ION REGULATION IN TWO SPECIES OF INTERTIDAL CRABS

by

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ABSTRACT

Estuarine animals are faced with environmental conditions which favour dilution of the body fluids by water uptake and ion loss. The mechanisms employed by two species of estuarine crabs, Hemigrapsus nudus and Hemigrapsus oregonensis, to maintain ionic stability in sea water of varying salinity were investigated. The concentrations of sodium, potassium, calcium and magnesium ions in the blood and urine of these species were measured at eight salinities (6-175‰ sea water), three temperatures (5°, 15° and 25°C.) and in summer and winter.

In dilute salinities (6-75‰ sea water), concentrations of sodium, potassium and calcium ions in the blood of both species were considerably hypertonic to the media concentrations. In high salinities (100-175‰ sea water), the concentrations of these ions approached isotonicity with the media. Hypertonic ion regulation appears to be primarily effected by extra-renal mechanisms since the urine-blood ratios (U/B) approximate unity. It is suggested that the principal site of ion uptake in dilute sea water may be the lamellar cells in the gill epithelium.

Magnesium ion concentrations in the blood were maintained in both species at constant, hypotonic levels in all salinities above 12‰ sea water. This appeared to result solely from kidney activity since the U/B magnesium ratios were markedly greater than unity over most of the salinity range. It is thought that low blood magnesium levels are necessary to facilitate neuromuscular impulse transmission.

Changes in blood and urine concentrations of sodium, potassium and calcium ions in 25, 75 and 125‰ sea water required at least 24 hours to be completed, whereas the response of blood concentrations of magnesium ion was comparatively rapid, the major changes occurring after 12 hours.

Although seasonal differences in the ability of Hemigrapsus to regulate sodium and potassium were not consistent, in dilute salinities calcium was found to be more effectively regulated by winter animals than by summer ones. Magnesium levels in the blood of both species were equivalent in the two seasons.

No effect of temperature on the body fluid concentrations of sodium, potassium or calcium was demonstrated at either season in Hemigrapsus. The magnesium regulatory mechanism in both species, however, appeared to be gradually impaired as the temperature increased from 5° to 25°C. This may have resulted from a decrease in available energy, since all other metabolic processes were accelerated.

Hemigrapsus oregonensis was more effective in regulating winter blood concentrations of sodium in high salinities than H. nudus. The ability to regulate blood magnesium levels was almost identical in the two species. Higher urine magnesium concentrations in H. oregonensis, as compared to H. nudus, were related to the more permeable exoskeleton in the former species.

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INTRODUCTION

Ion regulation, as an integral part of osmotic regulation, has been extensively investigated in decapod Crustacea. Of special interest are those studies dealing with animals from estuarine conditions, or from environments characterized by fluctuating salinity conditions, because of the variety of methods utilized by such forms in the maintenance of osmotic and ionic stability. Lockwood (1962), in a recent review of osmotic regulation in Crustacea, lists five principal mechanisms which may be utilized in regulation: active absorption and extrusion of ions, conservation or elimination of water and ions by the kidneys, regulation of water volume by mechanisms other than the kidneys, restriction of exoskeleton permeability, and regulation of the cellular ion concentration. The latter process, the maintenance of an appropriate ion balance in the cell, is presumably the end result of regulation of the blood osmotic concentration.

Replenishment of ions lost in the urine and from the general body surface in dilute sea water may be effected by absorptive activity in the gills (Koch, Evans and Schicks, 1954; Gross, 1957a; Flemister, 1959; Green, Harsch, Barr and Prosser, 1959), by uptake from the gut (Burger, 1957) or by reabsorption from the urine (Riegel, 1961; Riegel and Lockwood, 1961). Gross (1957a) has also found that the branchial epithelium lining the gill chamber in Pachygrapsus crassipes contributes slightly to ion and water exchange between the blood and external medium.

The presence of extra-vascular "salt pools", originally postulated by Hukuda (1932), has been proposed by Gross (1958) to account for sodium and potassium changes occurring between the medium and a source other than the blood when P. crassipes is subjected to an osmotic stress. These ion reservoirs would have special significance in animals subjected to fluctuating salinity conditions.

The antennary glands of crabs function primarily in ion regulation in sea water more concentrated than the blood. Thus, chloride excretion in concentrated sea water in Ocypode albicans (Flemister and Flemister, 1951) and regulation of major cations and anions in the fiddler crabs, Uca pugnax and Uca pugilator (Green et al, 1959) is by kidney activity. The principal function of the antennary glands, however, appears to be the regulation of magnesium, as shown for P. crassipes by Prosser, Green and Chow (1955) and Gross (1959), for Carcinus maenas by Robertson (1949), and for Uca by Green et al (1959).

The depressant effect of magnesium on muscle contraction in various crustaceans has been investigated (Bethe, 1929; Katz, 1936; Waterman, 1941; Boardman and Collier, 1946). Robertson (1953) has found that the state of activity in several species of crabs depends on the magnesium level in the blood. He has related this to the blocking effect of magnesium in neuromuscular transmission and to the general anaesthetic action of magnesium salts.

The effect of temperature on ion regulation in decapod Crustacea has not been treated extensively. Wikgren (1953) has found

that low temperatures ($1-2^{\circ}\text{C}.$) cause a loss of chloride ion in the crayfish, Potamobius fluviatilis, due to impairment of ion absorption. Williams (1960) has studied the influence of temperature on osmotic regulation in the shrimps, Penaeus duorarum and P. aztecus. These species exhibit a less effective regulatory ability in low temperatures ($9^{\circ}\text{C}.$ as compared with $17^{\circ}\text{C}.$ and $28^{\circ}\text{C}.$). No ion measurements were made.

The osmotic response of Hemigrapsus nudus and H. oregonensis to low and high salinities has been investigated by Jones (1941). He has found that although both species of Hemigrapsus effectively hyper-osmoregulate in dilute sea water, they are incapable of hyposmotic regulation in concentrated sea water, even after 72 hours. Recently, in a study of the osmotic response of the blood of summer and winter H. oregonensis and H. nudus to a series of temperatures and salinities, Dehnel (1962) has documented the inability of Hemigrapsus to hypo-osmoregulate (after 48 hours in 100-150% sea water) and has shown a seasonal variation in regulatory ability. A comparable study on the urine osmotic concentrations of these species has been performed by Stone (1962). He has found that hyperosmotic regulation in dilute sea water in Hemigrapsus is achieved in summer by extra-renal mechanisms; in winter, hyperosmotic regulation is accomplished by production of urine hyposmotic to the blood.

Dehnel (1960), in a study of whole animal respiration of summer and winter H. nudus and H. oregonensis at a series of temperatures and salinities, has found that weight-specific oxygen consumption increases

in dilute sea water, where the osmotic gradient between the blood and external medium is greatest. Oxygen requirement of isolated gill tissue of summer and winter H. nudus and H. oregonensis has been also related to the maintenance of an osmotic gradient between the blood and external medium (McCaughran, 1962).

This investigation determined the concentrations of the major cations in the blood and urine of H. nudus and H. oregonensis at a series of salinities and temperatures, and at summer and winter conditions.

MATERIAL AND METHODS

The two species of crabs used in this study are found in large numbers intertidally at Spanish Bank, Vancouver, British Columbia. The physical and biological characteristics of the habitat have been described (Dehnel, 1960). In this area marked seasonal temperature and salinity fluctuations occur as a result of variations in the flow of the nearby Fraser River. Measurements over a five year period show that in summer a high water temperature, low salinity combination exists (20°C., 35% sea water); in winter, low water temperature is associated with high salinity (5°C., 75% sea water).

Ion analysis experiments were performed at a series of temperatures and salinities, and at summer and winter conditions, on Hemigrapsus nudus and H. oregonensis. Summer experiments were conducted from July 1 to August 31, 1961 and winter experiments from

December 15, 1961 to February 28, 1962. The animals were kept in the laboratory in shallow plastic dishes (4 litre capacity), each containing 12-15 individuals. To eliminate sex and moulting as factors in the experiment, only male crabs with firm exoskeletons were used, and sizes ranged from 5-15 grams for H. nudus and 5-12 grams for H. oregonensis. Field salinity and temperature measurements were taken at each collection. To permit partial clearance of the gut and to reduce mortality accompanying a sudden temperature change from field to experimental laboratory conditions, the animals were held for 12 to 18 hours after collection in sea water matching that of the seasonal field salinity from which they were removed (for summer animals, in 35% sea water; for winter crabs, in 75% sea water) and, at the start of the holding period, at a temperature approximating that of the field. While still at the field temperature the animals were placed in a constant temperature environment room ($\pm 0.1^{\circ}\text{C.}$) preset at the desired experimental temperature. During the holding period the medium temperature gradually changed to the experimental temperature. At the start of the 24 hour experimental period the holding medium was replaced with sea water of the appropriate experimental salinity and temperature. At the end of 24 hours, blood and urine samples were taken and analyzed for concentration of sodium, potassium, calcium and magnesium ions. While in the laboratory the crabs were kept in complete darkness fully immersed in aerated water and were not fed. Within each season, the combination and order of exposure to the experimental factors were randomly determined.

Experimental temperatures used were 5°, 15° and 25°C. ($\pm 1.0^\circ\text{C}.$); experimental salinities used were 6, 12, 25, 75, 100, 125, 150 and 175‰ sea water, based on a normal 100‰ sea water of salinity 31.88‰ and chlorinity 17.65‰. The salinities were obtained by diluting, with glass distilled water, a stock 200‰ sea water made by adding to natural 75‰ sea water appropriate amounts of the following salts in the ratios given by Barnes (1954): NaCl, Na₂SO₄, KCl, CaCl₂·2H₂O and Mg Cl₂·6H₂O. A one-half dilution of this solution, when titrated with AgNO₃, gave a chlorinity reading of 17.8‰ and a calculated salinity of 32.16‰. The concentrations of the four major cations in 100‰ sea water were determined: sodium, 439 mEq./L; potassium, 10.7 mEq./L; calcium, 24.9 mEq./L; and magnesium, 100 mEq./L.

Blood samples were obtained by puncturing the damp-dried membrane proximal to the coxopodite of the fifth walking leg. The area was wiped dry after the puncture and two 50 lambda samples collected with micro-pipettes. These were placed together in a 25 ml. volumetric flask and diluted to a 1:250 ratio with glass distilled water. Rapid transfer of each sample from pipette to flask minimized blood clotting. For each combination of species, season, temperature and salinity, ten 100 lambda samples were analyzed for cation concentration and the mean for each ion determined.

Urine samples were obtained by applying gentle suction to a finely drawn glass capillary tube (O.D. 1.5 mm.) positioned beneath

the operculum at the entrance to the nephridiopore. Care was taken to avoid damaging the delicate tissues. Urine release from the bladder was usually effected by raising the operculum. From the capillary tube the urine passed through a short length of polyethylene tubing (I.D. 1.4 mm.) and was collected in a 2 ml. centrifuge tube by way of a short length of capillary tubing through a cork stopper. From a #18 hypodermic needle in the stopper a rubber tube led to a small compensation flask connected to an aspirator. A 100 lambda urine sample required collecting from both excretory pores of at least two animals (damp dried), especially those exposed to high salinity media. The urine sample was diluted with glass distilled water to a 1:250 ratio in a 25 ml. volumetric flask. Used collecting tubes, vial and stopper were replaced for each successive sample. For each combination of species, season, temperature and salinity ten 100 lambda samples were analyzed for cation concentration and the values averaged.

The samples of blood and urine were analysed for sodium, potassium and calcium ion concentrations with a Zeiss PF5 flame photometer. Duplicate readings were taken for each ion. Combined calcium and magnesium concentration was determined by a modification of the direct method of Schwarzenbach, Biedermann and Bangerter (1946). Here, duplicate 2 ml. aliquots of the blood and urine samples (buffered to pH 10 with ammonium hydroxide-ammonium chloride buffer) were titrated with 0.00005 M. ethylenediamine tetra acetic acid (EDTA)

with Eriochrome Black T as indicator. To obtain magnesium concentration the calcium value from the flame photometer was subtracted from the combined calcium-magnesium value determined by titration.

In experiments to determine the rate of ion response to salinity stress, measurements were performed on the body fluids of summer representatives of both species after 1, 3, 6, 12 and 24 hours exposure to three salinities (25, 75 and 125% sea water). The animals were held for 12 to 18 hours in 35% sea water. In this series, the results from five samples at each set of conditions were averaged. Body fluids of animals directly removed from field conditions were measured for ion concentrations and presented as baseline values for the summer population. No comparable winter experiments were conducted.

The ion measurement data obtained were suited to statistical treatment by Student's "t" test. Significance was considered at the 0.01 level of probability.

In this report the terms, hypertonic, isotonic and hypotonic refer to the concentrations of specific ions in a body fluid in relation to the concentration of the same ions in another body fluid or in the external medium. Similarly, the relative concentration of all osmotically active particles in solution, whether ions or non-electrolytes, is expressed by the terms, hyperosmotic, isosmotic and hyposmotic. The expression, osmotic regulation (or osmoregulation) refers to the maintenance of a blood osmotic concentration different from that of the medium. Volume regulation (water regulation) and ion regulation are complementary processes of osmotic regulation.

RESULTS

Blood - Hemigrapsus nudusIon concentration at seasonal conditions:

Blood ion concentration of summer and winter H. nudus as a function of salinity of the external medium is shown in Figure 1 (sodium and potassium) and Figure 2 (calcium and magnesium). These seasonal values are presented at experimental temperatures (15°C. and 5°C.) approximating field temperatures in summer and winter (17°C. and 5°C., respectively). In salinities from 12-100‰ sea water, sodium and potassium are maintained at relatively constant blood levels, hypertonic to the media. In salinities above and below this range, regulation becomes less effective and concentrations approach those of the media. In concentrated salinities (above 100‰ sea water), blood sodium concentrations of summer animals are isotonic to the media (Table 3). Winter animals in concentrated salinities, however, exhibit a limited hypotonic regulation of sodium (active maintenance of a blood ion concentration less than that of the medium). Similarly, blood potassium concentrations of summer and winter animals in salinities above 100‰ sea water are maintained hypotonic to the media. This hypotonic regulation, however, can be of little adaptive importance to these animals since field salinities greater than 75‰ sea water are rarely encountered in this area. The constancy of hypertonic calcium regulation in dilute salinities is demonstrated by animals in both seasons (Fig. 2). Considerable hypotonicity is maintained for magnesium over most of the salinity range; the values increase

Figure 1. Sodium and potassium ion concentrations in the body fluids of winter (5°C.) and summer (15°C.) Hemigrapsus nudus as a function of external salinity. Each point represents the mean value of ten measurements after exposure for 24 hours to each experimental salinity.

Table 1. Values of Student's "t" resulting from statistical comparison of ion concentrations in the blood and urine of Hemigrapsus nudus at winter and summer seasonal conditions and over a range of experimental salinities. Significance is considered at the P = .01 level ($t_{01} = 2.878$, d.f. = 18). Values marked with an asterisk* represent comparisons that are significant at this level.

		BLOOD				URINE			
		WINTER (5°C.)				SUMMER (15°C.)			
	Salinity % Sea- Water	Na	K	Ca	Mg	Na	K	Ca	Mg
BLOOD SUMMER (15°C.)	6	1.520	0.850	4.739*	3.144*	0.796	0.875	2.136	2.512
	12	3.216*	2.321	6.957*	6.419*	0.234	2.358	2.328	4.507*
	25	0.758	0.973	8.007*	3.508*	0.546	2.131	3.076*	4.250*
	75	2.704	2.622	11.128*	3.518*	5.153	0.204	1.121	4.886*
	100	2.864	7.774*	4.879*	0.562	0.775	5.858*	0.170	14.964*
	125	0.426	8.177*	8.055*	2.783	0.720	0.903	1.349	13.799*
	150	4.780*	4.315*	3.529*	1.517	1.381	3.458*	1.491	11.451*
	175	1.614	2.111	4.716*	0.512	3.203*	2.287	4.717*	7.660*
URINE WINTER (5°C.)	6	1.315	3.316*	1.295	0.343	3.274*	3.772*	5.007*	0.262
	12	5.014*	4.205*	2.396	5.738*	6.137*	11.026*	5.701*	6.416*
	25	12.878*	2.671	9.051*	2.591	14.754*	5.127*	26.505*	1.867
	75	7.177*	3.000*	0.449	8.116*	7.086*	5.656*	6.775*	3.445*
	100	6.870*	0.813	2.213	15.404*	5.223*	3.272*	7.239*	6.356*
	125	7.263*	2.432	3.799*	23.977*	5.165*	4.149*	7.808*	7.603*
	150	10.135*	1.000	9.158*	36.702*	5.539*	2.544	8.573*	13.612*
	175	5.805*	1.778	12.238*	11.787*	1.902	5.577*	5.118*	2.375

from only 19-52 mEq./L after exposure to a change in external magnesium concentration of 6-176 mEq./L. In media above 25% sea water, blood magnesium is regulated at about one third the medium concentration.

Statistical comparisons of seasonal blood ion concentrations are represented in Table 1 by Student's "t" values. These indicate that over the entire salinity range for sodium and over most of the salinity range for potassium, there is no significant seasonal difference (Fig. 1). In salinities from 100-150% sea water, summer potassium levels are significantly higher than winter levels. For calcium, however, winter crabs have a significantly higher blood concentration over the entire salinity range than summer crabs (Fig. 2). This winter calcium level, in dilute salinities in which the blood is hypertonic, is equivalent to sea water concentrations of 125-145%. Comparable summer calcium values are 95-115%. Blood magnesium concentrations are maintained at almost the same level regardless of the season (Fig. 2). Statistically significant seasonal differences in the dilute salinities reflect the lack of variation in the regulation of this ion, for the absolute magnitude of difference is small. Such slight differences are probably not meaningful from a biological standpoint.

Time response to external salinity change:

Change in blood ion concentrations over a 24 hour period in three salinities (25, 75 and 125% sea water) at 15°C. is recorded for

Figure 2. Calcium and magnesium ion concentrations in the body fluids of winter (5°C.) and summer (15°C.) Hemigrapsus nudus as a function of external salinity. Each point represents the mean value of ten measurements after exposure for 24 hours to each experimental salinity.

Table 2. Ion concentrations in blood and urine of summer Hemigrapsus nudus measured at intervals during a 24 hour period. Treatment involved exposure to sea water of three salinities (25, 75 and 125‰) at a temperature (15°C.) close to that of summer field conditions (17°C.). Each value represents the mean of five measurements. The baseline values at time zero are determined from crabs directly removed from summer field conditions (35‰ sea water and 17°C.).

		BLOOD (TIME-HRS.)					URINE (TIME-HRS.)					Baseline Values (Summer Field Conditions -35‰ Sea Water)	
	SALINITY ‰ mEq./L	1	3	6	12	24	1	3	6	12	24	BLOOD	URINE
Na (mEq./L)	25 110 75 329 125 549	407 437 443	406 422 473	396 418 484	399 408 521	395 403 544	401 474 482	455 462 498	392 437 503	419 420 534	401 442 552	435	391
K (mEq./L)	25 2.7 75 8.0 125 13.3	10.0 10.4 11.3	9.2 10.0 12.5	8.8 10.0 11.6	9.5 8.8 12.5	9.9 10.5 12.7	10.0 12.2 12.6	10.5 11.4 14.2	10.8 10.5 12.5	11.0 10.0 14.1	10.7 10.4 13.1	11.0	13.3
Ca (mEq./L)	25 6.2 75 18.7 125 31.1	26.0 27.3 25.8	25.4 26.9 27.7	25.5 24.5 24.4	26.2 24.3 26.7	22.7 23.6 29.0	23.6 27.7 27.8	26.4 26.1 25.9	23.9 25.5 26.1	24.6 23.3 28.2	20.1 25.0 31.1	24.7	23.6
Mg (mEq./L)	25 25 75 76 125 126	23 22 24	21 21 25	18 24 25	20 23 31	19 28 36	24 34 36	27 38 25	32 48 70	38 46 97	35 43 88	19	63

10b.

H. nudus in Table 2. The baseline values in Table 2 represent measurements performed on animals directly removed from summer field conditions (35‰ sea water and 17°C.) and for sodium, potassium and calcium are equivalent to sea water of 100%. Thus, in the natural environment these ions are strongly regulated. After 24 hours exposure to experimental salinities of 125, 75 and 25‰ sea water, blood sodium concentrations are equivalent to sea water of 125, 90 and 90% respectively. The blood concentrations of potassium after 24 hours are equivalent to sea water of 120, 100 and 95%; for calcium, the concentrations are 115, 95 and 90%. In 125‰ sea water, then, blood levels of these three ions approach isotonicity with the medium after 24 hours. In dilute salinities, strong hypertonic regulation is demonstrated by the maintenance of a considerable gradient between blood and external environment. Comparable magnesium values were equivalent to 36, 28 and 19‰ sea water. These results present additional evidence for the presence of an active mechanism for hypotonic regulation of this ion.

Effect of temperature:

The effect of temperature (5°C, 15°C and 25°C.) on blood ion concentrations of summer and winter H. nudus over a range of salinities is shown in Figure 3(sodium, potassium and calcium) and Figure 4 (magnesium). In dilute salinities, concentrations of sodium, potassium and calcium are maintained hypertonic to the media, apparently independent of experimental temperature or season. In concentrated salinities, limited hypotonic regulation is effective in maintaining the concentration

11a.

Figure 3. Relationship of sodium, potassium and calcium ion concentration in blood of summer and winter Hemigrapsus nudus at three experimental temperatures (5° , 15° and 25°C.) to a range of external salinities. Each point represents the mean value of ten measurements after exposure for 24 hours to each experimental salinity.

of these ions slightly below the media concentrations at almost all combinations of temperature and season. Potassium alone shows a consistent seasonal difference at the three temperatures, winter values tending to be lower than summer values in media above 25% sea water. Since no consistent effect of temperature on seasonal animals could be demonstrated it appears that the mechanisms important in maintaining ion hypertonicity in a dilute environment are remarkably tolerant to wide temperature variation.

This is not altogether true for the response of magnesium to temperature at each season. Table 10 shows, for H. nudus, that the range of magnesium concentrations in the blood (measured in 6 and 175% sea water) increases with increasing temperature in both seasons. Figure 4 shows that in 175% sea water the total variation in magnesium concentration for all combinations of temperature and season is only 49-68 mEq./L.

Urine-Hemigrapsus nudus

Ion concentration at seasonal conditions:

Urine ion concentrations of summer and winter H. nudus in relation to salinity are depicted in Figure 1 (sodium and potassium) and Figure 2 (calcium and magnesium). Statistical comparisons of seasonal urine ion values and comparisons of urine and blood ion concentrations at a given season are represented by Student's "t" values in Table 1. Winter urine sodium concentrations are significantly higher than summer values in most of the salinities (25-150% sea water). Also, in media

Figure 4. Relationship of magnesium ion concentration in the body fluids of summer and winter Hemigrapsus nudus at three experimental temperatures (5°, 15° and 25°C.) to a range of external salinities. Each point represents the mean of ten measurements after a 24 hour exposure for 24 hours to each experimental salinity.

above 12% sea water, winter urine sodium concentrations are hypertonic to winter blood sodium concentrations. This suggests that the antennary glands actively rid the blood of sodium, contributing to a winter blood sodium condition that is hypotonic to the medium in the concentrated salinities. This is presumably advantageous in that excessive concentrating of the blood in high salinities is lessened. In dilute media, however, sodium excretion by the kidneys tends to increase sodium loss by supplementing the passive outward diffusion of this ion (in salinities of 25-100% sea water, the blood sodium concentration is 400-450 mEq./L, equivalent to sea water of 85-100%). Thus, selective extra-renal sodium uptake from a dilute environment to maintain blood hypertonicity is opposed by a sodium loss due to antennary gland activity. Summer urine sodium concentrations show no significant difference from summer blood concentrations. This indicates that hypertonic sodium regulation in the blood in this season is by extra-renal mechanisms. The antennary glands are ineffective in hypertonic potassium regulation in summer animals for the urine concentrations are essentially isotonic with the blood concentrations. In winter crabs, however, maintenance of a blood potassium concentration hypertonic to the media is assisted by the production of a dilute urine. Hypotonic potassium regulation in media above 100% sea water is accomplished in both seasons extra-renally, for the urine is essentially isotonic to the blood (Table 1). A significant seasonal difference in urine potassium concentration is exhibited over most of the salinity range (summer higher than winter). Hypertonic

regulation of blood calcium is accomplished solely by extra-renal mechanisms in the summer for the urine concentrations are equal to the blood in most of the experimental media (Fig. 2 and Table 1). In winter animals, however, there is at least some participation of the kidneys in hypertonic calcium regulation in salinities of 100-150‰ for the urine is hypertonic to the blood. Thus, extra-renal absorption of calcium in dilute media appears to be gradually replaced in these concentrated salinities by calcium excretory activity of the kidneys. Winter urine calcium levels are significantly higher than summer levels over the entire salinity range.

Whereas regulation of the other ions appears to be largely a result of extra-renal processes, the hypotonic regulation of blood magnesium is effected almost entirely by antennary gland activity. Data presented in Figure 2 indicate that urine magnesium concentrations are significantly higher in both winter and summer crabs in salinities above 25‰ sea water than comparable blood magnesium values. Thus, regulation of magnesium in these salinities is by excretion in the urine. In salinities below 25‰ sea water, blood magnesium concentrations are hypertonic to the medium. Although urine-blood isotonicity minimizes magnesium loss in these dilute salinities, it is possible that absorptive processes also contribute to the maintenance of hypertonicity. In salinities above 25‰ sea water, summer urine magnesium is markedly hypotonic to the media (isotonic in 175‰ medium) and winter urine magnesium is slightly hypotonic, becoming hypertonic between 125-150‰ sea water.

14a.

Table 3. Values of the mean and standard error of urine ion concentrations of Hemigrapsus nudus measured at seasonal and experimental temperature (5°, 15° and 25°C.) conditions. Each mean value represents ten measurements after exposure for 24 hours to each experimental salinity.

		WINTER						SUMMER					
	SALINITY	5°C.		15°C.		25°C.		5°C.		15°C.		25°C.	
	% mEq./L	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Na (mEq./L)	6 27	263	13.3	260	6.6	281	8.6	287	11.1	327	14.2	321	14.2
	12 53	280	7.8	281	11.4	344	5.6	368	17.6	370	12.4	359	2.9
	25 110	562	6.5	375	16.9	397	6.6	383	12.3	402	8.7	350	5.4
	75 329	540	13.0	424	5.4	451	4.5	445	11.4	442	5.0	418	3.2
	100 439	545	12.5	459	7.1	484	3.0	493	12.9	477	3.9	446	16.8
	125 549	605	8.3	555	7.3	577	4.1	538	16.2	552	6.1	606	27.0
	150 658	731	12.1	629	21.0	659	5.5	610	21.9	649	8.4	699	12.2
	175 768	785	9.5	629	18.3	726	3.4	728	17.0	834	24.0	692	8.4
K (mEq./L)	6 0.6	6.2	.32	12.6	.92	10.3	.53	6.5	.71	8.6	.55	15.3	.88
	12 1.3	6.2	.21	11.6	.77	12.0	.42	5.9	.48	9.8	.25	15.3	.64
	25 2.7	8.4	.21	7.9	.27	9.2	.26	6.3	.31	10.9	.44	17.2	.50
	75 8.0	7.8	.33	9.0	.43	12.7	.47	7.4	.38	10.4	.32	15.4	.43
	100 10.7	9.8	.31	9.7	.34	13.6	.82	9.2	.30	10.9	.13	12.5	1.23
	125 13.3	11.0	.31	12.5	.37	11.7	.37	10.3	.31	13.1	.40	15.1	.62
	150 16.0	12.7	.24	14.4	.30	15.1	.29	11.6	.35	13.6	.26	18.7	.59
	175 18.7	14.0	.36	17.6	.40	15.8	.59	16.6	.46	16.8	.35	24.8	1.02
Ca (mEq./L)	6 1.5	26.8	1.00	28.9	.59	22.4	.76	31.4	.92	20.8	.66	17.7	.52
	12 2.9	28.6	1.13	24.3	1.13	23.7	.27	32.2	1.62	20.4	.89	17.9	.58
	25 6.2	36.7	.49	23.2	1.03	20.8	.51	31.8	.99	20.1	.39	17.4	.38
	75 18.7	33.6	.65	29.0	.55	27.6	.41	30.8	1.81	25.0	1.09	23.7	1.01
	100 24.9	35.8	1.02	33.5	.43	31.0	.82	31.3	.64	27.7	.46	27.6	1.10
	125 31.1	41.4	1.24	35.5	.41	39.4	1.24	32.6	.83	31.1	.45	32.6	1.87
	150 37.3	45.2	.62	43.8	1.24	38.0	.32	37.5	1.17	34.0	1.15	38.5	1.25
	175 43.5	51.3	.52	44.7	1.19	49.8	.98	49.5	.93	44.7	1.18	52.3	.69
Mg (mEq./L)	6 6	27	2.0	14	2.1	17	1.2	32	2.5	26	2.3	16	1.0
	12 12	74	7.4	23	1.7	17	.7	47	5.7	26	1.2	23	1.6
	25 25	42	1.3	68	2.4	16	1.0	38	2.0	35	3.8	20	.7
	75 76	56	2.7	73	4.1	46	2.5	51	3.3	43	2.8	74	1.2
	100 100	101	4.3	109	5.3	79	1.9	91	9.9	70	2.2	100	6.8
	125 126	116	2.9	168	7.5	72	3.4	120	8.9	83	3.3	108	8.3
	150 151	186	3.2	250	19.2	136	2.7	205	13.7	105	5.0	138	5.8
	175 176	230	15.0	353	15.9	195	2.9	217	7.4	177	16.3	188	4.0

Winter urine magnesium concentrations are significantly greater than summer values over most of the salinity range, even though the seasonal blood levels are almost identical. Possibly winter crabs, because of their seasonal acclimatization to higher environmental magnesium levels, are more effective in excreting magnesium when exposed to an excess in the experimental media. Summer animals, however, because they are accustomed to lower environmental levels of magnesium, must rely on accessory organs of excretion. Thus, summer urine is less concentrated.

Time response to external salinities:

Change in urine ion concentrations of summer H. nudus during 24 hours exposure to three salinities (25, 75 and 125% sea water) at 15°C. is given in Table 2. A continuous response over the 24 hour period is exhibited by sodium, potassium and calcium. Urine sodium is consistently higher than blood sodium in 75 and 125% sea water. This is true for potassium and calcium in 125% sea water. Urine-blood isotonicity is approached in most cases after 24 hours. Thus, in 125% media, a short-term ion regulation by the kidneys is evidenced which, after prolonged exposure, becomes less effective and isotonicity with the medium is adopted. Baseline values of blood and urine sodium, potassium and calcium, measured directly from 35% field salinity conditions, are closer to isotonicity than comparable values for magnesium. Thus, summer animals in the field appear to regulate magnesium

Figure 5. Relationship of urine-blood (U/B) ion ratios of summer and winter Hemigrapsus nudus to a range of external salinities. Each point represents a ratio of the means of ten measurements for blood and urine after exposure for 24 hours to each experimental salinity.

by excretion in the urine. In this experiment, urine magnesium increases rapidly to a peak concentration after 12 hours and in all three salinities remains hypertonic to the blood for the remaining 12 hours. These observations show that blood magnesium is actively regulated by the kidneys almost immediately upon exposure to a salinity stress.

Effect of temperature:

Table 3 gives urine ion values for seasonal H. nudus at three temperatures (5° , 15° and 25°C.). The values for sodium, potassium and calcium are comparable in magnitude and variation to those presented for the blood (Fig. 3) and no consistent effect of temperature on urine ion concentrations could be demonstrated.

Seasonal variation in urine magnesium concentration with temperature is shown in Figure 4. In media above 25% sea water, the urine is consistently hypertonic to the blood. Although an effect of temperature on this ion is not demonstrated, the variability exhibited in urine magnesium concentration at the three temperatures is twice that of the blood at a given season. Values for the other ions did not exhibit this difference in variability between the two body fluids.

Urine-blood ratio (U/B) - Hemigrapsus nudus

The urine-blood ion ratio is indicative of the extent of kidney activity in the regulation of a blood ion. Thus, if the U/B ratio for an ion equals, or approximates, unity, there can be no participation of the kidneys for the urine and blood are isotonic. Ratios greater than unity, however, indicate urinary excretion; ratios markedly less than

Table 4. Urine-blood (U/B) ion ratios for seasonal and experimental temperature (5°, 15° and 25°C.) conditions for Hemigrapsus nudus. Mean and standard error values are given for each ratio, representing the average of ten measurements of each body fluid after exposure for 24 hours to each experimental salinity.

	SALINITY % Sea Water	WINTER						SUMMER					
		5°C.		15°C.		25°C.		5°C.		15°C.		25°C.	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Na (mEq./L)	6	.92	.057	.81	.036	.96	.044	.96	.048	1.05	.064	1.05	.069
	12	.83	.031	.73	.032	.96	.021	1.07	.059	.99	.039	1.02	.027
	25	1.46	.049	.95	.044	1.03	.020	.03	.098	1.02	.028	1.00	.030
	75	1.26	.038	1.01	.015	1.12	.012	1.01	.027	1.09	.019	1.03	.013
	100	1.22	.034	1.03	.016	1.12	.009	1.04	.029	1.01	.014	1.00	.038
	125	1.12	.017	1.06	.014	1.06	.008	.99	.032	1.02	.022	1.20	.054
	150	1.22	.022	.98	.033	1.06	.012	1.01	.040	1.02	.016	1.06	.019
	175	1.09	.015	.88	.026	.96	.006	1.04	.027	1.12	.038	.90	.012
K (mEq./L)	6	.72	.066	1.71	.139	1.28	.100	.96	.110	1.07	.088	1.74	.115
	12	.76	.045	1.33	.093	1.34	.066	.86	.086	1.08	.034	1.58	.083
	25	.90	.038	.90	.043	.91	.046	.70	.078	1.10	.055	1.66	.058
	75	.85	.047	1.06	.056	1.36	.056	.69	.039	.99	.046	1.37	.054
	100	.97	.036	1.03	.045	1.29	.080	.78	.044	.88	.018	1.02	.103
	125	1.11	.043	1.06	.034	.90	.030	.76	.040	1.03	.035	1.12	.049
	150	.97	.030	1.06	.030	1.08	.025	.76	.029	.92	.022	1.11	.037
	175	.95	.030	1.22	.034	1.09	.044	.98	.036	1.07	.032	1.44	.063
Ca (mEq./L)	6	.94	.045	1.08	.041	.86	.038	1.18	.042	.91	.042	.78	.041
	12	.87	.049	.91	.049	.95	.041	1.17	.067	.89	.044	.74	.045
	25	1.22	.027	.85	.041	.83	.035	1.07	.079	.88	.034	.74	.025
	75	1.01	.027	.94	.027	.95	.019	1.10	.073	1.06	.053	.92	.053
	100	1.08	.037	1.07	.020	1.07	.032	1.02	.034	1.03	.045	1.05	.051
	125	1.14	.039	1.17	.017	1.26	.042	1.00	.032	1.07	.029	1.13	.069
	150	1.24	.029	1.28	.043	1.17	.017	.97	.036	1.08	.056	1.16	.043
	175	1.21	.019	1.06	.046	1.24	.029	1.17	.032	1.17	.037	1.43	.032
Mg (mEq./L)	6	1.03	.105	.82	.133	1.81	.171	1.12	.095	1.32	.137	1.05	.089
	12	2.46	.289	1.52	.146	1.76	.152	1.65	.214	1.34	.089	1.20	.164
	25	1.88	.114	4.37	.205	1.23	.160	1.18	.080	1.88	.209	.87	.122
	75	1.70	.096	2.83	.208	1.65	.139	1.44	.137	1.53	.119	2.30	.138
	100	3.16	.193	3.75	.217	1.78	.090	2.28	.296	2.14	.104	2.91	.234
	125	2.89	.121	4.90	.272	1.33	.091	2.65	.270	2.31	.110	2.63	.243
	150	3.93	.184	6.74	.544	2.16	.132	3.93	.303	2.45	.169	2.77	.134
	175	4.39	.299	7.22	.431	2.89	.098	4.27	.177	3.46	.344	3.16	.108

unity suggest renal absorption.

The U/B ion ratios for summer and winter H. nudus are depicted in Figure 5. As expected, U/B magnesium ratios deviate markedly from unity in both seasons. The higher winter ratio for magnesium, as noted previously, may reflect a greater potential for magnesium excretion in the winter population - necessary because the field concentration of magnesium in this season is twice that of the summer. If the absolute quantity of magnesium ion diffusing into summer and winter crabs when immersed in a comparable salinity (above 25‰ sea water) is equivalent, then the difference in the amount of magnesium excreted by summer crabs must be eliminated extra-renally since the blood magnesium concentration is identical in the two seasons.

The maintenance by winter crabs of a constant hypertonic blood sodium level in dilute salinities (Fig. 1) is accomplished even though the urine has a significantly high concentration of this ion, represented here by U/B sodium ratios greater than unity. Hypertonic sodium regulation in summer animals is effected by extra-renal mechanisms (U/B ratios near unity). A limited hypotonic sodium regulation in concentrated media by urinary excretion in winter crabs is suggested by the presence of ratios greater than unity. Hypertonic potassium regulation by winter crabs in dilute salinities is aided by production of urine less concentrated than the blood, indicated by U/B ratios less than unity. In summer, hypertonic regulation of potassium and calcium

17a.

Figure 6. Sodium and potassium ion concentrations in the body fluids of winter (5°C.) and summer (15°C.) Hemigrapsus oregonensis as a function of external salinity. Each point represents the mean value of ten measurements after exposure for 24 hours to each experimental salinity.

Table 5. Values of Student's "t" resulting from statistical comparison of ion concentrations in the blood and urine of Hemigrapsus oregonensis at winter and summer seasonal conditions and over a range of experimental salinities. Significance is considered at the P = .01 level ($t_{.01} = 2.878$, d.f. = 18). Values marked with an asterisk* represent comparisons that are significant at this level.

		BLOOD WINTER (5°C.)				URINE SUMMER (15°C.).			
	SALINITY % Sea Water	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>
BLOOD SUMMER (15°C.)	6	4.726*	2.357	8.265*	5.958*	1.350	1.931	0.129	6.636*
	12	2.572	3.230*	4.900*	5.129*	1.485	2.752	0.887	6.647*
	25	4.292*	3.407*	5.861*	4.468*	0.683	4.898*	0.898	2.222
	75	3.157*	3.201*	11.244*	0.993	0.255	6.889*	0.489	7.756*
	100	4.860*	8.061*	9.665*	2.914*	1.547	5.328*	1.481	5.656*
	125	7.031*	6.433*	3.300*	2.030	0.252	5.184*	0.939	13.946*
	150	10.137*	2.890*	6.247*	2.804	2.158	3.094*	6.135*	10.561*
	175	12.148*	2.017	5.404*	1.225	4.107	3.457*	4.041*	13.034*
URINE WINTER (5°C.)	6	2.964*	3.344*	4.672*	3.060*	6.973*	2.817	11.368*	3.313*
	12	2.171	2.846	6.322*	1.802	9.041*	1.635	9.516	0.942
	25	6.483*	2.938*	3.624*	21.041*	3.152*	1.068	12.156*	26.038*
	75	10.601*	6.246*	1.248	6.799*	9.861*	2.475	8.735*	0.824
	100	9.666*	4.391*	2.043	11.581*	6.305*	3.305*	3.862*	2.108
	125	16.229*	4.981*	0.653	20.398*	4.442*	10.605*	2.295	4.773*
	150	15.008*	5.929*	7.439*	15.835*	6.175*	9.375*	1.939	2.435
	175	10.918*	3.802*	5.431*	21.889*	4.878*	1.528	3.794*	5.033*

is by means other than the kidney. Hypotonic calcium regulation by winter crabs in concentrated media is assisted by calcium excretion in the urine.

The U/B ratios for each temperature (5° , 15° and 25°C.) at both seasons are presented for H. nudus in Table 4. These values are similar to the relationships presented for each season (Fig. 5) and no temperature effect could be demonstrated. Since the U/B ratios represent a compounding of the variability of two factors, blood and urine, the interpretation of such comparisons is difficult.

Blood - Hemigrapsus oregonensis

Ion concentration at seasonal conditions:

Figures 6 and 7 show the relationship of the blood ion concentrations of summer and winter H. oregonensis to the range of salinities. Hypertonic regulation of sodium, potassium and calcium in the blood is evident in dilute salinities. Sodium and potassium ion regulation becomes less effective in salinities below 25‰ and above 100‰ sea water. Winter crabs exhibit hypotonic regulation of blood sodium and potassium in salinities above 100‰ sea water (Fig. 6). Strong hypertonic calcium regulation in both seasons results in a relatively constant blood level in salinities 125‰ and below (Fig. 7). In salinities above 125‰ sea water, isotonicity with the media is approached in winter crabs. Summer crabs exhibit some hypotonic calcium regulation in salinities above 125‰ sea water. The constancy of hypotonic magnesium

18a.

Figure 7. Calcium and magnesium ion concentrations in the body fluids of winter (5°C.) and summer (15°C.) Hemigrapsus oregonensis as a function of external salinity. Each point represents the mean value of ten measurements after exposure for 24 hours to each experimental salinity.

regulation in this species permits an internal variation of only 17-56 mEq./L following exposure to media varying from 6-176 mEq./L.

Values of Student's 't' resulting from statistical comparisons of blood ion concentrations at each season are presented in Table 5. In dilute salinities, winter sodium concentrations are hypertonic to summer concentrations; in concentrated salinities, winter sodium values are hypotonic to summer values (Fig. 6). Summer potassium values are significantly higher than winter ones over most of the salinity range (except at 6‰ and 175‰ sea water); for calcium, the converse is true, winter values being significantly higher than summer ones. Although winter magnesium concentrations are significantly greater than summer values in salinities from 6-25‰ sea water, the actual difference is of only small magnitude and is probably not biologically important.

Time response to external salinity change:

Change in blood ion concentrations of summer H. oregonensis over a 24 hour period in response to salinities of 25, 75 and 125‰ sea water are illustrated in Figure 8 (sodium and potassium) and Figure 9 (calcium and magnesium). These data show that change in blood concentration of sodium, potassium and calcium in crabs from 35‰ field salinity is continuous over the 24 hour period of immersion. Animals directly removed from the field (time zero) have a blood sodium concentration equivalent to 95‰ sea water. Comparable values for potassium and calcium are 90‰ and 110‰. This maintenance of a relatively high blood calcium concentration over a wide range of

Figure 8. Sodium and potassium ion response to external salinity change in blood and urine of summer Hemigrapsus oregonensis at 15°C. Each point represents the mean value of five measurements. The baseline values at time zero are determined from crabs directly removed from the summer field conditions (35% sea water and 17°C.). A value representing the equivalent ion concentration in 100% sea water is indicated.

salinities is a constant feature of the ion regulation of both species.

After 24 hours exposure to 125, 75 and 25% sea water, blood sodium concentrations are equivalent to sea water of 125, 90 and 80%, respectively. Comparable blood potassium levels after 24 hours are equivalent to sea water of 125, 90 and 85%. For calcium, the blood concentrations are 120, 100 and 90%. In the two lower salinities, then, strong hypertonic regulation of these ions occurs. In the highest salinity, the blood approaches isotonicity with the media. Blood magnesium concentrations remain comparatively constant in H. oregonensis even in 125% sea water, over the entire 24 hour period of observation (Fig. 9).

Effect of temperature:

Data presented in Table 6, showing the blood ion concentrations of summer and winter H. oregonensis at the three temperatures, are comparable to the results shown graphically for H. nudus in Figures 3 and 4. No consistent differences could be demonstrated for temperature in the concentrations of sodium, potassium and calcium. For magnesium, however, as the temperature increases the range of concentration in the blood increases (Table 10), suggesting that the magnesium regulatory mechanism is impaired at higher temperatures. These results are comparable to those for H. nudus.

Urine - Hemigrapsus oregonensis

Ion concentration at seasonal conditions:

Figure 6 (sodium and potassium) and Figure 7 (calcium and magnesium) show, for summer and winter H. oregonensis, the relationship of the urine ion concentrations to external salinity. Statistical

Figure 9. Calcium and magnesium ion response to external salinity change in blood and urine of summer Hemigrapsus oregonensis at 15°C. Each point represents the mean value of five measurements. The baseline values at time zero are determined from crabs directly removed from the summer field conditions (35% sea water and 17°C.). A value representing the equivalent ion concentration in 100% sea water is indicated.

comparisons of seasonal urine ion concentrations and comparisons of urine and blood ion concentrations at a given season are represented by Student's "t" values in Table 5. Summer urine sodium concentrations are isotonic with summer blood concentrations and, except in 175% sea water, no significant differences were demonstrated. Winter urine sodium concentrations, however, are significantly hypertonic to winter blood concentrations in most salinities (not in 12% sea water). These results are comparable to those demonstrated for H. nudus. Winter urine sodium concentrations are hypertonic to the media over the total salinity range. In winter H. oregonensis, then, hypertonic sodium regulation in dilute media is accomplished even though sodium is actively excreted in the urine. In media above 100% sea water, however, this same mechanism contributes to the hypotonicity of blood sodium (Fig. 6). Summer urine sodium concentrations are significantly less than winter urine levels. For potassium, summer urine is hypertonic to winter urine in all salinities except 100% sea water. Summer and winter urine levels of potassium are hypotonic to the media above 100% sea water; in salinities above 12% sea water, urine potassium is significantly hypotonic to blood potassium in both seasons (except at 100% sea water). This hypotonic urine is a manifestation of renal absorption to maintain blood hypertonicity in dilute salinities. This does not explain, however, the hypotonic state of urine to blood in concentrated salinities, for renal absorption would tend to make the blood even more concentrated. Summer blood and urine calcium levels are isotonic in salinities from 6-125% sea

21a.

Table 6. Values of the mean and standard error of blood ion concentrations of Hemigrapsus oregonensis measured at seasonal and experimental temperature (5°, 15° and 25°C.) conditions. Each mean value represents ten measurements after exposure for 24 hours to each experimental salinity.

			WINTER						SUMMER					
			5°C.		15°C.		25°C.		5°C.		15°C.		25°C.	
			Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
			% mEq./L											
Na (mEq./L)	6	27	356	8.2	348	5.2	300	7.8	296	7.4	287	11.9	312	9.1
	12	53	366	8.2	387	3.2	322	9.7	315	11.4	323	14.7	327	14.7
	25	110	415	8.6	386	5.2	354	6.0	309	15.3	357	10.3	346	6.8
	75	329	426	4.2	399	2.9	398	3.4	393	10.5	406	4.7	394	3.4
	100	439	428	.9	413	3.7	396	3.0	438	4.8	448	4.1	474	5.3
	125	549	489	3.7	499	4.7	511	3.5	509	5.7	548	7.5	561	2.4
	150	658	551	5.9	617	5.4	611	5.8	607	8.7	644	7.0	674	3.2
	175	768	649	6.1	706	7.0	687	5.6	680	7.3	765	7.4	780	4.5
K (mEq./L)	6	0.6	6.9	.27	7.7	.28	6.1	.14	7.2	.26	7.9	.27	8.2	.46
	12	1.3	7.3	.22	8.1	.28	6.9	.31	6.9	.24	8.5	.26	9.0	.24
	25	2.7	7.8	.21	8.0	.19	7.3	.17	8.5	.39	9.5	.42	9.2	.34
	75	8.0	8.4	.25	9.4	.26	9.5	.22	9.7	.16	9.7	.32	10.9	.23
	100	10.7	8.1	.27	10.1	.20	11.1	.24	11.9	.61	11.5	.34	11.6	.19
	125	13.3	9.4	.29	11.1	.27	12.9	.29	12.3	.43	13.4	.55	13.6	.28
	150	16.0	13.1	.29	13.4	.22	15.3	.19	15.3	.68	15.0	.59	15.9	.29
	175	18.7	16.6	.37	14.1	.39	16.2	.22	16.0	.52	17.6	.33	16.7	.23
Ca (mEq./L)	6	1.5	29.1	.61	27.2	.72	24.7	.84	29.2	1.2	21.3	.72	23.5	.53
	12	2.9	28.5	.74	30.6	.64	26.9	.68	28.2	1.2	22.6	.95	24.2	1.02
	25	6.2	30.8	1.07	29.7	.31	30.2	.55	26.5	1.2	23.0	.82	23.9	1.12
	75	18.7	33.4	.35	28.7	.65	28.1	.57	28.7	.61	25.2	.65	26.5	1.07
	100	24.9	33.3	.40	30.4	.45	26.4	.36	31.0	.70	28.0	.39	28.8	.79
	125	31.1	34.2	.48	32.7	.63	30.3	.44	35.1	1.37	30.5	.60	30.1	.85
	150	37.3	37.0	.35	33.8	.24	32.8	.32	40.3	2.14	31.2	.86	33.3	1.10
	175	43.5	43.6	.39	36.6	.39	39.8	.61	41.6	1.45	38.1	.94	37.7	.73
Mg (mEq./L)	6	6	25	1.3	18	2.2	10	.6	23	1.0	17	.7	12	.9
	12	12	25	.9	18	2.1	10	.5	20	1.1	18	1.0	11	.7
	25	25	28	1.6	16	.6	11	.5	25	1.6	19	1.4	10	.5
	75	76	34	1.0	27	1.7	27	1.5	31	1.6	31	2.2	24	1.5
	100	100	34	.8	33	1.4	32	2.1	42	4.0	39	1.4	36	1.7
	125	126	41	1.4	38	1.5	48	1.4	54	4.3	46	2.2	40	1.5
	150	151	42	1.5	48	1.8	53	.9	53	3.9	50	2.3	42	1.7
	175	176	52	2.2	55	1.0	65	3.0	52	1.9	56	2.3	57	2.5

water (Fig. 7). In 150-175% sea water, however, as the blood becomes hypotonic to the media, urine concentrations become significantly hypertonic to the blood. Thus, while hypertonic regulation of calcium in dilute media is a result of extra-renal mechanisms, hypotonic calcium regulation in concentrated salinities is assisted by calcium excretion in the urine. Winter urine and blood calcium concentrations in 75-125% sea water are isotonic; above and below this range urine is more concentrated. However, since winter blood calcium levels are isotonic with the media in concentrated salinities, then no adaptive importance can be ascribed the higher urine concentrations. Nor can calcium excretion in dilute media assist in maintaining hypertonicity of this ion. Seasonal urine calcium levels differ significantly over most of the salinity range, with higher values in the winter - a situation similar to that demonstrated for H. nudus (Fig. 2).

Seasonal urine magnesium concentrations were found to differ significantly in some salinities, but no consistent trend could be demonstrated. Both winter and summer urine magnesium values are significantly greater than blood values in media above 25% sea water. This indicates that in this species, as in H. nudus, the antennary glands actively regulate the blood magnesium concentration at a constant hypotonic level.

Student's "t" values resulting from interspecific comparisons of seasonal ion concentrations in the blood and urine are given in Table 7. Winter H. oregonensis are shown to be somewhat more effective in hypotonic sodium regulation in salinities above 100% sea water than

Table 7. Values of Student's "t" resulting from interspecific comparison of ion concentrations in a given body fluid for each season over a range of experimental salinities. Significance is considered at the $P = .01$ level ($t_{01} = 2.878$, d.f. = 18). Values marked with an asterisk* represent comparisons that are significant at this level.

BLOOD								
Salinity % Sea Water	SUMMER (15°C.)				WINTER (5°C.)			
	<u>HEMIGRAPUS NUDUS</u>				<u>HEMIGRAPUS NUDUS</u>			
	x				x			
	<u>H. OREGONENSIS</u>				<u>H. OREGONENSIS</u>			
	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>
6	1.322	0.204	1.497	2.621	5.316*	2.272	0.669	0.280
12	3.027*	1.965	0.185	0.469	2.520	1.533	2.809	2.286
25	3.059*	0.601	0.270	0.140	2.026	4.314*	0.668	2.936*
75	0.456	1.636	1.795	1.301	0.380	1.932	0.427	0.446
100	3.552*	1.772	0.957	3.308*	2.563	5.952*	0.385	1.573
125	0.374	1.203	1.764	4.291*	9.333*	1.514	2.656	0.262
150	0.990	0.474	0.134	2.119	6.658*	0.231	0.744	2.200
175	1.257	4.285*	0.087	1.575	8.648*	4.148*	2.154	0.124
URINE								
Salinity % Sea Water	SUMMER (15°C.)				WINTER (5°C.)			
	<u>HEMIGRAPUS NUDUS</u>				<u>HEMIGRAPUS NUDUS</u>			
	x				x			
	<u>H. OREGONENSIS</u>				<u>H. OREGONENSIS</u>			
	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>
6	3.239*	2.393	2.034	0.558	7.052*	1.325	5.543*	2.496
12	4.823*	5.813*	0.519	1.472	12.022*	2.109	4.113*	5.435*
25	2.609	5.691*	1.818	2.864	15.835*	4.274	1.629	15.670*
75	3.408*	8.744*	0.437	2.977*	0.386	3.628*	1.060	0.248
100	4.651*	5.088*	1.776	3.983*	2.466	2.489	0.379	0.447
125	0.684	6.156*	0.506	8.787*	2.395	9.205*	5.860*	0.157
150	2.370	1.288	3.508*	7.343*	0.682	5.078*	4.922*	0.810
175	5.067	1.778	0.726	6.284*	0.440	1.100	3.636*	0.118

H. nudus (Figs. 1 and 6). No other consistent species differences in blood ion concentration could be demonstrated. Only with respect to magnesium can significance be ascribed to differences between the urine of the two species. Thus, urine magnesium of summer H. oregonensis is twice the concentration of that in H. nudus urine in salinities above 25% sea water (the values in 175% sea water are respectively, 350 and 175 mEq./L). Winter urine magnesium concentrations were almost identical in the two species in all salinities (Figs. 2 and 7, Table 7).

In summary, both species were found to effectively regulate blood concentrations of sodium, potassium and calcium in low salinities hypertonic to the media, with a limited hypotonic regulation in high salinities - H. nudus: hypotonic regulation of winter and summer potassium (Fig. 1), summer calcium (Fig. 2); H. oregonensis: hypotonic regulation of winter sodium and potassium (Fig. 6), summer calcium (Fig. 7). In media above 25% sea water, both species maintain blood magnesium at a level one third that of the medium concentration. In H. nudus, summer urine magnesium concentrations are mainly hypotonic to the media while winter values are isotonic (Fig. 2). In H. oregonensis, however, urine magnesium concentrations are isotonic to the media in winter and hypertonic in summer (Fig. 7).

Time response to external salinity changes:

Data presented in Figure 8 (sodium and potassium) and Figure 9 (calcium and magnesium) show, for summer H. oregonensis, change in urine ion concentrations over a 24 hour period in response to external

23a.

Table 8. Values of the mean and standard error of urine ion concentrations of Hemigrapsus oregonensis measured at seasonal and experimental temperature (5°, 15° and 25°C.) conditions. Each mean value represents ten measurements after exposure for 24 hours to each experimental salinity.

		WINTER						SUMMER					
	SALINITY	5°C.		15°C.		25°C.		5°C.		15°C.		25°C.	
	% mEq./L	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Na (mEq./L)	6 27	408	15.7	322	4.5	334	7.4	306	7.9	263	13.7	321	11.9
	12 53	386	4.2	383	5.1	358	5.5	345	7.7	298	8.2	355	23.1
	25 110	508	11.5	406	4.9	408	11.0	411	14.0	373	7.4	354	15.4
	75 329	545	10.4	405	7.6	450	6.0	449	18.1	404	9.9	388	10.6
	100 439	508	8.3	422	5.9	469	6.6	451	5.4	433	8.5	467	13.2
	125 549	583	4.4	483	7.9	570	5.5	585	14.8	546	7.1	511	9.3
	150 658	720	9.5	550	3.9	621	4.7	657	8.5	607	15.7	572	21.0
	175 768	792	11.6	677	7.8	745	8.3	725	11.0	665	23.4	634	9.8
K (mEq./L)	6 0.6	5.6	.32	8.4	.32	6.2	.12	6.5	.21	7.0	.38	8.4	.43
	12 1.3	6.6	.11	7.2	.52	8.7	.42	5.9	.35	7.3	.35	8.7	.54
	25 2.7	6.8	.31	7.0	.28	7.6	.23	6.9	.20	7.2	.21	8.2	.21
	75 8.0	6.4	.20	8.1	.18	11.0	.34	7.8	.30	7.1	.20	8.8	.47
	100 10.7	12.1	.87	8.0	.21	11.5	.24	8.2	.41	9.0	.35	11.0	.38
	125 13.3	7.7	.18	10.3	.15	13.0	.44	11.4	.51	10.4	.18	12.4	.25
	150 16.0	11.4	.09	10.9	.36	13.1	.27	19.8	2.11	13.2	.17	14.5	.29
	175 18.7	14.6	.41	14.9	.37	17.4	.15	17.5	.39	15.6	.51	16.0	.21
Ca (mEq./L)	6 1.5	33.9	.80	26.6	.35	25.0	.95	34.2	.39	22.6	.59	22.0	.70
	12 2.9	33.3	.17	28.1	.29	30.1	.32	34.0	1.42	21.2	1.26	25.4	1.44
	25 6.2	35.4	.63	27.7	.87	31.3	.64	34.7	.81	21.9	.91	22.4	.58
	75 18.7	35.0	1.15	30.9	.48	29.4	.43	35.1	1.37	24.5	.35	27.2	1.01
	100 24.9	35.3	.84	31.5	.55	32.2	.72	33.8	1.15	29.9	1.15	30.2	.63
	125 31.1	33.8	.38	33.6	.49	41.4	.46	39.3	2.03	31.6	.88	33.2	.74
	150 37.3	41.4	.46	38.2	.85	40.7	.88	55.7	5.16	39.3	.98	37.2	1.22
	175 43.5	48.1	.71	47.5	.67	51.0	.42	47.0	1.14	43.6	.95	41.5	1.00
Mg (mEq./L)	6 6	37	3.7	24	3.9	12	.7	33	3.3	25	1.0	15	1.5
	12 12	31	2.8	22	.9	10	1.5	46	3.8	28	1.0	18	2.1
	25 25	68	1.0	27	2.8	14	1.1	80	2.2	23	1.4	23	3.1
	75 76	55	3.0	80	6.7	50	1.5	63	4.2	52	1.6	55	4.2
	100 100	104	6.0	114	3.3	78	3.4	95	8.4	146	18.9	108	11.6
	125 126	116	3.4	149	5.4	97	2.0	137	11.5	155	7.5	162	14.1
	150 151	194	9.5	151	6.3	199	4.6	146	5.8	244	18.3	250	30.0
	175 176	232	7.9	165	14.3	180	7.9	196	5.0	352	22.6	352	12.0

salinity. In 125% sea water, urine sodium is hypertonic to the blood from 3-24 hours after immersion, indicating that sodium is excreted by the antennary glands. After six hours sodium excretion reaches a maximum and remains at this level for the next 18 hours. Urine-blood isotonicity is reached at 24 hours. A similar trend is observed for sodium in animals in 75% sea water - after a several hour period of urine hypertonicity, isotonicity is gradually approached. Urine potassium is hypotonic to the blood in the two lower salinities over most of the 24 hours, suggesting that potassium ions are absorbed from the urine to maintain a high blood concentration (85-90% sea water). Urine and blood potassium concentrations in 125% sea water are almost identical. Urine calcium is hypertonic to the blood from 1-12 hours after immersion in 125% sea water, indicating an attempt at regulation by the kidneys which terminates after 24 hours. In 25% sea water, urine calcium changes parallel blood calcium changes (Fig. 9).

Hypotonic magnesium regulation in this species, as in H. nudus, is operative almost immediately upon exposure to a salinity stress. Urine magnesium of animals in 125% sea water increases steadily in concentration, attaining a high value after 24 hours (150 mEq./L). Animals in 75% and 25% sea water have a urine magnesium concentration hypertonic to the blood. Magnesium concentration in the urine after 24 hours in 125% salinity is higher in H. oregonensis than in H. nudus (154 mEq./L and 88 mEq./L, respectively). This agrees with the seasonal measurements which demonstrate that summer urine magnesium of H. oregonensis is

24a.

Figure 10. Relationship of urine-blood (U/B) ion ratios of summer and winter Hemigrapsus oregonensis to a range of external salinities. Each point represents a ratio of the means of ten measurements for blood and urine after exposure for 24 hours to each experimental salinity.

hypertonic to that of H. nudus.

Effect of temperature:

The effect of temperature on the urine ion concentrations over a range of experimental salinities is presented for summer and winter H. oregonensis in Table 8. The results for the four ions are comparable with respect to variability and magnitude to those presented for H. nudus blood (Figs. 3 and 4) and urine (Fig. 4).

Data presented in Table 8 demonstrate for H. oregonensis the isotonic or considerably hypertonic state of urine magnesium to the media at all seasonal and temperature combinations in media above 75% sea water. For H. nudus, comparable magnesium values are hypotonic or only slightly hypertonic to the medium, except for the extremely high value in winter animals at 15°C. (Fig. 4). This interspecific difference in urine magnesium concentration may be attributed to a difference in exoskeleton permeability of the two species.

Urine-blood ratio (U/B) - Hemigrapsus oregonensis

The relationship of the U/B ion ratios to a range of salinities is shown for summer and winter H. oregonensis in Figure 10. Here, as for H. nudus, the dependence on the excretory activity of the antennary glands for regulation of blood magnesium in both seasons and nearly all salinities is indicated by U/B magnesium ratios greater than unity. Summer magnesium ratios are greater than winter ones in salinities above 25% sea water. This is opposite to that shown for H. nudus (Fig. 5) and therefore cannot be explained on the basis that winter animals, by

Table 9. Urine-blood (U/B) ion ratios for seasonal and experimental temperature (5°, 15° and 25°C.) conditions for Hemigrapsus oregonensis. Mean and standard error values are given for each ratio, representing the average of ten measurements of each body fluid after exposure for 24 hours to each experimental salinity.

	SALINITY ‰ Sea Water	WINTER						SUMMER					
		5°C.		15°C.		25°C.		5°C.		15°C.		25°C.	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Na (mEq./L)	6	1.15	.051	.92	.019	1.11	.038	1.04	.037	.91	.061	1.03	.048
	12	1.05	.026	.99	.015	1.11	.037	1.10	.046	.92	.049	1.09	.086
	25	1.22	.037	1.05	.019	1.15	.036	1.33	.080	1.04	.036	1.02	.048
	75	1.28	.027	1.01	.020	1.13	.018	1.14	.055	.99	.027	.98	.028
	100	1.19	.019	1.02	.017	1.18	.019	1.03	.016	.97	.021	.99	.030
	125	1.19	.012	.97	.018	1.12	.013	1.15	.031	1.00	.018	.91	.017
	150	1.31	.022	.89	.010	1.02	.012	1.08	.021	.94	.026	.85	.031
	175	1.22	.021	.96	.014	1.08	.015	1.06	.019	.87	.031	.81	.013
K (mEq./L)	6	.81	.057	1.08	.057	1.00	.031	.90	.045	.88	.057	1.02	.078
	12	.90	.032	.89	.071	1.25	.082	.84	.059	.85	.048	.96	.066
	25	.86	.047	.86	.040	1.04	.040	.80	.044	.75	.040	.89	.040
	75	.76	.033	.86	.031	1.15	.045	.79	.033	.73	.031	.80	.046
	100	1.48	.119	.78	.026	1.04	.031	.68	.049	.78	.038	.94	.036
	125	.81	.031	.92	.026	1.00	.040	.92	.053	.77	.034	.91	.026
	150	.87	.020	.81	.030	.85	.020	1.29	.148	.88	.036	.91	.024
	175	.87	.032	1.06	.039	1.07	.017	1.09	.043	.88	.033	.95	.018
Ca (mEq./L)	6	1.16	.036	.97	.028	1.01	.051	1.17	.050	1.06	.045	.93	.036
	12	1.17	.031	.92	.021	1.11	.030	1.20	.074	.94	.068	1.05	.074
	25	1.15	.044	.93	.031	1.04	.028	1.31	.069	.95	.052	.93	.049
	75	1.04	.036	1.07	.029	1.04	.026	1.22	.054	.97	.028	1.02	.056
	100	1.06	.028	1.03	.024	1.22	.032	1.09	.044	1.07	.044	1.05	.036
	125	.99	.018	1.03	.025	1.37	.025	1.12	.072	1.03	.035	1.10	.039
	150	1.12	.016	1.13	.026	1.24	.029	1.38	.147	1.26	.046	1.11	.052
	175	1.10	.019	1.30	.023	1.28	.022	1.13	.047	1.14	.037	1.10	.034
Mg (mEq./L)	6	1.47	.165	1.32	.272	1.24	.111	1.39	.154	1.49	.095	1.28	.165
	12	1.21	.121	1.23	.150	.96	.162	2.36	.237	1.51	.099	1.61	.211
	25	2.41	.144	1.67	.187	1.29	.125	3.20	.223	1.23	.122	2.24	.334
	75	1.64	.102	2.93	.307	1.86	.121	2.04	.177	1.68	.131	2.32	.234
	100	3.04	.190	3.47	.187	2.42	.195	2.27	.299	3.74	.505	3.04	.361
	125	2.84	.134	3.90	.216	2.02	.073	2.55	.295	3.36	.231	4.08	.386
	150	4.64	.287	3.10	.174	3.79	.113	2.74	.231	4.94	.440	5.94	.758
	175	4.45	.242	3.00	.268	2.78	.178	3.75	.168	6.30	.485	6.14	.348

seasonal acclimatization to a more concentrated environmental level of magnesium, are more capable of excreting magnesium in the urine.

The U/B sodium ratios in summer are equivalent to unity; in winter, the U/B sodium ratios are greater than unity over the entire salinity range. Although sodium excretion in winter crabs may contribute to sodium hypotonicity in concentrated salinities, it could not assist in hypertonic regulation in dilute salinities. In both seasons, the occurrence of U/B potassium ratios less than unity is a manifestation of renal absorption. However, this could only be of adaptive importance in dilute salinities. Deviations from unity of U/B calcium ratios in the high and low salinities in winter probably have no biological significance (see section on seasonal effects). In summer, however, U/B calcium ratios greater than unity in the concentrated salinities reflect the contribution of urinary excretion in the maintenance of blood hypotonicity of this ion.

Winter U/B magnesium ratios are comparable in the two species. Summer ratios, however, are higher in H. oregonensis than in H. nudus and in 100% sea water the ratios are, respectively, 3.7 and 2.1.

Table 9 gives the U/B ion ratios for each seasonal and experimental temperature combination over the external salinity range for H. oregonensis. These values show for each season the same general trends as presented for winter (5°C.) and summer (15°C.) and no clear effect of temperature could be demonstrated.

Table 10. Concentrations of magnesium ion (mEq./L) in the blood of Hemigrapsus nudus and Hemigrapsus oregonensis after 24 hours exposure to salinities of 6 and 175‰ sea water at three temperatures (5°, 15° and 25°C.) and two seasons. The smaller value of each pair corresponds to 6‰ sea water, the larger value to 175‰ sea water.

	<u>Temp.</u> °C.	<u>Summer</u>	<u>Winter</u>
H. Nudus	5	29 - 51	26 - 52
	15	20 - 51	17 - 49
	25	15 - 60	10 - 68
H. Oregonensis	5	23 - 52	25 - 52
	15	17 - 56	18 - 55
	25	12 - 57	10 - 65

DISCUSSION

Effect of Salinity

In dilute salinities (6-75% sea water), summer and winter blood sodium, potassium and calcium concentrations are hypertonic to the media in both species. In salinities above this, regulation is less effective and ion concentrations approach those of the media. The ability to maintain hypertonic blood ion concentrations is important to these species since in both seasons the field sea water is less concentrated than the blood.

Jones (1941) has demonstrated hyperosmotic regulation in dilute salinities in H. nudus and H. oregonensis but was unable, even after 72 hours, to show hyposmotic regulation in concentrated salinities. Gross (1957a), however, has found that Hemigrapsus effectively hypo-osmoregulates in 150% sea water for 20 hours, maintaining a 33% gradient between the blood and external medium. Hyposmotic regulation in an isolated population of H. oregonensis in a natural hypersaline lagoon has also been demonstrated by Gross (1961). After acclimation for several months to slowly increasing salinity, hyposmotic regulation was evident when the lagoon reached 160% sea water (maintained a 40% concentration gradient between blood and medium) and a few individuals were even regulating when the lagoon reached 175% sea water. At 180% sea water no H. oregonensis were regulating and death ensued at 190% sea water. Recent work on the effect of salinity and temperature on

the seasonal blood osmotic concentrations of H. nudus and H. oregonensis has shown that winter Hemigrapsus are more effective regulators in dilute salinities than summer ones (Dehnel, 1962). No hyposmotic regulation in concentrated sea water could be demonstrated. Stone (1962), in a comparable study on the urine osmotic concentrations of H. nudus and H. oregonensis, has found that hyperosmotic regulation in dilute salinities in summer is achieved in both species by extra-renal mechanisms, for the blood and urine are isosmotic. Hyperosmotic regulation in winter, however, is accomplished by production of urine less concentrated than the blood, presumably a result of ion reabsorption in the kidney tubules.

The results for total osmotic concentration of blood and urine in H. nudus and H. oregonensis may be compared with data from the ion experiments, using sodium concentration as indication of the degree and direction of major ion fluxes. Although it is not presumed that changes in sodium ion concentration reflect completely changes in total osmotic concentration, since it represents about 85% of the total internal cation concentration it is the most likely cation to do so.

Change in blood sodium concentrations in media of 25, 75 and 125% sea water occurs continuously in both species over the 24 hour period (Table 2 and Fig. 8). These data may be compared with the results obtained for blood osmotic concentration in which response to sea water of 6-150% is almost complete after the first 24 hours, with little change

during the following 24 hours (Dehnel, 1962). Similarly, the rate of change of urine osmotic concentrations in media of 6-150‰ sea water is considerably slower after 24 hours (Stone, 1962).

Agreement between sodium ion concentration and total osmotic concentration is less apparent in comparisons of blood and urine at seasonal conditions. For example, in both species, winter urine sodium concentrations are hypertonic to winter blood sodium concentrations in media above 12‰ sea water (Figs. 1 and 6). Comparable total osmotic concentrations, however, indicate that winter urine is hypotonic to blood. These results are clearly contradictory and cannot be explained at this time. Summer urine and blood osmotic concentrations are identical, as are summer urine and blood sodium concentrations. This suggests that the antennary glands are ineffective in hyperosmotic regulation in this season.

In both species, winter blood sodium concentrations in 150-175‰ sea water are hypotonic to the media (Figs. 1 and 6). Dehnel (1962), however, was unable to demonstrate hyposmotic regulation in concentrated salinities in either species. In H. oregonensis, winter blood sodium concentrations in dilute media are significantly greater than comparable summer concentrations. This corresponds to the results for total osmotic concentration in which winter crabs of both species were shown to be better regulators (maintain a greater gradient) in dilute salinities than summer crabs (Dehnel, 1962). However, no corresponding seasonal difference in blood sodium concentrations was shown for H. nudus in

dilute salinities (Fig. 1 and Table 1). Since discrepancies also exist in the comparison of seasonal urine sodium ion concentrations to urine total osmotic concentrations (Stone, 1962), it appears that sodium ion response to a range of salinities at winter and summer conditions does not necessarily reflect comparable total osmotic response. Possibly, measurement of the amount and direction of chloride ion movement in comparable experimental conditions will clarify these differences.

Effect of Temperature

No consistent temperature effect on the concentrations of sodium, potassium or calcium ions in the body fluids of either species at either season was demonstrated over the salinity range. The actual differences in the concentrations of these ions at each temperature (5°, 15° and 25°C.) are relatively small and are considered the result of uncontrolled variation (Fig. 3). For blood magnesium, however, both species exhibit an increase in range of concentration, as measured at the low and high salinities (6‰ and 175‰ sea water), with increased temperature (Table 10). A similar trend was evident in both seasons. This suggests that temperature increase gradually leads to a breakdown of magnesium regulation and results, in 6‰ and 175‰ sea water, in the approach of blood magnesium concentrations to isotonicity with the media. This impairment of magnesium regulation may be caused by a decrease in available energy since all other metabolic processes are accelerated. No comparable trend was demonstrated for urine magnesium concentrations.

Extra-renal Ion Regulation

The maintenance of a blood concentration greater than that of the medium means that ions will be lost, both in the urine and by diffusion through the body surface. This loss must be replenished, either by active absorption from the medium, by uptake from the food, by mobilization from extra-vascular "salt pools" or by ion reabsorption from the urine.

Nagel (1934) was one of the first to demonstrate active ion uptake in crabs in dilute sea water. Crabs of the genus Carcinus, kept in low salinity conditions until the blood concentration decreased, were placed in more concentrated sea water, but one which was still hypotonic to the blood. The blood concentration increased after transfer, indicating that ion uptake occurred. Flemister (1959) has reported that epithelial cells in the gill lamellae of the crab, Ocypode albicans, may function in salt absorption from the medium. Koch, Evans and Schicks (1954) have documented ion uptake in gills in the fresh water inhabiting Eriocheir sinensis. In this study, isolated gills were shown to take up sodium, potassium, calcium and chloride from dilute media. Measurements show that sodium chloride is absorbed by the isolated gills at a rate of 2.5 mg/ gram of tissue/ hour. In Pachygrapsus crassipes, Gross (1957a) has demonstrated salt and water exchange between the gills and the branchial fluid and has suggested that sodium, potassium and calcium ion regulation may be principally by the gills (Gross, 1959). In

Pachygrapsus, the branchial epithelium lining the gill chamber appears to contribute slightly to ion and water exchange (Gross, 1957a).

Flemister and Flemister (1951), however, have suggested that the branchial epithelium does not contribute significantly to chloride ion regulation in Ocypode albicans. In Hemigrapsus the maintenance of a hypertonic ion concentration in the blood in dilute salinities is accomplished by extra-renal mechanisms, possibly the gills, when the blood and urine concentrations of the ion are equal.

Extra-renal uptake of ions by active transport mechanisms appears to be independent for each ion. This permits the maintenance of ions in their appropriate relative proportions in the blood. For example, Krogh (1939) has demonstrated that active uptake of sodium may be from sodium chloride, sodium sulphate or sodium bicarbonate. In Eriocheir sinensis, chloride uptake may be from sodium chloride, potassium chloride or other chloride salts (Maluf, 1939).

The maintenance of blood hypertonicity in a dilute environment involves the participation of energy consuming processes. Flemister and Flemister (1951) have found that lowest oxygen consumption in Ocypode albicans occurs in media isotonic to the blood. Whole animal respiration of Hemigrapsus is at a maximum in dilute salinities where the osmotic gradient between blood and medium is large (Dehnell, 1960). Oxygen consumption of isolated gill tissue of summer H. oregonensis and H. nudus increases as the osmotic gradient between the blood and medium increases (McCaughran, 1962). Winter animals, however, do not exhibit increased

respiration in dilute salinities. This difference in summer and winter gill metabolism may be a result of the natural seasonal acclimation to low salinities in summer and high salinities in winter. Thus, summer animals, more accustomed to dilute sea water than winter animals, consequently exhibit more effective ion absorption by the gills - hence their higher gill metabolic rate.

Although summer blood and urine sodium concentrations are isotonic in dilute salinities, winter urine concentrations are significantly greater than blood concentrations (Figs. 1 and 6). There is not, however, a concomitant increase in oxygen consumption by isolated gills of Hemigrapsus in this season (McCaughran, 1962). Thus, if increased oxygen consumption is a reliable indicator of absorptive activity in the gills, this suggests that in these conditions the site of sodium uptake is not the gills, and other potential mechanisms of ion uptake from the environment must be investigated to account for this excess of excreted sodium.

The extra-vascular "salt pool" has been suggested by Gross (1958) as a reservoir from which ions may be mobilized to maintain blood hypertonicity in dilute sea water. When Pachygrapsus crassipes is exposed to an osmotic stress, potassium and sodium exchanges occur between the medium and a source other than blood, the "salt pool". According to Gross (1958), the "salt pool" has special importance in animals living in environments where the salinity fluctuates, permitting ion deficits in the blood, incurred at low salinity conditions, to be made

up from the "pool", with replenishment of the ions in the "pool" occurring at high salinity conditions. There is no indication from the present data that Hemigrapsus utilizes a similar source of ions.

Secretory activity of the epithelial cells in the gill lamellae may function to maintain hypotonicity in concentrated media (Flemister, 1959). For example, hypotonic blood potassium regulation in H. nudus in concentrated salinities is accomplished extra-renally in both seasons, possibly by potassium secretion by the gills, since the urine is hypotonic to the blood (Fig. 1 and Table 1). A similar mechanism may be responsible for hypotonic calcium regulation in H. nudus in the summer (Fig. 2). In H. oregonensis, gill secretion may contribute to hypotonic potassium regulation in concentrated salinities in the winter (Fig. 6).

Ion Regulation by the Antennary Glands

Ion concentrations in the blood are influenced, not only by the extent of ion absorption or secretion by the gills and by the degree of exoskeleton permeability to salts and water, but also by the effectiveness of the antennary glands in ridding the blood of excess ions in concentrated media or reabsorbing ions from the urine in dilute sea water. For example, Flemister and Flemister (1951) have shown that Ocypode albicans maintains an internal chloride ion concentration of 375 mM/L over an external range of 200-600 mM/L of chloride, and have suggested that this is accomplished by reciprocal mechanisms of chloride absorption (in gill lamellar cells) and chloride excretion (in renal tubule cells).

In Hemigrapsus, excess concentrating of the blood in high

salinities may be lessened by ion excretion in the urine. Examples of hypotonic ion regulation by this mechanism, excluding hypotonic magnesium ion regulation, are for H. nudus: winter blood sodium in salinities above 125‰ sea water (Fig. 1 and Table 1) and for H. oregonensis: winter blood sodium in salinities from 125-175‰ sea water (Fig. 6 and Table 5) and summer blood calcium in salinities above 125‰ sea water (Fig. 7 and Table 5).

Hypertonic ion regulation in dilute media by urinary absorption, involving the active uptake of ions from the urine against a concentration gradient is restricted to the potassium ion in Hemigrapsus. For example, in H. nudus, hypertonicity of winter blood potassium (Fig. 1 and Table 1) and in H. oregonensis, hypertonicity of blood potassium in both seasons (Fig. 6 and Table 5), is aided by the production of urine hypotonic to the blood with respect to potassium concentration. Ion reabsorption to replenish loss from the integument in dilute sea water presumably supplements ion uptake by the gills. Beadle (1957), in this respect, has suggested that although no fundamental difference exists between active uptake of ions across the gill surface and across the walls of an excretory tubule, the latter process is energy-saving in that ionic gradients between blood and urine are considerably less than those between the blood and external medium.

In sea water more concentrated than the blood, water is lost and ions are gained from the medium. Since no significant weight changes could be demonstrated in Hemigrapsus after exposure to concentrated salinities (Dehnel, 1962), then this water loss must be replenished,

either from the gut or withdrawal from the urine. Metabolic water may also make a small contribution. Green et al (1959) have shown that the principal site of water entry into the fiddler crabs, Uca pugnax and Uca pugilator, is the stomach. When the lobster, Homarus americanus, is immersed in normal sea water, 20% of the water lost in the urine can be attributed to uptake from the gut (Burger, 1957). Water withdrawal from the urine to replenish loss when in air or when exposed to concentrated salinities is well documented. For example, Flemister and Flemister (1951) have found that water reabsorption occurs in the antennary glands of Ocypode albicans when the crab is in air or in hypertonic solutions. Similarly, Flemister (1958) has found that Gecarcinus lateralis on dry sand filters across the antennary gland in 60 hours a volume equal to its total inulin space, withdrawing most of the water of the urine thus formed. Riegel and Lockwood (1961), have obtained direct evidence of water withdrawal from the urine of H. nudus. No details were given of this work.

Increased urine output in low salinity media to prevent dilution of the body fluids is reported for Carcinus by Prosser and Brown (1961). In Hemigrapsus, similarly, there must be a considerable increase in urine volume in low salinities, although no measurements were made, since no significant weight increase in animals exposed to low salinities could be demonstrated (Dehnel, 1962).

The principal function of the antennary glands appears to be the maintenance of blood magnesium at a constant, hypotonic level.

This is well illustrated for both species of Hemigrapsus (Fig. 4 and Table 6). In both seasons and over most of the salinity range, the U/B magnesium ratios are greater than unity for both species (Figs. 5 and 10). Before the absolute quantity of magnesium excreted by the kidneys can be known, the volume of urine produced in a given salinity will have to be determined.

In the prawn, Palaemonetes varians, increase in urine production in concentrated sea water (100%), over that in isosmotic conditions (65% sea water), is thought to be necessary to facilitate excretion of magnesium and sulphate ions (Potts, pers. comm.).

In Pachygrapsus crassipes, Gross (1959) has shown that U/B magnesium ratios increase from 5.6 in 50% sea water to 15.4 in 150% sea water. Prosser et al (1955) have found, not only that the antennary glands of P. crassipes effectively regulate magnesium, but also that the concentration of this ion in the urine is inversely related to sodium concentration. They suggest that in competing for transport across the kidney tubule linings, magnesium ions block the activity of the sodium transport system. In Uca, Green et al (1959) have found that while magnesium excretion by the antennary glands in concentrated media is markedly elevated, sodium excretion is significantly lower than that in normal sea water. This inverse sodium-magnesium relationship has not been demonstrated in Hemigrapsus.

High urine magnesium concentration in Hemigrapsus can result from either of two processes, and is most probably due to a combination

of both. After filtration, which is presumed to be the principal mechanism of urine formation, there may either be a selective reabsorption of water and sodium, potassium and calcium ions, leaving magnesium in a high concentration in the urine, or there may be simply an active secretion of magnesium ions from the blood into the urine. In Carcinus maenas, both these processes appear to contribute to the high urine magnesium level. When Carcinus is kept in air the urine magnesium concentration increases (Riegel and Lockwood, 1961). This is accompanied by a decrease in urine sodium concentration, such that the urine remains isosmotic with the blood (Lockwood, 1962). Since potassium and calcium ion concentrations do not increase as much as would be expected from the inulin U/B ratio (assumed to be entirely due to filtration), then it appears that these ions are withdrawn from the urine along with water. Also, since the magnesium U/B ratios are greater than the inulin U/B ratios, then the high urine magnesium concentration could not have resulted solely from water withdrawal. Thus, it appears as if magnesium is also secreted into the urine (Riegel and Lockwood, 1961). A similar mechanism may be functioning in Hemigrapsus. A magnesium transport system presumably operates in the kidney tubules to maintain the high concentrations of magnesium in the urine against a concentration gradient with the blood.

The control of ion regulation is at present not known. Experiments on this problem are currently being performed on H. nudus.

Aspects of Magnesium Regulation

Hemigrapsus maintains, by antennary gland activity, a blood magnesium concentration at approximately one third that of the medium concentration. This hypotonic regulation is independent of season (Figs. 2 and 7) and is equally effective in both species (Table 7). The response of blood magnesium concentration when exposed to a given salinity is comparatively rapid and in 125‰ sea water most of the change has occurred after 12 hours (Table 2 and Fig. 9).

As well as its effect on cell permeability and its importance in many enzyme reactions, magnesium is also a general anaesthetic. Several investigations into the depressant effect of magnesium in crabs have been performed. Bethe (1929), for example, has found that the blood magnesium concentration in Carcinus increases threefold when the environmental magnesium concentration is increased by the same factor, resulting in a lessening of muscular tone and impairment of normal reflex activity. Katz (1936) has demonstrated that a state of "curarization" can be induced in Carcinus maenas by excess magnesium concentration and, since the muscle responds normally to direct stimulation, suggests that this ion blocks the transmission of nerve impulses at the neuromuscular junction. An external magnesium concentration 2.5 times the normal blood concentration produces a state of complete (but reversible) neuromuscular block. Animals kept in magnesium-free artificial sea water were shown to exhibit increased excitability. Waterman (1941), in a comparative study on the effect of magnesium on the neuromuscular systems of three

decapods, Maia, Panulirus and Cambarus, has found that the amplitude of muscle contraction after nerve stimulation is inversely proportional to the magnesium concentration in the perfusing fluid. When the magnesium concentration reaches 4-5 times the normal blood magnesium level the isometric tension approaches zero. Further documentation of the effect of magnesium, also on nerve-muscle preparations of Carcinus maenas, is given by Boardman and Collier (1946). They have shown that a decrease in magnesium concentration in the perfusing fluid results in an enhancement of muscular contraction (increased amplitude) and suggest that neuromuscular transmission is facilitated by a reduction in concentration of magnesium. More recently, Robertson (1953) has related the plasma magnesium concentration of several crustaceans with their relative state of activity. He has found that a low plasma magnesium concentration (14-18%) is associated with a relatively high activity state (for example, in the portunids, Carcinus and Portunus and the grapsoid, Pachygrapsus) and a high plasma magnesium concentration (84-101%) is associated with slower movements (for example, in the spider crabs, Hyas and Maia and the lithoid, Lithodes). Robertson (1953) has suggested that the forms with high plasma magnesium levels are living in a condition of partial anaesthesia resulting from depressed neuromuscular transmission.

These observations suggest that the precise hypotonic regulation of blood magnesium in Hemigrapsus is necessary to facilitate impulse transmission across the neuromuscular junction. Both species of Hemigrapsus, living in the rocky intertidal zone, are comparatively active,

moving around rapidly when immersed by the tide.

Interspecific Difference in Ion Regulation

The only consistent interspecific difference in blood ion concentration involves winter sodium levels of H. oregonensis. This species more effectively maintains a hypotonic blood sodium concentration in salinities above 100‰ sea water than does H. nudus (Figs. 1 and 6, Table 7). No significant difference in blood magnesium concentrations of the two species could be demonstrated. Urine magnesium concentrations, however, are quite different in the two species. In H. oregonensis, urine magnesium levels are isotonic or considerably hypertonic to the media at most seasonal and temperature combinations in media above 25‰ sea water (Table 8). Comparable urine magnesium values of H. nudus (Fig. 4), however, are hypotonic or only slightly hypertonic to the media (except in winter at 15°C.). Since the blood concentrations of magnesium are equivalent in the two species then the higher urine magnesium concentrations in H. oregonensis appear to result from a greater amount of magnesium ions diffusing into the body. Gross (1957b) has found, in this respect, that H. oregonensis possesses a more permeable exoskeleton than H. nudus. This suggests that in a given salinity, more magnesium enters through the exoskeleton of H. oregonensis than in H. nudus. This excess is eliminated by urinary excretion in H. oregonensis to maintain a similar blood concentration to H. nudus.

SUMMARY

1. Concentrations of sodium, potassium, calcium and magnesium ions in the blood and urine of Hemigrapsus nudus and Hemigrapsus oregonensis have been measured at eight salinities (6-175‰ sea water), three temperatures (5°, 15° and 25°C.) and in summer and winter.

2. In dilute media (6-75‰ sea water), sodium, potassium and calcium ion concentrations in the blood of both species of Hemigrapsus were shown to be considerably hypertonic to the media concentrations. In high salinities (100-175‰ sea water), ion regulation became less effective and concentrations approached isotonicity with the media concentrations. The blood concentration of magnesium, however, was found to be regulated in both species at a precise, hypotonic level, approximately one-third that of the medium concentration, in all salinities above 12‰ sea water.

3. Since the field salinity in summer and winter (35 and 75‰ sea water, respectively) is considerably less concentrated than the blood, then the ability of these species to maintain hypertonic blood ion concentrations in low salinity conditions is important in minimizing dilution of the body fluids.

4. Changes in blood and urine concentrations of sodium, potassium and calcium ions in 25, 75 and 125‰ sea water were shown to occur continuously in both species over 24 hours exposure. The response of blood magnesium ion concentration in these salinities, however, was more rapid, and in 125‰ sea water the major change occurred after 12 hours.

5. Seasonal differences in ability of the two species to regulate sodium and potassium ions were not consistent and no general trend was apparent. Calcium ion concentrations in the blood of both species in dilute salinities, however, are higher in winter than in summer, which suggests that winter animals are more effective regulators of this ion. Concentrations of magnesium in the blood are almost identical in winter and summer Hemigrapsus.

6. No consistent effect of temperature on the blood and urine concentrations of sodium, potassium or calcium ions of either species at either season could be demonstrated. A gradual impairment of the magnesium regulatory mechanism in the two species, however, appeared to result from increased temperature, manifested by the approach of blood magnesium ion concentrations to isotonicity with the media concentrations at the extreme high and low salinities. It is suggested that the breakdown of magnesium regulation with increased temperature is a result of a decrease in available energy, since all other metabolic processes are accelerated.

7. Hypertonic regulation of sodium, potassium and calcium ions in the blood of both species in dilute sea water was shown to be primarily effected by extra-renal processes since the U/B ratios approximated unity. It is suggested that the principal site of ion uptake from the medium in Hemigrapsus may reside in the lamellar epithelium of the gills.

8. The principal function of the antennary glands in Hemigrapsus, with respect to cation regulation, was shown to be the maintenance of blood magnesium concentrations at precise, hypotonic levels, indicated by U/B magnesium ratios markedly greater than unity over most of the salinity range. The kidneys in both species appear to function also in a limited hypotonic regulation of sodium and calcium ions in high salinity media by excretion of these ions in the urine. In dilute salinities, both species exhibit active reabsorption of potassium ions in the kidney tubules in the winter, contributing to the maintenance of potassium hypertonicity to the media.

9. It is suggested that the precise hypotonic regulation of blood magnesium ion concentration in Hemigrapsus is necessary to facilitate neuromuscular impulse transmission, and appears to be a characteristic feature of ion regulation in active decapod Crustacea.

10. Ion regulatory ability was shown to differ only with respect to winter blood levels of sodium in the two species. Thus, H. oregonensis more effectively regulates this ion at a hypotonic blood level in concentrated salinities than does H. nudus. Urine concentrations of magnesium were found to differ in the two species, H. oregonensis excreting more magnesium than H. nudus. Since the blood magnesium concentrations in the two species were equivalent, then the higher concentration of magnesium in the urine of H. oregonensis may be related to the more permeable exoskeleton of this species.

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