A STUDY OF THE OSMOREGULATORY ROLE OF THE ANTENNARY GLANDS IN TWO SPECIES OF INTERTIDAL CRABS

by

DMITRY DAVID STONE

B. A., University of British Columbia, 1954

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

in the Department of ZOOLOGY

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April, 1962

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Zoology

The University of British Columbia,
Vancouver 8, Canada.

Date May, 1962

ABSTRACT

Two species of intertidal crabs, Hemigrapsus oregonensis and H. nudus occur in large numbers at Spanish Bank, Vancouver, British Columbia. The area is characterized by sea water of high temperature and low salinity in summer and low temperature and high salinity in winter. The crabs osmoregulate strongly in low salinities, keeping blood considerably hypertonic to the external medium. They do not regulate strongly in salinities higher than those normally found in the field in winter (70-80% sea water).

This study attempts to establish the role of the excretory organs (antennary glands) in osmoregulation. Effects on their function of seasonal adaptation, temperature, osmotic stress and body size were also investigated.

Experimental temperatures of 5°, 15° and 25° C and media of 6%, 12%, 25%, 75%, 100%, 125%, 150% and 175% sea water were used (100% sea water:31.88‰ salinity). Animals were brought into the laboratory and equilibrated in 75% sea water for 36-48 hours at the experimental temperature. After equilibration, groups of 10-15 animals were transferred to each of the experimental salinities. After 3, 24 and 48 hours, 10 urine samples were drawn from each group, sealed in separate capillary tubes and quick-frozen. Osmotic concentration was measured by the method of melting point determination. Identical series of experiments were carried out, summer and winter. Procedures

differed only in that summer animals were damp-dried and weighed before sampling.

For each species and experimental temperature, a series of urine osmotic response curves was drawn. Data for summer-adapted animals at 15° C and winter-adapted animals at 5° C were used for most comparisons. These approximated seasonal mean field temperatures. Osmotic gradients between urine and media at 48 hours formed the basis for comparison of seasonally-adapted responses.

Data were analysed for salinity and temperature effects and seasonal differences by means of Student's "t" test, which was used also to evaluate differences between urine and blood concentrations. Differences attributable to weight, and inter-specific differences in U/B ratios were analysed by means of Wilcoxon's Matched-Pairs Signed-Ranks test.

Concentration of urine was found to fall in dilute, and rise in concentrated media at rates, directly related to osmotic stress, which declined with time and were influenced by the seasonal adaptation of the animals and the experimental temperature. New equilibria were generally established by 48 hours, at levels, particularly in concentrated media (above 100% sea water), which were considerably higher in summer- than in winter-adapted animals.

Hyper-osmotic regulation was achieved in summer-adapted animals with the production of blood-isotonic urine, implicating extra-renal mechanisms. In winter-adapted animals, hyper-osmotic

regulation was enhanced by production of blood-hypotonic urine.

Summer-adapted animals appeared to resist blood change in 100-150% sea water by producing blood-hypertonic urine, and although this resistance was maintained longest in 100% and 125% sea water, blood soon became hypertonic. In general, cooling retarded, and warming stimulated salt absorption and regulation. Winter-adapted animals in high salinities did not effectively resist blood change, and both urine and blood quickly became hypertonic.

Effects on urine concentration of cooling or warming summer-adapted animals and warming winter-adapted animals were significant only in low and intermediate salinities.

Body size had, in some cases, significant effects on urine concentration. Small H. nudus, taken from summer field conditions, had urine significantly hypertonic to that of large animals. This was also true of H. oregonensis at 15° in concentrated media.

In winter-adapted animals, H. oregonensis had total osmotic U/B ratios significantly higher (nearer unity) than H. nudus for the whole range of experimental salinities at 5° C. In summer-adapted animals at 15° C, U/B ratios approached unity in both species.

Seasonal adaptation of osmoregulatory mechanisms in both species altered the balance of active processes so that urine was lower, both in absolute concentration and relative to blood, in winter than in summer.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. P. A. Dehnel for his guidance in the planning and execution of this study, and for his invaluable criticism of the manuscript in all stages of its preparation. Technical assistance of Mrs. Maureen Douglas is gratefully acknowledged. Appreciation is expressed to Drs. J. R. Adams, C. V. Finnegan and W. S. Hoar for their critical reading of the manuscript. This study was aided by grants from the National Research Council of Canada and the National Science Foundation of the United States.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Osmotic regulation in aquatic animals has been reviewed by Krogh (1939) and Beadle (1957) and discussed in detail more recently by Prosser and Brown (1961). Beadle (1957 Pg. 335), commenting on the evolution of osmotic regulation in Crustacea, postulated "that in marine crabs there are at least two sets of active processes at work, in the gills and in the excretory organs, which are responsible for the ionic imbalance between blood and sea water." He suggested that adjustments in the rates of these processes could have led, in appropriate environments, to the evolution of both hypo- and hyper-osmotic regulation. The method of urine formation in various Crustacea has been the subject of considerable research (Picken, 1936; Maluf, 1941; Robertson, 1957; Riegel and Kirschner, 1960; and the review of Martin, 1957). Osmotic behavior of nine species of eastern Pacific crabs was investigated by Jones (1941), who categorized Hemigrapsus oregonensis and H. nudus as hyper-osmotic regulators, without the capacity for hypo-osmotic regulation in high salinities. The mechanisms by which crabs establish and maintain osmotic and ionic gradients between their internal and external environments have been studied (Nagel, 1934; Green, Harsch, Barr and Prosser, 1955). In Carcinus maenas, a crab showing no hypo-osmotic regulation, Prosser and Brown (1961 Pg. 14) postulate three mechanisms which play a part in hyper-osmotic regulation: "low permeability to water and salts, increased fluid output, particularly of urine, and active salt absorption from the

medium." The participation of the antennary glands in hyper-osmotic regulation in Pachygrapsus crassipes, a doubly regulating intertidal species, is suggested by Prosser, Green and Chow (1955). Gross (1952) suggests that active absorption of water may be a method of hypo-osmotic regulation. The antennary glands have been considered to be more important in ionic than total osmotic regulation (Prosser, Green and Chow, 1955; Green, Harsch, Barr and Prosser, 1959; Prosser and Brown, 1961). Much of the evidence for this view has been drawn from the work of Nagel (1934), Webb (1940), Robertson (1949) and Parry (1954). In a semi-terrestrial crab, Coenobita perlatus, Gross and Holland (1960) demonstrated behavioral mechanisms for regulation of osmotic concentration of the blood. The antennary glands in this species were shown to contribute only to the regulation of potassium and not at all to total osmotic regulation. The ratio, Urine/Blood concentration (U/B ratio) for specific ions in a variety of both regulating and adjusting Crustacea, indicates that the antennary glands can act selectively to control certain ionic imbalances between blood and external media (Peters, 1935; Picken, 1936; Prosser, Green and Chow, 1955; Gross, 1959; Gross and Holland, 1960; Prosser and Brown, 1961).

Temperature acclimation of rate functions in poikilotherms has been documented (Bullock, 1955; Prosser, 1955), and the effects of temperature on osmotic regulation in aquatic organisms have been investigated and reviewed (Wikgren, 1953; Verwey, 1957). The effect of external salinity on animal activ-

ity, particularly on osmotic behavior and the flux of water and ions between body fluids and media, and concomitant weight changes have been studied (Jones, 1941; Robertson, 1949, 1953; Gross, 1954, 1955, 1957a; Prosser, Green and Chow, 1955; Dehnel, 1960).

Broekema (1941) studied the combined effects of temperature and salinity on seasonal migrations and osmotic relationships in the shrimp, Crangon crangon. In this species, and in Carcinides (Carcinus) maenas (Broekhuysen, 1936), it was found that both very low and very high salinities were best tolerated at high temperatures. Blood concentration in C. crangon held in a constant salinity of 15‰ was shown to fall as the temperature decreased by 10° C. In animals held in sea water of 25‰, a similar decrease in temperature resulted in a rise in blood concentration. The effect of both these changes was to reduce the difference between internal and external concentrations, as the animals were isotonic in salinities of 21‰ to 23‰, depending on temperature. Similar temperature effects and salinity tolerances were postulated for other Crustacea which show seasonal changes in distribution, from cold high salinity situations in winter to warm, low salinity situations in summer. By extension, this may also include intertidal species which do not migrate but are exposed in their usual habitat to similar seasonal changes in temperature and salinity (Verwey, 1957).

The osmotic behavior of H. oregonensis and H. nudus, has

been studied with respect to blood responses to a range of experimental temperatures and salinities (Dehnel, 1962). These two species are established in a geographic area with seasonal temperature and salinity characteristics similar to those discussed by Broekema (1941) and Verwey (1957). The activity of the antennary glands in osmotic regulation, as reflected in the total osmotic concentration of urine from similarly exposed animals, is herein considered as a further contribution to the understanding of the physiology of these animals. Evidence will be presented that the osmoregulatory responses of these species change significantly with seasonal field temperature and salinity, and that hypo-osmotic regulation does occur to some degree at least in summer-adapted animals, which agrees in part with the findings of Gross (1957a). Further, interspecific differences in osmotic response and U/B relationships will be presented which may be related to the different intertidal levels characteristically occupied by the two species in the study area and to their usual geographic distribution.

MATERIAL AND METHODS

Two species of shore crabs were used in these experiments, Hemigrapsus nudus (Dana) and H. oregonensis (Dana). The animals were collected at two seasons, summer and winter from the intertidal zone at Spanish Bank, Vancouver, British Columbia. The habitat and the seasonal fluctuations in temperature and salinity have been described (Dehnel, 1960).

The animals were collected in plastic pails and covered with damp seaweed. In the laboratory, they were rinsed with stock 50% to 80% sea water and distributed into plastic trays measuring 12 X 9⅛ X 4 inches. Usually, four groups of animals were selected, each containing a similar weight range. Depending on size, experimental salinity and temperature, the number per tray varied from 10 to 15 animals. Each group was intended to provide three sequential sets of 10 separate urine samples.

In order to bring the animals to a common osmotic level and to clear their intestines, each group was totally immersed in 4.0 liters of 75% sea water. This has been selected as a suitable intermediate salinity for equilibration (Dehnel, 1962). The trays were covered with perforated lids and placed in a darkened refrigerator or constant-temperature water bath, set at the current experimental temperature. The animals were not fed and the water was renewed once every 24 hours, both in the initial equilibration period and during the experiments.

Most of the change in osmotic concentration of the blood in both species occurred in the first 24 hours following the transfer of animals to new conditions of temperature and salinity (Dehnel, 1962). Based on this, an ample osmotic equilibration period of 36 to 48 hours in 75% sea water was selected for these experiments. Following the equilibration period, each group of animals was transferred to 4.0 liters of water at experimental temperature and salinity conditions. It was necessary to aerate the trays at high experimental temperatures and both high and low salinities.

The experimental temperatures used in both summer and winter series were 5°, 15° and 25° C. The experimental salinities were 6%, 12%, 25%, 75%, 100%, 125%, 150% and 175% sea water, based on 100% sea water: 31.88‰ salinity and 17.65‰ chlorinity. These salinities were obtained either by diluting stock sea water with dechlorinated tap water or by dissolving in it an appropriate amount of "Sea Salt", a product of the Leslie Salt Co. of San Francisco. Salinities were determined by means of a conductivity bridge, and alternatively by titration with $AgNO_3$.

For the summer series of experiments, weights ranged from 0.29 grams for both species, to about 15.0 grams for H. nudus and 10.0 grams for H. oregonensis. For most of this series, all animals were weighed immediately prior to urine sampling. For the winter series, to facilitate sampling, the smallest animals used were about 1.0 gram and the animals were not weighed. Only males were used and care was taken to avoid molting animals.

During the summer, when daytime collection was possible, groups of 10 animals of each species were set aside for immediate sampling. During the winter, night collections were carried out, and urine samples were not taken from animals removed directly from field conditions. In all other respects, the procedure followed in both seasons was the same.

In the summer experiments, the following procedure was used. After 3, 24 and 48 hours holding in the experimental conditions, a group of animals was removed. Each was damp-dried with cheese-cloth and weighed to the nearest 0.01 gram

on a Mettler Model K7T electric balance. The animals were then placed in numbered paper cups and covered.

Urine sampling was accomplished by means of glass capillary tubes, 0.40 mm. inside diameter and 1½ inches long, drawn to a fine tip and inserted into a small rubber pipette bulb. The tip of each tube was broken off at a diameter suitable to the size of the animal to be sampled. The crabs were held in the fingers and gently but thoroughly blotted dry with Kleenex tissue. They were then manipulated under a binocular microscope, so that the tip of a flattened and blunted needle, mounted on the microscope stage, could be inserted under one of the opercula covering the urinary pores. As the operculum was raised, the tip of the capillary tube was inserted beneath it. This usually caused the crab to expel sufficient urine for a sample to be drawn into the tube. If necessary, gentle pressure, exerted dorso-ventrally on the body of the crab would cause expulsion of urine. Care was taken to avoid tissue damage and contamination of the samples by water from the gill chambers or gut fluid, which was occasionally expelled through the mouth. If a sample were lost, a second one could generally be obtained from the other side of the animal. Usually, an excess of urine was drawn into the tubes and the samples reduced to approximately 3.0 mm^3 or a length of about 5.0 mm in the straight bore, by pressure on the bulb. The very fine tip was then broken off to reduce the surface tension holding the sample in the end of the tube, and the sample positioned centrally by withdrawing the

tube from the bulb while maintaining a slight pressure with the fingers. The entire tapered end of each tube was then broken off squarely with forceps. The tubes were sealed immediately with "Seal-Ease", placed in numerical order on labelled microscope slides and the samples quick-frozen on dry ice. Samples were then transferred to a brine tray held at -15° C, and kept in a frozen state until needed. The animals were returned to the experimental conditions after the 3-hour and 24-hour samplings, and discarded after 48 hours.

The same sequence of experiments was followed in both the summer and winter series: one species, H. nudus, was subjected to the 8 experimental salinities at 5° C, followed by H. oregonensis. The refrigerator or water bath was raised first to 15° C, then to 25° C, and the same sequence repeated each time.

Some experiments were repeated entirely or in part, both as a check on the technique and a verification of results.

Measurement of the total osmotic concentration of urine samples was accomplished by the method of melting point determination described by Jones (1941) and modified by Gross (1954).

The data were analysed for salinity effects, temperature effects and seasonal differences by means of Student's "t" test. The same test was applied to the differences between mean osmotic concentrations of blood (Dehnel, 1962) and urine from similarly treated animals. Differences in urine osmotic concentration due to weight, and interspecific differences in U/B ratios were

evaluated by means of Wilcoxon's Matched-Pairs Signed-Ranks test. Unless otherwise stated, statistical significance is attributed to P values $\leq$ 0.01.

Throughout this presentation, certain other terms are used with a specific connotation. Following Dehnel (1960, Pg. 223) acclimation refers to "intra- and inter-specific compensatory changes whether these changes be phenotypic or genotypic." Unless otherwise specified, experimental conditions refers to any of the 24 possible temperature-salinity combinations to which the animals were subjected in order to determine urine osmotic response. Osmotic regulation is considered to be the maintenance of some degree of imbalance between the total osmotic concentrations of the blood and the external medium, regardless of the concentrations of the constituent ions and non-electrolytes. Ionic regulation is considered to be the maintenance of an imbalance between blood and medium levels of particular ions. Volume regulation as such has not been discussed, but is recognized as a corollary of osmotic regulation in some soft bodied invertebrates (Scheer, 1948). In others, it is suggested that osmotic and volume regulation may be separate functions (Prosser and Brown, 1961). The term osmotic concentration refers to the total osmotic concentration of blood, urine or the medium. The equilibration or holding period is the time during which animals were held in 75% sea water at experimental temperatures in order to clear their intestines and bring their body fluids to a common osmotic level. The

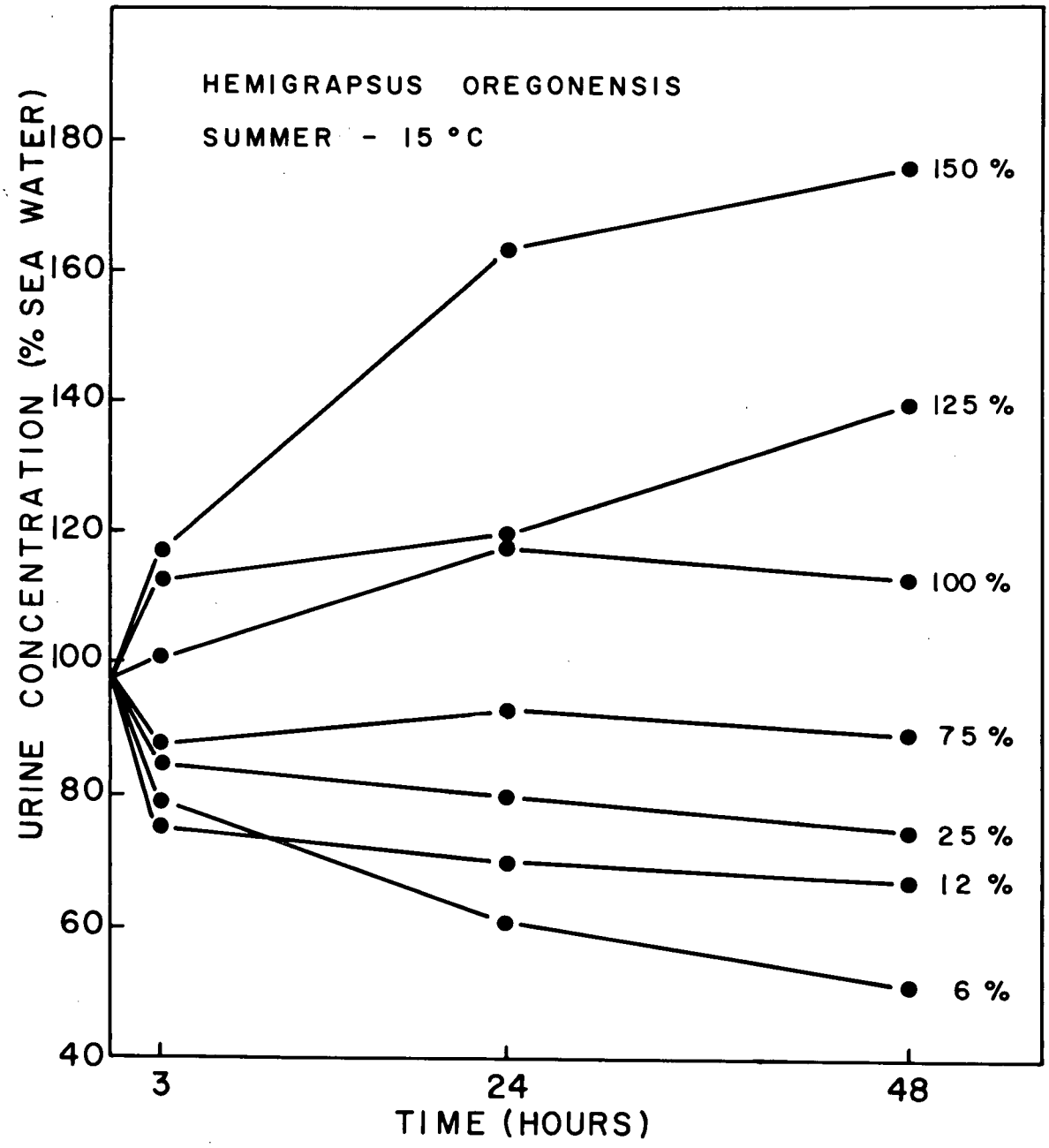
term gradient refers to a difference in total osmotic or particular ionic concentration between body fluids or a body fluid and an external medium, which are separated by a differentially permeable membrane. It may be experimentally imposed and transitory, or established and maintained by the osmoregulatory activity of the organism concerned.

RESULTS

EFFECTS OF SALINITY:

The osmotic responses of H. oregonensis and H. nudus to a range of experimental salinities, expressed as urine concentration (% sea water), are shown in Figures 1 to 4. Data for summer experiments, carried out at 15° C, and winter experiments, at 5° C, are presented. These temperatures are approximations of the seasonal field means (Dehnel, 1960). In each figure, urine concentration at time zero is the mean of all three-hour values used in the figure. This is the point of divergence (steady state value) for purposes of discussion. It will be noted that the 3, 24 and 48-hour values for animals in 75% sea water were obtained after 36 to 48 hours equilibration in that medium. Fluctuations in the 75% sea water curves are presumed to represent normal changes in steady state urine. Responses common to the four sets of curves are the rise in urine concentration with time in high salinities, and the fall in low salinities, at rates directly related to the gradients between media and the steady state urine concentrations. The sensitivity

Figure 1. Urine concentration changes in summer-adapted Hemigrapsus oregonensis exposed to a range of experimental salinities at 15° C.


HEMIGRAPSUS OREGONENSIS
SUMMER - 15 °C
URINE CONCENTRATION (% SEA WATER)
180
160
140
120
100
80
60
40
3
24
48
TIME (HOURS)
150 %
125 %
100 %
75 %
25 %
12 %
6 %

of both species to external sea water concentrations is demonstrated by the separation of response curves at 24 and 48 hours. Regulation of urine osmotic concentrations is demonstrated by the leveling of response curves for the media within the physiological limits of the mechanism.

Hemigrapsus oregonensis:

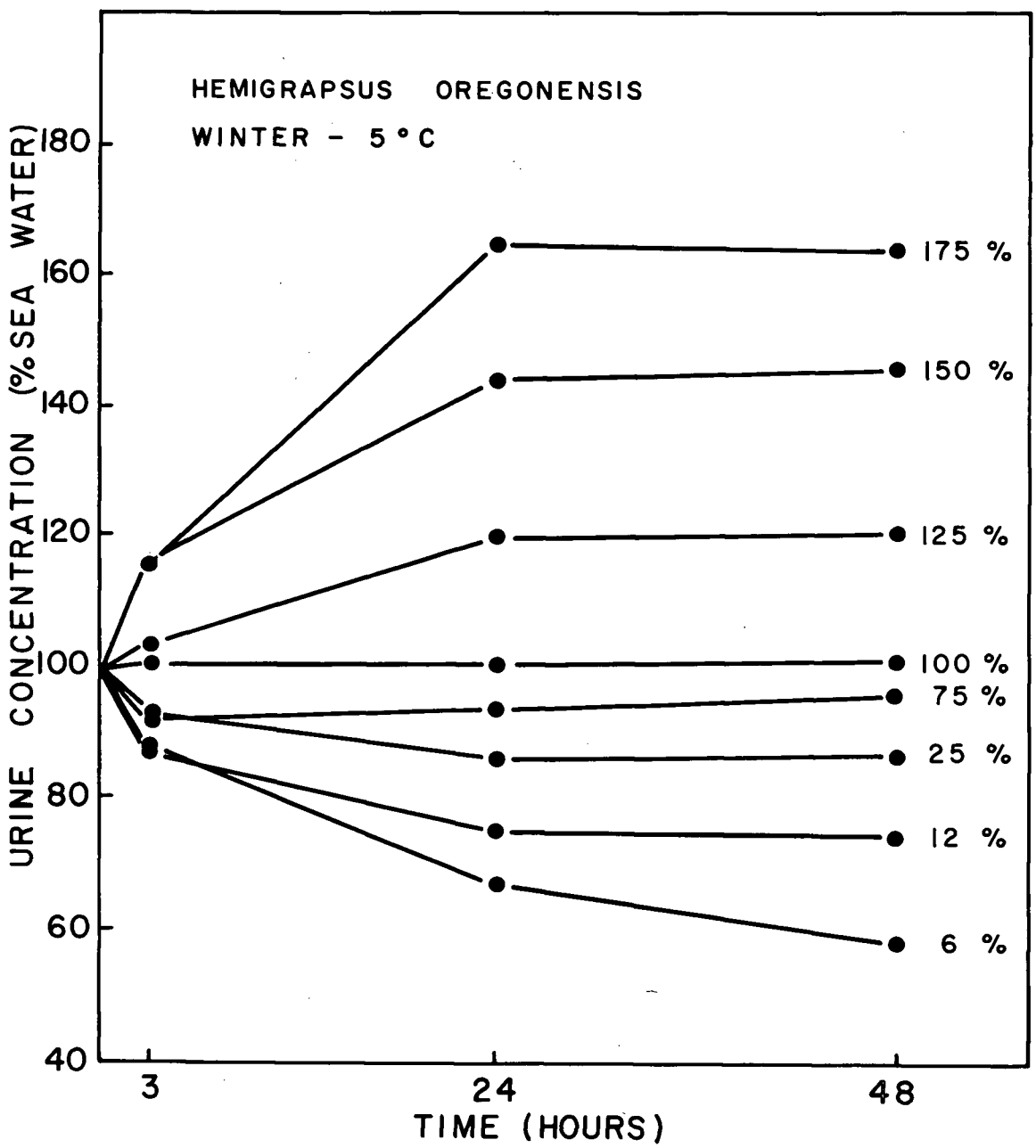
Summer animals (Fig. 1), did not survive 48 hours in 175% sea water. Regulation in lower salinities was demonstrated by the leveling of the curves after 24 hours. Only in 125% sea water did the urine concentration curve show an increased slope after 24 hours. This might have been due to experimental variation in the 24 and 48-hour determinations, as, even in 150% sea water, the leveling was distinct.

Winter animals (Fig. 2) survived 48 hours in 175% sea water, probably reflecting adaptation to high salinity field conditions. Regulation of urine concentration was again marked, particularly in high salinities. The curves for 125%, 150% and 175% sea water flattened after 24 hours. In hypotonic media, the curves, after falling markedly between 3 and 24 hours, flattened and demonstrated hyper-osmotic regulation.

The absolute difference between 48-hour urine concentrations in 6% and 150% sea water was 125% in summer and 88% in winter animals. The major portion of this difference was contributed by animals from high salinities, whose summer urine concentrations were significantly higher than corresponding winter ones.

It is shown that in summer, H. oregonensis maintained

Figure 2. Urine concentration changes in winter-adapted H. oregonensis exposed to a range of experimental salinities at 5° C.


HEMIGRAPSUS OREGONENSIS
WINTER - 5 °C
URINE CONCENTRATION (% SEA WATER)
180
160
140
120
100
80
60
40
3
24
48
TIME (HOURS)
175 %
150 %
125 %
100 %
75 %
25 %
12 %
6 %

urine concentrations hypertonic to high salinity media. This was very likely due to the continued absorption of salts by gill and gut tissues and a concomitant loss of water to the external media through the integument. In winter, in high salinities, 48-hour urine tended to be hypotonic to blood and media. In both seasons, hyper-osmotic regulation occurred in low salinities, even in 6% sea water, which is probably near the lower lethal limit for the species.

Reference to Table 1 gives the significance of observed departures of urine concentration from isotonicity with the media. It should be noted that in summer animals, all 48-hour urine concentrations for the three experimental temperatures, and salinities from 25% to 150% sea water were significantly different from those of the media. In winter animals, the 48-hour urine concentrations at 5° C in 100% and 125% sea water, at 15° C in 75% and 150% sea water, and at 25° C in 75% sea water did not differ significantly from the media.

Hemigrapsus nudus:

Summer animals (Fig. 3) survived 48 hours in 150% but not 175% sea water, as did H. oregonensis. The curves for all experimental salinities were again well separated, indicating sensitivity to external osmotic concentrations. The leveling of the curves and the existence of substantial gradients between urine and the media demonstrate hyper-osmotic regulation in dilute media, active absorption and reabsorption in concentrated media and the approach of urine to new equilibria. Some evidence

Figure 3. Urine concentration changes in summer-adapted H. nudus exposed to a range of experimental salinities at 15° C.

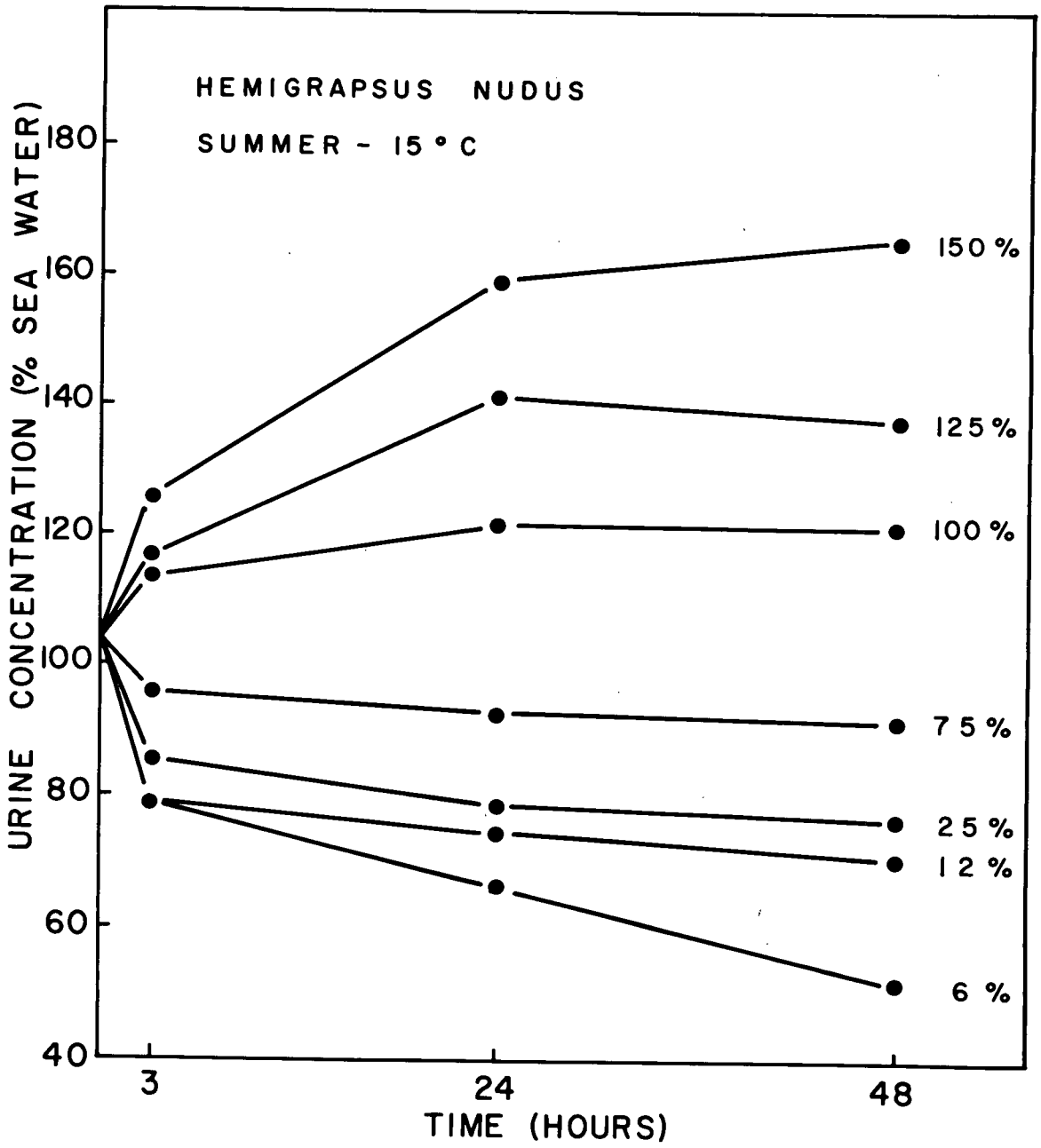

HEMIGRAPSUS NUDUS
SUMMER - 15 ° C
URINE CONCENTRATION (% SEA WATER)
180
160
140
120
100
80
60
40
150 %
125 %
100 %
75 %
25 %
12 %
6 %
3
24
48
TIME (HOURS)

Table 1: Comparison of 48-hour urine concentration with concentration of experimental media, summer and winter, at 5° , 15° and 25° C.

Species	T(°C)	25%	75%	100%	125%	150%
Summer						
Nudus	5	P<0.001	P<0.001	P<0.001	P 0.010	N.S.
Oreg.	5	P<0.001	P<0.001	P<0.001	P 0.001	I.D.
Nudus	15	P<0.001	P<0.001	P<0.001	P 0.005	I.D.
Oreg.	15	P<0.001	P<0.001	P<0.001	P 0.001	I.D.
Nudus	25	P<0.001	P<0.001	P<0.001	P 0.001	P<0.001
Oreg.	25	P<0.001	P<0.001	P<0.005	P 0.001	P<0.001
Winter						
Nudus	5	P<0.001	P<0.001	N.S.	P 0.001	P<0.005
Oreg.	5	P<0.001	P<0.001	N.S.	N.S.	P<0.010
Nudus	15	P<0.001	P<0.001	P<0.001	P 0.010	N.S.
Oreg.	15	P<0.001	N.S.	P<0.001	P 0.001	N.S.
Nudus	25	P<0.001	P<0.001	P<0.010	P 0.001	P<0.001
Oreg.	25	P<0.001	N.S.	P<0.001	P 0.001	P<0.001

I.D. = Insufficient data.

of efforts toward hyper-osmotic regulation is derived later, from U/B relationships.

In winter animals (Fig. 4), the separation of curves, especially in 75% and 100% sea water was not as clear as in the summer experiments. The animals survived 48 hours in 150% but not 175% sea water. The lower survival limit rose above the 6% summer level, and the 48-hour urine concentration in 12% sea water was significantly lower than the comparable summer value. Flattening of the 12% curve was less pronounced than in summer.

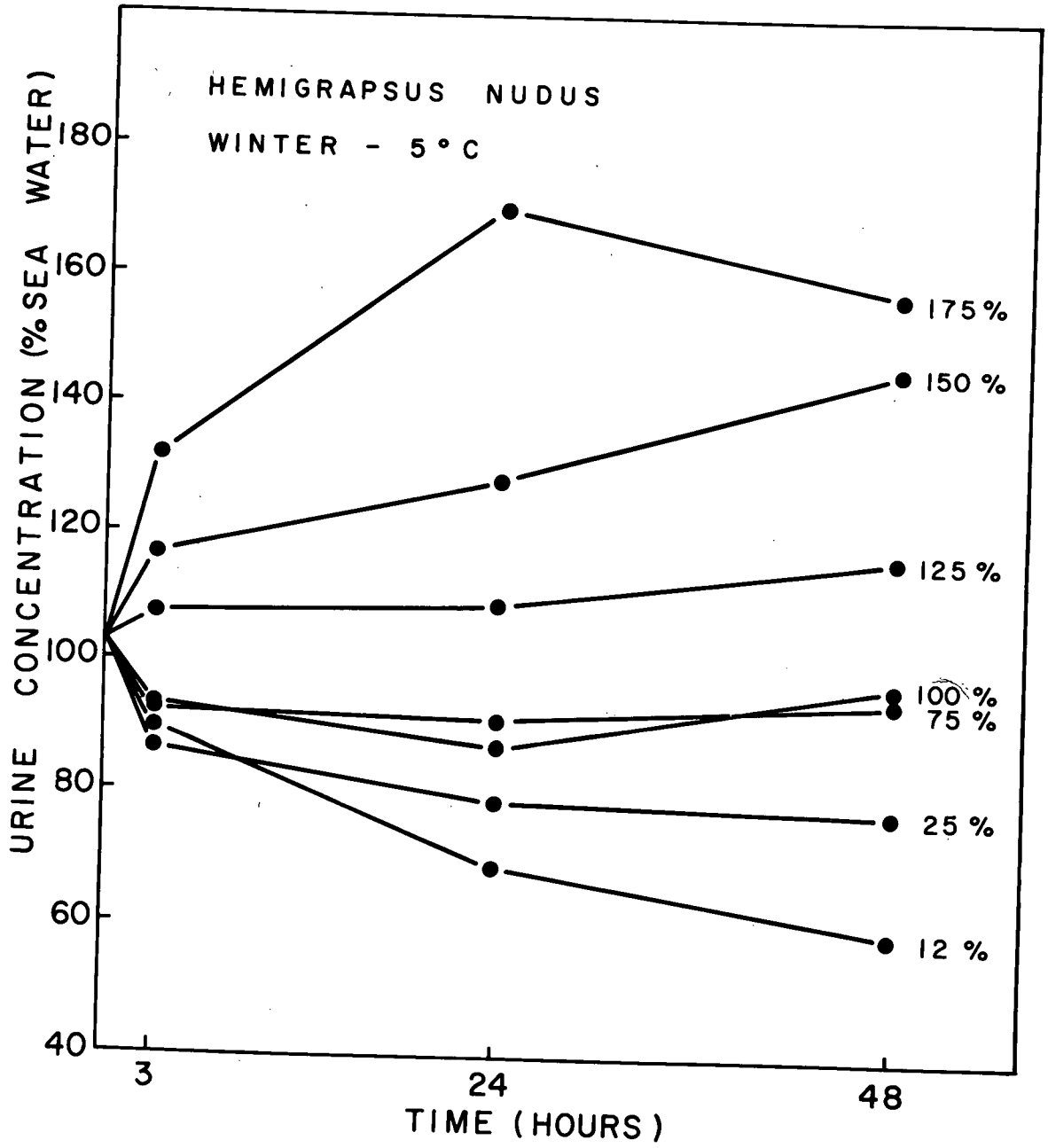
In 100%, 125% and 150% sea water in summer, 48-hour urine was hypertonic to the media. As in H. oregonensis, this might be due to continued salt absorption and water loss. In winter, at high salinities, blood concentrations were all hypertonic but urine concentrations became hypotonic to the media.

Due to high winter mortality in 6% sea water, the absolute difference between 48-hour urine concentrations in 12% and 150% sea water was considered. This was 92% in summer and 86% in winter. As in the case of H. oregonensis most of the difference was due to higher summer urine concentrations in media above 100% sea water.

Interspecific Comparison:

In summer, H. nudus showed a greater leveling than H. oregonensis of urine concentration curves in high salinities. This suggests that H. nudus potentially is the better regulator in high salinities. In low salinities, the abilities of both

Figure 4. Urine concentration changes in winter-adapted H. nudus exposed to a range of experimental salinities at 5° C.


HEMIGRAPSUS NUDUS
WINTER - 5°C
URINE CONCENTRATION (% SEA WATER)
180
160
140
120
100
80
60
40
3
24
48
TIME (HOURS)
175 %
150 %
125 %
100 %
75 %
25 %
12 %

species to hyper-osmoregulate were similar.

In winter, H. oregonensis exceeded H. nudus in ability to regulate in salinities lower than 75% sea water.

Reference to Table 2 shows that in winter, at 5° C, the 48-hour urine concentrations of the two species differed significantly in media below 75% sea water, with those for H. oregonensis being higher. In summer, at 15° C, they differed significantly only in 100% sea water, with H. nudus having the higher value.

SEASONAL EFFECTS OF SALINITY:

The abilities of the two species to establish and maintain osmotic gradients between their urine and the external media were observed to change from summer to winter. In order to evaluate seasonal effects, a set of gradient curves was derived from the data of Figures 1 to 4 and presented in Figure 5. The points on which the curves are drawn were obtained by subtracting the osmotic concentration of the experimental medium from each 48-hour urine osmotic concentration. Experimental temperatures for summer and winter animals were 15° C and 5° C respectively. The significance of the observed differences between summer and winter urine is given in Table 3.

Winter urine of both species (Fig. 5) tended to be slightly hypertonic to comparable summer urine in salinities up to 75% sea water, but was significantly hypotonic in more concentrated media. Reference to Figure 5 shows a marked divergence of

Figure 5. Osmotic gradients between urine and media for summer- and winter adapted crabs exposed for 48 hours to a range of experimental salinities.

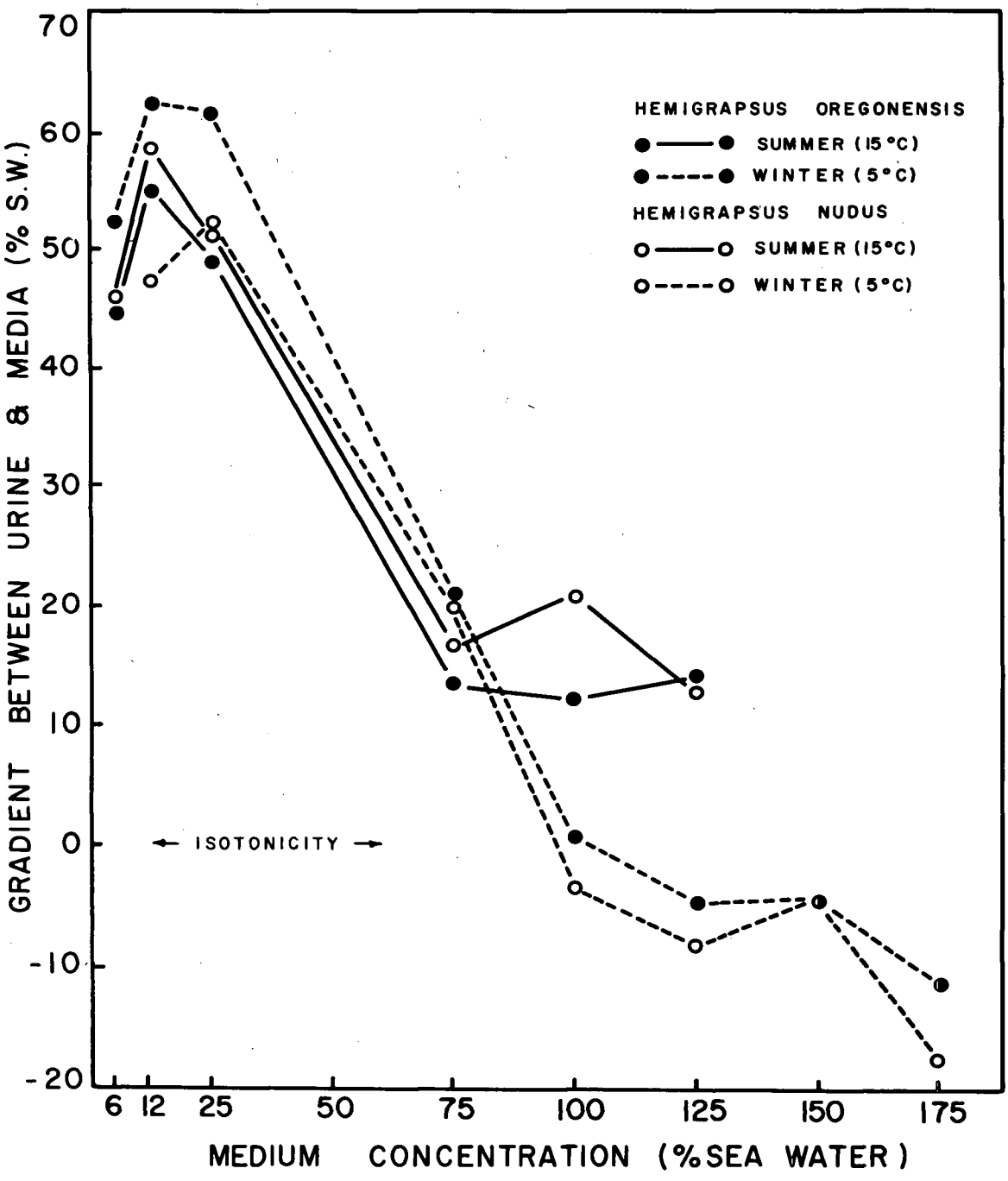

HEMIGRAPSUS OREGONENSIS
SUMMER (15°C)
WINTER (5°C)
HEMIGRAPSUS NUDUS
SUMMER (15°C)
WINTER (5°C)
GRADIENT BETWEEN URINE & MEDIA (% S.W.)
70
60
50
40
30
20
10
0
-10
-20
← ISOTONICITY →
6
12
25
50
75
100
125
150
175
MEDIUM CONCENTRATION (% SEA WATER)

Table 2: Comparison of 48-hour urine concentrations of Hemigrapsus oregonensis with those of H. nudus at 5°, 15° and 25° C. (Absolute values given in Table 4).

		Concentration of Media (% S.W.)							
	T(°C)	6%	12%	25%	75%	100%	125%	150%	175%
S	5	-	N.S.	N.S.	N.S.	N.S.	N.S.	-	-
W	5	-	P<0.001	P<0.001	N.S.	N.S.	N.S.	N.S.	N.S.
S	15	N.S.	N.S.	N.S.	N.S.	P<0.005	N.S.	-	-
W	15	-	N.S.	P<0.001	P<0.001	N.S.	N.S.	N.S.	-
S	25	-	-	N.S.	N.S.	P<0.001	N.S.	N.S.	-
W	25	N.S.	P<0.001	N.S.	P<0.001	P<0.001	N.S.	P<0.001	-

summer and winter gradient curves for both species in media above 75% sea water. Summer animals exhibited positive urine-to-medium osmotic gradients in excess of 45% sea water in low salinity media and of 12% to 21% in high salinity media. Winter gradients in low salinities remained in the same range as summer ones, but in the higher salinities, gradients from +1.0% to -18% sea water were shown.

Hemigrapsus oregonensis:

Significant seasonal differences in 48-hour urine osmotic concentrations were found in media of 25%, 75%, 100% and 125% sea water, but not in 12% sea water (Table 3 and Fig. 5). In media of 12%, 25% and 75% sea water, winter urine was hypertonic to summer urine. Above 75%, winter urine became hypotonic to summer urine, and above 100% sea water, to the media as well. Urine from winter crabs in 100% and 125% sea water was nearly isotonic with the media, but from crabs in 150% sea water, it was significantly hypotonic to the medium. Urine from summer crabs was significantly hypertonic to the media in all experimental salinities (Table 1). In 100% and 125% sea water, summer urine concentrations showed the greatest departure from comparable winter values (Table 4).

Hemigrapsus nudus:

The results for H. nudus in general paralleled those for H. oregonensis, with the difference that in 12% sea water, summer

Table 3: Comparison of 48-hour urine concentrations of summer-adapted animals (S) at 15° C with those of winter-adapted animals (W) at 5° C.

	Urine Concentration (% S.W.)									
Exp. Sal.	12%		25%		75%		100%		125%	
	S	W	S	W	S	W	S	W	S	W
Nudus	71	59	76	78	92	95	121	97	138	117
	P<0.010		N.S.		N.S.		P<0.001		P<0.001	
Oreg.	67	74	74	87	88	96	112	101	139	120
	N.S.		P<0.010		P<0.010		P<0.001		P<0.010	

urine was significantly hypertonic to winter urine (Table 3 and Fig. 5). In 25% and 75% sea water, urine of summer and winter animals was similar. In media above 75% sea water, summer urine remained significantly hypertonic to both winter urine and the media, while winter urine, which was not significantly different from the medium in 100% sea water, was significantly hypotonic in 125% and 150% sea water (Tables1 and 3).

Interspecific Comparison:

In summer, both species exhibited similar osmotic U-M gradients in media of 6% to 75% sea water. Urine from H. nudus was slightly hypertonic to that of H. oregonensis in these experimental salinities. In 100% sea water, H. nudus urine was significantly hypertonic to that of H. oregonensis but approached the same concentration in 125% sea water (Table 2). Winter urine osmotic concentrations for the two species differed significantly only in 12% and 25% sea water (Table 2). Poor survival of H. nudus in 6% sea water precluded comparison in this medium. In all media except 150% sea water, in which the urines had similar concentrations, H. oregonensis tended to have the higher values.

Both species were found to be capable of significant hyper-osmotic regulation in low salinities at both seasons. The hypertonicity of 48-hour summer urines to high salinity media, though of undoubted occurrence, appears to be largely an absorption phenomenon, as the animals do not normally encounter summer salinities much above 35% sea water (Dehnel, 1960). A slight

Table 4: Comparison of 48-hour urine concentrations of summer-adapted (S) and winter-adapted (W) animals at 5°, 15° and 25° C.

Urine Concentration (% S.W.)													
Exp.	Sal.	12‰		25‰		75‰		100‰		125‰		150‰	
S	(°C)	S	W	S	W	S	W	S	W	S	W	S	W
N	5	64	59	71	78	91	95	123	97	138	117	155	146
		N.S.		N.S.		N.S.		P<0.010		P<0.010		N.S.	
O	5	58	74	78	87	95	96	121	101	138	120	-	-
		P<0.010		P<0.010		N.S.		P<0.010		P<0.010		-	
N	15	71	45	76	84	92	94	121	91	138	111	-	-
		P<0.010		N.S.		N.S.		P<0.010		P<0.010		-	
O	15	67	54	74	73	88	77	112	91	139	112	-	-
		N.S.		N.S.		P<0.010		P<0.010		P<0.010		-	
N	25	60	48	76	74	89	84	123	97	138	112	-	-
		P<0.010		N.S.		N.S.		P<0.010		P<0.010		-	
O	25	71	57	76	76	93	78	109	88	135	112	174	138
		P<0.010		N.S.		P<0.010		P<0.010		P<0.010		P<0.010	

capacity to regulate in high salinities for short periods is, however, indicated and will be discussed. Production of hypotonic urine is carried on by winter animals of both species in high salinities.

SEASONAL EFFECTS OF TEMPERATURE:

Table 4 gives 48-hour urine concentrations at 5°, 15°, and 25° C for summer and winter animals held in experimental salinities of 12% to 150% sea water. The significance of observed differences between the mean values is included.

Hemigrapsus oregonensis:

At 5° C, in salinities below 75% sea water, winter animals showed significantly higher urine concentrations than summer animals. In 75% sea water, urine concentrations were alike, while in higher salinities, summer values significantly exceeded winter values. At 15° C, in low salinities, no significant difference was shown between summer and winter urines. In 75% sea water and higher concentrations, all summer values were significantly higher than their winter counterparts. At 25° C, summer values significantly exceeded winter values in all salinities but 25% sea water, in which they were the same. A rise in experimental temperature from 5° to 15° C was accompanied by a lowering from 75% to 25% sea water of the external concentration in which the seasonal means approached equality.

Hemigrapsus nudus:

At 5° C, in salinities of 75% sea water and less, no significant differences were observed between summer and winter urine concentrations. In 100% and 125% sea water, summer values significantly exceeded winter values. In 150% sea water, a similar but not statistically significant difference was observed. This lack of significance could be linked to a relatively high variance in the summer data for this salinity (S=9.46), and to a reduced sample size caused by mortalities (N=6.0). At 15° and 25° C, summer and winter urine concentrations were not significantly different in 25% and 75% sea water, but in both lower and higher salinities, summer values significantly exceeded winter values.

Interspecific Comparison:

Out of fifteen comparisons of seasonal mean urine concentrations carried out for each species, H. nudus showed no significant difference in seven cases, and H. oregonensis, in four cases. No statistical importance can be attributed to this ratio however, as the probability of its occurrence is above 50%. At best, there is a suggestion that H. oregonensis tends to show seasonal differences in urine osmotic concentration over a wider range of conditions than H. nudus. The principal differences in response between the two species occur in media of 12% to 75% sea water.

TEMPERATURE EFFECTS AT GIVEN SALINITIES:

Data were selected from Table 4 to show the effect of increasing temperature on 48-hour urine concentration for animals in given media. Low (12%), intermediate (75%) and high (125% sea water) salinities were chosen, and for each of these, the value at 5° C was compared with those at 15° C and 25° C, and the value at 15° C with that at 25° C. Significant results are shown in Figure 6 and Table 5.

It should be noted that the effects of temperature on osmotic concentration of urine, where they existed, were significant in low and intermediate salinities, but not in high salinities.

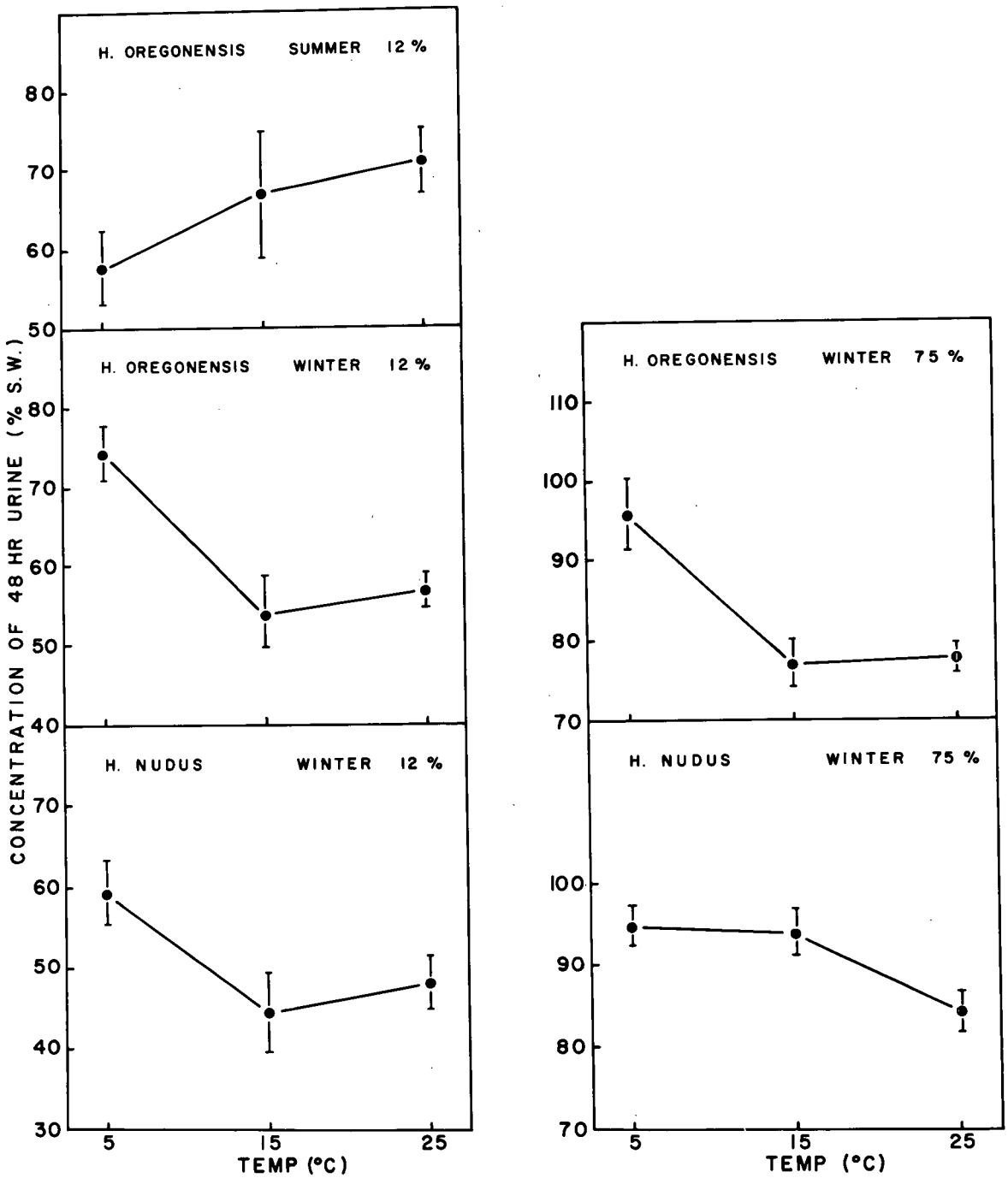
Hemigrapsus oregonensis:

In 12% and 75% sea water, winter animals showed a significant decline in urine concentration when experimental temperature was raised from 5° to 15° C (Fig. 6). No further significant changes occurred with the rise from 15° to 25° C. Summer animals in 12% sea water showed a significantly higher urine concentration at 25° C than at 5° C. Urine concentration at 15° C did not differ significantly from the values at 5° or 25° C. In 125% sea water neither summer nor winter urine concentrations changed significantly with changes in experimental temperature.

Hemigrapsus nudus:

In 12% sea water, winter animals of this species also showed a significant decline in urine concentration with an

Figure 6. Effect of temperature on 48-hour urine concentration in crabs exposed to selected salinities.


H. OREGONENSIS SUMMER 12 %
H. OREGONENSIS WINTER 12 %
H. NUDUS WINTER 12 %
H. OREGONENSIS WINTER 75 %
H. NUDUS WINTER 75 %
CONCENTRATION OF 48 HR URINE (% S.W.)
TEMP (°C)
TEMP (°C)

increase in experimental temperature from 5° to 15° C. In 75% sea water, a similar decline occurred with the increase of experimental temperature from 15° to 25° C. Summer animals showed no significant change with increased experimental temperature in any of the three media (Table 4). As in H. oregonensis, changes in experimental temperature had no significant effect on the urine concentration of animals in 125% sea water.

Interspecific Comparison:

In winter animals of both species in 12% sea water, and in H. oregonensis in 75% sea water as well, urine concentrations were significantly higher at 5° than 15° C (Fig. 6). Winter H. nudus showed no significant change between 5° and 15° C but urine concentrations at these temperatures were significantly higher than at 25° C. In the latter species, summer animals showed no significant changes in urine concentration with increased temperature, while in H. oregonensis in 12% sea water, a significant upward trend was observed with an increase in experimental temperature from 5° to 25° C.

EFFECTS OF TEMPERATURE ON RATES OF URINE AND BLOOD CONCENTRATION CHANGE IN HIGH SALINITIES:

Because salinity determinations on body fluids were made only at 3, 24 and 48 hours, rates of concentration change cannot precisely be gauged from the response curves. However, an indication of temperature effects can be obtained by considering

Table 5: Comparison of 48-hour urine concentrations at 5°, 15° and 25° C, in selected media.

Exp. Temp. (°C)	5°	vs	15°	15°	vs	25°	5°	vs	25°
Media Conc. (%S.W.)	12%	75%	125%	12%	75%	125%	12%	75%	125%
N-W	P<0.001	N.S.	N.S.	N.S.	P<0.001	N.S.	P<0.001	P<0.001	N.S.
O-S	P<0.001	P<0.001	N.S.	N.S.	N.S.	N.S.	P<0.001	P<0.001	N.S.
O-W	N.S.	N.S.	N.S.	N.S.	P<0.005	N.S.	P<0.001	N.S.	N.S.

the intervals in which rising blood and urine concentrations passed isotonicity. Both blood (Dehnel, 1962) and urine in summer-adapted Hemigrapsus were hypertonic to all experimental media at 5°, 15° and 25° C at 48 hours. In winter-adapted animals, blood of both species was hypertonic to all salinities. Urine of H. oregonensis was isotonic to 100% sea water and hypertonic to higher salinities. Urine of H. nudus was hypotonic to 100% sea water and higher salinities (Figs. 1 to 5 and data not presented). Blood and urine concentrations rose from equilibrated values at time zero and passed isotonicity at times directly related to the season, to concentrations of the media and experimental temperatures.

At 5° C, the blood of winter-adapted animals reached isotonicity with 100% sea water in the interval between 0 and 3 hours, with 125% and 150% sea water, between 3 and 24 hours, and with 175% sea water, between 24 and 48 hours. At 15° and 25° C the only change was in 175% sea water, where isotonicity was reached earlier, between 3 and 24 hours. At 5° C, urine was isotonic to 100% sea water by 3 hours, and did not become hypertonic to any higher salinity at 5°, 15° or 25° C.

In summer-adapted animals, at 15° C in 100% sea water, isotonicity was reached in blood at 3 hours, and in urine between 0 and 3 hours. Very slight hypotonicity of blood to urine and medium at 3 hours suggested a weak effort to regulate blood concentration in this salinity, which disappeared by 24 hours. In 125% sea water, blood reached isotonicity between 3 and 24 hours,

and urine, in the next interval. Again, slight hypotonicity of blood to urine and medium suggested weak regulation. In 150% and 175% sea water, both urine and blood passed isotonicity between 3 and 24 hours. In animals cooled to 5° C, blood and urine in 100%, 125% and 150% sea water reached the concentration of the media between 3 and 24 hours. In 100% sea water, 3-hour blood was hypotonic to urine and medium, in 125% sea water, to the medium only, and in 150% sea water, to both again. Blood concentration appeared to be regulated successfully in 100% sea water for over 3 hours, and with partial success (with production of blood-hypertonic urine) for 48 hours. In 125% sea water, regulation appeared to be somewhat successful up to 24 hours, at which time blood was still slightly hypotonic to urine, but not to the medium. Regulation was shown in 150% sea water for over 3 hours but it was not evident at 24 hours. When warmed to 25° C, these animals showed blood isotonicity in 100% sea water between 0 and 3 hours (earlier than at 15° C) and in 125% sea water, between 3 and 24 hours, as at 15° C. Urine became isotonic in 100% sea water between 3 and 24 hours, later than blood, and at 3 hours it was hypotonic to blood. At 24 and 48 hours, blood was hypotonic to urine, but not to the medium. This is some evidence of an effort towards hypo-osmotic regulation. In 125%, 150% and 175% sea water, urine passed isotonicity between 3 and 24 hours with no instance of blood-hypertonic urine being formed.

Hemigrapsus nudus:

Winter-adapted animals showed rates of blood concentration change like those of H. oregonensis at all temperatures and salinities except at 25° C in 175% sea water where the animals died sometime after 3 hours, and at which point blood was not yet isotonic. Urine did not become hypertonic at any temperature in media of 100% sea water or higher.

In summer-adapted animals, at 15° C, both blood and urine became isotonic with 100% sea water between 0 and 3 hours, and with 125% and 150% sea water, between 3 and 24 hours. In 100% sea water, 3-hour urine was hypertonic to blood (U/B=1.06) suggesting a slight effort toward hypo-osmotic regulation of blood concentration. In animals cooled to 5° C, blood isotonicity with 100%, 125% and 150% sea water was reached between 3 and 24 hours. Urine isotonicity was reached earlier in 100% sea water, and at 3 hours, a U/B ratio of 1.06 obtained, suggesting, as at 15° C, slight regulation in this medium. In 125% and 150% sea water, 3-hour U/B ratios were < 1.0. In 175% sea water, urine reached isotonicity between 3 and 24 hours. In summer-adapted animals warmed to 25° C blood passed isotonicity in 100% sea water between 0 and 3 hours, in 125% sea water, between 24 and 48 hours, and in 150% sea water between 3 and 24 hours. Urine became isotonic between 0 and 3 hours in 100% and between 3 and 24 hours in 125% and 150% sea water. Urine was hypertonic to blood in 100% sea water at 3, 24, and 48 hours, and in 125%

sea water at 3 and 24 hours, suggesting a considerable effort to regulate blood concentration in these salinities. In 150% sea water, however, the 3-hour urine was hypotonic to blood, with no evidence of regulation of blood concentration by the antennary glands.

EFFECTS OF SIZE ON OSMOTIC RESPONSES:

In order to determine whether small and large animals differed significantly in urine concentration under identical conditions, the mean urine concentrations of the smallest and largest summer animals were compared (Table 6). This was done first by grouping the urine concentrations of the four or more smallest, and an equal number of largest animals used in each determination, where weight data were collected, and determining the two mean values. These were treated as a pair of samples from one normally distributed population. Such pairs were calculated for both species, from urine concentrations of field animals and experimental animals after 3, 24 and 48 hours in experimental conditions, where at least 8 samples were used for the determinations. From the results, a table of differences was determined by subtracting the mean urine concentration of the largest from that of the smallest animals. It was then possible to group negative and positive differences according to experimental salinity, for each species. Groups of eight or more pairs were selected, in which all or most of the differences had the same sign. An evaluation of the ratios of positive to

Table 6: Differences in urine concentration between large (L) and small (S) summer-adapted animals. (Wilcoxon's Signed-Rank Test).

Source of Pairs			$\bar{x}$ Wt(Gm.)		No.of Pairs	O.C.S.-O.C.L. Differences		Smallest Sum of Signed Ranks	Probability
S	T(°C)	%S.W.	S	L		No.+	No.-		
N	F	F	2.0	7.8	8	8	0	0	P = 0.010
O	F	F	1.8	4.5	8	7	1	-6	P > 0.050
N	15°	6-25	2.5	6.4	8	1	7	+2	P = 0.020
O	15°	100-175	1.8	3.6	8	8	0	0	P = 0.010
N	25°	6-75	2.0	5.7	8	2	6	+12.5	P > 0.050
O	25°	6-75	1.5	3.6	14	9	5	-35.5	P > 0.050
N	25°	100-150	1.4	4.7	9	7	2	-5.5	P > 0.020
O	25°	100-175	1.2	4.0	12	11	1	-8	P > 0.010

F = Field Conditions (Summer)

O.C.S. = Mean osmotic concentration of urine of smallest animals

O.C.L. = Mean osmotic concentration of urine of largest animals

negative differences is given in Table 6. The Wilcoxon test takes into account the size of the differences between the mean urine concentrations of small and large animals and tests the null hypothesis that either group has an equal chance to have the higher concentration. The implications of the significant weight effects are not obvious. Some differences, while not significant at the 0.01 level, have P values < 0.05 and may suggest a tendency.

Small summer-adapted H. nudus, taken from field conditions, tended to have significantly higher urine concentrations than large animals. A similar, but not statistically significant tendency was shown by H. oregonensis. In high experimental salinities, the same tendency is suggested at 15° and 25° C for H. oregonensis, and 25° C for H. nudus. At 15° C, large H. nudus, in low experimental salinities, tended to have higher urine concentrations than small animals. At 25° C, in low salinities, neither species showed significant differences in urine concentration attributable to differences in weight.

There is an apparent anomaly in the fact that small H. nudus under summer field conditions of low salinity and high temperature showed significantly higher urine concentrations than large animals, while in experimental conditions approximating those of the field, the observed differences were reversed but not significant at the 0.01 level. This was probably due to the equilibration of experimental animals in 75% sea water before

exposure to low salinities in the laboratory, and the empirical grouping of data from a range of experimental salinities for comparison with those from more constant field conditions.

RELATIONSHIPS BETWEEN URINE AND BLOOD CONCENTRATIONS:

Consideration is given in Tables 7 and 8 to the relationship between urine and blood concentrations in animals exposed to the same experimental conditions. Blood data were provided by the experiments of Dehnel (1962). Forty-eight-hour urine values were taken from Table 4, 3-hour and 24-hour values from raw data. Table 7 gives total osmotic Urine/Blood ratios of previously equilibrated animals of both species, held for 48 hours in media of 12%, 75% and 125% sea water, summer and winter. Also included, are intraspecific Urine minus Blood concentration differences, and a statistical evaluation of their significance by means of Student's "t" test. Table 8 gives the significance of interspecific differences in U/B ratios of H. nudus and H. oregonensis in both seasons, over the entire range of experimental conditions. In order to apply the Wilcoxon test, all U/B ratios were converted to percentages and paired. Arbitrarily, for each experimental temperature, all values for H. oregonensis were subtracted from the corresponding values for H. nudus. The differences were ranked and the ranks given the signs of the differences. The positive and negative ranks were summed, and the smaller sum provided an entry into the tables of probability.

Table 7: Comparison of 48-hour urine and blood concentrations in summer- and winter-adapted animals at 5°, 15° and 25° in selected media.

		Summer								
Media Conc. (% S.W.)		12%			75%			125%		
	T(°C)	U/B	U-B	P	U/B	U-B	P	U/B	U-B	P
Nudus	5	0.84	-11.7	P<0.010	0.97	-2.4	N.S.	0.94	-8.3	N.S.
Oreg.	5	0.90	- 6.2	N.S.	0.99	-1.3	N.S.	-	-	-
Nudus	15	1.01	0.9	N.S.	0.99	-0.6	N.S.	0.98	3.3	N.S.
Oreg.	15	1.02	1.3	N.S.	0.95	-4.6	P<0.001	0.96	-5.7	P<0.010
Nudus	25	0.98	-1.0	N.S.	0.98	-1.7	N.S.	0.98	-2.4	N.S.
Oreg.	25	0.98	-1.1	N.S.	1.04	3.3	N.S.	1.00	-0.2	N.S.
		Winter								
Nudus	5	0.75	-19.3	P<0.001	0.83	-19.8	P<0.001	0.83	-24.3	P<0.001
Oreg.	5	0.83	-15.1	P<0.001	0.95	- 5.0	N.S.	0.93	- 9.4	P<0.005
Nudus	15	0.67	-21.9	P<0.001	0.97	- 2.5	N.S.	0.77	-33.6	P<0.001
Oreg.	15	0.63	-31.1	P<0.001	0.77	-22.9	P<0.001	0.86	-17.7	P<0.001
Nudus	25	0.63	-28.3*	-	0.87	-12.7*	-	0.82	-24.4	P<0.001
Oreg.	25	0.69	-25.1*	-	0.87	-12.1	P<0.001	0.88	-15.4	P<0.001

* = 24-hour blood values

U/B = Urine/Blood ratio

U-B = Urine-Blood gradient

Summer U/B ratios are shown in Table 7 to approach unity in both species. In most of the selected conditions, blood was more concentrated than urine. Where U/B ratios > 1.0, the departure from unity was not significant. Blood was significantly hypertonic to urine in H. oregonensis in 75% and 125% sea water at 15° C and in H. nudus, only in 12% sea water at 5° C.

Winter U/B ratios in both species in the selected conditions were all lower than comparable summer ratios. The absolute differences in concentration between urine and blood increased to statistically significant levels in most of these conditions. The increases were due to a generally larger net decrease in urine concentration than blood concentration from summer to winter in similar experimental conditions. Blood values were significantly higher than urine values in all conditions except for H. oregonensis, 5° C, and H. nudus, 15° C, in 75% sea water.

Table 8 shows that at 5° C, H. oregonensis had higher U/B ratios than H. nudus over the entire range of experimental salinities. The difference was due to relatively lower blood concentrations in H. oregonensis at this temperature. The discrepancy between ratios was significant in winter and approached significance in summer animals. At 15° and 25° C, in the same salinities, H. nudus ratios were higher, both in summer and in winter, but the difference was significant only in winter animals at 25° C.

Table 8: Wilcoxon's Signed Rank Test (two tailed) for inter-specific differences in U/B ratios; U/B ratios converted to % and paired for same conditions.

Source of Pairs			No. of	Smallest $\leq$ of	U/B	Probability
Season	T(°C)	% S.W.	Pairs	Ranked Diffs.	Trend	
	5°		22	+38.5	O>N	P<0.01
Winter	15°	6-175%	23	-77.4	N>O	P>0.05
	25°		19	-19.5	N>O	P<0.01
	5°		20	+43.5	O>N	P>0.02
Summer	15°	6-175%	22	+88.5	O>N	P>0.05
	25°		18	-45.5	N>O	P>0.05

DISCUSSION

Hyper-osmotic regulation of blood concentration in H. oregonensis and H. nudus was demonstrated by Jones (1941). Gross (1957a) was also able to show some degree of hypo-osmotic regulation in these species. Recent work (Dehnel, 1962), carried out for a full year, has demonstrated that the osmo-regulatory abilities of the two species changed significantly from summer- to winter-adapted animals. The results presented here support and complement the latter findings with details of urine osmotic responses.

EFFECTS OF SALINITY:

From an equilibrated or steady state at time zero (Figs. 1 to 4), the urine osmotic response curves fall in low and rise in high salinities at rates which in general decline with time and appear to reach new equilibria with media within the physiological limits of the species. Blood response curves for Hemigrapsus (Dehnel, 1962) and Pachygrapsus (Gross, 1957a) exhibit similar patterns. In the latter investigation, samples were drawn at shorter intervals (1, 3, 6 and 12 hours), so that stepwise changes towards new steady states are evident. As in the present case, most of the changes were complete by 24 hours immersion in the media. In Emerita, an adjuster, Gross (1957a) showed that all blood changes were complete after only two hours in a comparable range of experimental salinities.

The antennary glands of Pachygrapsus have been shown to

function mainly in the regulation of particular blood ions but not of total blood osmotic concentration (Jones, 1941; Robertson, 1949; Prosser, Green and Chow, 1955; Gross, 1957a, 1959). This conclusion was based on the isosmoticity of blood and urine in a variety of temperature and salinity combinations, and on high U/B ratios for magnesium (Gross, 1959). The prawns Palaemonetes varians, Leander serratus and L. squilla, in dilute media, produce urine isotonic to blood (Panikkar, 1941). Parry (1954) showed Mg and SO_4 to be lower in blood than in urine in L. serratus. In both species of Hemigrapsus, summer-adapted animals at least have total osmotic U/B ratios close to unity over the entire range of experimental temperature and salinity (Table 7). At the same time, large osmotic gradients between external media and body fluids (48-hour blood and urine) were obtained in salinities below 75% sea water (Fig. 5). The animals are thus shown to be effectively regulating hyper-osmotically. There was no significant weight increase such as would accompany a large influx of water from the hypotonic media (Dehnel, 1962). After an initial rapid drop in urine concentration, the rate of salt loss was reduced after 24 hours, so that a new state of equilibrium was approached. The demonstrated hypertonicity of 48-hour urine in summer animals exposed to concentrated media (Fig. 5) can have little adaptive importance, since salinities higher than 35% sea water are not as a rule encountered in this geographic area. Webb (1940) postulates that salt absorption is a continuous process under normal

conditions. The continued increase in urine concentration may thus be attributed to the activity of salt absorbing tissues in the gut and gills which, when adapted to high temperature and low salinity, continue to act as if they were aiding hyperosmotic regulation, within physiological limits, of blood concentrations. If this is the case, the leveling of response curves in high salinities may be the result of interference by increased blood ion concentrations, with the absorptive mechanism. Until the flux of sodium, magnesium, calcium, potassium, chloride and sulfate ions between concentrated media and the urine and blood is known, and the presence and influence of adaptive extravascular salt pools is established, a more complete explanation of this summer phenomenon cannot be undertaken.

Three major differences distinguish the urine osmotic responses of summer- and winter-adapted animals of both species, at their respective temperatures, to the same range of experimental salinities. The first is that over a given range of external concentrations, winter animals showed a smaller range of urine concentration than summer animals. This was markedly true for H. oregonensis (Figs. 1 to 4). Hemigrapsus nudus, in winter, showed a reduced tolerance for very low external salinity. Such a reduction, expressed by high mortality, was also shown for C. crangon, a migratory shrimp (Broekema, 1941). The second difference was that total osmotic U/B ratios for winter animals were in most cases significantly lower than summer ratios, that is, urine was more hypotonic to blood over the range of

experimental salinities (Table 7). This suggests winter participation of the antennary glands in hyper-osmotic regulation. The significance of low U/B ratios is not easy to see in relation to the third and most important difference between winter and summer responses: the production in winter of hypotonic urine in external salinities above 75% sea water for H. nudus and above 100% sea water for H. oregonensis.

While hypo-osmotic regulation of blood concentration has been well documented for a number of Crustacea from aquatic, intertidal, semi-terrestrial and terrestrial habitats (Jones, 1941; Broekema, 1941; Prosser, Green and Chow, 1955; Gross, 1957a and b; Riegel, 1959), it was not found in Hemigrapsus by Jones (1941), whose results have been widely cited. Gross (1957a) however, held that some degree of hypo-osmotic regulation of blood concentration occurred in Hemigrapsus from California, giving a value of up to 33% perfect regulation for 20 hours in 150% sea water. This value was obtained by dividing the sustained gradient between blood and medium at 20 hours by that at time zero. Recent work (Dehnel, 1962) has shown that both species of Hemigrapsus, equilibrated in 75% sea water, cannot maintain blood hypo-tonicity when transferred to experimental salinities of 100% to 175% sea water. Present results have indicated that although true regulation of blood concentration was not established in hypertonic media, increases in concentration may be resisted to some degree. It was shown that urine may differ in concentration from both blood and media, and that

seasonal changes occurred in urine as well as blood osmotic responses.

Summer-adapted Hemigrapsus in the field maintained their body fluids considerably hypertonic to summer salinities (25% to 35%). Urine was shown to be nearly isotonic with blood. Similar osmotic behavior is found in Carcinus in dilute sea water and Eriocheir in fresh water (Krogh, 1939). Webb (1940) has suggested that active water uptake is suspended and ion exchanges in gills and antennary glands are intensified under these conditions. The low permeability characteristic of the exoskeleton of regulating forms (Gross, 1957a) would aid the animals in resisting the influx of excess water with increasing osmotic gradients.

When exposed to increased or decreased experimental salinities, summer-adapted animals behave, osmotically, somewhat as if they were still in "normal" summer conditions, although the concentration of their body fluids follows changes in the external medium. In low salinities, both species maintained hypertonicity of blood and urine (Figs 3 and 5 and Dehnel, 1962). This was done without the production of blood-hypotonic urine, and might have been accomplished as Webb (1940) suggests. Another possibility is that during the experimental period, salts are mobilized from adaptive extra-vascular pools, whose existence was postulated by Hukuda (1932) and verified in Pachygrapsus by Gross (1958, 1959). No experiment as yet has

been conducted to establish the presence of these pools in Hemigrapsus. In 100% sea water, H. nudus showed a significantly larger gradient between 48-hour urine and the medium than H. oregonensis, but at higher salinities, there was no significant difference (Table 2). Urine was significantly hypotonic to blood in H. oregonensis in 75% and 125% sea water but was not so in H. nudus (Table 7). Urine osmotic response curves (Figs. 1 and 3) rose past isotonicity in most cases by 24 hours. At 48 hours, in salinities up to 150% sea water, new equilibria were approached, while in 175% sea water, lethal blood concentration was reached before 24 hours (Dehnel, 1962), at which time in the present experiments, urine was equivalent to 192% sea water in H. oregonensis. It is apparent from the above changes that osmoregulatory mechanisms which are adapted to high-temperature low-salinity conditions, seem to continue to operate in a similar way even when exposed to high experimental salinities at summer temperature. Summer-type regulation, characterized by active ion absorption and reduced water loss, and presumably accompanied by ion reabsorption in the antennary glands, is largely extra-renal and does not change after a period as short as 48 hours in experimental conditions. Parameters to which the regulatory mechanisms may become acclimated are temperature, salinity (and T/S combinations), light and desiccation, brought about by the seasonal progression of low tides from night in winter, to day in summer. The effect of temperature on osmotic responses of seasonally adapted animals will be considered in a later section.

The excretion of blood-hypotonic urine as a means of maintaining blood concentration above that of the medium has been well documented (see below). Winter-adapted Hemigrapsus in the field showed blood concentrations hypertonic to 70-80% sea water (Dehnel, 1962). Urine data for winter animals from the field are not available, but after 51 hours (48 equilibration plus 3 experimental) in 75% sea water at 5° C H. nudus had higher blood concentrations than H. oregonensis (Dehnel, 1962) while their urine concentrations were alike and hypotonic to the blood. For comparison with summer data, these values have been considered to approximate the urine and blood relationships in winter animals from field conditions. In summer animals from field conditions, H. nudus had both blood and urine concentrations higher than H. oregonensis. Mean winter and summer U/B ratios for H. nudus were 0.88 and 0.95, and for H. oregonensis 0.93 and 0.98, with urine-to-blood gradients of 13% and 6% and 7% and 2% respectively.

Winter animals of both species, in experimental media below average winter sea water concentration (70-80%) regulated their blood concentration with the production of blood-hypotonic urine. Absolute 48-hour blood and urine concentrations were significantly higher in H. oregonensis than H. nudus in 12% and 25% sea water, and 6% sea water was tolerated by H. oregonensis only (Figs. 2 and 4, Table 2, data not presented and Dehnel, 1962). The larger blood-to-medium gradients shown by H. oregonensis suggest a more active ion absorbing mechanism in this

species, perhaps correlated with its characteristically estuarine distribution. The active absorption of ions from hypotonic media has been demonstrated in a variety of regulating Crustacea, among them, a crayfish, Astacus, and the crabs Carcinus and Eriocheir, the latter related to Hemigrapsus (Schwabe, 1933; Nagel, 1934; Krogh, 1939; cited by Prosser and Brown, 1961). In crabs, the gills have been recognized as major sites of absorption (Nagel, 1934; Gross, 1957a). Excess water can enter the animals by diffusion and active absorption, together with ions, through the gills. Urine, if formed by filtration, at first may be isotonic with the blood and be rendered hypotonic by the reabsorption of specific ions. As long as the loss of ions in the urine is at least balanced by active absorption from the dilute media, the animals can achieve and maintain osmotic equilibrium (Webb, 1940). Increased urine output in dilute media has been shown to aid in elimination of excess water in Carcinus (Prosser and Brown, 1961). It has not been demonstrated in the present data but may be important in Hemigrapsus as well.

In media more concentrated than normal winter sea water, at 5° C, both species maintained blood hypertonicity and continued to excrete, at 48 hours, urine hypotonic to the blood and the media. As was the case in summer-adapted animals, the winter balance between active processes in the gills and antennary glands appeared to be maintained during short-term exposure of the animals to concentrated sea water. The general urine-to-blood

relationship established in average winter sea water conditions was retained, although absolute body fluid concentrations changed (Table 7). Gradients between blood and media tended to decrease with increasing external concentration above 75% sea water (Dehnel, 1962) to a minimum in 175% sea water for H. nudus and 125% for H. oregonensis. Above 125% sea water in the latter species, they again increased. Urine-to-medium gradients on the other hand tended to increase with increasing external concentrations, from a minimum in 100% sea water to a maximum in 175% sea water, for both species (Fig. 5). Renal excretion of magnesium and retention of sodium and potassium in dilute media has been demonstrated in Pachygrapsus (Prosser, Green and Chow, 1955; Gross, 1957a), and the extra-renal excretion of sodium in concentrated media has been suggested. Until ion determinations are available for local Hemigrapsus blood and urine, the precise activities of the antennary glands cannot be established.

EFFECT OF TEMPERATURE ON OSMOTIC RESPONSES OF SEASONALLY-ADAPTED ANIMALS:

Broekema (1941) reported that Crangon crangon maintained in sea water of 29‰ showed a gradual fall in blood concentration as experimental temperature was allowed to rise with the seasonal change from spring towards autumn (blood-medium gradient gradually increased). A reversal of these changes occurred when the experimental temperature was allowed to fall between autumn and winter. This species, in Dutch waters, winters offshore in water

of relatively high salinity and migrates shoreward into more brackish conditions in spring and early summer. Survival at low temperature was correlated with high salinity and high temperature increased tolerance to low salinity. Other species, with a reverse migratory pattern, appeared to tolerate low salinities better at low temperatures. These included a spider crab, Hyas araneus, a shrimp, Crangon allmani and a prawn, Pandalus montagui. A third group, represented by the crab Rhithropanopeus harrisi and the amphipod Gammarus duebeni had tolerances similar to Hyas but did not migrate seasonally (Verwey, 1957). The two species of Hemigrapsus combine tolerances similar to C. crangon, and non-migratory habits. In general, high temperatures increase and low temperatures decrease metabolic rates. Dehnel (1960) suggested that low salinities at high temperatures may impose a greater stress than high ones. This is compatible with observed osmotic gradients maintained by these species between blood and media and urine in high and low salinities.

Winter-adapted animals of both species in dilute (12‰) sea water showed significantly greater 48-hour hypertonicity at 5° C than at 15° C (Fig. 6). With the rise in temperature, the U/B ratio decreased because urine concentration decreased more than blood. A rise in experimental temperature from 15° C to 25° C caused no further significant change in urine concentration. Blood data from equivalent animals are not available for comparison. In 75‰ sea water, at 5° C, winter animals of

both species showed similar 48-hour urine concentrations but H. nudus had a higher blood concentration, hence a smaller U/B ratio (Table 7). At 15° C, H. nudus urine remained unchanged, blood concentration dropped, the U/B ratio rose, and regulation weakened. In H. oregonensis, however, blood did not change but urine concentration decreased giving a lower U/B ratio. The gradient between urine and medium dropped from 21% to 2% sea water. Thus, in experimental conditions approximating winter field temperature and salinity, H. oregonensis responded to a rise in temperature, increased permeability and metabolic rate, by a drop in urine concentration, while maintaining blood at the level found at 5° C. This is probably achieved by increased reabsorption in the antennary glands. A further rise in temperature to 25° C caused no significant change in urine concentration, but a rise in the U/B ratio from 0.77 to 0.87, was accompanied by a decrease in blood concentration. This species regulates less strongly in 75% sea water as temperature increases. In H. nudus, the rise in temperature from 15° to 25° C resulted in a significant decrease in urine, but not blood, concentration, hence a lower U/B ratio (Fig. 6). The 48-hour U/B ratios for the two species in 75% sea water were identical at 25° C but H. nudus had urine and blood values about 7% sea water higher than H. oregonensis, indicating stronger regulation.

Urine and blood concentrations were alike for summer-adapted H. oregonensis in 12% sea water, at 15° C, and the gradient between these fluids and the medium was 55% sea water.

Cooling the animals to 5° C reduced this gradient by 10% sea water for urine and 2% for blood. Blood osmotic concentration was regulated nearly as strongly as at 15° C, with a slight but not significant indication that the antennary glands were involved. Urine and blood concentrations were similar at 25° C but the gradient between them and the medium increased, indicating that summer adaptation favours stronger regulation at high temperatures and low salinities and emphasizes the resemblance of the temperature and salinity tolerances of this species to those of C. crangon.

Summer-adapted H. nudus at 5° C in 25% and 12% sea water, showed significant, and in 6% sea water, slight, hypotonicity of urine to blood (Table 7), suggesting here also that the antennary glands are taking part in the elimination of excess water and reabsorption of needed ions. At 15° C and 25° C, in dilute media, urine concentrations were not significantly different from those at 5° C, but blood-to-urine gradients were slightly reduced, suggesting that cooling of summer-adapted animals in low salinity conditions reduced their capacity for salt absorption and stimulated greater reabsorption in the antennary glands to compensate,

The effects of cooling or warming summer-adapted animals and of warming winter-adapted animals were pronounced only in low experimental salinities. In high salinities, similar changes in temperature caused no significant change in urine concentration in either species. It is probable that high

salinities pose less of an osmotic problem than low salinities (see gradients, Fig. 5), and that temperature changes consequently do not alter the balance between absorptive and reabsorptive activities as much in high as in low salinities.

In comparing summer and winter mean urine concentrations in the range of experimental salinities and at 5° , 15° and 25° C, (Table 4) it should be noted that each of the lower temperatures is foreign to one of the seasonally-adapted groups, and that 25° C is foreign to both. Therefore, differences between the results given in Tables 3 and 4 are attributable to temperature effects on the seasonal groups.

EFFECT OF TEMPERATURE ON RATES OF URINE AND BLOOD CHANGE IN HIGH SALINITIES:

Local Hemigrapsus, after equilibration in 75% sea water, showed rises in urine and blood concentration when placed in sea water of 100% or higher concentration. Rates of change were related to the season and experimental salinity and temperature. These animals do not regulate in the usual sense (Dehnel, 1962) at 48 hours, but give some evidence of resisting for a time upward changes in blood concentration. The antennary glands appear to be implicated in this resistance, and their activity, with that of absorptive tissues, is modified by changes in experimental temperature.

Hemigrapsus oregonensis:

Winter urine did not become hypertonic to concentrated

media, so that only blood changes are considered. A rise in experimental temperature from 5° to 15° or 25° C shortened the time taken for blood to become isotonic to 175% sea water. Presumably, salt absorption was enhanced in 100-150% sea water as well, but this effect was concealed in the relatively long intervals between samples.

In summer-adapted animals at 15° C, blood reached isotonicity with 100% sea water more slowly than in winter-adapted animals at any temperature used. The higher adaptation temperature appeared to favour stronger regulation. In 125% sea water, urine changed more rapidly than blood, and the antennary glands appeared to function in retarding blood change. In higher salinities, however, no such retardation was evident. Cooling the animals to 5° C reduced absolute urine and blood concentrations at 3 hours, suggesting that the lower temperature retarded all active processes involved in regulation. Warming the animals from 15° to 25° C raised 3-hour blood values by 8-22% in 125-175% sea water, while urine values increased less. These increases suggest that temperature elevation affects salt absorption more than salt excretion.

Hemigrapsus nudus:

Winter-adapted animals at 5° C in high salinities did not produce blood-hypertonic urine. Blood changes in 100-175% sea water were not obviously accelerated by increased experimental temperature.

In summer-adapted animals at 15° C, blood concentration exceeded 100-175% sea water by 3 hours. Blood-hypertonic urine, suggesting an attempt at regulation, was produced for 24 hours in 100% sea water, but apparently not in higher salinities. Cooling the animals 15° to 5° C tended to retard salt absorption, so that 3-hour blood concentration was lower at the lower temperature. Salt excretion was less affected, and urine was hypertonic to blood for 48 hours in 100% sea water and for over 3 hours in 125-175% sea water. In all media, however, both urine and blood passed isotonicity around 3 hours and attempts at regulation were weak. Warming the animals from 15° to 25° C tended to raise urine concentration. Although blood was not kept hypotonic to 100% sea water for as long as 3 hours, it was less hypertonic at the higher temperature at each sampling time. In 125% sea water, blood was hypotonic for over 24 hours with the production of markedly hypertonic urine. This suggests that the higher temperature enhanced regulation. In 150% sea water both urine and blood changes were more rapid at 25° C and no regulation was evident. It appears that, as in Pachygrapsus, wherever a degree of regulation is found, it commences very soon after exposure of the animals to the experimental conditions.

EFFECTS OF SIZE ON OSMOTIC RESPONSE:

Little work has been done on the effect of body size on osmotic behavior in invertebrates. Broekema (1941) compared blood concentrations of "young" and "old" C. crangon and found

no significant difference between them in media of 19.3‰ and 25.3‰ at 20° C and in 34.7‰ at 4°-5° C. Dehnel (1962) found no significant differences in blood concentration between small and large H. nudus and H. oregonensis either from field conditions or from a range of experimental conditions similar to that used in the present investigation.

Urine concentration in summer-adapted H. nudus taken from field conditions was significantly higher in small than in large animals (Table 6). A similar, but insignificant trend was shown in H. oregonensis. This may be an expression of proportionally higher rates of ion absorption in small animals, which allow more ions to pass out in the urine while adequate blood hypertonicity is maintained. Equilibration in 75% sea water at 15° C reduced osmotic stress, and in H. nudus at least, caused different responses in large and small animals when they were placed in low salinity media. In these conditions, at 15° and 25° C, the urine of large animals was slightly hypertonic to small animals. In experimental salinities up to 75% sea water at 25° C, H. oregonensis showed no significant size effect. In concentrated media, at 15° C, however, small H. oregonensis tended to have urine significantly hypertonic to that of large animals. A similar but insignificant relationship was shown at 25° C. It seems that neither size group regulates successfully against high external salinities, but small animals, by producing urine closer to blood concentration show a greater potential for regulation than large animals. This is true if

a criterion of hypo-osmotic regulation is the production of blood-hypertonic urine. The response of H. nudus to high external salinity at 25° C differed from that of H. oregonensis. The apparent size effect is the same in both species. Hemigrapsus nudus produced blood-hypertonic urine in concentrated media to a degree not shown by U/B ratios, and not shown by H. oregonensis. Hypertonicity of urine to blood is not sufficient to make this specie a successful hypo-osmotic regulator, but it suggests the presence of a greater potential than that possessed by H. oregonensis.

RELATIONSHIP BETWEEN URINE AND BLOOD CONCENTRATION:

Total osmotic U/B ratios in H. oregonensis and H. nudus changed seasonally in animals from the same experimental conditions (Table 7). Summer-adapted animals, which in the field regulated strongly against low salinities, produced blood-isosmotic urine. In low, intermediate and high experimental salinities at 5°, 15° and 25° C, U/B ratios approached unity in both species. Exceptions occurred in H. nudus in 12% sea water at 5° C, and in H. oregonensis in 75% and 125% sea water at 15° C, where urine was significantly hypotonic to blood (Table 7). In the first case, active ion absorption from the medium may be slowed down by the low temperature, necessitating a greater recovery of ions from the urine and causing a reduced total osmotic U/B ratio. The same order of difference between blood and urine occurred in H. nudus in 6% and 25% sea water as well, at this temperature. In the second case, H. oregonensis in concentrated sea water had blood hypertonic to the media. The

production of blood-hypotonic urine must have little value in regulation at these salinities, and the statistical significance attributed to it is partly the result of low variance in the salinity determinations on blood and urine. The disappearance of significant differences between urine and blood at 25° C, suggests that they are more apparent than real at 15° C. In experimental conditions, winter U/B ratios for both species depart considerably from unity in high and low salinities (Table 7) In 75% sea water, where osmotic conditions approach the seasonal norm, differences between urine and blood for H. oregonensis at 5° C and H. nudus at 15° C were not significant. Significant differences between urine and blood in low salinities were associated with strong hyper-osmotic regulation, and in high salinities with a possible persistence of a seasonally acclimated balance between ion absorption in the gills and reabsorption in the antennary glands.

Interspecific comparison of U/B ratios showed statistically significant temperature effects in winter-adapted but not summer-adapted animals. At 5° C for the salinity range 6% to 175% sea water, and for combined 3, 24 and 48-hour data, winter-adapted H. oregonensis showed a significant tendency to have U/B ratios nearer unity than H. nudus (Table 8). At 15° C, ratios tended to be nearer unity in H. nudus, but were not significant. At 25° C, ratios for H. nudus again tended to be nearer unity, showing significance at the 0.01 level. At 5° C, the tendency for U/B ratios to be significantly higher in H. oregonensis was

based on a large proportion of cases in which the urine of this species was hypertonic to urine of comparable H. nudus. At the same time, no trend was evident in blood concentration differences. At 25° C, the tendency for H. nudus to have higher U/B ratios was based on the fact that H. nudus urine and H. oregonensis blood were more frequently higher in concentration than the same fluids in the other species. The interspecific difference in U/B ratios for winter-adapted animals at 5° C suggests that reabsorptive processes in the antennary glands are more active in H. nudus than H. oregonensis at that temperature. Increasing temperature has a different effect on the two species, so that at 25° C, H. oregonensis shows on the average larger urine-minus-blood gradients than H. nudus. This, coupled with higher blood concentrations suggests that active absorptive sites are more temperature-sensitive in H. oregonensis.

In summer-adapted animals, total osmotic U/B ratios differed less between species than in winter-adapted animals (Table 8). At 5° C, the tendency for ratios in H. oregonensis to be higher than in H. nudus was based on the fact that blood in H. nudus and urine in H. oregonensis were more frequently higher in concentration than conversely. A rise in experimental temperature to 15° C increased the number of instances, in these comparisons, in which urine of H. nudus was hypertonic to that of H. oregonensis. Total osmotic U/B ratios in the latter were slightly higher than in H. nudus. Gradients between urine and blood, probably correlated with more active ion reabsorption, were greater in

H. nudus. At 25° C, the tendency was reversed and insignificant, and on the average, blood and urine differences between the two species were slight.

SUMMARY

1. Two species of shore crabs, Hemigrapsus oregonensis and H. nudus, from the Vancouver area, were equilibrated in 75% sea water for 36 to 48 hours and exposed to a range of experimental salinities from 6% to 175% sea water at 5°, 15° and 25° C. Urine samples were drawn after 3, 24 and 48 hours, and their osmotic concentration (% sea water) was measured by the method of melting point determination. Duplicate series of experiments were carried out, using animals adapted to summer and winter field conditions. All summer animals were weighed during the experiments, and representative groups were sampled at the time of collection.

2. Urine concentration was found to fall in dilute, and rise in concentrated media, at rates directly related to the gradients between media and equilibrated urine concentrations, and influenced by the seasonal adaptation of the animals and the experimental temperature. The rate of concentration change in a given medium was not continuous between time zero and 48 hours, but in most instances became slower after 24 hours. New equilibria were generally established by 48 hours.

3. Hyper-osmotic regulation in summer-adapted animals was achieved with the production of blood-isotonic urine, implicating extra-renal mechanisms. In winter-adapted animals, the production of blood-hypotonic urine indicated the participation of the antennary glands in hyper-osmotic regulation.

4. Evidence is presented which suggests that in summer animals, adapted to field conditions of low salinity and high temperature, the antennary glands function to some degree in retarding increases in blood concentration in media of 100% to 150% sea water. In general, cooling retarded, and warming stimulated salt absorption and regulation. The period of resistance to blood change, where demonstrated, was longest in 100% or 125% sea water and shorter in higher salinities. Hemigrapsus nudus appeared to have a greater potential for hypo-osmotic regulation than H. oregonensis.

5. The antennary glands were not shown to function in resisting upward changes in blood concentration in winter animals, adapted to field conditions of high salinity and low temperature.

6. Changes in experimental temperature revealed interspecific and seasonal differences in 48-hour urine concentration. Summer-adapted H. oregonensis in dilute (12%) sea water showed significantly higher urine concentration at 25° C than at 5° C, but H. nudus showed no temperature effects in low, intermediate or high salinities. Winter-adapted animals of both species showed significant decreases in urine concentration in low and intermediate, but not high salinities, when the experimental temperature was increased.

7. In both species, summer and winter adaptation tended to favour stronger hyper-osmotic regulation at the respective seasonal temperatures than at temperatures foreign to the seasons.

8. Body size was shown to have, in some circumstances, a significant effect on urine osmotic concentration. Small H. nudus, taken from summer field conditions, had significantly higher urine concentration than large animals, while H. oregonensis did not show similar tendency. In concentrated media, small H. oregonensis at their seasonal temperature had urine significantly hypertonic to that of large animals.

9. In winter-adapted animals, H. oregonensis had total osmotic U/B ratios significantly higher (nearer unity) than H. nudus for the whole range of experimental salinities at their seasonal temperature. Increasing the experimental temperature caused a rise in the ratios in H. nudus so that at 25° C, they were significantly higher than those in H. oregonensis. In summer-adapted animals, H. oregonensis had higher ratios at the seasonal and lower temperature but at 25° C, H. nudus again had ratios nearer unity. In summer animals however, these tendencies were not statistically significant.

10. Seasonal adaptation of osmoregulatory mechanisms in Hemigrapsus is shown to alter the balance of active processes so that for a given range of experimental conditions, urine is lower in winter animals than in summer animals both in absolute concentration and relative to the blood.

LITERATURE CITED

Beadle, L. C., 1957. Comparative physiology: osmotic and ionic regulation in aquatic animals. Ann. Rev. Physiol., 19: 329-358.

Broekema, M. M. M., 1941. Seasonal movements and the osmotic behavior of the shrimp Crangon crangon (L.). Arch. Neerl. Zool., 6: 1-100.

Broekhuysen, G. J., 1936. On development, growth and distribution of Carcinus maenas (L.). Arch. Neerl. Zool., 2: 257-399.

Bullock, T. H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev., 30: 311-342.

Dehnel, P. A., 1960. Effect of temperature and salinity on the oxygen consumption of two intertidal crabs. Biol. Bull., 118: 215-249.

Dehnel, P. A., 1962. Aspects of osmoregulation in two species of intertidal crabs. Biol. Bull., (in press).

Gross, W. J., 1952. Studies of response to stress in selected invertebrates. Ph.D. Dissertation, University of California at Los Angeles.

Gross, W. J., 1954. Osmotic responses in the sipunculid, Dendrostomum zostericulum. J. Exp. Biol., 31: 402-423.

Gross, W. J., 1955. Aspects of osmotic regulation in crabs showing the terrestrial habit. Amer. Nat., 89: 205-222.

Gross, W. J., 1957a. An analysis of response to osmotic stress in selected decapod Crustacea. Biol. Bull., 112: 43-62.

Gross, W. J., 1957b. A behavioral mechanism for osmotic regulation in a semi-terrestrial crab. Biol. Bull., 113: 268-274.

Gross, W. J., 1958. Potassium and sodium regulation in an intertidal crab. Biol. Bull., 114: 334-347.

Gross, W. J., 1959. The effect of osmotic stress on the ionic exchange of a shore crab. Biol. Bull., 116: 248-257.

Gross, W. J., and P. V. Holland., 1960. Water and ionic regulation in a terrestrial hermit crab. Physiol. Zool., 33: 21-28.

Hukuda, K., 1932. Change of weight of marine animals in dilute media. J. Exp. Biol., 9: 61-68.

Jones, L. L., 1941. Osmotic regulation in several crabs of the Pacific Coast of North America. J. Cell. Comp. Physiol., 18: 79-92.

Krogh, A., 1939. Osmotic regulation in aquatic animals. Cambridge at the University Press.

Maluf, N. S. R., 1941. Urine formation in crayfish. Biol. Bull., 81: 134-148.

Nagel, H., 1934. Osmoregulation in Crustacea. Zeitschr. Vergl. Physiol., 21: 468-491.

Panikkar, N. K., 1941. Osmotic behavior of fairy shrimp, Chirocephalus. J. Exp. Biol., 18: 110-114.

Parry, G., 1954. Ionic regulation in the palaemonid prawn Palaemon (=Leander) serratus. J. Exp. Biol., 31: 601-613.

Peters, H., 1935. Über den Einfluss des Salzgehaltes im Aussenmedium auf den Bau und die Funktion der Exkretionsorgane dekapoder Crustaceen. (Nach Untersuchungen an Potamobius fluviatilus und Homarus vulgaris). Zeitschr. f. Morph. u. Okol. d. Tiere., 30: 355-381.

Picken, L. E. R., 1936. The mechanism of urine formation in invertebrates. I The excretion mechanism in certain Arthropods. J. Exp. Biol., 13: 309-328.

Prosser, C. L., and F. A. Brown Jr., 1961. Comparative Animal Physiology. Philadelphia, Saunders.

Prosser, C. L., C. L. Green, and T. J. Chow, 1955. Ionic and osmotic concentration in blood and urine of Pachygrapsus crassipes acclimated to different salinities. Biol. Bull., 109: 99-107.

Riegel, J. A., 1959. Some aspects of osmoregulation in two species of sphaeromid isopod Crustacea. Biol. Bull., 116: 272-284.

Riegel, J. A., and L. B. Kirschner, 1960. The excretion of inulin and glucose by the crayfish antennal gland. Biol. Bull., 118: 296-307.

Robertson, J. D., 1949. Ionic regulation in some marine invertebrates. J. Exp. Biol., 26: 182-200.

Robertson, J. D., 1957. Osmotic and ionic regulation in aquatic invertebrates. Recent Advances in Invertebrate Physiology. University of Oregon Publications.

Scheer, B. T., 1948. Comparative Physiology. New York, Wiley.

Schwabe, E., 1933. Uber die Osmoregulation verschiedener Krebse (malacostracen). Zeitschr. vergl. Physiol., 19: 183-236.

Verwey, J., 1957. A plea for the study of temperature influence on osmotic regulation. Ann. Biol., 33: 129-149.

Webb, D. A., 1940. Ionic regulation in Carcinus maenas. Proc. Roy. Soc. London, Series B., 129: 107-136.

Wikgren, Bo-Jungar, 1953. Osmotic regulation in some aquatic animals, with special reference to the influence of temperature. Acta Zool. Fennica, 71: 1-102.