ASPECTS OF IONIC REGULATION IN *CANCER MAGISTER*, DANA

by

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B.Sc., University of Western Ontario, 1968

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the Department
of
Zoology

We accept this thesis as conforming to the
required standard

THE UNIVERSITY OF BRITISH COLUMBIA

DECEMBER, 1970

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ABSTRACT

Regulation of chloride, sodium, potassium, calcium, and magnesium ions was determined for hypo- and hypersaline conditions in the crab, Cancer magister, from an estuarine environment. Animals from both summer and winter were examined.

Chloride regulation in the blood was hypertonic in dilute salinities and hypotonic in concentrated salinities, with summer animals maintaining a greater gradient in the former and winter animals a greater gradient in the latter. Sodium in the blood is regulated hypertonically in all experimental salinities, with summer animals maintaining a greater gradient. Blood potassium is regulated hypertonically in dilute salinities, approaching isotonicity in hypersaline media. Summer animals maintain a greater gradient of potassium concentration. Blood calcium is regulated hypertonically in all experimental salinities, with summer animals maintaining a greater gradient in dilute salinities and winter animals a greater gradient in concentrated salinities. Magnesium is regulated at a pronounced hypotonic level in the blood over the entire experimental salinity range, with winter animals maintaining the greater gradient.

Major changes in the adaptation of blood ionic concentrations occur within a few hours of exposure to the experimental salinities, with half of the final equilibrated concentration values attained by twelve hours.

Animal weight was found to bear no significant relationship to the ionic regulatory activity observed.

Renal involvement in regulation has been shown for all the ions, with the production of a urine hypertonic to the blood for chloride and magnesium, and a urine hypotonic to the blood for sodium, potassium, and calcium. Renal regulation was greater in winter animals for chloride, and greater in summer animals for sodium and potassium.

Ionic regulation by the gills of summer and winter animals was investigated by potential difference measurements, and was suggested to occur for all ions. Chloride may have been regulated by the absorption from dilute media and excretion into concentrated media. Sodium may have been regulated by secretion into dilute media. The involvement of the gill in potassium, calcium, and magnesium regulation was implicated.

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ACKNOWLEDGEMENTS

I wish to expend my thanks to my supervisor, Dr. Paul A. Dehnel, for his support and critical evaluation of this study. Thanks are also due to Dr. T.H. Carefoot and Dr. J.E. Phillips for constructive reading of this thesis. I am indebted to Mr. G. Major, whose vessels and equipment were used for the collection of the research animals. I wish to acknowledge the receipt of financial support from the National Research Council of Canada. I extend my gratitude to my wife, Jennifer, for her enthusiasm and help in the preparation of this thesis.

INTRODUCTION

Crustaceans inhabit a wide range of environmental salinities, ranging from fresh water to greatly hypersaline conditions. Since crustaceans have probably evolved in the sea, and their tissues consequently tend to function most effectively when their total internal concentration is that of sea water, some regulation of body fluids becomes necessary in a non-marine environment such as the estuarine condition. Ionic concentrations of body fluids have to be maintained at a level not too far removed from intracellular concentrations, or else within a range where the cells themselves can regulate their concentration (Florkin, 1962 a, b; Shaw, 1958 a, b). Consequently, the concentration of these body fluids may be far removed from the ionic concentrations found in the particular environmental medium.

In the hyposaline estuarine environment, crustaceans can oppose reduced salinities by means of reducing the permeability of body membranes, as well as by regulating the ionic concentrations of body fluids, especially those of the blood. An increased production of urine serves to rid the body of excess water tending to flow into the animal from the hyposaline environment. The loss of salts accompanying such a urine flow must be compensated for by their active reabsorption (Martin, 1957).

There are several ways in which relative stability of body fluid ionic concentration is achieved in an estuarine crustacean.

Many researchers, past and present, have found the gill to be an ion regulating organ. Among these, Webb (1940) found that in the crab Carcinus maenas, ionic regulation in the blood is partly the result of active absorption by the gills of potassium, sodium, calcium, and chloride at a rate greater than that at which they are lost by diffusion. Active regulation of sodium at the gill surface has also been observed in the blue crab Callinectes sapidus (Habas, 1965; Habas and Prosser, 1963; Mantel, 1967), and in the mitten crab Eriochier sinensis (Koch, 1953; Koch et al, 1954). Potassium regulation has been observed to occur at the gill surface of the squat lobster Galathea squamifera (Bryan, 1965). Dall (1965) found that calcium regulation, as well, occurs at the gill surface of metapenaeid shrimp. Other researchers have used chloride ion regulation as a rough measure of the entire ion transport occurring in the gills, both for the uptake of ions, as in Astacus leptodactylus and Astacus astacus (Bielawski, 1964) and active secretion of ions, as in the ghost crab Ocypode albicans (Flemister and Flemister, 1951).

The antennary gland, as an excretory system combined with the urinary bladder, is able to produce a urine hypertonic to the blood with regard to such ions as magnesium and sulphate, and in this way maintains the blood concentrations of these ions hypotonic to the medium. This has been shown, for example, in fiddler crabs, Uca pugnax and Uca pugilator by Green, et al (1959), in the squat lobster Galathea squamifera by Bryan (1965), and in a shore crab, Pachygrapsus crassipes by Gross (1959 a, b).

Two other species of shore crab, Hemigrapsus nudus and Hemigrapsus oregonensis have been shown to regulate blood magnesium levels by selective urinary secretion (Dehnel, 1967; Dehnel and Carefoot, 1965). The antennary gland also serves to regulate sodium, potassium, and calcium in some crustaceans, such as the lobster Homarus sp. (Burger, 1956 a,b; 1957), the brachyuran crabs Cardisoma sp., Sesarma sp., and Varuna sp. (Gross, et al, 1966), and the crab Carcinus maenas (Riegel and Lockwood, 1961; Webb, 1940).

Other sites have been found to be involved in ionic regulation by estuarine crustaceans. In the lobster Homarus sp. (Burger, 1956 a; 1957), the gut is effective in the regulation of magnesium and sulphate. The mid-gut gland has been suggested by Green, et al (1959) as having some regulatory function in Uca pugnax and Uca pugilator.

Seasonal differences in the degree of ionic regulation of body fluids have been demonstrated in several species. For example, recent work on the shore crabs Hemigrapsus nudus and Hemigrapsus oregonensis showed an interrelationship of season and calcium ion regulation, these animals being better regulators of this ion in winter at low salinities (Dehnel, 1967; Dehnel and Carefoot, 1965). Temperature was found by Dehnel and Carefoot (1965) to affect the degree of magnesium regulation by impairing it at high temperatures of 25^{o} C.

This study is undertaken to determine the occurrence and extent

of ionic regulation in the body fluids of the crab Cancer magister, taken from an estuarine habitat in different seasons and exposed to a range of hypo- and hypersaline experimental concentrations. Seasonal differences in ionic regulation are related to seasonal concentration and temperature changes in the habitat. The antennary gland system and the gills are examined as potential sites of regulatory activity.

MATERIALS AND METHODS

Source of Research Animal:

The animal used in this study was the Pacific or common edible crab, Cancer magister Dana, which ranges from Unalaska, Alaska to Magdalena Bay, Lower California (Schmitt, 1921).

Male intermolt C. magister were obtained from an estuarine environment, the Roberts Banks area in the Strait of Georgia off the mouth of the south arm of the Fraser River (Fig. 1). This collecting area is designated as fishing area 29 A and B by the Department of Fisheries of Canada. The animals were caught in standard commercial crab traps, set at depths of 30 to 60 feet, mean tide level, using as bait dead flounder and other ground fish caught in the same area. The traps were laid for a distance of about 15 miles along the coast, and animals were taken from the entire length. Collecting was done in both summer and winter in the same area. Summer animals were collected on June 6 and August 17, 1969 and July 30, 1970. Winter animals were collected February 24, 1970. On August 17, 1969 and February 24, 1970, a sample of sea water was taken in the area at the depth of each trap for later ionic concentration measurements. At the same time, temperature measurements were obtained from the same depth of water. Summer temperatures were found to be 14 - 15° C. and winter temperatures were 7.5 - 8° C.

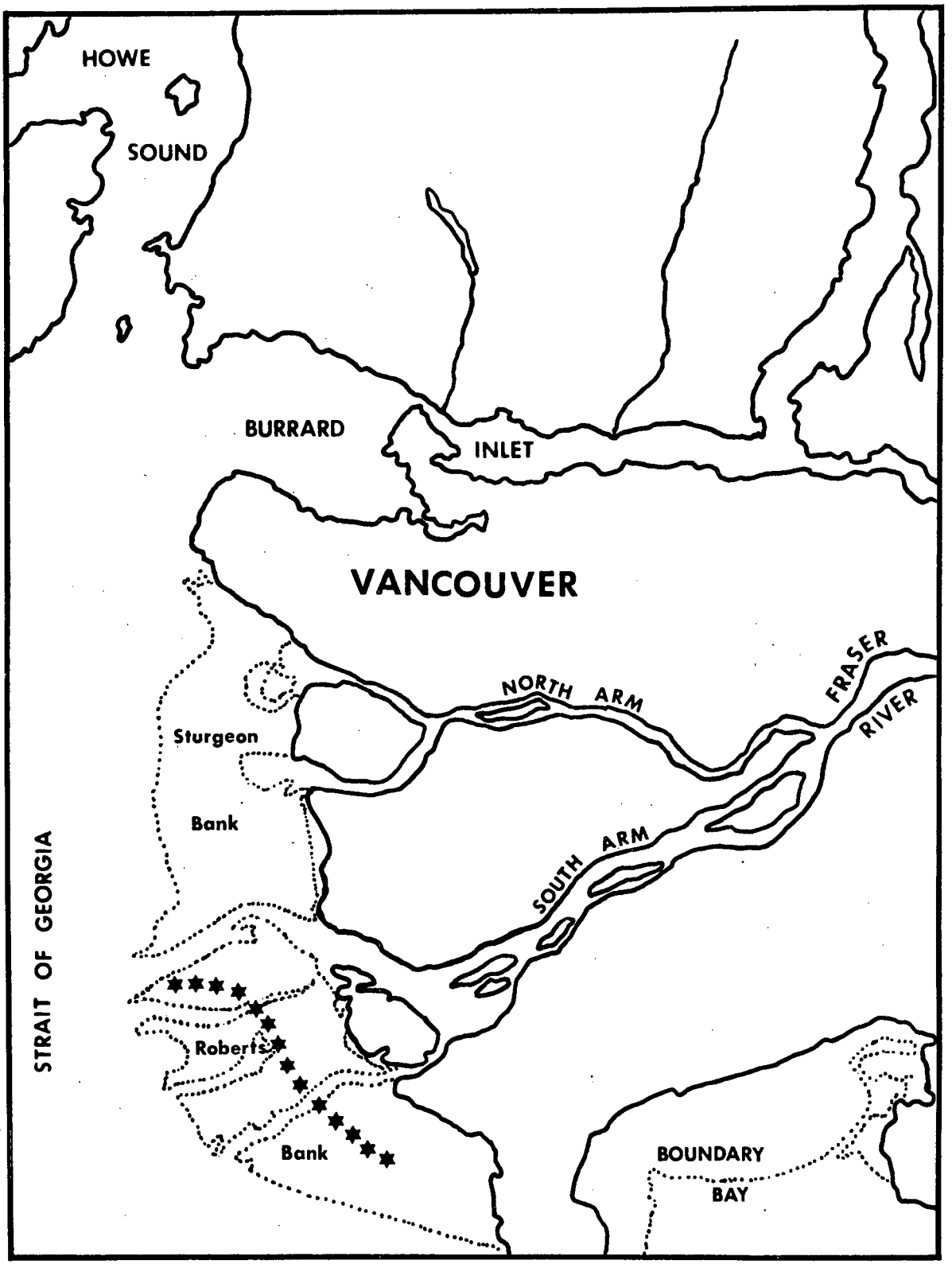
Both field and experimental concentrations were expressed as

FIGURE 1

Map of collecting area off the south arm of the Fraser River, British Columbia (from Department of Mines and Technological Surveys, Canada; No. 92 G).

Scale 1:250,000

✱✱✱✱ - collecting sites


HOWE
SOUND
BURRARD
INLET
VANCOUVER
NORTH ARM
FRASER
RIVER
Sturgeon
Bank
SOUTH ARM
STRAIT OF GEORGIA
Roberts
Bank
BOUNDARY
BAY

percentages of sea water, where 100% sea water has been determined in this laboratory to have a salinity of 31.88 $^{o}/_{oo}$ and a chlorinity of 17.65 $^{o}/_{oo}$ at 25^{o} C. (Dehnel, 1960, 1962, 1966). Concentrations of the major ions in 100% sea water, measured in mEq/L, are then determined to be 497 for chloride, 433 for sodium, 10.13 for potassium, 25.6 for calcium, and 97.9 for magnesium.

Ion analyses of field sea water showed the concentration of chloride, sodium, potassium, calcium, and magnesium to be lower in the summer on August 17, 1969 than in the winter on February 24, 1970 (Table 1). A greater variation was present in the summer samples than in the winter samples. Conversion of chloride concentration to total salinity by the Knudsen equation (Strickland and Parsons, 1968, p. 11) gives a value for the summer condition of 27.3 $^{o}/_{oo}$ and for the winter condition of 29.4 $^{o}/_{oo}$, both below the standard value of 31.88 $^{o}/_{oo}$. These results are similar to the analyses given by Waldichuk, et al (1968 a, b) for the Fraser River estuary in the Roberts Banks area.

Experimental Design:

Holding Conditions

After collection from the traps, the crabs were transferred to holding boxes, where they were kept in damp sea weed (Fucus sp.)

TABLE 1

Field ionic concentrations in summer (August 17, 1969) and winter (February 24, 1970) in the Roberts Banks area off the Fraser River. These values are averages of 12 collecting stations in summer and 10 collecting stations in winter (± S.D.).

Ion	Summer		Winter	
	mEq/L	% sea water	mEq/L	% sea water
Cl^-	426 ± 37	86	458 ± 14	92
Na^+	370 ± 29	85	418 ± 5	96
K^+	8.2 ± 0.9	81	8.3 ± 0.2	82
Ca^{++}	16.1 ± 1.8	63	18.3 ± 0.4	71
Mg^{++}	83.8 ± 6.8	86	94.6 ± 3.1	96

or in sea water wetted excelsior. They were then transferred to laboratory holding tanks, no longer than 3 - 7 hours following collection.

In the laboratory, the crabs were kept in a controlled temperature environment room, at 15 ± 1° C. for summer and at 7.5 ± 1° C. for winter conditions. No more than 12 animals were kept in any one of the covered holding tanks. These tanks had a floor area of 3.14 m^2 and were filled with at least 50 L. of aerated sea water. The water was changed daily until all digested and undigested food had been voided from the animals, then changed every second day. The sea water used for the eight-day holding period was from Burrard Inlet (Fig. 1). The concentrations of ions in this stock sea water were found to be essentially the same as those of the summer and winter field conditions in both relative and absolute amounts. This indicated the suitability of stock sea water for use during the holding periods.

Experimental salinities were determined as follows: hyposaline media were obtained by dilution of stock sea water with glass distilled water, while hypersaline media were obtained by the dilution of a 200% artificial sea water (based on 100% sea water of 31.88 $^{o}/_{oo}$ salinity). The 200% sea water was made up by the addition of NaCl, Na_2SO_4, KCl, $CaCl_2$, and $MgCl_2$ to stock sea water (Barnes, 1954).

A concentration range of 50, 75, 100, and 125% sea water was expected, but subsequent measurement showed this to be the case only for chloride, sodium, and magnesium. Potassium and calcium

varied from the expected range as a result of relatively lower values of these two ions in stock sea water, and, in the case of calcium, wetness of the reagent salt to depress final experimental salinity values (Table 2).

After the eight-day holding period, 12 crabs were placed into each of the experimental media, and this time noted as 0 hours of exposure to these salinities. Subsequent unaccountable deaths occurred, but left at least 8 animals in each salinity, summer and winter, which survived the entire experimental period. 5 animals were also used as controls for both summer and winter and were placed into stock sea water at the same time.

Sampling of Blood and Urine

Blood and urine samples were taken at 0, 6, 12, 24, 48, 72, and 96 hour time intervals from individual animals, placed at 0 hours into the experimental salinities. The controls were sampled the same way to determine that the eight-day holding period was of sufficient length to allow the animals to maintain stable blood ionic concentrations, and further, to test any effect of the sampling operation on blood ionic concentrations.

A group of summer animals was sampled first, and for the additional time periods of 120, 144, 168, and 216 hours, to determine the time at which regulatory activity achieved a constant level. Since this point had occurred by the 96 hour time interval, winter animals were sampled subsequently only to the 96 hour time period.

TABLE 2

Measured ionic concentrations in experimental media.

Ion	Experimental Salinities							
	Medium 1		Medium 2		Medium 3		Medium 4	
	mEq/L	% sea water	mEq/L	% sea water	mEq/L	% sea water	mEq/L	% sea water
Cl^-	249	50	373	75	497	100	621	125
Na^+	217	50	325	75	433	100	542	125
K^+	3.9	38	6.7	66	10.1	100	12.7	125
Ca^{++}	8.8	34	14.3	56	20.8	81	26.8	105
Mg^{++}	48.9	50	73.4	75	97.9	100	122.3	125

Blood samples of approximately 1.5 ml. were drawn with a No. 22 hypodermic needle and syringe through the membrane proximal to the coxopodite of the second, third, or fourth pairs of pereiopods. Urine samples were obtained by lifting the operculum covering the ureter from the antennary gland. This lifting action was usually sufficient to stimulate the flow of urine from the ureter, of which approximately 1.5 ml. were collected by aspiration. Urine samples were drawn at the same time as the blood samples, and from the same animals. Any contamination of the urine sample with blood, as a result of rupture of the ureter, was readily determined since the contaminated sample lost its clear appearance to take on a turbidity due to the clotting of the blood contaminant. Such samples were discarded. Aliquots of the experimental sea water were also taken during the sampling period, to be analyzed as a check on the specific experimental salinities.

Once all blood, urine, and medium sampling at the particular time period was completed, individual samples were sealed in glass vials with parafilm (American Can Company) and refrigerated at 1.0 $\pm$ 0.5^{o} C. to await later analyses.

Wet weights of animals used in the salinity experiments were recorded at the end of the experimental period.

Analyses of Ion Concentrations:

Blood, urine, and medium samples were treated uniformly in the

analyses.

The samples were first agitated using a Vortex mixer, which served to break up the clot in the blood samples. Subsequently, they were centrifuged at 1600 x G. In the time required for these operations, the samples attained the room temperatures necessary to carry out the dilutions for the ion analyses.

Chloride concentrations were determined as a measure of the regulation for this ion, as well as a rough indication of the total ionic regulation occurring in these animals since a large proportion of the cations in sea water are in solution as their chloride salts (Pearse and Gunter, 1957). Total chloride concentration was obtained by coulometric-amperometric titration with silver ions using a Buchler-Cotlove chloridometer with direct readout (Buchler Instruments, Inc.) and following the method designated by Cotlove, et al (1958 a, b) and Cotlove (1963). A 100 lambda aliquot of a 100 mEq/L solution of NaCl was used to standardize the instrument. This standard, as well as 100 lambda aliquots of 1:4 dilutions of blood, urine, and medium samples were titrated at high rate, coulometrically delivering about 0.25 microequivalents of silver per second, in a 4 ml. solution of 0.1 N HNO_3, 10% glacial acetic acid, and 0.025% gelatin reagent (Buchler Instruments, Inc.). Multiplication of the readout value by a factor of four then gave total chloride concentration. Fresh polishing of the electrodes, analysis of at least six standard samples at the start of each set of chloride analyses, as well as restandardizing the instrument every twenty samples, if required, and use of identical volumes of reagent

and sample in each case assured minimal variability (± 1%) due to method.

Concentrations of sodium, potassium, and calcium ions were determined by flame photometry using a Zeiss PF 5 flame photometer, and the method described by Hoefert (1962). At the start of the analysis for each of the three ions, the instrument was calibrated using a blank solution, and a series of standard solutions of NaCl, KCl, and $CaCl_2$. Potassium and calcium calibration solutions were corrected for flame background by the addition of 1.5 mEq/L of NaCl. The values obtained for each set of calibrations were plotted as a calibration curve, from which readout values of 1:250 dilutions of blood and urine, as well as medium, samples yielded concentration values for sodium, potassium, or calcium in mEq/L. These were multiplied by a factor of 250 to give final ionic concentrations.

Magnesium concentrations were found by determining combined calcium-magnesium concentrations by the EDTA-titration method for divalent cations of Schwarzenbach, et al (1946). 2.0 ml. aliquots of the same 1:250 dilutions used for flame photometry were prepared for titration with 5×10^{-5}M EDTA by the addition of three drops of NH_4OH - NH_4Cl buffer (pH 10) and four drops of Eriochrome Black T indicator (Hartman Leddon Company). Constant agitation and heating of this mixture to 38° C. was found to sharpen the titration end point. The colour change was from a clear magenta to a clear aquamarine. Since EDTA at pH 10 combines with both calcium and magnesium, the volume of EDTA

solution used to bring about the titration end point indicated the total amount of both ions present. The concentration of magnesium alone was determined by subtracting the corresponding concentration value for calcium in the same sample from the product of the number of ml. of EDTA required, multiplied by a conversion factor of 12.5, based on the 1:250 dilution.

In all of the above analyses, only the samples drawn from animals which survived the whole experimental period were used.

Analyses of blank solutions for chloride, sodium, potassium, calcium, and magnesium ions using the same methods as described for blood, urine, and sea water samples were carried out to determine the leaching of these ions from the glassware used in the experiments. Sodium was found to contribute no more than 1% to the final experimental concentration values, and thus was ignored. No indication of leaching of the four other ions could be found.

U/B Ratios:

Urine to blood (U/B) ratios were calculated using the concentration values obtained by the above methods. U/B ratios greater than unity were interpreted as evidence of regulatory activity by the antennary gland by the production of a hypertonic urine. This may have occurred by an active secretion of ions into the urine, or by the reabsorption of water from it. Conversely,

U/B ratios less than unity indicate a hypotonic urine, produced by selective reabsorption of an ion from the urine, or an active excretion of water.

Gill Activity Measurements:

Both summer and winter conditions were examined, with summer animals collected on July 30, 1970, and winter animals collected on February 24, 1970. These animals received the same treatment as those used for determination of blood and urine concentrations. This included a holding period of eight days, and 96 hours of exposure to the experimental concentrations, at which time the potential difference measurements were carried out.

Individual gills, 3 cm. in length, measured from the distal end, of the fourth to the ninth gill pairs were used. Each gill was flushed and perfused with a total of 10 ml. of a single salt solution, the concentration of its specific cation corresponding to the experimental salinity from which the crab had been removed. Sucrose was added to bring the total osmotic pressure of these solutions equal to that of the experimental salinities. The single salt solutions used for both summer and winter animals were of NaCl, KCl, $CaCl_2$, and $MgCl_2$. Further, the gills of summer animals were exposed to solutions of choline chloride, where the chloride concentration corresponded to the experimental salinities, depending on the treatment given the crab. The gill was expected to be impermeable to the choline radical (Mantel, 1967). The gills of winter animals were additionally treated

with solutions of sodium acetate and sodium sulphate. These particular sodium sodium salts were chosen since the sulphate and acetate radicals were probably not transported (Mantel, 1967; Shaw, 1960 a). In each case, the concentration of the sodium ion was equal to its respective experimental salinity.

All of these solutions were buffered with 0.2 M Tris-HCl (Sigma Chemical Company) to pH 7.4.

Once filled with the experimental solution, the single gill preparation was attached to a frit junction calomel electrode (No. 39071, Beckman Instruments, Inc.) and immersed in approximately 50 ml. of the same solution, which was aerated. The same type of electrode was placed into this external solution to act as a reference electrode in the circuit. These electrodes were connected to a pH-meter (Model 1019, Beckman Instruments, Inc.) to measure potential differences in millivolts. Assymetry potentials measured for the electrodes were subtracted from the potential differences obtained with the gill preparations. 5 to 8 separate gill preparations were measured for each solution at each salinity and season.

All potential difference measurements for gills from summer crabs were carried out at 15 $\pm$ 1° C., and winter crabs at 7.5 $\pm$ 1° C.

Statistical Analysis of Data:

A large part of the preliminary calculation of the data was

done with the aid of the computing services of the Biology Data Centre at the University of British Columbia.

Statistical analyses were carried out using Student's t-test to determine significance at a 5% level ($P \leq 0.05$) of blood concentrations, urine concentrations, U/B ratios, and potential differences, as well as of seasonal differences in blood concentrations, U/B ratios, and potential differences.

RESULTS

Since blood concentration values for chloride, sodium, potassium, calcium, and magnesium ions at 0 and 96 hours in stock sea water were significantly equal for both summer and winter crabs (Fig. 2), it may be assumed that the eight-day pre-experimental holding period was of sufficient length for blood ion levels to stabilize following the collection of the crabs. Further, it may be assumed that the sampling operations themselves had no significant effect on blood ionic concentrations.

Since blood ionic concentrations of summer C. magister attained at 96 hours in all salinities remained significantly constant when compared with the final measurement at 216 hours, it was assumed that maximum regulation of each ion in the blood was reached by 96 hours.

The change in blood concentration of chloride over the 96 hour exposure period in 50 and 125% sea water can be seen in Figure 3. Plotted curves of similar shape were observed also in the cations, and in all the experimental salinities. Blood concentrations for all ions had attained about half of their 96 hour level of regulation after 12 hours of exposure.

Chloride:

Significant regulation of blood chloride occurred in all of

FIGURE 2

Concentrations of 5 ions, expressed as a percentage of the medium, in blood of summer and winter Cancer magister, as a function of time of exposure, in hours, to stock sea water.

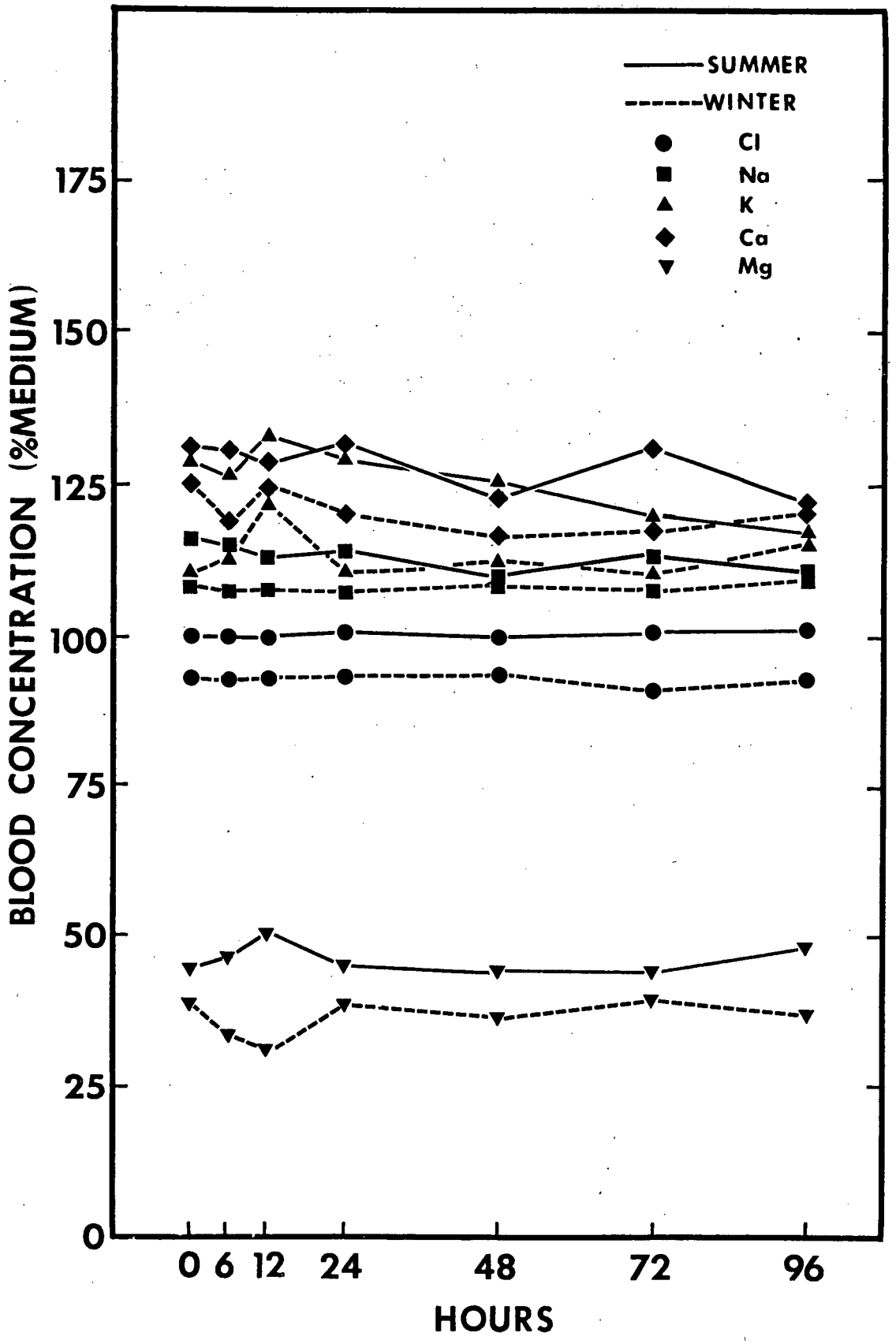
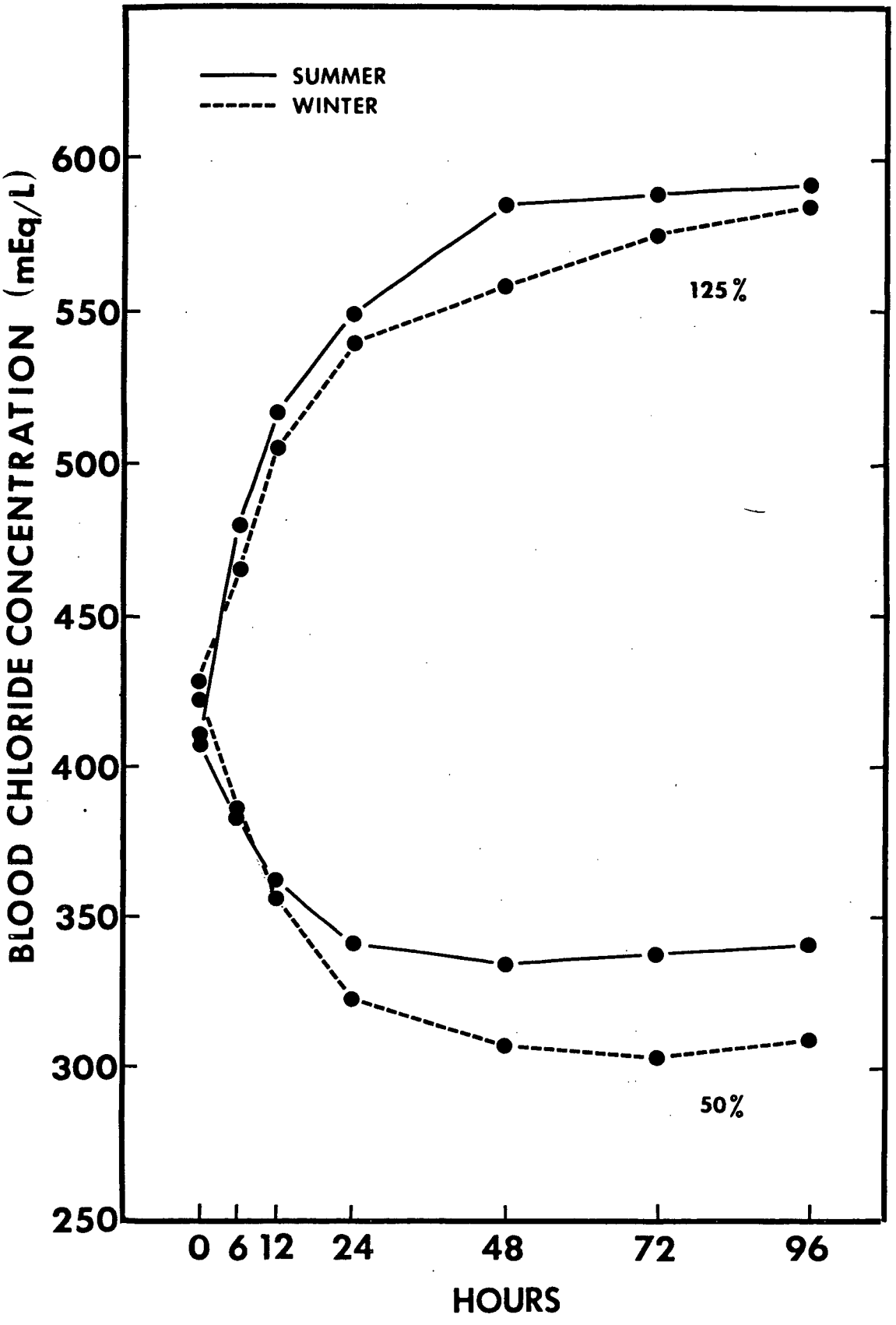

SUMMER
WINTER
Cl
Na
K
Ca
Mg
BLOOD CONCENTRATION (%MEDIUM)
175
150
125
100
75
50
25
0
0 6 12 24 48 72 96
HOURS

FIGURE 3

Blood chloride concentration, in mEq/L, of summer and winter *Cancer magister*, as a function of time of exposure, in hours, to experimental salinities of 50 and 125% sea water.


SUMMER
WINTER
600
550
500
450
400
350
300
250
BLOOD CHLORIDE CONCENTRATION (mEq/L)
125%
50%
0 6 12 24 48 72 96
HOURS

the experimental salinities, with hypertonic regulation in 50 and 75% sea water, and hypotonic regulation in 100 and 125% sea water (Fig. 4), in both summer and winter animals. Summer and winter crabs were significantly different in the degree of chloride regulation they maintained in 50 and 100% sea water. Summer animals maintained the higher gradient in 50% — 46% more hypertonic than the winter animals, and winter animals maintained the greater gradient in 100% — 39% more hypotonic than the summer animals.

Since the concentration of chloride ion in the urine of both summer and winter animals in 50% sea water was found not to differ significantly from that of the corresponding blood chloride, regulation to achieve the hypertonic blood chloride at this experimental salinity is by an extra-renal route. A U/B ratio equal to 1.0 at this salinity (Fig. 5) documents this. When both summer and winter animals were exposed to 75, 100, and 125% sea water, however, urine chloride was significantly higher than that of the blood, and the U/B ratios were significantly greater than 1.0, indicating the involvement of the antennary gland in regulation. If the measurement of total chloride may be assumed the sum of the regulation of chloride salts, this would indicate that regulation of overall blood ion concentration, in any but the most dilute salinity, occurs at least in part in the antennary gland. Summer and winter animals were not found to be significantly different in this, except for animals in 100% sea water, where the summer U/B ratio was significantly less than that of the winter, indicating less involvement of the antennary gland, at least in this salinity.

FIGURE 4

Chloride ion concentration, in mEq/L, at 96 hours in blood and urine of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.

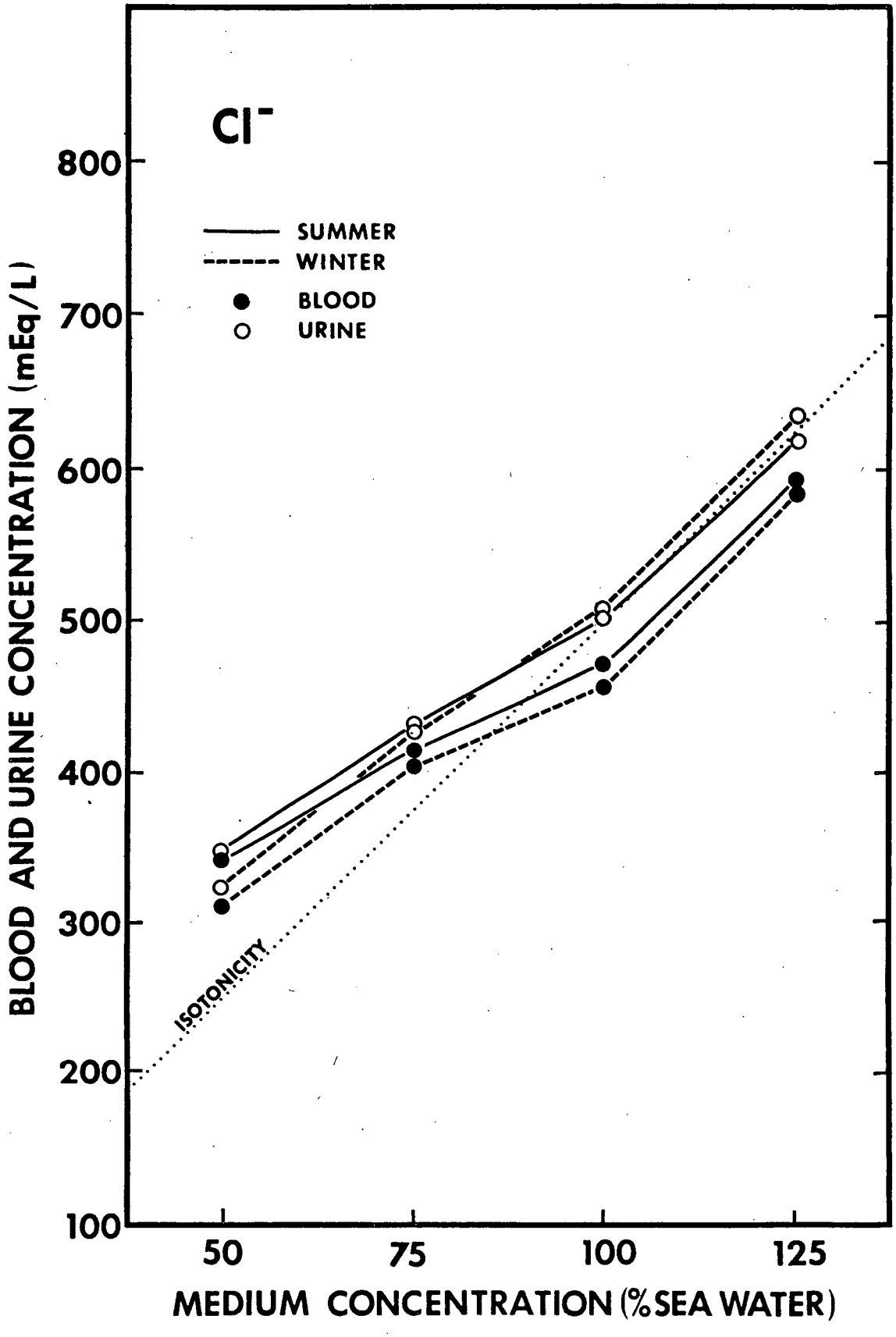
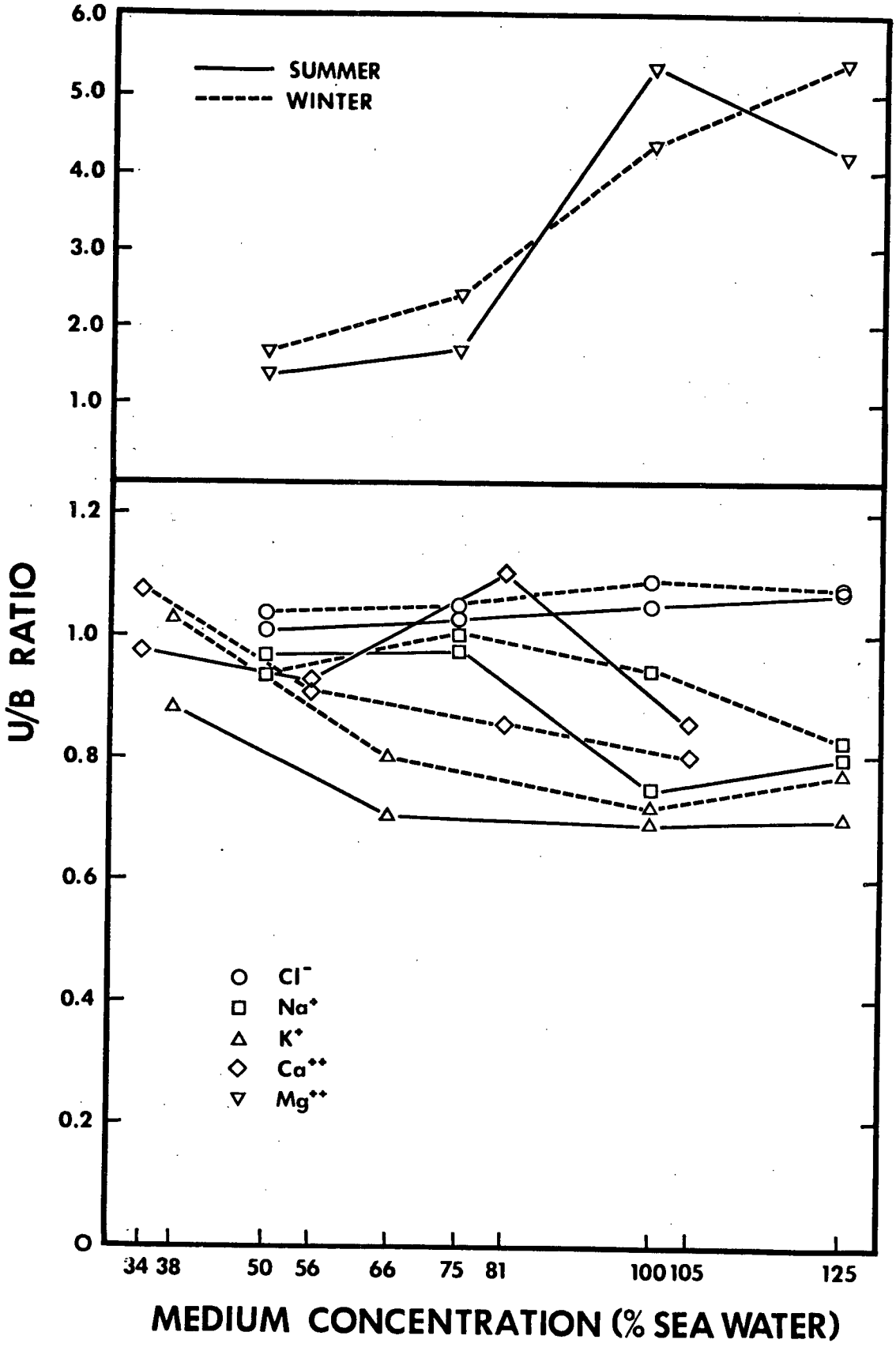

Cl⁻
SUMMER
WINTER
BLOOD
URINE
ISOTONICITY
800
700
600
500
400
300
200
100
50
75
100
125
BLOOD AND URINE CONCENTRATION (mEq/L)
MEDIUM CONCENTRATION (%SEA WATER)

FIGURE 5

Urine - blood (U/B) ratios of 5 ions of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.


6.0
5.0
4.0
3.0
2.0
1.0
SUMMER
WINTER
1.2
1.0
0.8
0.6
0.4
0.2
0
U/B RATIO
Cl⁻
Na⁺
K⁺
Ca⁺⁺
Mg⁺⁺
34 38
50 56
66
75 81
100 105
125
MEDIUM CONCENTRATION (% SEA WATER)

This would have contributed to the winter chloride concentration in the blood of C. magister being more hypotonic than the summer.

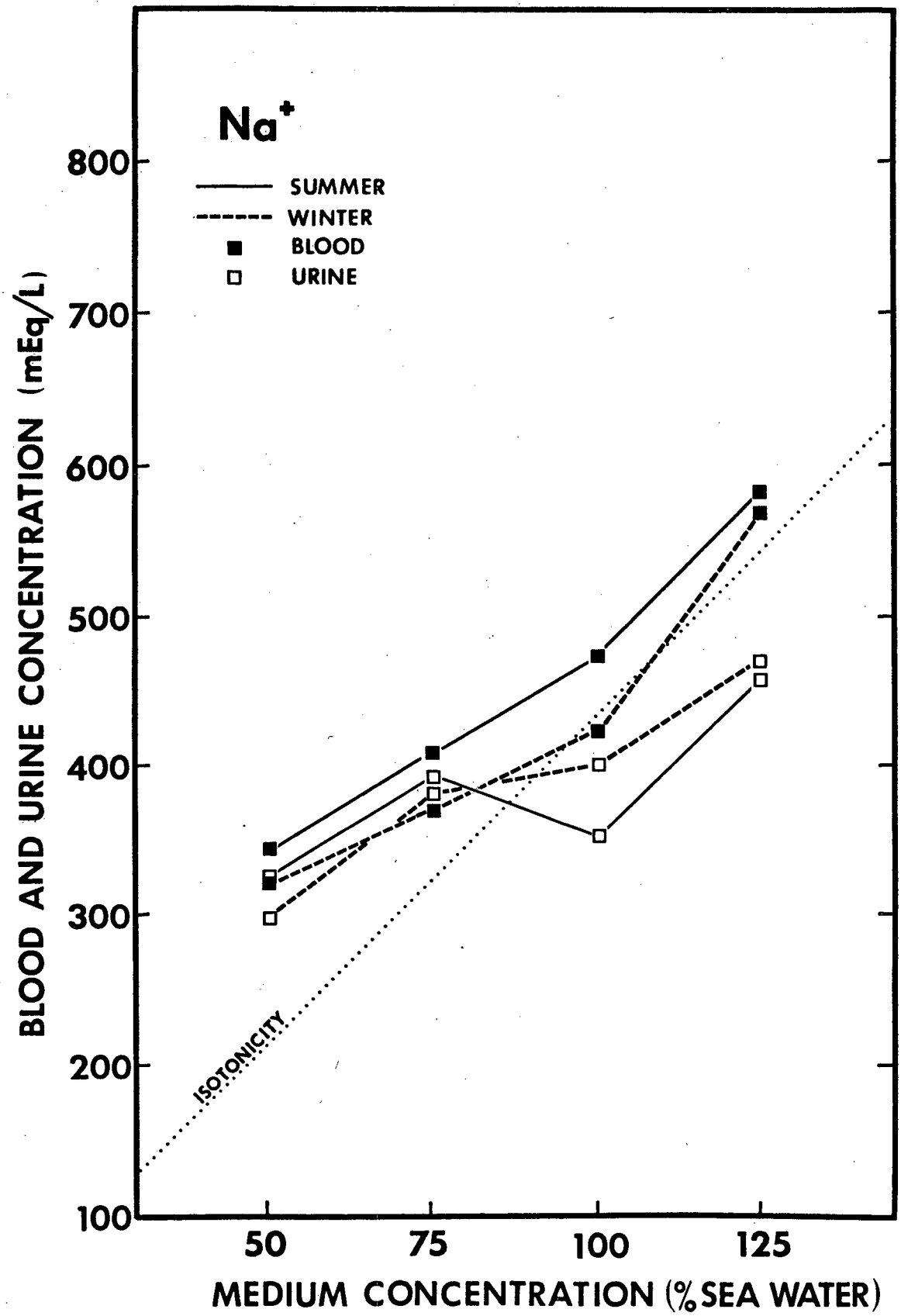
Sodium:

Summer C. magister was observed to be a hyper-regulator of sodium in all of the experimental salinities, hypo- and hypersaline (Fig. 6). The winter animals were also observed to regulate hypertonically to a significant degree in 50, 75, and 125% sea water, while hypotonic in 100%. This last result was only barely significant, and thus may have been aberrant, particularly in view of the hypertonic regulation observed in winter crabs at the 125% salinity. Summer crabs maintained a higher gradient of sodium than winter animals, being 22, 67, and 38% more hypertonic in 50, 75, and 125% sea water, respectively.

Sodium concentration in the urine of summer animals was found to be significantly less than that of the corresponding blood sodium in all of the experimental salinities, except at 75%, where the results were not significant. Accordingly, U/B ratios (Fig. 5) in 50, 100, and 125% sea water were significantly less than unity, indicating a renal involvement in the regulation of hypertonic blood sodium. In experimental salinities of 50 and 75%, the urine sodium concentration in winter animals indicated no significant involvement of the antennary gland in blood sodium regulation, and the U/B ratios in these salinities were not significantly different from 1.0. At the 100 and 125% salinity, however, the urine sodium was lower than that of the

FIGURE 6

Sodium ion concentration, in mEq/L, at 96 hours in blood and urine of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.


Na+
SUMMER
WINTER
BLOOD
URINE
800
700
600
500
400
300
200
100
BLOOD AND URINE CONCENTRATION (mEq/L)
ISOTONICITY
50
75
100
125
MEDIUM CONCENTRATION (% SEA WATER)

blood, and U/B ratios less than 1.0 occurred in winter animals as well. Thus, at higher salinities, the antennary gland of winter animals plays a significant role in the regulation of blood sodium, but this may be less effective than that of the summer animals since the U/B ratio of summer animals at 100% is significantly less than that of the winter animals at this salinity.

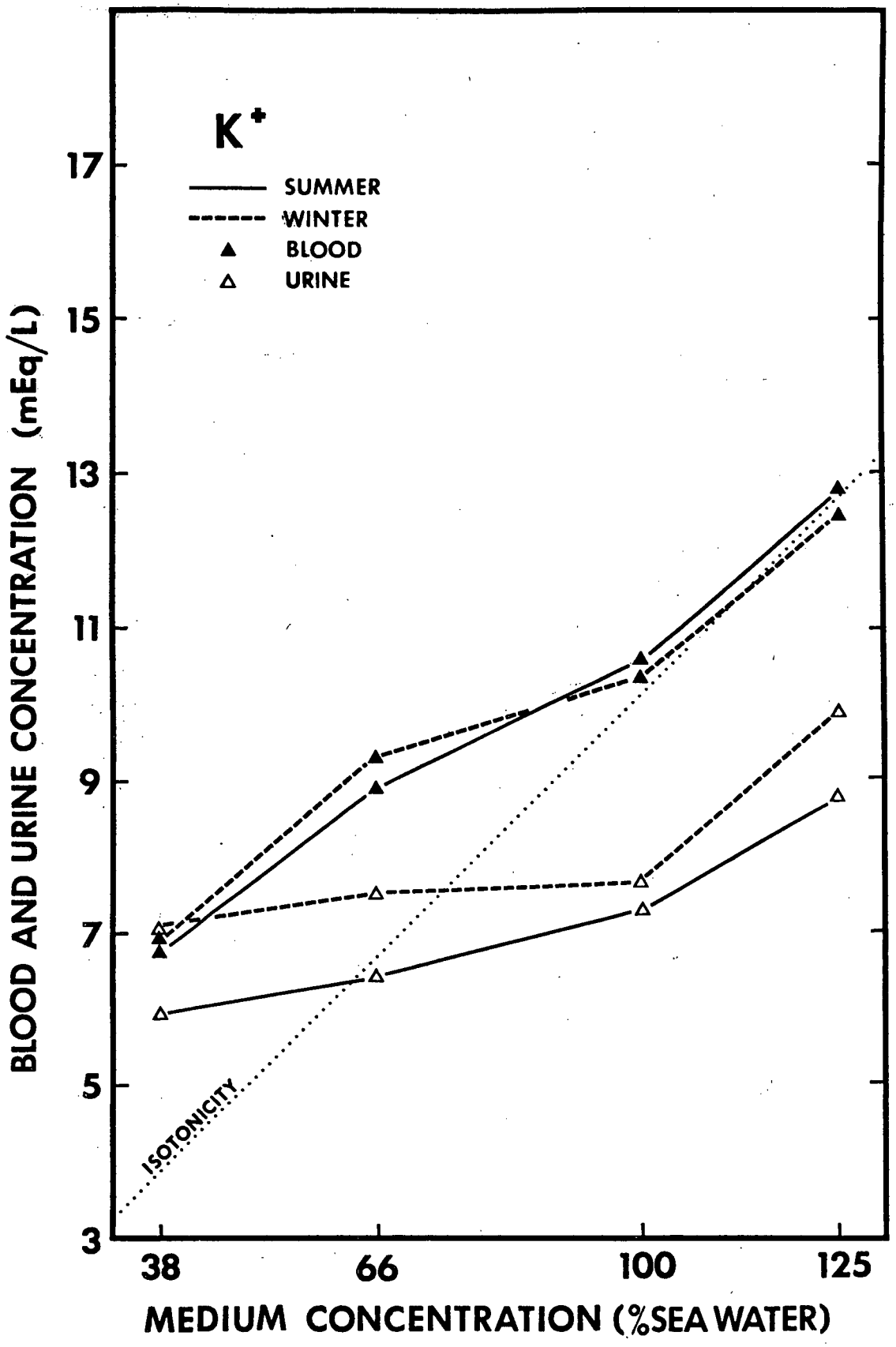
Potassium:

Summer C. magister maintained potassium concentrations in the blood significantly hypertonic in 38, 66, and 100% sea water, becoming isotonic with the medium in 125% (Fig. 7). In winter crabs, blood concentrations of potassium were higher than the experimental salinity only in 38 and 66% sea water, and hypertonic regulation failed in the 100 and 125% salinities where the blood became isotonic. In 38, 66, and 125%, the blood potassium of summer animals was not significantly different from that of the winter animals. In 100% sea water, however, summer animals maintained the hypertonic gradient significantly higher, by 160%, than winter animals, exemplifying a possibly greater ability for hyper-regulation in the summer animals.

Potassium concentration in the urine was always significantly lower than that of the corresponding blood, except for winter crabs at 38%, when it was the same. The U/B ratios for both summer and winter animals (Fig. 5) were similarly less than unity, except for winter animals at 38%, when the ratio was 1.0.

FIGURE 7

Potassium ion concentration, in mEq/L, at 96 hours in blood and urine of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.


K+
SUMMER
WINTER
BLOOD
URINE
17
15
13
11
9
7
5
3
ISOTONICITY
BLOOD AND URINE CONCENTRATION (mEq/L)
38
66
100
125
MEDIUM CONCENTRATION (%SEA WATER)

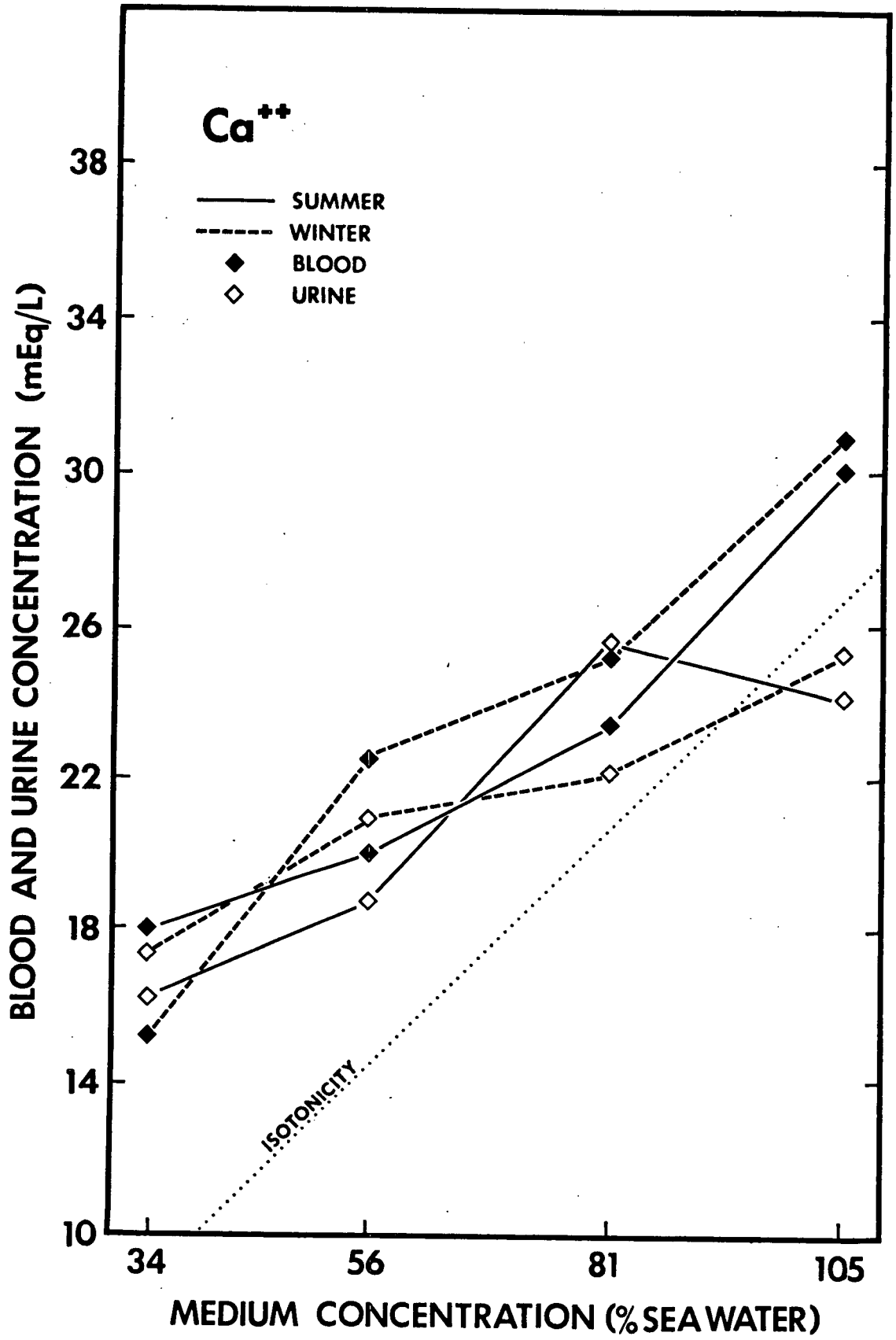
Consequently, the antennary gland was involved in the hypertonic regulation of blood potassium observed. At higher experimental salinities, the activity of the antennary gland was not sufficient to maintain a hypertonic state, and blood concentrations approached isotonicity. Summer animals showed a significantly lower U/B ratio at 66 and 100% sea water than winter animals. This, combined with the fact that the winter animals showed no antennary gland activity in 38% sea water while those of the summer condition did, indicated that the summer antennary gland involvement in the regulation of blood potassium levels is greater in all salinities except 125% than that of the winter condition.

Calcium:

Calcium ionic concentration in the blood of both summer and winter C. magister was regulated significantly hypertonically in all experimental salinities (Fig. 8). At 34%, the summer crabs maintained a significantly hypertonic gradient 46% greater than that of the winter animals at this salinity. This condition became reversed in experimental salinities of higher calcium ion concentration, where the winter animals maintained the greater gradient, significantly more hypertonic by 34% in 56%, by 77% in 81%, and by 28% in 105% sea water. Thus, the winter animals seemed to regulate to a greater degree at these three higher salinities, while the summer animals were better regulators in 34% sea water.

FIGURE 8

Calcium ion concentration, in mEq/L, at 96 hours in blood and urine of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.


Ca++
SUMMER
WINTER
BLOOD
URINE
38
34
30
26
22
18
14
10
BLOOD AND URINE CONCENTRATION (mEq/L)
ISOTONICITY
34
56
81
105
MEDIUM CONCENTRATION (% SEA WATER)

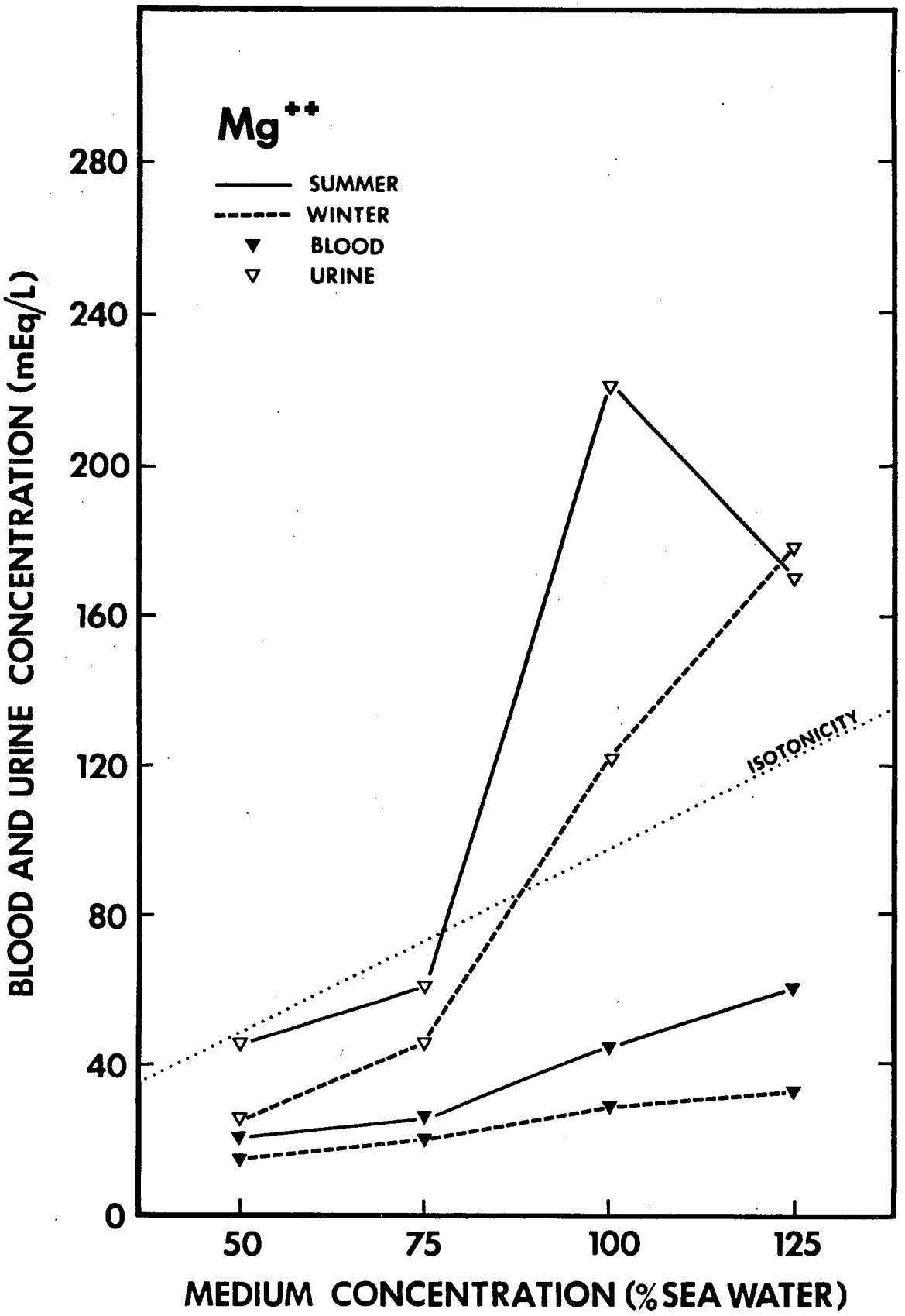
Involvement of the antennary gland in the maintenance of the hypertonic calcium levels observed above was indicated by urine calcium concentrations significantly less than those of the blood in 105% for summer and 81 and 105% for winter animals, as indicated by U/B ratios significantly less than 1.0 at these salinities (Fig. 5). No explanation is offered for the apparently aberrant high urine concentration observed in summer animals at 81%, resulting in a U/B ratio greater than unity. In the two lower experimental salinities, the U/B ratios were found to be significantly equal to unity, indicating that the antennary gland was not involved in the maintenance of hypertonic blood calcium at these salinities, but that some extra-renal site had to be active. With the exception of the aberrant 81% result, U/B ratios of summer and winter animals were not significantly different, nor, consequently, was the effective regulatory activity of their antennary glands.

Magnesium:

Regulation of magnesium in the blood of C. magister was the strongest of any of the ions observed, as illustrated by the large gradient maintained between the blood and the experimental salinity (Fig. 9). Both summer and winter animals regulated hypotonically, with the winter animals being significantly better hyporegulators for this ion in all of the experimental salinities. Winter animals kept their blood magnesium about one third of the experimental salinities, while summer animals maintained their blood magnesium concentration at about half

FIGURE 9

Magnesium ion concentration, in mEq/L, at 96 hours in blood and urine of summer and winter Cancer magister, as a function of medium concentration, as expressed in per cent sea water.


Mg++
SUMMER
WINTER
BLOOD
URINE
ISOTONICITY
BLOOD AND URINE CONCENTRATION (mEq/L)
0
40
80
120
160
200
240
280
50
75
100
125
MEDIUM CONCENTRATION (% SEA WATER)

of the experimental salinities.

The greatest degree of renal ionic regulation was for magnesium. In both summer and winter crabs, the urine concentration remained much higher than that of the blood, with this gradient increasing with increasing salinity, so that at 125% the antennary gland was approximately three times as active in the excretion of magnesium than at 50%. The U/B ratio, always significantly greater than 1.0, showed a similar trend (Fig. 5). No significant difference in the degree of renal magnesium regulation was determined between summer and winter animals, which may have been the result of the greater variability of the urine magnesium concentration values, as compared to that of the blood values. Otherwise, some other site of regulation was indicated to account for the greater gradient maintained in winter animals.

Animal Weight:

No significant relationships could be determined between the weight of individual animals and the degree of ionic regulation maintained in their blood.

Gill Activity Measurements:

Potential differences across a gill surface <u>in vitro</u> were measured to investigate the role of the gill as an organ of ionic regulation. The results obtained by these methods were by no

means unequivocal since the potential differences observed, interpreted as an indication of ion movement from on side of the gill to the other, may have been the result of exchanges of ions between the cells of the gill epithelium and the inside or outside medium, for instance. Notwithstanding the lack of conclusive evidence for regulatory involvement by the gill, these results are presented as a possible indication of such regulation, and as a preliminary to further work in this area.

Winter gill preparations of C. magister placed into solutions of NaCl, corresponding in salinity to 50, 75, and 100% sea water with respect to the sodium ion, showed a significant negative potential on the inside, relative to the outside solution (Fig. 10 a). Gills obtained from summer animals also showed such a negative potential, but only at 50%. In both summer and winter preparations, the potential difference decreased with increased sodium chloride concentration, to a point at 125%, where no significant potential difference was observed. The summer and winter gill preparations were not found to differ significantly in their potential difference readings.

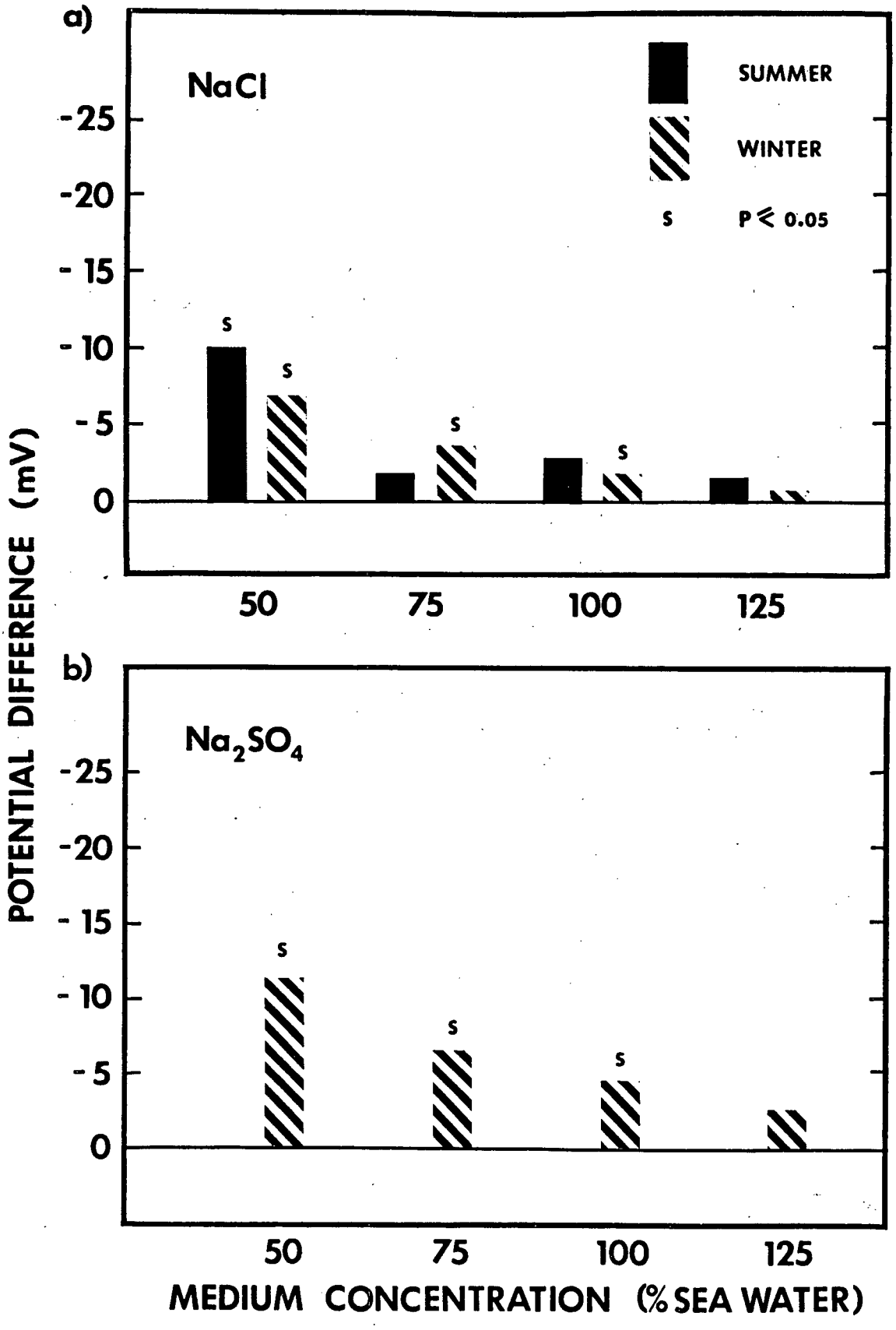
A further experiment using preparations of winter crab gills and chloride-free sodium salt solutions resulted in a significant negative potential difference, inside relative to the outside, in solutions of 50, 75, and 100% sea water of Na_2SO_4. The potential difference was not significantly different from zero at 125% (Fig. 10 b). A significant negative potential was found also using 50 and 100% salinities of sodium acetate, and of the

FIGURE 10

Potential differences, in mV, of *in vitro* gill preparations of summer and winter *Cancer magister*, as a function of medium concentration, as expressed in per cent sea water.

a. In NaCl, where concentration is based on sodium ion.

b. In Na_2SO_4, where concentration is based on sodium ion.


a)
NaCl
SUMMER
WINTER
S
P ≤ 0.05
-25
-20
-15
-10
-5
0
S
S
S
S
50
75
100
125
b)
Na2SO4
-25
-20
-15
-10
-5
0
S
S
S
50
75
100
125
POTENTIAL DIFFERENCE (mV)
MEDIUM CONCENTRATION (% SEA WATER)

same polarity as above. The potential difference readings obtained for 75 and 125% solutions of sodium acetate were not significantly different from zero (Fig. 11 a).

Using solutions of choline chloride and summer gill preparations, a significant negative potential difference, inside relative to the outside, resulted with the 50% salinity solution. In 125%, a significant positive potential difference resulted, inside relative to the outside. In 75 and 100% salinities, the potentials were not significant (Fig. 11 b).

When single salt solutions of KCl were used, significant potential differences, also negative on the inside, were obtained for gill preparations of both summer and winter animals, for all salinities. Winter potential differences were significantly greater in the 38% salinity, but no significant differences were found in the other concentrations (Fig. 12 a).

Similarly, significant negative potential differences were found in single salt solutions of $CaCl_2$ when the salinity for calcium was 34% for summer and winter preparations, and 105% for the winter preparation only. No significant differences could be determined between summer and winter preparations (Fig. 12 b).

The potential differences obtained using solutions of $MgCl_2$ were negative on the inside, relative to the outside, and signifor summer preparations in 50, 75, and 100% salinities, and winter preparations in all but 100%. The gill activity of summer and winter animals was determined to differ significantly only in

FIGURE 11

Potential differences, in mV, of *in vitro* gill preparations of summer and winter *Cancer magister*, as a function of medium concentration, as expressed in per cent sea water.

a. In Na-Acetate, where concentration is based on sodium ion.

b. In Choline-Cl, where concentration is based on chloride ion.

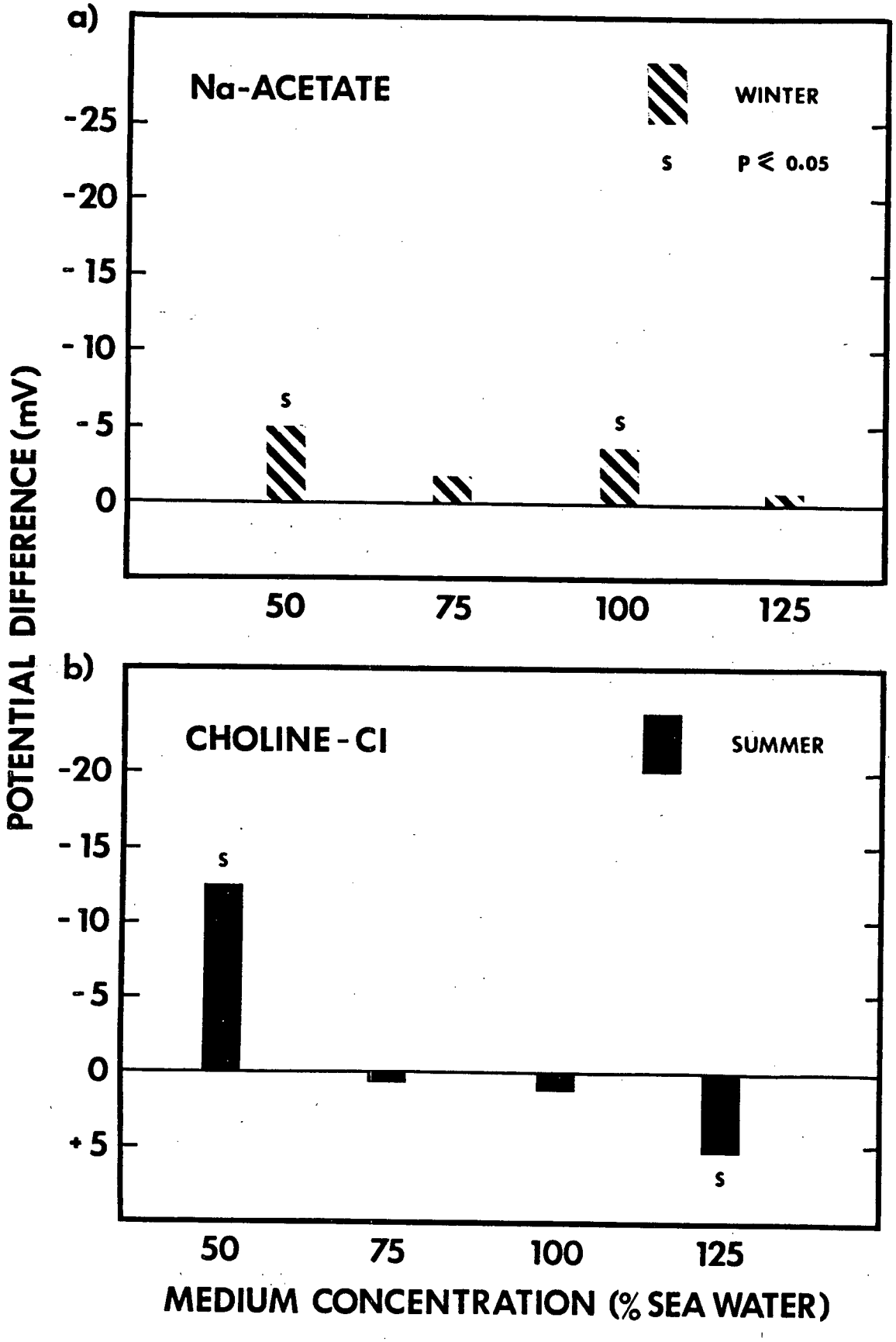
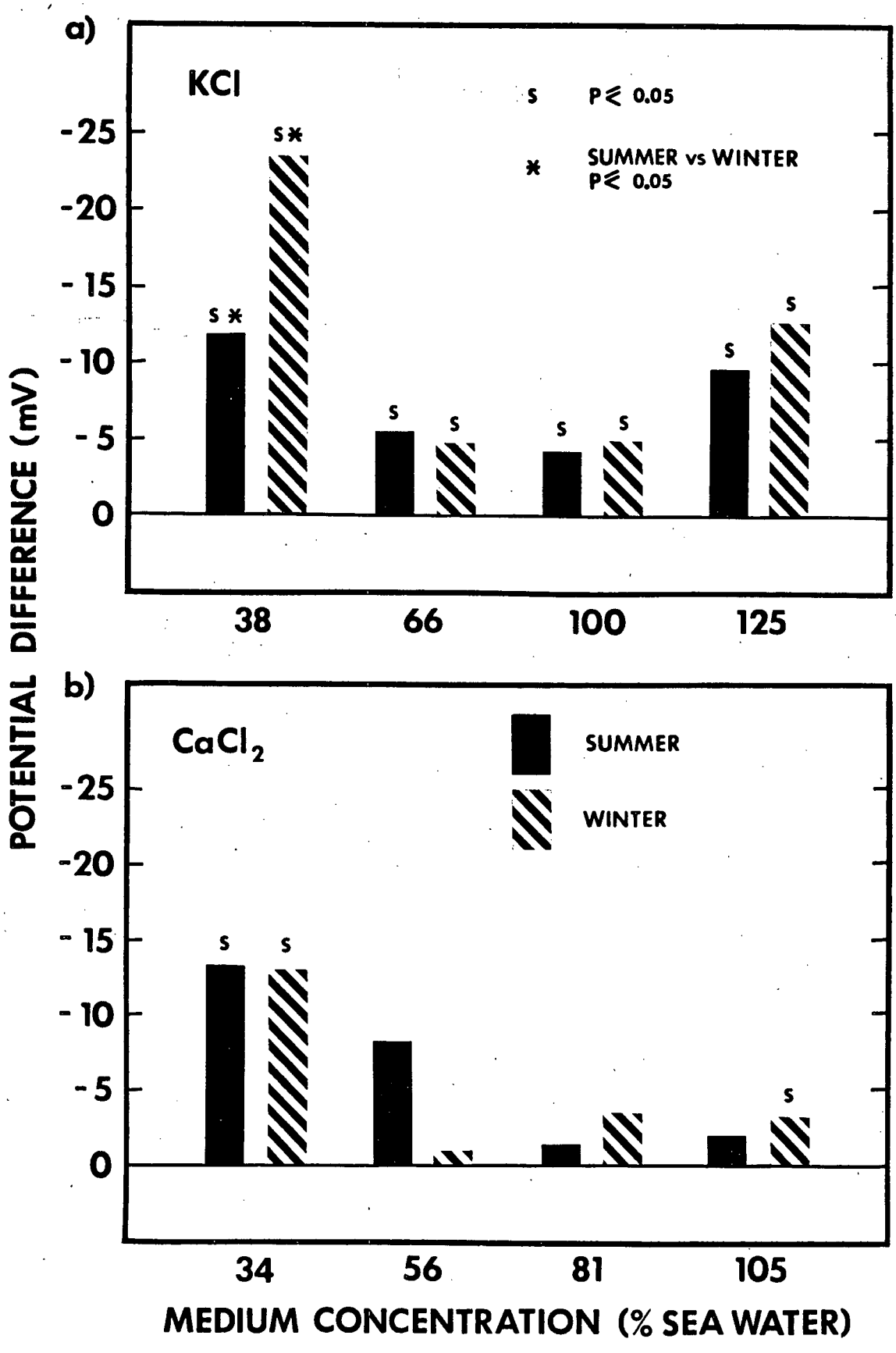

a)
Na-ACETATE
WINTER
S
P ≤ 0.05
-25
-20
-15
-10
-5
0
S
S
50
75
100
125
b)
CHOLINE - Cl
SUMMER
-20
-15
-10
-5
0
+5
S
S
50
75
100
125
POTENTIAL DIFFERENCE (mV)
MEDIUM CONCENTRATION (% SEA WATER)

FIGURE 12

Potential differences, in mV, of *in vitro* gill preparations of summer and winter *Cancer magister*, as a function of medium concentration, as expressed in per cent sea water.

a. In KCl, where concentration is based on potassium ion.

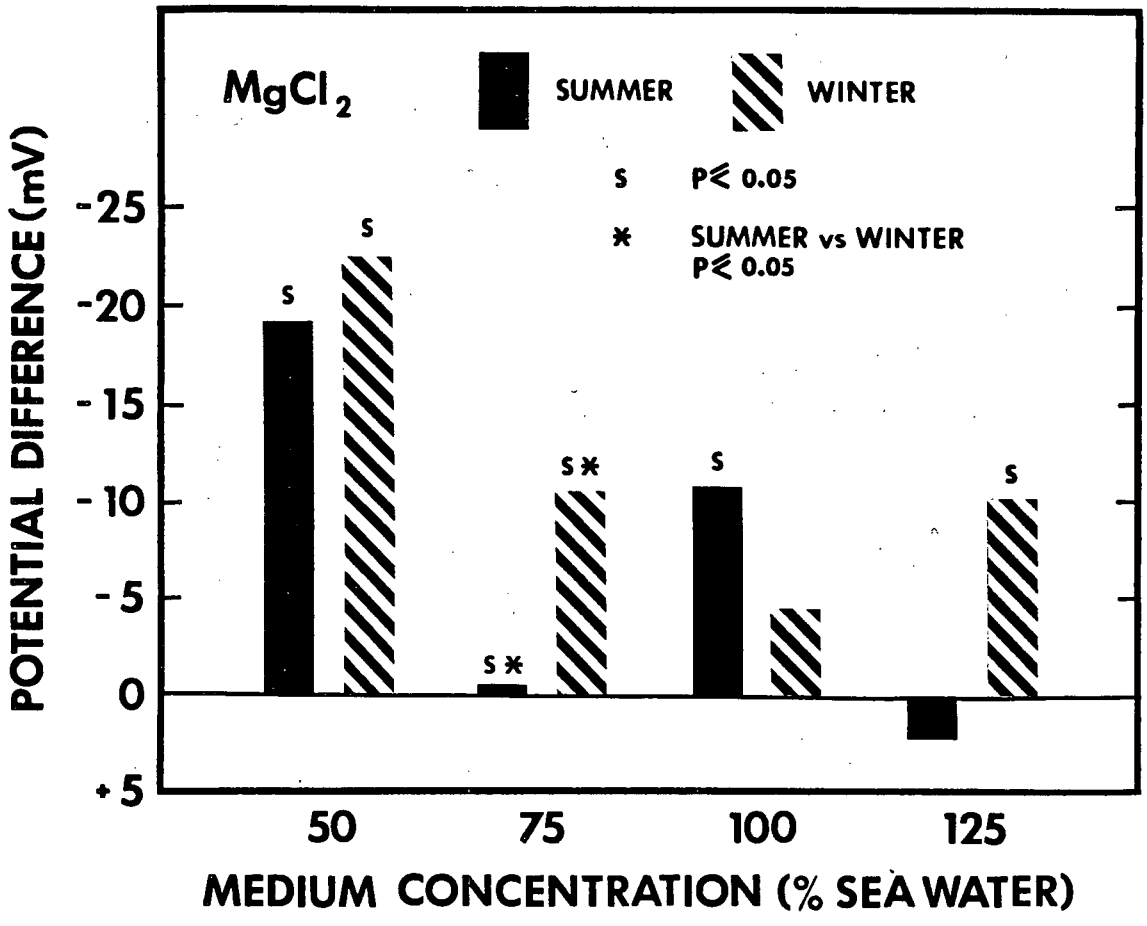
b. In $CaCl_2$, where concentration is based on calcium ion.


a)
KCl
S P ≤ 0.05
* SUMMER vs WINTER P ≤ 0.05
-25
-20
-15
-10
-5
0
S *
S *
S
S
S
S
S
S
38
66
100
125
b)
CaCl2
SUMMER
WINTER
S
S
S
34
56
81
105
POTENTIAL DIFFERENCE (mV)
MEDIUM CONCENTRATION (% SEA WATER)

the 75% salinity, where that of the winter was more negative (Fig. 13).

FIGURE 13

Potential differences, in mV, of *in vitro* gill preparations of summer and winter *Cancer magister*, as a function of magnesium concentration, as expressed in per cent sea water, in solutions of $MgCl_2$.


MgCl2
SUMMER
WINTER
S P≤ 0.05
* SUMMER vs WINTER P≤ 0.05
POTENTIAL DIFFERENCE (mV)
-25
-20
-15
-10
-5
0
+5
S
S
S*
S*
S
S
50
75
100
125
MEDIUM CONCENTRATION (% SEA WATER)

DISCUSSION

The Estuarine Environment:

Estuarine crustacean fauna is derived almost exclusively from marine species, and as such has amplified greatly the capabilities for ionic regulation of its marine precursor, enabling the estuarine forms to be euryhaline. Although marine crustaceans are stenohaline, limited to a narrow range ofosmotic and ionic concentrations (Gross, 1957; Habas, 1965; Jones, 1941; Kalber and Costlow, 1968; Mantel, 1967), they are capable of active regulation of those ions contributing to the total isosmotic concentration of the blood. Generally, increased values, relative to sea water, of sodium, potassium, and calcium and lowered values of magnesium are found in the blood. Sodium and chloride concentrations are found to vary least from the medium, while calcium tends to be regulated strongly hypertonically and magnesium strongly hypotonically (Prosser, 1955). Extreme variation in the concentration of any one ion away from its optimum results in decreased survival as a result of an alteration of the compensatory effects the ions have on each other when present at optimum concentrations (Pora, 1958, 1960). In a few cases where the blood tonicities do not correspond to the above general scheme, concomitant changes in the regulation of other major ions occur. For example, in the lobster Homarus gammarus and the shrimp Nephrops norvegicus, potassium is regulated hypotonically to sea water (Robertson, 1949, 1960). This can be related to increased concentrations of blood sodium, where the

hypertonic sodium is in turn a compensation for extremely low hypotonic blood magnesium concentrations. This same sort of selective regulation of ion content in the blood of marine crustaceans is enhanced in their related estuarine species, or in the estuarine population of a species normally considered a marine form, as is the case with Cancer magister in this study. Estuarine fauna is additionally modified in that it is euryhaline, able to withstand wide environmental salinity fluctuations. It seems that physical factors such as fluctuating osmotic and ionic concentrations are involved in determining the population dynamics of a species (Kinne, 1967). Those species not able to compensate for the increased metabolic demands of life in low salinities cannot survive for extended periods in an estuarine environment. Survival depends, of course, not on the average environmental condition, but on the most extreme condition, and adaptation to several extremes is usually involved (Prosser, 1955).

Estuaries, as areas of transition between the more stable environments of fresh water and the neighbouring sea, may show radical departures in the relative and absolute concentrations of specific ions. The Fraser River is a good example in that, while it has depressed values for all the major sea water ions, calcium and potassium are depressed relatively more than the other ions (Table 1). The calcium and potassium regulatory abilities of C. magister are consequently stressed to a greater degree. Such fluctuations are dependent on diverse geological factors, such as river bed composition and the terrain through which the river flows, picking up its specific load of ions. Absolute changes in the salinities of estuaries, as a result of dilution by fresh river water, may

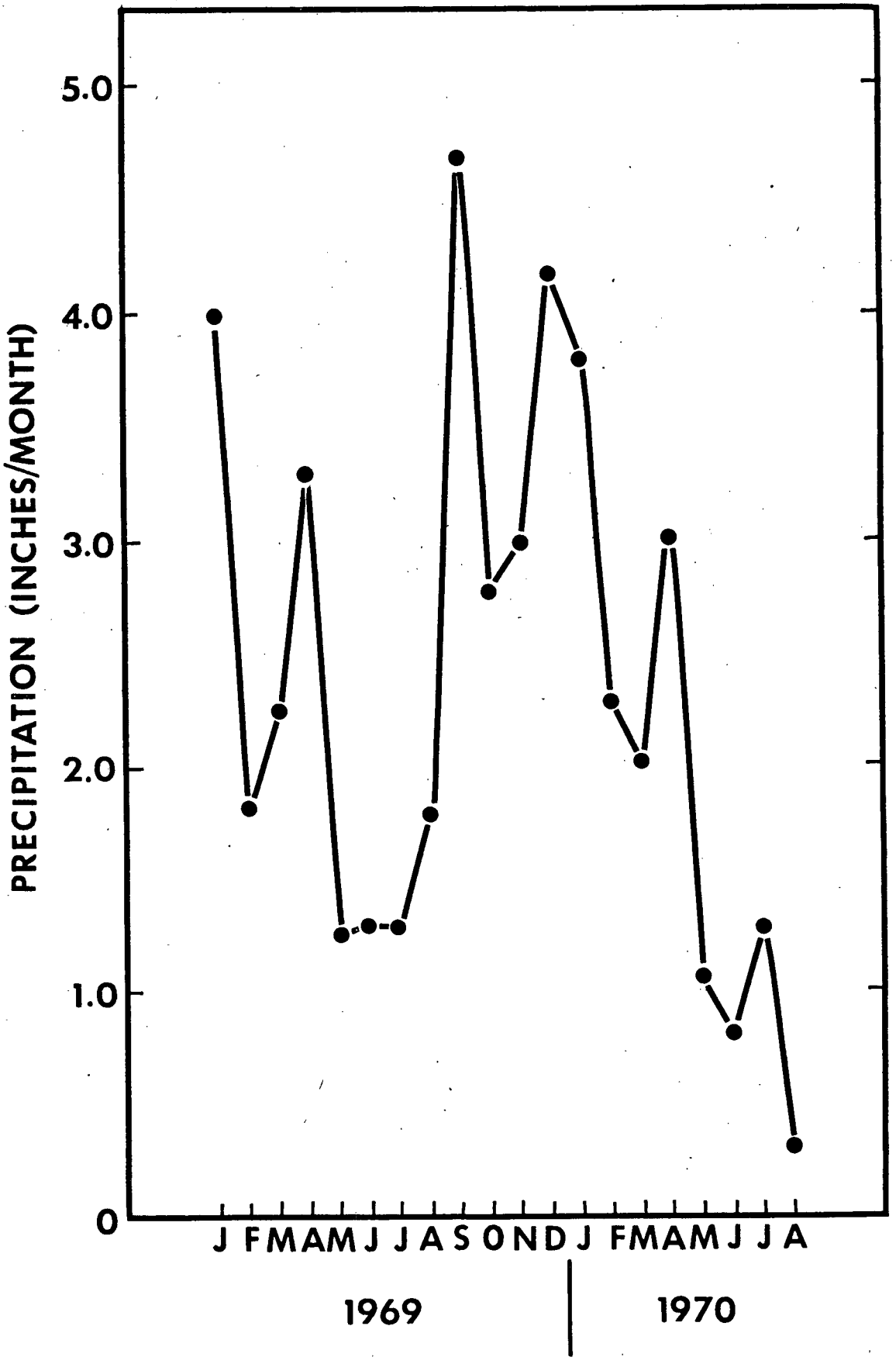
vary seasonally as well. Positive correlation can be found between seasonal salinity changes (Table 1) and precipitation in the Fraser River Basin as shown in Figure 14 (Vancouver Weather Office, personal communication). Low salinities in the months of June, July, and August are mainly the result of the melting of high altitude ice and snow, deposited in the winter months, causing a greater fresh water discharge by the Fraser River into its estuary. Reduced precipitation in the months of February and March, as well as reduced melting in the winter months, allows high field salinities in February and March.

Several processes are involved in the maintenance of blood ions at concentrations which differ from those of the medium. These include: 1) reduction in the permeability of the body surface, 2) tolerance at the cellular level of variations in blood concentration, and 3) enhanced ability to transport inorganic ions against a concentration gradient across the body surface (Lockwood, 1962).

In the lower estuarine salinity, regulation by the active uptake or secretion of ions is coupled with physiological adjustment by lowering the effective gradient that has to be maintained. This reduces the metabolic input required to maintain optimum ion concentration (Croghan, 1961). Potts (1954) considers this reduction as the single most important means whereby an animal entering brackish water can lessen the strain on its regulatory mechanisms. It is probably this requirement which becomes the limiting factor to determine the penetration of a species up the estuary. Penetration down the estuary, toward

FIGURE 14

Average precipitation in inches per month for the Fraser River Basin, from January 1969 to August 1970, based on monthly meteorological reports from Abbotsford, Hope, Lytton, Quesnel, Vancouver, and Williams Lake, British Columbia.


PRECIPITATION (INCHES/MONTH)
5.0
4.0
3.0
2.0
1.0
0
J F M A M J J A S O N D J F M A M J J A
1969
1970

the sea again, does not seem to be a limiting factor, at least in some grapsoid crabs (Barnes, 1967), indicating that estuarine crustaceans do not necessarily lose their ability to live in sea water. C. magister reacts similarly, being able to survive in 100% sea water.

While often regarded as a stenohaline marine decapod (Jones, 1941), C. magister was found to be euryhaline in this study, regulating the ionic concentrations of its blood in experimental salinities as low as 34% and as high as 125% sea water. Preliminary studies showed that the survival of C. magister was much reduced in experimental salinities of 25% and 150%, indicating that the effective regulatory range of this crab is between these two extremes. On the basis of this, and using the classification of estuarine fauna proposed by Carrikker (1967), it may be concluded that this decapod is a euryhaline marine species, extending from the sea into the upper dilute reaches of an estuary, as distinct from: 1) stenohaline marine, able to inhabit only the lower reaches of an estuary, such as Cambarus virilis (Prosser, 1955), 2) euryhaline marine migrants, able to move up the estuary through the entire mixohaline range of salinities before returning to the sea, such as the blue crab Callinectes sapidus (Tan and van Engel, 1966), or 3) true estuarine, in the middle and lower salinities of an estuary, spending its whole life there, but so adapted to the dilute conditions, that they are not able to tolerate marine salinities. An example of this is the brachyuran decapod Rithropanopeus harrisii (Jones, 1941).

Size:

No significant correlation could be found in C. magister between ionic regulation and the size of the animal. This situation is similar to that found in Hemigrapsus nudus and Hemigrapsus oregonensis (Dehnel, 1959, 1960), and Ligia oceanica and Ligia granulosa (Todd, 1963).

The lack of correlation may not be conclusive since a relationship between size and salinity optima may exist. This has been postulated by Broekema (1941) for the estuarine shrimp Crangon crangon, where only the younger animals were able to tolerate the most dilute environments. Thus, C. magister in the collecting area may all have been of one size range, determined by a possible size dependent tolerance of lower salinities. The field crab Paratelphusa sp. shows a similar relationship (Padmanabhanaidu and Ramamurthy, 1961).

Sex:

Although only male C. magister were used in this study, the relationship of sex of the animal and its body fluid regulation deserves mention. Repeated observations indicate that the females of many species of crustaceans have reduced abilities of ionic regulation. This occurs in Carcinus maenas (Gilbert, 1959 a), in Paratelphusa sp.(Padmanabhanaidu and Ramamurthy, 1961), and also in Callinectes sapidus (Tan and van Engel, 1966), where the females show reduced sodium regulatory ability. This has

been extended to include reduced chloride regulatory ability in female crustaceans, as in Carcinus maenas (Gilbert, 1959 b). Examination of female C. magister may reveal differences in their ion regulatory abilities, as compared to the male animals examined in this study.

Chloride:

Regulation of chloride ion, both hyper- and hypotonically in dilute sea water and hypotonically in concentrations of 100% sea water and higher, has been observed in many crustaceans. Marine species maintain their blood chloride levels close to that of the medium, regulating only slightly above or below ambient concentration. Cancer pagurus, for instance, has a chloride level 97% of medium concentration (Robertson, 1939), while the spider crabs Hyas araneus and Maia squinado maintain a hypertonic gradient equivalent to 102% sea-water (Robertson, 1953).

Measurement of chloride concentration is an indication of the osmotic concentration in the animal, and, thus, of osmoregulatory ability. This is true for hypersaline and hyposaline conditions down to about 50% sea water. In Penaeus setiferus, Penaeus aztecus, and Trachypenaeus similis, for instance, the total ion concentration accounts for 94 to 97% of the total osmotic pressure in the above salinity range (McFarland and Lee, 1963). At salinities less than half that of sea water, the role of chloride salts in the maintenance of osmotic equilibrium decreases

but is compensated by the the addition of amino acids, effecting a Donnan equilibrium (Potts and Parry, 1963, pp. 27-32). Thus, the measurement of chloride concentration in the blood of C. magister in the experimental salinity range was a measure of total ion regulation of chloride salts, as well as of osmoregulation.

In salinities below 100% sea water, C. magister regulates chloride hypertonically, as much as 138% of the experimental salinity in 50%. As such, it is similar to the decapods Homarus americanus (Burger, 1956 b, 1957) and Hemigrapsus nudus and Hemigrapsus oregonensis (Dehnel, 1966), the amphipod Corophium volutator (McLusky, 1968), the isopod Mesidotea entomon (Lockwood and Croghan, 1957), and the stomatopod Squilla empusa (Lee and McFarland, 1962).

Correlating with the chloride tonicity of their marine ancestry, brackish water crustaceans will regulate chloride in 100% sea water either hyper- or hypotonically. C. magister does the latter, regulating at about 93% of 100% sea water. In this, it is similar to the crab Rithropanopeus harrisii (Smith, 1967) and the estuarine shrimp Palaemon serratus (Parry, 1954) and Metapenaeus monoceros (Panikkar and Viswanathan, 1948). This condition is distinct from that observed in the intertidal brackish water crustaceans, such as Ligia oceanica (Parry, 1953), Corophium volutator (McLusky, 1968) or Hemigrapsus nudus and Hemigrapsus oregonensis (Dehnel, 1966, 1967), which maintain a hypertonic blood chloride concentration in 100% sea water.

Hypotonic chloride regulatory ability is often observed in estuarine crustaceans in hypersaline media. For example, the shrimp Palaemon serratus (Parry, 1954) reacts this way. C. magister behaves similarly, regulating its blood chloride hypotonically at about 94% of the experimental salinity of 125% sea water.

Although Burger (1956 b, 1957) demonstrated that Homarus americanus is not able to regulate chloride by urinary activity, this ability has been demonstrated in several other crustaceans. In Hemigrapsus nudus, for instance, Dehnel (1966) found that the antennary gland can excrete a hypertonic urine, 140% of the blood chloride concentration. The estuarine Palaemon serratus, as well, produces a hypertonic urine (Parry, 1954). These studies of C. magister have illustrated that this crab has a similar ability to concentrate chloride in the urine, at least in a concentration range of 75 to 125% sea water. Excretion of a urine more concentrated than the blood in 125% is instrumental in keeping the blood chloride hypotonic. At 50% sea water, the concentration of chloride in the urine is not sufficiently different from that in the blood to indicate antennary gland activity, but it aids the animal in maintaining a hypertonic blood chloride with reduced loss of chloride by way of the urine. Examination of the fresh water crayfish Austropotamobius pallipes and Oronectes virilis (Riegel, 1963) shows that they produce a hypotonic urine with respect to blood chloride. This may be an extension of the production of an isotonic urine observed in C. magister to conserve chloride further and aid in the maintenance of a hypertonic blood. In this same paper,

Riegel also implicates the bladder component of the antennary gland as being the specific tissue involved in the regulation of urinary chloride. The large amounts of urinary bladder tissue observed in the body cavity of C. magister may function similarly.

Most of the regulation of chloride has been attributed to the crustacean gill, paricularly active uptake in dilute salinities (Burger, 1956 b; Flemister and Flemister, 1951; Webb, 1940). Histologically, the gill epithelium seems to be a secretory type (Flemister, 1959), filled with mitochondrial and osmiophilic materials (Copeland, 1963, 1968).

Measurements of the potential difference between the inside of a regulating gill and the outside medium may possibly suggest an active transport of ions in C. magister, with an uptake of chloride ions from the choline chloride solutions at dilute salinities and a possible secretion of chloride to the outside in concentrated salinities. Essentially the same results were obtained by Mantel (1967) with in vitro preparations of Callinectes sapidus gills. In C. sapidus, chloride seems to be transported independently of cations such as sodium. Shaw (1960 a, b, c) has shown this to occur also in the crayfish Astacus pallipes, where chloride uptake into the gills was independent of and about one third of the rate of sodium uptake.

It may be that shrimp and crabs show a different system of chloride excretion, since Cl^{36} flux measurements in the shrimp Metapenaeus bennettae indicated chloride to be excreted exclusively by way of the gut, and not the gills (Dall, 1967).

This is in contrast to C. magister excreting chloride in hypersaline condition both by way of the antennary gland and the gills. Croghan (1958 a, b) also invokes the gut as a chloride regulatory organ in Artemia salina, implicating it as a site of active uptake of chloride. This may be occurring in C. magister as well to keep the blood hypertonic in dilute salinities.

Sodium:

Like most brackish water crustaceans, C. magister regulates blood sodium hypertonically in dilute salinities. C. magister maintains a level of sodium in the blood 156% that of the 50% experimental salinity. The intensity of regulation is somewhat less than that observed at this salinity for the shore crabs Pachygrapsus crassipes (Gross, 1959 a) and Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965). Regulation of sodium is greater than that in the estuarine prawn Palaemon serratus, which regulates sodium at only 105% of the experimental 50% salinity (Parry, 1954).

In 100% sea water, sodium regulation by C. magister is much like that of most marine decapods (Burger, 1956 b; Robertson, 1939, 1949), with a maintenance of the blood at about 110% of the environmental concentration, at least in animals from the summer condition. Pachygrapsus crassipes (Gross, 1958; Prosser et al, 1955), Hemigrapsus nudus (Dehnel, 1967; Dehnel and Carefoot, 1965), Palaemon serratus (Parry, 1954), and the isopod Ligia oceanica (Parry, 1953) are all brackish water crustaceans

exhibiting the same slight degree of hypertonicity of sodium in 100% sea water.

While C. magister shows agreement with most other brackish water species with respect to sodium regulation in sea water of 100% or less, in the hypersaline condition of 125% it is unusual in that the blood is still hypertonic at 107% of the medium concentration. This was not found for any of the species above, where blood sodium concentration either approached isotonicity, as in Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965), or where hyporegulation of sodium occurred, as in Pachygrapsus crassipes (Gross, 1958; Prosser et al, 1955) and Palaemon serratus (Parry, 1954). The hypertonicity of sodium in C. magister may be correlated with the extensive degree of hyporegulation of magnesium occurring at this salinity of 125% sea water (Gifford, 1962; Prosser, et al, 1955).

Associated with hypertonic regulation of sodium in C. magister is the production of a urine hypotonic to the blood, particularly at the 100% and 125% salinities. The antennary gland is the site most commonly attributed to carry out sodium regulation in crustaceans. Measurement of urine tonicity with reference to blood sodium concentration has revealed that estuarine crustaceans such as the amphipod Gammarus duebeni (Lockwood, 1961 a, b, 1965; Sutcliffe, 1967 a, b) and the shrimp Palaemon serratus (Parry, 1954) show a similar capacity for renal sodium regulation. Intertidal crustaceans also carry out renal regulation of this ion, as shown in Hemigrapsus nudus and orego-

nensis by Dehnel (1967) and Dehnel and Carefoot (1965), and in Pachygrapsus crassipes by Prosser, et al (1955). P. crassipes shows a hypotonic urine at high salinities (170% sea water) and, interestingly, a hypertonic urine in 50% sea water, distinct from the antennary gland activity observed in C. magister. This may be an indication of better adaptation to dilute salinities on the part of Pachygrapsus crassipes, which maintains a much lower optimum blood sodium level by the production of this hypertonic urine than C. magister.

Gill activity is commonly associated with a hypertonic blood sodium. An active uptake of sodium by the gill epithelium in dilute media is found in the fresh water Eriochier sinensis (Koch, 1953, 1954; Koch and Evans, 1956; Koch, et al, 1954). In the euryhaline estuarine migrant Callinectes sapidus, as well, sodium shows a net influx into the gill in dilute salinities. It is postulated that in this animal the chloride influx exceeds that of sodium to create the measured negative potential differences inside the gill and to facilitate further sodium influx in this way (Habas, 1965; Habas and Prosser, 1963; Mantel, 1967). If the negative potential differences observed in C. magister gill preparations using Na_2SO_4 and Na-Acetate are indicative of an outward movement of sodium from the gill, sodium regulation by the gill of this crab would consequently not fit into the scheme proposed above for Eriochier sinensis and Callinectes sapidus. Further, since the possible transport of sodium by the gill of C. magister occurs in the absence of chloride and other cations, it may be independent of them. This was also suggested by Shaw (1960 a, b) for Astacus pallipes. He suggested

also that when acetate and sulphate radicals are used in the formation of sodium salts, it is possible that sodium ions exchange for either ammonium or hydrogen ions.

The hypothesis that some site of sodium regulation other than the gill or antennary gland exists in crustaceans finds support in the work of Mantel (1968) on Gecarcinus lateralis, where the foregut of this land crab may be able to regulate sodium, moving it into and out of the blood as needed.

Potassium:

Potassium is hypertonic in C. magister in 38, 66, and 100% sea water, maintained at gradients of 174, 144, and 105% of the experimental salinities, respectively. Pronounced hypertonicity in this animal at low salinities can be related to a relatively lower potassium level in the estuary (Table 1), stressing a well-developed hypertonic regulation of this ion.

The hypertonicity of blood potassium in C. magister is similar to that found in other estuarine or littoral crustaceans, such as in Pachygrapsus crassipes (Gross, 1959 a; Prosser, et al, 1955), in the shrimp Palaemon serratus (Parry, 1954), and in the shore crabs Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965).

In 100% sea water, C. magister is capable of a slight degree of

hyper-regulation, but this is not comparable to the pronounced hypertonicity observed in Pachygrapsus crassipes (Gross, 1958), in Palaemon serratus (Parry, 1954), or in strictly marine decapods (Robertson, 1939, 1949, 1953). It would seem that C. magister falls more into a category of isotonicity in 100% sea water and above, similar to the shore crabs Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965).

The antennary gland in C. magister is strongly involved in potassium regulation in all but the most dilute salinities, to produce a urine hypotonic to the blood. While not as active in hypertonic regulation of the blood as the fresh water crab Potamon niloticus (Shaw, 1959) or crayfish (Riegel, 1965), renal involvement is greater than that of marine crustaceans, in particular the decapods Cancer pagurus, Homarus vulgaris (Robertson, 1939, 1949), Homarus americanus (Burger, 1956 b), and Galathea squamifera (Bryan, 1965). It is comparable to that shown by brackish water crustaceans such as Palaemon serratus (Parry, 1954), as well as that shown by the littoral crab Carcinus maenas (Riegel and Lockwood, 1961; Webb, 1940).

Using the findings of Shaw (1960 c) that the uptake of chloride from solutions of KCl is slight, or non-existent, to interpret the negative potentials observed in the in vitro gills of C. magister, an indication of an outward movement of potassium through the gill epithelium may exist. Potassium transport, if this is what has been observed in the gill preparations, may be independent of the presence of other ions, as indicated by

Harvey and Nedergaard (1964) for sodium independent active transport of potassium in the Cecropia sp. midgut.

Calcium:

Hypertonic regulation of blood calcium in an estuarine crustacean such as C. magister may be expected from the reduced calcium levels found in estuarine waters, and is associated with the need for calcium in the seasonal moult cycle (Robertson, 1937). Only in the intermoult phase is the calcium level at all constant (Hayes, et al, 1962). Blood calcium concentrations in C. magister are hypertonic in all experimental salinities, averaging 192, 148, 117, and 113% of medium calcium concentration in experimental salinities of 34, 56, 81, and 105% sea water, respectively. Calcium tends to be regulated hypertonically in strictly marine crabs as well (Robertson, 1939, 1949; Gross, 1964).

The ability to regulate calcium demonstrated by C. magister over the entire experimental salinity range corresponds almost exactly to that of Palaemon serratus (Parry, 1954) and to that of the shore crabs Pachygrapsus crassipes (Prosser, et al, 1955) and Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965). Hypertonic calcium regulation would seem to be a common feature of estuarine crustaceans. Hypotonic regulation in high salinities exists in some crustaceans, as in Pachygrapsus crassipes (Prosser, et al, 1955) and in the fiddler

crabs Uca pugnax and pugilator (Green, et al, 1959).

The antennary gland in C. magister seems to be involved in the maintenance of hypertonic blood calcium levels only in the 81 and 105% salinities. Here, a urine hypotonic to the blood is produced. Similar regulation of calcium was observed in the terrestrial crab Cardisoma armatum (deLeersnyder and Hoestlandt, 1963), in the lobsters Homarus americanus (Burger, 1956 a, b) and vulgaris (Robertson, 1939), and in the estuarine shrimp Palaemon serratus (Parry, 1954). The littoral crabs Pachygrapsus crassipes (Gross, 1959 a) and Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965) do not show any renal involvement in calcium regulation in this salinity range, indicating that an extra-renal mechanism must be active, possibly the gills. These species do show hypotonic blood regulation, however, with the production of a hypertonic urine, in hypersaline sea water. No hypertonic urine production was observed in C. magister, but may have been found if the experimental salinity range were extended to include more of the upper hypersaline concentrations. Fiddler crabs definitely produce a hypertonic urine in high calcium concentrations (Green, et al, 1959), which correlates with their pronounced hypotonic blood regulation.

Since urine concentrations of calcium in C. magister are equal to those of the blood in 34 and 56% experimental salinities, the antennary gland is not involved in the maintenance of hypertonic blood calcium at these salinities. The source of the calcium producing this hypertonicity is probably not the calcified

exoskeleton, since in the lobster Homarus americanus the source for hypertonic blood calcium was found to be not the exoskeleton, but derived through gill activity. The gill in the lobster actively takes up calcium when the internal state becomes hypocalcaemic (Hayes, et al, 1962).

If the potential differences observed in the in vitro gill preparations of C. magister are indicative of calcium transport through the gill, some involvement of the gill in the regulation of blood calcium is implied in this animal as well, particularly in dilute salinities, where the potential differences were the largest. Some other undetermined site may be involved in the regulation of a hypertonic blood calcium level. Burger (1956 b), for instance, indicated that divalent ions entered by way of the stomach in Homarus americanus. In the amphipod Corophium volutator (McLusky, 1970), as well, a large part of the ion uptake is by way of the gut. Further experimentation may clarify the situation in C. magister.

Magnesium:

Hyporegulation of magnesium is the most universal feature of ionic regulation in crustacean blood. A definite correlation seems to exist between the locomotory activity of a particular species and its blood tonicity for magnesium. Those with low values of magnesium are more active and capable of faster movement than those with high levels of magnesium (Lockwood, 1962, 1968 p. 11; McFarland and Lee, 1963; Potts and Parry, 1963 pp.

100-101; Robertson, 1949, 1953, 1960). This leads to the supposition that magnesium levels are related to the speed of neuromuscular impulse transmission. C. magister is considered as a fairly active crustacean, being a scavenger and moving about readily on the sea bottom, and correspondingly has a magnesium tonicity half or less than that of the experimental salinities. C. magister is similar in this respect to fairly active crustaceans such as the decapods Cancer pagurus, Homarus vulgaris (Robertson, 1939) and gammarus (Robertson, 1960), which also have magnesium levels at least half of the medium concentration. By way of comparison, the slower moving spider crabs Maia squinado and Hyas araneus have higher blood magnesium levels, only slightly hypotonic to the medium (Robertson, 1953).

The antennary gland is involved in the reduction of blood magnesium tonicity in C. magister. This is achieved by production of a hypertonic urine. The pronounced increase in the tonicity of urine at 100 and 125% salinity may indicate a disproportionately greater net influx in these salinities. The degree of magnesium regulation carried out by the antennary gland in 100% sea water is comparable to that found in Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965), while larger than that of the marine lobsters Galathea squamifera (Bryan, 1965) and Homarus vulgaris (Robertson, 1949). It is, however, not as large as in the estuarine shrimp Palaemon serratus (Parry, 1954), which also maintains a lower blood magnesium concentration than C. magister.

Interactions of magnesium and sodium are possible, as indicated

by Gifford (1962) in Uca pugnax and Ocypode albicans. A similar situation may exist in C. magister. While C. magister regulates for a hypotonic magnesium level, it maintains its blood sodium hypertonic, so that compensatory regulation may be occurring here. Prosser, et al (1955) indicate that regulation by the antennary glands to produce a hypertonic urine with respect to magnesium in some way effectively reduces the concentration of sodium in the urine to make it hypotonic for sodium. Thus, increased urine hypertonicity for magnesium in 100 and 125% sea water correlates with, and may be a function of, increased urine hypotonicity of sodium at these salinities.

The gill of C. magister may also be involved in the regulation of magnesium over the whole experimental salinity range, and this possibly by an extrusion of magnesium ions to lower blood tonicity.

Other organs may contribute to the maintenance of hypotonic blood magnesium. Indications of this have been found in Callinectes sapidus and Ocypode albicans (Gifford, 1962), as well as the lobster Homarus americanus. In H. americanus, the cycle seems to involve an uptake of magnesium from the gut, with an excretion by way of the antennary gland (Burger, 1956 a, b).

Season:

The effects of season seem to extend to the regulation of all

ions studied in C. magister. The process involves acclimation, where this term is defined as "any demonstrable compensatory change over some length of time" (Bullock, 1955). Salinity acclimation is observed in C. magister. Temperature acclimation is involved as well, since temperature varies with season and thus will have a demonstrable effect on the degree of ionic regulation carried out by crustaceans. The effects of temperature on ionic regulation in C. magister have not been studied independently, but may be inferred since temperature varied with season.

Chloride in winter blood is lower at 50% and higher at 100% than summer blood. That is, winter animals maintain a lesser gradient at low salinities, but a higher one at high salinities. This may be correlated with changes in chloride concentrations in the summer and winter environments, where summer animals become acclimated to lower salinities and have a greater ability to maintain hypertonicity in the lowest experimental salinity than winter animals. The reverse holds for the higher salinities. Figure 3 shows that summer animals have significantly lower blood chloride concentrations than the corresponding winter animals at 0 hours. The principle involved here may be one suggested by Beadle (1943) for fresh water animals — the lower the blood concentration initially, the lower the concentration of brackish water to which they can be adapted. The converse seems to hold for the hypersaline conditions. Temperature effects, as well, may be involved in chloride regulation, similar to the situation found in Potamobilis fluviatilis, a fresh water crayfish, where low temperatures decreased the

absorption of chloride (Wikgren, 1953). The results for chloride regulation in C. magister are not similar to those obtained for summer and winter comparisons of regulation in Hemigrapsus nudus (Dehnel, 1966) or in Callinectes sapidus (Ballard and Abbott, 1969), where the winter blood was maintained hypertonic to the summer blood at all salinities. Similar results were obtained, however, for the chloride gradients of urine to blood, all showing greater antennary gland activity in the winter months.

The concentration of sodium is also higher in summer than in winter C. magister. Again the situation is the reverse of that found in Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965). Greater hypertonicity of summer sodium in C. magister can be related directly to a greater degree of renal regulation of sodium, as indicated by a greater gradient maintained between blood and urine of summer animals as compared to winter. Lower winter sodium values may be related as well to a situation found in Asellus aquaticus, where a fall in temperature caused a decrease in blood sodium as a result of decreased uptake (Lockwood, 1960; 1961 a, b; 1962). This may occur at the antennary gland or some other regulatory site such as the gut.

Potassium concentration in summer and winter blood differed only in the 100% salinity, where that of the summer was the greater, and the blood was hypertonic as compared to isotonic winter blood. This related to greater activity of the antennary gland of summer C. magister.

With regard to the regulation of calcium, summer blood levels are higher only in the lowest experimental salinity of 34% sea water, while at 56, 81, and 105% sea water, the calcium of winter animals was regulated more hypertonically. This may be attributable to a higher calcium concentration in the winter field condition, resulting in acclimation and a higher optimum blood calcium level in all but 34% sea water, where summer animals carry out more effective regulation while that of winter animals, acclimated to higher salinities, breaks down. Higher winter calcium levels are also observed in Hemigrapsus nudus and oregonensis (Dehnel, 1967; Dehnel and Carefoot, 1965). Since no significant seasonal differences are obtained for the regulatory activity of gill or antennary gland, some other site such as the gut may be more effective in winter animals.

Magnesium regulation in C. magister is similar in that the winter animals maintain a greater effective gradient than those of the summer. The greater degree of hypotonicity observed for the winter animals may be the result of a greater loss rate at the body surface, possibly the gill. It could not be determined as a function of increased antennary gland activity. Acclimation is involved here, as well, with summer animals maintaining a lower magnesium gradient as a result of acclimation to a lower environmental salinity. The reverse situation exists for animals from the winter condition.

SUMMARY

1) Concentrations of chloride, sodium, potassium, calcium, and magnesium ions in the blood and urine of Cancer magister have been measured in four experimental salinities: chloride, sodium, and magnesium in 50, 75, 100, and 125% sea water; potassium in 38, 66, 100, and 125% sea water; calcium in 34, 56, 81, and 105% sea water.

2) Blood and urine ion values were determined for summer animals at an experimental temperature of 15° C. and for winter animals at 7.5° C.

3) Major changes in the adaptation of blood ionic concentrations to dilute or concentrated salinities occur within a few hours of exposure, half of the final equilibrated concentration achieved by twelve hours.

4) Animal weight was found to bear no significant relationship to the ionic regulation observed.

5) Chloride was determined to be regulated hypertonically in hyposaline media and hypotonically in hypersaline media. Summer animals maintain a greater gradient in dilute salinities, and a lesser gradient in concentrated salinities, than winter animals. Regulation at 50% is extra-renal. At higher salinities, the antennary gland actively excretes chloride by way of a hypertonic urine. Renal regulation is greater in winter animals.

6) Sodium is regulated hypertonically in the blood at all experimental salinities, with summer animals maintaining the greater gradient. Renal sodium regulation occurs at all salinities to produce a hypotonic urine. Winter animals show less renal activity than summer animals.

7) Potassium is maintained hypertonic in dilute salinities, with summer animals maintaining the greater gradient. The antennary glands are active in regulation, producing a hypotonic urine. Summer animals show greater renal regulation than winter animals.

8) Calcium is regulated hypertonically at all medium concentrations. Summer animals are the better regulators in the most dilute medium of 34% sea water, while winter animals are better hyper-regulators in the higher salinities. Except at 34%, the antennary gland actively regulates calcium to produce a hypotonic urine.

9) Regulation of magnesium is strongly hypotonic, at about half the medium concentration in summer animals and one third the medium concentration in winter animals. Renal involvement in magnesium regulation is pronounced with the production of a hypertonic urine. Summer and winter animals showed no difference in their degree of renal regulation.

10) Ion regulatory activity by the gills of summer and winter animals was investigated by potential difference measurements of <u>in vitro</u> gill preparations using single salt media for each

of the five ions of this study. Chloride is suggested to be regulated by absorption at 50% and secretion at 125%. Sodium may be transported outwards, especially in dilute salinities. The involvement of the gill in the regulation of potassium, calcium, and magnesium is implicated. Seasonal differences in the degree of regulatory activity of the gill were determined for potassium and magnesium, with winter preparations showing greater activity.

11) Seasonal acclimation is related to changes in the concentrations of constituent ions in summer and winter of the estuarine environment.

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