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Title: ASPECTS OF OSMOREGULATION IN AN INTERTIDAL SHORE CRAB, HEMIGRAPSUS NUDUS (DANA)

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Abstract approved: Dr. Austin W. Pritchard

Seasonal measurements of osmotic and ionic concentrations in the intertidal crab, Hemigrapsus nudus, were made after 24 and 48 hours exposure to a range of five salinities from 25% to 150% sea water (100% = 32‰). Blood and urine were analyzed for osmotic concentration and sodium, potassium, chloride, calcium and magnesium ion concentrations. Chela muscle was analyzed only in winter animals for the above ions and for free amino nitrogen.

In dilute sea water, blood is hyperosmotic to the media for winter and summer crabs. Urine concentrations differ seasonally with a hypo-osmotic urine in winter and an isosmotic urine in summer. In winter, urine sodium and chloride is maintained hypoionic to the blood.

No blood hypo-osmotic regulation is shown, although some blood hypoionic regulation of potassium and considerable hypoionic regulation of magnesium occurred. Magnesium is excreted in

copious quantities in 100% and 150% sea water.

The total of ions measured in chela muscle is greater than the total of ions in the blood. In dilute sea water, muscle potassium is regulated. In concentrated, but not dilute, media, sodium and chloride are regulated. Amino nitrogen is not regulated. Apparently, H. nudus is able to tolerate fluctuations in intracellular osmotic concentrations.

In comparing these results with those of other workers, there is some suggestion that latitudinal variations in osmotic responses may exist in this species on the Pacific coast.

# ASPECTS OF OSMOREGULATION IN AN INTERTIDAL SHORE CRAB, HEMIGRAPSUS NUDUS (DANA)

by

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TABLE OF CONTENTS

LIST OF FIGURES

# LIST OF TABLES

# ASPECTS OF OSMOREGULATION IN AN INTERTIDAL SHORE CRAB, HEMIGRAPSUS NUDUS (DANA)

## INTRODUCTION

The osmoregulatory capacity of decapod crustaceans has received considerable attention because of the variety of habitats these animals occupy from marine to fresh water. Extensive reviews of the literature have been compiled by Krogh (1939), Prosser and Brown (1961), Lockwood (1962), and Potts and Parry (1964). From the numerous studies it is apparent that a variety of patterns of osmotic and ionic regulation are present in crustaceans. Various species have been found capable of maintaining their internal osmotic concentrations at levels different from those of the external environment, i. e. osmoregulation. This capability has distinct advantages when the species inhabits either dilute sea water or a more concentrated medium. In the former case, hyperosmotic regulation (maintenance of an internal body fluid concentration greater than the surrounding medium) may occur. When the internal body fluids are less concentrated than the external environment, hypo-osmotic regulation is said to take place. Equilibrium between the body fluids and the external medium is termed osmoconformity.

Studies on the blood concentration in decapods have shown several patterns of osmoregulation in response to a salinity stress.

For example, Uca and Pachygrapsus show both hypo- and hyperosmotic regulation; Hemigrapsus exhibits weak hypo- and good hyperosmotic regulation, while another species, Cancer pagurus, remains isosmotic over the entire salinity range (Potts and Parry, 1964). The osmoregulatory patterns reflect one or more of three mechanisms employed to maintain cellular function against osmotic stress; they are (1) change in permeability of the body wall and boundary membranes, (2) active uptake and extrusion of selected ions by both renal and extra-renal routes and (3) osmotic and ionic regulation in the tissues themselves. A given species may utilize one or a combination of these. When these mechanisms are employed for either hypo- or hyperosmotic regulation a concentration gradient is maintained between the blood and the medium. Cellular processes which actively maintain the balance between the body fluids and the external environment require energy to carry out their function.

Changes in permeability to water and salt have been confirmed as a means of osmoregulation for brackish water decapod species. Gross (1957a) has shown the permeability of the exoskeleton parallels osmotic regulation in various species which inhabit a wide range of sea water concentrations. Brackish water species with wide regulatory capacities are most impermeable when compared to osmoconformers from a marine environment. For example, a gradation of permeability occurs in which Pachygrapsus crassipes, an estuarine

crab, is more impermeable to salt and water loss than Hemigrapsus nudus. Likewise, H. oregonensis is more permeable than H. nudus but more impermeable than Cancer, a marine dweller. Similar findings have been obtained in the estuarine species, Carcinus, versus the marine Maia (Huf, 1936; Nagel 1934).

Active uptake or extrusion of ions is another widely used mechanism of osmoregulation. Ionic imbalance between the blood and the medium may be maintained by an active uptake of salt across the body surfaces. An active influx of salt has been shown in Carcinus (Nagel, 1934; Shaw, 1961), Ocypode (Flemister, 1959), and Pachygrapsus (Gross, 1957a, 1958). Such a mechanism may account for replenishment of ions lost both by the general body surface and by the urine. Active transport of ions may be found in both isosmotic and hyperosmotic species and may involve either uptake at the surface boundaries and/or reabsorption from the urine. The gill epithelium has been implicated as a principal site for active absorption (Green et al., 1959; Gross, 1957a; Flemister, 1959; Shaw, 1961).

The antennary glands have been shown to have little function in low salinity media but do have an important function in concentrated sea water. Analyses have shown selected secretion of magnesium ions by the kidney in Pachygrapsus (Prosser et al., 1955; Gross, 1959; Gross and Capen, 1966), Carcinus (Robertson, 1959), Uca (Green et al., 1959), and Hemigrapsus (Gross, 1964; Dehnel and

Carefoot, 1965). Limited regulation of sodium and calcium occurs in a concentrated medium. Hypo-osmotic regulation may be further aided by the active absorption of water as suggested for Pachygrapsus by Gross (1957a). Green et al. (1959) have shown water uptake by the stomach in Uca. This phenomenon of drinking sea water has not been widely demonstrated.

In general, urine osmotic concentration in most decapods remains isosmotic to the blood when animals are subjected to low salinities. Carcinus, for example, looses water through increased urine output, but this is not accompanied by a change in urine concentration, i.e. the urine remains isosmotic (Nagel, 1934; Shaw, 1961). However, Dehnel and Stone (1964) have found a seasonal difference in urine production exists for Hemigrapsus. Hyperosmotically regulating Hemigrapsus produce an isosmotic urine in the summer, while winter crabs produce a hypo-osmotic urine. The former case implies an extra-renal mechanism for regulating in dilute sea water.

Tissues themselves must be able to adapt osmotically and ionically or must be capable of tolerating large blood:tissue concentration gradients. For example, tissues of brackish water species can withstand considerable fluctuations in blood osmotic concentration (Potts and Parry, 1964). Upon dilution of the blood, a net uptake of water would be expected in the tissues. Shaw (1955b) has shown in Carcinus an increase in water content of muscle fibers

when the crab is transferred from 100% to 40% sea water. A similar increase in cell volume was found in Pachygrapsus (Gross and Marshall, 1960). Upon transfer to low salinity water Carcinus shows a reduction in amounts of intracellular sodium, potassium, and chloride (Shaw, 1955a, b and 1958a,,b). Larger reductions are found in organic compounds, namely free amino acids, trimethylene oxide, and betaine (Shaw, 1958a, b). Similar findings have been reported by Camein et al (1951) for Astacus; Duchateau and Florkin (1955) for Eriocheir; Kermack et al. (1955) for Homarus; and Robertson (1961) for Nephrops. Regulation of specific amino acids, proline, glycine, and glutamic acid apparently accounts for the major decrease in organic compounds and, thereby, a decrease in intracellular osmotic pressure (Duchateau and Florkin, 1955). Shaw (1958b) has concluded that the amino acids are either transported out of the muscle, combined, or converted into other components.

Gross (1957) has suggested the possible existence of extravascular salt pools which could be reservoirs to prevent ion deficits during salinity fluctuations. In low salinity water, mobilization of ions from the salt pools could permit concentration of the blood. Replenishment of the ions in such pools could occur in a more concentrated environment. The existence of such pools has not been well established; however, Lockwood (1959) has shown in Asellus aquaticus two glands which might serve this purpose.

The osmotic and ionic regulatory patterns for the genus Hemigrapsus have received additional study since the pioneer work by Jones (1941). Subsequent studies have shown hyperosmotic regulation in low salinity water and little or no hypo-osmotic regulation, in general, confirming Jones' work (Gross, 1957 and 1964; Dehnel, 1960 and 1962; Dehnel and Stone, 1964; Dehnel and Carefoot, 1965). Gross (1955, 1957a), however, has obtained evidence indicating hypo-osmotic regulation between 100% and 150% sea water in H. oregonensis, but Dehnel and Stone (1964) more recently have reconfirmed Jones' observations for this species. In addition, Dehnel and Stone (1964) have reported seasonal differences in the magnitude of the blood-to-medium gradient, winter animals maintaining a larger gradient than summer crabs. Apparently, latitudinal variations exist between the populations sampled by Dehnel (British Columbia) and Gross (southern California).

Because Hemigrapsus nudus has been shown to regulate hyperosmotically it was thought that it might be of interest to determine if variation in intracellular osmotic constituents accompanies such regulation in this species. In the present investigation an analysis of intracellular ionic and organic components was made in addition to measurements of osmotic and ionic data for the blood and urine. A series of sea water concentrations from 25% to 150% was used. Data were collected in both winter and summer to

determine if seasonal differences in the regulatory ability occurred as was reported by Dehnel for the British Columbia population of this species.

# MATERIALS AND METHODS

Collections of Hemigrapsus nudus were made during summer and winter at the low tide zone from two semi-protected, rocky beaches on the Oregon coast. Agate Beach, four miles north of Newport (lat. 44° 39.1'N), and Seal Rock State Park, eleven miles south of Newport, were chosen as collection sites because of their proximity to the laboratory and similarities in habitat.

Only male, intermolt (stage C) crabs were obtained in order to reduce sex and molting as factors in the experiments. Intermolt (stage C) animals were judged by the hardness of the carapace (Drach, 1939; Kincaid and Scheer, 1952). Animals were transported in plastic buckets to the Oregon State University Marine Science Center at Newport, Oregon. Upon return to the Marine Science Center summer-collected crabs were kept in fiberglass holding tanks with a continuous-flow sea water system. The system draws water from a salt "wedge" in the Yaquina Bay estuary and the water characteristics are approximately those of the open coast during summer months. Summer laboratory salinities ranged from 25-32‰ and temperatures ranged from 12-18°C. Hydrographic data from the Oregon coast off Newport for July and August, 1960, show a salinity range from 30.82 to 33.26‰ and a temperature range from 11.2 to 14.4°C. (Wyatt and Kujala, 1961).

Winter hydrographic measurements for the Oregon coast off Newport from December, 1960, through March, 1961 (Wyatt and Kujala, 1961), show a salinity and temperature range from 31.4 to 31.5‰ and 10.2 to 10.8°C respectively. The influence of fresh water runoff in the estuary caused laboratory salinity to range as low as 5‰; thus, the laboratory salinity was too far removed from open coast conditions to be used for acclimation. Instead, winter crabs were held in plastic trays containing 75% sea water (approximately 26‰) and the trays were immersed in holding tanks where water temperatures ranged from 5 to 7°C.

Both summer and winter animals were acclimated to their respective laboratory temperature and salinity conditions for one week before being placed under experimental conditions.

Experimental studies were performed at laboratory sea water seasonal temperatures (summer, 12-18°C; winter, 5-7°C). All experimental salinities were expressed as percentage sea water based on a standard sea water (100% sea water) of 32‰ salinity (19 mg/ml chlorinity). Twenty-five, 50, 75, 100 and 150% sea water concentrations were used. Dilutions of 25, 50, and 75% were prepared by adding distilled water. The 150% sea water was made by diluting a 200% artificial sea water solution with laboratory sea water. Artificial sea water was made by the ratios given in Nicol (1958)

as follows:

| | |
|---|---|
| NaCl | 24.087 g |
| KCl | 0.681 g |
| $CaCl_2$ | 1.131 g |
| $MgCl_2$ | 5.111 g |
| $Na_2SO_4$ | 4.019 g |
| $NaHCO_3$ | 0.197 g |
| $H_2O$ | to 1000 ml |

Quantities of each salinity were mixed in a twenty gallon plastic garbage can and the concentration determined by use of a RS5-3 portable salinometer (Industrial Instruments, Inc.).

After one week of acclimation animals were placed in one-half and one pint plastic freezer containers with the desired experimental salinity. Each container held one crab immersed in aerated sea water. Five to ten animals were run at each sea water concentration for each time unit of sampling in the experiment.

Blood and urine were sampled at twenty-four and forty- eight hours. In the winter experiments a muscle sample from the chelipeds was also obtained. Blood and urine samples were analyzed for sodium, potassium, calcium, magnesium, and chloride. Muscle was analyzed for the above ions and for alpha-amino nitrogen.

Before blood extraction each crab was blotted with paper toweling to prevent contamination from excess water. Samples of

blood were drawn from the membrane at the base of the coxopodite of the first walking leg. The membrane was punctured with a "Dispo" pipet and, when necessary, suction was applied through a mouth tube connected to the pipet. The blood was transferred to a test tube and immediately frozen for future analysis.

A "serum" suitable for ion analysis was obtained from the blood by the following technique. Frozen blood was macerated with a glass rod to break the clot. After complete thawing each sample was filtered through a 1.2 micron membrane filter placed in the bottom of a 2.5 ml glass syringe. A 25 microliter sample of the serum was diluted 1:400 and used for all ion analyses. Separate samples were obtained for determination of osmotic pressure.

Urine was sampled from crabs previously blotted dry and placed on the stage of a binocular dissecting microscope to facilitate the operation. Urine was withdrawn from the nephridiopore with a "Dispo" pipet drawn to a fine tip and connected to a mouth tube. Manipulation of the pipet beneath the operculum at the entrance of the nephridiopore caused the release of urine from the bladder. The microscope aided in distinguishing clear urine from the more opaque, bluish blood. From the 'Dispo" pipet urine was delivered into micro-test tubes and frozen for future analyses.

A 10 microliter sample of the urine was diluted 1:1000 with deionized water and analyzed for sodium, potassium, calcium,

magnesium, and chloride. Separate samples were used for the determination of osmotic pressure.

Muscle samples were obtained from the chelipeds. Both chelae were removed and immediately placed on a glass tray submerged in an ice bath. After removal of the chitinous exoskeleton and pigment layer the muscle was teased from the underlying cartilage. Each sample was lightly blotted and weighed on a Sauter milligram balance to 0.1 milligrams. After drying at 60°C for at least 48 hours a dry weight was recorded. From wet and dry weights the muscle water content was calculated.

The dried muscle was ground to a fine powder and approximately 20 milligrams of the powder was used for determination of alpha-amino nitrogen. The remainder of the powdered muscle was digested for cation analysis.

Digestion of the muscle samples was carried out in 2 ml of 30% hydrogen peroxide (superoxal) for four hours, filtered, and diluted to 10 ml with distilled water. A one ml aliquot was analyzed for tissue chloride by the method of Schales and Schales (1941) using a Beckman Spinco microtitrator. Another aliquot of the diluted digest was further diluted 1:50 for ion analysis. These ions were measured in a similar manner to those for the blood and urine. Intracellular ion concentrations were expressed in mEq/kg muscle water.

Free intracellular amino acids of the muscle were measured using the quantitative ninhydrin positive method as described by Rosen (1957). To eliminate any reaction with the terminal groups on proteins precipitation with TCA preceded color development with ninhydrin. Samples were read against a D-L leucine standard at 525 mμ on a Bausch and Lomb Spectronic 20. The results were expressed in mM/kg muscle water.

A Coleman Flame Photometer was used for analysis of sodium, potassium, and calcium ions. Magnesium ion concentrations were determined with a Perkin-Elmer Atomic Adsorption Spectrophotometer, model 303, at 285 mμ. Blood and urine chloride analysis was made with a Buchler-Cotlove chloridometer. Results for blood and urine ion analyses were expressed in mEq/l.

Osmotic pressure determinations were made from undiluted blood and urine samples and measured with a Mechrolab Vapor Pressure Osmometer. Milliosmolar concentrations of sodium chloride were utilized as standards. The technique was adapted for microliter quantities and results were expressed as milliosmoles per liter.

# RESULTS

## Osmotic Regulation

Seasonal comparisons were made of the osmotic responses of Hemigrapsus nudus exposed for 24 and 48 hours to a range of experimental salinities. Results for winter animals were determined at 5-7°C and those for summer at 12-18°C. These temperatures approximate seasonal field conditions.

Prior acclimation to 75% sea water for a period of one week was used for all animals before transfer to the experimental salinity stress. It was assumed that a steady state with respect to this acclimation medium was achieved in this time. A 75% sea water acclimation salinity was utilized for the following reasons: (1) the ease of obtaining this concentration of sea water during most of the year, (2) this concentration represented a medium close to the crabs' ambient conditions for the major part of the year and (3) it is a concentration in which they could survive indefinitely. The blood and urine concentrations at time zero in Figs. 3 and 4 represent a mean of ten animals from the 75% sea water control medium prior to experimental stress. Figures 3 and 4 are plotted to show the time course of acclimation to the salinity stresses imposed, while Figures 1 and 2 represent only osmotic responses after 48 hours in the experimental media.

Major changes were assumed to have occurred within the 48 hour period following application of the experimental salinity. However, it cannot be said for certain that a final steady state had been achieved, For example, Gross (1963) has demonstrated hypo-osmotic regulation in Hemigrapsus oregonensis after prolonged acclimation, both in the laboratory and in the field. Thus, an "acclimation state" may be achieved after prolonged exposure to either a dilute or a concentrated salinity medium.

In general, blood and urine osmotic concentrations for both winter and summer are maintained hyperosmotic to the medium over the range of experimental salinities, with greater hyperosmotic regulation in the lower salinities. The blood osmotic concentrations for both winter and summer animals (Figs. 1 and 2) are regulated similarly over the salinity range. In 150% sea water summer H. nudus (Fig. 1) maintains a greater blood:medium gradient than winter crabs (Fig. 2). In the dilute salinities, the blood:medium gradient for the 50% sea water stress in the winter is slightly greater than for the summer.

There is a difference in the urine concentration between summer and winter seasons for H. nudus. Summer urine (Fig. 1) was slightly hyperosmotic to the blood in 25% sea water (not statistically significant), whereas the urine of winter animals was hypo-osmotic to the blood in 25% and 50% sea water ($P<.05$). The urine osmotic

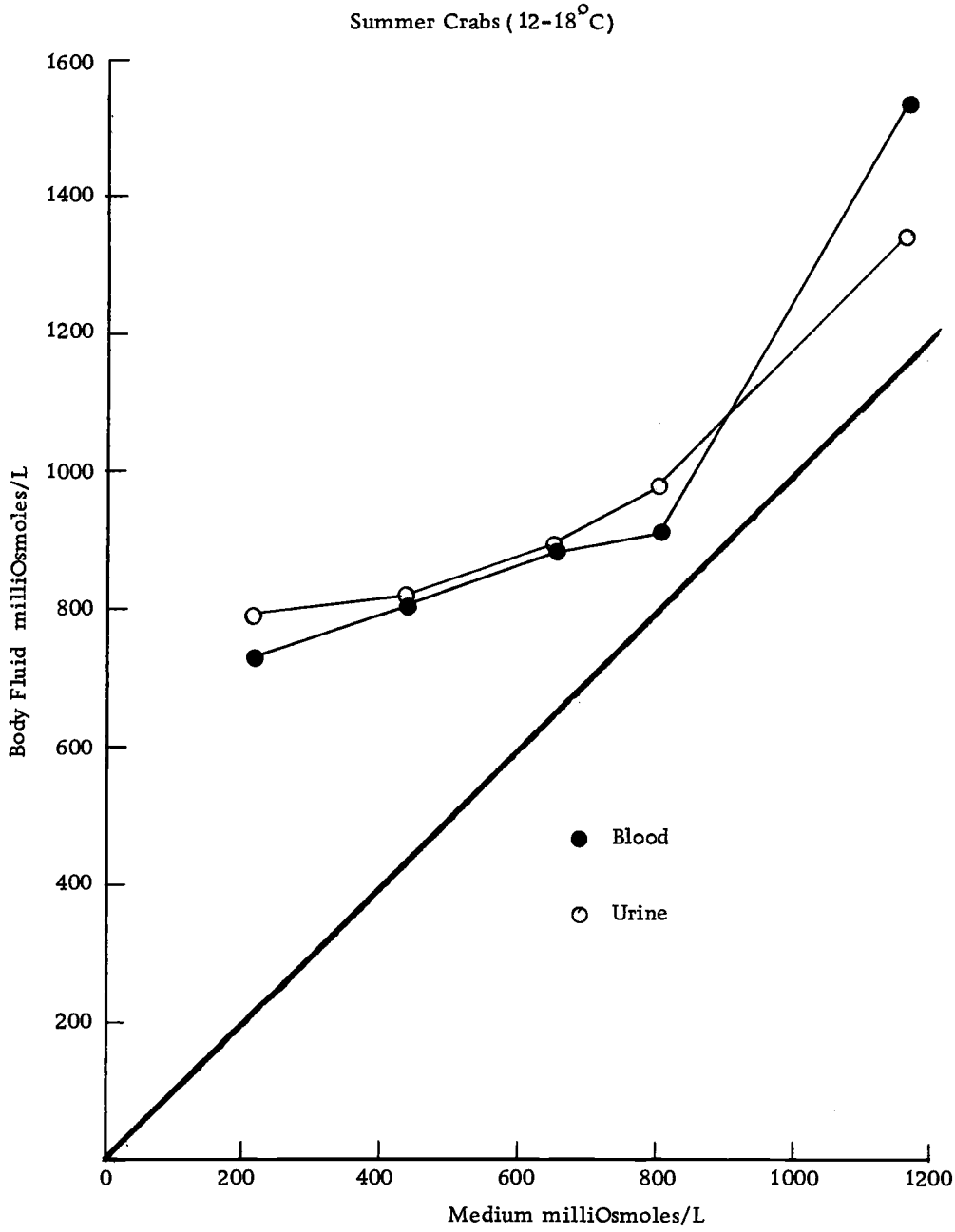



Figure 1. Summer osmotic concentrations in blood and urine after 48 hours exposure to the experimental salinity. Each point represents the mean value of approximately ten animals, and is expressed in milliosmoles per liter.

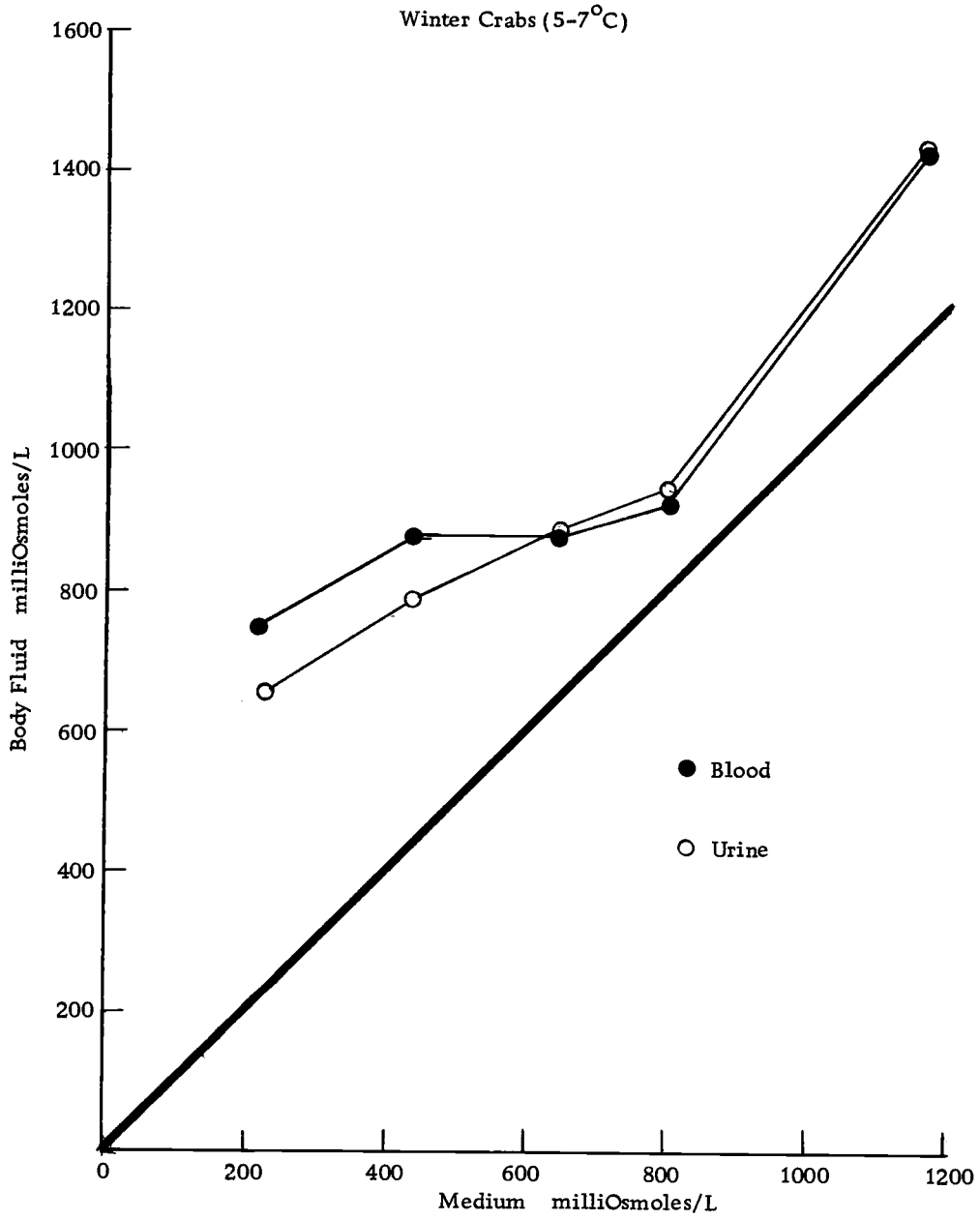



Figure 2. Winter osmotic concentrations in the blood and urine after 48 hours exposure to the experimental salinity. Each point represents the mean value of approximately ten animals and is expressed in milliosmoles per liter.

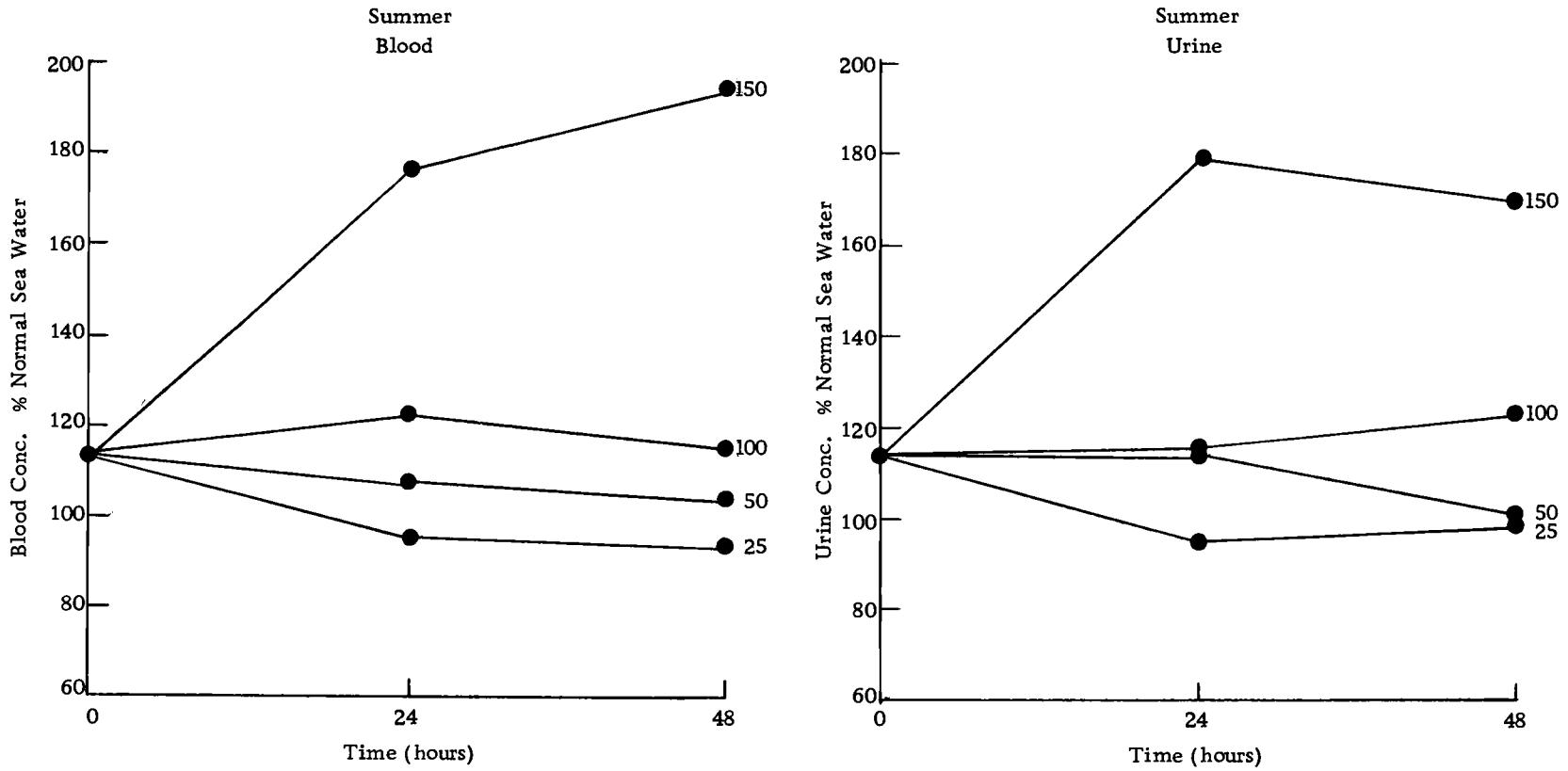



Figure 3. Summer osmotic concentrations in the blood and urine as a function of time of exposure to the experimental salinity. Values represent the blood or urine concentration as a percentage of normal sea water (800 mOsmoles).

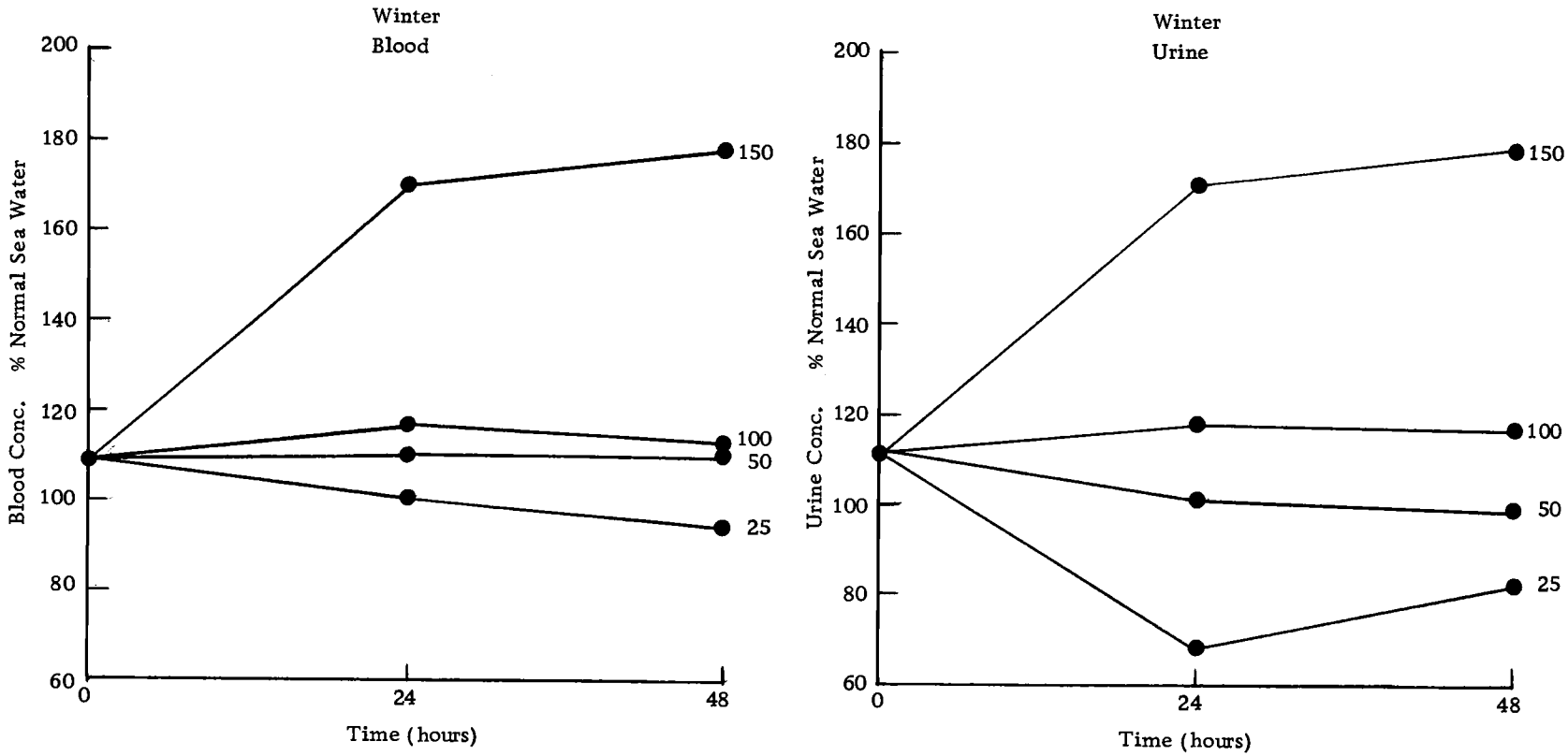



Figure 4. Winter osmotic concentrations in the blood and urine as a function of time of exposure to the experimental salinity. Values represent the blood or urine concentration as a percentage of normal sea water (800 mOsmoles).

concentrations were maintained blood isosmotic in the winter in 100% and 150% sea water, while the summer H. nudus maintained a slightly hypo-osmotic urine in 150% sea water (Fig. 1). The summer animals present a curious picture for the urine osmotic regulation in 150% sea water. After 24 hours the urine was blood isosmotic. This has been the result observed by other investigators (Gross, 1964; Dehnel, 1962; and Dehnel and Stone, 1964) after this length of immersion. However, at the end of 48 hours the urine was hypo-osmotic to the blood. As previously stated, the values at 48 hours are assumed to represent a more nearly true steady state with respect to the medium. Many authors have assumed that the achievement of a steady state has occurred after 24 hours. The time course adjustment will provide an interesting subject for further investigation.

## Ionic Regulation - Seasonal Responses

The results of blood and urine measurements of sodium, potassium, chloride, calcium and magnesium ions in the winter and summer animals are shown in Figures 5 through 9. Unfortunately, no measurements were made at the 25% sea water stress in the summer. The absence of data does not allow meaningful comparisons to the winter data for this medium.

## Sodium

Both winter and summer crabs regulate their blood and urine sodium ion concentrations well. Winter H. nudus appear to be somewhat better able to regulate blood sodium in dilute media than summer crabs (Fig. 5). Urine sodium was found to be blood hypoionic in all situations except in winter animals in 50% sea water and summer animals in 75% sea water, where it was very slightly blood hyperionic. In 150% sea water the summer urine sodium was more markedly hypoionic to the blood than in winter crabs. The urine sodium was significantly less than blood sodium ($P < .01$) in 100% and 150% sea water.

## Chloride

The pattern of blood and urine chloride regulation in summer and winter crabs (Fig. 6) is roughly similar to that of sodium regulation (Fig. 5) with the exception of the 150% sea water stress. In this salinity the summer blood chloride is slightly hypoionic to the medium in contrast to sodium which is hyperionic. In addition, the winter blood chloride in the higher salinities (100% and 150% sea water) is more nearly isoionic to the medium than is the blood sodium which is slightly hyperionic.

In contrast to urine sodium, the urine chloride is blood

isoionic in the winter at 100% and 150% sea water and blood hyperionic in the summer at the same stresses.

## Potassium

Blood potassium is poorly regulated in both the winter and summer animals. In summer animals blood potassium is hypoionic to the medium except in 50% and 75% sea water where it is nearly isoionic. Winter potassium values differ in the 100% and 150% sea water in that the blood is roughly isoionic with respect to the medium. Thus, some hypoionic regulation is shown in the higher salinities for summer crabs.

The urine potassium is hyperionic to the blood in the summer over the entire range tested. In the winter, however, the urine is hyperionic to the blood in the dilute salinities (25% to 75% sea water) and hypoionic to the blood in 100% and 150% sea water.

## Magnesium

Blood magnesium for both summer and winter *H. nudus* is regulated hypoionic to the medium over the entire range, agreeing with the previous findings of Dehnel and Carefoot (1965). Urine magnesium is hyperionic to the blood over the salinity range tested ($P < .01$ at all stress levels). Summer animals have a much greater output of magnesium in the urine than winter crabs (Fig. 8).

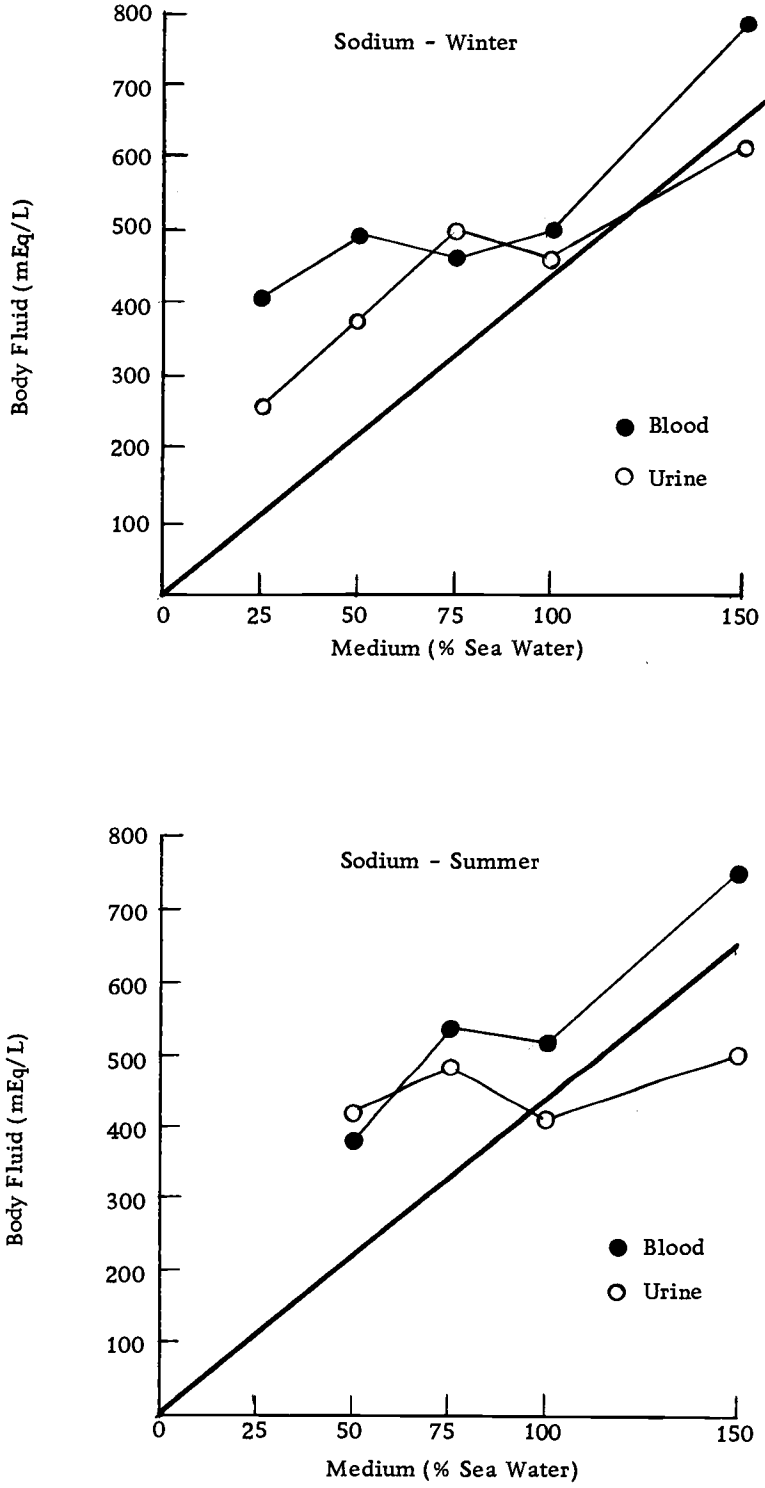



Figure 5. Sodium ion concentrations in the blood and urine of winter (5-7°C) and summer (12-18°C) Hemigrapsus nudus as a function of salinity. Each point represents the mean value of ten measurements after exposure for 48 hours to each experimental salinity.

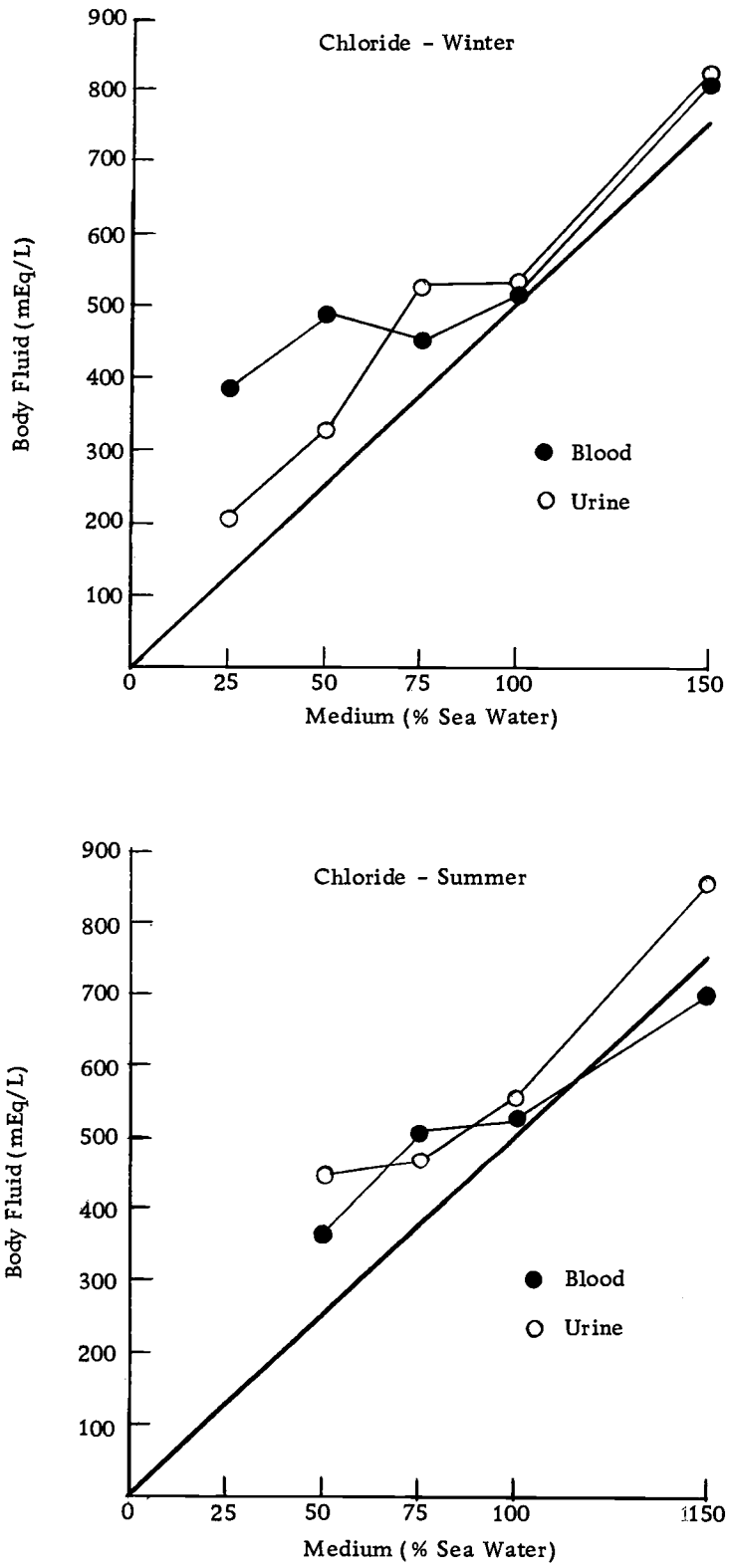



Figure 6. Chloride ion concentrations in the blood and urine of winter (5-7°C) and summer (12-18°C) Hemigrapsus nudus as a function of salinity. Each point represents the mean value of ten measurements after exposure for 48 hours to each experimental salinity.

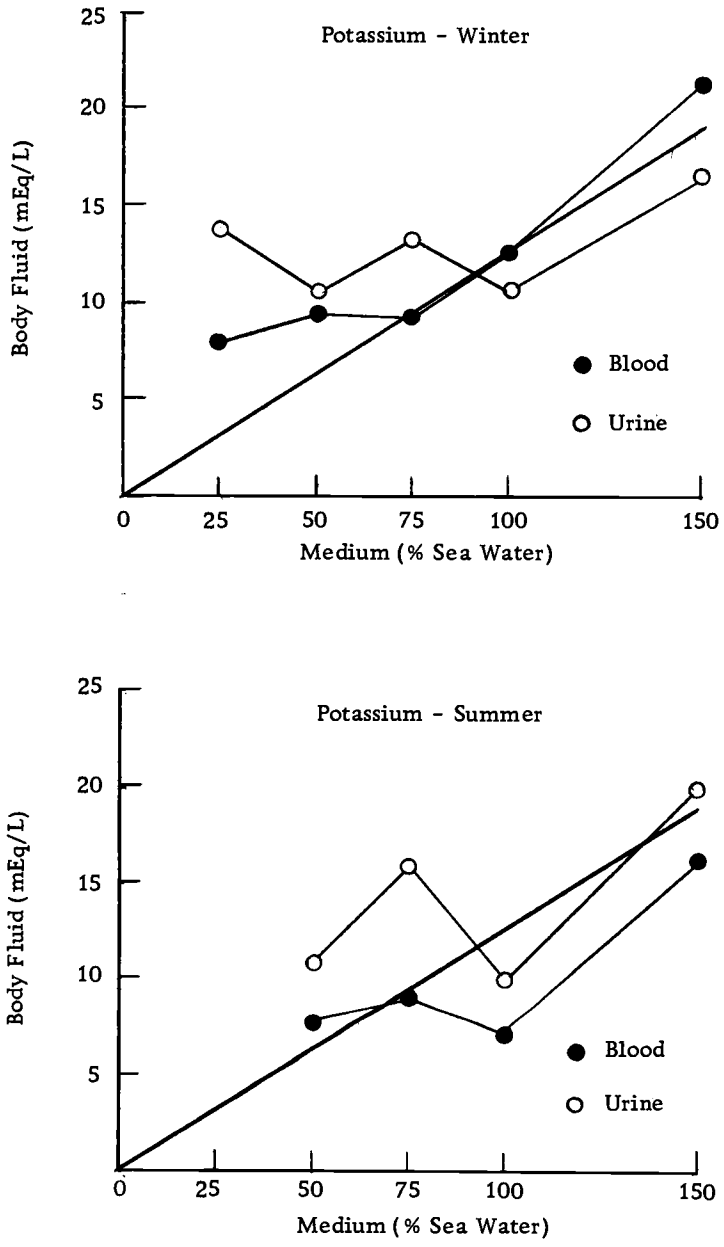
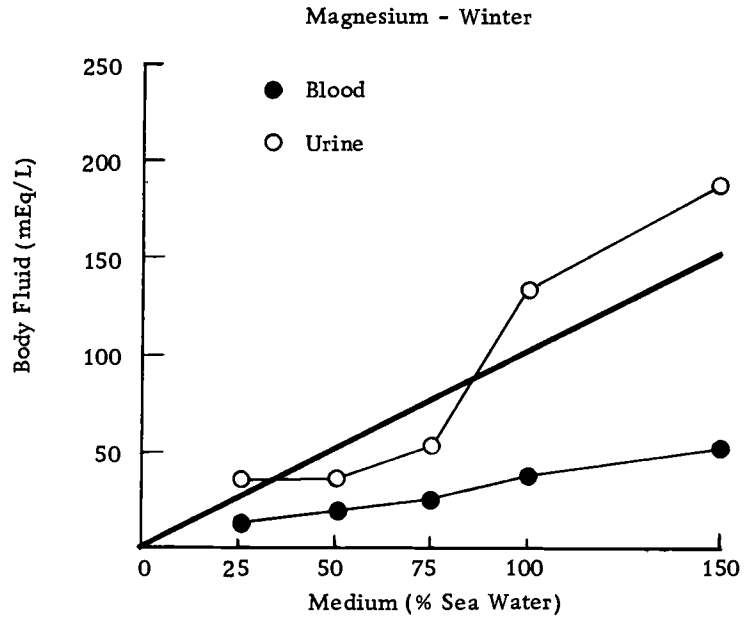



Figure 7. Potassium ion concentrations in the blood and urine of winter (5-7°C) and summer (12-18°C) Hemigrapsus nudus as a function of salinity. Each point represents the mean value of ten measurements after exposure for 48 hours to each experimental salinity.



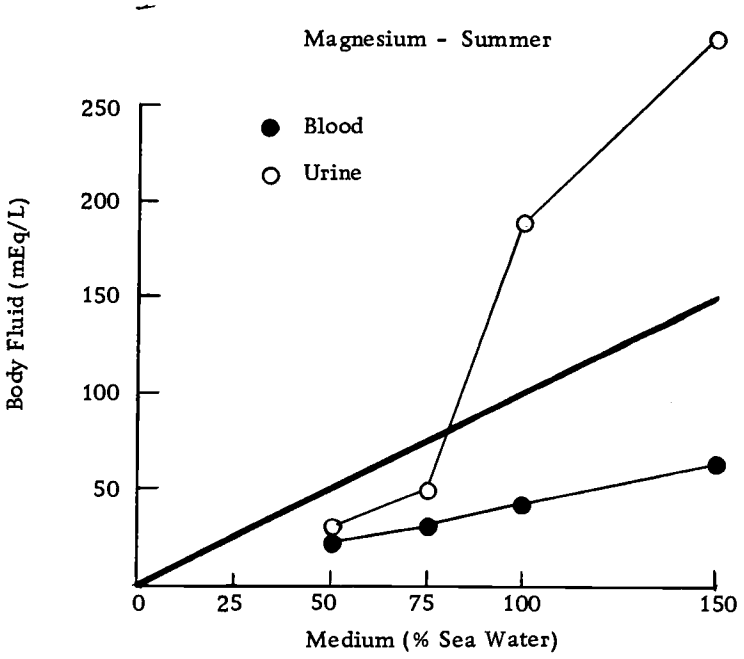




Figure 8. Magnesium ion concentrations in the blood and urine of winter (5-7°C) and summer (12-18°C) Hemigrapsus nudus as a function of salinity. Each point represents the mean value of ten measurements after exposure for 48 hours to each experimental salinity.

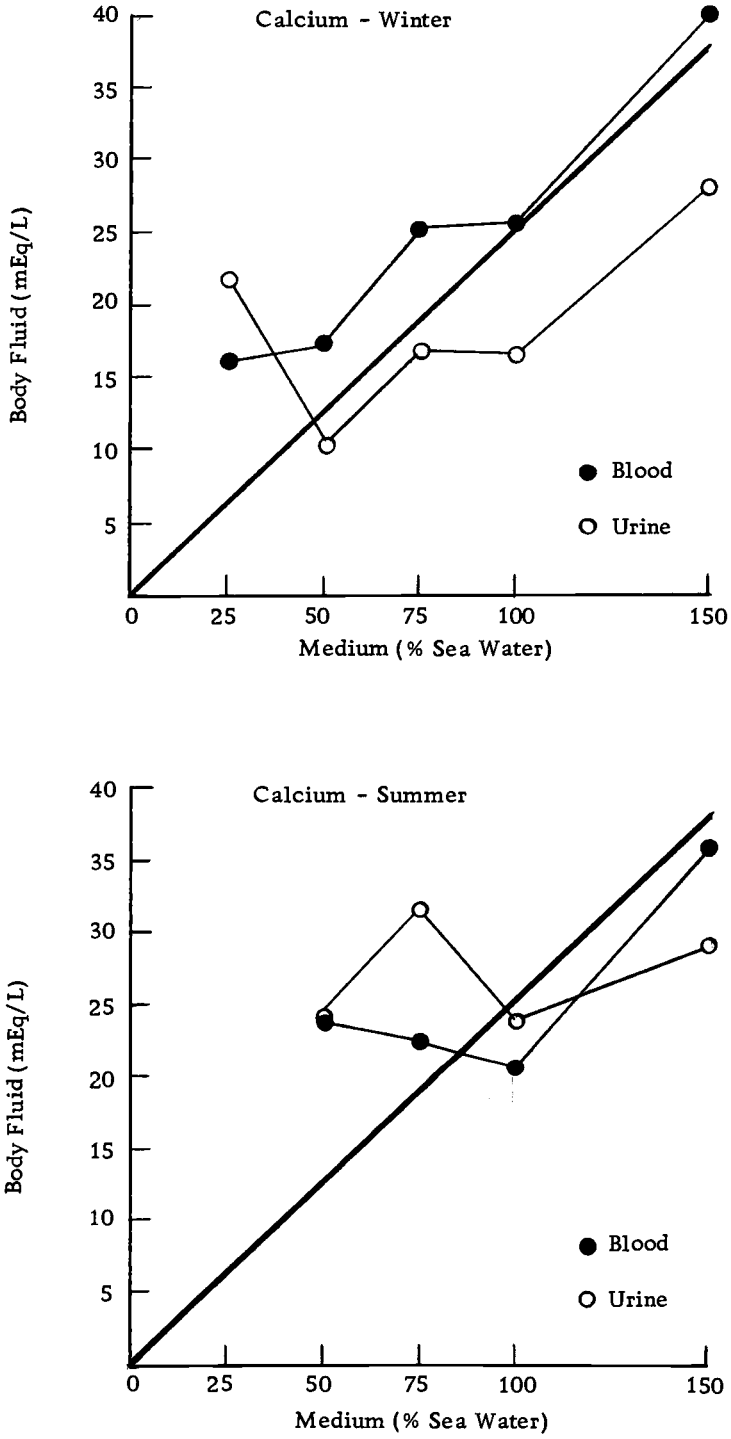



Figure 9. Calcium ion concentrations in the blood and urine of winter (5-7°C) and summer (12-18°C) Hemigrapsus nudus as a function of salinity. Each point represents the mean value of ten measurements after exposure for 48 hours to each experimental salinity.

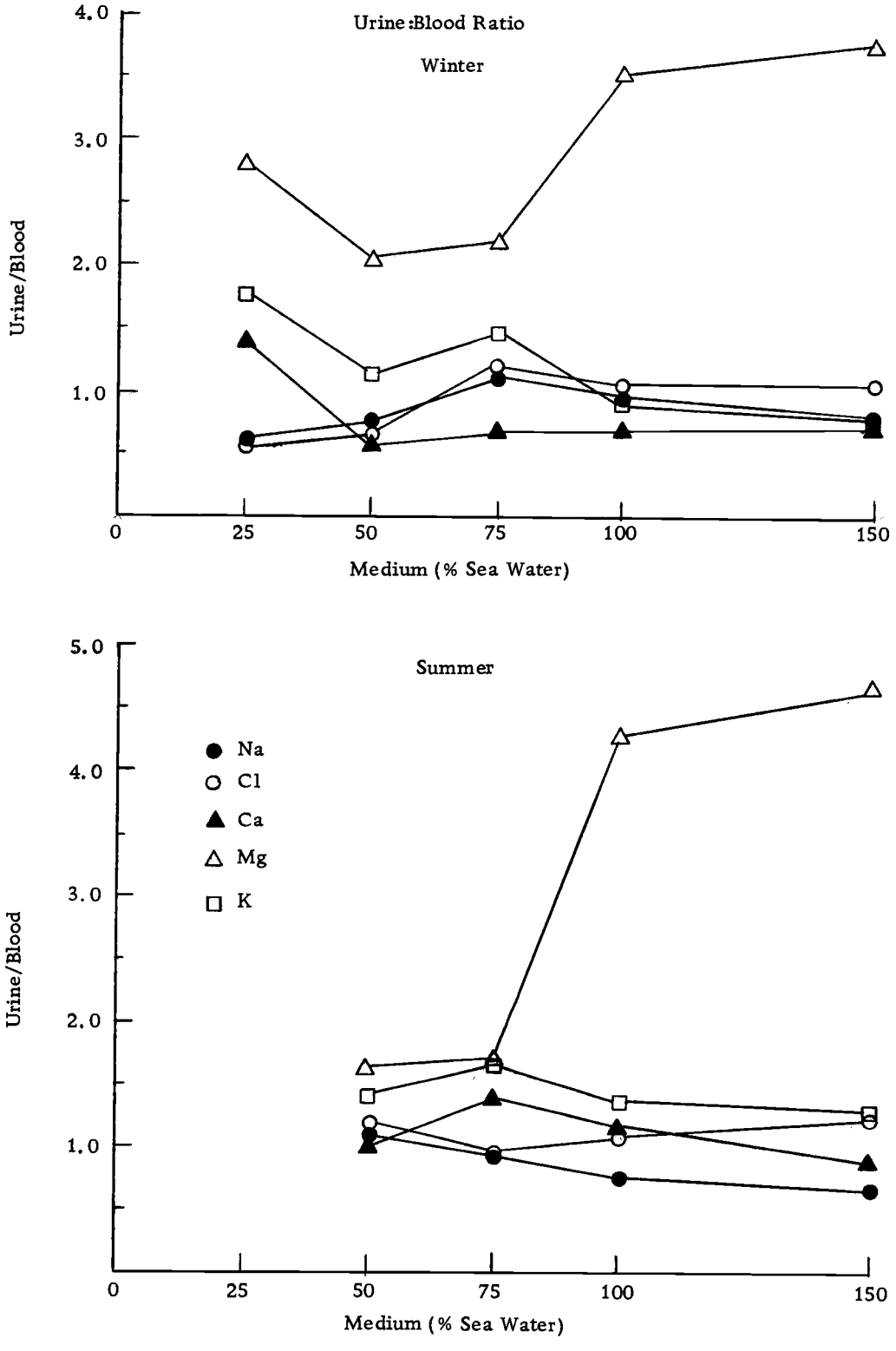



Figure 10. Urine:blood ion ratios of winter and summer Hemigrapsus nudus over a range of salinities. Each ratio represents the ratio of the ion concentration after 48 hours to each experimental salinity.

## Calcium

In general, the regulation of calcium is poor over the range tested. Winter H. nudus maintain their blood nearly isoionic over the salinity range except in 25% sea water. Summer animals appear to regulate blood calcium better in the lower salinities; but in the absence of data at 25% sea water, comparison with winter animals is less meaningful.

In the winter, urine calcium was found to be hypoionic to the blood from 50% to 150% sea water. Strangely, urine calcium for summer crabs was much greater in concentration at all salinities except 150% sea water in comparison with winter crabs. It might be that these values reflect a difference in the molt condition of the crabs. However, inaccuracies in the method of analysis employed (flame photometry) lessen confidence in all calcium measurements.

## Muscle Ion Analysis

The measurements of muscle ions and total alpha-amino nitrogen were performed, unfortunately, only on winter animals. Since muscle ion concentrations were expressed in terms of muscle water content they are not strictly comparable to the blood ions which are expressed in milliequivalents per liter of solution.

In general, the ion concentrations in the muscle appear to

fall off in dilute sea water and rise to levels greater than the control levels (75% sea water) in the more concentrated media (Table 1). There appears to be little regulation of muscle sodium and chloride in Hemigrapsus nudus; only in the case of animals stressed to 50% sea water does there appear to be regulation of these two ions. At this salinity the values approach the controls (75% sea water) after 48 hours.

The magnesium concentrations of the muscle in both 100% and 150% sea water media rise during the first 24 hours but fall in the second 24 hour period to a level which approaches the control level. In the light of the urine and blood magnesium regulation pattern, this condition is most interesting. Urine magnesium approaches two to three times the concentration in the blood in 100% and 150% sea water (Fig. 8).

At all salinities the muscle calcium values show a dramatic and unexplained fall after 48 hours. This fall is below the control level for all samples which were measured.

A decrease in the muscle potassium is found with a reduction in the salinity of the medium (Table 1), but proportionately not to the same degree as blood potassium. This is reflected in the blood:tissue ratios (Table 2). Therefore, some regulation of muscle potassium is apparent in dilute salinities and might involve a change in permeability.

Although the standard errors are large, the values for muscle alpha-amino nitrogen do not appear to differ greatly from the 75% sea water controls at any of the salinity stresses.

Table 1. Chela muscle ion concentrations.

| Medium (% sea water) | Time of exposure | Ions (mEq/kg muscle water) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Na | Cl | K | Ca | Mg | Amino Nitrogen |
| 25 | 24 | 139 ± 16 | 113 ± 21 | 147 ± 4 | 103 ± 16 | 46 ± 3 | 211 ± 14 |
| | 48 | 116 ± 5 | 97 ± 7 | 146 ± 2 | 79 ± 6 | 43 ± 0.6 | 273 ± 45 |
| 50 | 24 | 128 ± 4 | 92 ± 6 | 133 ± 0.9 | 139 ± 16 | 51 ± 3 | 320 ± 33 |
| | 48 | 183 ± 22 | 138 ± 16 | 127 ± 10 | 88 ± 19 | 49 ± 5 | 256 ± 24 |
| 75 | Control | 177 ± 8 | 149 ± 6 | 132 ± 8 | 164 ± 24 | 61 ± 5 | 239 ± 25 |
| | | | | | | | 255 ± 29 |
| 100 | 24 | 178 ± 5 | 114 ± 12 | 157 ± 14 | 173 ± 44 | 59 ± 5 | 194 ± 9 |
| | 48 | 257 ± 19 | 170 ± 16 | 138 ± 6 | 150 ± 32 | 66 ± 4 | 222 ± 19 |
| 150 | 24 | 337 ± 16 | 229 ± 40 | 162 ± 3 | 180 ± 32 | 78 ± 5 | 204 ± 35 |
| | 48 | 367 ± 10 | 322 ± 11 | 217 ± 15 | 78 ± 2 | 64 ± 3 | 278 ± 20 |

Ion concentrations and total alpha-amino nitrogen measurements of the chela muscle in Hemigrapsus nudus for the winter. Acclimation for one week at 75% S. W. was used as a control. Mean values (± S. E.) are expressed in terms of mEq/kg muscle water, and are given for 24 and 48 hours exposure to each of the experimental salinities.

Table 2. Blood:tissue ion ratios

| Medium (% Sea Water) | Blood Ions/Muscle Ions | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Na | | Cl | | K | | Ca | | Mg | |
| | conc | ratio | conc | ratio | conc | ratio | conc | ratio | conc | ratio |
| 25 | 418/116 | 3.60 | 386/98 | 3.94 | 8/146 | .055 | 16/79 | .203 | 13.1/43 | .304 |
| 50 | 493/183 | 2.69 | 486/138 | 3.52 | 9.4/127 | .074 | 17.4/88 | .198 | 17.6/49 | .36 |
| 75 | 465/177 | 2.62 | 452/149 | 3.04 | 9.2/132 | .069 | 25.4/164 | .154 | 25.5/61 | .42 |
| 100 | 507/257 | 1.97 | 514/170 | 3.02 | 12.6/138 | .092 | 25.9/150 | .173 | 38/66 | .575 |
| 150 | 784/367 | 2.13 | 808/322 | 2.51 | 21.2/217 | .098 | 39.8/78 | .51 | 51.4/64 | .804 |

Relationship of the blood:tissue ion ratios to a range of salinities. Each value represents the ratio of the ion concentration after 48 hours to each experimental salinity. Blood ions are expressed as mEq/l; muscle ions as mEq/kg muscle water.

Table 3. Sum of blood ions:sum of muscle ions.

| Medium (% Sea Water) | Time of Exposure | Sum of Blood Ions (mEq/l) | Sum of Muscle Ions (mEq/kg muscle water) | Blood:Muscle Ratio |
|---|---|---|---|---|
| 25 | 24 | 888 | 759 | 1.17 |
| | 48 | 841 | 754 | 1.12 |
| 50 | 24 | 943 | 863 | 1.09 |
| | 48 | 1023 | 841 | 1.22 |
| 75 | control | 977 | 938 | 1.04 |
| 100 | 24 | 1018 | 875 | 1.16 |
| | 48 | 1098 | 1003 | 1.09 |
| 150 | 24 | 1499 | 1190 | 1.26 |
| | 48 | 1704 | 1326 | 1.29 |

Relationship of the total blood:total tissue ion ratios after 24 and 48 hours to a range of salinities. The total blood ions represents the sum of all the ions measured while the total tissue ions represent the sum of all the muscle ions and the total alpha-amino nitrogen.

Table 4. Comparison of the sums of muscle ions and blood ions to blood osmotic concentration as a function of salinity.

| Medium (% Sea Water) | Time of Exposure | Muscle Ion / Blood m0s Conc | Muscle Ion / Blood m0s Ratio | Blood Ion / Blood m0s Conc | Blood Ion / Blood m0s Ratio |
|---|---|---|---|---|---|
| 25 | 24 | 759/810 | .935 | 888/810 | 1.09 |
| | 48 | 754/754 | 1.00 | 841/754 | 1.12 |
| 50 | 24 | 863/885 | .975 | 943/885 | 1.07 |
| | 48 | 841/880 | .955 | 1023/880 | 1.17 |
| 75 | Control | 938/872 | 1.07 | 977/872 | 1.12 |
| 100 | 24 | 875/939 | .934 | 1017/939 | 1.08 |
| | 48 | 1003/908 | 1.105 | 1097/908 | 1.21 |
| 150 | 24 | 1190/1361 | .874 | 1498/1361 | 1.10 |
| | 48 | 1326/1426 | .932 | 1704/1426 | 1.19 |

Muscle ions are in mEq/kg muscle water, blood ions in mEq/l, and blood osmotic concentration in milliOsmoles.

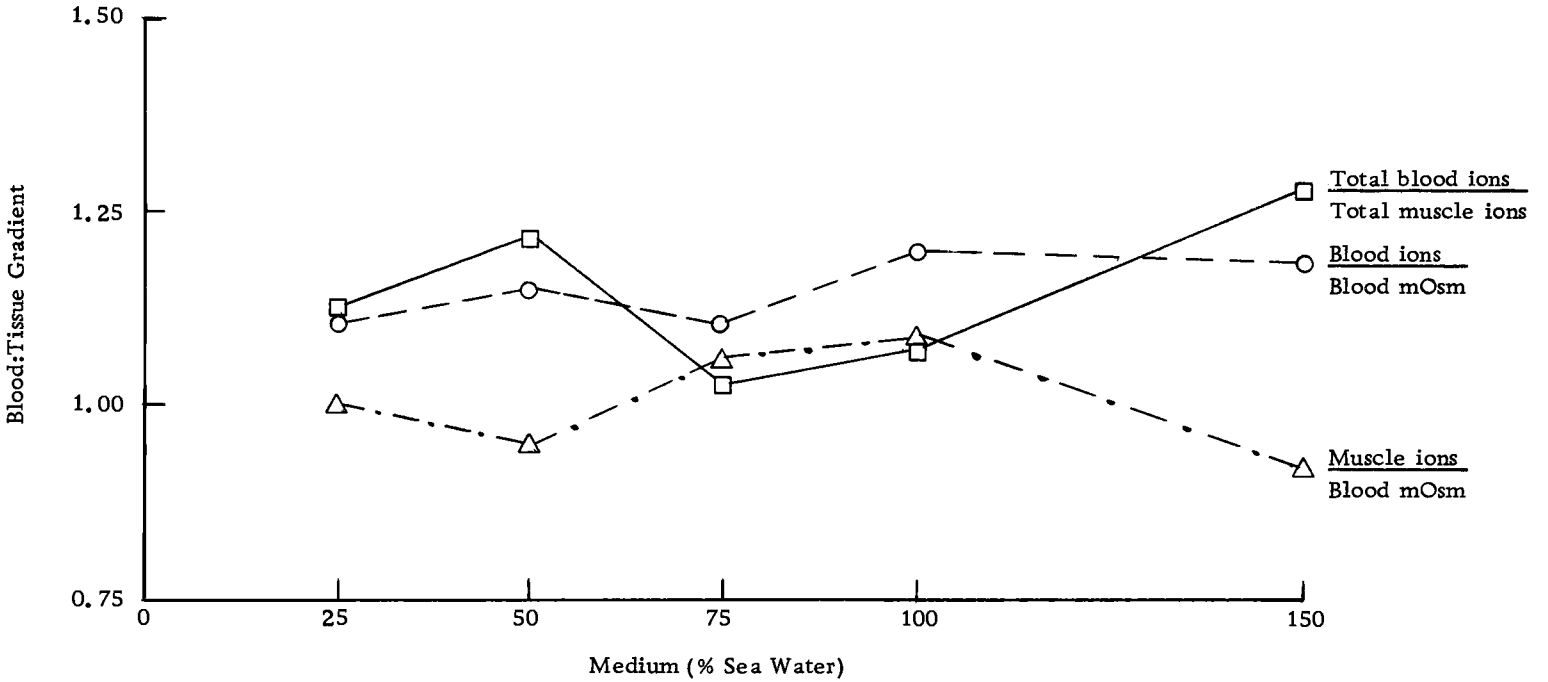



Figure 11. The blood:tissue gradient as a function of the salinity of the external medium. Graphical representation of the relationships presented in Tables 2, 3, and 4; namely total blood ions:total tissue ions, blood ions:blood osmotic concentration (mOsm), and muscle ions:blood osmotic concentration (mOsm).

# DISCUSSION

The responses of Hemigrapsus nudus to salinity stress may be characterized as follows: in dilute sea water, the osmotic and ionic concentrations in both blood and urine are maintained at levels considerably greater than the medium. Thus, the ability of this species to regulate hyperosmotically is demonstrated. However, blood and urine concentrations are approximately equal to the medium when the animals are stressed to the higher salinities. Although blood hypo-osmotic regulation is not shown, some blood hypoionic regulation of potassium, and considerable hypoionic regulation of magnesium, are evident. In muscle tissue, the ion concentrations differ from their concentrations in the blood. When the ion concentrations are lowered in the blood, decreases are also shown in the tissues. Muscle tissue appeared to lack regulating ability and apparently tolerates fluctuations in intracellular osmotic concentrations. The ability to tolerate changes in the tissue ion composition is important in an environment characterized by fluctuations in salinity, such as that to which Hemigrapsus is subjected in brackish water.

## Hyperosmotic Regulation

In dilute sea water Hemigrapsus nudus has been shown

capable of maintaining its blood hyperosmotic to dilute media (Jones, 1941; Dehnel, 1962; Dehnel and Stone, 1964; and present study). Such hyperosmotic regulation would be of adaptive significance during exposure to brackish water.

A seasonal difference in the hyperosmotic regulatory mechanisms is evident from a comparison of winter and summer stressed crabs. Dehnel (1962) and Dehnel and Stone (1964) have shown in a seasonal comparison that H. nudus regulates by producing a hypo-osmotic urine in the winter, while summer crabs produce an is-osmotic urine. Similar results were obtained in the present study. Here, in dilute salinities, 25% to 75% sea water, both winter and summer blood osmotic concentrations were hyperosmotic to the medium. However, the urine osmotic concentrations in the summer are roughly isosmotic to the blood, while in the winter the production of a hypo-osmotic urine was shown.

A seasonal comparison for sodium and chloride shows similar patterns of regulation. In dilute salinities, Dehnel and Carefoot (1965) have reported winter urine sodium and chloride to be regulated hyperionically to winter blood. In contrast to Dehnel and Carefoot's study, the present work showed urine sodium and chloride to be hypoionic to winter blood. Thus, the present results indicate regulation occurs through some reabsorption of sodium and chloride from the urine in the winter-stressed crabs. Dehnel (1966)

explains the seasonal difference in his study in terms of changes in protein content of the blood seasonally as well as with salinity. Summer results of the present study, on the other hand, are similar to the reported values of Dehnel and indicate an isosmotic urine.

Renal mechanisms for hyperosmotic regulation apparently differ seasonally. In the winter, reabsorption of sodium and chloride from the urine is evident, as well as maintenance of a hypo-osmotic urine. Summer animals, however, do not show reabsorption of sodium and chloride, and this is reflected in the production of an isosmotic urine. Thus, an extra-renal influx mechanism for absorption of ions in dilute media in the summer may be present.

Extra-vascular salt pools, as postulated by Gross (1958), may significantly contribute toward the maintenance of hyperionic ion levels in dilute sea water. Such reservoirs could supply the ions necessary under dilute salinities and be replenished in more concentrated media. Further evidence for the validity of the existence of the salt pools has been reported by Lockwood (1959) in Asellus aquaticus. Lockwood found concentrations of sodium to be stored in Zenker's organs in this insect.

## Hypo-osmotic Regulation

The maintenance of blood hypo-osmotic to the external medium has been shown in decapod crustacea which tend to be

amphibious or to be found in hypersaline pools (Jones, 1941; Gross, 1961). Thus, hypo-osmotic regulation might be of adaptive significance by preventing concentration of the fluids in the branchial chamber by evaporation and thereby preventing the blood from reaching a harmful osmotic pressure. Gross (1955) has shown the branchial fluid chamber in Pachygrapsus to contain too small a volume to sufficiently effect a change in blood osmotic concentration. However, under conditions of desiccation, Gross (1955) has shown salt exchange takes place in the branchial chamber; perhaps there is some control over water entering the gill chamber which may aid regulation (Gross, 1957a). Gross feels the latter idea is pertinent only for hyperosmotic regulation.

Gross (1961) has found a population of H. oregonensis in a hypersaline lagoon to effectively regulate hypo-osmotically. With further experimentation, Gross (1963) was able to demonstrate hypo-osmotic regulation under both laboratory step-wise acclimation and after three days in concentrated sea water (160% sea water) without any prior acclimation. He cautioned that the hypo-osmotic regulatory response could be easily obliterated by improper laboratory handling and holding conditions. However, the marine habitats of H. oregonensis and H. nudus are not normally characterized by hypersaline salt pools. Thus, the acclimation response observed by Gross (1963) for H. oregonensis is not normally found in nature.

Hypo-osmotic regulation has not been shown in Hemigrapsus nudus in concentrated salinities (Jones, 1941; Dehnel, 1962; Dehnel and Stone, 1964). No blood hypo-osmotic regulation was found in the present study which confirms the results of both Jones and Dehnel. Results of the current work showed both blood and urine osmotic concentrations to approach the line of isosmocity and remain parallel and slightly hyperosmotic to it.

A comparison of our data with that of shore crabs from British Columbia shows possible latitudinal variation. In 150% sea water, Dehnel and Stone (1964) found summer H. nudus produced a urine isosmotic to the blood, while in the winter a slightly hypo-osmotic urine was produced. In contrast, present results showed an opposite situation in 150% sea water. Winter H. nudus maintain the urine isosmotic to the blood; however, summer crabs produce a hypo-osmotic urine. Our determinations were made after 48 hours exposure to the experimental salinity, whereas Dehnel and Stone's were made after 24 hours. Curiously, the present study showed the summer urine osmotic concentration was isosmotic to the blood after 24 hours but became hypo-osmotic by the end of 48 hours. Thus, the possibility of latitudinal variation in the hypo-osmotic regulatory pattern may exist, as well as an acclimation effect.

In concentrated sea water the patterns of regulation of sodium and chloride are not similar. Urine sodium for winter and

summer is hypoionic to the blood in both 100% and 150% sea water. Urine chloride, however, is isoionic in the winter and hyperionic to the blood in the summer. Looking at blood concentrations it is evident that blood sodium concentrations for summer animals in 150% sea water were parallel to the line of isoionicity but slightly hyperionic, whereas chloride values for blood were slightly hypoionic to the medium in 150% sea water (Fig. 6). Thus, the low summer blood chloride levels may be explained by the production of a urine hyperionic to the blood.

In concentrated salinities, reduced urine sodium (urine hypoionic to blood ) has been shown for Carcinus (Riegel and Lockwood, 1961), Uca (Green et al., 1959), and Pachygrapsus (Prosser et al., 1955; Gross and Marshall, 1960). Riegel and Lockwood (1961) were able to show on Carcinus a decrease in the urine sodium in concentrated salinities and, at the same time, an increase in the inulin urine:blood ratio which indicates reabsorption of water. In a cursory study on Hemigrapsus nudus, Riegel and Lockwood (1961) were able to demonstrate an inulin urine:blood ratio greater than unity in 100% and 150% sea water and in air. Thus, Hemigrapsus nudus appears capable of reabsorbing water from the urine under these conditions. If this is the case, then it is indeed surprising to find a decrease in the urine sodium concentration. Interestingly, while the urine sodium decreases in concentrated salinities, the

magnesium concentration in the urine rises. Perhaps volume changes in the blood space may occur as suggested by Gross and Marshall (1960), or the excretion of magnesium by the urine may compensate for the sodium retention by the antennary gland.

Magnesium is excreted in copious quantities in both the summer and winter when H. nudus are placed in 100% and 150% sea water. The urine:blood ratio for this ion ranges from 3 to 6. This phenomenon has been well documented for decapods and well reviewed (Prosser and Brown, 1961; Lockwood, 1962; Potts and Parry, 1964). More recently, Gross and Capen (1966) have examined in detail the mechanisms controlling the ability to produce high concentrations of magnesium in the urine. They find the concentration of magnesium in the urine is not a function of the magnesium influx or of the concentration of the ion in the external medium. Magnesium excretion, however, is a function of the salinity of the medium. In concentrated salinities the urine is held in the bladder for extended periods of time allowing an increase in the magnesium concentration. Hyperosmotically regulating crabs, on the other hand, release urine from the bladder too frequently for the magnesium concentration to build up. Electro-chemical balance in the transport of magnesium is probably achieved by direct exchange with sodium and some chloride. The transport of magnesium, however, is not coupled to the transport mechanism for sodium (Gross and Capen, 1966). The direct

exchange with sodium found by Gross and Capen (1966) correlates with the observed decreases in urine sodium in 150% sea water both in the present study and in theirs.

## Tissue Regulation

All ions, in general, were found in greater concentration in the muscle tissue than previously reported in similar studies on crabs (Robertson, 1961; Shaw, 1958b). Of particular interest is the relatively large amount of sodium present in the chela muscle of H. nudus. A primitive fish, Myxine, a hagfish, has a similar order of magnitude of sodium and potassium as that observed for H. nudus in this study (Potts and Parry, 1964). The reason for this high concentration of sodium in the tissues compared to the amount of potassium is not clear. In a majority of the other animals which have been examined there has been reported an unequal concentration difference between sodium and potassium in the tissues with potassium being seven to ten times higher in concentration.

In the muscle tissue calcium was on the order of one hundred times more concentrated in H. nudus than in Carcinus (Shaw, 1958b). It has been shown more recently in crayfish (Van der Kloot, 1966) that action potentials may be propagated by calcium activation without the necessity for sodium. If such is the case for H. nudus, it might explain the relatively high concentrations of calcium in the

chela muscle tissue.

The present study showed a muscle magnesium concentration value which was twice the value reported for Carcinus (Shaw, 1958b). This high concentration does not correlate with the large amount of magnesium excreted in the urine in 150% sea water. If, as Dehnel and Carefoot (1965) suggest, the excretion of magnesium in concentrated salinities facilitates the transmission across the neuromuscular junction because of its anaesthetic effect, then the high muscle magnesium concentration observed may be normal for Hemigrapsus, thus contradicting Dehnel and Carefoot's suggestion.

Amino acids in the tissues have been implicated as a major contributor to the total intracellular osmotic pressure. In Homarus muscle (Kermack, Lees and Wood, 1955) the amino acids amount to 256 mM/ kg wet tissue, in Eriocheir (Lockwood, 1962) 239 mEq/kg muscle water, in Nephrops (Robertson, 1961) 476 mM/kg muscle water, and in Carcinus (Shaw, 1958b) 434 mg ions/kg muscle water. Shaw(1958b) has shown in Carcinus the participation of muscle free amino acids in regulation in dilute salinities. Although the data from the present study do not demonstrate regulation of muscle free amino acids with salinity changes in Hemigrapsus nudus (Table 1 ), the role of the free amino acids as osmotic particles and in Donnan ratios should not be neglected. The relatively low total amino nitrogen (240 mEq/kg muscle water) correlates with the unusually high

concentrations of ions in the chela muscle in H. nudus, as shown above. Thus, the total intracellular osmotic pressure would be about the same as in other crustaceans.

Changes in the blood:tissue ratio of ions may indicate changes in cell permeability to ions. Thus, a decrease in the blood:tissue ratio would indicate some maintenance of the intracellular compartment by a change in permeability. An increase in the blood:tissue ratio, on the other hand, would indicate a lack of cellular regulation and a blood:tissue gradient which favors the blood. It is apparent from the present analyses that the blood:tissue gradient for sodium and chloride decreases in 150% sea water (Table 2). However, large increases in the blood:tissue ratio are found for potassium, magnesium, and calcium in 150% sea water. Thus, some intracellular sodium and chloride regulation may be indicated in concentrated sea water. In addition, while the ratio of the sum of blood ions:blood osmotic pressure remains fairly constant in 100% and 150% sea water, there is a decrease in the ratio of the sum of muscle ions: blood osmotic pressure (Tables 3 and 4) in concentrated sea water. Thus, the gradient favors the blood.

In dilute salinities, the blood:tissue ratio for potassium is found to decrease in 75% to 25% sea water. A very slight decrease was also noted in the magnesium. However, increases were observed in the ratios for sodium and chloride. Thus, it appears

that potassium, and possibly magnesium, may be regulated intracellularly in dilute sea water.

In all cases, the sum of the blood ions was greater than the sum of the muscle ions and amino nitrogen (Table 3 ). Although the ratios between the blood and tissues were determined, the units of concentration used to express each are not directly comparable. This was due to the failure to measure the water content of the blood. Likewise, dialysis of the blood samples was not measured and binding of the ions could not be assessed. However, it is interesting to note that if the amount of blood water were taken into account, the sum of the blood ions would, perhaps, be even greater.

## Conclusion

When Hemigrapsus nudus is exposed to a range of experimental salinities (25% to 150% sea water) hyperosmotic regulation with little hypo-osmotic regulation is observed. This picture is similar to the observations made by Dehnel in British Columbia with certain exceptions in the sodium and chloride balance (Figs. 5 and 6). As previously suggested, a latitudinal variation in the osmoregulatory response may exist. Further, a difference in the acclimation response to salinity variations in local habitats between British Columbia, Oregon, and, perhaps, California may prevail.

The existence of hyperosmotic regulation in dilute salinities is advantageous to Hemigrapsus considering its existence in an estuarine habitat. Tolerance or regulation of the tissue constituents is of paramount importance for acclimation or adaptation to the environment. The results obtained in the present study appear to indicate a tolerance by the muscle tissue to fluctuations in intracellular osmotic pressure with the exception of the muscle potassium in dilute salinities (Table 2 ). Thus, changes in the intracellular permeability may provide an avenue for aiding the general osmoregulatory response.

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