, particularly the point of insertion of the capillary and the adjacent area were thoroughly dried with absorbent tissue. Approximately 5 to 10 microlitres of haemolymph were collected from each prawn, depending on the side of animal. The haemolymph sample was stored under liquid paraffin in a siliconized watch glass and kept until required. Between sampling operations

the micropipette was cleaned in chromic acid, rinsed by repeated filling and emptying the deionized water and finally dried with acetone. Care was taken to ensure that no traces of acetone remained in the pipette when the next haemolymph sample was taken since acetone causes coagulation of the haemolymph.

7. Urine sampling.

Obtaining samples of urine was considerably more difficult. Repeated trials showed that the following method gave the best results. The prawn, after the adhering water had been wiped off, was laid on its back on a glass plate under a long arm microscope. By manipulation, the openings of the antennary glands at the base of the antennary peduncles was located. To ensure that there was no possibility of contamination by fluid from the branchial chamber, the outside border of the antennary peduncles was dried with strips of absorbent paper. The tapered cannula , with an attached rubber tube was then inserted into the excretory pore and the urine content drawn into the pipette. The hydrostatic pressure of the urine inside the bladder was sufficiently high to fill the pipette without much effort.

8. Determination of sodium.

A Unicam SP 900 flame spectrophotometer was used to determine the sodium concentration of the haemolymph, urine, and medium samples. This instrument has a detectability limit of about 0.0001 ppm for the sodium ion, but all samples used in this study were well above this value. The wavelength, slit adjustment, air pressure and gas pressure used, were those recommended in the

instruction manual, viz. air pressure 28 lb per square inch, gas pressure 14 lb per square inch, slit 0.8 mm, wavelength 586 mμ . The samples were run alternately with standard solutions of known concentration to ensure that no change in sensitivity occurred between each series of determinations.

Samples for sodium determination, together with standard sodium chloride solutions were taken up in a 1 μl micropipette. The pipette was filled completely before being blown out. Identical and constant volumes of unknown samples and standard solutions could be collected in this way. The tip of micropipette was then wiped and the sample blown out into a polythene specimen-tube containing 10 ml of deionized water. The solution was then stirred with a stream of air and the concentration read off on the flame spectrophotometer. Repeated samples of a known standard were accurate to a standard deviation of ± 2 per cent; this value included both pipetting and instrument error.

It was necessary to ensure that when taking up samples from under liquid paraffin none of the paraffin contaminated the sample. This could be detected by the differing refractive indexes and immiscibility of the two fluids. Between each sampling operation the pipette was cleaned as before. Triplicate determinations on each sample from one animal were carried out.

9. Determination of chloride.

Chloride was measured by the first method of Ramsay, Brown, and Croghan (1955). The method was slightly modified in that the titration was carried out in a drop of acetone and glacial acetic acid (v/v, 50:50) on the hydrofuge surface of a piece of teflon.

In titration of chloride with silver nitrate, the concentration of chloride ions determines the potential of a silver/silver chloride electrode which dripped into the titration droplet. The potential is measured with a reference electrode which is sealed into the delivery tube of the micro-burette. The chloride is titrated against standard silver nitrate solution of N/25. The standard silver nitrate was introduced into the droplet with an Agla micrometer syringe burette with a micrometer delivery control. Thorough mixing of the titration droplet was necessary for a consistent reading on the Pye pH meter. This was effected by a jet of air playing on the surface of the drop. As silver chloride is precipitated, the potential of the electrode declines. The point of inflection of the curve relating potential to concentration is taken as being the end-point of the titration.

The sample to be titrated was taken up in a 1 µl micropipette, similar to the one used in the determination of sodium, and blown out into the acetic acid/acetone drop. The sample was measured in triplicate and was standardized against the reading given by the identical volume of standard solution of sodium chloride from the same micropipette.

The end-point for the titration was taken as that point where the potential change of the drop was most rapid. The presence of acetone reduced the solubility of silver chloride. The potential at which the end-point occurred depends on the pH of the drop and this solution acts as a buffer and ensures that the end-point is reached at approximately the same potential each time. Repeated measurements on a standard gave a standard deviation of $\pm$ 2.5%.

10. Determination of water content.

As a basis for determination of water content, the dependability of wet weight and dry weight measurements is important. Considerable controversy has occurred over the temperature necessary for drying animals and as a consequence dry weights quoted in the literature depend on the methods adopted. According to the literature temperatures in the range from 60° to 110°C have been used for determining dry weights of animals by different investigators. It is necessary to achieve a compromise between the maximum amount of water removed and the least loss of organic materials. Lovegrove (1962) suggested 60°C as the most suitable drying temperature as final values were comparable with those from a desiccator. Carl (Lovegrove, 1962) criticized the practice of drying plankton samples at 100°C, since this temperature caused volatilization of some lipids and amines as well as denaturing many proteins. In this study a temperature of 70°C was used. This was found to be most suitable for at this temperature volatilization of lipid components is probably minimal (Raymont et al., 1964). Kharoof (1970) found good agreement between samples dried at this temperature and similar samples dried in a desiccator.

10.1 Wet weight of dead prawns.

Prawns were removed from the acclimating medium, and quickly rinsed in deionized water to remove salts adhering to the body surface. They were dried with an absorbent paper to absorb the water adhering to the surface of the animals. Final traces of adherent water were removed by sprinkling the animals with a few drops of acetone and drying them quickly with an air jet.

Each prawn was weighed to the nearest 0.01 mg on an aluminium foil weighing vessel. Although it was impossible to remove all the water adhering to the surface of the animals, particularly between the appendages, it was hoped that weighings would be constant if the procedure was standardized. This was tested by immersing, drying off and weighing the same animals several times. Twelve replicate weighings of a dead prawn yielded a coefficient variation ± 0.83 per cent (Appendix 1).

10.2 Dry weight.

Prawns, after blotting and weighing as described above, were transferred to a sintered crucible and dried to a constant weight at 70°C for 36 hours. They were then placed in a desiccator to cool, weighed and replaced in the oven for another 12 hours. After this second drying there was usually little change in weight, indicating that all free water had been removed.

10.3 Ash.

Grove et al. (1961) found that for samples of animal tissues of size range from 1 to 5 g most of the carbon is burnt at 450° to 500°C in a 24 hours period without causing any loss of the relatively volatile ions, i.e. sodium and potassium. In the experiment, dried prawns were ashed in a muffle furnace at 480°C for 12 hours. The ash was ground to a fine powder with a piece of glass rod, and taken up in 2 ml of N/5 hydrochloric acid. Five microliter of both samples and standards were taken in a micro-pipette and the sodium was estimated by flame photometry.

11. Estimation of wet weight of live prawns.

A standard procedure was followed in all experiments where the weight of living prawns was required. Animals were removed from the acclimating medium and carefully dabbed with an absorbent paper to remove as much surface moisture as possible. When no more moisture could be taken up on the tissue paper, the dried animals were carefully wrapped in a fresh dry tissue to restrain its movements and weighed. The tissue alone was then weighed. The weight of the animal was obtained by subtraction. From 12 replicated weighings of a live prawn, with reimmersion between weighing, a coefficient of variation of 0.17% was obtained (Appendix 1). Another source of error of weighing might be due to the stress imposed on the animals by the weighing procedure if this causes release of urine or gut content. This source of error can be minimised by subjecting the prawns to the same procedure at the beginning and end of any experiment.

12. Estimation of urine production.

12.1 Weight-gained method.

A modification of a method first described by Peters (1935) and later used by Lienemann (1938), Maluf (1941b), and Bryan (1960a), was used to estimate the quantity of urine produced by the prawn. The area around the antennal gland openings was dabbed with absorbent paper and the urine aspirated from the bladder by inserting a small glass tube through the antennal pore and applying a slight vacuum. De Trey Poly-F dental cement was applied to the excretory pores so as to form a solid block covering both

papillae. After blocking, the animal was returned to the acclimatisation medium to recover. Before using an individual for the experiment, the nephropores were reexamined under a binocular microscope to ensure the effectiveness of the blockage. The weight as fraction of the original weight of the animals were determined after 1, 3, 6, 12, and 24 hours.

12.2 Clearance of Cr-51 EDTA.

As shown in experiment 2 of chapter XI, the loss of Cr-51 EDTA (ethylene diamine tetraacetic acid) from the haemolymph is due only to production of urine. Thus, it is justifiable to calculate the urine production rate of the prawn by following the procedure of Binns (1969a) who calculated directly from the change in haemolymph activities of crabs where the initial and final haemolymph activity levels were known. When Cr-51 EDTA is injected into the prawns, the fall in haemolymph activity level follows a curve. A semi-logarithmic plot showing the log of haemolymph activity against time gives a straight line which means that the curve showing haemolymph activity against time is exponential. The equation for such a curve is

$$y = Ae^{-t/T} \qquad \text{..... (3.1)}$$

where y is the activity at time t,

A is a constant, and

T is the time constant for the curve.

When t = 0, then the constant A = y_o, the activity at time = 0

$$y = y_o e^{-t/T}$$

$$\log_e y = \log_e y_o - 1\ t/T$$

$$\log_e y_o - \log_e y = 1\ t/T$$

$$(\log_e y_o - \log_e y)/t = 1/T = \text{GRADIENT}$$

when T = t;

$$\log_e y_o - \log_e y = 1$$

$$\log_{10} y_o - \log_{10} y = 0.4343$$

By subtracting 0.4343 from $\log_{10} y_o$, the time constant (T) is found for the curve showing the fall in haemolymph activity of each prawn against time. The time constant (T) is rate at which chromium is cleared from the haemolymph. The rate of primary urine production was taken to be equal to the clearance rate. The definitive urine production is taken as being the clearance divided by the U/H ratio for EDTA. The urine production rates are expressed as percentage of body wt/day.

For each prawn used the haemolymph activity levels were recorded at the beginning and the end of the experiment. From the initial activity level the EDTA volume was calculated and the time constant (T) found from the plot of the logs of the initial and final haemolymph activities against time. The following is an example of this method of calculating urine production knowing these two factors:

Prawn no. 26 (weight 1.35 g) kept in 100% SW at 15°C

Duration of experiment	36	hours
Activity injected	144,776	counts/100 sec
Initial haemolymph activity	614	counts/100 sec/1µl
Final haemolymph activity	65	counts/100 sec/1µl

Haemolymph volume		17.92	% body weight
Time constant (T)		16	hours
Clearance rate (24x100/16)	=	150	% haemolymph volume/day
Urine production $(\frac{150 \times 17.92}{100 \times 2.34})$	=	11.48	% body wt/day

12.3 Efflux of Cr-51 EDTA.

Urine flow may be determined indirectly in prawns by measuring the filtration rate and dividing it by the chromium urine to haemolymph ratio. If the Cr-51 EDTA is uniformly distributed in the EDTA space, and if urine flow is constant, the total amount of Cr-51 EDTA loss from the whole animal will be exponential. The rate constant for chromium excretion can be calculated by plotting the activity in the whole animal and time. The rate constant for the excretion of chromium can then be used to calculate the filtration rate in the bladder (ml $kg^{-1}hr^{-1}$) by using the equation:

$$F = \frac{K \times V}{Wt} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.2)$$

where K = the rate constant of Cr-51 EDTA from the whole animal,

V = the EDTA space (ml),

Wt = is the wet weight (kg), and

F = the filtration rate in the bladder (ml $kg^{-1}hr^{-1}$)

13. Determination of drinking rate.

13.1 Under a steady state experiment.

The experimental animals were placed into a loading medium with an activity of approximately 35,000 counts/100 sec/ml. After

loading for two hours, the animals were placed in a non-radioactive bath for one hour to remove the Cr-51 EDTA from the gill chamber. The salinity was the same in all baths for a particular experiment. The volume of water ingested was estimated. The Cr-51 EDTA activity in the animal was counted using a Panax well-typed crystal (c5 SH), and scintillation counter (USC-BP1). The activity of 1 ml of the medium was also measured. From the activity of the medium, the volume of water ingested was estimated, on the assumption that no excretion had occurred.

$$Dr = \frac{At \times 100}{A_o \times t \times Wt} \quad \text{.....} \quad (3.3)$$

where Dr is the drinking rate (% body weight/hr),

At is the total radioactivity disintegrate in the animal at time t (counts/100 sec),

A_o is the mean concentration of Cr-51 EDTA of the external medium per ml (counts/100 sec),

t is the duration of the measurement (hr), and

Wt is the weight of the prawn (g)

13.2 A sudden transfer experiment.

The main procedure of this experiment is similar to those in 13.1 except that the experimental prawns were loading at 1, 3, 6, 12, and 24 hours in loading media of designed salinity. The volume of water ingested was then estimated from equation ....(3.3)

14. Estimation of haemolymph volume.

The radioactive tracer employed was Cr-51 EDTA. Cr-51 EDTA is a metabolically inert substance, easily filtered and is not

taken up rapidly into body cells. It is readily counted and therefore can be used to estimate haemolymph volume by isotopic dilution.

Initially the experimental prawns were blotted and weighed. An injection of Cr-51 EDTA was made with a 0.1 ml Hamilton syringe. The needle was inserted through the abdominal segment and 2 µl of Cr-51 EDTA was injected into the abdominal artery at the sixth abdominal segment. The syringe was held in place for 15 sec, then gently withdrawn through a wad of absorbent tissue whose function was to minimized bleeding and also to absorb any leakage. This volume of injected solution, about 2 µl, was calculated not to increase the expected haemolymph volume of the prawn by more than two per cent. After injection, the prawns were returned to the medium which they had previously been acclimated and left for an hour to allow both recovery from the operation and also tracer mixing throughout the extra-cellular fluid. The animal was then removed from the medium and placed in a counting tube. For all determinations of radioactivity the whole animal was counted over a 400 second period and the average of counts for 100 seconds was recorded.

Haemolymph samples were taken at one hour after injection. The haemolymph was blown out of the pipette into a watch glass of liquid paraffin. Ten µl of haemolymph was taken up by capillarity using Drummond micro-pipettes. The Drummond pipettes were discharged into 1 ml of distilled water in a counting tube. The radioactive content of the haemolymph was then counted over a 400 second period.

The volume of haemolymph of each prawn was calculated from the equation:

$$V_1 = Q/S \quad \text{..... (3.4)}$$

where V_1 = haemolymph volume (µl),

Q = the amount of radioactivity in the whole animal after injection, and

S = an average concentration of tracer substance in 1 µl taken an hour after injection

In order to convert the haemolymph volume into the percentage of body weight, the haemolymph volume obtained from equation (3.4) was used for further calculation in equation (3.5).

$$V_2 = (1.03 \times V_1 \times 100)/Wt \quad \text{..... (3.5)}$$

where V_2 is the haemolymph volume (% body weight),

1.03 is the specific gravity of haemolymph of prawn (Parry, 1954),

V_1 is the haemolymph volume obtained from equation (3.4) and expressed in terms of ml, and

Wt is the initial wet weight (g)

15. Estimation of bladder volume.

Prawns were injected with 2 µl of Cr-51 EDTA. The initial activity of the injected Cr-51 EDTA was recorded. After one hour the haemolymph samples were taken for the haemolymph volume estimation. At the end of the experiment the total activity of labelled chromium in the whole animal, in the haemolymph, and in the urine were recorded. The volume of the bladder then can be calculated by assuming that the space occupied by extrahaemolymph chromium is equivalent to the volume of the urinary bladder.

16. Sodium-22 efflux.

The use of radioactive tracers to measure ionic fluxes is now widespread. It is based on the principle that the radio isotope employed behaves chemically exactly like the corresponding natural element. The rate of fluxes from a compartment A, to another, B, containing initially no isotope is accurately measured by the rate of appearance of the isotope in B over a short but finite period of time during which the back diffusion of the isotope from B to A is small and negligible by comparison with errors from other sources. The method is of enormous value since it permits the measurement of rate of fluxes even when there is no chemically analysable change in the contents of either component.

The isotope sodium-22 was used in preference to sodium-24 since the latter has a half-life of only 14.9 hours compared with the 2.6 years of the former. The sodium-22 was supplied by the Radiochemical Centre, Amersham, as sodium chloride in aqueous solution, specific activity 1.21 mCi/mgNa.

Owing to the fact that radioactive disintegration is a random process with reference to time, the accuracy of any count will be related to the number of disintegrations observed. The following equation relates the size of the standard deviation of the means to the count size expressed as a percentage.

$$\frac{\sqrt{\text{No. of counts observed}}}{\text{No. of counts observed}} \times 100 = n\ \%SD \quad \ldots\ldots \quad (3.6)$$

For a standard deviation of $\pm 1\%$ of the mean, it can be seen from the above equation that a count 10,000 is necessary. Any reduction in the size of the observed count will increase the

deviation from the mean and reduce accuracy. Specific activities and count times were selected to give not less than ±2% accuracy.

Sodium-22 has a long half-life for decay, 2.6 years. As most of the experiments were run for only a few days, the problem of correction for decay during the experiment did not arise. The total count of a radioactive sample is related to the inverse square of the distance from the detector and sample constant when a series of samples is being counted, or the same sample recounted over a period of time.

16.1 The counting of radioactive sodium.

Counts were recorded on Panax scaler with a preset time. The EHT voltage, window width and attenuation settings were selected to obtain the maximum count with minimum background. The counting characteristics of the equipment are affected by external factors such as atmospheric pressure and temperature. In order to compensate for any such change a sodium-22 standard was counted regularly during experiments and the appropriate corrections made to the experimental count when necessary.

16.2 Measurement of the sodium efflux.

Sodium effluxes were measured either in prawns which had reached a steady state or in prawns subjected to rapid transfer from a medium of one salinity to another (a nonsteady state prawn). Fully loaded animals were used to determine the rate constant for loss of sodium in experimental media. Experimental animals were removed from the loading medium and thoroughly washed in deionized water or inactive medium to remove tracer adhering to the body surface. The washing out experiment was performed in a special

circulation apparatus. This allowed continuous monitoring of the radioactivity remaining in the animal with time, without removing the animal from the experimental medium.

The apparatus consisted of a large reservoir of two litres capacity and an animal counting chamber. A piece of narrow bore polythene tubing carried the washing medium from the reservoir to the counting chamber which contained the experimental animals. Another polythene tube carried the medium back to the reservoir, thus, forming a continuous circulation system. A stream of air produced by a small aquarium pump was used to drive the circulation. The counting chamber was placed in the well of the scintillation counter. Counts were taken at zero time and at 15 minute intervals. The rate of constant for the sodium efflux was calculated from the equation ..... (3.7)

$$C_t = C_o e^{-Kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.7)$$

where C_o is the initial counts of the whole animal,

C_t is the counts of the whole animal after time t(hr), and

K is the rate constant for loss of sodium from the body which is the fraction of the total sodium lost by all routes per hour.

Multiplication of K by 100 give the percentage of total sodium loss from the prawn per hour. From equation (3.7), K can be derived:

$$\frac{C_t}{C_o} = e^{-Kt} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (3.8)$$

By taking the logarithm of each side this becomes

$$2.303 \log_{10} \frac{C_t}{C_o} = -Kt$$

or $$2.303 \log_{10} \frac{C_o}{C_t} = Kt$$

Therefore $$K = \frac{2.303}{t} \log_{10} \frac{C_o}{C_t} \quad \ldots\ldots\ldots (3.9)$$

When C_t has fallen to half the value of C_o, exchange is half completed and, since $\frac{C_o}{C_t} = 2$

So $$Kt_{\frac{1}{2}} = 2.303 \log_{10} 2$$

$$K = \frac{0.693}{t_{\frac{1}{2}}} \quad \ldots\ldots\ldots\ldots (3.10)$$

17. Statistical treatment of the data.

Values obtained in the present investigations were submitted to appropriate statistical test. The results for any two groups were analysed by "Student's" t-test. Any significant effect due to interactions between the two factors such as temperature and salinity was determined by the analysis of variance. In the present study the following probability for significant differences were assigned:

$P > 0.05$, not significant;

$P < 0.05$, significant;

$P < 0.001$, highly significant.

In the tables,

n = number of observation;

$\bar{X}$ = the arithmetic mean;

SD = the standard deviation.

The vertical line in the figures represent a ±standard deviation of the mean.

18. Notations and terms used in the text.

Several possible ambiguities in terms need to be clarified. The terms in question are here set off by quotation marks, hereafter, difficulties in the connotation of these terms should be resolvable by reference to qualification set out in the present section.

The terms "shrimp" and "prawn" are used throughout the text as common names. There is no universally accepted differences between these terms, and no taxonomic affinities distinguish of the animals under consideration. Prawn, however, usually indicates a species of the family Palaemonidae. Shrimp refers to member of the family Penaeidae, and Crangonidae. Pandalus montagui, the English pink shrimp, is one of several exceptions.

The term "haemolymph" used in this thesis refers to the whole blood taken straight from the animal, and its volume is defined operationally as a tracer dilution volume. "Blood" is used as equivalent to haemolymph, and used interchangeably with the term haemolymph.

"The equilibrium concentration" is the external sodium concentration at which a steady state is reached between the solution and the animals. The term "fully loaded animal" is applied to one which has been allowed to exchange its sodium with a medium containing tracer for a time sufficient to ensure that the specific activity is the same in both animal and medium.

"Loading medium" refers to a solution containing one or more radioactive ions.

The terms "molt" and "ecdysis" are used interchangeably in this thesis. "Time course" of a process signifies that successive measurements of a designated variable were made at intervals during the process. The object of determining time courses was to allow prediction of time to completion of an event in an individual which was sampling during the process.

The term "gradient" is used to indicate the difference in concentration between body fluids and the medium (Riegel, 1959).

The term "acclimation", "acclimatization", and "adaptation" were defined after Fry (1958). Actually there is by no means universal agreement in the literature as to how these terms should be defined. For instance, Fry (1958) makes a distinction between all three terms. He defined acclimation as a short-term non-genetic change in response to a change in a single environmental factor. Acclimatization is a long-term response operating throughout life and possibly influencing future generations through non-genetic means. By adaptation, he referred to change at the phylogenetic level, including mechanisms for the production of direct responses and mechanisms for anticipation of seasonal changes that have been fixed in the heritage of the species. On the other hand, Bullock (1955) did not distinguish between acclimation and acclimatization but preferred the former term. Kinne (1964) considered adaptation as often consisting of a genetic plus a non-genetic (acclimation) component and did not separate acclimation and acclimatization. Other authors, for instance, Grainger (1958) and Bowler (1963)

have defined acclimation, and acclimatization in more specific ways. The term acclimation, as used here, includes any non-genetic adjustment by an organism as a direct response to a change in a single environment factor, salinity or temperature, in the laboratory.

IV. EFFECTS OF TEMPERATURE AND SALINITY ON THE ACCLIMATION OF THE PRAWN, *PALAEMON SERRATUS*

Before studying the ionic behaviour in different salinities and different temperatures, preliminary experiments were necessary to determine the time course of acclimation for the prawn to a change in salinity and temperature. When a prawn in a steady state with its medium is transferred to a new medium, there will be a net gain in ions by the animal if the new medium is more concentrated than the old, and a net loss if it is more dilute. The animal will reach a new steady state when influx of ions from the external medium is balanced by a similar efflux from the body. The period for the attainment of a new steady state will be referred to in this thesis as the acclimation time. In order to assess the time taken for the prawn to adapt to a change in temperatures and salinities the following experiments were undertaken.

1. Influence of temperature on acclimation.

Two sets of experiment were designed to measure the rate of change of the concentrations of sodium in the haemolymph of prawn when temperature is changed. The salinity of the external medium was kept as constant as possible and the temperature varied. Preliminary studies indicated that a rapid and considerable change in temperature is inimicable to the prawns. Experimental temperature changes were, therefore, limited to $10^{o}C$ and changes in temperature were introduced gradually over a period of about 30 minutes.

1.1 A rapid increase of the temperature of the external medium from 15° to 25°C.

After four days acclimation in 100% SW at 15°C, forty prawns were subjected to an increase in temperature from 15° to 25°C. Five animals were subsequently removed from the experimental tank at 3, 6, 12, 24, 48, 72, and 96 hours. Haemolymph samples were taken and the concentration of sodium measured by flame spectrophotometry. The results are given in Table 4.1, and in Figure 4.1. The value at zero time represents the haemolymph sample from animals maintained at 15°C for four days.

The prawns show a rapid decrease in concentration of sodium in the haemolymph from 365 ±7.1 mM/l (n=18) to 341 ±2.5 mM/l (n=5) within 12 hours. After this period the sodium concentration rises again until at 48 hours the level in the haemolymph has stabilized. The differences in the sodium concentration in the haemolymph between those at 48 hours, 350 ±2.6 mM/l (n=5) and those at 96 hours, 352 ±3.3 mM/l (n=5) are not statistically significant. This suggests that in normal sea water when the temperature increases from 15° to 25°C it takes the prawns about 48 hours to reach the new steady state.

1.2 A sudden decrease of temperature of the external medium from 15° to 5°C.

Prawns acclimated in 100% SW at 15°C for seven days were subjected to a decrease in temperature to 5°C. The results are shown in Table 4.1 and in Figure 4.1. The decrease in temperature resulted in an initial increase in the average concentration of sodium in the haemolymph. The increase in mean concentration of

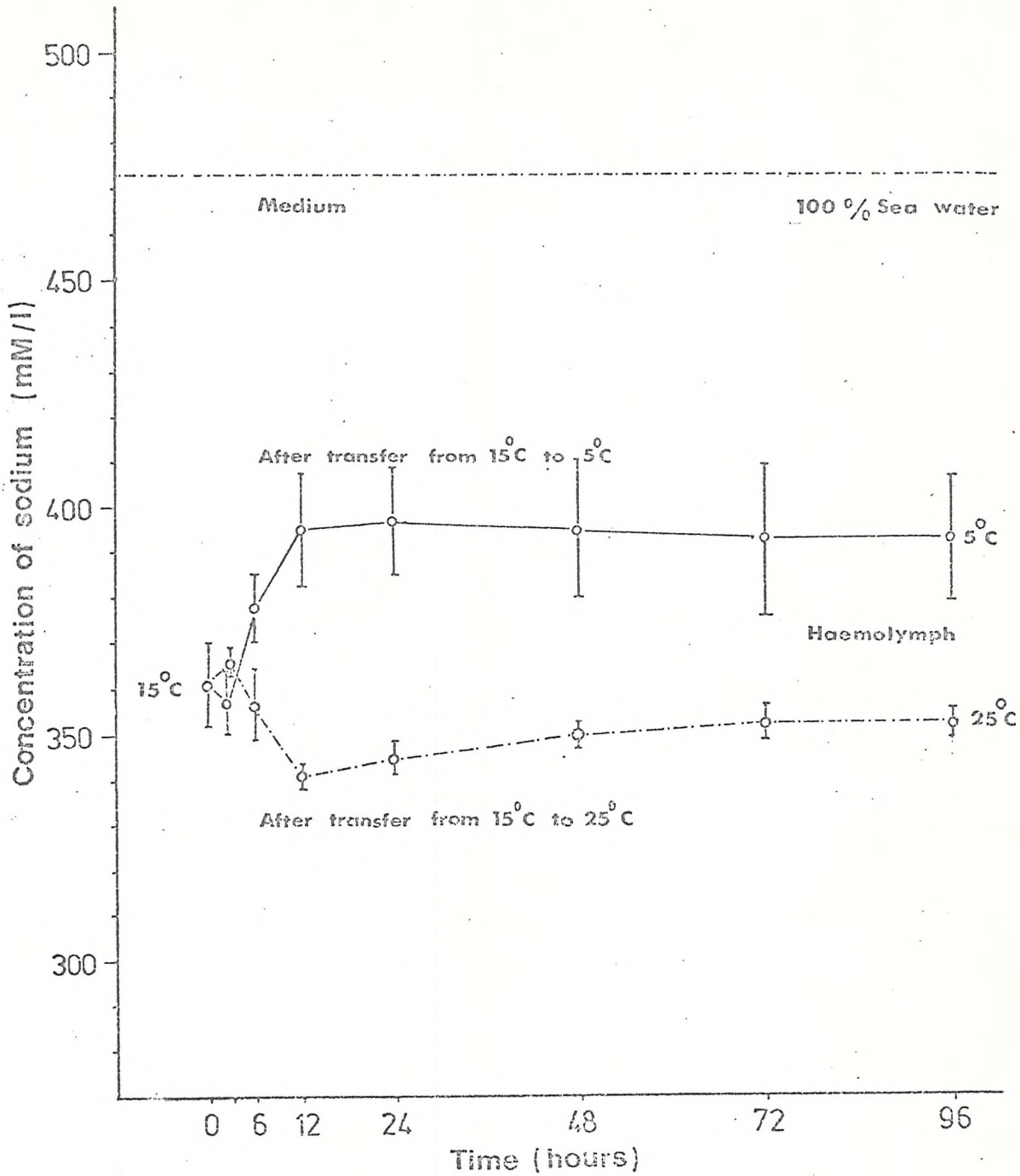

Figure 4.1 The changes in sodium concentration in the haemolymph of prawns, kept in normal sea water after transfer from 15° to 5°C, and from 15° to 25°C.

sodium in the haemolymph was rapid during the first 12 hours, from 365 ±7.1 mM/l (n=18) to 395 ±13.1 mM/l (n=5). This phase was followed by a more gradual decline in concentration from 395 ±13.1 mM/l (n=5) to 393 ±14.1 mM/l (n=5) over the next 12 hours. The differences between the mean sodium concentration in the haemolymph at 12 hours and those at 96 hours are not statistically significant.

2. Adaptation of prawns to a change of external medium salinity.

The time course of adaptation of the haemolymph to changes of external medium salinity at a constant temperature has also been studied in experiments involving either increase or decrease in salinity.

2.1 A rapid transfer from 100% to 50% SW at 15°C.

Forty prawns were acclimated for four days in 100% SW at 15°C, and then were transferred to the test medium, 50% SW. Groups of five prawns each were sampled at 3, 6, 12, 24, 48, 72, and 96 hours after transfer. The results are shown in Table 4.2, and in Figure 4.2. The value at zero time represents the haemolymph samples taken from animals maintained at 15°C in 100% SW for four days.

The prawns show a rapid decrease in concentration of sodium in the haemolymph from 365 ±7.1 mM/l (n=18) to 332 ±7.4 mM/l (n=5) within six hours followed by a period in which the sodium in the haemolymph gradually decreased to the equilibrium level. The decrease of sodium concentration in the haemolymph was slow after six hours and tended towards the steady state after 48 hours.

2.2 A sudden transfer from 100% to 125% SW at 15°C.

This experiment was undertaken in the same manner as that described in experiment 2.1. The results are shown in Table 4.2, and in Figure 4.2. Both in 100% and 125% SW the sodium concentration in the haemolymph of prawns was hypo-ionic to the medium. When the concentration of medium was increased from 100% to 125% SW, the concentration of sodium in the haemolymph rose rapidly from 365 ± 7.1 mM/l (n=8) to 389 ± 16.2 mM/l (n=5). After 12 hours the sodium concentration in the haemolymph decreased again and approached a steady level at around 382 ± 4.0 mM/l (n=3).

3. Discussion.

The temperature as well as the salinity has been shown to influence the rate of acclimation of the prawns. The present data show that it takes between 12 to 48 hours to reach the new steady state, as indicated by steady state haemolymph concentration, after exposure to a changed temperature or salinity. In order to ensure full acclimation of the experimental animals used in the subsequent experiments three days were allowed for acclimation to a new medium or new temperature.

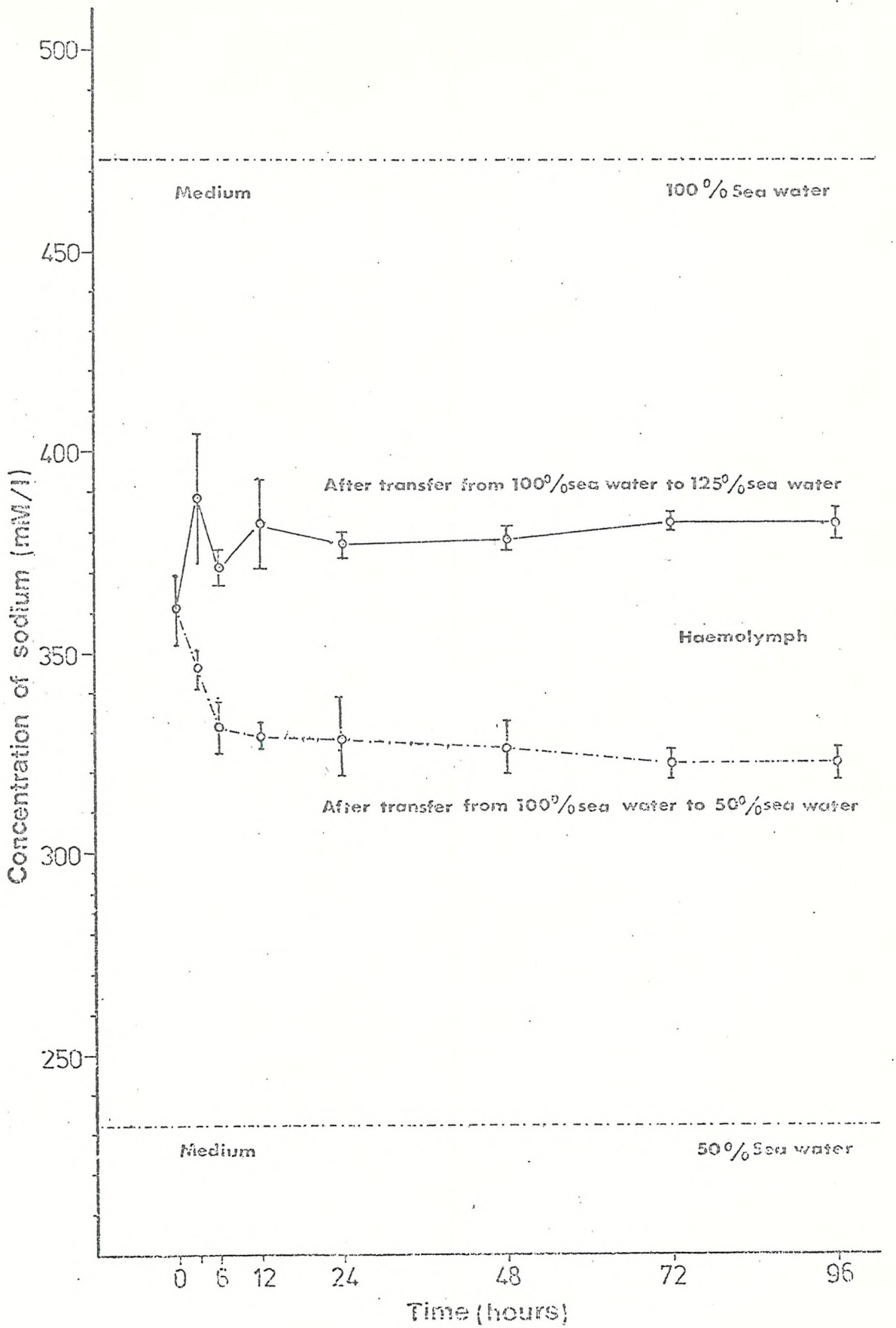

Figure 4.2 Effects of transfer from 100% to 50% SW, and from 100% to 125% SW, at 15°C, on sodium concentration in the haemolymph of prawns.

Table 4.1 Concentrations of sodium of the haemolymph of prawns in 100% SW after transfer from 15° to 5°C and 15° to 25°C

Time (hours)	Concentrations of sodium in haemolymph (mM/l) 15°C to 5°C	15°C to 25°C
0	364.78 ± 7.14 (18)	364.78 ± 7.14 (18)
3	357.00 ± 7.58 (5)	366.40 ± 3.58 (5)
6	378.00 ± 7.58 (5)	356.00 ± 7.87 (5)
12	395.20 ± 13.08 (5)	341.25 ± 2.50 (5)
24	397.00 ± 12.55 (5)	345.75 ± 3.95 (4)
48	395.25 ± 15.61 (4)	350.40 ± 2.61 (5)
72	393.20 ± 17.36 (5)	352.20 ± 5.31 (5)
96	393.20 ± 14.40 (5)	352.40 ± 3.29 (5)

Table 4.2 Concentrations of sodium of the haemolymph of prawns after transfer from 100% to 50% SW, and 100% to 125% SW, at 15°C

Time (hours)	Concentrations of sodium in haemolymph (mM/l) 100% to 50% SW	100% to 125% SW
0	364.78 ± 7.14 (18)	364.78 ± 7.14 (18)
3	346.00 ± 5.48 (5)	388.80 ± 16.21 (5)
6	331.60 ± 7.40 (5)	371.40 ± 4.90 (5)
12	329.00 ± 3.08 (5)	382.00 ± 11.31 (4)
24	328.50 ± 10.14 (4)	376.75 ± 3.50 (4)
48	326.50 ± 7.14 (4)	378.33 ± 2.89 (3)
72	322.40 ± 3.71 (5)	381.76 ± 9.60 (4)
96	322.33 ± 4.13 (6)	382.33 ± 4.04 (3)

V. EFFECTS OF TEMPERATURE ON THE CONCENTRATION OF THE HAEMOLYMPH OF THE PRAWN, *PALAEMON SERRATUS* IN MEDIA OF VARIOUS SALINITIES

The evidence described in the chapter IV led to the conclusion that the ionic behaviour of the prawn is affected by temperature and salinity. In the light of this, an experiment was designed to study the influence of temperature on the concentration of the haemolymph of the prawn in different salinities.

Prawns were acclimated to four levels of salinity, 50%, 75%, 100% and 125% SW. Each series of experiments was repeated at 5^{o}, 10^{o}, 15^{o}, 20^{o}, and 25^{o}C under the same conditions as described in the method section. Prawns were initially acclimated for three days in each salinity, and temperature in order to ensure that they were in a steady state with their medium and temperature.

1. Sodium and chloride concentrations of the haemolymph of prawns kept in normal sea water at different temperatures.

The concentrations of sodium and chloride in the haemolymph of prawns kept in normal sea water at temperatures ranging from 5^{o} to 25^{o}C were measured. The results are shown in Table 5.1. In general, the response of the experimental animals to the test temperatures in normal sea water was similar in respect of both sodium and chloride. The concentration of these ions in the haemolymph was maintained hypoionic to the medium. The degree of hypotonicity depended on the temperature (Figure 5.1).

The sodium concentrations of the haemolymph decreased with increasing temperature, going from 391 $\pm$18.9 mM/l (n=18) at 5^{o}C

to 344 ±9.2 mM/l (n=18) at 25°C. Statistical analysis showed that in normal sea water there were significant differences between the sodium concentrations of the haemolymph of prawns kept at a temperature ranging from 5° to 25°C (Table 5.2). The chloride ion followed the same pattern as those of sodium, decreasing with increasing temperature, from 410 ±8.1 mM/l (n=15) at 5°C to 358 ±5.5 mM/l (n=15) at 25°C (Table 5.1)

2. Sodium concentrations of the haemolymph of prawns immersed in 50% SW at different temperatures.

In 50% SW the sodium concentration of the haemolymph is maintained hyperionic to the medium. It apparently showed an irregular increase with increasing temperature (Figure 5.2) rising from 265 ±10.6 mM/l (n=18) at 5°C to 307 ±2.2 mM/l at 15°C and then decreasing down to 297 ±6.9 mM/l (n=18), and 297 ±8.3 mM/l (n=18) at 20° and 25°C respectively.

3. Sodium concentrations of the haemolymph of prawns kept in 75% SW at different temperatures.

For prawns kept in 75% SW at 5°, 10°, 15°, 20°, and 25°C the sodium concentrations of the haemolymph were 336 ±10.2 mM/l (n=18), 335 ±11.0 mM/l (n=18), 339 ±3.3 mM/l (n=18), 333 ±10.5 mM/l (n=18), and 343 ±8.6 mM/l (n=18) respectively. A trend of increasing sodium concentrations in the haemolymph with increasing temperature was seen, but the differences were not statistically significant (Table 5.4).

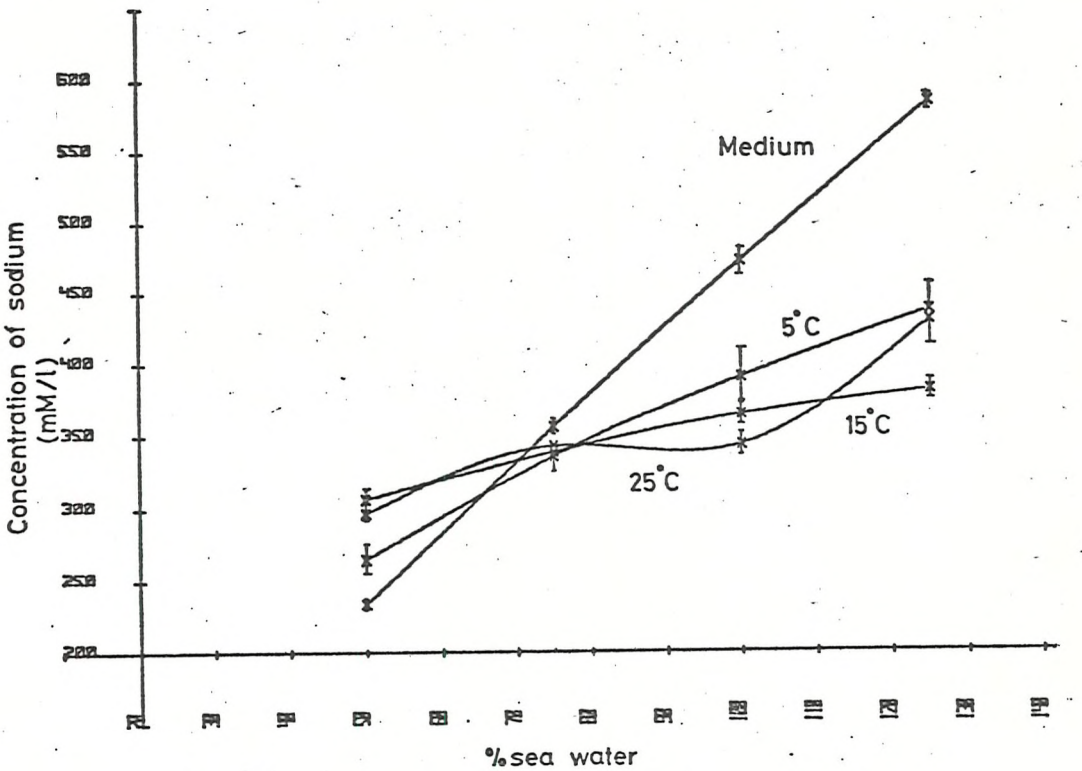

Figure 5.1 Haemolymph sodium concentration of prawns in varying sea water concentrations.

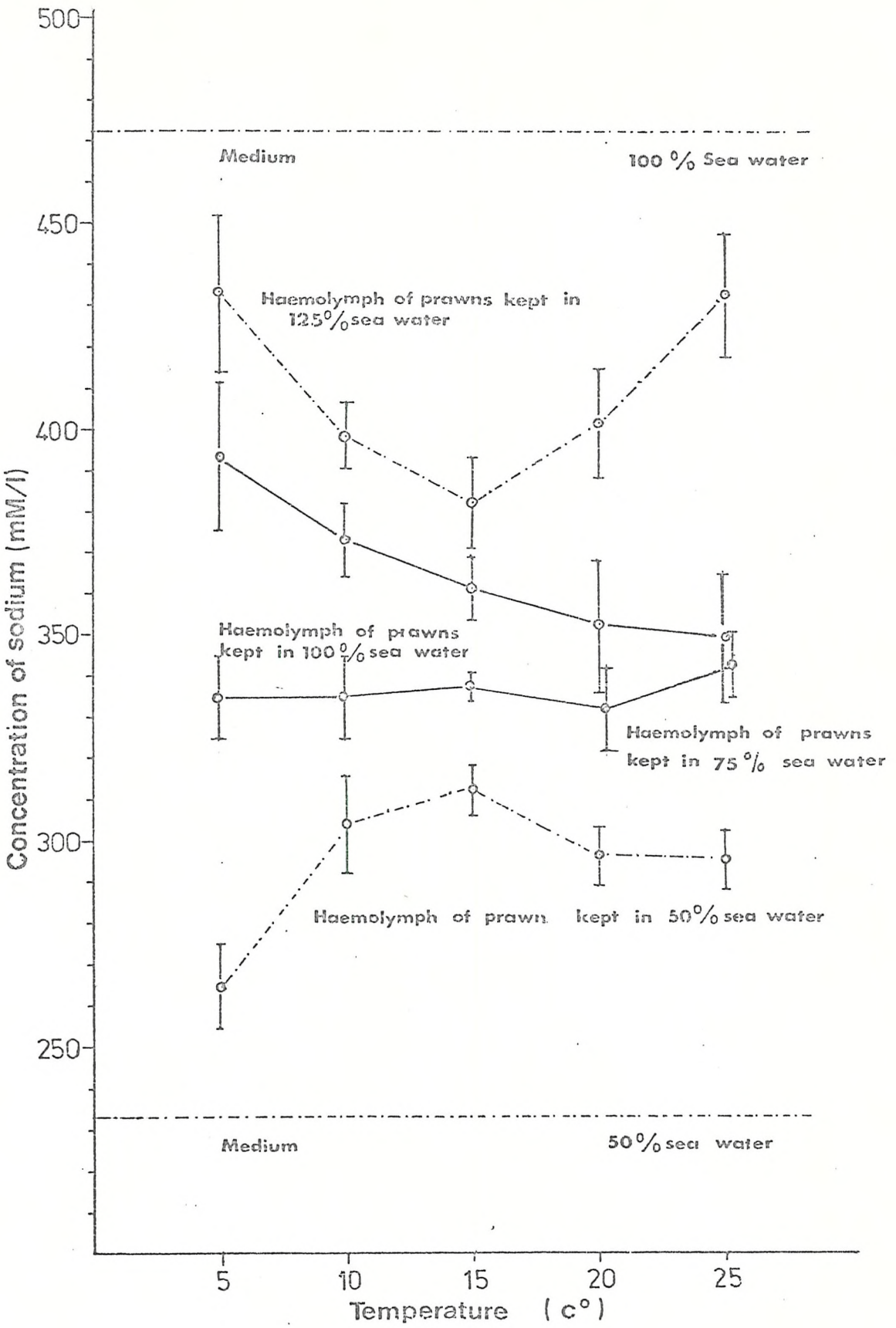

Figure 5.2 The concentrations of sodium in the haemolymph of prawns in four levels of salinity at different temperatures.

4. Sodium concentrations of the haemolymph of prawns kept in concentrated sea water, 125% SW, at different temperatures.

In 125% SW the sodium concentration of the haemolymph was about 438 ± 14.0 mM/l (n=18) at 5°C. There was a decrease in the sodium concentrations with an increase of temperature to a minimum value at about 15°C, 382 ± 8.2 mM/l (n=18), and then elevated to 406 ± 10.2 mM/l (n=18) at 20°C, and to 431 ± 14.8 mM/l (n=18) at 25°C. In other words, the differences between the internal and the external osmotic concentration were greatest at 15°C (Figure 5.2).

5. Discussion.

The results presented in Figures 5.1, 5.2, and in Tables 5.1 - 5.5 suggest that the prawn is an excellent osmoregulator. The animal regulates hyperosmotically in media below approximately 70% SW. Conversely it regulates hypoosmotically in sea water concentrations above 70% SW.

It appears that salinity and temperature interact to alter the ionic balance in the prawn. In 50% SW prawns acclimated to five temperatures, 5°, 10°, 15°, 20° and 25°C, maintained the haemolymph sodium above that of the medium, apparently independently of the temperature. The regulation of sodium ion in the haemolymph becomes less effective at 10°C, and still less at a temperature of 5°C. As shown in Figure 5.2, at low temperature the concentration of the sodium in the haemolymph of the animal in 50% SW decreases towards the medium concentration. It is possible that at low temperature the uptake efficiency of the gills is impaired, and a lesser amount of sodium is taken in to compensate for sodium loss in urine (see further experiments in chapter X, XII).

In dilute media over the normal range of temperature, the uptake of salts is balanced by excretion. If the intensity of the environmental stresses increases, such as cold plus a dilute salinity, the rate of salt uptake cannot balance losses through the extra-renal and renal routes. Therefore, the haemolymph sodium concentration falls until balance is again achieved. In low salinity environments, low temperature appears to be much more of a threat to the osmotic and ionic regulatory mechanisms in the prawn than are higher temperatures in so far as the water content increases and the degree of regulation declines (Figure 5.3). The maintenance of a hyperosmotic state in dilute media is an energy consuming process. The decrease observed at low temperature in haemolymph sodium of prawns kept in dilute medium might, therefore, give some energetic advantage by reducing the energy requirement for osmoregulation though, of course, the reduced gradient may itself represents the inability of the prawn to regulate because of failure of adequate metabolic provision of energy.

Within the range of the test temperatures, the animals show isotonicity to the medium in salinities ranging from 65-70% SW (Figure 5.1). The point of isotonicity varies with temperature. At 5^{o}C the isotonic point is found in a salinity of 65% SW, whilst it is at a salinity of 70% SW at 15^{o} and 25^{o}C.

In salinities above 70% SW the animals exhibit hypoionic regulation of sodium. This capacity is more marked when the prawns are kept in more concentrated sea water. At a salinity of 125% SW, the sodium concentration in the haemolymph was higher at 5^{o}C and 25^{o}C than at 15^{o}C. This suggests that the ability of the prawn

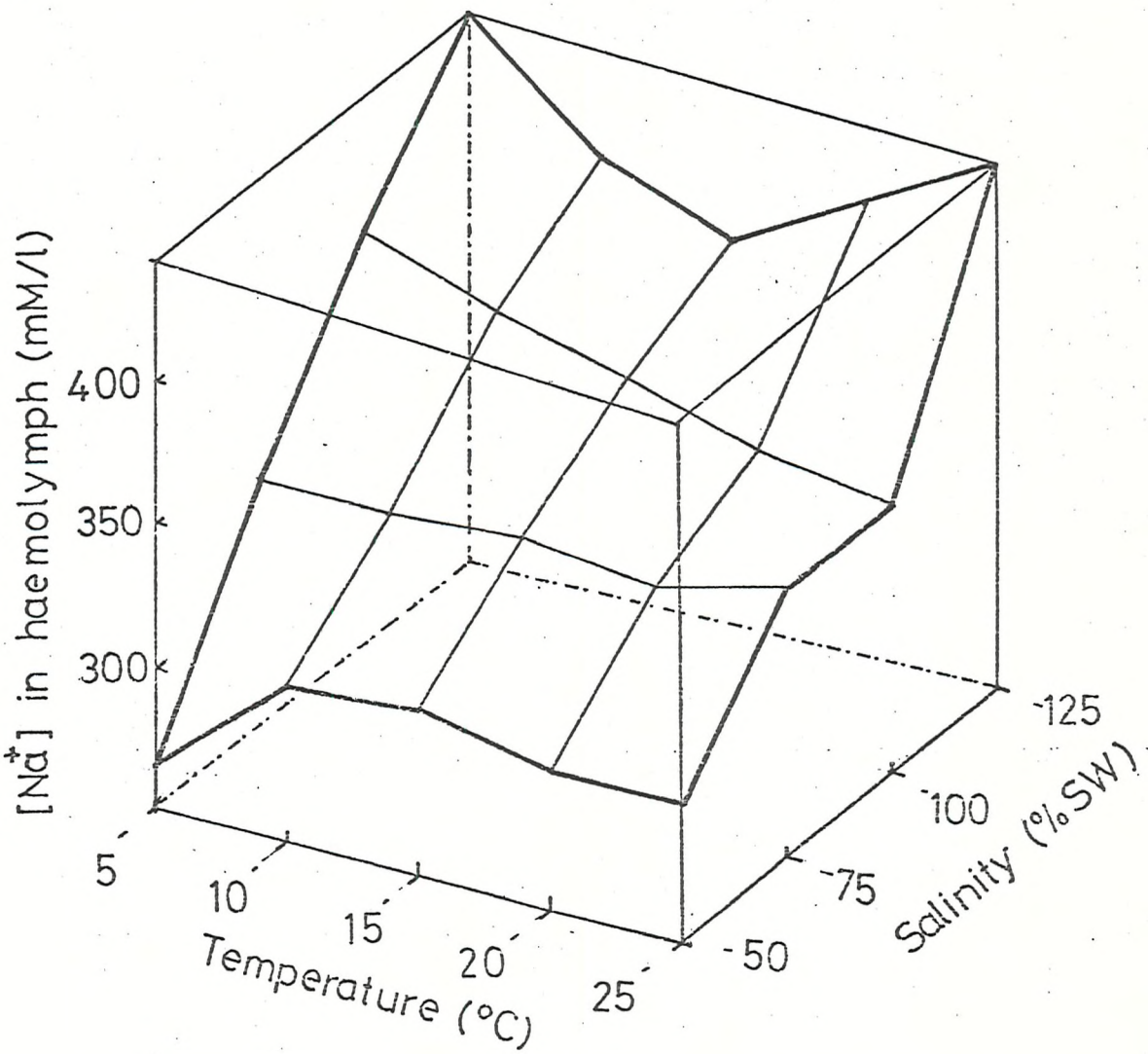

Figure 5.3 The combined effects of temperature and salinity on sodium concentration of prawns.

to maintain a large gradient of hypotonicity declines at high and low temperature.

In normal sea water when the prawns are subjected to low temperature, the sodium concentration in the haemolymph is increased as the temperature gets lower. The results of this experiment agree with the observations of Panikkar (1941) on P. serratus and Palaemonetes varians, with those of Broekema (1942) on Crangon crangon and with those of Todd (1963) on the isopods, Ligia oceania, and Idotea granulosa.

Similarly, animals in dilute medium tend to show a decrease at least initially on exposure to low temperature as Lockwood (1960) showed in Asellus aquaticus. He suggested that the eventual increase in haemolymph sodium ion concentration in A. aquaticus associated with a fall in temperature might be due to the accumulation of metabolites in the cells followed by a shift of water from the haemolymph. Riegel (1959) compared the weights of sphaeromid isopods at temperatures of $5^{o}C$ and $16^{o}C$ in both low and high salinity media and reported a weight gain in the low salinities at the lower temperature. Along with the change in weight which he explained as an upset in matabolism, there was also a shorter survival period at the low temperature.

Verwey (1957) interpreted the temperature effect as an attempt by the animals to maintain a constant osmotic pressure, measured in atmospheres, by increasing osmotic concentration of the body fluids at the lower temperature. His calculations certainly showed a reasonably constant osmotic pressure of the haemolymph of C. crangon based on Broekema's (1942) work, but could not explain

the lower osmotic concentration of the haemolymph at the low temperature in low salinity. The reversal effects of the temperature on C. crangon (Weber and Spaargaren, 1970) and Penaeus aztecus and P. duorarum (Williams, 1960) occurred within the natural range of temperature conditions suggesting that any question of a breakdown of osmoregulation at the low temperature is unlikely. Moreoever, there is experimental evidence suggesting that these changes represent a readjustment of the level of the new steady state of the body fluids regulation rather then osmotic failure.

Only the interdependence of temperature and salinity can explain the effects in the prawns as observed in the present study. The increase of haemolymph sodium of prawns kept in normal sea water at low temperature could be due to either an increase in integumental sodium permeability or a decrease the net rate of extrusion of sodium. The first hypothesis is unlikely to be responsible for the increase in sodium concentrations of the haemolymph, since low temperature tends to cause a decrease in effective cell membrane permeability rather than increase. This, therefore, leaves the second hypothesis, a decrease in the net rate of excretion of the sodium ion, as the possible cause.

It can be seen in Table 7.6 of chapter VII that the drinking rate is reduced to about half that of the control while the sodium efflux (experiment 2 of chapter XI) is only one third of the rate at 15^{o}C. Furthermore, the decrease in urine flow at low temperature as shown in experiment 12 of chapter X also suggested that the urinary sodium loss rate is reduced. The increased sodium concentration of the haemolymph also decreases the osmotic gradient thus reducing water outflux. This, in turn, minimises the osmotic work for the prawn in such circumstance.

Table 5.1 Concentrations of chloride of the haemolymph of prawns kept in 100% SW at different temperatures.

Treatment	$[Cl^-]$ mM/l	group	Analysis of t-test df	t-value	probability
A, 5°C	409.87 ± 8.12 ($\bar{15}$)	A,B	28	9.1267	$P < 0.001$
B,10°C	385.07 ± 6.69 ($\bar{15}$)	B,C	28	7.2164	$P < 0.001$
C,15°C	365.87 ± 7.84 ($\bar{15}$)	C,D	28	2.9882	$P < 0.01$
D,20°C	358.73 ± 4.91 ($\bar{15}$)	D,E	28	0.5947	NS
E,25°C	357.60 ± 5.51 ($\bar{15}$)	E,A	28	20.6196	$P < 0.001$

Table 5.2 Concentrations of sodium of the haemolymph of prawns kept in 100% SW at different temperatures.

Treatment	$[Na^+]$ mM/l	group	Analysis of t-test df	t-value	probability
A, 5°C	391.39 ± 18.94 ($\bar{18}$)	A,B	34	3.2096	$P < 0.005$
B,10°C	375.17 ± 10.06 ($\bar{18}$)	B,C	34	3.5726	$P < 0.005$
C,15°C	364.78 ± 7.14 ($\bar{18}$)	C,D	34	4.2112	$P < 0.001$
D,20°C	351.44 ± 11.38 ($\bar{18}$)	D,E	34	2.2040	$P < 0.05$
E,25°C	343.83 ± 9.23 ($\bar{18}$)	E,A	34	9.5773	$P < 0.001$

Table 5.3 Concentrations of sodium of the haemolymph of prawns kept in 50% SW at different temperatures.

Treatment	$[Na^+]$ mM/l	group	Analysis of t-test df	t-value	probability
A, 5°C	264.56 ± 10.55 ($\bar{18}$)	A,B	34	10.6770	P< 0.001
B,10°C	303.33 ± 11.24 ($\bar{18}$)	B,C	34	1.3791	NS
C,15°C	307.06 ± 2.21 ($\bar{18}$)	C,D	34	5.9928	P< 0.001
D,20°C	296.78 ± 6.93 ($\bar{18}$)	D,E	34	0.1092	NS
E,25°C	296.50 ± 8.25 ($\bar{18}$)	E,A	34	10.1202	P< 0.001

Table 5.4 Concentrations of sodium of the haemolymph of prawns kept in 75% SW at different temperatures.

Treatment	$[Na^+]$ mM/l	group	Analysis of t-test df	t-value	probability
A, 5°C	335.50 ± 10.17 ($\bar{18}$)	A,B	34	0.1101	NS
B,10°C	335.11 ± 10.99 ($\bar{18}$)	B,C	34	1.2741	NS
C,15°C	338.56 ± 3.28 ($\bar{18}$)	C,D	34	2.3424	P< 0.05
D,20°C	332.50 ± 10.47 ($\bar{18}$)	D,E	34	3.3624	P< 0.005
E,25°C	343.22 ± 8.57 ($\bar{18}$)	E,A	34	2.4632	P< 0.05

Table 5.5 Concentrations of sodium of the haemolymph of prawns kept in 125% SW at different temperatures.

Treatment	$[Na^+]$ mM/l	group	Analysis of t-test df	t-value	probability
A, 5°C	438.44 ± 14.43 ($\bar{18}$)	A,B	34	11.1291	P< 0.001
B,10°C	399.28 ± 3.83 ($\bar{18}$)	B,C	34	7.8981	P< 0.001
C,15°C	382.44 ± 8.19 ($\bar{18}$)	C,D	34	7.5116	P< 0.001
D,20°C	405.61 ± 10.20 ($\bar{18}$)	D,E	34	6.0104	P< 0.001
E,25°C	431.11 ± 14.83 ($\bar{18}$)	E,A	34	1.5036	NS

VI. WATER CONTENT OF THE PRAWN, *PALAEMON SERRATUS*, ACCLIMATED AT DIFFERENT TEMPERATURES AND IN DIFFERENT SALINITIES

It has been shown in chapter V that prawns regulate their haemolymph hypertonic to the medium in dilute media, and hypotonic in normal sea water as well as in concentrated sea water. In dilute media, water tends to enter the body along the osmotic gradient, conversely the animals tend to lose water in hypertonic media. Thus changes in the internal water content may occur which influence osmotic concentration of the haemolymph. On the other hand the prawn possesses a body surface with low permeability to water and salts which is capable of limiting water gain in a hypotonic medium to some extent. There is also the possibility that the antennal glands might play an important role in regulation of water content of the prawn either in hypotonic or hypertonic media.

The above considerations led to the investigation of whether any effective regulation of the water content of prawns occurs in the temperature range from 5^{o} to 25^{o}C when the animals are kept in salinity range from 50% to 125% SW. In order to study the possible combined effect of temperature and salinity on the water content of the animals, a basic factorial experimental design was used. The design was a 3 x 3 factorial in which three levels of temperature and salinity were studied, making a total of nine different combinations of the experimental conditions. Groups of 18 prawns were used in the analysis. The total water content was determined by drying the animal at 70^{o}C to a constant weight after

obtaining the wet weight. The water content was calculated as the difference between the wet weight and dry weight of the prawn.

1. Total water content of prawns in relation to size.

The water content of prawns ranging in size from 0.27 to 2.90 g was determined. Figures 6.1, 6.2, and 6.3 represent the water content of animals in 50%, 100%, and 125% SW at 5^{o}, 15^{o}, and 25^{o}C respectively. A straight line was fitted to the data by the method of least squares. There was no evidence that size had any effect on the water content. This is clearly apparent in the Figures 6.1, 6.2, and 6.3, showing the positive linear relationship that exists between the wet weight of the prawns and their body water content. The linear regression relating the water content (W) to size (S) in prawns acclimated at different temperatures and in different experimental salinities is summarized in Table 6.1. The correlation coefficient (r) of the regression for the values shown in Table 6.1 are close to one, the water content can, therefore, be considered constant irrespective of size of the animal.

2. Water content of prawns in relation to salinity and temperature.

2.1 Salinity effect.

The total water content was studied in a salinity range from 50% to 125% SW at three temperatures, 5^{o}, 15^{o}, and 25^{o}C. The results are given in Table 6.2. At 5^{o}C, the difference in the body content of prawns acclimated in 50% and 125% SW as well as the animals kept in 100% and 125% SW were statistically significant ($t = 7.5841$, $P < 0.001$; and $t = 4.6440$, $P < 0.001$ respectively; see Appendix 3), but the difference between the water content of

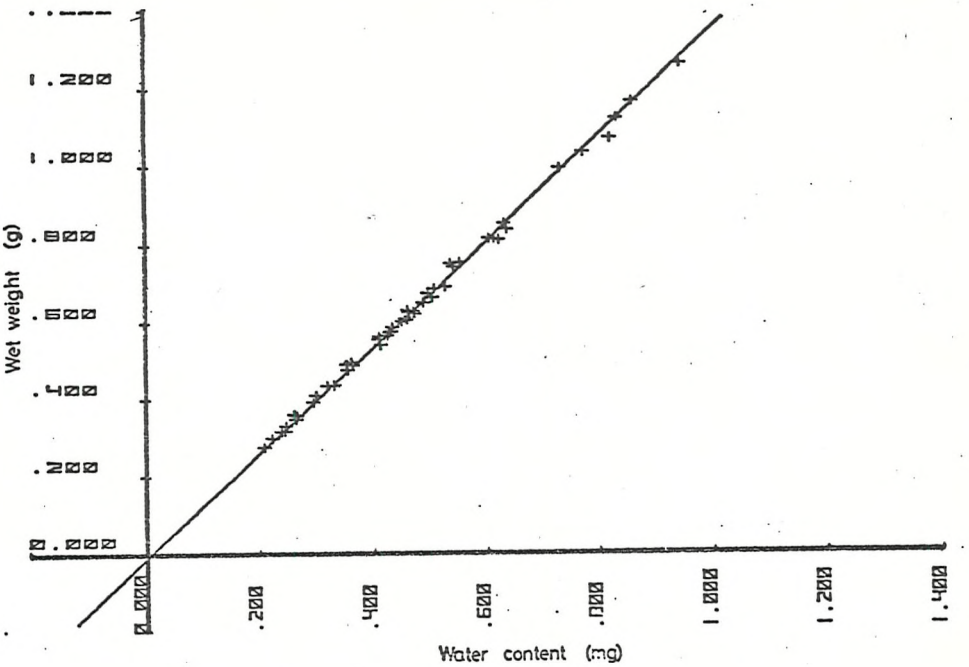

Figure 6.1 The relationship between water content and body weight of prawns kept in 100% SW at 5°, 15°, and 25°C.

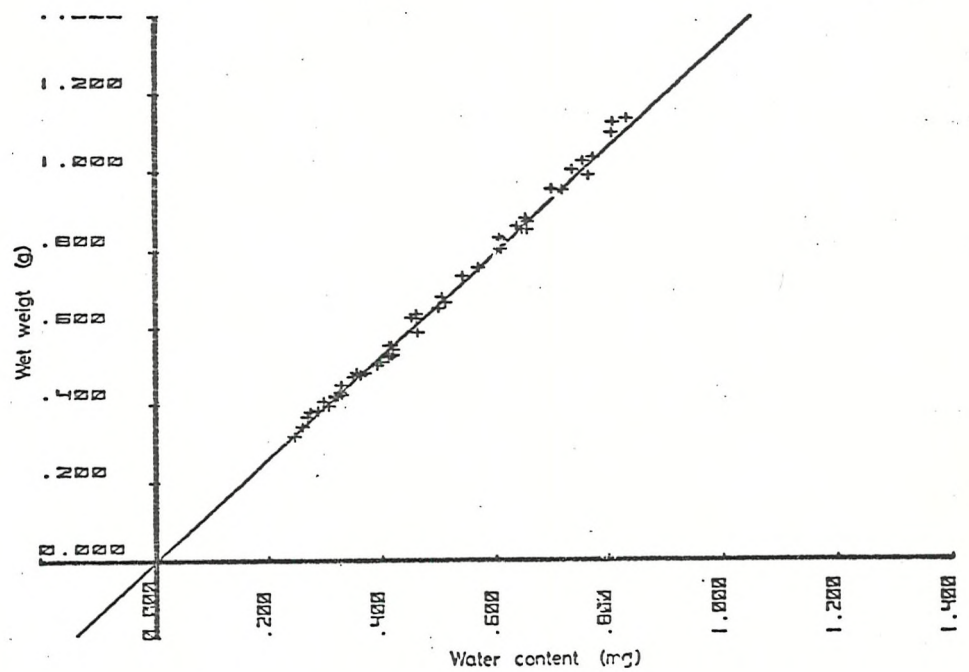

Figure 6.2 The relationship between water content and body weight of prawns kept in 50% SW at 5°, 15°, and 25°C.

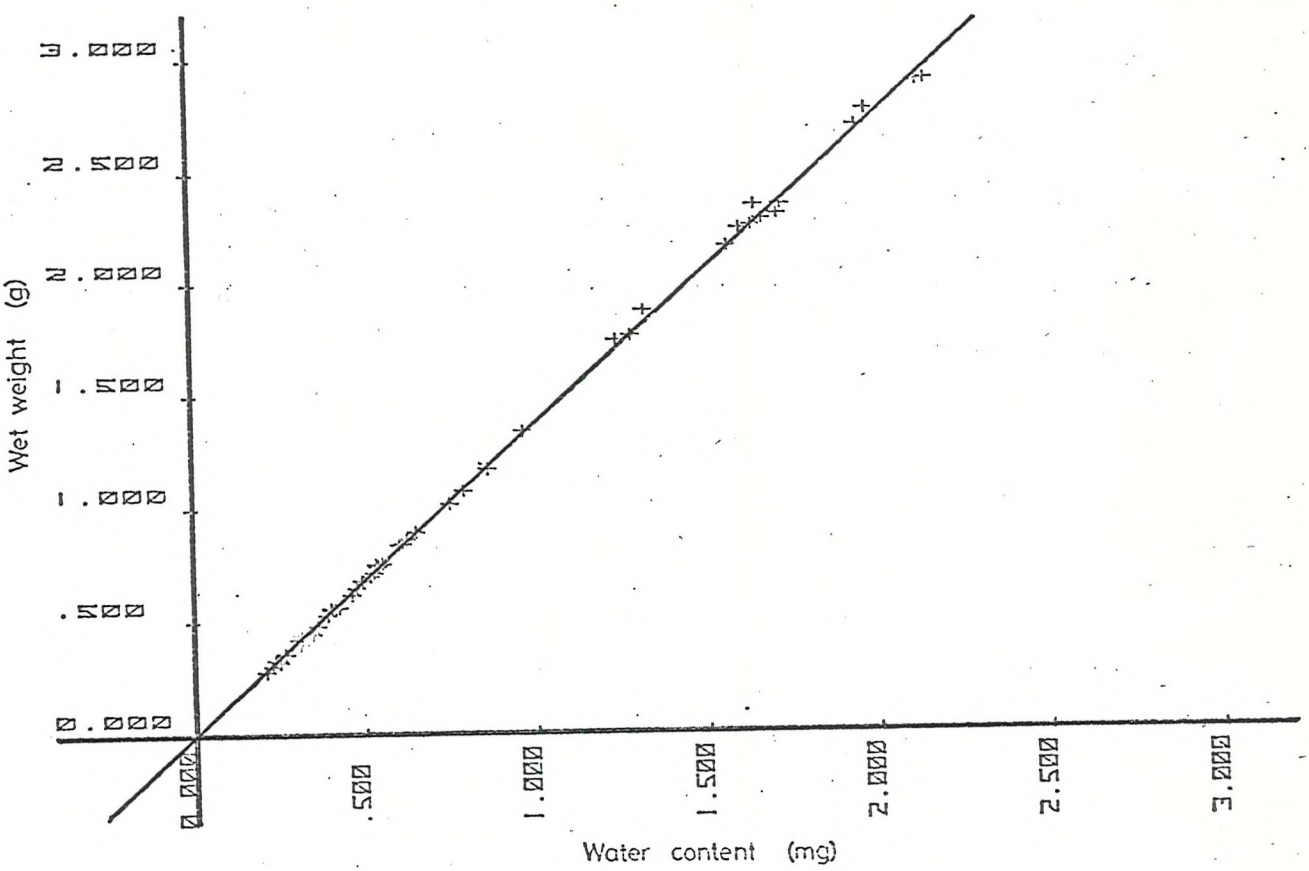

Figure 6.3 The relationship between water content and body weight of prawns kept in 125% SW at 5^{o}, 15^{o}, and 25^{o}C.

Table 6.1 Linear regression equations of water content of prawns acclimated at different temperatures and salinities.

Salinity (% SW)	Temperature (^{o}C)	Equation	r
50	5	W = 0.7421 S + 0.0095	0.9985
	15	W = 0.7163 S + 0.0174	0.9939
	25	W = 0.7230 S + 0.0147	0.9984
100	5	W = 0.7308 S + 0.0063	0.9962
	15	W = 0.7468 S - 0.0019	0.9987
	25	W = 0.7132 S + 0.0239	0.9985
125	5	W = 0.7178 S + 0.0122	0.9985
	15	W = 0.7109 S + 0.0173	0.9937
	25	W = 0.7352 S + 0.0003	0.9985

Table 6.2 Water content of prawns acclimated to different temperatures, and salinities. Data expressed as the percentage of water content of the whole animal per wet weight.

Salinity (% SW)	Temperature (^{o}C) 5	15	25
50	75.70 ± 1.20 (18)	75.34 ± 2.24 (18)	74.71 ± 2.30 (18)
100	74.84 ± 1.59 (18)	74.56 ± 1.42 (18)	74.36 ± 0.74 (18)
125	72.65 ± 1.21 (18)	74.97 ± 2.10 (18)	73.23 ± 1.12 (18)

prawns acclimated in 50% and 100% SW was not significant. This means that when the prawns are subjected to a change of salinity from 50% to 125% SW or vice versa, the change in water content is significant ($F = 24.4963$; $P < 0.01$; Appendix 4).

At 15°C, the total body water content of prawns over the range from 50% to 125% SW was relatively constant. An analysis of variance (single classification with temperature held constant) showed that the differences in water content in each salinity were not significant (Appendix 4). This suggests an effective regulation of the amount of water in the prawns at this temperature over the range of salinity from 50% to 125% SW. When the salinity decreases to 10% SW the water content of the prawns increases to 79 ± 1.1% body wt ($n=18$). This increase is statistically significant ($t = 9.5840$, $P < 0.001$).

Table 6.3 Effect of low salinity on water content of prawns at 15°C.

Treatment	Weight range (g)	Water content (% body weight)	Analysis of t-test df	t-value	probability
100% SW	0.06 ± 1.07	75 ± 1.4 ($\bar{18}$)	34	9.5840	$P < 0.001$
10% SW	0.20 ± 0.44	79 ± 1.1 ($\bar{18}$)			

At 25°C, the differences in water content of prawns acclimated to low salinity, 50% SW, and to high salinity, 125% SW, as well as the animals kept in 100%, and 125% SW were significant ($t = 2.4390$, $P < 0.05$; and $t = 3.5617$, $P < 0.001$ respectively). However the water content of prawns acclimated in 50% and 100% SW was not significantly different.

2.2 Temperature effect.

In dilute medium, 50% SW, as well as in normal sea water, the animals showed a trend toward an increase in water content at a low temperature. As showed in Appendix 5, the body water content of the prawns at the three temperatures, 5^{o}, 15^{o}, and 25^{o}C were only slightly different. No significance was found.

In concentrated medium, 125% SW, the water content of prawns acclimated at 5^{o} and 25^{o}C was decreased when compared with the animals kept at 15^{o}C. Differences significant at the 1% level ($F = 10.9191$) or better existed between the prawns kept over the range of temperature from 5^{o} to 25^{o}C.

2.3 Temperature - salinity effect.

In the hope of gaining some understanding concerning the possible interactions between the temperature and salinity, the variance of water content of prawns acclimated to different temperatures and different salinities was analysed. The results are shown in Table 6.2.

Temperature as well as salinity influenced the water content, especially at the lower and the upper range of the limit of osmoregulatory capacity. The combination effects of these two parameters was shown by the significance of the temperature - salinity interaction ($F = 4.0200$, $P < 0.01$). This evidence suggests that the temperature and salinity are interdependent in exerting an influence on the water content of the whole animal.

3. Discussion.

The water content of prawns kept in normal sea water at temperature ranging from 5^{o} to $25^{o}C$ is fairly uniform. This suggests that within the osmoregulation range the prawns possess a mechanism to regulate their water content effectively. When the animals are exposed to concentrated sea water, they show signs of body dehydration especially when they are exposed to extremes of temperature. In contrast, the animals show some tendency to increase water content when they are exposed to very dilute media. However, for animals in 50% SW the increase is not large enough to be significantly different when compared with those kept in normal sea water. This suggests that within the range of temperature 5^{o} to $25^{o}C$ the prawns are still able to regulate their body fluid effectively when they are exposed to 50% SW.

VII. WATER DRINKING

The study of the ability to regulate the body fluids hypo-osmotically to sea water by some marine organisms dates back to 1930 when Smith proposed that teleosts continually drink sea water, absorb monovalent ions and water through the gut wall and simultaneously excrete excess ions extra-renally, mainly through the gills, in order to maintain a constant haemolymph concentration lower than that of the external sea water. Later several workers (Keys, 1933; Mullins, 1950; House, 1963) have performed *in vivo* ion transport experiments which confirmed Smith's view.

It is still unclear to what extent the conclusions drawn from the investigation of the teleosts can be extended to crustaceans like the prawn, *P. serratus* which are also hypoosmotic to sea water. Fox (1952) showed that both anal and oral drinking of the medium occurs in a number of crustaceans. Work on oral drinking in the isopods, *Oniscus asellus*, *Porcellio scaber*, and *Armadillidium vulgare* has been reviewed by Lockwood (1962). Edney and Spencer (1955) demonstrated, by using eosin marked media, that *Ligia oceania* takes up water primarily through the anus. For *P. serratus*, Panikkar (1941), using vital red and bromo-cresol green, observed water drinking occuring in both hyper- and hypo-tonic conditions but failed to quantify the actual drinking rates over a range of salinity. In an effort to extend the physiological study of osmoregulatory mechanisms of the prawn, the water drinking and reverse peristalsis of the prawn over a range of salinity were observed.

1. Staining properties of prawns and motility of the gut.

To detect possible sites of salts and water absorption prawns were placed in saturated Amaranth solution (0.4 g/100 ml) in the appropriate test salinity. After one hour in the medium at 15°C they were examined under a dissecting microscope. Three salinities, 50%, 70%, and 100% SW were selected. In each salinity, 20 prawns were examined. The number of gulps of water intake per minute were recorded. The counts for each prawn, were made over a five minute period.

In all three experimental media oral drinking always took place, but the dye entered the gut more rapidly in 100% SW. In normal sea water, the hepatopancreas, the foregut and the antennal glands were bright red within an hour. However, the dye seldom reaches the hind gut or even the posterior half of the mid gut. Quantitative estimation of drinking rates will be discussed in section 2.3 of this chapter.

In the examination for reverse peristalsis in the hind gut only conspicuous examples were construed as positive. Cardiac agitation of the gut and stationary contractions were disregarded. The anal intake of water by rhythmic antiperistaltic movements of the rectum was clearly seen. In some individuals it went on for long periods with few interruption while in others it occured only in occasional bursts of rhythmic rectal contractions. In some cases the anal swallowing movements were interrupted at intervals by pseudo-defaecation of water, whether the gut was full or empty of food. For fed prawns defaecation was frequently observed. When defaecation occured, the faeces were pushed quickly

down the intestine and extruded through the anus. In some prawns the swallowing movements stopped for a few moments after each defaecation, in other cases their rate doubled just before defaecation and slowed just afterwards.

Fox (1952) described the functional process of reverse peristalsis in the hind gut of a variety of small crustaceans. After discounting the hypothesis that the function is respiratory, he proposed that reverse peristalsis is a kind of natural enema.

In the present study the observation of reverse peristalsis in fasting animals with absolutely empty hind guts seem to indicate that the anal intake of water has a second independent function additional to that of enema. The evidence in Table 7.1 suggests the possibility of a difference in response in some ways dependent upon the osmotic environment (see more discussion in section 2.5 of this chapter).

2. Water drinking.

The rate of water drinking of prawns can be quantified by dissolving a compound in the surrounding water and measuring its accumulation in gut contents of the animal over a given time. The compound must, however, be non-toxic, water soluble or able to form a colloidal suspension and of a molecular size too large to pass through the gut wall or gill membrane. It should also not be digestible and preferably not associated with any of the ions in the medium. In early studies phenol red was utilized to determine the drinking rate of marine teleosts by Smith (1930), and Croghan (1958b) with A. salina, while Panikkar (1941) has

Table 7.1 Reverse peristalsis in the hind gut of prawns kept in different salinities at 15°C.

Group	Acclimation medium (% SW)	n	Gulps per minute fed prawn	Gulps per minute fasted prawn
A	100	20	14.6 ± 4.1	11.8 ± 3.7
B	70	20	8.2 ± 2.5	7.3 ± 2.4
C	50	20	9.5 ± 3.2	8.1 ± 2.3

Analysis of t-test

Source of variation	df	t-value	probability
Fed prawn			
A,B	38	5.9380	P< 0.001
B,C	38	1.4047	NS
C,A	38	4.4043	P< 0.001
Fasted prawn			
A,B	38	4.6149	P< 0.001
B,C	38	1.0110	NS
C,A	38	3.8848	P< 0.001

employed vital red, and bromo-cresol green with P. serratus. These water soluble technical dyes are of limited use unless reasonable quantities can be recovered from the gut. Use of this principle, but utilizing a colloid labelled with radio-isotope, permits accurate measurements to be made in small animals. Among the suitable compounds are inulin C-14 (Evans, 1967; Potts and Evans, 1967), sulphate-35 (Potts et al., 1967), polyvinylpyrrolidone I-125 (Evans, 1968), silver chloride colloid, Ag-110 (Dall, 1967), and polyethylene glycol (Shehadeh and Gordon, 1969).

In the present study quantitative estimation was carried out using Cr-51 EDTA to ascertain whether drinking formed a significant part of water intake. The experiment was designed to determine the drinking rates in the absence of food and thus the reported rates are considered to be independent of feeding activity.

2.1 Time course of drinking rates in 100% SW.

This experiment was designed to determine time course of drinking rates of prawns in 100% SW. Figure 7.1 gives the general evolution of this parameter as measured over 24 hours period. It is likely that within 24 hours the drinking rate of the prawns was approximately linear with time. However to make sure that no appreciable amount of Cr-51 EDTA is extruded through the anus, the drinking rates of prawns were only measured within two hours after immersion in the experimental medium when results were being obtained to relate to water losses from the animals.

For comparison of oral and anal drinking, it was necessary to extend the period of test, and the anus of the animals was sealed with dental cement. The results obtained could also be used

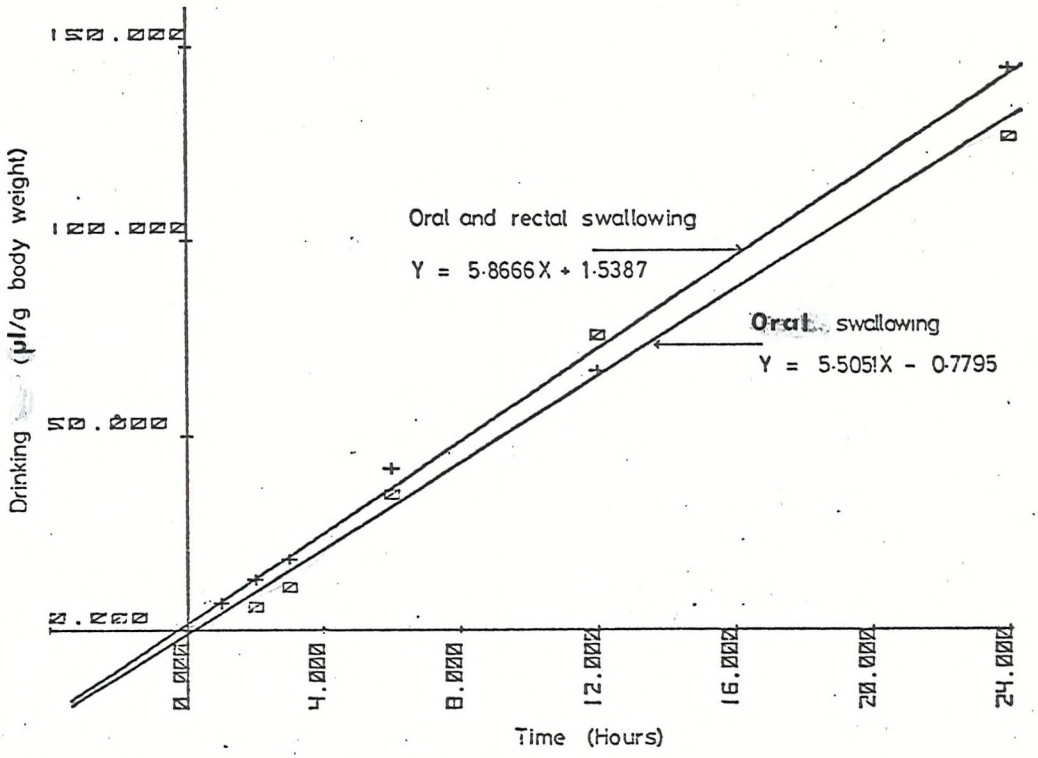

Figure 7.1 Time course of drinking rate of prawns kept in 100% SW at 15°C.

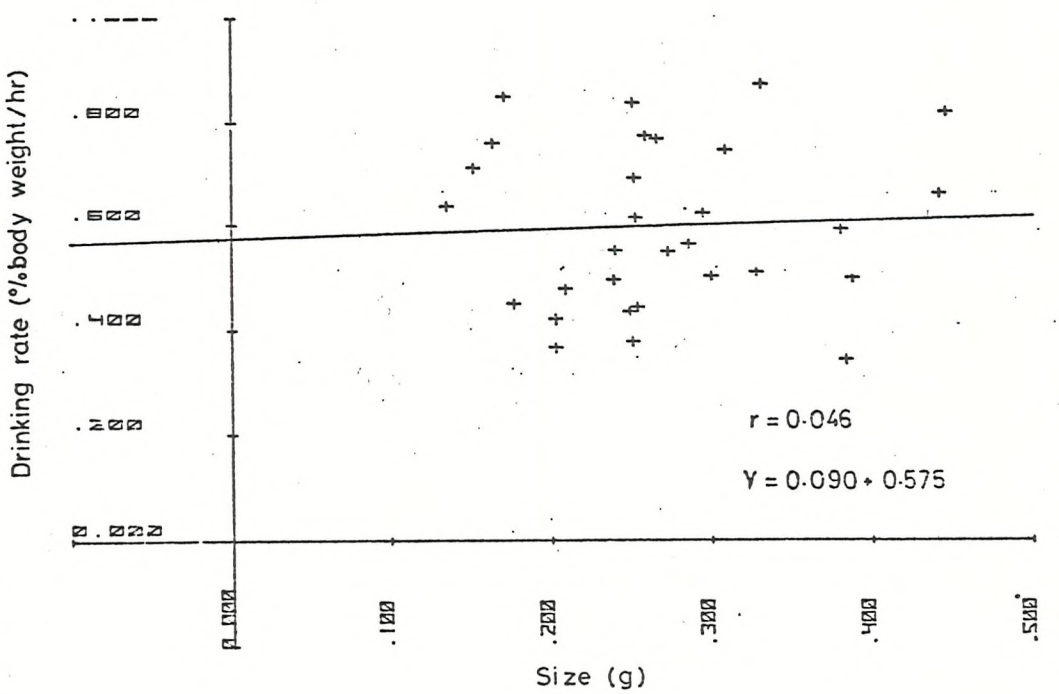

Figure 7.2 The relationship between the drinking rate and body size of prawns kept in 100% SW at 15°C.

for a comparison time course of drinking rate with the normal prawns as well as providing additional information on the function of the anus.

The results are given in Figure 7.1. It is clear that the volume of water swallowed by the anus-ligatured prawns increases with time. The low drinking rate during the first three hour are possibly due to stress from the operative treatment. After three hours the volume of water swallowed increases with time and is approximately linear.

2.2 Drinking rate and body size.

Ralph (1965) showed for Neomysis integer that there was a relationship between body size and drinking rate. However, Harris (1967) found no such relationship for Sphaeroma rugicauda. In order to determine the effect of body size, if any, the drinking rate of prawns ranging from 0.13 g to 0.45 g was determined. The relationship between drinking rate and body size can be expressed by the linear regression equation shown in Figure 7.2. It is apparent that size has little effect, especially within the range of animals used in the experiment ($t = 0.0901$, $df = 28$, the correlation is not statistically significant).

2.3 Drinking rate of prawns under steady state conditions at 15°C in different salinities.

As mentioned in section 1, the prawns drink the water in all three experimental media, 50%, 70%, and 100% SW. Water drinking is generally continuous, and not necessarily related to feeding activity, but the rate of drinking was significantly reduced with

Table 7.2 Time course of drinking rate of prawns after transfer to 100% SW with an addition of Cr-51 EDTA. The experimental prawns have the anus sealed.

Hours after transfer	control fluid intake (ul/g body weight)	experimental prawn fluid intake (ul/g body weight)
1	7.1 ± 3.2 (10)	-
2	13.1 ± 3.3 (10)	6.2 ± 3.4 (5)
3	18.5 ± 4.3 (10)	11.4 ± 2.6 (5)
6	41.8 ± 16.7 (10)	35.4 ± 5.0 (5)
12	66.4 ± 28.0 (6)	75.7 ± 24.9 (5)
24	144.0 ± 41.1 (10)	126.3 ± 22.7 (5)

Table 7.3 Drinking rates of prawns, under steady state, kept in different salinities at 15°C.

Group	Acclimation medium (% SW)	n	Weight range (g)	Drinking rate (% body wt/hr)
A	100	12	0.13 - 0.33	0.69 ± 0.13
B	70	12	0.21 - 0.41	0.42 ± 0.08
C	50	12	0.13 - 0.32	0.26 ± 0.07

Analysis of t-test

Source of variation	t-value	probability
A,B	6.1252	$P < 0.001$
B,C	5.2300	$P < 0.001$
C,A	9.9011	$P < 0.001$

decrease in external salinity (Table 7.3). In normal life, of course, the animal might in addition be expected to take a certain amount of preformed water in the food.

Drinking rate of the prawns kept in 100% SW was about 0.69% body wt/hr, and this value agrees with those reported by Dall (1967) on M. bennette, 0.6-0.7% body wt/hr. Drinking rate of P. varians in 100% SW is more than treble, 1.90% body wt/hr (Ralph, 1965), and 2.5% body wt/hr (Potts and Parry, 1964b), the observed drinking rate in P. serratus. This would be expected both because the former is an estuarine species with a higher degree of hypotonicity than that shown by P. serratus and because of its smaller body size when adult.

It is probable that in 100% SW the animals compensate for the water lost to the environment in the urine and by osmosis across the gills by drinking and that the water is absorbed across the gut epithelium in association with the active transport of sodium. The possibility of solute linked water flow playing a role in this uptake from the gut will be discussed further in chapter X.

2.4 Effect of sudden transfer from 100% SW to dilute media on drinking rates.

If the animals continued to drink at the rate normal to individuals in a steady state after they had been transferred to a more dilute medium, it is probable that this would result in an increase in osmotic load. It was therefore considered that the prawns might adapt to osmotic changes by restricting their drinking activity during the period of re-acclimatization following a sudden change in the osmolarity of the medium in order to reduce the immediate water load.

Experiments were designed to determine

(a) whether the prawn modified its drinking rate following a sudden change in the external salinity, and

(b) the time course of such changes.

Figure 7.3 gives the general evolution of the drinking rates of prawns subjected to the sudden change of salinity as measured over a period of 24 hours. The drinking rates of prawns subjected to sudden transfer from 100% to 70% SW decreased significantly during the first hour, after transfer and then gradually decrease towards the equilibrium at 12 hours. For the prawns subjected to sudden transfer from 100% to 50% SW the drinking rate is reduced to the same level as those in 70% SW during the first hour after transfer and then there is a gradual reduction to a steady value of 0.26% body wt/hr. In both groups, the differences in drinking rates between the initial and one hour following an abrupt change in the external salinity were statistically different. This suggests that the prawns reduced their drinking rate rapidly as they were subjected to the sudden change to the low external salinity. However comparison with the data in Table 7.3 that the drinking rate in animals fully acclimatised to 50% and 70% SW is in fact less than that in the first few hours after transfer from 100% to either 70% or 50% SW. This is the opposite to the situation which might be expected if the animals are directly relating drinking rate to body water load.

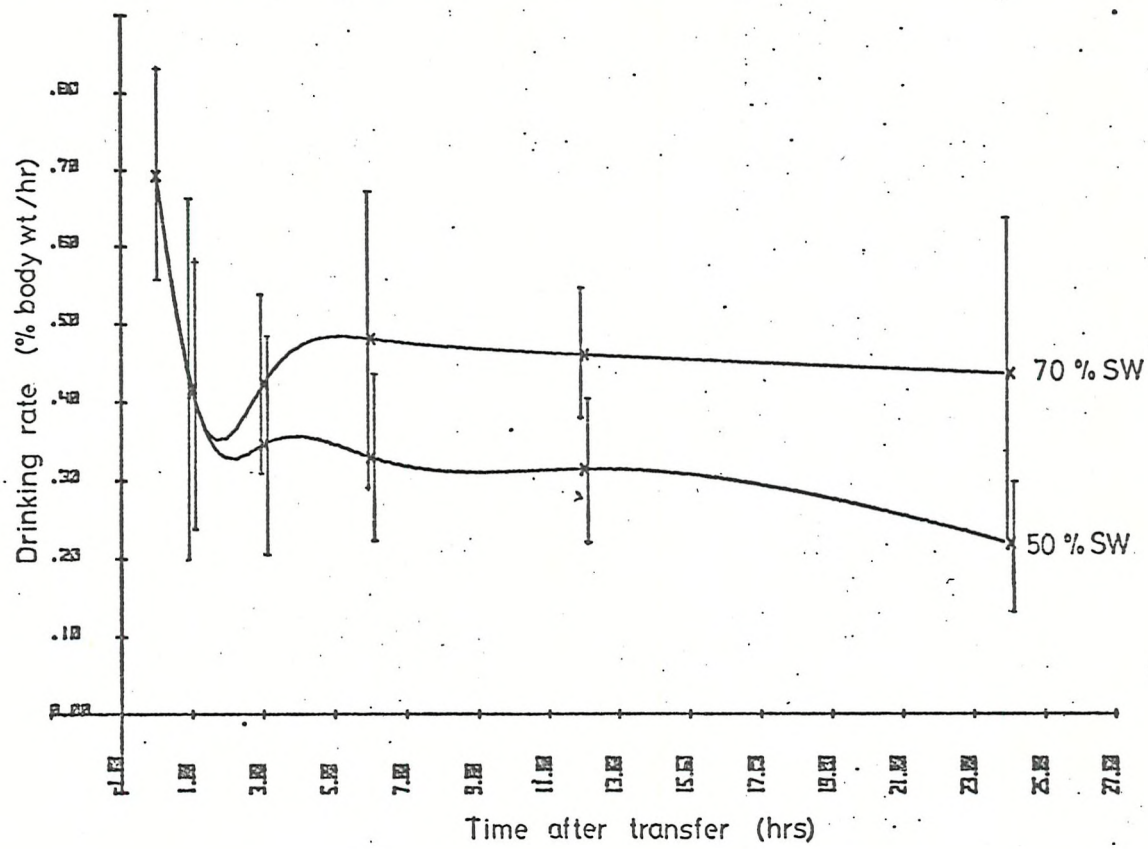

Figure 7.3 Drinking rate of prawns after subjection to a sudden change of salinity from 100% to 70% SW, and 100% to 50% SW.

Table 7.4 Non steady state drinking rate of prawns following transfer from 100% SW to low salinity, expressed as a % body wt/hr.

Hours after transfer	Initial drinking rate	Drinking rater after transfer 70% SW	50% SW
1	0.69 ± 0.13 (n=12)	0.41 ± 0.17 (n=12)	0.42 ± 0.23 (n=12)
3		0.42 ± 0.12 (n=12)	0.35 ± 0.13 (n=12)
6		0.48 ± 0.18 (n=12)	0.33 ± 0.11 (n=12)
12		0.46 ± 0.08 (n=12)	0.31 ± 0.10 (n=12)
24		0.43 ± 0.18 (n=12)	0.22 ± 0.08 (n=12)

Analysis of t-test

Source of variation	Drinking rate (% body wt/hr)	df	t-value	probability
Initial drinking rate	0.69 ± 0.13 (n=12)	22	4.4887	P< 0.001
1 hr after transfer to 70% SW	0.41 ± 0.17 (n=12)			
Initial drinking rate	0.69 ± 0.13 (n=12)	22	3.5755	P< 0.005
1 hr after transfer to 50% SW	0.42 ± 0.23 (n=12)			

2.5 Drinking rates of prawns with ligatured anus kept in 100% SW at 15°C.

The drinking rate of prawns, as shown in section 2.3 represents the amount of water taken into the body both through the mouth and the anus. An assessment of the role of oral drinking alone was made by repeating the drinking experiments with animals in which the anus was ligatured. The results are shown in Table 7.5. It appeared that the amount of water drunk through the mouth by the prawns is about 0.59 ± 0.07 (n=15)% body wt/hr whereas in the case of controls, taking the water into the body both through the mouth and the anus, it is about 0.69 ± 0.13 (n=12)% body wt/hr. Thus the rate of rectal swallowing is approximately 0.10% body wt/hr, assuming that there is no compensation for rectal ligaturing.

Fox (1952) discussed at some length the function of the water taken into the gut through the anus. He believed that most, perhaps all, of the water taken in by prawns through the anus is expelled through the same aperture at defaecation or at pseudo-defaecation. The necessity for a certain amount of water for the purpose of defaecation is unquestionable, but if all of the water taken in through the anus serves only that purpose, the drinking rate of the prawns with ligatured anus should not be significantly different from those of controls. If Fox's view is correct all of the marker, or nearly all, that is taken in through the anus should be extruded with the faeces, and there should be no net uptake of water via this path. The results of experiment 2.5 also provided a quantitative estimation of the rectal swallowing, and can be used as additional evidence to confirm the finding in experiment 1 of

this chapter. This suggests that the net uptake of water through the anus of prawns kept in 100% SW at a rate of 0.10% body wt/hr is a supplementary means of water uptake into the gut.

2.6 Effect of low temperature on drinking rate of prawns kept in 100% SW.

As shown in chapter V, the sodium concentration of the haemolymph of prawn in 100% SW is increased with decreasing temperature. This change, or the decreased temperature itself, might be expected to result indirectly in changes in the drinking rate. In order to determine the ability of the prawn to maintain their haemolymph homeostasis at low environmental temperature, experiments were conducted to determine if the prawn can alter the drinking rate in response to a change of temperature.

The results are given in Table 7.5. It appeared that reducing the temperature from 15^{o} to 5^{o}C resulted in reduction in drinking rate from 0.69 $\pm$ 0.13 (n=12)% body wt/hr to 0.35 $\pm$ 0.20 (n=15)% body wt/hr. The differences were statistically significant ($t = 5.0265$, $P < 0.001$). Similar reduction in drinking rate induced by low temperatures has also been shown in some teleosts (Motais & Isaia, 1972). This probably results from inactivation, and a low rate of metabolism of the animal at low temperature.

Table 7.5 Drinking rate of anus-ligatured prawns kept in 100% SW at 15°C.

Treatment	Drinking rate (% body wt/hr)	df	Analysis of t-test t-test	probability
Normal prawn	0.69 ± 0.13 (n=12)	25	2.6700	P< 0.025
Anus-ligatured prawn	0.59 ± 0.07 (n=15)			

Table 7.6 Effect of low temperature on drinking rate of prawns.

Treatment (°C)	Drinking rate (% body wt/hr)	df	Analysis of t-test t-test	probability
15	0.69 ± 0.13 (n=12)	25	5.0265	P< 0.001
5	0.35 ± 0.20 (n=15)			

VIII. HAEMOLYMPH VOLUME

The haemolymph volume is intimately related to total body water. Changes in total internal body water are normally reflected equally throughout the body-water compartments though variations in the level of inorganic ions in the haemolymph can initiate variations in the distribution of water between compartments. One of the major functions of the haemolymph system in animals is to provide the cells with a relatively constant internal environment, despite the fluctuations of the external environment. In consequence control of the extra-cellular volume plays an important role in euryhaline animals during non-genetic adaptation to fluctuating salinities. Control of variations of haemolymph volume potentially provide a certain buffer capacity which, by delaying the change in internal concentrations, may partly alleviate short term osmotic stresses (Spaargaren, 1972; Lockwood and Inman, 1973). The regulation of the extra-cellular volume itself is achieved by epithelial structures such as the gills, gut, and antennal glands. Like the movement of salts, the net transfer of water in the prawn in dilute media and normal sea water are opposite in direction.

A series of experiments were carried out to investigate the magnitude and variations of extra-cellular space in prawns exposed to various osmotic stresses. The marker dilution technique was used to estimate haemolymph volume.

1. Haemolymph volume of prawns kept in different salinities.

The haemolymph volumes of a number of animals subjected to a range of salinities are summarized in Table 8.1. The results

indicate that prawns which are fully acclimated to the test salinities have remarkably constant haemolymph volumes. This agrees well with the results observed in chapter VI, confirming that the prawns possess an efficient means of maintaining their haemolymph water content.

2. Haemolymph volume of prawns kept in 100% SW and stressed by exposure to low temperature.

It has been shown in chapter V, that in 100% SW the sodium concentrations of the haemolymph of prawns rise with a decrease of temperature. No such variations occur in haemolymph volume in these conditions (Table 8.2). At low temperature, in normal sea water, the prawns are still able to regulate their haemolymph volume as effectively as those kept at 15°C. In other words, the haemolymph volume of prawns kept at 5°C is not significantly different from those kept at 15°C. The results agree well with those described in chapter VI in connection with the water content of the total body of prawns kept in normal sea water when exposed to low temperature. This means that the increase in sodium concentrations of the haemolymph of prawns as observed at low temperature is mainly due to the increasing amount of the ion.

3. Effect of some inhibitors on haemolymph volume of prawns.

The observations in this section are part of experiment 7, chapter X. It seemed appropriate, however, to put these data in the present chapter, since they provided evidence showing the effect of some inhibitors on salts and water balance of prawns which are pertinent to the discussions in subsequent experiments.

Table 8.1 Haemolymph volume of prawns kept in different salinities at 15°C.

Group	Acclimation medium (% SW)	n	Weight range (g)	Haemolymph volume (% body weight)
A	100	35	0.61 - 2.45	19.4 ± 2.3
B	70	25	0.40 - 1.35	20.0 ± 2.3
C	50	20	0.51 - 1.29	20.4 ± 3.1
D	10	8	0.93 - 1.71	21.5 ± 4.8

Analysis of t-test

Source of variation	df	t-value	probability
A,B	58	0.8839	NS
B,C	43	0.5998	NS
C,D	53	1.3839	NS
D,A	41	1.8497	NS

Table 8.2 Effects of low temperature on haemolymph volume of prawns.

Treatment	Haemolymph volume (% body weight)	df	t-value	probability
Control	19.4 ± 2.3	48	0.4832	NS
Experimental prawns	19.8 ± 2.7			

Table 8.3 Effects of some inhibitors on haemolymph volume of prawns.

Treatment	Haemolymph volume (% body weight)	group	Analysis of t-test df	t-value	probability
100% SW					
A, control	19.4 ± 2.3	A,B	45	7.3060	P< 0.001
B, 10^{-5}M thionine	25.4 ± 2.8	B,C	25	5.8000	P< 0.001
C, 10^{-4}M amiloride	19.8 ± 2.2	C,A	48	0.5129	NS
50% SW					
D, control	20.4 ± 3.1	D,E	30	0.1665	NS
E, 10^{-5}M thionine	20.3 ± 2.8	E,F	16	2.9770	P< 0.010
F*, 10^{-5}M thionine	26.6 ± 6.4	F,D	24	3.3429	P< 0.005

Table 8.4 Haemolymph volume of prawns during the first hour after a sudden transfer from 100% to 50% SW.

Treatment	Weight range (g)	Haemolymph volume (% body weight)	Analysis of t-test df	t-value	probability
100% SW (control)	0.61 - 2.45	19.4 ± 2.3 (n=35)			
			43	2.5075	P< 0.025
transfer from 100 to 50% SW	0.70 - 2.10	17.2 ± 3.3 (n=10)			
			28	2.7014	P< 0.025
50% SW (control)	0.51 - 1.29	20.4 ± 3.1 (n=20)			

* Prawns did not survive throughout the experiment.

Table 8.3 shows that an addition of external thionine to the 100% SW medium increases the haemolymph volume of prawns from 19.4 ± 2.3 (n=35)% body weight to 25.4 ± 2.8 (n=12)% body weight. The difference is statistically significant ($t = 7.3060$, $P < 0.001$). When amiloride was added to the external medium there was no effect on the haemolymph volume of the prawns (the relevant discussions are given in experiment 6.2, 6.3, 7.1, and 7.3 of chapter X).

When thionine was added to 50% SW medium only 67% of the experimental prawns survived throughout the 24 hour-period of the experiment. Those that cannot survive through the 24 hour-period showed a significant increase of haemolymph volume from 20.4 ± 3.1 (n=20)% body weight to 26.6 ± 6.4 (n=6)% body weight, while those surviving through the experiment showed no significant increase in the haemolymph volume Table 8.3). This implies that the prawns can survive as long as they are able to maintain the concentrations of their body fluids within a minimum limit. Presumably when the sodium pumps cannot keep up with the sodium loss owing to the action of the drug, the continuing dilution of the body fluids due to the passive influx of water, will give rise to impairment of the osmoregulatory mechanisms (further discussion is given in experiment 7.2, chapter X).

4. Haemolymph volume of prawns during the first hour after a sudden transfer from 100% to 50% SW.

The haemolymph volume of prawns during the first hour after a sudden transfer from 100% to 50% SW was determined. The results are given in Table 8.4. When prawns are subjected to a sudden transfer from 100% to 50% SW, the haemolymph volume is reduced

from 19.4 ± 2.3% body wt (n=25) to 17.2 ± 3.3% body wt (n=10) during the first hour after the transfer. The reduction is statistically significant ($t = 2.5075$, $P < 0.025$). The value is also significantly lower than the value when the prawns reached a steady state, 20.4 ± 3.1% body wt (n=20).

5. Haemolymph volume and body size.

Prosser and Weistein (1950) showed for Cambarus virilis as did Shaw (1959b) for Astacus pallipes that there was a relationship between body size and haemolymph volume. However, Bryan (1960a) found no such relationship for Astacus fluviatilis. In order to determine the effect of body size, if any, the haemolymph volume of a number of animals ranging from 0.61 to 2.45 g was determined and the results are illustrated in Figure 8.0. It is apparent that there is no direct relationship between the haemolymph volume and the body weight P. serratus at least within the range of animals used in this experiment ($t = 0.077$, $df = 33$).

6. Discussion.

The values obtained here for the haemolymph volume of P. serratus agree well with those obtained by Binns (1969b) on Carcinus maenas, and by Flemister (1958) on Gecarcinus lateralis but are somewhat lower than Spaagaren's (1972) values on Crangon crangon and Donoghue's (1971) value on P. serratus. The latter were determined by estimation of dilution of I-131 sodium diatrizoate.

It must be borne in mind that the apparent haemolymph volume of animals may vary according to the method used for measurement,

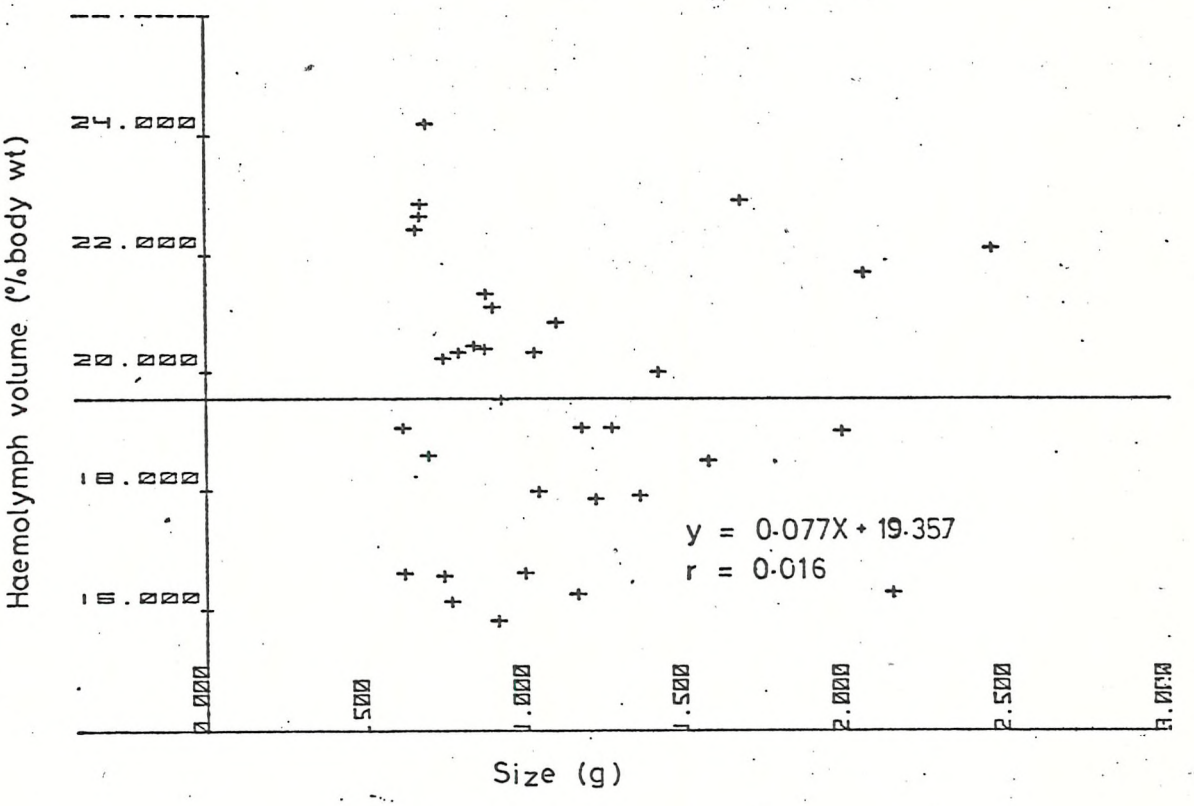

Figure 8.0 The relationship between the haemolymph volume and body size of prawns kept in 100% SW at 15°C.

the molecular size of the marker used (Barr & Marvin, 1968), and physiological state of the experimental animals such as molting, starvation (Lockwood & Inman, 1971). In view of this, comparison with other works on haemolymph volume is of limited value. If only for interest, then Table 8.5 is given for the comparison of the haemolymph volume of a selected decapods.

Flemister, 1958, found inulin occupied only about two-thirds of the distribution space of sodium thiocyanate in three species of crabs, and believed that the difference in distribution may be due to a functionally closed circulation of inulin within a larger extra-cellular compartments. The chromium method will give a low value for the haemolymph volume, if it does not penetrate the entire blood system. Since it is not certain exactly what space is being estimated by Cr-51 EDTA distribution, the so-called haemolymph volume is more accurately referred to as the EDTA space. This compartment was used for calculations of urine production rate in experiment 3.2, chapter X, and was determined in individual animals at the start of an experiment.

Table 8.5 Comparison of haemolymph volume in selected decapods in regard to species and method of determination.

Species	Salinity (% SW)	Haemolymph volume (% body wt)	n	Method	Reference
Crangon crangon	100	27.0	78	Chloride space	Spaagaren (1972)
" "	100	22.0	28	electrolite space	"
Pachygrapsus crassipes	100	18.7 ± 3.5	11	C-14 Sucrose	Gross & Marshall(1960)
Cambarus virilis	100	25.1	11	Evans blue	Prosser & Weinstein(1950)
Carcinus maenas	35 - 175	33.1	48	Amaranth	Siebers & Lucu (1973)
" "	35 - 175	17.9	41	C-14 Inulin	" "
" "	100	19.4 ± 5.0	28	"	Binns(1969b)
" "	100	19.7 ± 3.0	8	"	Riegel et al. (1974)
Ocypoda		21.5		"	Flemister(1958)
Goniopsis		20.0		"	" "
" "		30.4		Thiocyanate	" "
Gecarcinus		19.0		C-14 Inulin	" "
G. lateralis	moistened sand	22.0 ± 1.7	12	C-14 Sucrose	Skinner(1965)
Palaemon serratus	100	22.9 ± 8.3	12	Sodium diatrizoate	Donoghue(1971)
" "	100	19.4 ± 2.3	35	Cr-51 EDTA	present study
" "	70	20.0 ± 2.3	25	"	"
" "	50	20.4 ± 3.1	20	"	"
" "	10	21.5 ± 4.8	8	"	"

IX. BLADDER VOLUME

Riegel *et al.* (1974) have shown that the bladder volume also has an influence on marker U/H ratios of the shore crab, *Carcinus maenas*. In an attempt to examine whether this parameter affects U/H ratios of prawns, an experiment was performed to obtain the bladder volume. Seven prawns were injected with exactly 2 µl of Cr-51 EDTA. The initial activity of the injected Cr-51 EDTA was recorded. After one hour to allow mixing haemolymph samples were taken for haemolymph volume estimated by simple proportion. At the end of the experiment the total activity of labelled chromium in the whole animal, in the haemolymph and in the urine were recorded. From knowledge of the haemolymph volume, the U/H ratio of Cr-51 EDTA and the total Cr-51 EDTA in the animal, the bladder volume can be calculated from:

$$\frac{\text{Total count animal} - \left(\frac{\text{haemolymph count}}{\text{unit volume}} \times \frac{\text{haemolymph volume}}{\text{unit volume}}\right)}{\text{U/H for Cr-51 EDTA}}$$

The results are shown in Table 9.0. The volume of the bladder varies tremendously from close to nil to 5.17% body weight. The bladder volume obtained from this estimation is based on two assumptions. First that the volume of the haemolymph does not change over the two days of the experiment. Second that the space occupied by extra-haemolymph EDTA is equivalent to the volume of the urinary bladder. If the EDTA enters the cells or is bound to them the volume of the bladder calculated in this way will be overestimated. The values for bladder volume shown in Table 9.0 are, therefore, the maximum volume. The average value for the

bladder was 2.6 ± 1.8% body weight or 13.0 ± 10.6% haemolymph volume which was the same magnitude with the values reported by Riegel *et al.* (1974) on *C. maenas*, 11.0 ± 8.0% haemolymph volume and on *M. depurator*, 12.1 ± 5.0% haemolymph volume.

Table 9.0 Bladder volume of prawns kept in 100% SW at 15°C.

No.	Size (g)	Bladder volume (% body weight)	(% haemolymph volume)
1	1.3546	5.17	32.85
2	1.1708	4.00	21.69
3	1.0181	2.88	9.56
4	0.8134	2.81	10.75
5	1.2658	0.87	4.68
6	1.2142	1.36	7.85
7	0.9208	0.65	3.41
$\bar{X}$		2.61	12.97
SD		± 1.83	± 10.58

X. URINE PRODUCTION

In all concentrations of sea water, the prawn produces urine which is isotonic with the haemolymph (Parry, 1954). Hence, the urine plays no part in conservation of inorganic ions in general. If the antennal glands of the prawn are of little importance in salt conservation, they must have some other function. In crustaceans which produce a urine isotonic with the haemolymph, the antennal glands are generally most active in the secretion of magnesium and sulphate ions and in the reabsorption of potassium ions. This selective excretion is augmented as the external concentration of these ions rises (Parry, 1954). In an attempt to elucidate further the function of the antennal glands of the prawn, the following experiments were performed.

1. Mode of discharge of urine.

1.1 Discharge of urine of prawns kept in 100% SW.

To determine whether Cr-51 EDTA would appear in the medium at a constant rate or whether the labelled chromium would be released in irregular surges, nine prawns were injected with 5 µl of Cr-51 EDTA. Samples of medium were taken at 2, 4, 6, 12, 24, and 36 hours after injection. The results are shown in Table 10.1. The rate of increase of the labelled chromium in the bath declines exponentially. This implies that the Cr-51 EDTA appeared in the bath at a constant rate. Figure 10.1 also suggests that the mode of urination of prawns in normal sea water follows a regular and consistent pattern. Parry (1955) suggested that *P. varians* expells its urine by contraction of the epigastric sac,

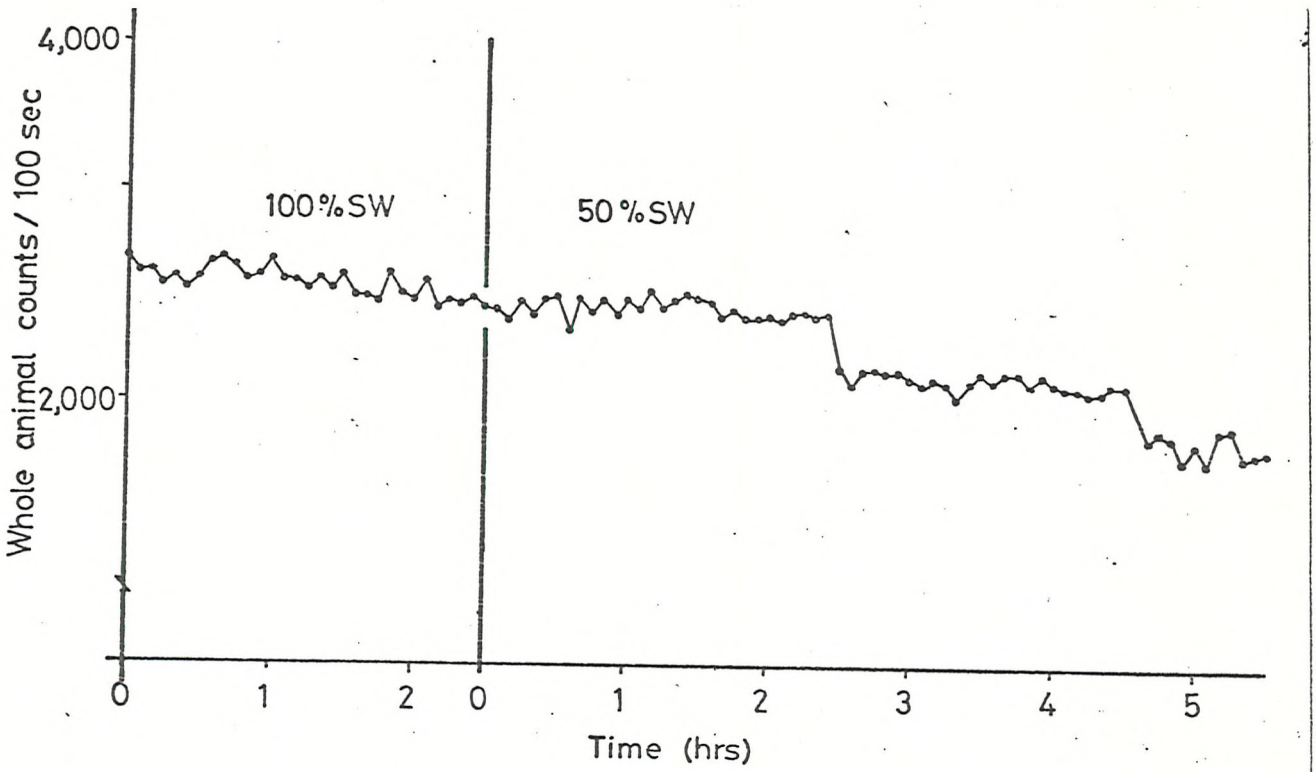

Figure 10.1 Total efflux of Cr-51 EDTA of prawn kept in 100% SW, and then subjected to a sudden change to 50% SW.

which formed the fusion of two backward projections of the bladder. P. serratus probably expells its urine in a similar fashion to P. varians. If this is the case, the frequency of contraction of the epigastric sac of prawns kept in normal sea water, must be high and consistent to make the disappearance of Cr-51 EDTA from the whole animals occur in a continuous rather than an intermittent pattern.

1.2 Discharge of urine of prawns kept in 50% SW.

When the animals are suddenly transferred to 50% SW, the periodicity of extrusion of Cr-51 EDTA becomes more pronounced. This implies that the animals increase the rate of urine flow by a periodic emptying of the bladder as shown by the discontinuous increases in the efflux of labelled chromium (Figure 10.1). This pattern of irregular discharge of urine found in the prawn is similar to that of Crangon crangon and C. allmanni (Spaargaren, 1971).

2. Fate of injected Cr-51 EDTA.

When using Cr-51 EDTA to estimate the urine production, two assumptions are made in assessing the validity of the method. First it is assumed that the labelled chromium is not escaping from the animal through any route other than the antennal glands; secondly, that the combination of EDTA with the chromium molecule is not metabolized by the experimental animal. Before attempting quantitative work it was necessary to investigate whether Cr-51 EDTA is an inert molecule when injected into prawns. This was done by measuring the appearance of Cr-51 EDTA in the external medium.

Table 10.1 Appearance of radioactivity in the bath after injection of Cr-51 EDTA. Values are expressed as counts/ml/100 sec.

No.	Size (g)	Hours after injection 2	4	6	12	24	36
1	1.35	255	305	401	782	1258	1558
2	1.17	112	277	292	707	1476	1724
3	0.96	49	249	366	893	1453	1586
4	0.81	370	597	624	865	982	1221
5	1.01	165	198	197	250	335	394
6	1.34	51	81	116	140	236	256
7	1.27	67	96	160	322	428	456
8	0.92	151	204	250	273	666	746
9	1.21	185	256	363	468	757	814

Table 10.2 Changes in activities of haemolymph and medium when labelled chromium was injected into prawns.

No.	Size (g)	Total activity in the haemolymph (counts/100 secs) Initial	At 36 hrs	Decrease in total activity in haemolymph	Increase in total activity in medium	% loss from total initial haemolymph
1	1.35	142,319	15,380	126,938	126,000	0.66
2	1.17	163,316	31,249	132,068	131,600	0.29
3	0.96	156,584	17,962	138,622	134,400	2.70
4	0.81	186,078	56,102	129,975	113,400	8.91
5	1.35	47,271	16,817	30,454	29,400	2.23
					$\bar{X}$	2.96

Animals were injected with a known amount of Cr-51 EDTA and placed in a large volume of medium to wash off any tracer which might have leaked onto the surface. A 10 µl sample of haemolymph was taken from each prawn one hour after the injection and the chromium space determined by calculating the dilution of the injected chromium. Prawns were then placed individually in 70 ml of the 100% SW and samples of the medium were taken at 2, 4, 6, 12, 24, and 36 hours after injection of Cr-51 EDTA. The experiment was allowed to run for about 36 hours. At the end of the experiment, the level of activity in the haemolymph was again estimated and the final sample of medium was taken.

Comparison of the loss of labelled chromium from the haemolymph with the increase in medium activity was made using five prawns. Details of changes in haemolymph and medium activity for all the animals are shown in Table 10.2. The change in total haemolymph activity over 36 hours was calculated knowing the EDTA volume, the initial, and the final haemolymph level of chromium. The change in activity of the medium over 36 hours was calculated. The rate of increase of activity in the medium, due to the elimination of urine and the change in haemolymph activity were then compared.

The changes in the activity in the haemolymph of prawns and surrounding medium are shown in Table 10.2. The correspondence between haemolymph and medium activity change is good, the loss of activity from the haemolymph being balanced by an increase in activity in the medium. In most cases, there was little difference between the two values. The discrepancy, the difference between

the two figures expressed as a percentage of the larger one, was small. This means that nearly all of the labelled chromium is cleared from the haemolymph due to the production of urine, and only a small percentage has been lost. If the haemolymph volume of the experimental animals is constant, the loss could be either by binding of the Cr-51 EDTA within the tissues, or extra-renal loss. On the basis of these results it is probably safe to assume that Cr-51 EDTA is a suitable molecule to use for the study of the mechanism and the rate of urine production.

3. Urine production.

The rate of urine production of decapod crustaceans has been measured by several methods. Herrmann (1931), Scholles (1933), Peters (1935), Lienemann (1938), Maluf (1941b), Parry (1955), Bryan (1960a), Riegel (1961), and Kamemoto et al., (1966) estimated the urine flow by determining the change in the weight of animals, after the occlusion of the nephropores had prevented the elimination of urine. The increase in weight over a period of time, presumably due to the influx of water, was taken as equivalent to the amount of urine normally produced by an individual in as much as no change in weight is seen in animals when the nephropores are not plugged.

A second method in use is the estimating of excretion rate of injected non-metabolized, and non-reabsorbed substances such as inulin, chromium, and various dyes (Parry, 1955; Shaw, 1959b; Binns, 1969b; and Lockwood & Inman, 1973). A third method employed is a direct cannulation. The cannulation of the urinary bladder is of preference for recording urine production rate in teleosts. Because

of the ventro-anterior position of the excretory openings of decapod crustaceans, direct collection of urine is nearly impossible. In macruric forms, cannulation has been attempted with some success (Kamemoto and Ono, 1968), but the caridic form of P. serratus and many other decapods create additional problems, besides the obvious mechanical difficulties, the small size and sensitivity to handling of the experimental animals make this method impractical.

It was demonstrated in the previous chapter that the prawns regulate the sodium concentrations of the haemolymph, hyperionic in dilute media and hypoionic in normal sea water. In dilute media the osmotic entry of water is considerable and the removal of excess water and uptake of sodium ion require energy, especially for those prawns that produce an isotonic urine. In sea water the entry of water will be reflected by the volume of urine produced. In other words, urine production has to be adjusted to osmotic influx or outflux of water so that no dilution of body fluids is caused by the net intake of water and no dehydration of body fluids occurs due to net osmotic outflow of water. In order to investigate this aspect of osmoregulation, the first two methods of estimation of urine production were used to measure the urine output of the prawn when stressed by different salinities and temperatures.

3.1 Weight-gained method.

Bryan (1960a) found that the values of urine production by crayfish obtained by weighing the animals eight hours after plugging the nephropores were higher than those obtained if he weighed the animals 24 hours after plugging. This is not surprising since the kidney of the crayfish is a filtration system composed

of a number of connected anatomical parts without intervening valves (Maluf, 1939, 1941a), and any back pressure caused by an excess of urine in the bladder would result in a back pressure that could reduce the rate of filtration. Back pressure would be expected to cause kidney failure and turgor pressure in the animal could therefore result in a decrease in the osmotic flow of water into the animals.

The antennal glands of the prawn are similar to the kidney of the crayfish, consisting of an internal end-sac and excretory tubule which leads into a bladder (Weldon, 1891; Allen, 1892). The back pressure that reduces the rate of filtration in the crayfish, when the excretory openings were blocked for a certain period, must also exist in the prawn. As shown in Table 12.1-12.3(P.145) the increase in weight per hour of prawns in the first hour after blocking the urinary openings was relatively greater than those obtained at 3, 6, 12, and 24 hours. The fast increase in weight during the first hour may reflect the effect of handling, whereas the relatively small increase in weight during the latter period, probably arises from the increasing turgor pressure of the animal, which tends to prevent the entry of more water. In order to decrease the possible interference of the handling effect, turgor pressure, and defaecating capabilities of the animals, the volume of urine produced was taken as the increase in weight over the first three hours and expressed as % body wt/hr.

Table 10.3 summarises the results of the measurements of urine production, estimated by the weight-gained method, of prawns in three salinities. In 50% SW prawns produced the urine at a rate of 0.84% body wt/hr, whilst the rate in 70% SW is about

Table 10.3 Rate of urine flow in prawns measuring by weight changes after blocking the excretory pores.

Treatment (% SW)	% increase in body weight (three hours after blocking) Excretory pores open	Blocked excretory pores	Urine production % body wt/hr	% body wt/day
100	0.36 ± 0.75 (15)	1.43 ± 0.82 (15)	0.36	8.64
70	0.49 ± 0.72 (19)	1.14 ± 1.11 (19)	0.22	5.28
50	0.13 ± 0.50 (17)	2.64 ± 0.92 (15)	0.84	20.16

Table 10.4 U/H ratios for Cr-51 EDTA in prawns exposed to different salinities.

Treatment (% SW)	Size range (g)	U/H ratio	U/H ratio* after correction of blood protein
100	1.17 - 6.00	2.67 ± 1.10 (10)	2.34
70	3.18 - 6.59	2.47 ± 0.44 (6)	2.16
50	3.11 - 6.16	2.02 ± 0.33 (6)	1.77
10	2.99 - 5.23	1.40 ± 0.58 (5)	1.22

* U/H values in this column will be used for a calculation of urine production in Table 10.5

** Blood protein of prawn is 12% (Parry, 1955)

0.22% body wt/hr. In 100% SW prawns produce urine at a rate of 0.36% body wt/hr which is higher than those kept in 70% SW, but considerably less than that of prawns kept in 50% SW.

3.2 Clearance of Cr-51 EDTA from the haemolymph.

In order to provide relevant data for calculating the urine production by the method of clearance of Cr-51 EDTA, the U/H ratios of Cr-51 EDTA of prawns exposed to different salinities were determined. The results are given in Table 10.4.

The urine production measured from the disappearance of Cr-51 EDTA from the haemolymph of prawns exposed to different salinities is shown in Table 10.5. It appears that the values obtained from this method were slightly larger than those obtained from the weight-gained method. The possible explanation is that the second method of measuring urine production required an injection of a marker fluid. The injection of 5 µl of the marker solution into the experimental prawns resulted in an immediate increase of the haemolymph volume by one to two per cent. Since the prawn regulates its internal concentration of body fluids precisely, the rapid rate of urine production as seen in the second method may perhaps be attributed to the process of elimination of the excess fluid volume injected. This effect is seen to be more pronounced for prawns kept in 70% SW, since in this media, the main outlet by which the excess water is excreted is the excretory pores. However, the rate of urine flow of prawn kept in 70% SW obtained from the weight-gained method might also be an under estimate, since blocking the antennary openings to prevent the urination might create an adverse physiological reaction by

Table 10.5 Urine production calculated from disappearance of Cr-51 EDTA from the haemolymph.

Group	Medium (% SW)	Weight range (g)	Clearance rate (% body wt/day)	Urine production % body wt/hr	Urine production % body wt/day
A	100	0.67 - 1.65	24.71 ± 3.84 ($\bar{18}$)	0.44	10.56
B	70	0.56 - 1.21	23.30 ± 4.96 ($\bar{11}$)	0.45	10.79
C	50	0.44 - 1.29	39.52 ± 7.88 ($\bar{15}$)	0.93	22.33

Analysis of t-test for clearance rate

Source of variation	df	t-value	probability
A,B	27	0.8589	NS
B,C	24	5.9923	$P < 0.001$
C,A	31	7.0483	$P < 0.001$

Table 10.6 Effect of a sudden change of salinities on the rate of urine production in prawns measuring by weight changes after blocking the excretory pores.

Treatment (% SW)	% increase in body weight (three hours after blocking) Excretory pores open	Blocked excretory pores	Urine production % body wt/hr	Urine production % body wt/day
100 to 70	0.48 ± 1.09 ($\bar{17}$)	2.01 ± 0.44 ($\bar{18}$)	0.51	12.24
100 to 50	0.92 ± 0.86 ($\bar{17}$)	5.79 ± 1.84 ($\bar{15}$)	1.62	38.88

decreasing the transport rate of water across the gut or reducing the drinking rate, thus in turn decreasing the amount of water transported across the gut. To test the latter possibility, a number of animals were acclimated to 70% SW, and the drinking rates of prawns were measured after their antennary openings were blocked for 6 hours. It appeared that the drinking rates of prawns with blocked antennary pores were not significantly different from the control (Table 10.7). This suggests that the preventing of urination by blocking the excretory pores probably also creates an adverse physiological reaction which results in a slower rate of water transport across the gut.

4. Effect of a sudden transfer from normal sea water to dilute media on urine production.

The rates of urine production of prawns subjected to a sudden decrease to low salinities, from 100% to 70% SW, and from 100% to 50% SW, were also measured. The results (Table 10.6) indicate that the antennal glands play a significant part in water regulation. Their importance is demonstrated by the spectacular change in urine volume when the prawns are suddenly transferred from 100% SW to a dilute medium. When prawns are suddenly transferred from 100% to 50% SW, their rates of urine production are increased to a level twice that of those in a steady state in 50% SW. This suggests that when the prawns are subjected to sudden changes from normal sea water to dilute sea water, the animals are able to excrete the excess water out of the body as rapidly as its enters until equilibrium has been reached.

5. Effects of some metabolic inhibitors on weight changes.

It has been shown that the prawns drink sea water and can alter the drinking rate according to the salinity. It is considered that the active uptake of water across the gut epithelium might be mediated by some forms of ion transport, responsible for the eventual net uptake of water, so any substance affecting the rate of ion transport might also influence the weight changes of prawns kept in sea water.

5.1 Effects of an external addition of ouabain on weight changes.

This experiment was designed to determine whether an addition of ouabain to normal sea water would cause a blocking of sodium transport across the gut of prawns kept in that medium with consequent loss of weight. The results are given in Table 10.8, and Figure 10.2. When 10^{-5}M ouabain was added to the medium, 100% SW, the prawns showed a lesser increase in weight than the control. The effect is not significantly different except for those at 12 hours after transfer (Table 10.8). This one would expect, since ouabain is known to have an asymmetric action functioning only on the side of the membrane from which K^+ is actively transported (Kirschner, 1969; Maetz, 1971). The effect of the drug on sodium transport, when it is added to the external medium as in this case, must be less when compared with its effect on the sodium-excreting pump on the outer border of the branchial epithelium as shown in experiment 4.3 of chapter XI.

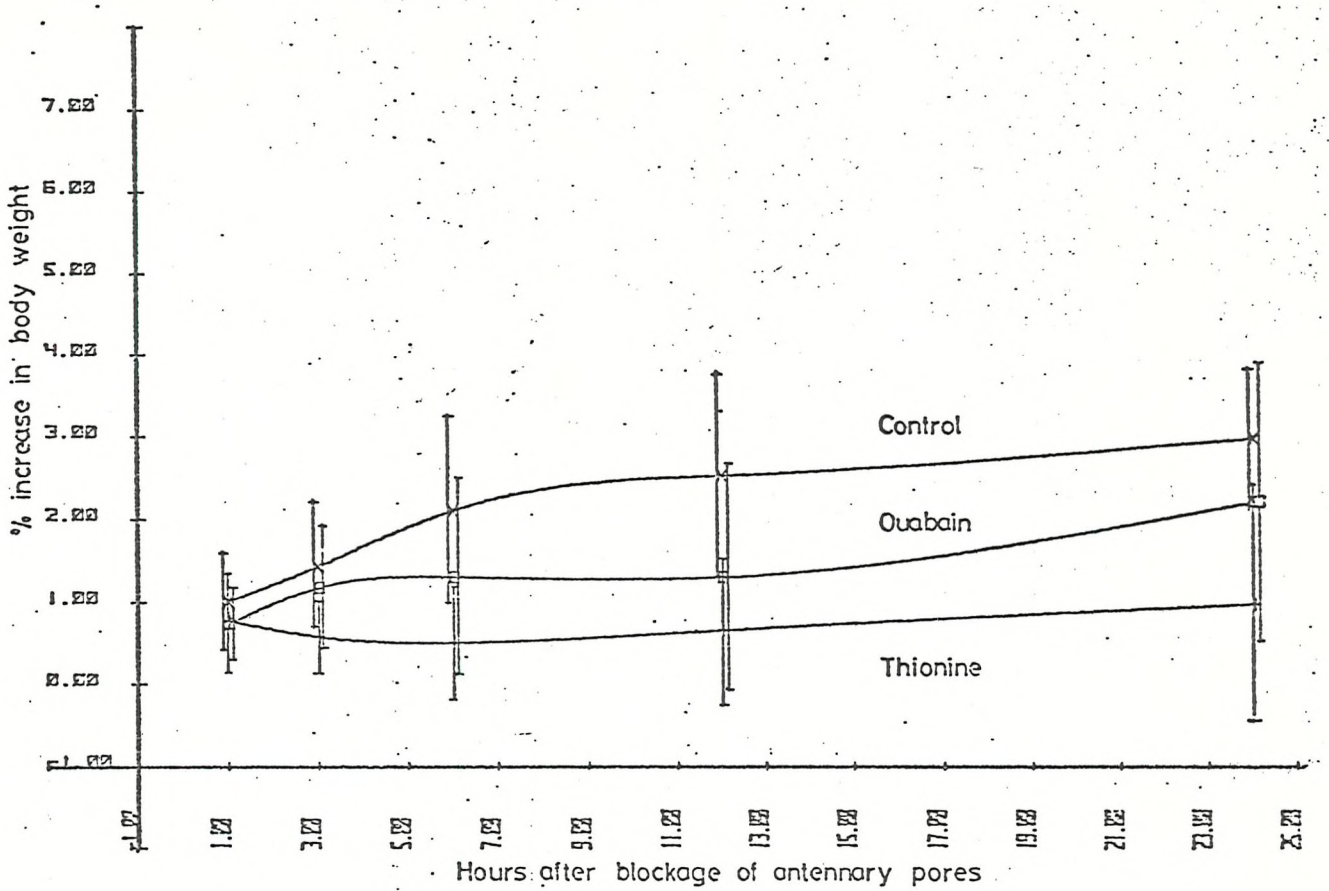

Figure 10.2 Changes in weight of prawns after subjection to 100% SW with addition of ouabain and thionine.

Table 10.7 Effects of blocking the antennary openings on drinking rates of prawns kept in 70% SW, at 15°C.

Treatment	Weight range (g)	Drinking rate (% body wt/hr)	Analysis of t-test df	t-value	probability
Control	0.21 - 0.41	0.42 ± 0.08 ($\bar{12}$)	15	1.1275	NS
Experimental prawns	0.25 - 0.61	0.42 ± 0.15 ($\bar{5}$)			

Table 10.8 Effect of 10^{-5}M ouabain on weight increase of prawns in 100% SW, at 15°C.

Hours after blockage	Prawn in 100% SW	Prawn in 100% SW + ouabain	Analysis of t-test df	t-value	probability
1	1.01 ± 0.63 ($\bar{15}$)	0.74 ± 0.44 ($\bar{26}$)	39	1.4852	NS
3	1.43 ± 0.82 ($\bar{15}$)	1.17 ± 0.73 ($\bar{26}$)	39	1.0720	NS
6	2.10 ± 1.23 ($\bar{15}$)	1.30 ± 1.27 ($\bar{26}$)	39	1.9544	NS
12	2.52 ± 1.34 ($\bar{10}$)	1.30 ± 1.44 ($\bar{26}$)	38	2.3079	$P < 0.05$
24	2.97 ± 0.83 ($\bar{6}$)	2.02 ± 1.8 ($\bar{24}$)	28	1.2364	NS

5.2 Effects of external thionine on weight changes.

Similarly to ouabain, the basic dye thionine has been shown to inhibit active uptake of sodium in crabs, both in the whole animals and in the isolated gills (Koch, Evans, and Schicks, 1954; Koch and Evans, 1956), in Gammarus duebeni (Lockwood and Andrews, 1969), and in P. serratus (Donoghue, 1971). The experiment was performed in an effort to further substantiate the claim that water transport across the gut epithelium is coupled with sodium.

The results are illustrated in Table 10.9, and in Figure 10.2. It can be seen that thionine is a very effective inhibitor for sodium transport, as well as inhibiting the water that is taken into the body. The effect is prominent in the first hour after transfer. The animals showed a gradual decrease in weight in the subsequent periods of the experiment. The decrease is statistically significant (Table 10.9).

6. Effects of external addition of thionine in 100% SW on the concentration of sodium in the haemolymph.

It has been shown in chapter VIII that the haemolymph volume of prawns kept in 100% SW with an addition of 10^{-5}M thionine is increased by about 30.61% of the control. An increase in the extracellular fluids might be due to

(1) the water influx being in excess of the water outflux,

(2) the passive withdrawal of water from the intracellular compartment if the concentration of sodium in the haemolymph is increased, and there is no other physiological processes countering it, and

(3) the sodium pump that is located in the bladder epithelium might be stimulated by an increase of sodium concentration in the haemolymph so that more water in the bladder is transported back into the extracellular compartment.

The first possibility is unlikely, since the magnitude of water entry should be reflected by the volume of urine produced, (see some relevant data in the experiment 8.1 of this chapter) and a decrease in weight of prawns kept in 100% SW with an addition of thionine, as shown in the experiment 5.2 of this chapter, does not support the possibility. Hence it leaves the second and the third suggestion for the possible causes.

An experiment was designed to determine the level of sodium concentration of the haemolymph of prawns after subjection to 100% SW with an addition of 10^{-5} thionine. The results are shown in Table 10.10. The sodium concentration of the haemolymph of prawn is raised from 365 $\pm$ 7.1 (n=18) to 381 $\pm$ 7.9 (n=8) mM/l, and stabilized to this level throughout the experimental period, 24 hours. The increase is statistically significant ($t = 5.3012$, $P < 0.001$).

7. Effects of external addition of thionine in 100% SW on the total water content of the whole animal, at 15°C.

Groups of 18 prawns were used in each treatment. Three treatments were designed, one set of animals was acclimated in 100% SW for a control, the other two sets were kept in 100% SW with addition of 10^{-3}M and 10^{-5}M thionine respectively. After 24 hours in the acclimated media, the wet weight and the dry weight of prawns were determined.

Table 10.9 Effect of 10^{-5}M thionine on weight increase of prawns in 100% SW at 15°C.

Hours after blockage	Prawn in 100% SW	Prawn in 100% SW + thionine	Analysis of t-test df	t-value	probability
1	1.01 ± 0.63 ($\bar{15}$)	0.78 ± 0.65 ($\bar{15}$)	28	0.9752	NS
3	1.43 ± 0.82 ($\bar{15}$)	0.58 ± 0.48 ($\bar{15}$)	28	3.1891	P< 0.01
6	2.10 ± 1.23 ($\bar{15}$)	0.51 ± 0.70 ($\bar{12}$)	25	3.8840	P< 0.001
12	2.52 ± 1.34 ($\bar{10}$)	0.66 ± 0.98 ($\bar{12}$)	20	3.7457	P< 0.005
24	2.97 ± 0.83 ($\bar{6}$)	0.97 ± 1.59 ($\bar{12}$)	16	2.8740	P< 0.025

Table 10.10 Effect of external addition of thionine in 100% SW on the concentration of sodium of the haemolymph.

Group	Hours after transfer	Na^+ (mM/l)	Group	Analysis of t-test df	t-value	probability
A	0	364.78 ± 7.14 ($\bar{18}$)				
B	1	381.36 ± 7.90 ($\bar{8}$)	A,B	24	5.3012	P< 0.001
C	3	382.50 ± 8.12 ($\bar{8}$)	A,C	24	5.6300	P< 0.001
D	6	379.38 ± 6.78 ($\bar{8}$)	A,D	24	4.8807	P< 0.001
E	12	380.00 ± 7.07 ($\bar{8}$)	A,E	24	5.0306	P< 0.001
F	24	381.86 ± 7.04 ($\bar{8}$)	A,F	24	5.6576	P< 0.001

The results are given in Table 10.11. Only ten prawns in 100% SW with an addition of 10^{-3}M thionine survived throughout the experiment. The total water content of prawns treated with 10^{-3}M thionine is not much different than those in 10^{-5}M thionine, but it is significantly lower than that of the controls.

The results confirm the findings of experiment 5.2, since the amount of water taken in via the gut epithelium of the experimental prawns is less than that of the controls due to the action of thionine. The increase in haemolymph volume of prawns kept in 100% SW with an addition of thionine must be due to the movement of water either from the intracellular compartment or from the urinary bladder. To test the latter possibility, thionine 10^{-5}M was added to the external medium, 70% SW, and U/H ratios for Cr-51 EDTA of the prawns were determined. This salinity was selected, since it is isotonic to the haemolymph. It is assumed that, if an increase of the sodium concentration of the haemolymph activates the sodium pump along the bladder epithelia, and results in a higher rate of water turnover from the urinary bladder, the U/H ratio for Cr-51 EDTA of the experimental prawns should be higher than those of the controls.

The results are given in Table 10.12. There is no significant difference between the U/H ratios for Cr-51 EDTA of the experimental animal and the control.

8. Effects of some metabolic inhibitors on the rate of urine production of prawns kept in 100% SW.

In order to strengthen the view that the uptake of water of prawns in normal sea water is mediated by some forms of ion

transport, two series of experiments were carried out to test the effect of some metabolic inhibitors on urine production of the prawn.

Table 10.11 Water content of prawns kept in 100% SW with an addition of thionine.

Treatment	Weight range (g)	Water content (% body wt)	Analysis of t-test df	t-value	probability
Thionine 10^{-3}M	0.35 - 0.60	74.80 ± 0.90 (10)	16	2.9151	P< 0.025
Control	0.24 - 0.61	76.09 ± 1.21 (18)			
Thionine 10^{-5}M	0.35 - 0.91	74.73 ± 1.51 (18)	34	2.9942	P< 0.010

Table 10.12 U/H ratios for Cr-51 EDTA of prawns kept in 70% SW with an addition of 10^{-5}M thionine.

Treatment	Weight range (g)	U/H ratio	U/H ratio*	Analysis of t-test df	t-value	probability
Control	3.18 - 6.59	2.47 ± 0.44 ($\bar{6}$)	2.16	10	0.1314	NS
Experimental prawns	3.14 - 4.26	2.43 ± 0.75 ($\bar{6}$)	2.13			

* U/H ratio after corrected the blood protein

Table 10.13 Effect of thionine on urine flow calculated from the clearance of Cr-51 EDTA from the haemolymph of prawns kept in 100% SW, at 15°C.

Treatment	Weight range (g)	Urine production (% body wt/day)	Analysis of t-test df	t-value	probability
Control	0.67 - 1.71	10.56 ± 1.64 (18)	26	4.4185	$P < 0.001$
Thionine 10^{-5}M	1.03 - 1.46	7.28 ± 2.27 (10)			

8.1 Effect of external addition of 10^{-5}M thionine.

When 10^{-5}M thionine was applied to the external medium the urine production of the prawns was markedly slower than that of the controls. It was reduced from 10.56 ± 1.64% body wt/day to 7.28 ± 2.27% body wt/day. The reduction is statistical significant ($t = 4.4185$, $P < 0.001$). This implies that the thionine influences the rate at which water was taken up into the body and also the rate at which it leaves via the urine.

8.2 Effect of external addition of amiloride.

The diuretic compound amiloride (N-amidino-3, 5-diamino-6-chloropyrazinecarboxamide) has an inhibitory property on sodium transport in a number of vertebrate epithelia including isolated frog skin (Nagel and Dörge, 1970), and toad bladder (Bentley, 1968). Its effect is shown in very low concentrations (10^{-5}M to 10^{-4}M) when applied to the fresh water system (Kirschner et al., 1973). Hence, it seems that amiloride might be useful in trying to show the influx of water during sodium uptake of prawns in

sea water. In addition it was thought that the amiloride might be an additional tool, besides thionine and ouabain, to examine the behaviour of animals in sea water system.

Two levels of concentration of amiloride, 10^{-3}M, and 10^{-4}M, were used. The results are shown in Table 10.14. It appears that the inhibition by amiloride observed in the present experiment is not so effective as when applied to the fresh water animals by Kirschner et al. The reason for this difference is not clear, especially since, when higher concentrations of amiloride are applied, the effect is reversed.

9. Effect of adding thionine to 50% SW on the rate of urine production of prawns.

In 50% SW where the body fluids of prawns are hypertonic and of higher ionic concentration than the environment, the animals need to combat an osmotic influx of water and outward diffusion of salts. The former problem is counteracted by producing a large quantities of urine, and the latter problem by active uptake of both sodium and chloride ions at the gills.

In an attempt to elucidate certain aspects of the regulatory processes and to gain some understanding of the water movements between the prawn and the hypoosmotic external environment, the experiment was performed as before, but the 10^{-5}M thionine was added in 50% SW. The results of the experiment are shown in Table 10.15. It appeared that the drug had no effect when it was added to 50% SW. This suggested that in 50% SW the major component of water influx is passively through the body surface. The water that is taken into the body by coupling with sodium ion through

Table 10.14 Effect of amiloride on urine production calculated from the clearance of Cr-51 EDTA from the haemolymph of prawns kept in 100% SW at 15°C.

Group	Treatment	n	Weight range (g)	Urine production (% body wt/day)
A	Control	18	0.67 - 1.71	10.56 ± 1.64
B	10^{-4}M amiloride	10	0.98 - 1.44	9.17 ± 2.66
C	10^{-3}M amiloride	5	1.16 - 1.33	15.45 ± 1.88

Analysis of t-test

Source of variation	df	t-value	probability
A,B	26	1.6780	NS
B,C	13	4.6341	P< 0.001
C,A	21	5.7307	P< 0.001

Table 10.15 Effect of thionine on urine flow calculated from the clearance of Cr-51 EDTA from the haemolymph of prawns kept in 50% SW at 15°C.

Treatment	Urine production (% body wt/day)	Analysis of t-test df	t-value	probability
Control	22.33 ± 4.41 (15)	25	1.3510	NS
10^{-5}M thionine	24.76 ± 4.87 (12)			

the gut is small when compared with influx due to the osmotic gradient through the gills. The inhibition of sodium uptake mechanisms by the thionine seems to affect the overall regulatory process of the prawn. It seems likely that the animal solves the problem of passive influx of water by increasing the rate of urine production. Since prawns produce isotonic urine, the increase in rate of urine production is a disadvantage to the prawns especially when the uptake mechanism by which the prawn takes up salts to compensate the loss is inhibited by the thionine. The effect of thionine in this connection can be seen from the large haemolymph volume of those prawns which cannot survive through the experiment (experiment 3, chapter VIII).

It is clear, therefore, that prime responsibility for regulation of the volume and composition of the extra-cellular fluids in the prawn in dilute media lies with the gills and the antennal glands. The two mechanisms are probably controlled by endocrine systems that permit very fine adjustments in body fluid composition. The problem of maintaining the composition of the extra-cellular fluids in the prawn is acute, since ions and water tend to move passively along diffusion and osmotic gradients between the external and internal media. As seen in this experiment active transport of ions across the gills takes place sharing the importance of antennal glands in salts and water regulation (see more discussion experiments 5, 6, and 7 of chapter XII).

10. Sealing the mouth.

In an attempt to provide more direct evidence of water uptake across the gut epithelium, efforts have been made to seal the mouth

of prawns by using dental cement. It is assumed that if the major route of influx of water is blocked, the water uptake across the gut would decrease and result in a low rate of urine production. Unfortunately only one prawn out of six survived throughout the experimental period, 24 hours. The urine flow of this prawn was 9.16% body wt/day whereas the control was 10.56 ± 1.64 (n=18). This experiment was not pursued further. Besides the technical difficulty, the limiting of supply of prawns, and the high mortality of the experimental animals, the animal that survived throughout the experiment also showed a diuresis which is probably due either to excessive handling or general damage.

11. Urine production of prawns kept in 10% SW.

The urine flow obtained from this study is determined indirectly from measuring the efflux of Cr-51 EDTA from the whole animals. The results are given in Table 10.16. It appeared that in changing the medium from 50% to 10% SW, the flow of urine of prawns is increased to nearly twice that of those kept in 50% SW. This pattern of urine production is similar to that of P. varians (Parry, 1955), but it contrasts with C. crangon which is able to restrict its rate of urine flow to a maximum rate (Spaargaren, 1971). The difference between P. serratus and C. crangon in terms of the pattern of urine flow may be due to the fact that the P. serratus possesses a more effective uptake mechanism than C. crangon when the animals are exposed to a very dilute salinity.

The rate of urine flow obtained from this experiment cannot be compared with those in experiment 3.2 of this chapter, since the values obtained from the present study may be under-estimates.

The disadvantage of this method is that the measurement of the total efflux of Cr-51 EDTA from the whole animal involves not only counting the labelled chromium in the haemolymph but also the marker both in the intracellular compartments and in the bladder. The latter compartment probably causes a big error for urine flow obtained from this method, especially as the prawn stores its urine in the bladder and releases it intermittently rather than a continuous discharge as shown in section 1 of this chapter.

The indirect method of measuring urine flow, although not so reliable or accurate as the clearance of Cr-51 EDTA from the haemolymph, is very valuable in situations where the measurement of urine production by clearance of Cr-51 EDTA from the haemolymph is impractical.

12. Effect of low temperature on urine production.

Two experiments were carried out to determine the effects of temperature on urine production. The first was done in 100% SW at 15°C as a control. The second experiment was undertaken in 100% SW at 5°C. Prawns used for both experiments were acclimated to the given temperature during the two weeks prior to experiments. Urine production obtained from this study is determined by clearance of Cr-51 EDTA from the haemolymph.

The results are shown in Table 10.17. The urine production of prawns kept in 100% SW at 5°C ranged from 2.4 - 7.4 % body wt/day. The mean value is 4.6 ± 1.4 % body wt/day whereas the control is 10.6 ± 1.6 % body wt/day.

The temperature effect may be expressed in Q_{10} value. The formula used to calculate a Q_{10} value for a particular reaction

Table 10.16 Urine production calculated from the rate constant for Cr-51 EDTA efflux from the whole animal kept in 10% SW, at 15°C.

Treatment (% SW)	Weight range (g)	Urine production (% body wt/hr)	Analysis of t-test df	t-value	probability
50	1.46 - 5.40	0.4871 ± 0.1170 ($\bar{8}$)	12	3.0943	$P < 0.010$
10	1.50 - 1.91	0.7854 ± 0.2563 ($\bar{6}$)			

Table 10.17 Urine flow calculated from the clearance of Cr-51 EDTA from the haemolymph of prawns kept in 100% SW at two temperatures.

Treatment (°C)	Weight range (g)	Urine production (% body wt/day)	Analysis of t-test df	t-value	probability
15	0.67 - 1.71	10.56 ± 1.64 ($\bar{18}$)	31	11.2355	$P < 0.001$
5	1.02 - 1.88	4.60 ± 1.35 ($\bar{15}$)			

can be written as:

$$\log Q_{10} = \frac{10}{T_2 - T_1} \log_{10} k_2/k_1$$

where k_2 is the rate at the higher temperature,

T_2, and k_1 is the rate at lower temperature, T_1.

The Q_{10} for a particular biological function is not constant but varies depending on the temperature at which it is measured. Q_{10} values for urine production obtained here was about 2.4 which is within the usual range for metabolic reactions.

XI. SODIUM EFFLUXES IN THE PRAWN, *PALAEMON SERRATUS*, IN RELATION TO TEMPERATURE AND SALINITY

The investigation of the exchange of the amount of sodium between the prawns and its environment is facilitated by using sodium-22 as a tracer. In an attempt to gain an insight into the mechanism stabilizing the internal concentration of sodium in the prawn, sodium-22 was used to determine the sodium exchange and to measure the response of the prawn to sudden change of temperature and salinity.

1. Influence of internal sodium concentration in the haemolymph on the sodium efflux in prawns.

As shown in chapter V, a decrease in temperature from 15° to 5°C results in an increase of the mean haemolymph sodium from 365 ± 7.1 (n=18) mM/l to 391 ± 18.9 (n=18) mM/l. In order to examine whether the sodium concentration in haemolymph affects the rate of loss of sodium, two experiments were set up for comparison.

1.1 Loss of sodium-22 from prawns acclimated to 15°C washing out into 100% SW at 15°C.

Prawns which had been acclimated to 15°C in 100% SW for three days were blotted and weighed. A single prawn was loaded with sodium-22 by placing it in 200 ml of sea water containing 50 microcuries (μCi) of sodium-22 with the appropriate aeration. The prawn remained in this solution for 36 hours, which was sufficient time for a steady state between the prawn and the medium

to be achieved. The fully loaded prawn was subsequently removed from the loading medium and briefly washed in sea water to remove excess tracer adhering to the body surface and the gills. The prawn was then placed in the animal counting chamber and washed out in inactive 100% SW at 15°C.

The efflux experiment was performed in a special circulation apparatus. This allowed continuous monitoring of the radioactivity remaining in the animal with time, without the necessity for removing the animal from the experimental medium. The apparatus consisted of a plexiglass aquarium of 13 litres capacity and animal counting chamber. The animal was placed in a pyrex glass specimen tube of 12 mm diameter which fitted into the well of the scintilation counter. The tube was sealed by a rubber bung through which passed polythene inlet and outlet tubes. The inlet tube carried the washing medium from the reservoir to the counting chamber which contained the experimental prawn. The outlet tube carried the medium back to a beaker on the bench at a rate of about 15 ml/min. A stream of air produced by a small aquarium pump was used to drive the circulation.

The advantage of this apparatus was that the washing medium could be changed easily and rapidly without disturbing the experimental prawn in the counting chamber. The temperature and salinity of the circulatory medium could be varied as required. With the animal *in situ* counts were made at zero time and at five-minute intervals.

Two factors can influence the accuracy of the count, the number of disintegrations observed and the geometry of counting.

In efflux experiments, the number of counts recorded was sufficient to keep the statistical error under ±2 per cent. The second potential source of error was due to the change in position of the prawn within the animal chamber affecting the geometry. However this source of error was minimized by developing the chamber so that movement of the animal was restricted. In the experiment, prawns size ranging from 1.8 to 3.7 g were used. The mean rate constant for the efflux of sodium, and the half time values of prawns acclimated to 5° and 15°C and washing out at 15°C in 100% SW are presented in Table 11.1.

1.2 Loss of sodium-22 from prawns acclimated to 5°C washing out into 100% SW at 15°C.

Prawns were acclimated to 5°C in 100% SW for three days prior to loading in 100% SW containing sodium-22 at 5°C. The experimental arrangement, the loading procedure, and the washing out technique used were the same as those in the experiment 1.1.

The rate constant for the efflux, and the half time values are given in Table 11.1. In comparison with the results in the experiment 1.1, prawns acclimated to 5°C tend to have a faster rate of sodium efflux than those acclimated to 15°C. The difference is statistically significant ($t = 2.8641$, $P < 0.01$). The results suggest that the increase in internal sodium in haemolymph from 365 ± 7.1 (n=18) to 391 ± 18.9 (n=18, section 1, chapter 5) results in a rising total efflux rate from 0.4455 ± 0.0866 to 0.5446 ± 0.0871 hr^{-1}. This means that when the equilibrium of the haemolymph sodium is disturbed by a sudden transfer from 5° to 15°C the prawn adjusts the rate of loss of sodium, both by extra-

Table 11.1 Rate constant and half time value for sodium efflux of prawns acclimated to 5°, and 15°C, washing out at 15°C in 100% SW.

No.	Prawn acclimated to 5°C			Prawn acclimated to 15°C		
	Wet weight (g)	K (hr^{-1})	$t_{\frac{1}{2}}$ (hr)	Wet weight (g)	K (hr^{-1})	$t_{\frac{1}{2}}$ (hr)
1	2.18	0.6499	1.07	2.19	0.5545	1.25
2	1.83	0.6254	1.10	2.05	0.5942	1.17
3	2.15	0.6116	1.13	1.98	0.5199	1.33
4	2.12	0.4673	1.48	3.41	0.4159	1.67
5	2.07	0.6208	1.12	2.60	0.5472	1.27
6	1.83	0.5041	1.37	2.36	0.4159	1.67
7	2.05	0.5298	1.31	1.98	0.4288	1.62
8	2.45	0.3554	1.95	2.93	0.3554	1.95
9	2.57	0.6398	1.08	1.83	0.3616	1.92
10	3.29	0.5041	1.36	2.20	0.3585	1.93
11	3.73	0.5199	1.33	2.10	0.4424	1.57
12	3.39	0.5074	1.37	1.91	0.3524	1.97
$\bar{X}$		0.5446	1.31		0.4455	1.61
SD		± 0.0871	±0.25		± 0.0866	±0.30

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
Acclimated at 5°C	1.83 - 3.73	0.5446 ± 0.0871 ($\bar{12}$)	22	2.8641	$P < 0.01$
Acclimated at 15°C	1.83 - 3.41	0.4455 ± 0.0866 ($\bar{12}$)			

renal and renal routes, also the rate of uptake across the gut (section 2.6, chapter VII) as well as the rate of urinary water losses via the excretory pores (section 11, chapter X).

2. Effect of a sudden change of temperature from 15° to 5°C on the rate of sodium efflux from prawns kept in 100% SW.

While considering the question of ionic regulation of the prawn, it is essential to know how factors other than salinity might affect the process of ionic adjustment. Among these external factors, the temperature is important. As shown in chapter V, temperature has some influence on the sodium concentration in the haemolymph of the prawn; thus one would expect temperature changes to affect the rate of movement of sodium ions between the prawn and its medium.

This experiment was carried out with the object of clarifying the nature of sodium efflux of prawns with special reference to the effect of a sudden change of temperature. Prawns were acclimated in 100% SW at 15°C for three days prior to loading in 100% SW containing sodium-22 at 15°C. The experimental arrangement, the loading procedure, and the washing technique were the same as those used in experiment 1.1 accept that at the end of two hours of washing out at 15°C in 100% SW the medium was changed to 100% SW at 5°C. The effect of temperature on efflux was assessed by comparison of the efflux of sodium at 15°, and 5°C on the same animal.

The rate constants of sodium efflux at the two temperatures are given in Table 11.2. Each pair of values of K represent the

Table 11.2 Relative efflux of sodium from prawns kept in 100% SW at $15^{o}$and 5^{o}C.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1}) 15^{o}C	5^{o}C	Relative efflux (% of 15^{o}C)	Half time efflux 15^{o}C (hr)	5^{o}C (hr)
1	2.79	0.4159	0.2772	66.65	1.67	2.50
2	2.59	0.3409	0.2446	71.75	2.03	2.83
3	2.06	0.2929	0.1386	47.34	2.37	5.00
4	2.37	0.3923	0.1907	48.61	1.77	3.63
5	2.14	0.3524	0.2683	76.13	1.97	2.58
6	2.66	0.4332	0.1934	44.64	1.60	3.58
7	2.56	0.3327	0.2536	76.22	2.08	2.73
$\bar{X}$		0.3657	0.2237	61.63	1.93	3.26
SD		$\pm$ 0.0494	$\pm$ 0.0500	$\pm$ 14.23	$\pm$ 0.28	$\pm$ 0.89

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
15^{o}C	2.06 - 2.79	0.3657 $\pm$ 0.0494 ($\bar{7}$)	12	5.3787	P 0.001
5^{o}C	2.06 - 2.79	0.2237 $\pm$ 0.0500 ($\bar{7}$)			

rate constant from the same animal at two temperatures. When the prawns were subjected to a sudden change of temperature from 15° to 5°C, the rate constant for efflux of sodium was reduced from 0.3657 ± 0.0494 to 0.2237 ± 0.0500 hr^{-1}. The reduction on the average was about 38.87 per cent of the value at 15°C. It has been shown in experiment 12, chapter X, that there is a significant reduction of urine production from 10.6 ± 1.6 % to 4.6 ± 1.4% body wt/day when the animals were transferred from 15° to 5°C. This suggests that, when a steady state of sodium in the prawns is disturbed by a sudden change of temperature from 15° to 5°C, the rate of loss of ions from the gills as well as from the excretory pores is reduced. If such thermal disharmonization exceeds the ability of the animal to compensate for the effect it will lead finally to a complete breakdown of the regulatory process.

3. Effect of the concentration of the external sodium on the efflux of sodium from prawns kept at 15°C.

The ability of certain species of prawn and shrimp to withstand sudden changes of salinities is a matter of interest to shrimp farmers and biologists alike. Most marine prawns which are suitable for culture are hypotonic to sea water and hypertonic to brackish water. In their natural habitat, these prawns are subjected to wide fluctuations in salinity, not only on a seasonal basis but also diurnal variations. In the latter case the animals do not have time to approach their acclimated state. Relatively large changes in salinity also tend to occur in shrimp farms in tropical countries in which the animals may be subjected to heavy rain during the summer, so that the water of the pond is suddenly changed

from high salinity to low salinity. To survive in such variable environments, the animal must have mechanisms to respond to fluctuations in the medium so as to maintain the haemolymph osmolarity above or below that of the surrounding medium as appropriate. The purpose of this investigation is to determine the role of external salinity on the outflux of sodium from the prawn.

3.1 Efflux of sodium-22 from prawns after a rapid transfer from 100% to 50% SW.

If adjustment to dilution of the medium is partly or totally achieved by reducing the total efflux in dilute salines, either by reducing the body surface permeability to sodium or by reducing the loss of sodium in the urine, this should be revealed when the rate constants of sodium efflux from animals washing out into 100% SW and 50% SW are compared.

The experimental arrangement and the loading technique used were the same as those in the previous experiments. The rate of sodium efflux from prawns in 50% SW was measured. The results obtained were compared with the losses into 100% SW. The rate constant of sodium efflux and the half time for sodium loss in this experiment are given in Table 11.3. Each pair of K values represent the rate constant of efflux for the same animal in the two media. The sodium efflux of prawns acclimated to 15^{o}C and washing out in 50% SW is about 0.1870 $\pm$ 0.0141 hr^{-1} or 49.22% of those kept in 100% SW.

3.2 Efflux of sodium-22 from prawns after a sudden transfer from 100% to 70% SW.

Prawns which had been fully acclimated to 100% SW for

Table 11.3 Relative efflux of sodium from prawns in 50% and 100% SW at 15°C.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	50% SW		100% SW (hr)	50% SW (hr)
1	2.10	0.4424	0.1769	39.98	1.57	3.92
2	1.82	0.3616	0.1856	51.32	1.92	3.73
3	2.20	0.3585	0.1777	49.56	1.93	3.90
4	1.91	0.3524	0.1856	52.66	1.97	3.73
5	2.89	0.3616	0.1777	49.14	1.92	3.90
6	3.15	0.4159	0.2189	52.63	1.67	3.17
$\bar{X}$		0.3820	0.1870	49.22	1.83	3.72
SD		± 0.0374	± 0.0141	± 4.76	± 0.17	± 0.28

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW	1.82 - 3.15	0.3820 ± 0.0374 $(\bar{6})$	10	13.8297	$P < 0.001$
50% SW		0.1870 ± 0.0141 $(\bar{6})$			

three days, were transferred to a loading medium of 100% SW to which sodium-22 has been added to give the required specific activity. The loading technique and the washing out experiment used were the same as those in the previous experiments except that after two hours washing out into 100% SW, the washing medium was changed to 70% SW. The results of sodium efflux of the experiment are given in Table 11.4. The rate of sodium efflux in 70% SW was 0.2383 $\pm$ 0.0264 hr^{-1} or about 65.52 per cent of the control efflux in 100% SW.

3.3 Efflux of sodium-22 from prawns after a rapid transfer from 100% to 10% SW.

In preceding experiments the results suggested that the sodium efflux is likely to be dependent on the sodium concentration in the external medium. This experiment was designed for further observation on the sodium efflux in a very dilute salinity, 10% SW.

Prawns used in the experiment were loaded in 100% SW containing sodium-22 at 15^{o}C. The loading procedure and washing out techniques are the same as those in experiment 3.1 except that after two hours washing out in 100% SW, the medium was changed to 10% SW. the rate constant of efflux, and the half time values in two media are given in Table 11.5. The rate of efflux of sodium in 10% SW is very slow. It is only 24.96 $\pm$ 8.06% of the efflux in 100% SW.

Table 11.4 Relative efflux of sodium from prawns in 70% and 100% SW at 15°C.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	70% SW		100% SW (hr)	70% SW (hr)
1	2.55	0.3640	0.2772	75.98	1.90	2.50
2	3.35	0.3713	0.2363	63.64	1.87	2.93
3	2.51	0.3409	0.2189	64.21	2.03	3.17
4	2.31	0.3616	0.2038	56.36	1.92	3.40
5	2.02	0.3781	0.2418	63.95	1.83	2.87
6	2.58	0.4621	0.2712	58.68	1.50	2.55
7	4.39	0.2888	0.2189	75.79	2.40	3.17
$\bar{X}$		0.3666	0.2383	65.52	1.92	2.94
SD		± 0.0509	± 0.0264	± 7.67	± 0.27	± 0.33

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW	2.02 - 4.39	0.3666 ± 0.0509 $(\bar{7})$	12	6.4150	$P < 0.001$
70% SW		0.2383 ± 0.0264 $(\bar{7})$			

Table 11.5 Relative efflux of sodium from prawns washing out in 100%, and 10% SW at 15°C.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	10% SW		100% SW (hr)	10% SW (hr)
1	2.06	0.3706	0.0885	23.88	1.87	7.83
2	3.12	0.3787	0.1093	28.86	1.83	6.33
3	2.51	0.3414	0.0660	19.33	2.03	10.50
4	2.74	0.3787	0.1123	33.72	1.83	6.17
5	2.34	0.3466	0.1093	31.53	2.00	6.33
6	2.06	0.5059	0.0630	12.45	1.37	11.00
$\bar{X}$		0.3869	0.0914	24.96	1.82	8.03
SD		± 0.0600	± 0.0223	± 8.06	± 0.24	± 2.20

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW	2.06 - 3.12	0.3869 ± 0.0600 ($\bar{6}$)	10	12.1106	$P < 0.001$
10% SW		0.0914 ± 0.0223 ($\bar{6}$)			

Knowing the Cr-51 EDTA clearance rate (Table 10.5), U/H for Cr-51 EDTA (Table 10.4), U/H ratio for sodium (Appendix 12), and the total sodium efflux (Appendix 13) the rate of urinary sodium loss for P. serratus in different osmotic environments can be calculated from the following equation:

$$\frac{\text{Cr-51 EDTA} \times \text{U/H for sodium} \times \text{haemolymph } Na^+ \text{ mM/l}}{\text{U/H for Cr-51 EDTA} \times 100} \quad \ldots\ldots \text{(Lockwood \& Riegel, 1969)}$$

The rates at which sodium is lost from the body in terms of mM Na^+ lost/l haemolymph/hr are listed in Table 11.6.

Table 11.6 The urinary loss of sodium of prawns acclimated in three salinities.

Medium % SW	Total sodium loss mM/l haemolymph/hr	Urinary sodium loss mM/l haemolymph/hr	Urinary sodium loss % total sodium loss
100	139	1.1	0.79
70	81	1.1	1.40
50	55	2.5	4.50

Urinary sodium loss in P. serratus in 100% SW is very small, 0.79% of the total sodium loss. In isosmotic medium the prawn loses its sodium in urine about 1.4% of the total sodium loss (measured as efflux) which agrees with the rate of urinary loss in P. varians, about 1.4% of its total sodium loss (Potts and Parry 1964b), but it is less than those in C. maenas, about 3% of the total sodium efflux (Shaw, 1961). In 50% SW the urinary loss of sodium from the prawn is about 4.5% of the total sodium loss.

4. Sodium-potassium pump.

It has been shown in section 3 that the sodium efflux of the prawn is reduced with decreasing salinity which seems to suggest that the sodium extrusion is sensitive to environmental potassium

concentration, the external $[Na^+]$ competes with $[K^+]$ on the potassium site. In an effort to extend the physiological study of sodium extrusion mechanisms to P. serratus the following experiments were performed.

4.1 Effect of external potassium ion on sodium efflux.

Prawns were acclimated to 15°C in 100% SW for three days, then loaded in medium containing sodium-22 as described in previous experiments. After 36 hours in loading medium, the prawn was transferred to the animal chamber and washed with potassium free sea water. The results are given in Appendix 7, and Figure 11.1 is a typical washing out experiment.

It can be seen that when the potassium influx was abolished by removing all the potassium normally present in the medium the sodium efflux from the prawn was reduced. The reduction is about 27.33% of its normal value. The results support the idea of sodium movement coupled with potassium moving in the opposite direction (Stein, 1967). Thus for each sodium ion that is extruded to the outer medium without an anion, potassium must enter to maintain electro-neutrality. Thus under normal condition in sea water there is an Na-K exchange. This conclusion receive more support from the results in experiments 4.2, and 4.3. The efflux of sodium that occurred in the absence of potassium ion is attributed to the sum of the system of exchange sodium for sodium exclusively and to loss of sodium in the urine.

4.2 Efflux of sodium from prawns in 10 mM-K/l saline.

From the results of experiment 4.1 it was shown that the efflux of sodium from prawns showed a marked reduction if all of

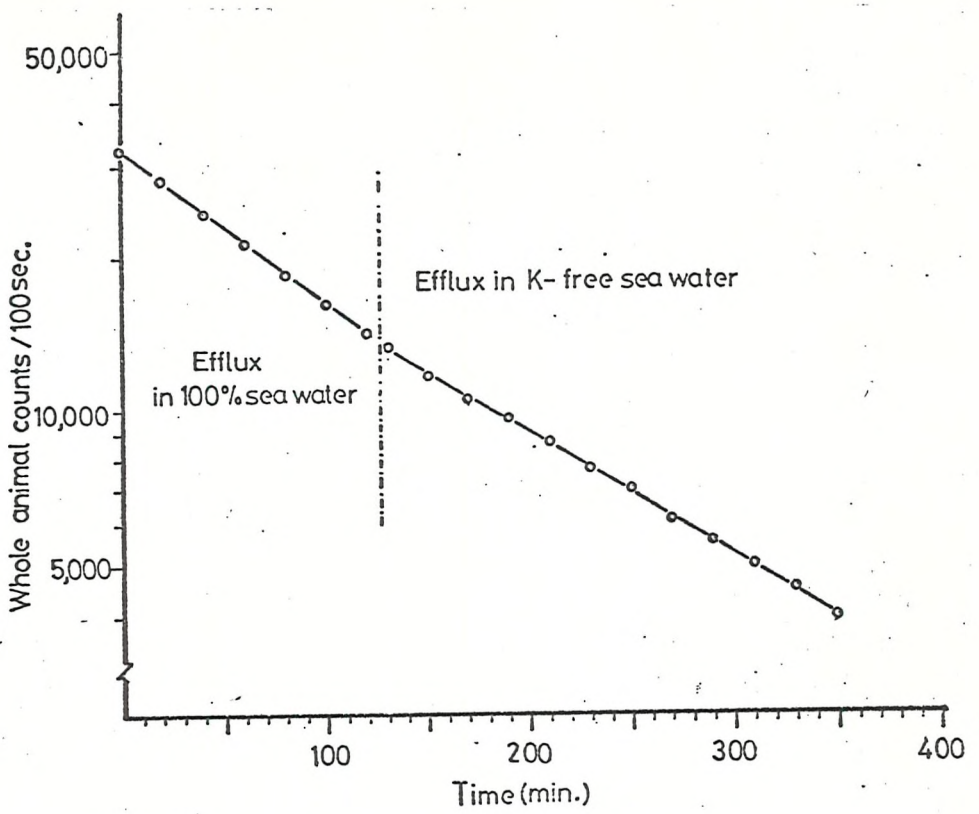

Figure 11.1 The total efflux of sodium from a prawn washed out into 100% SW for two hours and then the medium was changed to K-free sea water.

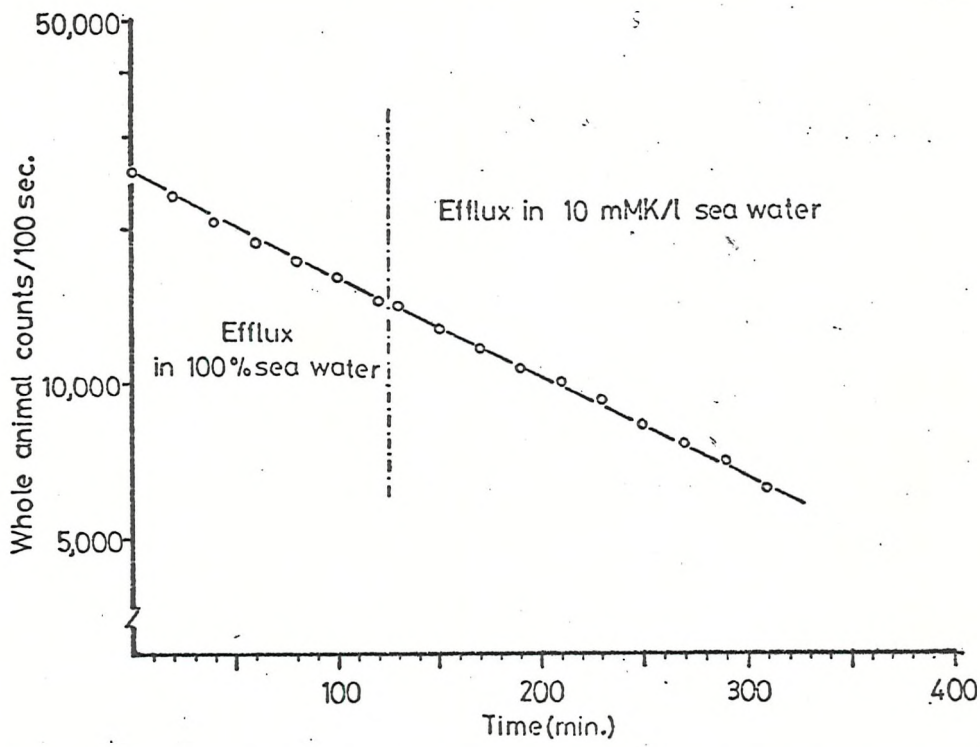

Figure 11.2 The total efflux of sodium from a prawn washed out into 100% SW for two hours and then the medium was changed to 10 mM-K/l saline.

the potassium ion in the medium was removed. Another experiment was, therefore, carried out to observe whether the efflux of sodium would return to the normal rate if 10 mM/l potassium was added to the potassium free sea water.

Prawns were acclimated and loaded in 100% SW containing sodium-22 at 15^{o}C. After two hours washing out in 100% SW the medium was changed to 10 mM-K/l saline. The typical washing out experiment is shown in Figure 11.2. The results in Appendix 8 show that when 10 mM/l potassium is added to the K-free sea water medium the efflux of sodium is increased to 98.3% of the normal rate shown in sea water control. This suggests that the addition of the external potassium ion fully restores the capacity of the animal to extrude sodium.

The effect of K-free saline on sodium efflux is similar to that observed in the fat sleeper, Dormitator maculatus, by Evans et al. (1973). In this experiment, the results suggested that in normal sea water sodium is extruded against its electro-chemical gradient. Potassium may then flow in by a passive movement to replace sodium. The experiment has shown that the efflux of sodium from prawns was reduced about 27.33% when all of the external potassium concentration was removed.

4.3 Effect of ouabain on sodium efflux of prawns kept in normal sea water.

The cardiac glycosides, Strophathin-G (ouabain), first shown to inhibit ion transport by Schatzmann(1953) and later by Kayner & Weatherall (1957), Glynn (1957), and Weatherall (1962), is known to affect sodium transport by blocking transport while

having no discernible effect on cellular energy metabolism. An experiment was designed to study the effect of ouabain on the sodium efflux in the prawn.

Prawns were acclimated to 100% SW at 15°C and loaded with sodium-22 by the same procedure as those in experiment 4.1. After two hours washing out in 100% SW, the medium was changed to sea water containing 10^{-5}M ouabain. The results of the experiment are given in Appendix 9. A typical washing out experiment is shown in Figure 11.3.

When 10^{-5}M ouabain is added to the external medium, 100% SW, there is a significant reduction of sodium efflux. The effect takes some minutes for its full manifestation. By comparison with the rate constant of sodium efflux in 100% SW, the reduction amounts to 19.43%. This is a value closely comparable to the reduction occuring in the absence of potassium from the external medium which suggests that the Na : K extrusion system has been inhibited by the ouabain. The present results are in agreement with that reported by Evans et al. (1973) in the fat sleeper, Dormitator maculatus. This evidence seems to support the concept suggested by Kirschner (1969), and Maetz (1971), that the ouabain sensitive pump is located on the external membrane with the K^+ site facing outward. Ouabain would operate through preventing Na^+ : K^+ exchange, probably by physically occupying a critical place on the membrane. This pump seems to be responsible for a substantial fraction of the sodium transport in the prawn as Motais and Isaia (1972) suggested in euryhaline eel, Angilla angilla, and in the fat sleeper, D. maculatus by Evans et al. (1973).

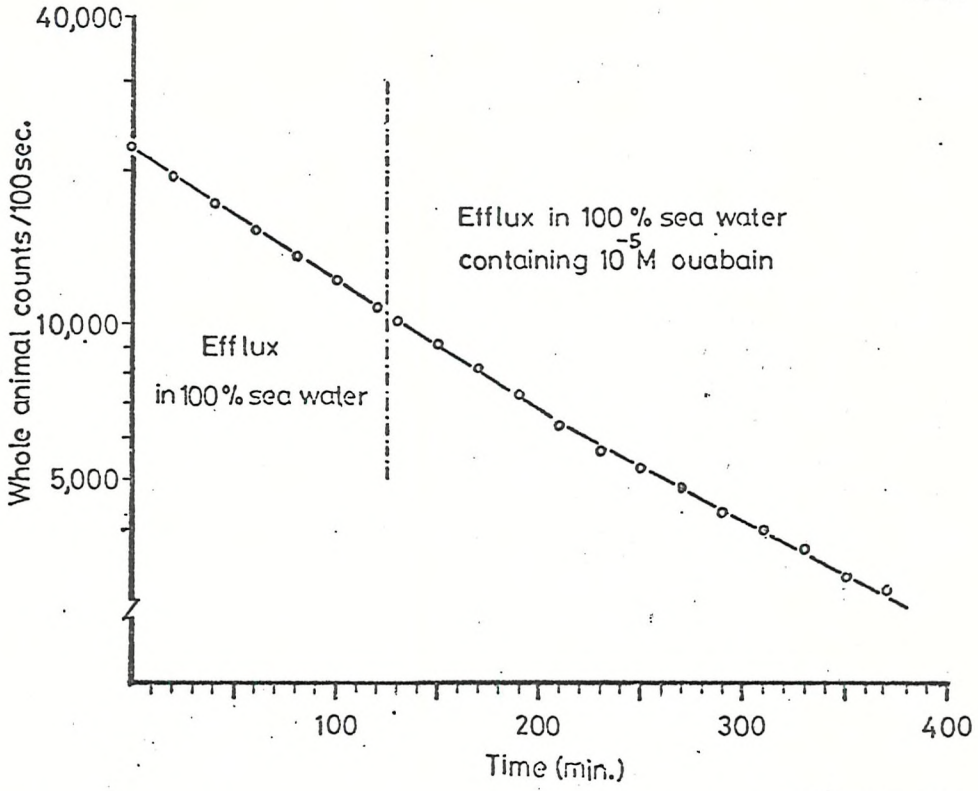

Figure 11.3 The total efflux of sodium from a prawn washed out into 100% SW for two hours and then the medium was changed to 100% SW containing 10^{-5}M ouabain.

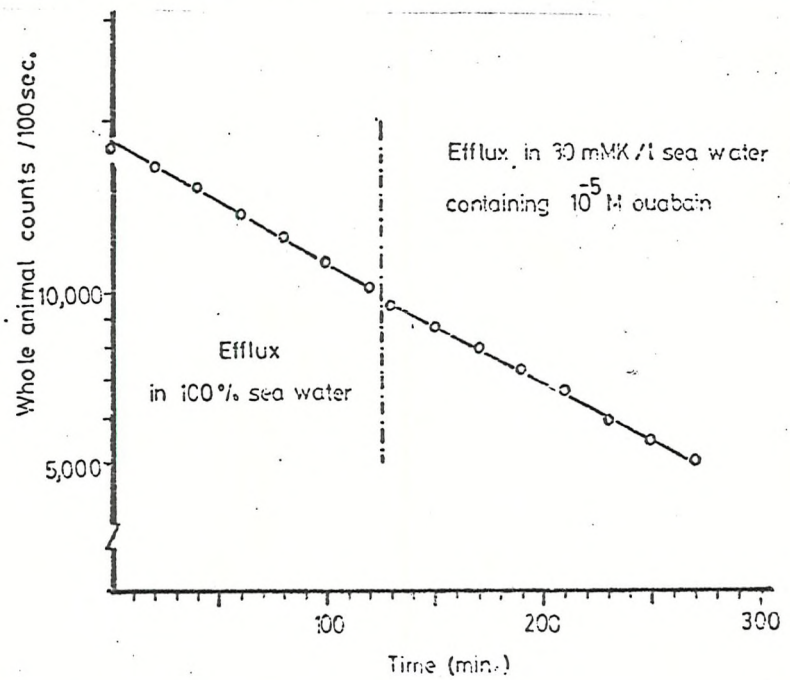

Figure 11.4 The total efflux of sodium from a prawn washed out into 100% SW for two hours and then the medium was changed to 30 mM-K saline containing 10^{-5}M ouabain.

4.4 Effect of ouabain on sodium efflux from prawns in normal sea water with addition of 30 mM-K/l.

The experiment 4.3 has shown that when the 10^{-5}M ouabain was added to the external medium the sodium efflux from the prawn was reduced by about 19.43% of the control efflux. However, Glynn (1957) working on the human red corpuscle found that the inhibition by cardiac glycoside can be reversed by increasing the external potassium concentration. This behaviour would be expected if potassium and ouabain competed for the same sites so that excess potassium displaced ouabain and reversed the inhibition. To examine whether this is true in case of the prawn, the following experiment was performed.

A similar procedure was carried out as experiment 4.3. A single fully load prawn was washed out in inactive 100% SW for a period of two hours, the medium was then changed to 100% SW with addition of 30 mM-K/l, and 10^{-5}M ouabain. The washing procedure continued for a further three hours. The results are presented in Appendix 10, and a typical washing out experiment is shown in Figure 11.4. The addition of 30 mM-K/l to normal sea water with 10^{-5}M ouabain stimulated the efflux sodium. The rate of efflux of sodium is expressed as a percentage of the mean rate of the control efflux. The reversible effect of ouabain in excess potassium medium may be considered to support the concept of ouabain-K competition. The evidence suggest that the site of action of the drug appears to be the ion pump itself. This agrees with Glynn's (1957) observation that digoxin inhibition of K^+ influx into red cells was inversely proportional to the saline K^+ concentration.

5. Discussion.

The most obvious explanation for reduction in the sodium efflux, when an external ion is removed, is that there is some form of coupling between the outward movement of sodium ions and the inward movement of the ion in question. The results of the preceding experiments are able to show a feature of sodium transport, the coupled sodium and potassium exchange. This form of sodium transport is illustrated by the results of the experiments in 4.1, and 4.2 where it can be shown that the external potassium is needed for transport of sodium.

The second feature of the sodium transport in the prawn is its asymmetry with respect to the actions of the sodium and potassium ions. The best illustration of this asymmetry is shown in the experiment 4.3 which showed that ouabain inhibits sodium efflux from the animal when the drug is present in the external medium. The site of action of the drug appears to be the ion pump itself, since it has been shown in experiment 4.4 that the effect may be reduced by increasing the potassium concentration in the external medium.

The evidence, therefore, suggests that when in 100% SW *P. serratus* utilised in part a system of forced exchange of potassium for sodium during the active extrusion of sodium.

XII. NEUROENDOCRINE INVOLVEMENT IN SODIUM AND WATER REGULATION

Crustacean endocrinology has its beginnings in the latter half of the 1920's. The early efforts in this area were essentially directed toward identification of the source of the hormones and their functions. Of major significance to investigation in this field is the fact that many of the crustacean hormones are neurosecretory products. They are produced by nerve cells and transported along their axons for ultimate release into the haemolymph instead of being produced by non-neuronal glandular structures (Fingerman, 1970).

It has been demonstrated by many investigators that a number of diverse processes in decapod crustaceans, molting, ovarian developing stage, and shell colour changes, are under hormonal control (Brown and Jones, 1949; Carlisle, 1953a, 1953b; Demeusy, 1962; Echalier, 1955, 1956; Lenel and Veillet, 1951; and Passano, 1951, etc.). While much experimental work has been done by the above authors on those fields, little attempt has been made to investigate the hormonal control of ionic transport in the decapod crustaceans.

The hormonal control of the water economy of crustaceans was first indicated by bilateral eyestalk ablation experiments. Abramowitz and Abramowitz (1940) observed that eyestalkless Uca pugilator were larger after molting than normal controls. Using precise techniques Scudamore (1942, 1947)found that the removal of eyestalks or sinus glands, in the crayfish, Cambarus immunis resulted in increased weight and a greater water content. These

changes were prevented by the implantation of the sinus gland in eyestalk ablated animals. The evidence suggested the presence of a neuroendocrine factor in the sinus glands which regulated water content of the animal during the molting phase. The neuroendocrine involvement in water metabolism was further suggested by Guyselman (1953). He reported a daily rhythmic fluctuation in the weights of Uca, and assumed weight increases were directly due to water uptake. After the removal of eyestalks, the fluctuations in weight became arhymthmic. Consequently, he postulated that the diurnal rhythm of water uptake in Uca was under control of a hormone from the X organ - sinus gland complex.

Koch (1952) has shown that the greater increase in volume following molts in eyestalkless animals is not due to greater formation of new tissue, but simply to greater water intake. Carlisle (1956), working with the shore crab, Carcinus maenas, reported results similar to those of Scudamore (1947) in that removal of eyestalks resulted in greater increases in both weight and water content. These changes were prevented by injection of an aqueous extract of the sinus glands. The distinction between the molt inhibiting hormone and the water balance hormone was later substantiated by Passano & Jyssum (1963) in the same species of crab. They transplanted activated Y organs from eyestalkless crabs into juvenile crab stages and obtained precocious molting in the recipients. More recently, Rangaroa (1966) isolated and characterized the molt inhibiting hormone of the crab Ocypode macrocera. He demonstrated its effectiveness in inhibiting molt in eyestalkless animals, but found it to be without effect on premolt water uptake.

Using the land crab, Gecarcinus lateralis, Bliss et al. (1966) concluded that the central nervous system produced a diuretic hormone, probably released by the sinus glands that, after molting, causes an output of the previously retained water. These investigators also postulated that an antidiuretic hormone is responsible for the original uptake and retention of the water.

The presence of a neuroendocrine factor in the haemolymph of crustaceans is also supported by the work of Dandrifosse (1966). He found that the water flux across the integument of the crab Maia squinado can be increased by the addition of haemolymph from an animal undergoing ecdysis. Such a hormone may arise from the ventral ganglion since in in vitro preparations of the foregut of land crab, Gecarcinus lateralis, the addition of ventral ganglionic extracts to the haemolymph side results in an increased permeability of the foregut to water and salts (Mantel, 1967).

The results of other experiments suggest that the water economy of the intermolt animal is also under the control of a hormone released from the eyestalk. For example, nephropore plugged, eyestalk ablated fresh water crayfish, Procambarus clarkii, showed a significant increase in weight over controls when placed in tap water (Kamemoto, et al., 1966). The same results have been obtained for an intertidal crab, Metapograpsus messor. That is, plugging the nephropores of destalked animals causes them to gain weight faster than normal controls when placed in 25% SW (Kato & Kamemoto, 1969). This conclusion was confirmed by Kamemoto and Ono (1968) who showed that the urine flow increases from 3.6% body wt/day in normal animals to 6.1% in eyestalkless individuals.

Bilateral eyestalk ablation in Chinese wool-handed crab, Eriochier sinensis, caused the urine flow to increase from 18.6% to 47.1% of the body wt/day in fresh water and from 11% to 21% in sea water (De Leersnyder, 1967).

Therefore, there is good support for the hypothesis that there is a factor in the eyestalk that affects the urine flow in decapod crustaceans. Kamemoto et al. (1966) believed that the increase in urine flow is a secondary result of an increase in integumental water permeability. However, the only experimental results directly supporting this hypothesis are those reported by Thompson (1967) where, following eyestalk ablation, there was an increase in the tritiated water influx in a fresh water crab, Pseudothelphusa jouyi.

One criticism of the urine flow data is that the animals were not given sufficient time to recover from the operation. The data from Kamemoto and his associates were collected for the first 24 hours immediately following operation or ligation. De Leersnyder (1967) let the animals recover for only 24 hours. Therefore, many other operation-associated factors could have contributed to the observed changes in urine flow. A further complication is that the operation removes only the release site of hormones most of which are produced in the central nervous system. Therefore, continued production would be expected.

In interpreting some of this data Lockwood (1968) suggested that the apparent action of eyestalk removal on the water balance may be an artefact due to the precipitated molt and not any factor actively mediating cuticular permeability. For example, the increase in urine flow may be due to increased drinking, which is

a common method for water uptake prior to molting in many crustaceans.

Changes in the haemolymph electrolyte and osmotic concentrations following eyestalk ablation have also been studied. In a grapsoid crab, Metapograpsus messor, the operation results in a decrease in the haemolymph osmotic concentration when the animals are placed in a hypoosmotic medium, and an increase in haemolymph concentration when they are placed in a hyperosmotic medium (Kamemoto et al., 1966). In other words, the haemolymph concentration tends towards that of medium. In the fresh water crayfish, Procambarus, the same operation results in a decrease in the haemolymph chloride concentration of about 3% (Kamemoto et al., 1966), and a drop in the haemolymph osmotic concentration of about 3.9% (Peterson and Loizzi, 1970). Bryan (1960a) observed an 11% drop in the haemolymph sodium concentration following eyestalk ablation in the fresh water crayfish, Astacus fluviatilis. He found no change, however, in the net uptake of sodium in eyestalk ablated animals over controls, and suggested that the eyestalks do not exert any direct influence over sodium balance. Ramamurthi and Scheer (1967), on the other hand, have demonstrated the existence of a substance in the cephalothorax of the shrimp, Pandalus jordani which decreases the sodium efflux in a shore crab, Hemigrapsus nudus.

Recently, attempts have been made to isolate the osmotically active substances. Kamemoto and Tullis (1972), using Sephadex G-50 chromatography, have partially isolated the substance from the brain of a fresh water crayfish, P. clarkii, that causes increases in both chloride and sodium influx rates. This is suggested by

increased haemolymph concentration for the former ion and increased radioactive count uptake for the second ion when the animals are in 100 mM NaCl baths.

In many tissues the enzymatic basis for the active transport of the cations Na^+, K^+ is a Na^+, K^+ activated, Mg^{++} dependent, ouabain sensitive ATPase system. Such an enzyme system has been found in the gills of the land crab, Cardisoma guanhumi (Quinn and Lane, 1966), a grapsoid crab, Metapograpsus messor (Kato, 1968), and a brine shrimp, Artemia salina (Augenfeld, 1969). In Metapograpsus the enzyme activity increases as the salinity of the medium decreases. In theory this change in ATPase could be related to the increasing Na^+ gradient since the animal is very hyper-osmotic in dilute water. In Artemia gills the enzyme activity is the reverse, i.e., it is five times higher at 400% SW than at 50% SW.

In Metapograpsus bilateral eyestalk ablation decreases the enzyme activity and injections of brain homogenate restores it. In the crayfish P. clarkii the enzyme is 3 to 4 times as active in the tubule as in the labyrinth or the coelomosac. Eyestalk removal decreases the activity while injections of eyestalk extracts increase the activity and brain extracts decrease the activity (Kamemoto and Tullis, 1972).

The present investigation was conducted to determine the possibility of the involvement of neuroendocrine systems on sodium and water regulation.

1. Effect of eyestalk removal on weight changes of prawns.

To determine the effects of eyestalk removal on the weight of prawns, the eyestalks of prawns from 100% SW were ligatured at

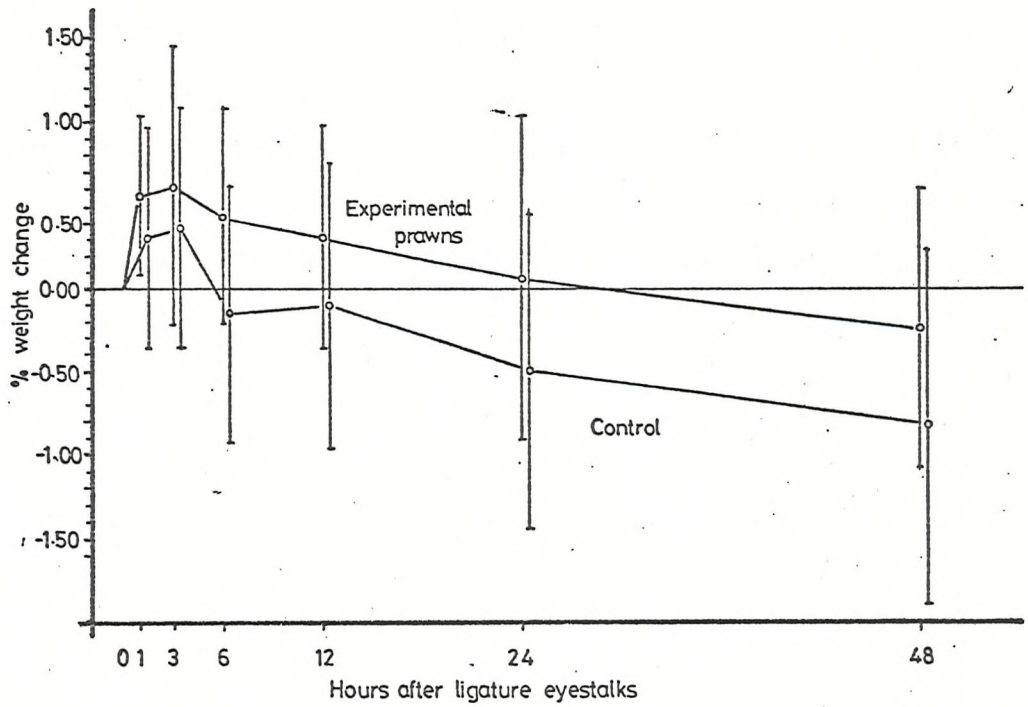

Figure 12.1 Weight change of prawns in 100% SW following ligaturing of the eyestalks and their excretory openings were not blocked.

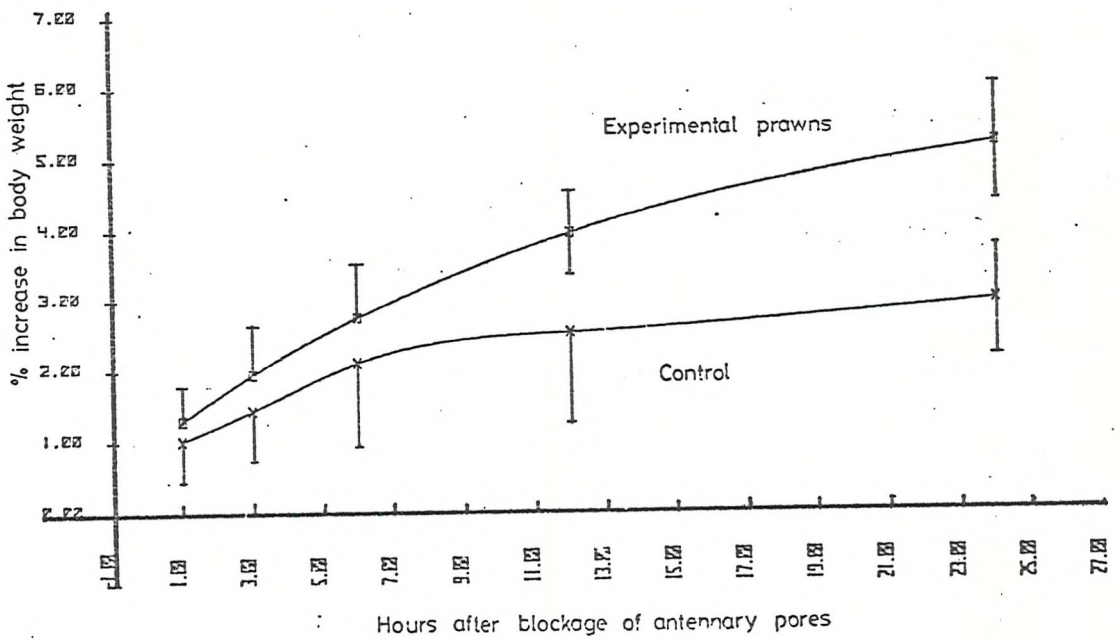

Figure 12.2 Changes in weight in normal and eyestalk-ligatured animal kept in 100% SW at 15°C after occlusion of excretory pores.

their bases and the prawns were then weighed and replaced in the medium. Subsequent weighings were taken periodically.

The results are presented in Figure 12.1. There is an increase in weight of the prawns with ligatured eyestalks as well as in the control during the first three hours, but the weight decreases to normal over the six hour-period. The initial increase in weight seems to be due either to water uptake which is subsequently eliminated by the antennal glands, as suggested by the results presented in Figure 12.2, or the animals stop releasing urine during that period as a result of handling effects.

A greater increase in weight of animals with ligatured eyestalks, and unblocked antennal pores when compared with the control as shown in Figure 12.1 accords with the findings of Scudamore (1942, 1947) in the crayfish, Cambarus immunis, which suggests that the removal of eyestalk caused a greater influx of water. In theory, the eyestalks could release an antidiuretic factor into the haemolymph which, when removed from the circulation by eyestalk ablation, results in an increase in the rate of water reabsorption probably both across the gut and the urinary bladder.

2. Changes in weight of prawns in 100% SW after occlusion of excretory pores.

Following occlusion of the excretory openings, both normal and eyestalk ligatured prawns showed an increase in weight. The serial increase in weights of these animals, when kept in 100% SW are illustrated in Figure 12.2. The experimental animals appeared to experience a faster and greater weight increase than the normal

control. After 12 hours the weight increase of the former was significantly higher than that of the latter (Table 12.1). The increase in weight of P. serratus in sea water with their eyestalk ligatured as shown in the present study suggests that it is not due to a purely passive movement of water, but it is likely to involve an active process of water transport across the gut (further discussion is given in section 7, chapter XIII).

3. Effect of transfer of prawns from normal sea water to 70% SW.

Immediately following transference from normal sea water to 70% SW, all animals, both prawns with ligatured eyestalks and the normal controls showed an increase in weight. This is of interest, since in 70% SW the osmotic difference between the internal and external medium is nil, so in this case there is neither osmotic inflow nor osmotic outflow of water.

It has been shown that a lowering of the haemolymph concentration brings about an activation of the processes responsible for uptake of ions at the body surface of fresh water and the brackish water animals (Shaw, 1958, 1959a, 1960, 1961; Shaw & Sutcliffe, 1961; and Lockwood, 1961). The faster increase in weight of prawns with ligatured eyestalks as seen in the present experiment suggests that active intake of water is stimulated by eyestalk removal rather than just an increase in permeability of body surface to water. The possibility is that the sodium uptake mechanism of prawns at the gills is controlled by an eyestalk factor which, when it is removed from the circulation by eyestalk ablation, the rate of sodium uptake increases, and in turn elevates the rate of water intake.

Table 12.1 Weight increases of normal and experimental prawns kept in 100% SW, expressed as the percentage of the initial body weight, after occlusion of excretory pores.

Hours of blockage	Normal prawn	Eyestalk-ligatured prawn	t-value	probability
1	0.96 ± 0.60 (18)	1.21 ± 0.50 (18)	1.3204	NS
3	1.41 ± 0.75 (18)	1.80 ± 0.72 (18)	1.5897	NS
6	2.10 ± 1.23 (15)	2.74 ± 0.82 (14)	1.6062	NS
12	2.51 ± 1.34 (10)	3.94 ± 0.66 (5)	2.2137	$P < 0.05$
24	2.97 ± 0.83 (6)	5.20 ± 0.90 (5)	4.2467	$P < 0.005$

Table 12.2 Weight increases of normal and experimental prawns, expressed as the percentage of the initial body weight, after occlusion of excretory pores, and transfer from 100% to 70% SW.

Hours of blockage	Normal prawn	Eyestalk-ligatured prawn	t-value	probability
1	1.49 ± 0.38 (15)	1.75 ± 0.67 (15)	1.4080	NS
3	2.01 ± 0.44 (14)	2.84 ± 1.02 (15)	2.7970	$P < 0.01$
6	2.63 ± 0.53 (13)	3.91 ± 1.21 (12)	3.4692	$P < 0.005$
12	3.80 ± 0.87 (11)	5.47 ± 1.33 (12)	3.5344	$P < 0.005$
24	5.51 ± 1.29 (11)	7.95 ± 2.09 (12)	3.2956	$P < 0.005$

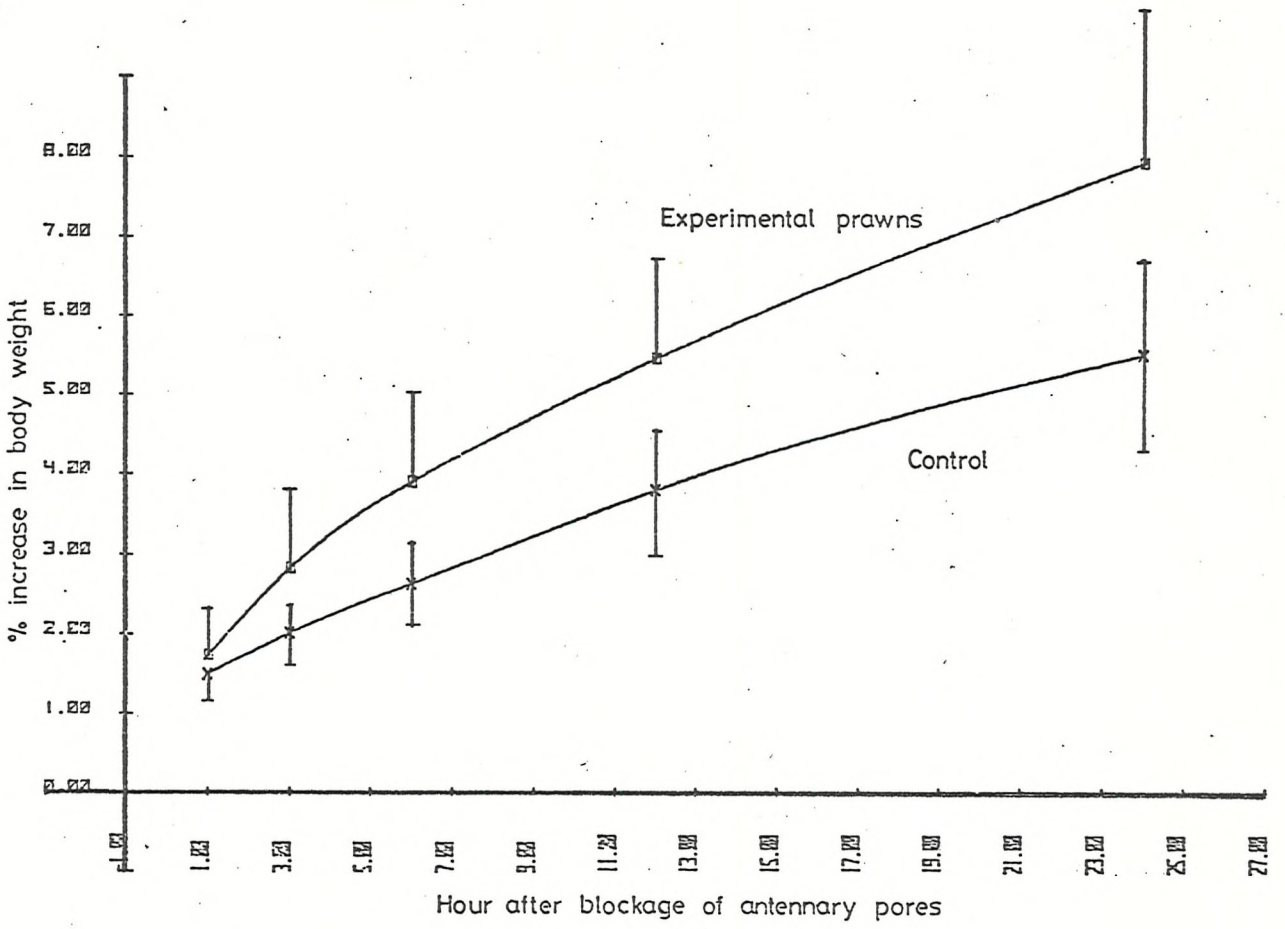

Figure 12.3 Changes in weight in normal and eyestalk-ligatured prawns after occlusion of excretory pores following transfer from 100% to 70% SW.

4. Effect of transfer of prawns from normal sea water to 50% SW

It has been shown in experiment 6 of chapter X that in dilute media the major route of the osmotic inflow of water is across the body surface, so if bilateral eyestalk ablation results in changes in water permeability of the body surface, differences in weight will be expected between the control and the experimental animals after sudden transfer from 100% to 50% SW.

The results are shown in Table 12.3 and in Figure 12.4. When the animals were subjected to a sudden change of salinity from 100% to 50% SW, the increase in weight, both of control and experimental animals, was not significantly different. This evidence suggests that ligaturing the eyestalks does not increase the net osmotic flux of water, and hence that the surface permeability is not substantially increased.

5. Urine production of eyestalk-ligatured prawns kept in 100% SW at 15^{o}C.

5.1 Weight-gained method.

The results are given in Table 12.4. The prawns with ligatured eyestalks showed a higher rate of increase in weight (15.35% body wt/day) than the control, 8.64% body wt/day.

5.2 Clearance of Cr-51 EDTA from the haemolymph.

The average urine flow of the normal prawns was about 10.56% body wt/day (SD $\pm$ 1.64, n = 15) while the ligatured animals produced urine at the rate of 9.87% body wt/day (SD $\pm$ 2.59, n = 18). The difference was not statistically significant (t = 0.9542, Table 12.5).

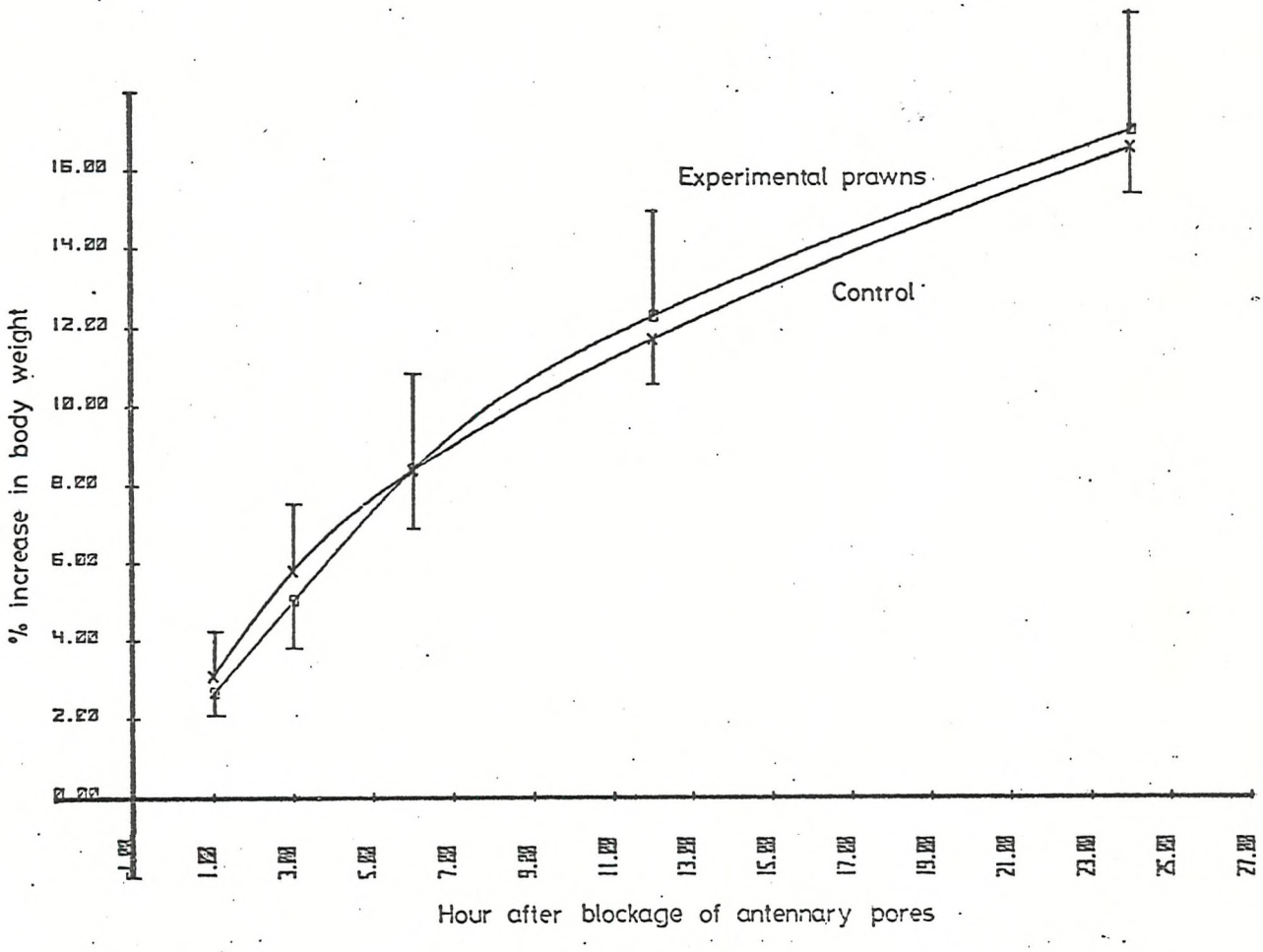

Figure 12.4 Changes in weight in normal and eyestalk-ligatured animals after occlusion of excretory pores following transfer from 100% SW to 50% SW.

Table 12.3 Weight increases of normal and eyestalk-ligatured prawns, expressed as a percentage of the initial body weight, after occlusion of excretory pores and transference from 100% to 50% SW.

Hours of blockage	Normal prawn	Eyestalk-ligatured prawn	Analysis of t-test df	t-value	probability
1	3.09 ± 1.02 ($\bar{15}$)	2.68 ± 0.66 ($\bar{15}$)	28	1.2763	NS
3	5.79 ± 1.83 ($\bar{15}$)	5.04 ± 1.03 ($\bar{15}$)	28	1.2763	NS
6	8.37 ± 2.59 ($\bar{15}$)	8.42 ± 1.67 ($\bar{15}$)	28	0.1091	NS
12	11.56 ± 1.69 ($\bar{13}$)	12.29 ± 2.80 ($\bar{9}$)	20	0.7576	NS
24	16.55 ± 1.67 ($\bar{5}$)	17.01 ± 3.13 ($\bar{6}$)	9	0.2929	NS

Table 12.4 Rate of urine flow of prawns with eyestalks ligatured, measuring by weight changes after blocking the excretory pores, and kept in 100% SW at 15°C.

Treatment	% increase in body weight (three hours after blocking) Excretory pores open	Blocked excretory pores	Urine production % body wt/hr	% body wt/day
Control	0.36 ± 0.75 ($\bar{15}$)	1.43 ± 0.82 ($\bar{15}$)	0.36	8.64
Eyestalk-ligatured prawn	0.01 ± 0.79 ($\bar{15}$)	1.94 ± 0.70 ($\bar{15}$)	0.64	15.35

The contrast between the rates of urine flow of prawns with ligatured eyestalks obtained from the weight-gained method, and the clearance of Cr-51 EDTA from the haemolymph as shown in Table 12.4 and 12.5 is of interesting. The faster increase in weight of the animals with ligatured eyestalks when they are in normal sea water as shown in experiment 5.1 is not due to an increase in their integumental water permeability as Kamemoto et al. (1966) interpreted in M. messor, but is rather due to a higher rate of active uptake of water across the gut.

6. Total efflux of Cr-51 EDTA from the whole animal.

Experiment 4, using the weight change method, indicated that there is no significant different between control and experimental animal in respect of weight increase following transfer from 100% to 50% SW. Further confirmation of this conclusion has been obtained from the results of the present experiment by comparison the total efflux of Cr-51 EDTA from the whole animal between prawns with ligatured eyestalks and the normal controls.

The method used in this study is similar to that in experiment 8, of chapter X. The results are shown in Table 12.6. The efflux of Cr-51 EDTA from the experimental prawns was not significantly different from that of the control. This result therefore also suggests that the urine production rate of the experimental prawns exposed to 50% SW is similar to those observed in the control.

7. Haemolymph sodium concentration of prawns after exposure to a sudden change of salinity from 100% to 50% SW.

The haemolymph sodium concentration for animals subjected

Table 12.5 Effect of eyestalk ligature on urine production of prawns kept in 100% SW at 15°C, measuring by clearance of Cr-51 EDTA from the haemolymph.

Treatment	Weight (g)	Clearance rate (% body wt/day)	Urine production (% body wt/day)	Analysis of t-test t-value	probability
Control	0.67±1.65	24.71±3.87 (15)	10.56±1.64 (15)	0.9542	NS
Ligatured	1.08±1.76	29.07±7.70 (18)	9.87±2.59 (18)		

U/H for Cr-51 EDTA of the control is 2.34

U/H for Cr-51 EDTA of prawns with ligatured eyestalks = 2.94

Table 12.6 Effect of ligaturing the eyestalks of prawns on efflux of Cr-51 EDTA from the whole animal in 50% SW.

Treatment	Rate constant efflux (hr^{-1})	Analysis of t-test df	t-value	probability
Control	0.0439 ± 0.0118 (8)	16	0.0000	NS
Ligatured	0.0398 ± 0.0177 (8)			

to a sudden change from 100% to 50% SW are shown in Figure 12.5 and in Table 12.7. Both normal prawns and eyestalk-ligatured prawns showed an increase in haemolymph sodium concentration within 1-2 hours. The highest value of sodium concentration is about 375 ± 5.5 mM/l (n=10) for the control, and about 384 ± 5.7 mM/l (n=10) for the experimental animals. After the maximum haemolymph sodium concentration had been reached the sodium in the haemolymph decreased slowly towards the steady state value. As in normal animals, a new steady state of the haemolymph of the ligatured animals is achieved within 6 hours after transfer. However, the animals with eyestalks ligatured maintained their sodium in the haemolymph at higher concentrations than the control.

The results of the experiment suggest that

(i) either the animal with eyestalks ligatured is passively resistant to salt loss because of its morphological constitution and/or

(ii) the movement of salts and water down the concentration gradient is counteracted by the opposing systems which eliminate excess water and actively take up salts from the medium.

It was demonstrated in the previous experiments (Figure 12.4, Tables 12.3 and 12.6) that the prawn, P. serratus, is not impervious to water influx. Thus there must be physiological processes counteracting salt depletion. These were demonstrated as follows:

8. Effect of eyestalk removal on the sodium concentration of the haemolymph of prawns in a dilute medium, under steady state conditions.

Table 12.7 Changes in sodium concentration of haemolymph of prawns after a sudden transfer from 100% to 50% SW.

Hours after transfer	Sodium concentration in haemolymph Control (mM/l)	Ligatured (mM/l)	Analysis of t-test t-value	probability
0	360 ± 6.2 (10)	364 ± 5.8 (10)	1.2999	NS
1	375 ± 5.5 (10)	384 ± 5.7 (10)	3.8000	P< 0.005
3	349 ± 4.1 (10)	354 ± 9.1 (10)	1.7465	NS
6	300 ± 3.9 (10)	338 ± 12.0 (10)	9.6081	P< 0.001
12	301 ± 12.2 (10)	349 ± 12.5 (10)	8.0506	P< 0.001
24	303 ± 8.6 (10)	347 ± 10.0 (10)	10.4401	P< 0.001

Table 12.8 Sodium concentration of haemolymph of prawns after acclimation to 100% and 50% SW for 96 hours.

Medium (% SW)	Control (mM/l)	Ligatured (mM/l)	Analysis of t-test df	t-value	probability
100	365 ± 7.1 (18)	363 ± 6.2 (15)	31	0.8952	NS
50	307 ± 2.2 (18)	341 ± 4.0 (15)	31	30.6573	P< 0.001

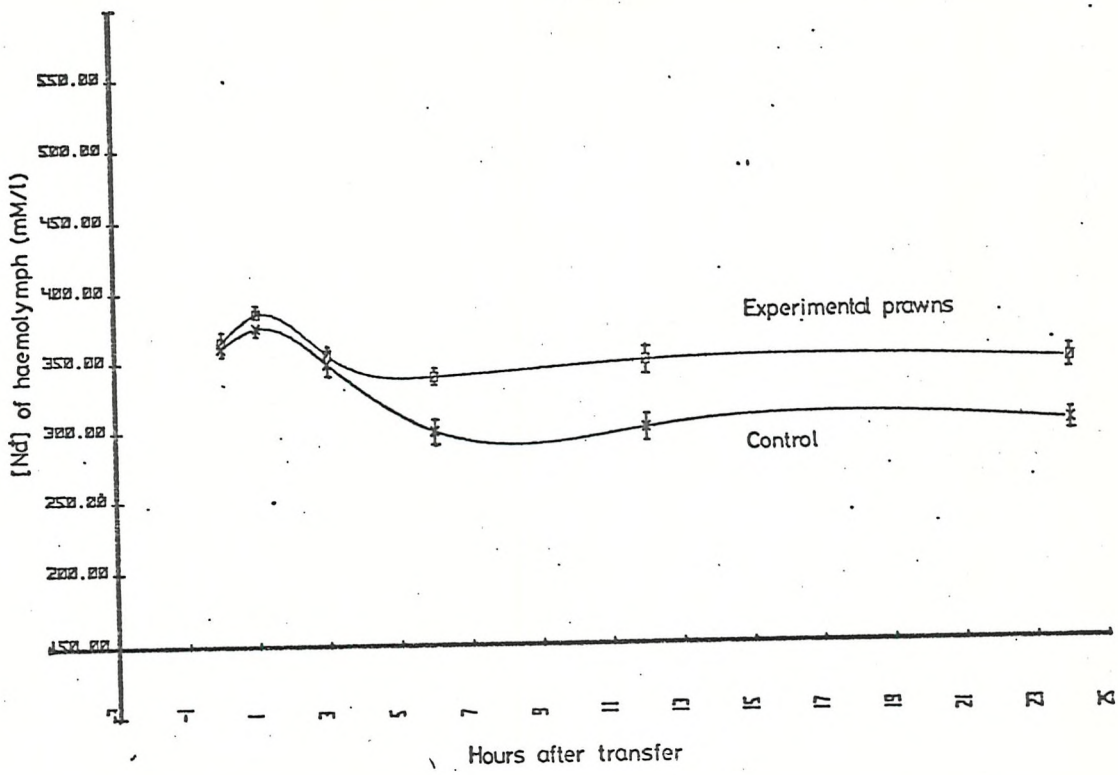

Figure 12.5 Changes in haemolymph sodium concentration of normal and eyestalk-ligatured prawns after subjection to a sudden change from 100% to 50% SW.

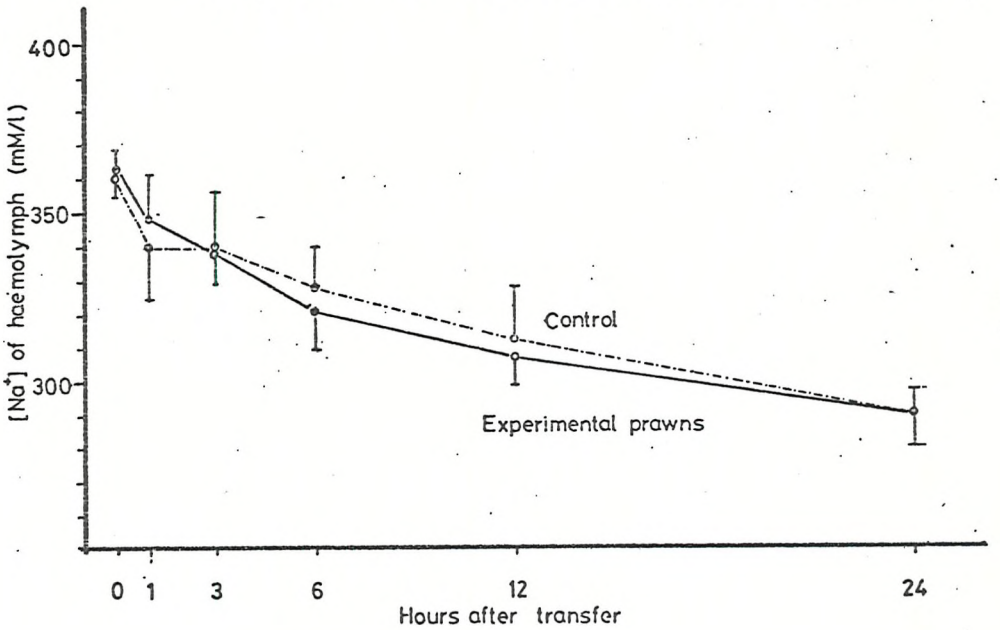

Figure 12.6 Changes in haemolymph sodium concentration of normal and eyestalk-ligatured prawns after subjection to a sudden transfer from 100% to 50% SW with addition of 10^{-5}M thionine.

The changes in sodium concentration of the haemolymph were determined from two batches of animals subjected for 96 hours to 50% and 100% SW respectively. The results are summarized in Table 12.8. The data show that in normal sea water the haemolymph sodium concentration of the experimental prawns is not significantly different from that of the control. In 50% SW the haemolymph sodium concentration of both control and experimental animals decreased, but in this case the difference is statistically significant, the eyestalkless animals having a substantially higher haemolymph sodium concentration.

9. Effect of a sudden transfer of prawns from 100% to 50% SW with addition of 10^{-5}M thionine.

The preceding experiment indicates that prawns with eyestalks ligatured have a higher haemolymph sodium concentration than the normal prawns when the animals are in dilute media. An experiment was designed to test whether this difference is due to an active or passive process.

The procedure and methods used in this experiment are similar to those in experiment 7 of this chapter except that the 50% SW medium contained 10^{-5}M thionine. The results are summarized in Table 12.9. It appeared that the haemolymph sodium of both controls and experimental animals decreased at the same rate (Figure 12.6). This suggests that the higher haemolymph Na^{+} level of the eyestalk-ligatured animals observed in section 8 is due to an active uptake of sodium; an uptake which can, however, be inhibited by thionine.

Table 12.9 Changes in sodium concentration of haemolymph of prawns after a sudden transfer from 100% to 50% SW containing 10^{-5}M thionine.

Hours after transfer	Sodium concentration in haemolymph Control (mM/l)	Ligatured (mM/l)	Analysis of t-test t-value	probability
0	360 ± 6.2 (10)	364 ± 5.8 (10)	1.2999	NS
1	341 ± 15.2 (10)	349 ± 13.6 (10)	1.2746	NS
3	341 ± 17.0 (10)	339 ± 9.0 (10)	0.1647	NS
6	328 ± 12.0 (10)	321 ± 12.2 (9)	1.2492	NS
12	313 ± 16.7 (10)	308 ± 8.8 (10)	0.8387	NS
24	300 ± 7.2 (10)	301 ± 9.3 (10)	0.0804	NS

Table 12.10 Changes in sodium concentration of haemolymph of prawns subjected to a sudden change of salinity from 50% to 100% SW.

Hours after transfer	Sodium concentration in haemolymph Control (mM/l)	Ligatured (mM/l)	Analysis of t-test t-value	probability
0	307 ± 2.2 (18)	341 ± 4.0 (15)	30.6573	P< 0.001
1	329 ± 15.8 (6)	358 ± 14.3 (6)	3.2902	P< 0.025
3	343 ± 6.8 (6)	351 ± 15.4 (6)	1.1122	NS
6	361 ± 7.4 (6)	374 ± 17.7 (6)	1.7017	NS
12	338 ± 8.2 (6)	365 ± 15.8 (6)	3.7804	P< 0.010
24	350 ± 15.6 (6)	370 ± 10.3 (5)	2.3843	P< 0.050

10. Effect of a sudden transference of prawns from 50% to 100% SW.

As shown in the preceding experiments (experiments 7, and 9), both the control and experimental prawns possess the necessary sodium uptake mechanism to compensate for the renal and extra-renal loss of sodium which occurs when they are kept in dilute media. The results of those experiments suggested that the animals with ligatured eyestalks have a faster rate of sodium uptake per unit time than the controls.

In this experiment another technique was used to examine the rate of uptake of sodium of the prawns. It was hoped that the results obtained would provide some understanding of the mechanism, and would allow for a comparison to be made between the normal prawns, and the animals with ligatured eyestalks.

Prawns acclimated to 50% SW at 15^{o}C were suddenly transferred to 100% SW at the same temperature. Haemolymph sodium concentrations were determined at 0, 1, 3, 6, 12, and 24 hours. These measurements are listed in Table 12.10. It appeared that a new steady state for sodium concentration of the animals, both the controls, and experimental animals, is reached within 6 hours. The rate of uptake of sodium as observed in this experiment is probably the sum of sodium that is taken in from both the gills and the gut. This makes the results rather difficult to interpret.

Another factor, that makes the results difficult for comparison, is the difference in sodium concentration in the haemolymph between the control and the experimental animals at the zero hour. Since the animals with ligatured eyestalks tend to maintain their haemolymph higher than that of the controls when they are kept in

a dilute medium, this inevitably results in a higher sodium concentration in the haemolymph of the experimental animals, 341 ± 4.0 mM/l (n=15) whereas the sodium concentration in the haemolymph of the controls is 307 ± 2.2 mM/l (n=18).

11. Effect of bilateral eyestalk ablation on drinking rate.

It was suggested by Lockwood (1968, P.96) that the apparent action of eyestalk removal on the water balance could be an artefact due to the precipitated molt and not any factor actively mediating cuticular permeability. If this was the case the increase in urine flow could be due to an increase in drinking activity. An experiment was therefore designed to investigate this parameter.

The results are shown in Table 12.11. Eyestalk-ligatured prawns had a drinking rate of 0.45 ± 0.18% body wt/hr compared with 0.69 ± 0.13% body wt/hr for the normal prawns. The low drinking rate of water of the prawns with ligatured eyestalks might in some way be connected with an inactive state which is always seen in the animals with ligatured eyestalks. This might lead to the decrease in the ventilatory movement of the gills as well as the water flow around the animal itself and consequently decreased water flow through the mouth and anus. In any event it is clear that increased drinking does not occur in the eyestalk-ligatured animals.

Another possible explanation is that the effect might be a physiological response of the animal itself to reduce the amount of sodium that is transported in across the gut, since an eyestalk factor probably controls sodium pump mechanisms on the gut epethelium which, when it is removed, allows the rate of transport to be increased. Increased ion transport is accompanied by increased

water transport. A larger haemolymph volume of prawns with ligatured eyestalks as observed in experiment 12 of this chapter also is indirect evidence indicating that the water is transported in the animals with ligatured eyestalks at a faster rate than in the normal controls. A decrease in drinking rate probably is a secondary response to the water-load in the animal.

Table 12.11 Effect of bilateral eyestalk ablation on drinking rate of prawns kept in 100% SW at 15°C.

Treatment	Weight range (g)	Drinking rate (% body wt/hr)	Analysis of t-test df	t-value	probability
Control	0.13 - 0.29	0.69 ± 0.13 ($\bar{12}$)	22	3.8466	$P < 0.001$
Ligatured	0.24 - 0.78	0.45 ± 0.16 ($\bar{12}$)			

Table 12.12 Effect of eyestalk ligature on haemolymph volume of prawns kept in 100% SW at 15°C.

Treatment	Weight range (g)	Haemolymph volume (% body wt)	Analysis of t-test df	t-value	probability
Control	0.62 - 2.45	19.44 ± 2.28 ($\bar{35}$)	63	10.5792	$P < 0.001$
Ligatured	0.56 - 2.47	25.94 ± 2.67 ($\bar{30}$)			

12. Effect of eyestalk ligature on the haemolymph volume.

Table 12.12 summarizes the data on haemolymph volumes of prawns with ligatured eyestalks, and of control animals. In normal prawns the haemolymph volume is about 19.44 ± 2.28 (n=35)% body wt whereas for prawns with ligatured eyestalks it is about 25.94 ± 2.67 (n=30) % body wt. Factors which could be involved included the increased water uptake in the body by ligatured animals (section 9), and also the possibility that there is a higher rate of water reabsorption from the bladder of prawns with ligatured eyestalks (section 14).

13. Effect of eyestalk ablation on the sodium efflux of prawns.

Changes in the haemolymph electrolyte and osmotic concentrations following eyestalk ablation have been studied in various crustaceans by several investigators (Kamemoto *et al.*, 1966; Bryan, 1960a; and Peterson & Loizzi, 1970). In *P. serratus* the operation results in an increase in the sodium concentration of haemolymph when it is placed in a dilute medium, but the animal shows no significant difference in sodium concentration when it is placed in 100% SW (see experiment 8, this chapter). These results suggested that the investigation of the effect of eyestalk ligature on sodium efflux in animal acclimated to 50% and 100% SW or on transfer from 100% to 50% SW might shed light on the question of whether the gills and the antennal glands are acting passively in haemolymph concentration changes or whether both are responding directly to hormonal control.

The results of such experiments are presented in Table 12.13. The data show that in 100% SW the sodium efflux of prawns with

ligatured eyestalks are not significantly different from those in normal prawns. In contrast to the efflux in 100% SW, the sodium efflux in 50% SW is slower in the experimental prawns as compared to that in the control, and the difference is significant ($t = 2.9000$, $P < 0.025$).

A slower efflux of sodium-22 as observed in the experimental prawns, when compared with the control, in this case probably is mainly due to a secondary effect of a higher rate of sodium uptake across the gills in the experimental prawns which results in more dilution of sodium-22 in the haemolymph so that in turn a smaller amount of sodium-22 diffuses out from the body.

With respect to the animals in normal sea water, a comparison of rate of urine production, haemolymph sodium concentration, drinking rate, haemolymph volume, and sodium efflux of intact and eyestalkless animals provides evidence that extirpation may stimulate renal reabsorption (see also further studies in experiment 14 of this chapter).

14. Neuroendorine involvement in water reabsorption in the bladder of prawns.

In order to follow the changes in haemolymph and urine levels of EDTA, equal amounts of Cr-51 EDTA were injected into normal prawns and animals with ligatured eyestalks, of approximately the same size. The relative concentration of the tracer in the haemolymph and the urine were determined at intervals. The results of analysis of haemolymph and urine after the injection of tracer are shown in Table 12.14 and expressed in the form of a graph in Figure 12.7 and 12.8.

Table 12.13 Effect of ligaturing the eyestalks of prawns on sodium efflux in 50% SW, at 15°C.

Treatment	Wet weight (g)	Rate constant of efflux (hr^{-1})	Analysis of t-test df	t-value	probability
100% SW					
Control	1.82 - 3.15	0.3820 ± 0.0370 ($\bar{6}$)	9	0.0390	NS
Ligatured	2.20 - 2.58	0.3809 ± 0.0608 ($\bar{5}$)			
50% SW					
Control	1.82 - 3.15	0.1870 ± 0.0141 ($\bar{6}$)	9	2.9000	$P < 0.025$
Ligatured	2.20 - 2.58	0.1580 ± 0.0244 ($\bar{5}$)			

Table 12.14 EDTA U/H ratio at time intervals after injection of Cr-51 EDTA into prawns kept in 100% SW at 15°C.

Time after injection	U/H ratios of Cr-51 EDTA control	experimental prawn
3	0.79 ± 0.23 ($\bar{6}$)	1.11 ± 0.38 ($\bar{6}$)
6	1.53 ± 0.54 ($\bar{6}$)	1.79 ± 0.47 ($\bar{7}$)
12	2.31 ± 0.35 ($\bar{6}$)	2.70 ± 0.88 ($\bar{7}$)
24	2.67 ± 1.10 ($\bar{10}$)	3.36 ± 0.96 ($\bar{12}$)
36	3.11 ± 0.63 ($\bar{6}$)	3.48 ± 1.67 ($\bar{5}$)
48	3.18 ± 0.80 ($\bar{6}$)	3.48 ± 1.75 ($\bar{9}$)

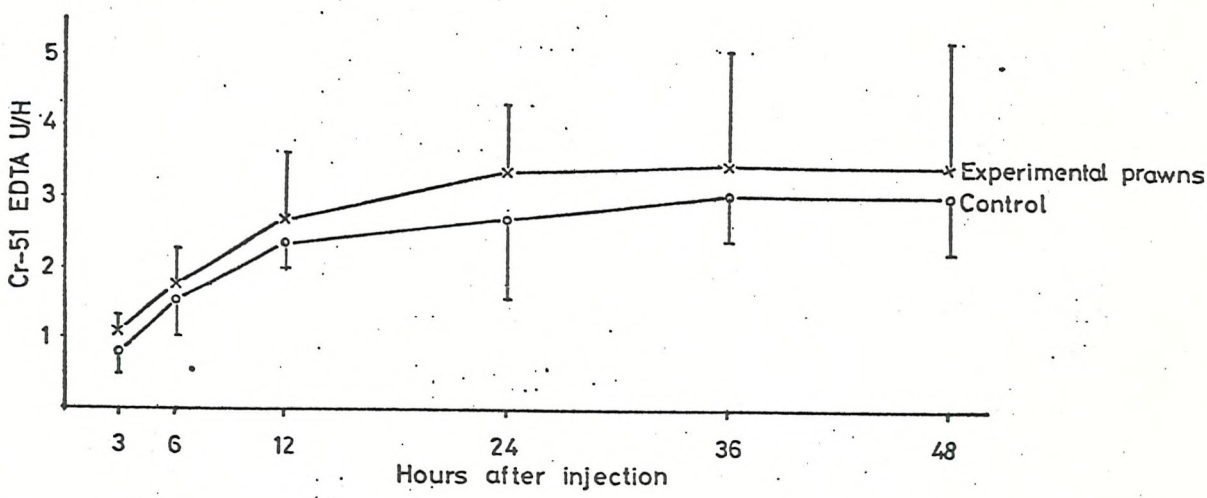

Figure 12.7 Concentrations of Cr-51 EDTA in urine and haemolymph at time intervals after injection of the labelled chromium into prawns with ligatured eyestalks and controls in 100% SW at 15°C.

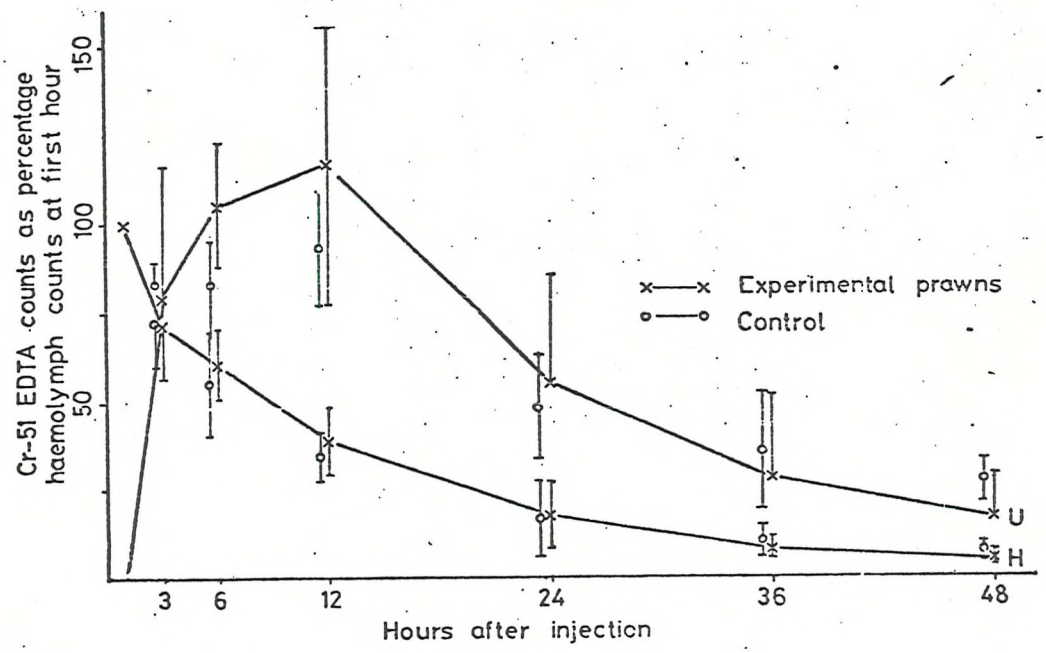

Figure 12.8 The relationship between the concentration of Cr-51 EDTA in the haemolymph and urine of prawns, both with eyestalk ligatured and controls, in 100% SW at 15°C. All values are expressed as per cent of unit volume of haemolymph at zero time, U = urine, H = haemolymph.

The haemolymph chromium concentration level fell as the marker passed into the antennal glands and at the same time the concentration of chromium in the bladder began to increase due to the continuous production of urine. As in the normal prawns, the U/H ratio of the eyestalks ligatured prawns approached equilibrium at 12 hours after injected Cr-51 EDTA. After 24 hours the U/H ratios of the normal prawns is about 2.67 $\pm$ 1.10 (n=10) whereas that of the experimental prawns is about 3.36 $\pm$ 0.96 (n=12), Table 12.14. This implies that a proportion of water in the primary urine is reabsorbed before release of the definitive urine from the bladder (further discussion in chapter XIII, section 4).

The U/H ratio for Cr-51 EDTA obtained from this experiment is of the same magnitude as that reported by Franklin, 1975 (2.95) but it is slightly higher than the U/H ratio for inulin that Bryan and Ward (1962) showed in the same species. This would be expected, since a marker with a smaller molecular weight like Cr-51 EDTA, a molecular weight about 500, tends to have a higher U/H ratio than inulin where the molecular weight is about 5,000 because of molecular sieving effects.

In order to test whether the high U/H ratio of Cr-51 EDTA as observed in the present experiment results from a lack of equilibrium between the haemolymph and urine in the bladder with respect to Cr-51 EDTA concentration or to a water reabsorption, the theoretical model of Riegel *et al.* (1974) also applied in a similar manner. In an ideal excretory system, primary urine is produced at a rate R from a space (haemolymph) of constant volume V_1 and passes into a bladder of constant volume V_2. If an inert,

filterable substance (marker) is introduced into V_1 at time t = 0 then its concentration in the haemolymph C_1 will decline at a rate governed by the clearance of the primary urine and loss through other routes, R. This proposition may be expressed mathematically as follows:

$$\frac{d}{dt}(C_1V_1) = -(R + \alpha R)C_1, \quad \ldots\ldots\ldots\ldots\ldots \quad (12.1)$$

where RC_1 is the extra-renal loss. The rate of change of the concentration of the marker in the urinary bladder will be described as follows:

$$\frac{d}{dt}(C_2V_2) = R(C_1 - C_2), \quad \ldots\ldots\ldots\ldots\ldots \quad (12.2)$$

where C_2 is the concentration of the marker in the bladder. Combining equations (12.1) and (12.2) and solving (see Riegel et al., 1974) provides a description of the effect on the ratio of the concentration of marker in the haemolymph and bladder (C_2/C_1) at t = 1 :

$$\frac{C_2}{C_1} = \frac{1}{1 - \frac{V_2}{V_1}(1 + \alpha)}\left[1 - \exp - \left(1 - \frac{V_2}{V_1}1 + \alpha\right)\frac{Rt}{V_2}\right]\ldots \quad (12.3)$$

Equation (12.3) will apply provided $1 - V_2/V_1\ (1 + \alpha) > 0$. (It is highly unlike that this condition will fail to be true).

As $t \rightarrow$, $\frac{C_2}{C_1} \rightarrow \frac{1}{1 - V_2/V_1\ (1 + \alpha)}$,

Therefore, utilizing estimated volumes of the haemolymph and urinary bladder, and the rates of renal clearance and limits of extra-renal loss of marker, it is possible, using equation (12.3),

to calculate theoretical limits to the marker U/H (i.e. C_2/C_1) which can occur in the absence of water reabsorption.

Figure 12.9 compares the observed U/H with values calculated from equation (12.3), assuming no water uptake but different sizes of urinary bladder volume and extra-renal loss of marker. In both examples, shown in Figure 12.9, the clearance of marker from the haemolymph is taken as the same, 117.71% of the haemolymph per day. In prawn (a) if it assumed that the extra-renal loss of marker is nil, and the bladder volume is 17% of the haemolymph volume, the marker U/H of the prawn will gradually rise to a maximum value, 1.2, before 12 hours. In prawn (b) assuming that its bladder volume is small (2% of the haemolymph volume), but loss of marker to the tissues is large (1.24 times renal clearance). In the later case, the marker U/H reaches a maximum value very rapidly, but the maximum U/H ratio is only slightly in excess of one. In the present studies, the prawn has the bladder volume about 12.57% of the haemolymph volume, the extra-renal loss ia about 0.02 times of the renal clearance which is intermediate between (a) and (b), but its U/H ratio for Cr-51 EDTA is 2.67 after 24 hours. This suggests that the high value of U/H is not due to the purely mechanical factors.

Families of curves may be constructed using equation (12.3) and a variety of values of renal clearance, bladder volume, and loss of marker by extra-renal routes (Figures 12.10, 12.11 and 12.12). As shown in Figures 12.10 and 12.11, the larger size of the bladder, the greater will be the steady state of U/H. Furthermore, for any given bladder size the magnitude of U/H will be

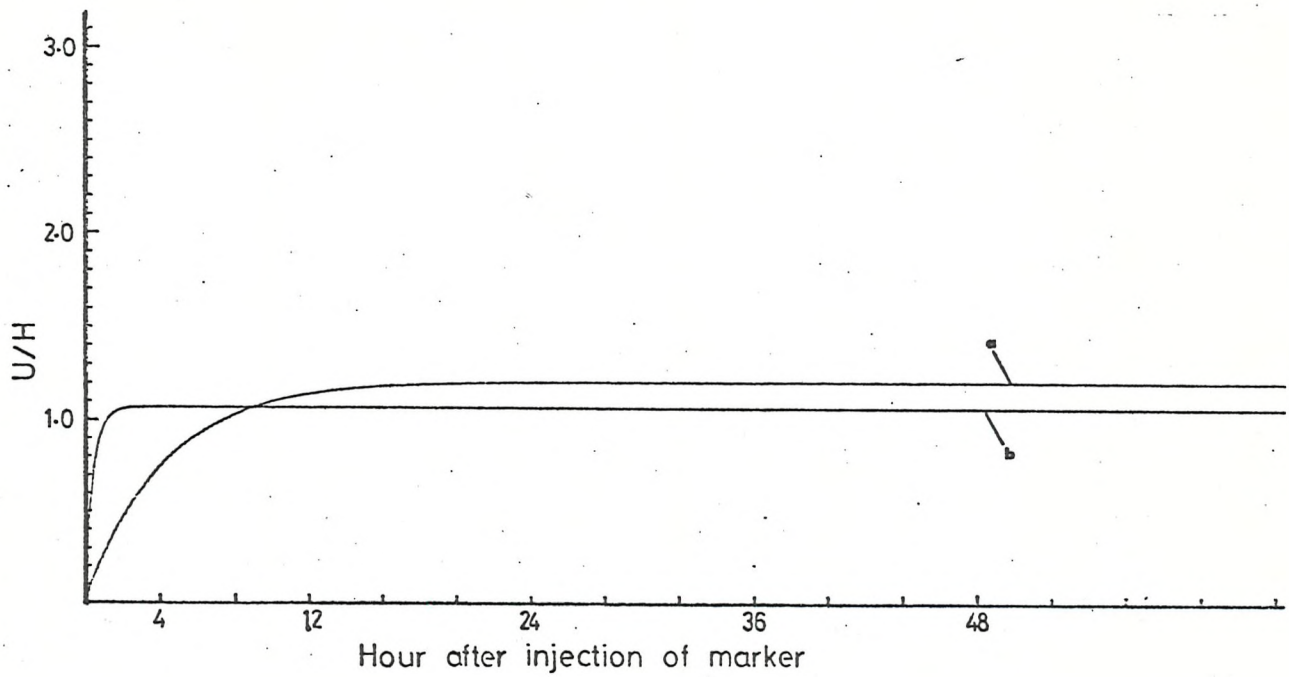

Figure 12.9 Theoretical plot of the change of U/H with time after the injection of marker, assuming that there is no water reabsorption form the urine. In (a) clearance is 117.71% of the haemolymph volume per day, the bladder volume is 17% of the haemolymph volume, and the loss of marker to the tissue (extra-renal loss) is zero. In (b) clearance is 117.71% of the haemolymph per day, the bladder volume is 2% of the haemolymph, and loss of marker to the tissue is 1.24 times the loss from the haemolymph by clearance to the excretory organ.

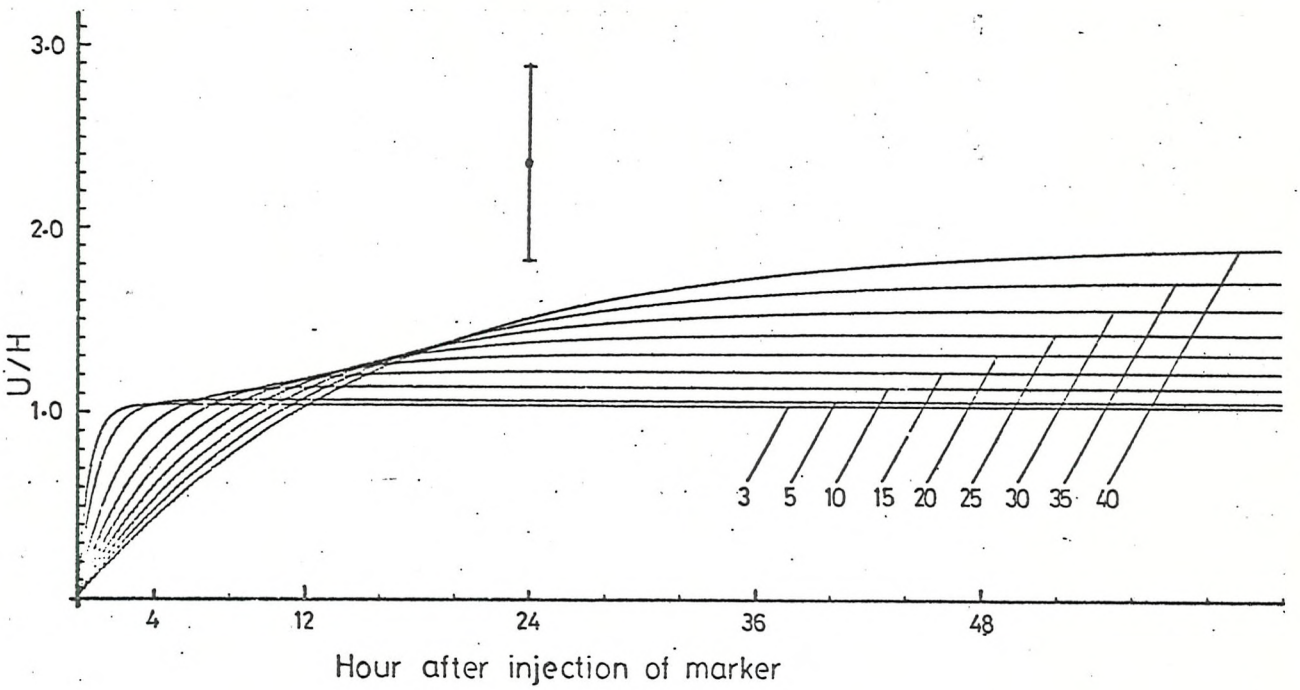

Figure 12.10 Theoretical plots illustrating the influence of bladder volume on marker U/H. For all plots the values are : clearance, 117.71% of the haemolymph volume per day; extra-renal loss, 0.2 times the clearance. Bladder volume, variable between 3 and 40% of the haemolymph volume. Note that the observed Cr-51 EDTA U/H (.) 24 hours after injection lies outside the range of any of the predicted levels of U/H even though the theoretical plots include bladder volume values in excess of those possible.

increased further by any loss of marker from the haemolymph via extra-renal routes. As shown in Figure 12.12 increased renal clearance rates shorten the time taken for the U/H to reach a maximum but they do not alter the final value.

As shown above, mathematically, purely mechanical factors can give rise to differences in concentration of marker in haemolymph and bladder. It is clearly shown that U/H ratios, as observed in the present studies, are in excess of those predicted from purely physical effects. The reabsorption of water from the primary urine must be the prime cause of U/H values in excess of those predicted by lag effects.

With respect to effect of eyestalk ligature on water reabsorption, the results obtained in this experiment also suggests the possibility that the animals with ligatured eyestalks have the ability to reabsorb the water in primary urine faster than the normal prawns. However, before a conclusion can be drawn, further studies to delineate the action of neuroendocrine on the rate of water reabsorption in the bladder of the prawns are needed.

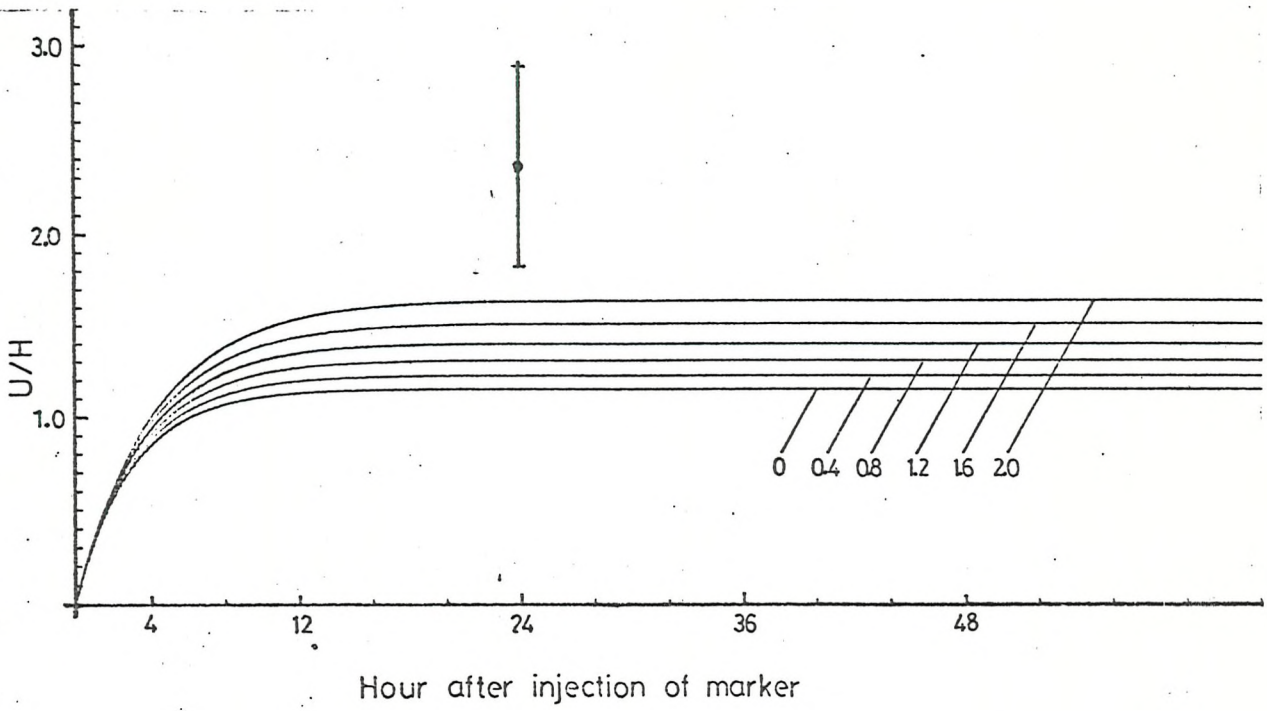

Figure 12.11 Theoretical plots illustrating the influence of various rates of extra-renal loss of marker. Parameters are : Cleaeance, 117.71% of the haemolymph volume per day; bladder volume 12.97% of the haemolymph volume; extra-renal loss, varies from zero to twice the loss of marker due to clearance to the renal organ. Note that the observed U/H (.) 24 hours after injection of Cr-51 EDTA exceeds the range of predicted levels.

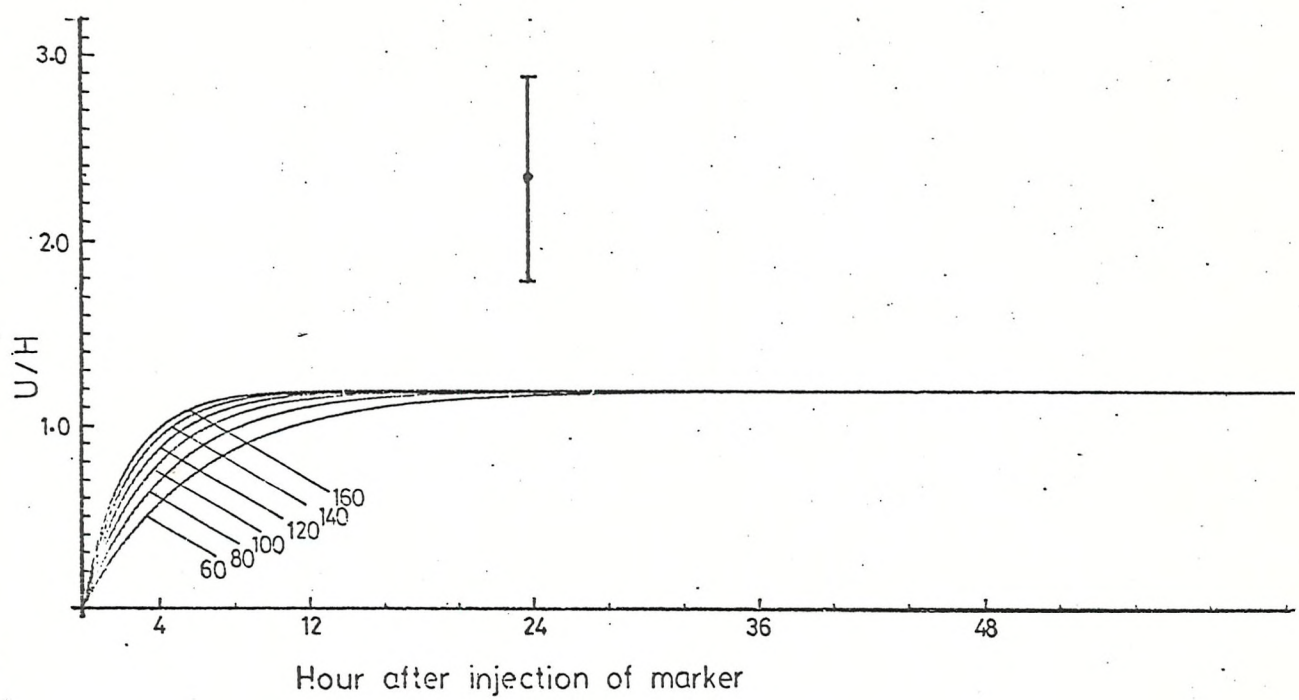

Figure 12.12 Theoretical plots illustrating the influence of clearance rate on the marker U/H. Parameters are bladder volume, 12.97% of the haemolymph volume; extra-renal loss, 0.2 times the renal clearance; variable rate in range from 60 to 160% of the haemolymph volume per day. Note that the observed Cr-51 EDTA U/H 24 hours after injection (.) exceeds the range of predicted values.

XIII. GENERAL DISCUSSION

A comprehensive picture of osmoregulation in the common prawn, Palaemon serratus (Pennant) can be drawn from the findings of the present investigation. The results of individual experiments have been briefly discussed in the appropriate chapter. These will be reviewed and brought together to give an overall picture of the mechanisms employed by prawns in the regulation of salts and water under fluctuating environmental conditions.

The family Palaemonidae has a cosmopolitan distribution, its habitats ranging from fresh water to marine (Holthuis, 1950). According to the response of their haemolymph to changes in the external medium, the palaemonid prawns can be divided into two groups. First, species which regulate their blood concentration hyper-osmotically in all salinity. This group includes the majority of fresh water prawns. Among them are Palaemonetes paludosus (Dobkin and Manning, 1964) and Macrobrachium australiense (Denne, 1968). Second, those species which are capable of hyper-osmotic regulation in dilute media and hypo-osmotic regulation in concentrated media. This group is made up of mostly marine and brackish water species, including Palaemon macrodactylus (Born, 1968), Palaemonetes varians (Panikkar, 1941), P. intermedius (Dobkin and Manning, 1964), and Macrobrachium equidens (Denne, 1968). P. serratus is in the second group, and is able to maintain the sodium in its haemolymph hypotonically in normal and concentrated sea water and hypertonically in media of salinity below 70% SW.

The haemolymph sodium concentrations reported here for *P. serratus* are substantially lower than those reported for *Crangon vulgaris* (Hagerman, 1971; Grimm, 1969), but are higher than those of *Palaemonetes varians* (Potts and Parry, 1964b) for media within the range used in the present study. Although the concentration of sodium in the haemolymph of *P. serratus* is somewhat different from that of other species, the ionic behaviour of *P. serratus* in general resembles that of other species of prawn and shrimp for instance, *P. varians* (Panikkar, 1941), *Panaeus setiferus* and *P. aztecus* (McFarland and Lee, 1963), and *Crangon vulgaris* (Hagerman, 1971).

The osmotic and ionic properties of the haemolymph of *P. serratus* have been studied in detail by Panikkar (1941), Parry (1954), and Spaargaren (1971). The first author was principally interested in the influence of salinity on the osmotic behaviour of the haemolymph, while Parry (1954) elucidated the osmoregulatory mechanism of the prawn by analysing the inorganic composition of blood and urine, and Spaargaren (1971) used freezing point depressions and conductivity measurements on the concentration of substances in the blood with a view to observing the influence of temperature and salinity on the osmoregulation of the prawn.

The results of the present work agree with those of Panikkar (1941), Parry (1954), and Spaargaren (1971) with regard to the behaviour of sodium in the haemolymph. Since sodium is the major cation one would expect it to follow rather closely any change in the haemolymph osmotic concentration. On quantitative assessment, sodium concentration in the haemolymph of the prawn is 365 $\pm$7.1 mM/l

at 15°C and 392 ± 19 mM/l at 5°C (Table 5.2), not substantially different from the values of Parry (1954), 394 ± 12 m.equiv./l.

For chloride ion in the haemolymph of prawns kept in normal sea water, the values, 366 ± 8 mM/l at 15°C and 410 ± 8 mM/l at 5°C (Table 5.1), obtained from the present investigation are lower than those reported by Parry (1954), 430 ± 4 m.equiv./l. The differences in this case is probably due to the difference of techniques and method of determination of chloride.

In its ability to regulate its haemolymph concentration in both concentrated and dilute sea water, P. serratus resembles some other brackish water invertebrates for instance Pachygrapsus crassipes (Gross, 1957), Gnorimosphaeroma oregonensis and Sphaeroma pentodon (Riegel, 1959), and Artemia salina (Croghan, 1968a). The maintenance of a relatively constant blood concentration must be considered one adaptive mechanism to life in a changing environment. This homoismotic regulation protects the cells from osmotic and ionic injuries.

1. Possible mechanisms of hyper-osmotic regulation.

Hyper-osmotic regulation is well known to occur widely in decapods occupying brackish water (Lockwood, 1962, Potts and Parry, 1964a). Animal placed in a dilute medium will gain water and lose salts. The mechanisms developed to counter such effects may include

(1) the production of urine hypo-osmotic to the haemolymph,

(2) a reduction of permeability of the body surface to salts and/or water,

(3) the active uptake of salts by the gills from the external environment into body fluids to compensate for the renal and extra-renal loss of sodium.

Not all these mechanisms are, however, necessarily developed in an individual species.

On the basis of the present work it is possible to assess the relative importance in the prawn of the various osmoregulatory control systems proposed by earlier workers for other organisms which are regulating hypertonically. The first of these mechanisms, production of urine hypo-osmotic to the haemolymph does not occur in the prawn, since it has been established that in all concentrations of sea water, prawns produce urine isotonic to the haemolymph (Parry, 1954). If the antennal glands of P. serratus are of little importance in salt conservation, they must have some other functions. The experimental evidence has shown that in the prawn, as in other estuarine species, the antennal glands prove to be of prime importance in removal of excess water. Their importance is demonstrated by a spectacular change in volume of urine production when the prawns are suddenly transferred to dilute media. When the antennary pores are sealed with a dental cement, an obvious swelling occurs within six hours of the transfer at the junction of the abdomen and the thorax. Such animals rarely survive more than 36 hours.

Under a steady state, the prawn is also able to increase its urine flow with decreasing salinity, even in a very dilute medium, 10% SW, which is probably a lower limit of its tolerance

(experiment 11, chapter 10). Whether this is due to an active removal of water by the antennal glands or the result of purely physical forces is not known.

Low permeability of the body surface to salts and /or water is also an aid in the osmoregulatory process. On the basis of urine production, the prawn has a higher body surface permeability than that of C. maenas, and much higher than those of A. fluviatilis (Table 13.0). The ability to vary the permeability of the body surface has not been investigated in the present work but, if we accept the similarity between P. serratus and P. varians, the animal probably does not alter its body surface permeability when exposed to different salinities (Parry, 1955; Rudy, 1967). If low permeability and permeability changes to salts and water cannot account for the ability of the prawn to regulate its haemolymph concentration, the animal must have other mechanisms to counteract the osmotic inflow of water as well as the osmotic outflow of salts.

Active uptake of salts from the surrounding water into the haemolymph across body surfaces is another widely used mechanism for osmoregulation. For the prawn, this mechanism seems to play a major role in maintenance of salt and water balance between the haemolymph and the medium when the animals are in a dilute medium. The mechanism can be inhibited by addition of thionine in 50% SW as clearly shown in experiment 8, and 9, chapter XII). The ability to take up salts against a concentration gradient as shown in the prawn has been shown in C. maenas (Nagel, 1934; Shaw, 1961), and also other euryhaline forms Pachygrapsus (Gross, 1957; Rudy, 1966), Ocypode (Flemister, 1958), Eriochier (Shaw, 1961), Uca (Green et al.,

1969), and Callinectes (Mantel, 1967). Such a mechanism can account for the replenishment of ions lost by both renal and extra-renal routes. For P. serratus, the animal seems to possess a more effective uptake mechanism than C. crangon, since the former still increases its urine flow even if exposed to 10% SW which probably is a lower limit of tolerance for this species, whereas (obviously) the latter does not increase its urine flow when it is acclimated to a concentration below the salinity at which the maximum rate of urine production has been reached (Spaargaren, 1971). Although P. serratus and P. varians show the same pattern in their mode of urine production, an increase in the rate with decreasing salinity, the former, a marine species, probably possesses a less effective uptake mechanism than the latter which is an estuarine species surviving in water with a concentration as low as 0.50% SW (Potts and Parry, 1964b). It seems reasonable to propose that the other palaemonid prawns are restricted to sea water, not because they lack the sodium uptake mechanism, but because this mechanism is not sufficient to balance the sodium loss caused by net diffusional and renal losses.

2. Possible mechanisms of hypo-osmotic regulation.

The maintenance of blood hypo-osmotic to an external medium has been shown in a limited number of decapod crustaceans which inhibit either a semi-terrestrial or hyper-saline environment (Jones, 1941; Gross, 1964). In hyper-osmotic media, animals tend to lose water and gain salts passively. Hypo-osmotic regulation in such conditions will depend upon the concurrent uptake of water

and excretion of excess salts. The results of the present study indicate that the prawn maintains its sodium concentration in the haemolymph at a lower level than the medium when it is kept in a concentration higher than 70% SW.

Crustaceans capable of hypo-osmotic regulation include Pachygrapsus crassipes (Jones, 1941; Rudy, 1966); Uca pugnax (Jones, 1941; Green et al., 1959); Palaemonetes varians (Panikkar, 1941), Sphaeroma pentodon (Riegel, 1959); Artemia salina (Croghan, 1958a), Haloniscus searlei (Bayly and Ellis, 1969); Palaemonetes paludosus and P. intermedius (Dobkin and Manning 1964); P. antennarius (Parry, 1957); Penaeus duorarum, and P. aztecus (Williams, 1960); Metapenaeus monoceros (Panikkar and Viswanathan, 1948); M. dobsoni, Penaeus indicus, and P. carinatus (Panikkar, 1950); P. setiferus, Trachypeneus similis, and Sicyonia dorsalis (McFarland and Lee, 1963); Metapenaeus bennette (Dall, 1967); Palaemon macrodactylus, and Synacaris pacifica (Born, 1968); and Macrobrachium equidens (Dene, 1968). These may be divided into two groups, those which maintain their haemolymph osmolality relatively constant over a wide range of salinities such as A. salina and H. searlei, and those which maintain a relatively constant osmotic gradient between haemolymph and external medium, for example, U. pugnax and P. varians. Those crustaceans which maintain a relatively constant haemolymph concentration depend upon regulation at the body surface, while those of the latter group must either tolerate changes in haemolymph concentration or regulate at the cellular level. Regulation of body fluids at a relatively constant concentration is dependent on an ability of the animals to excrete excess salts and take up

water, in order to compensate for the osmotic and ionic imbalance between the internal and external environment. Maintenance of constant osmotic and ionic gradients between haemolymph and environment requires only a limited ability to excrete salts and take up water, but necessitates that the cells are capable of functioning over a wide range of osmotic and ionic conditions. In spite of these differences the mechanism of hypo-osmotic regulation in crustaceans is generally considered to be the same (Potts and Parry, 1964a) involving the maintenance of water balance by ingesting the external medium, taking up both salts and water across the gut epithelium, and excreting excess salts from the gills.

The osmotic regulation of P. serratus in hyper-osmotic media indicates that it maintains a relatively constant gradient between haemolymph and external medium. The mechanisms involved in hypo-osmotic regulation in the prawn, which are similar to those proposed for other crustaceans capable of hypo-regulation, include three aspects : lowered permeability of the body surface, active extrusion of ions at the gills, and drinking and anal uptake of water.

2.1 Decreased permeability of the body surface to salt and water.

On the basis of urine production, P. serratus probably has a permeability of the body surface similar to P. varians, but higher than C. maenas (Table 13.0). However, the low permeability alone cannot account for the capacity for hypo-osmotic regulation as shown in the prawn, so it must also depend on other mechanisms.

2.2 Active extrusion of ions at the gills.

The prawn maintains the haemolymph sodium in a steady state by the active extrusion of ions across the gills. Maetz (1969) suggested that the sodium extrusion pump is a sodium potassium pump. The first demonstration that this is also the case in the prawn is given in section 4, chapter XI where it is shown that removal of external potassium significantly lowers sodium efflux. Confirmation of the role of the gills in ion regulation is also indicated by the fact that the inhibitor, thionine, when placed in the external medium causes a rise in haemolymph concentration when the animal is in 100% SW (experiment 6, chapter X).

In normal sea water, sodium ion tends to enter the haemolymph by diffusing across the permeable gill membranes and, to a limited extent, the general body surface. In addition, there is active uptake of sodium from the gut, which is an essential element of the osmoregulatory mechanism shown in the present investigation because it is associated with the uptake of water from the gut. This influx of ion is offset by active extrusion in the gills and losses in the urine.

2.3 Drinking and anal uptake of water.

The prawn compensates for water loss by drinking and absorbing water and salts across the gut epithelium, in a fashion similar to A. salina, and the marine teleosts. Drinking is generally continuous and not necessarily related to feeding activity. In species which continuously swallow sea water, but feed discontinuously like the penaeid shrimp, M. bennette (Dall, 1967),

Table 13.0 Urine flow in some selected decapods and method of determination.

Medium (% SW)	Species	Urine flow (% body wt/hr)	Method of measuring	Reference
100	C. maenas	4.40	Clearance of inulin from haemolymph	Binns, 1969b.
100	P. varians	9.60	Weight changes after blocking the excretory pores	Parry, 1955
100	P. serratus	8.64	"	Present study
100	P. serratus	10.56	Clearance of Cr-51 EDTA from haemolymph	Present study
75	C. maenas	11.60	Clearance of inulin from haemolymph	Binns, 1969b.
70	P. varians	8.16	Weight changes after blocking the excretory pores	Parry, 1955
70	P. serratus	5.28	"	Present study
70	P. serratus	10.79	Clearance of Cr-51 EDTA from haemolymph	Present study
50	C. maenas	17.40	Clearance of inulin from haemolymph	Binns, 1969b.
50	P. varians	6.72	Weight changes after blocking the excretory pores	Parry, 1955
50	P. serratus	20.16	"	Present study
50	P. serratus	22.33	Clearance of Cr-51 EDTA from haemolymph	Present study
Fresh water	A. fluviatilis	8.22	Weight changes after blocking the excretory pores	Bryan, 1960a.

the brine shrimp, A. salina (Croghan, 1958b), the crangonid shrimp, C. vulgaris (Ralph, 1965), and the palaemonid prawns, P. varians, and P. serratus, swallowing would be the dominant means of water uptake into the gut. In the present study the drinking rates of P. serratus were determined in the absence of food, and thus the reported rates are also considered to be independent of feeding activities.

Swallowing is not, however, the only means of uptake of fluids into the gut. Fox (1952) has shown that many invertebrates are also capable of anal drinking. In the prawn the intake of water is taking place both at its mouth, about 0.59% body wt/hr, and anus, 0.10% body wt/hr (experiment 2.5, chapter VII). For convenience in subsequent discussion the term drinking is used to describe total water intake via both routes.

Drinking rates in P. serratus in a steady state were significantly reduced in dilute sea water. Total water ingestion by P. serratus in normal sea water, as measured by Cr-51 EDTA, is about 0.69% body wt/hr. The value agrees with those reported by Dall (1967) on M. bennette using colloidal silver (Ag-110), 0.6-0.7% body wt/hr. It is also comparable with the maximum water ingestion rates of teleosts in sea water, 0.70% body wt/hr (Smith, 1930).

The drinking rate of P. serratus was found to be lower than that of P. varians for which Ralph (1965), using amaranth dye, obtained the value of 1.90% body wt/hr, and Potts and Parry (1964b), on the basis of urine losses and water lost by exosmosis calculated the value of 2.5% body wt/hr. This would be expected, since P. varians is an estuarine species with a higher degree of hypotonicity than that shown by P. serratus.

As suggested by Smith (1930), the uptake of water from the gut replaces that lost by exosmosis to the environment. A similar mechanism has been proposed for A. salina (Croghan, 1958b), Uca pugnax (Green et al., 1959), and Pachygrapsus crassipes (Rudy, 1966). Croghan (1958b) proposed that the brine shrimp, A. salina ingests the medium and that sodium and chloride are then actively transported across the gut wall resulting in a decrease of osmotic pressure in the gut fluid which, in turn, reduces the osmotic pressure difference to a level at which active water absorption from the gut into the haemolymph can be undertaken; thus providing the net gain of water to offset exosmotic and excretory losses. Following the analogy with teleost fish, Croghan (1958b) suggested that the excess sodium and chloride taken up into the haemolymph were excreted via the gills.

There are some inconsistencies concerning the part played by the gut in the hypo-osmotic regulation in Artemia. Bayly (1972) analysed some of Croghan's (1958b) data and calculated that when Artemia was in sea water the osmotic pressure of the gut fluid was approximately 680 mOsm/kg H_2O while the total osmotic equivalent of the amounts of Na^+, K^+, and Cl^- in the gut was only about 200 mOsm/kg H_2O. This leaves a deficit of some 480 mOsm/kg H_2O. Croghan (1958c) suggests that much of the deficit is made up by the divalent ions Mg^{++}, Ca^{++}, and SO_4^{++}, and that organic substances may contribute appreciably to the gut osmotic pressure. No determinations, however, have been made to demonstrate high concentration of these substances in the gut.

Potts and Parry (1964a), and Bayly (1972) have pointed out that the Croghan model of osmoregulatory mechanisms differs from that of teleosts in that it involves the absorption of water from the gut against an osmotic gradient. The osmotic pressure of the gut is as much as 600 mOsm/kg H_2O greater than that of the haemolymph when Artemia is in a concentrated brine solution. This means that the mechanism by which water is actively moved across the gut is unlikely to involve simple osmosis unless the ion transport system can reverse the gradient locally in the gut wall.

In the light of the present study, the mechanisms of hypo-regulation in P. serratus are in general agreement with those outlined by Croghan (1958c) and so endorse the validity of Croghan's model. The mechanism by which water is actively transported across the gut wall against an osmotic gradient can be explained in terms of water transport coupled with sodium. As clearly shown in the present study, the addition of thionine to 100% SW resulted in a decrease of urine production rate and increase in the sodium concentration in the haemolymph. This line of evidence does not support Dall's (1967) theory that the gut is a site of salt extrusion in hypo-osmotically regulating prawns. If salts are removed in the gut and only water absorbed across the gut epithelium as suggested by Dall (1967), there should be no significant difference in urine production between those prawns kept in 100% SW plus thionine and the controls. The increase in sodium concentration in the haemolymph of prawns kept in 100% SW with an addition of thionine also suggests that the branchial active extrusion mechanism of sodium is impaired by the action of the drug.

Additional criticisms have been made of Croghan's model of the mechanism of hypo-osmotic regulation in A. salina by Dall (1967) He suggested that, because animals ligatured at the neck and anus did not dehydrate in sea water plus 15% glycol, it could be concluded that the gills were capable of resisting exosmosis. This observation was in line with his theory that the gills played an absorptive rather than an excretory role, a view contrary to the Croghan model of the osmoregulatory mechanism. However, Geddes (1975) has recently ligatured the Australian brine shrimp, Parartemia zietziana in a similar manner to that of Croghan (1958b) and found that the ligatured animals lose about 33% of their weight after 24 hours in sea water. The fact that the ligatured A. salina did survive a 23-hour exposure to sea water plus 15% glycol may therefore perhaps be explained, either by the animals having an unusually low water permeability, or by the possibility that some water was absorbed from the fluid already in the gut and that this enabled the animals to survive in a dehydrated condition. The first possibility was confirmed by Stewart (1974) who demonstrated that Artemia has a body water exchange rate of only 8.8% body wt per hour at 15°C in 100% SW; a value which is very low when compared with C. maenas, 236% body water exchanged per hour (Smith, 1970). No direct evidence is available in regard to the second possibility but it may be recalled that there is good evidence to suggest that water uptake from the gut is a normal process by animals hypotonic to the medium.

3. Adaptation to a change in salinity.

Adaptation to salinity changes has been studied both by

following changes in haemolymph sodium concentration, and by measuring the rate of sodium efflux from the prawn. Prawns transferred from 100% to 50% SW required about 12 hours for the haemolymph to reach a new steady state. The result corroborates those presented for the same species by Panikkar (1941), and for P. squilla by Lucú et al. (1972).

An overshoot of sodium concentration, occurs during the first hour after the prawn are suddenly transferred from 100% to 50% SW (Figure 12.5). Two possible mechanisms might produce this effect

(1) activation of sodium uptake at the gills following reduction of the haemolymph volume (Table 8.4) or

(2) delayed inactivation of sodium transport across the gut wall into the blood due to the gut being full of sea water prior to the transfer to dilute medium.

The first possibility may be responsible in part but not in total for the brief rise in blood concentration since the time required for full activation of the sodium pump is probably longer than the period when the rise in the sodium concentration of the haemolymph is taking place. Sodium uptake at the gills is, therefore, not at its maximum rate when the overshoot occurs.

The second possibility is the more likely explanation, as can be shown by comparing the drinking rate and urine production during the first hour by prawns in a nonsteady state and in a steady state. During the first hour after transfer from 100% to 50% SW the prawns drink water to the extent of approximately 0.42% body wt, and produce urine at a rate of 1.62% body wt, whereas

prawns in a steady state drink about 0.26% body wt/hr in 50% SW, and produce urine at a rate of 0.93% body wt/hr. If we assume that, under a steady state condition, the water influx equals the outflux, then in 50% SW the osmotic inflow of water is (0.93-0.26) 0.67% body wt/hr. When the prawns are suddenly transferred from 100% to 50% SW they should produce circa 1.09% body wt (0.67 + 0.42) urine during the first hour, but in fact the animals produce a urine volume of approximately 1.62% body wt so a deficit of 0.53% body wt is left for water balance. If the prawns have no source of water to balance the deficit, the animals will lose weight to the value of some 0.53% body wt during the first hour. By looking at the weight-gained experiment (Appendix table 11), it is seen that the animals with unblocked antennary pores gain about 0.54% of the initial weight during the first hour. This means that there must be some source of water that the animals can obtain to balance the deficit. Possibly therefore uptake of salt and water from the gut may continue for a period after transfer from sea water to a more dilute medium. The sodium taken in with the water across the gut wall during this period cannot be extruded via the gills since in a dilute medium the gills' ion pump operates in the reverse direction. This effect probably gives rise to the high concentration of sodium in the haemolymph, as seen during the first hour after a sudden transfer from 100% to 50% SW (Figure 12.5).

A similar explanation can be applied to the situation where the animals are suddenly transferred from 100% to 70% SW. The water that is taken in across the gut epithelium during the first hour should equal the urine flow in the same period (0.51% body wt/hr),

since in this medium the osmotic gradient between the haemolymph and the external medium is nil, and there is no osmotic inflow of water. Hence, the fraction of water that has been in the gut prior to transfer, and is transported into the haemolymph in this case equals (0.51-0.41) 0.10% body wt. It can be seen that the water reabsorption across the gut of the prawn during the first hour after transfer into 70% SW equals a fraction that has been in the gut, plus a new component of water that the prawn drinks a total of 0.51% body wt, which is less than for those animals in 50% SW (0.95% body wt/hr). This would be expected because the ionic and osmolar concentrations of the two media are very different, so the conditions for the reabsorption of salts and water are not equivalent.

As shown in Table 8.4, the haemolymph volume of the prawn during the first two hours after transfer to 50% SW is reduced significantly from 19.4 $\pm$ 2.3 to 17.2 $\pm$ 3.0% body wt. Potentially therefore the sodium pump at the gills could be initially activated by the reduction of the haemolymph volume, since it can secondly be activated by the haemolymph concentration as this rises initially (experiment 7, chapter XII). The apparent link between the reduction of the haemolymph volume and the active uptake of sodium is also shown in experiment 4, chapter VIII, and experiment 9 of chapter XII. When the prawn is transferred suddenly from high to low salinity, the animal loses sodium from its haemolymph quickly. If the haemolymph sodium is decreasing faster than the intracellular concentration can be adjusted, water will pass from the haemolymph to the cells. The volume of haemolymph will, therefore,

be reduced if the uptake mechanism cannot take in enough sodium to compensate for the sudden loss or alternatively, if adjustment of cell amino acid concentration fails to maintain isotonicity between intra- and extra-cellular compartment. The instantanous reduction of haemolymph volume upon the transfer of prawn to dilute medium, as shown in experiment 4, chapter VIII, would be expected to stimulate the sodium pump resulting in an increased sodium uptake as Lockwood (1970) has shown in the amphipod, Gammarus duebeni.

When prawns are transferred from 50% to 100% SW the sodium concentration in their haemolymph reaches an equilibrium within 6 hours, but when the prawns are transferred from 100% to 125% SW, a new steady state of sodium in the haemolymph is only restored after 12 hours. Obviously, P. serratus can sustain a sudden fall in salinity from 100% to 50% SW better than a sudden increase of salinity from 100% to 125% SW. In the former case the sodium concentration in the haemolymph decreases slowly, since the animal possesses an active uptake mechanism counteracting the loss through the renal and extra-renal routes. In the latter situation, the inflow of sodium and the outflow of water disturbs the balance with an initial rise in sodium concentration in haemolymph (Figure 4.2) as a consequence of the sudden transfer from normal to more concentrated sea water.

4. Urine flow, urinary bladder function, and reabsorption of water.

Estimates of urine production in prawns stressed by decreasing salinities were accomplished by the weight-gained method and

clearance of Cr-51 EDTA from the haemolymph. The urine flow obtained from the latter method is slightly greater than the value obtained from the former. It must be borne in mind that neither of these methods give an exact value for the rate of urine production. The primary criticisms of the estimation of urine flow by the weight-gained method are

(1) the possible inhibition of urine formation due to back pressure,

(2) the risk of a handling shock effect producing anomalous urine production rates over the short period of test, and

(3) the adverse physiological reactions that may ensue.

The second method of measuring urine production has the advantage that handling can be reduced to a minimum, but it requires some exogeneous tracer substance to be administered, hence causing at least some stress which probably results in an increase in urine flow. Probably the most reliable measurement of urine flow in the prawn is the second method. Besides the fact that the handling effect can be reduced to a minimum, it is also possible to measure the rate of urine flow for a considerable period, 24 hours at least, which is long enough to obtain a reliable value for urine flow.

In 100% SW prawns produce urine at the rate of 0.44% body wt/hr. If this were continued without some compensatory intake the animals would become dehydrated and haemolymph volume and water content would decrease. The results of experiment 2.3 in chapter VII suggested that a major route by which the prawns obtain water is

by drinking. In full strength sea water the prawn drinks water at a rate of approximately 0.69% body wt/hr, therefore, if all of the water entering the gut is absorbed, there would be a surplus over the urine production rate of 0.25% body wt/hr for the osmotic outflow of water across the body surface.

As might be anticipated, another feature of adaptation by the prawn to the osmotically unstable environment in which it lives is a modification of its drinking activity according to the osmolality of the medium. When the prawn is in 50% SW, its drinking rate is about 0.26% body wt/hr, and the rate increases with increasing salinity, to circa 0.42%, and 0.69% body wt/hr in 70% and 100% SW respectively. The prawn is also able to reduce its drinking rate from 0.69% to 0.41% and 0.42% body wt during the first hour after the animal is subjected to a sudden change of salinity from 100% to 70% SW, and from 100% to 50% SW. In the latter medium, the prawn further reduces its drinking rate to a steady state level, 0.26% body wt/hr within 12 hours.

The U/H ratios for the inert urinary marker Cr-51 EDTA of prawns in 100% SW is 2.34 and it decreases with decreasing salinity from 2.34 to 2.16, 1.77, and 1.22 for prawns kept in 70%, 50%, and 10% SW respectively (Table 10.4). Since Cr-51 EDTA is presumably not transported across the bladder, the high U/H ratios for this marker could be either the result of active secretion or alternatively of water reabsorption from the primary urine. The decrease in U/H ratio with decreasing salinity suggests that a lesser proportion of water in the primary urine is reabsorbed before release of the definitive urine from the bladder when the prawns were kept in dilute media. The rapid clearance of Cr-51 EDTA

from the haemolymph as seen in the prawn when compared with crustaceans such as C. maenas probably results from the higher rates of water turnover across the bladder and the gut.

When exposed to a more dilute medium (10% SW) the animals show an ability to further increase their urine flow to compensate for a higher osmotic inflow of water. Flow increases to nearly twice that produced in 50% SW. This ability of P. serratus is similar to P. varians (Parry, 1955), but it is different from C. crangon which Spaargaren (1971) has shown, does not increase its urine flow when the salinity is decreased to nearly the lower limit of its range of tolerance. The difference in the mode of urine flow between P. serratus and C. crangon may be due to the fact that P. serratus possesses a more effective uptake mechanism than C. crangon.

Measurements of the rate of urine production of prawns which have just been subjected to a sudden decrease in salinity also show some evidence that the antennal glands play a significant part during the adjustment of the regulatory process. Their importance is demonstrated by the spectacular change in urine volume already mentioned when the prawns are suddenly transferred from 100% to 50% SW. The animals increase their urine flow to a level more than twice that of those acclimated to 50% SW. This suggests that when the prawns are subjected to a sudden changes from normal sea water to dilute sea water, the animals are able to excrete the excess water out of the body as rapidly as it enters until equilibrium has been reached and also confirms that urine production rate is determined by internal parameters rather than by the external salinity.

The volume of urine that the prawn produced in sea water (2.5 $\mu l/hr^{-1}g^{-1}$) possibly represents the minimum amount required for excretion of sufficient magnesium, sulphate, and calcium ions to keep the haemolymph level of these ions constant. This line of evidence is supported by the fact that the concentration of magnesium, sulphate, and calcium in the final urine of prawns is higher than in the haemolymph (Parry, 1954). Magnesium is a co-factor in several important enzyme systems and its regulation seems to be essential (Engback, 1952). Calcium is an important constituent of the skeleton in prawns so the regulation of haemolymph calcium levels must be precise. Control of haemolymph magnesium, calcium, and sulphate ions would seem to be a major function of the antennal glands.

In 70% SW the rate of urine flow measured by the weight-gained method was found to be 0.22% body wt/hr, whereas the value obtained from the clearance of Cr-51 EDTA was 0.45% body wt/hr. As has been discussed in section 3.2, chapter X, the urine flow obtained by the former method is probably an under-estimate, whereas the value determined from the clearance of Cr-51 EDTA may slightly over-estimate production. Although the value obtained from Cr-51 EDTA clearance is the same magnitude as the drinking rate of prawns in 70% SW (0.42% body wt/hr) it is unlikely that all the water in the gut of prawns in this medium, in which very little transport of solute is presumed to occur, will be transported into the internal milieu.

In 50% SW the rate of urine production by the prawn is increased by a factor of two when compared with that of animals

kept in 70% SW. This indicates that the antennal glands are capable of excreting large amounts of water through the excretory openings. The water which enters at an increasing rate, as the salinity in the medium decreases, can be excreted by increasing the rate of urine production.

Since the antennal glands are presumably the major site of water loss in hypo-osmotic medium, the rate of urinary water loss is assumed to be equivalent to the total net water uptake. The total water uptake of prawns acclimated to 50% SW is the sum of the osmotic inflow of water across the body surface and that due to drinking (0.26% body wt/hr). The net water uptake is balanced by an equivalent loss due to the urinary water (0.93% body wt/hr). Thus the water inflow osmotically across the body surface is about 0.67% body wt/hr.

Morphological examination of the antennal glands by Weldon (1891) has shown that in P. serratus these structures consist of an end-sac and excretory tubule which leads into the bladder. The end-sac is a small, compact, specialized structure composed of an outer layer of connective tissue containing blood spaces and an inner layer, thrown into a fold which projects into the lumen of the sac. The bladder, besides receiving the opening of the nephroperitoneal ducts, gives off on the one hand the ureter, and on the other a system of excretory tubules of the green glands. The excretory tubules forming the glandular plexus of the labyrinth, anastomose freely with one another, but they communicated by means of a common opening with the end-sac and by several openings in the bladder. Large clear vacuoles at the apices of cells lining the distal tubule were interpreted by Maluf (1939) as indicating

absorption of water from the haemolymph. Parry (1954) believed that the antennal glands of palaemonid prawns lack any segment such as the tubule of fresh water crayfish which might perform a salt-absorbing or water regulating function. Water reabsorption in the prawn probably does not occur in the nephidial tubule. Peters (1935) and Maluf (1939) found that the antennal artery of the crayfish sends a branch direct to the coelomic sac of the antennal gladd. A similar arrangement has been found in the blood supply to the end-sac of the prawn, where it suddenly splits up into numerous fine vessels which are lost in the walls of the end-sac. Neither the labyrinth nor any other part of the gland appears to have direct blood supply, although the connective tissue of the labyrinth has numerous blood lucunae, and all the parts of the antennal gland lie within the haemocoel (Parry, 1955). This arrangement of blood vessels suggests that at least the necessary apparatus is present for the production of primary urine in the coelomic sac by a filtration process.

A number of observations have been made on the mode of discharge of urine by the prawn (section 1, chapter X). In normal sea water the prawn releases its urine in a regular and consistent pattern whereas in 50% SW the animal tends to have a pattern of irregular discharge of urine. If the urine of P. serratus is expelled by contraction of the epigastric-sac as Parry (1955) has suggested is the case in P. varians, rates of the contraction of the epigastric-sac of prawn kept in normal sea water must be high and consistent which will make the disappearance of Cr-51 EDTA from the whole animal occur in a continuous rather than an intermittent pattern. If this is the case, a large portion of urinary

fluid must be retained in the bladder all the time. This suggests the possibility that the urinary bladder can function as a storage organ when the prawn is in 100% SW. Potentially, therefore, the bladder could act as a water reserve if water reabsorption is possible.

It is still not possible to state categorically whether secretion or filtration is the means by which primary urine is produced in P. serratus or in other marine crustaceans, although on the basis of the evidence available, the tendency to favour the hypothesis of filtration and subsequent water reabsorption is strong. The following discussion will serve to describe and then briefly assess the evidence now available.

A number of crustaceans have now been shown to excrete inulin and other polymers in the urine. Among them are the crayfish (Maluf, 1941a; Riegel & Kirshner, 1960), Homarus americanus (Burger, 1957), and Carcinus maenas (Riegel and Lockwood, 1961). Maluf (1941b) as a result of his cytological work and experiments using inulin, xylose, and dyes proposed that all the components of urine, including water, are secreted. Riegel & Kirshner (1960) repeated the experimental work and obtained similar results. Although they considered Maluf's work did not justify the conclusion that the urine is produced by secretion, their own could not be used as evidence that the urine of the crayfish is produced by filtration.

In the present work, there is some evidence to suggest that active secretion might be involved in the process of primary urine formation. As shown in chapter XII, in 100% SW the prawns with ligatured eyestalks have a higher Cr-51 EDTA U/H ratio (about 2.94) than the normal prawns (about 2.34) whereas the urine flow

of the former is slower (9.87 ± 2.59% body wt/day) than the latter (10.56 ± 1.65% body wt/day). This would be expected, if the primary excretory fluid was produced by a mechanism other than filtration, since the higher the rate of urine production the less the amount of marker which will accumulate in the bladder and the lower will be the U/H ratio.

There is some evidence for secondary modification of the urine after its initial formation. Thus the high U/H ratio for Cr-51 EDTA as shown in Table 10.4, can be interpreted as being due to water reabsorption from the primary urine. The theorectical model used by Riegel et al. (1974) for C. maenas has also been employed to test whether the high U/H ratio of Cr-51 EDTA found in the present study is due to a lack of equilibrium between the haemolymph and urine in the bladder or to water reabsorption. As shown in Figure 12.9-12.12, when acceptable limits are used for bladder volume, renal clearance rate and possible loss of marker to the tissue, the resulting U/H which can be attributed to a lag effect falls short of that observed. It is, therefore, necessary to postulate either that active secretion of marker occurs or that water reabsorption from the urine contributes to the rise in U/H.

When the prawn was injected with Cr-51 EDTA, the concentration in the bladder increased as urine production continued. After the maximum urine concentration of labelled chromium had been reached, the U/H ratio was maintained, at about 2.34 since the haemolymph and urine activity levels fell at the same rate. This also is a a good indication that urine production is a filtration mechanism.

Two additional factors suggest that either water reabsorption or secretion of marker contributes to the rise in U/H ratio.

Firstly, urine Cr-51 EDTA concentrations rise in a number of individual prawns to a level exceeding the concentration present initially in the haemolymph and this effect is more pronounced when the eyestalks of the prawns are removed (Figure 12.8). Secondly, the Cr-51 EDTA U/H ratio in prawns treated with ethacrynic acid is significantly lower than that of controls (Franklin, 1975) and the ratio reduces to near unity by the sixth day following such treatment.

5. Volume regulation of the prawn.

It has been shown in the preceding chapter that the prawn regulates its haemolymph volume as well as its body water content fairly constant in the salinity range 50% to 125% SW. The constancy of the fluid content shown implies that the animal regulates its body volume accurately in its normal range of salinity.

The regulation of total body volume when the animal is subjected to a sudden change from high to low salinity must be a function of the antennal glands (experiment 4, chapter X). The results of the present investigation in chapter X (Table 10.4) have shown that prawns control the rate at which water is removed from the body both by regulating the rate at which primary urine is formed and by reabsorbing fluid during the passage of urine through the excretory organs. The ability of the prawn to increase its urine flow with decreasing salinity also illustrates the importance of the function of the antennal glands. Volume regulation, however, it is not solely a question of elimination or gain or water. The osmotic and ionic levels of the haemolymph must be maintained despite the fluctuations in the volume (Lockwood, 1970).

An analysis of the haemolymph volume of prawns exposed to 100% SW plus thionine showed an increase compared with controls from 19.4 ± 2.3 to 25.4 ± 2.9% body wt. The result corresponds with the rates of urine production of these prawns which was reduced from 10.6 ± 1.6 to 7.3 ± 2.3% body wt/day. A possible explanation of this effect is that when the prawns are kept in 100% SW with an addition of thionine, there is inhibition both of sodium uptake across the gut epithelium and sodium extrusion via the gills. The inhibition of sodium transport across the gut, since it is probably associated with water uptake, in turn results in a lesser amount of urine production. The increase in the concentration of sodium in the haemolymph probably results from the failure of the gills to extrude sodium adequately in the presence of thionine. The increase in blood volume, as shown in experiment 3 of chapter VIII, must be a secondary response to the salt load of the prawn. The burden of ionic adjustment appears to be handled by the increase in haemolymph volume in order to maintain the homeostasis of the haemolymph.

6. Influence of temperature on osmotic and ionic regulation.

It appears that both salinity and temperature interact to alter the salt and water balance in the prawn. In dilute media the sodium concentration of the haemolymph decreases as the temperature is reduced. A possible explanation is that in dilute media the prawn relies on the sodium pump at the gills to compensate for the sodium loss via the renal and extra-renal paths and the antennal glands to excrete the excess water from its body.

As the temperature decreases the effectiveness of the sodium uptake mechanisms declines and results in less sodium intake. The effect is pronounced at 5°C, when the sodium uptake rate is impaired and the urine production is reduced from 10.6 ± 1.6 % to 4.6 ± 1.4 % body wt/day and is indicated by an increase in water content of the whole body (Table 6.1, and 6.3, chapter IV). The prawn can only survive for as long as the cells are able to tolerate the decrease in sodium concentration. When the haemolymph sodium concentration becomes too dilute for the cells to maintain the ions at an appropriate level the prawn dies.

In normal sea water, the sodium concentration of the haemolymph is increased when the temperature falls. The probable explanation is that in normal sea water the animals rely on the mechanisms that takes the water in across the gut epithelium and excretes the excess salts via renal and extra-renal routes. Since low temperature tends to decrease the net rate of extrusion of sodium this in turn results in increased sodium concentration in the haemolymph. This would also apply in the case of prawns kept in concentrated sea water within the temperature range 5° to 15°C.

For those kept in concentrated sea water in the temperature range from 15° to 25°C, the situation is reversed. In extreme conditions, high salinity and high temperature, the sodium concentration of the haemolymph increases toward that of the external environment (Figure 5.2, chapter V).

The results obtained in the present investigation in respect of the seasonal affects on osmotic regulation are comparable with those of Panikkar (1941) on P. serratus, and P. varians. He

found that prawns have a higher haemolymph osmotic level in winter than in summer. Broekema (1942) found the same phenomenon in C. crangon, the shrimp having a higher blood osmotic concentration at 4°C than at 21°C in 100% SW. Williams (1960) showed a like reversal of temperature effect in Panaeus aztecus and P. duorarum when results from the highest and lowest experimental temperatures (28.5° and 8.8°C) are compared.

The data obtained so far in crustaceans with regard to the nature of temperature effects on the osmotic concentration of body fluids can be related to their range of ecological adaptation. For example Gammarus duebeni and Rithropanopaeus harrisi (Kinne and Rotthauwe, 1952), can both be physiologically adapted to fresh water, even though they live primarily in brackish water. They have in common a tendency to maintain a higher osmotic concentration of the blood in fresh water when at a low temperature. Marine or brackish water species, such as Ligia oceanica and Idotea granulosa (Todd, 1963), C. crangon (Broekema, 1942), P. aztecus and P. duorarum (Williams, 1960), P. serratus, and P. varians (Panikkar, 1941), on the other hand, have a low blood osmotic concentration when low temperatures are combined with low salinities and show the reverse temperature effect when in 100% SW.

Although no satisfactory explanation can be offered here to account for the variety of response between different species with respect to temperature and salinity conditions, the results justify the inference that there is a physiologically effective temperature-salinity interaction. The three important properties of the medium are, therefore, the salinity, temperature, and their combination.

7. The involvement of neuroendocrine control in salt and water balance.

The maintenance of a constant gradient between the internal and external environments of the prawn depends on the regulation of both salts and water. The burden of compensation for movements of water and salts caused by external salinity changes is borne by specialized epithelia separating internal and external media, situated in effector organs.

The effector organs that play a major role in salt and water balance in P. serratus in dilute media are the gills and the antennal glands, while in normal sea water the gills, the gut, and the antennal glands operate simultaneously.

7.1 Water balance.

As stated in the introduction of chapter XIII, several investigators (Kamemoto et al., 1966; Mantel, 1967; Carlisle, 1956; etc.) believe that water permeability in crustaceans is under hormonal control. In support of this hypothesis, it has been found that following eyestalk ablation the urine flow increases (Kamemoto et al., 1966; De Leersnyder, 1967; Kato and Kamemoto, 1969; Kamemoto & Ono, 1969). These data have been interpreted as indicating that the operation results in an increase in integumental water permeability (Kamemoto et al., 1966), but the increase in urine flow could also result from either an increased filtration and/or a decreased reabsorption rate in the urinary bladder.

The hypothesis that ligaturing of the eyestalks increases the water surface permeability is not supported by the results of the present work on P. serratus, since there is no significant

difference in either increase in weight or the rate of clearance of Cr-51 EDTA between prawns with ligatured eyestalks and the controls when they are kept in 50% SW. In 70% and 100% SW the experimental prawns show a faster increase in weight than the controls (Figure 12.2, and 12.3) whereas if Kamemoto's conclusions were correct a slower rate of uptake would be expected in ligatured animals in 100% SW. The increase in weight of the experimental prawns in 70% SW, where the osmotic difference between the internal and external environment is nil and in 100% SW where the osmotic gradient favours the loss of water, must involve a process of active intake of water.

One interpretation of the above observations is that in 70% SW, even though the animal is isotonic to the medium, the sodium pump at the gills operates to take sodium in and the excess water is excreted through the antennary openings. The faster increase in weight of the eyestalk-ligatured prawns kept in 70% SW (experiment 3, chapter XII) suggests that the sodium uptake mechanism at the gills is controlled by an eyestalk factor. When this is removed from the circulation by eyestalk ablation, there is an increased rate of sodium uptake which in turn elevates the rate of water intake. The evidence that prawns with ligatured eyestalks have a faster rate of sodium uptake than the control is shown in experiment 8, and 9 of chapter XII.

At the moment, it seems likely that an eyestalk factor also controls the rate of sodium transport across the gut epithelium, for when the factor is removed, the rate of sodium transport is increased and this is accompanied by increased water intake. The lower rate of drinking in prawns with ligatured eyestalks

(0.45% body wt/hr) in 100% SW, when compared with controls (0.69% body wt/hr) is probably a physiological compensatory reaction to reduce the amount of the water that is transported in via the gut wall.

Ligaturing the eyestalks of prawns results in a higher U/H ratio for Cr-51 EDTA (experiment 14 of chapter XII) indicating a higher rate of water reabsorption than in the normal prawn. This suggests that water reabsorption from the urinary bladder is also controlled by an eyestalk factor. Whether or not the eyestalk factor is the same as the eyestalk anti-diuretic factor remains to be established.

A faster increase in weight of prawns with ligatured eyestalks and blocked antennary openings, as shown in experiment 2.3, chapter XII, when kept in 100% SW is presumably due to a higher rate of water uptake across the gut epithelium by comparison with those kept in 70% SW. The mechanism by which water is actively transported across the gut epithelium of prawns in 100% SW has been discussed in section 2 of this chapter. The faster rate of water transport across the gut epithelium and the higher rate of water turnover across the bladder in the experimental prawns definitely results in a larger increase in body water. The larger haemolymph volume of prawns with ligatured eyestalks (Table 12.12) confirms this point.

7.2 Salt balance.

The haemolymph electrolyte and osmotic concentration may also be under hormonal control as suggested by changes in these parameters following bilateral eyestalk ablation (Bryan, 1960a;

Kamemoto et al., 1966; Peterson & Loizzi, 1970). In the present study, there is no significant difference in the sodium concentration in the haemolymph or the rate of sodium extrusion at the gills between the animals with ligatured eyestalks and the controls when acclimated to 100% SW. This suggests that animals are still able to control their sodium balance although some parameter, such as drinking rate and urine production, is altered by the extirpation of the eyestalks.

In 50% SW the haemolymph $[Na^+]$ of the animals with ligatured eyestalks is higher than that of the controls. One possible explanation is that the sodium pumps at the gills are controlled by a factor which is released into the haemolymph from the eyestalks. When the factor is removed from the circulation by eyestalk ablation, it results in an increased rate of sodium uptake. This explanation is supported by the results of experiment 9, chapter XII which clearly showed that when the basic dye thionine, which is known to partially inhibit active sodium uptake, is applied to the external medium the sodium concentration in the haemolymph of both experimental and control animals falls by the same magnitude when they are transferred from 100% to 50% SW.

The higher rate of sodium efflux, as seen in the animals with ligatured eyestalks, probably is a secondary response to the higher concentration of the sodium in the haemolymph. This would be expected, since P. serratus has a tendency to regulate its haemolymph sodium constant at an appropriate level.

Kamemoto and Tullis (1972) have recently obtained results with Procambarus clarkii which accord with the present results.

They found that injection of brain homogenate into P. clarkii adapted to an isosmotic 0.1 M NaCl solution, causes the sodium influx rate to almost double.

There is now sufficient evidence to conclude that neuro-hormonal factors secreted by the eyestalks protect the prawn from excessive water turnover through the gut and the antennary bladder when in normal sea water and increase the sodium uptake by stimulating the transport mechanisms at the gut epithelium when the animal is kept in 100% SW and at the gills when it is exposed to dilute media.

8. Implications for prawn farming.

Physiological studies like those reported here are conducted in the laboratory because it is more convenient to study an animal under controlled conditions. In so doing the investogator may unknowingly eliminate environmental factors which are significant and hence extension of conclusions from laboratory studies to field situations should be undertaken with some caution. In the present study, P. serratus, was found to survive in media with salinities between 10% and 125% SW and temperatures ranging from 5^{o} to $25^{o}C$. The ability of the prawn to withstand a wide range of salinities and temperatures appears to depend primarily upon physiological compensation and to a lesser extent upon tolerating changes in haemolymph sodium concentration. It appears therefore to be well adapted to the sudden changes in salinity characteristic of prawn ponds. When the pond is fairly dilute, for example due to rainfall in early spring, the prawn could osmoregulate by actively taking up ions from the pond water to replace those lost

through the renal and extra-renal routes. As the season progresses, and the salinity of the pond increases, the prawn could maintain osmotic and ionic balances by reducing the rate of sodium uptake across the body surface and increasing the rate of water taken in across the gut epithelium. When the salinity of the pond water increases to a level higher than the normal sea water, the active uptake of sodium across the gills could be diminished, and the rate of water transport across the gut is increased as well as the rate of sodium extrusion via the gills. The prawn could only survive further concentration of the pond water as long as its cells were able to tolerate the increased haemolymph sodium concentration. When this became too great for the cells to maintain ionic balance the prawn would die.

This also applies to shrimp farms in the far east such as in Indonesia, Malaysia, the Philippines, and Thailand, where the culture of species like Penaeus monodon, P. merguiensis, is practised in outdoor ponds. In the first case, dilution of pond water always occurs during the rainy season when the medium may be diluted down to 10% SW during periods of heavy rain. For the second case, the concentration of pond water in summer will rise to more than 150% SW if no protection is undertaken.

For shrimp farms where the salinity can be controlled a knowledge of the isotonic conditions for the culture species will be of considerable value. When prawns are isotonic the energy required for osmotic work and the oxygen consumption of the animals are both minimal. The latter parameter has been demonstrated in P. varians (Lofts, 1956). The implication is that under isosmotic

conditions the natural mortality due to low oxygen tension is reduced, thus, the largest number of organisms can be cultured in a given volume of water. However, before a model of the optimum conditions of prawn ponds necessary to permit maximum growth for economic aquaculture can be proposed, the basic information concerning the effects of salinity in combination of temperature on feeding levels, food conversion, growth, and survival rate of the cultured species are required.

XIV. SUMMARY

1. The prawn, P. serratus regulates its haemolymph sodium hyper-ionic, and hypo-ionic in sea water concentrations below and above 70% SW respectively.

2. In normal sea water there is an inverse correlation between the haemolymph sodium concentration and temperature. In 50% SW the concentration increases irregularly with increasing temperature. In 125% SW, the sodium concentration has a minimum value at 15°C (382 ± 8.2 mM/l) and it increases with a change in temperature.

3. Within the salinity range 50% to 100% SW and temperature range 5° to 25°C the prawn regulates its water content very efficiently to between 74 ± 0.7 and 76 ± 1.2% body wt. At 15° to 25°C, when the salinity increases from 100% to 125% SW, the prawn is still able to regulate its water content within the range 75 ± 2.1 to 73 ± 1.2% body wt i.e. approximately the same as prawns in 100% SW at these temperatures (75 ± 1.4 to 74 ± 0.7% body wt). At 5°C, the water content of the prawns in 100% SW decreases significantly from 75 ± 1.6 to 73 ± 1.2% body wt. When the salinity decreases from 100% to 10% SW, at 15°C, the water content of the prawn increases from 75 ± 1.4 to 79 ± 1.1% body wt.

4. Reverse peristalsis in the hind gut and water drinking by prawns in different media were observed. In normal sea water the drinking rate was 0.69% body wt/hr (SD ± 0.13, n=12), and it was reduced significantly to 0.42% body wt/hr (SD ± 0.08, n=12) in 70% SW, and to 0.26% body wt/hr (SD ± 0.07, n=12) in 50% SW.

Reduction of temperature from 15° to 5°C resulted in a decrease in the drinking rate by about a half.

5. The haemolymph volume of prawns acclimated to different salinities has been measured. Within the range 10% to 100% SW the prawns regulated their haemolymph volume between 21.52 ± 4.76% and 19.44 ± 2.28% body wt.

6. The bladder volume of the prawn was measured. An average value of 2.61% body weight or 12.97% of haemolymph volume was recorded.

7. Urine production of prawns in various media has been determined. In 100% SW the prawn produced urine at the rate of 0.44% body wt/hr whilst the rate in 50% SW was 0.93% body wt/hr. In an isotonic medium (70% SW) the rate was 0.34% body wt/hr. At a salinity nearly at the lower limit of its tolerance, the prawn is still able to increase its urine flow to partially compensate for the osmotic inflow of water, but its water content increases. When the temperature is decreased from 15° to 5°C, the urine flow rate of prawns in 100% SW decreased from 0.44% to 0.19% body wt/hr.

8. Cr-51 EDTA U/H ratios of prawns kept in different salinities have been measured and there is evidence that water reabsorption occurs in the bladder. U/H ratios of 2.34, 2.16, 1.77, and 1.22 were observed in prawns kept in 100%, 70%, 50%, and 10% SW respectively.

9. The influence of salinity and temperature on the osmoregulatory response of the prawn was determined and the mechanisms which permit

the animal to adjust its internal fluids to changes in environmental salinity and temperature are discussed.

10. In a steady state condition the ionic regulation of the prawn in 100% SW involves continuous ingestion of medium and absorption of univalent ions and water by the gut epithelia. The excess ions are extruded through the gills and this gives a net uptake of water to balance the passive osmotic loss. The antennal glands serve to excrete divalent ions and reabsorb water. The possibility that the urinary bladder can function as a storage organ is suggested. In dilute salinities, the prawn swallows very little medium, ions are actively absorbed through the gills, and the osmotic inflow of water is balanced by the production of large quantities of isotonic urine.

11. The sodium extrusion across the body surface of the prawns exposed to various degrees of osmotic stress has been measured. A feature of sodium transport, Na-K exchange, is demonstrated. The transport system is located on the external membrane since it is inhibited by ouabain added in the external medium. The Na-K process is ouabain sensitive, but K^+ can antagonize the inhibitory action of the glycoside.

12. The possible hormonal control of salts and water balance in the prawn was studied by bilateral eyestalk ablation experiments. In 100% SW the haemolymph sodium concentration and the rate of urine flow of animals with ligatured eyestalks are similar to the controls, but there are significant differences in the rate of water drinking, the haemolymph volume, and the rate of water

reabsorption in the bladder. Eyestalk ablation elevates the U/H ratio for Cr-51 EDTA of the prawn from 2.34 to 2.94 which indicates a higher rate of water reabsorption from the bladder than the normal prawn. This implies that water reabsorption from the urinary bladder is controlled by an eyestalk factor. When prawns were subjected to a sudden change from 100% to 50% SW the haemolymph sodium concentration of animals with ligatured eyestalks and normal animals both reached a new steady state within 6 hours after the transfer but the animals with their eyestalks ligatured maintained a sodium value higher than the controls. In 50% SW, eyestalk ablation caused no change in rate of urine flow or haemolymph volume, but it raises the concentration of sodium in the haemolymph and also the rate of sodium efflux.

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XVI. APPENDIX

Appendix 1. A coefficient of variation of the replicate weighings of a prawn with reimmersion between weighings.

Replicate	Dead prawn (g)	Replicate	Live prawn (g)
1	0.8318	1	2.5415
2	0.8301	2	2.5299
3	0.8228	3	2.5299
4	0.8150	4	2.5234
5	0.8148	5	2.5266
6	0.8185	6	2.5276
7	0.8225	7	2.5322
8	0.8130	8	2.5188
9	0.8209	9	2.5256
10	0.8143	10	2.5186
11	0.8112	11	2.5226
12	0.8127	12	2.5244
$\bar{X}$	0.8189	$\bar{X}$	2.5258
S.D.	$\pm$ 0.0068	S.D.	$\pm$ 0.0045

Coefficient of variation = 0.83% Coefficient of variation = 0.17%

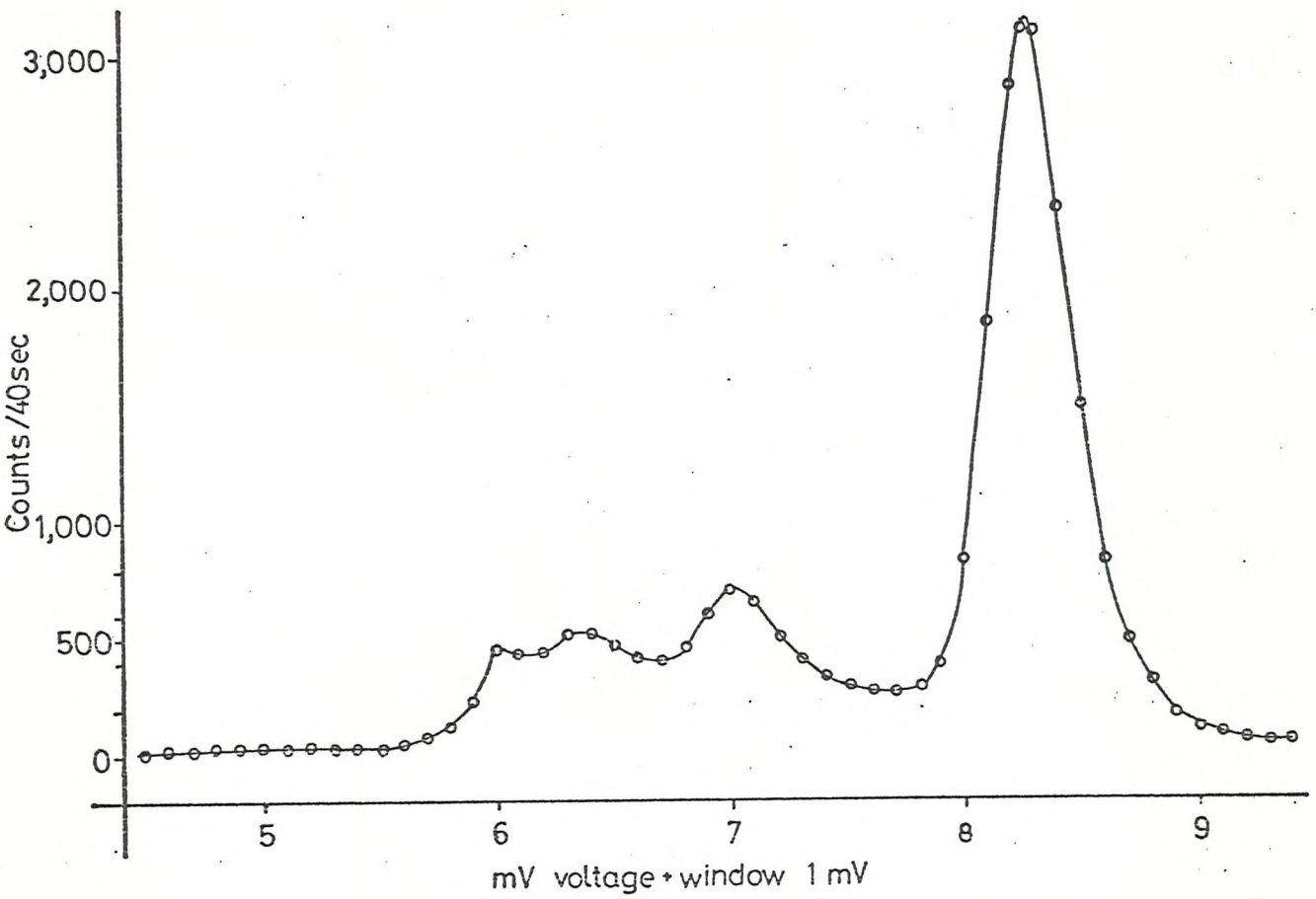
EHT = 950

Attenuation = 64

Fine attenuation = 2

Low window = 7.7

Upper window = 9.0



Appendix 2. Cr-51 EDTA gamma counting spectrum.

Appendix 3. Levels of significance of the salinity induced variation in water content of the prawn.

Temp. (°C)	Sal. (‰ SW)	n	Water content (% body wt weight)	S.D.	df	t-value	Probability
5	50	18	75.70	± 1.20	34	7.5841	P< 0.001
	125	18	72.65	± 1.21			
	50	18	75.70	± 1.20	34	1.8273	NS
	100	18	74.84	± 1.59			
	125	18	72.65	± 1.21	34	4.6440	P< 0.001
	100	18	74.84	± 1.59			
15	50	18	75.34	± 2.24	34	0.5138	NS
	125	18	74.97	± 2.10			
	50	18	75.34	± 2.24	34	1.6290	NS
	100	18	74.56	± 1.42			
	125	18	74.97	± 2.10	34	0.6783	NS
	100	18	74.56	± 1.42			
25	50	18	74.71	± 2.3	34	2.4390	P< 0.05
	125	18	73.23	± 1.12			
	50	18	74.71	± 2.3	34	0.6084	NS
	100	18	74.36	± 0.74			
	125	18	73.23	± 1.12	34	3.5617	P< 0.001
	100	18	74.36	± 0.74			

Appendix 4. Analysis of variance. The analysis is based on the percentage of water content of the whole animal per unit wet weight.

Temp. (^{o}C)	Variation due to	df	Sum of squares	Mean square	F	Probability
5	Sal	2	89.1078	4.5539	24.4963	$P < 0.01$
	Residuals	51	92.7612	1.8188		
	Total	53	181.8690			
15	Sal	2	5.4635	2.7317	0.7173	NS
	Residuals	51	195.1846	3.8271		
	Total	53	200.6481			
25	Sal	2	21.6134	10.8067	4.5024	$P < 0.01$
	Residuals	51	122.4152	2.4002		
	Total	53	144.0286			

Appendix 5. Analysis of variance. The analysis is based on the percentage of water content of the whole animal per unit wet weight.

Sal (% SW)	Variation due to	df	Sum of squares	Mean square	F	Probability
50	Temp	2	9.0487	7.0487	1.7866	NS
	Residuals	51	201.2119	3.9453		
	Total	53	210.2606			
100	Temp	2	2.1051	1.0525	0.6163	NS
	Residuals	51	87.0887	1.7076		
	Total	53	89.1938			
125	Temp	2	52.2659	26.1329	10.9191	$P < 0.01$
	Residuals	51	122.0603	2.3933		
	Total	53	174.3262			

Appendix 6. Analysis of variance. The analysis is based on the percentage of water content of the whole animal per unit wet weight.

Variation due to	df	Sum of squares	Mean square	F	Probability
Sal	2	73.0582	36.5291	13.6200	$P < 0.001$
Temp	2	20.2926	10.1463	3.7831	$P < 0.05$
Sal x Temp	4	43.1271	10.7817	4.0200	$P < 0.01$
Residuals	153	410.3609	2.6820		
Total	161	546.8388			

Appendix 7. Effect of transfer to potassium free-sea water on sodium efflux from prawns.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	K-free SW		100% SW (hr)	K-free SW (hr)
1	2.06	0.4332	0.3300	76.17	1.60	2.10
2	2.14	0.3466	0.2888	83.32	2.00	2.40
3	1.83	0.3466	0.2599	75.01	2.00	2.67
4	1.91	0.3150	0.1808	57.39	2.00	3.83
5	3.15	0.3466	0.2632	75.93	2.00	2.63
6	2.89	0.3466	0.2363	68.17	2.00	2.93
$\bar{X}$		0.3557	0.2598	72.67	1.97	2.76
SD		$\pm$ 0.0400	$\pm$ 0.0500	$\pm$ 9.89	$\pm$ 0.20	$\pm$ 0.59

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW		0.3557 $\pm$ 0.0400 ($\bar{6}$)			
	1.83 - 3.15		10	3.9303	$P < 0.005$
K-free SW		0.2598 $\pm$ 0.0500 ($\bar{6}$)			

Appendix 8. The relative efflux of sodium from prawns after transfer from 100% SW to 10 mM-K/l saline.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	10 mM-K/l		100% SW (hr)	10 mM-K/l (hr)
1	2.06	0.1764	0.1764	100.00	3.93	3.93
2	3.12	0.4415	0.4333	98.00	1.57	1.60
3	2.51	0.2773	0.2773	100.00	2.50	2.50
4	2.74	0.3109	0.3109	100.00	2.23	2.23
5	2.34	0.2888	0.2853	98.79	2.40	2.43
6	20.6	0.3610	0.3349	92.77	1.92	2.07
$\bar{X}$		0.3093	0.3030	98.28	2.43	2.46
SD		± 0.0883	± 0.0836	± 2.81	± 0.81	± 0.78

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW		0.3093 ± 0.0883 $(\bar{6})$			
	2.06 - 3.12		10	0.1288	NS
10 mM-K/l		0.3030 ± 0.0836 $(\bar{6})$			

Appendix 9. Effect of transfer to sea water containing ouabain on sodium efflux from prawns.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1})		Relative efflux (% SW control)	Half time efflux	
		100% SW	100% SW + ouabain		100% SW (hr)	100% SW + ouabain (hr)
1	2.03	0.3648	0.2599	80.02	1.90	2.67
2	3.07	0.3466	0.2736	78.93	2.00	2.53
3	2.52	0.3466	0.2772	79.97	2.00	2.50
4	1.89	0.3035	0.2666	87.84	2.28	2.60
5	2.34	0.4077	0.3300	80.94	1.70	2.10
6	2.90	0.3923	0.2971	75.73	1.76	2.33
$\bar{X}$		0.3602	0.2840	80.57	1.94	2.46
SD		$\pm$ 0.0360	$\pm$ 0.0244	$\pm$ 3.99	$\pm$ 0.21	0.21

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW		0.3602 $\pm$ 0.0360 ($\bar{6}$)			
	1.89 - 3.07		10	4.4046	$P < 0.005$
100% SW + ouabain		0.2840 $\pm$ 0.0244 ($\bar{6}$)			

Appendix 10. Effect of ouabain on sodium efflux from prawns kept in normal sea water with addition of 30 mM-K/l.

No.	Wet weight (g)	Rate constant of efflux (hr^{-1}) 100% SW	100% SW +30mM-K/l +ouabain	Relative efflux (% SW control)	Half time efflux 100% SW (hr)	100% SW +30mM-K/l +ouabain (hr)
1	4.09	0.4332	0.3851	88.99	1.60	1.80
2	2.39	0.2971	0.2736	92.09	2.33	2.53
3	2.40	0.2310	0.2143	92.77	3.00	3.23
4	2.40	0.3175	0.2970	93.54	2.18	2.33
5	1.96	0.3999	0.3961	99.04	1.73	1.75
6	2.27	0.2971	0.2632	88.58	2.33	2.63
$\bar{X}$		0.3293	0.3048	92.48	2.20	2.38
SD		$\pm$ 0.0741	$\pm$ 0.0714	$\pm$ 3.8076	$\pm$ 0.50	$\pm$ 0.56

Analysis of t-test

Treatment	Weight range (g)	Rate constant of efflux (hr^{-1})	df	t-value	probability
100% SW	1.96 - 4.09	0.3293 $\pm$ 0.0741 ($\bar{6}$)	10	0.5946	NS
100% SW + 30mM-K/l + ouabain		0.3048 $\pm$ 0.0714 ($\bar{6}$)			

Appendix 11. Weight changes of normal prawns after a sudden transfer from 100% to 50% SW.

Hours after transfer	Weight increase (% initial weight) excretory pores open	blocked excretory pores
1	0.54 ± 0.83 (n=17)	3.09 ± 1.03 (n=15)
3	0.92 ± 0.86 (n=17)	5.79 ± 1.89 (n=15)
6	1.35 ± 0.98 (n=17)	8.37 ± 2.59 (n=15)
12	1.74 ± 1.51 (n=17)	11.56 ± 1.69 (n=13)
24	1.05 ± 0.85 (n=17)	16.55 ± 1.67 (n=5)

Appendix 12. Concentrations of sodium in total body, haemolymph, and urine of prawns in different salinities after at least three days acclimation.

Sea water (% SW)	(Na^+)	Concentration of sodium (mM/l) Total body	Haemolymph	Urine	U/H ratio	$\frac{\text{Total body}}{\text{Haemolymph}}$
100	473±9.3	146±11.0 (n=18)	364±7.0 (n=18)	316±5.0 (n=8)	0.71	0.40
75	356±7.0	137±10.0 (n=8)	339±3.0 (n=18)	294±6.0 (n=8)	0.75	0.42
50	233±3.0	134± 8.0 (n=18)	307±2.0 (n=18)	268±7.3 (n=18)	0.87	0.43

Appendix 13. Efflux of sodium from prawns acclimated in three salinities at 15°C.

Salinity (% SW)	Weight range (g)	Rate constant of efflux (hr^{-1})
100	1.82 - 3.41	0.3817 ± 0.0346 (n=30)
70	2.02 - 4.39	0.2383 ± 0.0264 (n=7)
50	1.82 - 3.15	0.1870 ± 0.0141 (n=6)