SALT AND WATER BALANCE IN THE DUNGENESS CRAB,

CANCER MAGISTER DANA (DECAPODA, BRACHYURA)

by

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A DISSERTATION

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CHAPTER 1

INTRODUCTION

The freshwater-seawater interface created in coastal areas by freshwater runoff and freshwater rivers and streams provides a myriad of environments of varying osmotic concentration. Because marine invertebrate animals are generally in osmotic equilibrium with seawater, their survival in brackish water and freshwater depends in part upon their ability to cope with the osmotic problems invoked by the hypoosmotic environment. Life in any environment is dependent upon several physicochemical exchanges with the external environment. It is likely, therefore, that a permeable or semipermeable barrier exists between the internal and external milieux. In a hypoosmotic medium, a permeable organism will incur a net loss of electrolytes and a net absorption of water through a summation of diffusional and electrochemical processes; thus life in brackish water or freshwater is contingent upon an organism's control of or tolerance to net movements of water and salt. Some organisms temporarily exposed to a hypoosmotic environment can prevent water and salt exchanges for short periods by behavioral regulation (e.g. Mytilus, Potts and Parry, 1963, p. 121). Freshwater crabs of the family Potamonidae are believed to be relatively impermeable to water, but permeable to certain solutes (Shaw, 1959b).
Many brackish water invertebrates survive because of their tolerance to environmental salinity fluctuations. The tissue osmotic concentration is controlled by an adjustment in the amino acid content of the cells (Florkin and Schoffeniels, 1965). Other brackish water and freshwater species rely upon osmoregulatory processes that produce an inside-hyperosmotic gradient between the internal and external environments. It is readily observed that mineral requirements for physiological and biochemical processes and the tolerance to fluctuations in the osmotic and ionic concentrations of the blood and urine vary substantially (Potts and Parry, 1963).

The mechanisms by which an animal osmoregulates have been a primary subject of examination, and crustaceans have been extensively studied. It has been demonstrated that euryhaline crustaceans, when compared to stenohaline forms, generally exhibit a reduced permeability to both solutes (Nagel, 1934; Gross, 1957) and water (Rudy, 1967). Also, freshwater crustaceans are usually less permeable than crustaceans inhabiting brackish water. The antennary gland of crustaceans assists in osmoregulatory functions, although reabsorption of solute has been observed in relatively few species (e.g. Bryan, 1960; Werntz, 1963). Besides providing for the expulsion of water, the antennary gland is also important for the excretion of magnesium and sulfate in brackish water crustaceans (Potts and Parry, 1963). Mechanisms for the active uptake of salts are complexly involved in osmotic regulation, and quantitative analyses of sodium and chloride balance have been performed using radioisotopic tracers (e.g. Shaw, 1959a, 1960a, 1961a,b; Potts and
In the study of the sodium and chloride transport mechanisms in aquatic animals, several lines of investigation have been pursued: a correlation of habitat with the affinity of the uptake mechanism for sodium ions has been advanced (e.g. Shaw, 1961a,b; Shaw and Sutcliffe, 1961); the effects of other ions upon sodium and chloride exchanges have been studied, providing information about the nature of the sodium and chloride exchanges (Stobbart, 1971; Kirschner et al., 1973; Maetz, 1973); and the role of sodium-potassium activated ATPase in ion transport is a subject of intense examination (Kamiya and Utida, 1968; Krishnamoorthy and Venkatramiah, 1969). In crustaceans, preparations of isolated perfused gills have provided a tool for a more controlled study of salt balance in this organ (Bielawski, 1964; Smith and Linton, 1971).

The aim of this study was to determine the osmoregulatory capabilities of Cancer magister Dana, thus establishing a basis for a more comprehensive analysis of various aspects of salt and water balance in this relatively large and abundant crustacean of the Pacific coast of North America. The Dungeness crab is distributed from Unalaska, Alaska, to Magdalena Bay, Mexico. This species inhabits shallow waters (2-20 fathoms), and is found primarily in sandy-bottomed areas. It is common in bays and estuaries along the Pacific coast (MacKay, 1942). Little information concerning the occurrence of the animal in waters of reduced salinity is available, however. In Coos Bay, along the south-central Oregon coast, crabs have been collected from brackish water having a
salinity of 12-15 °/oo (Tom Wayne, personal communication). Cleaver (1957) has reported that the salinity tolerance of the Dungeness crab ranges from 12-32 °/oo, but a preliminary examination of osmotic regulation in Cancer magister indicated that the animal is a relatively "weak" osmoregulator when compared to other euryhaline brachyurans (Jones, 1941). A comprehensive account of certain aspects of osmotic and ionic regulation has recently become available (Alspach, 1972). In this study, the capacity of the animal for osmotic and ionic regulation in isomotic and hypoosmotic environments was determined, followed by a comprehensive analysis of water and sodium balance using radioisotopic tracers.
CHAPTER 2

GENERAL PROCEDURES

Collection of Animals

*Cancer magister* was collected from the South Slough, Coos Bay, Oregon (Lat. 43°20.5', Long. 124°19.3') from November, 1969 to December, 1972. Baited crab rings (ring mesh size = 3 inches) were used to collect larger animals (100-600 g); an otter trawl (3/4 inch mesh) was employed to seine smaller crabs (less than 100 g). Both sexes were studied, and the weight range utilized was 40-200 g. Although premolt individuals are not readily recognizable, postmolt (softshell) animals were rejected in an attempt to focus upon the statics and dynamics of salt and water balance in the intermolt animal.

Upon collection, the crabs were transported to the Oregon Institute of Marine Biology, Charleston, Oregon, and maintained in large aerated holding tanks (1000 l.) of flowing seawater. The seawater in these tanks is pumped directly from Coos Bay, and fluctuations in the chemical and physical parameters of the bay water are inherent in the laboratory water. The water temperature in the Coos Bay region is reasonably constant for a particular time of the year, ranging from 9 to 12°C.

¹This seawater will be hereafter termed Coos Bay seawater.
through the wet season, November-April, and from 13-16°C. during the dry season, May-October. The salinity of Coos Bay also varies throughout the year. In the wet season, salinity averages approximately 30 °/oo, while during the dry season it averages 33 °/oo.2

Preparatory Experimental Procedures

The research will be presented as three interrelated investigations: (1) a static assessment of osmotic and ionic regulation; (2) an analysis of water movements between exterior and interior pools; and (3) a study of the dynamics of sodium regulation. All three studies were conducted in both isosmotic and hypoosmotic environments. Throughout the entire study one basic procedure was followed in the preparation of animals for experimentation. After a 24 hour period in Coos Bay seawater, the animals were either directly exposed or acclimated to isosmotic and hypoosmotic test media. Coos Bay seawater was the isosmotic test medium, and its osmotic concentration ranged from 950-1000 mOsm/kg H2O. The hypoosmotic media were prepared by dilution of Coos Bay seawater with tapwater in the 1000 liter holding tanks. Preliminary exposure of Cancer magister to a particular hypoosmotic environment varied from 72-96 hours before the final experimental

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1 Unpublished data from records of the Fish Commission of Oregon, Charleston laboratory.
2 Unpublished data from the records of the Oregon Institute of Marine Biology, Charleston, Oregon
3 mOsm/kg H2O.
procedures commenced.

For the assessment of the osmotic and ionic regulatory capabilities, test media of the following osmotic concentrations were employed: 1000, 750, 500, and 300 mOsm. In the analyses of water and sodium balance, two test media, the 1000 mOsm and the 300 mOsm, were used primarily.

Animals were directly exposed to the 100%, 75%, and 50% seawater solutions. The mortality rate in these environments was quite low—less than 5%. The initial experiments indicated that mortality was high (greater than 33%) for animals directly exposed to 30% seawater. Subsequently, crabs in this medium were acclimated 24-48 hours by a gradual influx of freshwater until the desired osmotic concentration was attained. This process reduced the mortality rate to less than 5%.

The temperature at which osmotic and ionic regulation was examined varied from 9 to 13°C. The seasonal variation of both air and water temperatures encountered in the Coos Bay region was responsible for this fluctuation. Because temperature variations can have considerable impact upon biological rate phenomena, experiments designed to study the dynamics of water and sodium balance were conducted at 10 ± 0.5°C., by maintaining the animals in constant-temperature chambers upon completion of the pre-experimental adaptation procedure.

1In this dissertation, 100% seawater and seawater having an osmotic concentration of 1000 mOsm/kg H₂O are synonymous terms.
Procurement of Blood and Urine Samples

Samples of body fluids and the external bathing medium were collected and analyzed for the total osmotic concentration, and sodium, potassium, calcium, magnesium, chloride, and sulfate ion concentrations. Blood and urine samples were obtained with pipettes molded from lengths of glass tubing. The solutions were either examined immediately, or frozen for later study. As blood taken from Cancer magister clots rapidly, the serum was analyzed for the osmotic and ionic components. Centrifugation was not required.

Blood samples were procured by puncturing the arthrodial membrane of a walking leg with the pipette tip and applying suction with a piece of attached rubber tubing. Urine was obtained by lifting the nephropore operculum with a hooked dissecting needle, gently inserting a finely drawn pipette tip, and applying suction. Contamination by the gill chamber fluid was prevented by obstructing the opening with tissue paper. To prevent disruption of the thin-walled excretory bladder and the ensuing infusion of blood, the pipette tips were fire-polished. The permeation of the colorless urine with blood results in a visibly turbid solution; these samples were rejected.

In most instances, analyses of the osmotic and ionic constituents were possible with a single blood or urine sample. Reagent grade chemicals were used in the ionic analyses and standardization procedures. A Mettler H20T Analytical Balance was employed for most chemical weight measurements and for determination of the percentage of water in the body fluid and seawater samples. In some chemical weight
determinations, and in the estimation of wet animal weights, a Mettler P1200 top-loading balance was used.

Statistical Treatment of the Data

To test hypotheses that two mean expressions are equivalent, the Student's t test was employed. In this study, the following probabilities for significant differences were assigned: \( P > 0.05 \), not significant; \( P < 0.05 \), significant; \( P < 0.001 \), highly significant. For paired data comparisons, linear regression analyses were performed. The assigned significance of the correlation coefficient, \( r \), was governed by the above probabilities. The regression lines were formulated by the method of least squares. In the tables, \( n \) = the number of observations; \( \bar{x} \) = the mean; and s.d. = the standard deviation. The vertical lines in the figures represent a ± standard deviation of the mean.
CHAPTER 3

OSMOTIC REGULATION

Methods

Osmotic concentrations of the seawater test media were estimated by refractometry. Both a TS meter (specific gravity scale, Amer. Optical Inst. Co., Model 10400) and a Goldberg T/C refractometer (salinity as °/oo scale, Amer. Optical Inst. Co., Model 10423) were employed in these initial measurements. Final seawater, and urine and serum osmotic concentrations were determined by vapor pressure osmometry, using a Hewlett-Packard Model 302B Vapor Pressure Osmometer.

Sodium chloride calibrating solutions were prepared from the osmolality data of Jacobsen et al. (1962). The standard deviation from the mean of a triplicate analysis of a 1000 mOsm solution of NaCl was ± 6 mOsm.

Results

Osmotic regulation by Cancer magister is demonstrated in Fig. 1 and Table 1. The information is based upon a static assessment of the osmotic concentrations of serum, urine, and seawater. The animals were exposed solely to isosmotic and hypoosmotic experimental environments,

\[ 32.2 \text{ g NaCl/kg H}_2\text{O} = 1000 \text{ mOsm/kg H}_2\text{O}. \]
Fig. 1. Serum osmotic concentrations for crabs in environments of varied osmotic concentration. Diagonal line is the Isosmotic Line.

**SERUM CONCENTRATION**

(mOsm/Kg H$_2$O)

MEDIUM CONCENTRATION (mOsm/Kg H$_2$O)
Table 1. Serum and urine osmotic concentrations for animals in isosmotic and hypoosmotic media.

<table>
<thead>
<tr>
<th>Medium</th>
<th>n</th>
<th>x ± s.d.</th>
<th>n</th>
<th>U/S</th>
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Medium and body fluid concentrations expressed as mOsm/kg H₂O

U/S = urine-serum ratio

P t = significance of t test
as these media are representative of the natural habitat of this species. Seawater with an osmotic concentration of 1000 mOsm simulates Coos Bay seawater during the period of lowered freshwater influx, June-August, and is termed 100% seawater. Even though osmotic concentration is measured as milliosmoles per kilogram water in this investigation, it can be conveniently expressed as a percentage of Coos Bay seawater.

Crabs were immersed in media approximating 100%, 75%, 50%, and 30% Coos Bay seawater. Because of the difficulty involved in obtaining the exact desired osmotic concentration, intermediate concentrations were encountered. In the 100% seawater media (1000 and 980 mOsm), the serum of Cancer magister is essentially isosmotic to the external medium. In the dilute environments (750, 730, 520, 480, 325, 300 and 275 mOsm), hyperosmotic regulation is achieved. In increasingly hypoosmotic environments the osmotic differential between serum and seawater is increased. The increase can be observed in Fig. 1 by the difference in slope between the lines of regulation and isosmoticity and in Table 2 by the mole fraction differences between the external and internal fluid compartments. The mole fraction of water in either pool is equal to \[ \frac{55.56}{55.56 + \text{the number of moles of solute}} \]. The sustained osmotic differential is apparently maximal at external concentrations approaching 300 mOsm (Table 2). The regulatory curve seems to parallel the isosmotic line in extremely dilute environments (Fig. 1).

Throughout the investigation of osmotic and ionic regulation in Cancer magister, several qualitative observations indicated that smaller
Table 2. Solvent mole fraction differences between external and internal milieux.

<table>
<thead>
<tr>
<th>Medium (mOsm)</th>
<th>M.F. of Seawater</th>
<th>M.F. of Serum</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>750</td>
<td>0.9866</td>
<td>0.9845</td>
<td>0.0021</td>
</tr>
<tr>
<td>730</td>
<td>0.9870</td>
<td>0.9847</td>
<td>0.0023</td>
</tr>
<tr>
<td>520</td>
<td>0.9907</td>
<td>0.9864</td>
<td>0.0043</td>
</tr>
<tr>
<td>480</td>
<td>0.9914</td>
<td>0.9878</td>
<td>0.0046</td>
</tr>
<tr>
<td>325</td>
<td>0.9941</td>
<td>0.9890</td>
<td>0.0051</td>
</tr>
<tr>
<td>300</td>
<td>0.9946</td>
<td>0.9896</td>
<td>0.0051</td>
</tr>
<tr>
<td>275</td>
<td>0.9950</td>
<td>0.9910</td>
<td>0.0040</td>
</tr>
</tbody>
</table>

M.F. = Solvent Mole Fraction

M.F. = $55.56 \div (55.56 + n \text{ moles of solute})$

animals may have a greater capacity for osmoregulation than larger animals. An abundance of primarily smaller crabs (less than 200 g) in the estuarine habitat was observed in the collection of animals in the Coos Bay area. The fluctuating salinity of this estuary has been characterized (pp. 4,6). Additional evidence for the existence of a body weight-osmoregulation phenomenon was provided through: (1) a higher observed mortality rate for larger crabs in experimental media of reduced salinity; and (2) a cursory examination of the serum osmotic concentration data for crabs of varied body weights, indicating that a greater osmotic differential is maintained by smaller animals.

The results of a more thorough study of the hypothesis are presented in Figures 2 and 3 and Tables 3a and 3b. Three weight classes
Fig. 2. Serum osmotic concentrations of three weight classes of crabs in different osmotic environments. Dashed diagonal line is the Isosmotic Line.

SERUM CONCENTRATION

(mOsm/Kg H₂O)

- • = small crabs
- □ = medium crabs
- ▲ = large crabs

MEDIUM CONCENTRATION (mOsm/Kg H₂O)
Fig. 3. Linear regression plot of two variables, serum osmotic concentration and body weight, for animals in 35% seawater.
Table 3a. Comparison of serum osmotic concentrations for three weight classes of *Cancer magister* in hypoosmotic media.

<table>
<thead>
<tr>
<th>Seawater (mOsm)</th>
<th>Small (S) (14-63 g)</th>
<th>Medium (M) (81-190 g)</th>
<th>Large (L) (211-664 g)</th>
<th>S vs M</th>
<th>S vs L</th>
<th>M vs L</th>
</tr>
</thead>
<tbody>
<tr>
<td>775</td>
<td>25 902 48</td>
<td>20 911 41</td>
<td>13 865 39</td>
<td>&gt;.05</td>
<td>&lt;.05</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>520</td>
<td>17 776 74</td>
<td>17 739 47</td>
<td>15 740 49</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>350</td>
<td>25 664 68</td>
<td>20 621 40</td>
<td>9 577 48</td>
<td>&lt;.02</td>
<td>&lt;.01</td>
<td>&lt;.02</td>
</tr>
</tbody>
</table>

*Pt = Significance of t test*

Table 3b. Linear regression analysis of body weight (x) with serum osmotic concentration (y).

<table>
<thead>
<tr>
<th>Medium (mOsm/kg H₂O)</th>
<th>Slope ≠ zero (Pt)</th>
<th>Pr</th>
<th>Regression Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>775</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
<td>y = -0.037x + 901.34</td>
</tr>
<tr>
<td>520</td>
<td>&gt;.05</td>
<td>&gt;.05</td>
<td>y = -0.106x + 773.47</td>
</tr>
<tr>
<td>350</td>
<td>&lt;.01</td>
<td>&lt;.01</td>
<td>y = -0.279x + 664.20</td>
</tr>
</tbody>
</table>

*Pr = Significance of the correlation coefficient*
of animals were studied: small crabs weighing 20-70 g; medium-sized crabs weighing 90-190 g; and large animals with a body weight greater than 200 g. These weight classes are not totally artificially chosen. Crabs weighing less than 100 g have endured several early molt cycles, and comprise animals of more than one distinct weight class. However, these individuals are generally less than a year in age. The medium-sized class includes animals between one and two years of age, surviving one additional molt. Crabs larger than 200 g are older than two years, although the weights may span several distinct classes. The animals were subjected to media of three particular osmotic concentrations, 775, 520 and 350 mOsm. Because the larger crabs displayed a tendency to prey upon the smaller ones during confinement and partial starvation, the three weight classes were each maintained in separate experimental tanks with similar environmental conditions. It was difficult, however, to produce precisely equivalent osmotic concentrations in all three systems. The t-test data in Table 3a indicate several differences in the serum osmotic concentration among crabs of varied body weights. Both small and medium crabs exhibited a significantly higher mean serum osmotic concentration when compared to larger animals in the 775 mOsm medium. Although there were no apparent differences at 520 mOsm, in 35% seawater the differences were distinct. Both small and medium crabs maintained higher serum osmotic concentrations than the larger crabs, and the small crabs regulated at a significantly higher level than the medium-sized animals.

A more accurate representation of the body weight-serum osmotic
concentration relationship can be achieved by comparing the independent variable, body weight, with the possible dependent variable, serum osmotic concentration, in a linear regression analysis. The results are tabulated in Table 3b. In one medium, 35% seawater, the correlation is significant. The regression line is illustrated in Fig. 3.

Table 4 demonstrates the serum osmotic concentration of male and female Dungeness crabs subjected to an isosmotic and several hypo-osmotic environments. There were no significant differences between male and female osmotic concentrations in any of the selected environments.

Table 4. Comparison of serum osmotic concentrations between male and female crabs in hypoosmotic media.

<table>
<thead>
<tr>
<th>Seawater (mOsm)</th>
<th>Males (mOsm)</th>
<th>Females (mOsm)</th>
<th>( P_t )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>54</td>
<td>1002</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>990</td>
<td>36</td>
</tr>
<tr>
<td>750</td>
<td>25</td>
<td>867</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>861</td>
<td>42</td>
</tr>
<tr>
<td>500</td>
<td>17</td>
<td>715</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>756</td>
<td>62</td>
</tr>
<tr>
<td>300</td>
<td>59</td>
<td>572</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>562</td>
<td>62</td>
</tr>
</tbody>
</table>

\( P_t = \text{Significance of t test} \)

Mean values for the urine osmotic concentrations of crabs immersed in isosmotic and hypoosmotic media are compared with the serum concentrations in Fig. 4 and Table 1. The serum and urine are isosmotic in crabs exposed to seawater or brackish water.
Fig. 4. A comparison of seawater, serum, and urine osmotic concentrations.

OSMOTIC CONCENTRATION (mOsm/Kg H₂O)

- W = medium
- S = serum
- U = urine

EXPERIMENTAL MEDIA (as Percent Coos Bay Seawater)
Discussion

The Dungeness crab maintains its body fluids in osmotic equilibrium with seawater, but hyperosmoregulates in less concentrated media. This pattern of osmotic behavior is not unusual for marine decapods (Potts and Parry, 1963), and has been described and documented previously for this species (Jones, 1941; Alspach, 1972). The latter examination was comprehensive, whereas the initial demonstration was somewhat superficial, embodying only three measurements. When converted to mOsm, Jones' freezing point depression serum values are 20-50 milliosmoles lower than the measurements expressed in Table 1 for animals immersed in similar experimental environments. For crabs bathed 24 hours in 75%, 50% and 25% seawater, Alspach's (1972) osmolality measurements are relatively equivalent to the information procured in this investigation. However, when the time of exposure was extended to 48 hours, the serum osmotic concentrations of crabs exposed to these media were notably lessened (10-20%). Consequently, limited survival was recorded for animals retained in 25% and 50% seawater for a time period greater than 48 hours (Alspach, 1972).

Animals used in this study were exposed to an experimental medium for a minimum of 72 hours before the osmotic concentrations of the serum and urine were determined. No survival problems were encountered upon direct exposure to 75% and 50% seawater; after a 24-48 hour gradual acclimation to 30% seawater, mortality in this medium was greatly

---

1 Osmolality = freezing point depression divided by 1.86.
diminished (see p. 7). The probability that larger crabs were utilized in the Alspach experiments (1972), coupled with the indication that smaller animals may possess a greater capacity for osmoregulation (Figs. 2, 3), provides a partial explanation for these inconsistencies. The "acute stress" to which the Alspach animals were subjected may have affected their osmoregulatory performance and survival in 25% seawater.

In hypoosmotic media, the osmotic differential maintained by Cancer magister is somewhat less than that exhibited by many other euryhaline decapods. Pachygrapsus crassipes maintains a relatively stable blood osmotic concentration (900-1000 mOsm) in media ranging in concentration from 50% to 100% seawater (Jones, 1941; Gross, 1957). In experimental media less concentrated than 500 mOsm, a decrease in the external osmotic concentration is paralleled by a decline in the concentration of the serum. Similar results have been obtained for Hemigrapsus nudus and H. oregonensis (Jones, 1941; Dehnel and Stone, 1964), and Metapograpus messor (Kamemoto and Kato, 1969). The European green crab, Carcinus maenas, does not retain this degree of constancy in 50% and 75% seawater, but the osmotic differential is perceptibly greater than that displayed by Cancer magister (Nagel, 1934; Smith, 1970). The blood osmotic concentration of Callinectes sapidus (Ballard and Abbott, 1969), Eriocheir sinensis (Krogh, 1938), and Rhithropanopeus harrisi (Jones, 1941; Smith, 1967) fluctuates, but these crabs are capable of indefinite survival in freshwater, whereas the species discussed above are not. It is apparent that different modes of osmoregulation have evolved within the Brachyura. Both the capacity for osmoregulation (evaluated by the sustained osmotic
gradient), and the tolerance to hypoosmotic environments displayed by the Dungeness crab seem to be intermediate when compared to other euryhaline species.

If one can assume that the metabolic energy required to sustain an osmotic gradient is proportional to this gradient, as Gross (1957) has proposed, then the relaxed osmoregulatory posture incorporated by Cancer magister and Carcinus maenas may be an energetically less expensive system than that employed by the intertidal grapsid crabs. The type of exposure must also be considered, however. Salinity fluctuations in the intertidal habitat are often instantaneous, whereas estuarine shifts in salinity may occur over extended time periods. An increased sensitivity of the osmoregulatory apparatus of the grapsids may be a necessary adaptation for their intertidal existence.

The lack of precision in the regulation of the extracellular osmotic concentration by Cancer magister in hypoosmotic environments must certainly elicit some degree of osmotic accommodation in the tissues, if the cells are to remain osmotically intact. This regulatory process has been termed isosmotic intracellular regulation, and in marine crustaceans it is believed to be principally effected through an adjustment in the concentration of free amino acids (Duchateau and Florkin, 1956). This adaptation has been demonstrated for both osmo-conforming crustaceans tolerating extreme dilutions of the internal body fluids, and euryhaline crustaceans tolerating more moderate reductions in the osmotic concentration of the internal milieu (Schoffeniels, 1970).
In response to a reduction in the external medium from 100% to 40% seawater, *Carcinus maenas* reduces the free amino acid concentration of the muscle from 436 to 191 mM/kg fiber water (Shaw, 1958). A portion of the amino acid efflux from the cells can be attributed to an increase in the hydration of the muscle tissue. However, Shaw (1958) has calculated that there is a 40% decrease in the free amino acid concentration which is initiated in response to the osmotic stress.

One might expect a similar response from *Cancer magister*, as this species demonstrates a comparable osmoregulatory behavior. But Alspach (1972), with observations based upon results from animals exposed to 25% seawater, suggested that the exchange of free amino acids is not a significant factor in the isosmotic intracellular regulation of this organism. Careful examination of Alspach's data for animals exposed to 50% seawater does, however, indicate a marked increase in the amino acid concentration. This reduction exceeds that which can be predicted from the increase in water content of the muscle, and varies from 20% to 30% (or 60-90 mM/kg muscle water) of the total free amino acid concentration. One might conclude that the amino acid exchange could indeed have a profound effect upon the final osmotic concentration of the tissues of the Dungeness crab in hypoosmotic media.

When subjected to isosmotic and hypoosmotic environments, *Cancer magister* produces urine that is not osmotically distinguishable from its serum (Table 1). Because the internal milieu-external milieu interface in the Dungeness crab is permeable to water (Table 5, Chapter 4), the water concentration gradient thus imposed by life in a hypotonic
solution results in a superfluous influx of water. This surplus is abated through excretory processes. The expulsion of water by micturition is accompanied by an unavoidable loss of osmotically active solute, an obvious impediment to the osmoregulatory mechanism.

Such an anomalous isosmotic relationship between the serum and urine of an animal inhabiting a hypotonic medium seems to be characteristic for euryhaline brachyurans and anomurans (Jones, 1941; Prosser et al., 1955; Gross, 1957; Smith, 1967; Thompson and Pritchard, 1969). The ineffectiveness of the antennary gland as a salt-conserving device is not without explanation. For a semipermeable animal, Potts (1954) has calculated that the reabsorption of solute through the excretory system is energetically advantageous only when the osmotic gradient is of considerable magnitude—a medium osmotic concentration less than 500 mOsm. Potts' (1954) assumption that brackish water and freshwater animals are semipermeable (permeable to water but relatively impermeable to salts) impairs the comprehensive application of his hypothesis (Shaw, 1959b). Studies using radioisotopes of sodium have quantitatively confirmed the observations of Nagel (1934) and Krogh (1938), that many decapod crustaceans transport significant quantities of this ion across membranes exposed to the external milieu (Shaw, 1959a,b, 1969a,b; Bryan, 1960; Rudy, 1966). The amount of sodium eliminated through the isosmotic urine of several salt-permeable brachyurans is only a fraction of the total sodium efflux, and varies from 15-25% (Shaw, 1961a,b; Rudy, 1966). The exact amount is dependent upon the sustained osmotic differential, and the permeability of the animal to
water and sodium ions. Shaw (1959b) has emphasized that any applied effort by freshwater or brackish water brachyurans to produce a dilute urine would be inconsequential, because the urinary efflux component is only a portion of the total flux.

Crayfish, however, appear to be appropriate but exceptional examples for Potts' (1954) theoretical model. Astacus pallipes, a crayfish possessing a very effective solute reabsorptive mechanism in the antennary gland (Scholles, 1933), is relatively impermeable to sodium ions, when compared with the freshwater crab, Eriocheir sinensis, and the brackish water species, Carcinus maenas (Shaw, 1961b). Excretion of urine with a sodium concentration equivalent to that of the serum would invoke an extraordinary and perhaps energetically disastrous sodium uptake load upon the medium-exposed tissues of the crayfish.

It is evident from the information presented above that Cancer magister, a brachyuran exhibiting a comparatively moderate permeability to sodium ions (Chapter 6), does not require the production of a hypo-osmotic urine for the proper functioning of the osmoregulatory machinery, when immersed in hypoosmotic media. This premise does not necessarily signify the absence of, or the inability to stimulate the operation of, renal salt-conserving mechanisms. The production of hypoosmotic urine by winter populations of Hemigrapsus nudus exposed to hypoosmotic media has been recorded, although the physiological and ecological significance of this phenomenon is not known (Dehnel and Stone, 1964; Alspach, 1967). Alspach (1972) has suggested that the excretory fluid of Cancer magister is slightly less concentrated than
the serum of hyperosmoregulating individuals, thus attributing a "weak" osmoregulatory role to the antennary gland of this animal. His conclusions are not statistically substantiated, and the findings of this study do not support his observations. Studies concerning ionic regulation in decapod crustaceans have demonstrated the capacity for the reabsorption and secretion of certain ions, particularly $K^+$, $Ca^{++}$, $Mg^{++}$, and $SO_4^{--}$ (Potts and Parry, 1963). Some reabsorption of sodium ions by hyperosmoregulating Cancer magister will be illustrated in this presentation. It seems probable, therefore, that the assessment of the antennary gland's contribution to the entire osmoregulatory process is not possible solely through the measurement of one variable, total osmotic concentration. Additionally, as Thompson and Pritchard (1969) have asserted, the antennary gland of decapod crustaceans osmoregulating in hypoosmotic environments most certainly functions as an osmoregulatory device for the removal of water entering the animal through osmotic processes.

Environmental effectors and intraspecific differences should be considered in an examination of osmotic regulation. Temperature changes can affect the blood osmotic concentration of many aquatic animals (Verwey, 1957). An increase or decrease in the environmental temperature most often results in the opposite transformation of the blood osmotic concentration (Broekema, 1941; Dehnel, 1962; Ballard and Abbott, 1969). Although the significance of the correlation is not well understood, this phenomenon has been utilized in explaining seasonal migrational responses (Verwey, 1957; Ballard and Abbott, 1969), and the
successful colonization of brackish water and freshwater habitats in the
tropics by certain decapod crustaceans (Panikkar, 1940). Verwey (1957)
has also provided an explanation based upon energetic considerations
for the existence of a temperature-osmotic concentration relationship
in non-migrating forms. Sufficient field and laboratory information is
not available to warrant a conjecture concerning salinity-temperature
induced physiological or behavioral responses by the Dungeness crab.

Intraspecific variations in osmoregulatory capacity have been
recorded for several decapod crustaceans. Male Callinectes sapidus
sustain a lower serum osmotic concentration in hypoosmotic media than do
females of the same species (Ballard and Abbott, 1969). Gilbert (1959a)
has discovered a contrasting situation in Carcinus maenas, in which the
males are stronger osmoregulators than the females in all test media
employed. In a study of natural Friocheir sinensis populations, De
Leersnyder (1967a) detected no variations in serum osmotic concentration
that were dependent upon gender; male and female animals did, however,
exhibit differences in the regulation of several ionic constituents.
Although the number of observations of male animals outnumbered those
for females in this investigation, a comparison of osmoregulatory
capacity between male and female Cancer magister was conducted. The
results of Table 4 indicate that osmoregulation is a phenomenon charac-
teristic of both sexes, and that the osmotic gradient sustained in
several hypoosmotic environments varies independently of gender. It is
possible that any existent sex variations may be behaviorally adaptable
for a particular species. Ballard and Abbott (1969) have suggested that
the osmoregulatory differences between male and female blue crabs may be important when the observed preference of male animals for the brackish water habitat is considered (Gunter, 1967).

Body weight provides another dimension of experimental approach for the analysis of osmoregulatory behavior within a species. Insufficient development of the osmoregulatory mechanism may be a prominent cause for the observed limited tolerance of larval crabs (Costlow and Bookhout, 1959), larval nereids (Bogucki, cited in Beadle, 1957), and juvenile salmonids (Parry, 1958), to an imposed osmotic stress. For adult animals that demonstrate a distinct body size-osmoregulatory capacity relationship, the serum osmotic concentration varies inversely with body weight. Gilbert's (1959a) examination of Carcinus maenas, and Bogucki's (cited by Beadle, 1957) work with nereids have conclusively established this correlation. The results presented in Tables 3a and 3b provide evidence for such a correlation in the Dungeness crab. The osmoregulatory performance of smaller individuals (less than 100 g) significantly surpasses that exhibited by the larger animals. A statistically significant correlation between body weight and serum osmotic concentration was demonstrated for crabs in 35% seawater. A similar analysis has established a parallel relationship for hyper-osmoregulating Eriocheir sinensis (De Leersnyder, 1967b).

The absence of the correlation in 75% and 50% seawater for Cancer magister is puzzling, and may be a result of the experimental procedure. However, because the increasing slope of the osmoregulatory curve through the 75% and 50% seawater range (Fig. 1) indicates that maximal
regulatory capacity is not achieved in these media, one might not expect intraspecific differences in the ability to cope with an osmotic stress to be apparent in more compatible environments.

The greater osmoregulatory capacity exhibited by smaller individuals of some species can be explained. The gill surface area for several species of brachyurans has been calculated by Gray (1957). In two species, Callinectes sapidus and Libinia dubia, the gill surface area was correlated with body weight; the number of gill lamellae and the gill surface area decreases with increasing body weight. If the gills are the primary sites of osmoregulation in crustaceans, as Webb (1940) has theorized, then it is obvious that a body weight-gill surface area correlation might be paralleled by a significant body weight-osmoregulatory capacity relationship, unless of course the osmoregulatory site is morphologically specific. Another explanation, not necessarily contrary to the first but perhaps ecologically supplementary, is possible. This account is somewhat whimsical in nature, and is based upon qualitative observations. In captivity, larger crabs have been observed to prey regularly upon smaller individuals. While energetically expensive, an increased osmotic differential could extend the range an animal in brackish water, thus providing an escape from a possible predatory threat.
CHAPTER 4
WATER BALANCE

Methods

Assessment of the Rate of Urine Elimination

In the course of this investigation, a technique was developed for the continuous recording of the micturition rate of *Cancer magister* in situ. This method is similar to the inulin clearance technique used in vertebrates to determine the glomerular filtration rate. The procedure employs a radioisotopic tracer, $^{125}$iodine-labeled sodium iothalamate (Glofil, Abbott Laboratories, Radiopharmaceuticals Division). Although Glofil has a lower molecular weight than inulin, most biological membranes are impermeable to both compounds (Elwood *et al.*, 1967).

The initial collection and preparation of the animals for experimentation has been previously described (pp. 5-7). $^{125}$I-labeled Glofil (10 µc in 50 µl) was introduced into the circulatory system by injection with a Hamilton microliter syringe. Monitoring of the release of labeled urine from the crab was delayed 24-36 hours to allow for equilibration of the compound in the body fluids.¹

¹Equilibration time is defined and discussed on p. 40.
The experimental system is illustrated in Fig. 5. Because several instruments were required for the measurement, it became impractical to perform the experimentation in a constant temperature chamber. To maintain the temperature at $10 \pm 0.5^\circ C$, a Lauda refrigerated Circulator (model K-2/R, Brinkmann Instruments) was adjoined to the system. The medium bathing the crab (500 ml in a one l. beaker) was aerated and stirred to insure homogeneous distribution of the released label. A portion (25 ml) of the bath was continually circulated through the counting apparatus by a variable speed peristaltic pump (model 1215, Harvard Apparatus). The pumping volume was approximately 180 ml/min.

The counting device included a scintillation well counter containing a thorium-activated sodium iodide crystal, Pulse Height Analyzer, and a Dual Ratemeter (model 628-046, Picker Nuclear Corp.). Released activity was registered with a Rectilinear Recording Millimeter (Texas Instruments, Inc.). For each animal, 100 ml samples of serum and urine were procured and counted before and after each experiment. To allow for geometrical errors in counting, the samples were diluted with 25 ml of non-radioactive bathing medium. The osmotic concentrations of the serum and urine were determined.

An expression of the eliminated urine as a percentage of the crab's body weight per hour was calculated with the following equation:

\[ U/V = \frac{\text{bath volume} \times \text{cpm/ml bath}}{\text{time} \times \text{weight}} \]

$U$ is equal to the activity of the urine in cpm/ml, and $V$ is the micturition rate. Even though the activity in the urine changes with time, this decrease can be minimized by experiments of short duration.
Fig. 5. Diagram of the apparatus employed in the measurement of micturition rates.
(3-6 hrs.). In the present study longer experimental periods were necessary; the simplicity of the mathematical treatment could therefore yield an inaccurate representation. This problem will be treated in greater detail in subsequent discussions.

**Water Permeability Measurements**

The water permeability or total water turnover of *Cancer magister* was examined using the radioactive tracer, tritiated water (THO, New England Nuclear Corp.). After the initial collection and acclimation, the crabs were maintained at 10 ± 0.5°C in a constant-temperature chamber. Two different techniques were practiced in the administration of the tracer and the subsequent experimentation.

**Method A:** the "open" system. The crabs were exposed to the tritium in a 5 liter glass aquarium containing two liters of the "hot" solution with an activity of 0.5-1 μc/ml. At time zero, the animals were introduced to the medium, and exposed for 15 minutes. After the loading period, a crab was removed and rinsed in three washes of non-radioactive medium. Samples of the blood and loading solution were collected for counting and the determination of the total osmotic concentration (see Rudy, 1967).

**Method B:** the "closed" system. This technique was performed in conjunction with the measurements of urine elimination. Prior to the termination of the experimental period, tritiated water was added to the 500 ml bathing medium. Enough isotope was used to insure a specific activity of 0.5 μc/ml. A five second delay allowed for proper
distribution of the tracer in the medium. Following a 15 minute exposure, the animal was removed from the bath and rinsed three times in non-radioactive fluid. Blood and seawater samples were procured.

To prepare the samples for scintillation counting, the water was removed by dry ice-acetone "cold" traps through freeze drying. A high-vacuum pump apparatus described by Rudy (1967) was employed. Fifty µl aliquots of serum and loading solution samples were pipetted into scintillation vials containing four ml of a solution with the following composition: 7.54 g Fluoralloy, Formula TLA, Beckman Inst., Inc.; 31 ml Bio-Solv Solubilizer, Formula BBS-3, Beckman Inst., Inc., and 900 ml reagent grade toluene. Triplicate samples were prepared, and the radioactivity was monitored with a Beckman Model LS-150 Liquid Scintillation System.

The water influx constant, $K_1$, was calculated using the following equation (Rudy, 1967):

$$K_1 = \frac{1}{T} \times \ln \left( \frac{C_\infty}{C_r} - \frac{C_r}{C_\infty} \right)$$

$K_1 = $ influx constant (hr$^{-1}$)

$C_r = $ specific activity of the internal water after time $T$ (15 minutes)

$C_\infty = $ specific activity of the loading solution

$K_1$ is expressed as the fraction of the exchangeable body water which is exchanging per hour.

Results

Two of the major pathways of water balance, total water influx and urinary afflux, were comprehensively analyzed in an effort to gain some
understanding of the water movements between Cancer magister and the isosmotic or hypoosmotic external environment. The results of the study of the tritiated water-monitored influx are presented in Tables 5a and 5b. Two experimental setups were employed, and these have been briefly defined and described as "open" and "closed" systems. The primary differences were: (1) a physical manipulation of the crabs preceding the 15 minute exposure to the loading solution—an element incorporated into the "open" system; and (2) the restriction of animal movements that prevailed in the "closed" system. The "closed" system was stirred, whereas the "open" system was not, but repetitive sampling of the "open" loading solution indicated a homogeneous distribution of the tracer.

With the "open" system (Table 5a), it is evident that the influx constant ($K_1$), an expression representing the fraction of exchangeable body water that is transported per hour, is of greater magnitude for crabs in 100% seawater. In this medium 215% of the exchangeable body water pool is renewed per hour, compared with a 112% exchange by crabs exposed to 30% seawater. This difference is highly significant. The standard deviations for the two experimental populations are dissimilar, however. Even though the water exchange rate may be dependent upon body weight, it can be readily seen that the tested weight ranges for the two experimental environments are reasonably uniform (Table 5a). In the "open" system, the reduction in the rate of water turnover is apparently caused by a decrease in the salinity of the medium.

Conversely, utilization of a "closed" method yielded water influx constants approximating 0.80 for animals in both the isosmotic and
Table 5a. The effect of salinity upon the water influx constant ($K_i$) of *Cancer magister* in an "open" system*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100% Seawater</th>
<th>30% Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>s.d.</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Body Weight Range (g)</td>
<td>22-178</td>
<td>29-165</td>
</tr>
<tr>
<td>Average Weight (g)</td>
<td>85.6 $\pm$ 43.6</td>
<td>73.0 $\pm$ 36.6</td>
</tr>
<tr>
<td>Serum Osmotic Conc. (mOsm)</td>
<td>988 $\pm$ 16</td>
<td>629 $\pm$ 70</td>
</tr>
<tr>
<td>$K_i$ (hr$^{-1}$)</td>
<td>2.15 $\pm$ 0.51</td>
<td>1.12 $\pm$ 0.16</td>
</tr>
</tbody>
</table>

*The "open" system is Method A, Materials and Methods.

Table 5b. The effect of salinity upon the water influx constant ($K_i$) of *Cancer magister Dana* in a "closed" system.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100% Seawater</th>
<th>30% Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\bar{x}$</td>
<td>s.d.</td>
</tr>
<tr>
<td>Number of Animals</td>
<td>10</td>
<td>35</td>
</tr>
<tr>
<td>Body Weight Range (g)</td>
<td>68-95</td>
<td>31-121</td>
</tr>
<tr>
<td>Average Weight (g)</td>
<td>80.9 $\pm$ 7.6</td>
<td>67.8 $\pm$ 23.9</td>
</tr>
<tr>
<td>Serum Osmotic Conc. (mOsm)</td>
<td>1013 $\pm$ 28</td>
<td>554 $\pm$ 67</td>
</tr>
<tr>
<td>$K_i$ (hr$^{-1}$)</td>
<td>0.80 $\pm$ 0.18</td>
<td>0.82 $\pm$ 0.20</td>
</tr>
</tbody>
</table>

**The "closed" system is Method B, Materials and Methods.
hypoosmotic media (Table 5b)—the results were statistically indistinguishable. The rate constants obtained for the animals in this system, although significantly lower, approach the range of measurements observed for crabs exposed to the "open" hypoosmotic environment. The standard deviations encountered in the "closed" milieux are similar, as are the experimental weight ranges (Table 5b). Standard deviations for both sets of "closed" system experiments are low when compared to the "open" 100% seawater examination—perhaps an indication of the homogeneity of the "closed" system.

The functional relationship between body weight and the rate of water influx is illustrated in Figures 6a and 6b for the set of all animals tested in a "closed" system. The correlation coefficient for the regression analysis of body weight and \( K_i \), the water influx constant, is significant. As body weight increases, the water influx constant decreases (Fig. 6b).

\( K_i \) can be expressed as milliliters water exchanged per hour, if the body weight and the size of the internal water pool are known. The amount of water was measured in six crabs by determining the wet weight of the animals, determining the dry weight after a 24 hr. period at 110°C, and calculating the percentage water. The average for animals in 50% and 75% seawater was 72% of the wet weight with a range of 70-75%. To use this factor in determining the quantity of water transported by a crab of prescribed body weight in an hour, two assumptions must be advanced: (1) all of the water in the crab, 72% of the body weight, is exchangeable with the external milieu; and (2) a single rate constant
Fig. 6a. The relationship between the water turnover rate and body weight in the "closed" system (Method B).

Fig. 6b. The relationship between the water exchange constant ($K_i$) and body weight in the "closed" system (Method B).

\[ y = 0.443x + 8.52 \]

\[ y = -0.003x + 1.028 \]
governs the turnover rate of the entire water pool. The rate of
exchange in ml/hr. is equal to body weight times 0.72 times $K_1$. This
rate is compared to the body weight in Fig. 6a for animals in a
"closed" system. The slope of the regression line was calculated to be
0.45. It should be emphasized that the body weight range encompassed
was not extensive (30-120 g). Because this study was directed toward a
determination of the effects of salinity upon the water influx con-
stant, unrelated variables were kept at a minimum. Therefore, the body
weight-water influx correlation is strictly applicable only over a
limited size range (30-120 g).

To measure the rate of micturition of Cancer magister, a procedure
using $^{125}$I-labeled sodium iothalamate (Glofil) was employed. Table 6
is a summary of data that indicates the suitability of the technique.
The equilibration time is an approximation of the period required for
the attainment of consistently equivalent urine-serum ratios for the
tracer. It was determined for several crabs in each environment by
following the time course of Glofil distribution as observed in serum
and urine samples. The shortening of this time with decreasing salinity
is obviously due to the varied rates of water movement through the
antennary gland in response to the external-internal osmotic dif-
ferential.

Upon equilibration, the U/S for Glofil is greater than one in all
of the test environments, suggesting the involvement of water reabsorp-
tion or a secretory process in the formation of the urine. The U/S
displayed by animals in 30% seawater is significantly lower than the
Table 6. Analysis of the employment of $^{125}$I-labeled sodium iothalamate (Glofil) for the measurement of micturition rates in Cancer magister.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100% Seawater</th>
<th>75% Seawater</th>
<th>50% Seawater</th>
<th>30% Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td>U/S for Glofil</td>
<td>15 1.45 0.26</td>
<td>14 1.34 0.21</td>
<td>10 1.32 0.16</td>
<td>75 1.20 0.14</td>
</tr>
<tr>
<td>Equilibration Time</td>
<td>36 hr.</td>
<td>24 hr.</td>
<td>16 hr.</td>
<td>16 hr.</td>
</tr>
<tr>
<td>% Decline in Activity</td>
<td>20%</td>
<td>11%</td>
<td>17%</td>
<td>15%</td>
</tr>
<tr>
<td>Experimental Period</td>
<td>24 hr.</td>
<td>12 hr.</td>
<td>8 hr.</td>
<td>6 hr.</td>
</tr>
</tbody>
</table>
ratio expressed by animals in 100%, 75%, and 50% Coos Bay seawater. The more important consideration for this ratio in discussing the compatibility of the method, is its degree of stability throughout the duration of each experiment. Initial and final samplings of the serum and urine for crabs in all applied media demonstrated a very acceptable reproducibility. The average difference between the initial and final U/S was 12%.

To apply the simplified mathematical treatment in calculating the micturition rate, the urine concentration of the tracer must be relatively constant through the experimental period. For very short experiments (less than 3 hr.) such a requirement is satisfied. However, a problem was encountered in this examination because of the mode of excretion. Micturition in Cancer magister is intermittent rather than a continuous discharge (Fig. 7). A rate calculated from one urination would be unsatisfactory, and so it was proposed that a minimum of three urinations be required for a proper assessment. The experimental period for animals in the four concentrations of Coos Bay seawater was therefore variable (Table 6). The extended time periods consequently affected a decrease in the initial urine concentration of Glofil. This decrease in activity is expressed as the average % decline in activity in Table 6. Because a calculation based on the application of the initial urine concentration would yield a low urination rate, and that calculated from the final tracer concentration in the urine would be high, the average of the initial and final urine Glofil activities was applied in an effort to correct the unavoidable error, at least
Fig. 7. A chart recording of micturition in the Dungeness crab.

Chart speed is 3 inches per hour.
Another minor problem was discovered in the employment of \textsuperscript{125}I-labeled Glofil as a tracer: pulsative discharge was not the sole mode of its release. The increase in activity of the bathing medium detectable between urinations is demonstrated in Fig. 8. Such a leakage, although slight, was displayed by the majority of the animals. To determine its origin, the nephridiopore opercula of two animals exhibiting the atypical release of tracer in 30\% seawater were sealed with dental cement for a period of six hours. During this time, the gradual increase in activity was observed with no urinary pulses. After six hours, the plugs were removed, and micturition proceeded in the normal manner. It was concluded that the slow emission of tracer from the animal was not due to leakage through the opercula, but an extrarenal loss of activity that was presumed to be free iodine, probably diffusing through the gills. When detectable, this increase in activity was subtracted from the total activity of the bath prior to calculation of the micturition rate.

The micturition rates of \textit{Cancer magister} in environments of varied osmotic concentration are expressed in Table 7 as a percentage of the body weight excreted per hour and per day. In the isosmotic environment, the micturition rate is low, due to the absence of a large osmotic gradient. The observed rate may be high, however, as the handling of the animals that preceded the monitoring of activity quite often evoked a urination at the onset. Nevertheless, the rate of urination displayed by crabs in dilute media is substantially greater.
Fig. 8. A chart recording demonstrating leakage of the tracer during intermittent micturition in the Dungeness crab.

Chart speed is 1.5 inches per hour.
Table 7. The effect of salinity upon the micturition rate of *Cancer magister*, monitored with 125I-labeled sodium iothalamate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>100% Seawater</th>
<th>75% Seawater</th>
<th>50% Seawater</th>
<th>30% Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td>s.d.</td>
<td>n</td>
</tr>
<tr>
<td>Serum Osmotic Conc. (mOsm)</td>
<td>10</td>
<td>1013</td>
<td>28</td>
<td>8</td>
</tr>
<tr>
<td>Urine Osmotic Conc. (mOsm)</td>
<td>7</td>
<td>995</td>
<td>24</td>
<td>8</td>
</tr>
<tr>
<td>Mole Fraction Diff.*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>Micturition Rate</td>
<td>10</td>
<td>0.06</td>
<td>0.02</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>% B.W.** (hr.⁻¹)</td>
<td>1.38</td>
<td>0.54</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>% B.W.** (da⁻¹)</td>
<td>1.38</td>
<td>0.54</td>
<td>5</td>
</tr>
</tbody>
</table>

*Solvent mole fraction difference

**B.W. = Body Weight
In 75% seawater the micturition rate is 5% of the body weight per day, and this quantity is doubled in 50% seawater. One might expect a further increase in 30% seawater, but this is not the case. The rates for crabs exposed to 50% and 30% seawater are identical. Two primary factors govern the rate of urine formation for animals in a hypotonic situation: (1) the rate of water movement between the external and internal environments; and (2) the developed osmotic differential (solvent mole fraction difference) between the seawater and serum compartments. The mole fraction differences exhibited in each medium are also listed in Table 7. The figures were not derived from the mean osmotic concentrations, but were instead computed for the individual animals and averaged. It is readily observed that the mole fraction differences maintained by animals in 50% and 30% seawater are equivalent.

The relationship between the solvent mole fraction difference and the urination rate for all animals observed in hypotonic environments is illustrated in Fig. 9 by a linear regression plot. The correlation is highly significant, but it is probably not absolute as $K_1$, the water influx constant, can modify the micturition rate. In fact, the rate can be predicted for an animal if $K_1$, the solvent mole fraction difference, and the fraction of body weight representing the total body water are known (see Rudy, 1967; Potts and Parry, 1963, p. 23). Such an estimation has been termed the diffusional permeability, or $P_d$. This fraction, multiplied successively by the water influx constant, 0.72, and 24, yields the net water influx in terms of the body weight.
Fig. 9. Linear regression plot of two variables, micturition rate and body weight, for crabs exposed to different concentrations of seawater.

**Micturition Rate (Y)**
(% Body Weight/Day)

\[ y = 2660x + 1.065 \]

- ○ = 75% SW
- + = 50% SW
- ● = 30% SW

**Mole Fraction Difference (X)**
per day. This quantity must be discharged to maintain water balance. In Table 8 the calculated diffusional permeabilities exhibited by the animals used in the Glofil experiments are compared with the corresponding osmotic permeabilities ($P_{os}$), the observed rates of micturition. Water influx constants were not determined for the Dungeness crab in 75% and 50% seawater. As urine production measurements were performed in the "closed" system, in which salinity did not affect $K_1$ (Table 5b), it was assumed that 0.80 was a close approximation to $K_1$ in these media. The solvent mole fraction differences in the two environments are mean values for individual computations. The diffusional permeabilities determined for crabs tested in the 30% seawater urine production experiments resulted from the application of $K_1$ and the mole fraction difference measured for each animal. In all three environments the observed rate of micturition exceeded the predicted rate. The ratio of $P_{os}$ to $P_d$, is approximately 2 in all of the hypoosmotic media.

The continuous recording of urine discharge provided a basis for the study of other aspects of the physiology of the antennary gland of decapod crustaceans. The frequency and volume per urination for animals exposed to isosmotic and hypoosmotic solutions are presented in Table 9. The frequency of urination apparently dictates the observed urination rate, as this parameter is observed to increase with an increasing osmotic differential. The volume of a distinct urination (Fig. 7), given as a percentage of the body weight, was readily calculated by modifying the equation given on p. 32. The volume per urination is equal to the increased activity of the bath divided by the
Table 8. Comparison of the osmotic permeability ($P_{os}$) with the diffusional permeability ($P_d$) of the Dungeness crab in hypoosmotic media.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>75% Seawater</th>
<th>50% Seawater</th>
<th>30% Seawater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>$\bar{x}$ ± s.d.</td>
<td>n</td>
</tr>
<tr>
<td>$p^*$</td>
<td>9</td>
<td>5.28 ± 1.54</td>
<td>5</td>
</tr>
<tr>
<td>$p^{**}$</td>
<td></td>
<td>2.35***</td>
<td></td>
</tr>
<tr>
<td>$P_{os}/P_d$</td>
<td></td>
<td>2.25</td>
<td></td>
</tr>
</tbody>
</table>

* $p_{os}$ = the micturition rate as % body weight per day

** $p_d$ = the calculated net water influx as % body weight per day

*** These values are based upon a predicted $K_i$ of 0.80, and the mean solvent mole fraction differences.

Table 9. The effect of salinity upon the urination frequency and the urination volume of the Dungeness crab.

<table>
<thead>
<tr>
<th>Treatment (%) Seawater</th>
<th>100</th>
<th>75</th>
<th>50</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (hr$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>6</td>
<td>5</td>
<td>32</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>0.10</td>
<td>0.4</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>± s.d.</td>
<td>0.05</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Volume (% B.W.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>33</td>
</tr>
<tr>
<td>$\bar{x}$</td>
<td>.58</td>
<td>.79</td>
<td>.67</td>
<td>.49</td>
</tr>
<tr>
<td>± s.d.</td>
<td>.24</td>
<td>.31</td>
<td>.25</td>
<td>.14</td>
</tr>
</tbody>
</table>

B.W. = Body Weight
activity per ml urine (U), and multiplied by 1/body weight. There are no significant differences in the urination volumes exhibited by animals in 100%, 75%, and 50% Coos Bay seawater. However, the volume per urination demonstrated by crabs in 30% seawater is significantly lower than the observed measurement in 100%, 75%, and 50% seawater. Because the frequency (Table 9) and micturition rate (Table 7) displayed by animals in 50% and 30% seawater are equivalent, this urination volume discrepancy is difficult to explain. The sample size for the 50% class is small, and the significance of the difference is marginal. Urination volume could conceivably be an important factor controlling the micturition rate of 30% seawater-adapted crabs.

Discussion

Because the Dungeness crab hyperosmoregulates in brackish water, the diffusional gradient for water molecules thus created supplies the animal with an abundance of water that must be dissipated through excretory processes. Assuming that excretion of waste materials requires energy, any physiological mechanism reducing the net amount of water entering an animal would obviously be advantageous. The net osmotic inflow of water in this decapod inhabiting a hypoosmotic environment is a function of the osmotic gradient and the permeability of the exposed surfaces.

Tolerance to a dilution of the internal salt pool by a species must indeed be complexly associated with the invasion of a certain habitat, but the subject of major concern in this examination was the
permeability of the externally-exposed membranes of Cancer magister. An increase in the resistance of the crab's membranes to the osmotic inflow of water would lessen the excretory load in dilute media. Varied permeability to water for different species from a variety of habitats has been previously examined. By exposing excised discs of carapace to dilute seawater, Gross (1957) has demonstrated that crabs capable of osmoregulating in dilute media possess an integument with a lower permeability to both water and salts than that of osmoconforming crustaceans. A similar study correlating the retardation of evaporative water loss with a terrestrial existence has been executed using whole animals and excised integument (Herreid, 1968, 1969).

The availability of tritiated and deuterated water has provided a tool for a more dynamic and direct assessment of water movements across membranes (e.g. Chinard and Enns, 1954). Employing tritiated water, Rudy (1967) has reported that the influx constant for the freshwater crayfish, Astacus fluviatilis ($K_i = 0.20$), is considerably lower than that exhibited by the marine brachyuran, Macropipus depurator ($K_i = 2.39$). The rate constant of the brackish water-dwelling Carcinus maenas is intermediate in magnitude ($K_i = 0.79$). Another brackish water crab, Hemigrapsus nudus, displays a tritiated water-monitored $K_i$ of 0.94 in seawater (Smith and Rudy, 1972). The temperature at which these studies were conducted was 10°C. The recorded $K_i$ of 0.80 for the Dungeness crab in a "closed" system is similar to the established
values for brackish water crabs.\textsuperscript{1} The diffusional permeability constant for three species of the semi-terrestrial fiddler crab, \textit{Uca}, is quite low ($K_1 = 0.30$, Hannan and Evans, 1973), while it has been inferred, on the basis of urine production experiments, that the freshwater crab, \textit{Potamon niloticus}, is essentially impermeable to water (Shaw, 1959b). A lowered permeability of the medium-exposed surfaces may be adaptable for the inhabitation of hypoosmotic environments by many crustacean species.

For a euryhaline animal, differential rates of diffusional transport of water and salt across the exposed membranes may be appropriate for subsistence in waters of changing osmotic concentration. The transition from freshwater to seawater, or vice versa, elicits a water permeability change in some species of euryhaline fishes (Potts \textit{et al.}, 1967; Evans, 1969a; Motais \textit{et al.}, 1969), although the existence and pattern of the phenomenon is species dependent (Evans, 1967c, 1969a,b). The establishment of the presence of such a rate change in euryhaline crustaceans has been more controversial. A decline in the rate of passive water influx was postulated for \textit{Carcinus maenas} in brackish water (Webb, 1940). Such an adaptive reduction in the "apparent" water permeability of a crustacean was initially demonstrated in the ubiquitous crab of the Zuider Zee, \textit{Rhithropanopeus harrisi} (Smith, 1967). The water influx constant, as monitored with deuterated water (DHO), sequentially increases with salinity in environments ranging from 1\% to

\textsuperscript{1}0.80 is assumed to be the more acceptable value, because of the homogeneity of the "closed" system.
95% seawater. In his study of several crustacean species, Rudy (1967), however, recorded salinity-independent influx constants for the euryhaline crustaceans *Carcinus maenas* and *Palaemonetes varians*. Conversely, Smith (1970), with the application of another technique (DHO) and a higher temperature regime, was able to demonstrate the water exchange reduction in *Carcinus maenas*. Additionally, his temperature-compensated rate constants were much higher than Rudy's (1967). Because the two experiments suggested an isotope or temperature effect, the two workers concurrently studied water balance in *Hemigrapsus nudus*, employing the two tracers and similar experimental temperatures (Smith and Rudy, 1972). The influx measurements with THO were slightly lower than the corresponding DHO values (4%), and both methods yielded results indicating the occurrence of a reduction in the water exchange rate when this species is exposed to 60% seawater. *In vitro* examinations of isolated membranes have shown that there is little difference in the rates of penetration of DHO and THO in these systems (King, 1969; Chinard and Enns, 1954). An "isotope effect" is probably minimal, and an explanation for the disparate results for *Carcinus maenas* based upon this factor or a "temperature effect" is difficult to visualize. Another element must be controlling the varied response.

More recent studies have been directed at resolving the controversy developed over the existence of an adaptive reduction of the water influx rate in crustaceans; but in fact, they have not. The chelicerate, *Limulus polyphemus*, reduces the rate of THO uptake when exposed to brackish water, whereas three species of the fiddler crab,
**Uca**, do not (Hannan and Evans, 1973). **Uca** is an extremely capable osmoregulator (Green et al., 1959), but **Limulus polyphemus** exhibits a rather poor homeosmotic performance in hypoosmotic media (Robertson, 1970). Lockwood, Inman, and Courtenay (1973) have recorded a progressive reduction in the water permeability of **Gammarus duebeni** exposed to salinities ranging from 150% to 2% seawater. Interestingly, it is suggested that the effect is not hormonally controlled. Any conclusions concerning the effect of salinity upon the water influx rate of the euryhaline Crustacea as a group would be premature. It appears that a permeability reduction phenomenon may be species dependent, and in some instances the perception of such a change can be controlled by prescribed experimental conditions.

The results of this investigation impart credence to the latter idea. Method A and Method B produced results (Tables 5a,b) which lead to widely disparate conclusions about the effect of salinity upon the water exchange rate. The homogeneity of Method B (Table 5b) yielded the more consistent results, because of (1) the absence of any physical manipulation of the animals before termination; (2) continuous stirring of the medium insuring thorough distribution of the tracer; and (3) the confinement restricting unnecessary activity, created by the container-animal size ratio. The only variables in this system, aside from the crab, were the time required for the homogeneous distribution of tritiated water throughout the system, and the time necessary for removal of the animal from the loading solution following the 15 minute exposure. Introduction of methylene blue into the "closed" system
resulted in a rapid dispersal. Because of the large size (12-15 cm. across the carapace), the removal of a crab for sampling procedures was unpredictable. However, care was taken to reduce the time lag to a minimum.

The principal effector producing differential rates of water turnover in the "open" system has not been identified. Some differential response altering the water exchange rate is evidently triggered by salinity in this system. Evans (1969a) has studied the effects of several factors upon water permeability in fishes, and Hannan and Evans (1973) have similarly examined a chelicerate and several crustaceans. Physical "stress" increased the water influx of Salmo trutta and Salmo gairdneri in freshwater, while evoking a decrease in the flux across the exposed membranes of Platichthys platessa in seawater (Evans, 1969a). "Stress" had no effect upon the integumental water permeability of Limulus polyphemus, Penaeus duorarum, or Uca pugilator (Hannan and Evans, 1973). Additionally, feeding movements and walking leg autotomy in Uca, and the degree of mineralization of the shell of Limulus, do not affect the water influx rate constant (Hannan and Evans, 1973). The imposition of "stress" in these two studies was a procedure similar to Method A of this examination. The applied "stress" involved the transferal of animals into a loading solution, rather than the normal introduction of the tracer into the bathing medium (Evans, 1969a; Hannan and Evans, 1973). Curiously, the methods used by Smith (1967, 1970), Rudy (1967), Smith and Rudy (1972), and Lockwood, Inman and Courtenay (1973) apparently included this element of inflicted "stress."
Such a factor may be involved in producing the high $K_i$ for Cancer magister in the "open" seawater solution. But a salinity-induced alteration of $K_i$ in the "open" 30% seawater environment created by a differential response to the "stress" stimulus seems incongruous. The significant increment of difference in influx rate observed between animals in the 30% "open" environment and that exhibited in a "closed" system may result from "stress," but the permeability change observed in the "open" system probably does not.

The effect of activity upon the turnover rates of water and salt in the rainbow trout, Salmo gairdneri, has been elucidated (Randall, et al., 1972; Wood and Randall, 1973a,b,c). Shifts in the permeability of the gills to water during activity were indirectly evaluated by recording weight and urine production changes (Wood and Randall, 1973c). A distinct increase in the rate of urine accumulation was correlated with increased activity. Activity could conceivably be the effector of the change in the rate of water exchange seen in Cancer magister in an "open" system. In the "closed" system, in which an alteration in the apparent water permeability does not occur, extensive activity of the crabs was inhibited. Although the opportunity for movement exists in the "open" environment, no concrete evidence is available indicating any relationship between activity and the environmental salinity. However, subjective observations of crabs in holding tanks and the crabs exposed to the "open" system suggest a greater tendency for activity in 100% seawater, especially after physical manipulation. Lockwood, Inman, and Courtenay (1973) also observed an increase in permeability when they
exposed gammarids to mannitol solutions of varied osmotic concentrations. The substitution of mannitol for NaCl has proven to be very "uncomfortable" for the Dungeness crab, quite often eliciting a violent response upon initial exposure. Such a burst of activity may be involved in producing the observed permeability change in gammarids exposed to the mannitol milieu.

The mechanism for the variation in the water turnover rate in Cancer magister exposed to an "open" system is not immediately obvious. Smith (1970) has submitted that the permeability transformation may be induced by a structural change in the integumental barrier, an increase in the ventilation rate, or a circulatory response. Hannan and Evans (1973) have dismissed the latter two hypotheses, since the fraction of water flowing across the gills, relative to the quantity of haemolymph flowing through the gills or to the ventilation volume, is minute. Richards and Fromm (1970) have detected little change in the net sodium uptake across the isolated perfused gills of Salmo gairdneri by altering the pattern and flow rate of perfusion fluid. However, the activity-induced augmentation of the diffusional flow of water and sodium ions in the gills of the rainbow trout is accompanied by marked intensification of certain ventilatory and cardiovascular functions. Ventilatory frequency and amplitude, the blood pressure in the dorsal aorta, and cardiac output, all increase in response to swimming activity (Wood and Randall, 1973a). The level of circulating catecholamines rises when rainbow trout are physically disturbed (Nakano and Tomlinson, 1967). These compounds decrease the vascular resistance to blood flow through
the gills (Randall et al., 1972). It was therefore postulated that diffusional permeability changes are mediated by a transformation in the pattern of blood flow across the gills that either increases the "functional" area available for the transfer of gases, water, and salts, or effectively reduces the diffusional distance (Randall et al., 1972).

The situation in the crustacean gill and circulatory system during activity has not been comprehensively analyzed. The ventilation volume of some decapods increases in response to a diminution of oxygen in the external milieu (Larimer, 1961; Arudpragasam and Naylor, 1964; Seaton and Rehm, 1972). In Carcinus maenas, this response not only results in a sharp increase in the oxygen consumption rate, but also elicits an increase in the efficiency of extraction (Arudpragasam and Naylor, 1964). Branchial permeability has been characterized in fishes as a "dynamic compromise between respiratory and osmoregulatory demands" (Wood and Randall, 1973a), thus ventilatory modifications increasing the oxygen transfer in the gills of crustaceans must surely influence the movement of water and salts. Perhaps a change in the gill chamber pressure that has been observed during increased ventilation is involved (Seaton and Rehm, 1972). Arudpragasam and Naylor (1964) have demonstrated that the frequency of the periodic backward flow of water in the gill chamber of Carcinus maenas, created by a reversal in the beat of the scaphognathite, is directly proportional to the rate of forward transport. The ventilation rate is in turn dependent upon activity and the oxygen requirement. This process is believed to facilitate bathing
of the posterior gills, as the inhalant Milne-Edwards openings are located ventro-medially to the gill chamber (Arudpragasam and Naylor, 1966). This current reversal significantly increases the pressure in the gill chamber of *Carcinus maenas*, and may increase the respiratory efficiency (Blatchford, 1971). It is conceivable that pressure shifts in the gill chamber of *Cancer magister* during activity could enhance the exchanges of gas, solute and water.

Although a distinct correlation between salinity-induced activity and an increase in the water turnover rate of *Cancer magister* was not established in this study, such a phenomenon would provide a solution to much of the controversy concerning the adaptive reduction of the apparent water permeability seen in some euryhaline crustaceans. The possible relationship between activity, salinity, and water movements in the natural environment therefore presents an intriguing problem. The "preferred" experimental conditions may be an unnatural imposition construed by the experimenter, thereby yielding illusory information. Nevertheless, on the basis of experiments performed in both "closed" and "open" systems, the water influx constant of the Dungeness crab in natural waters of reduced salinity is apparently low when compared to stenohaline crustaceans. This circumstance may constitute an inter-specific adaptation for life in brackish water.

The effects of temperature and body size upon the water influx rate in crabs is also important in a consideration of water balance. The $Q_{10}$ for the diffusional permeability constant of water is approximately 2 for *Limulus polyphemus* and *Uca pugilator* (Hannan and Evans, 1973), 1.9
for three species of teleost (Evans, 1969a), and 1.6 for Hemigrapsus nudus (Smith and Rudy, 1972). The flux of water is also related to the 0.8-0.9 power of the body weight in fishes (Potts et al., 1967; Evans, 1969a). Smith (1967) reported extremely low slopes for the body weight-influx rate relationship in Rhithropanopeus harrisi. The rate of influx varied with the -0.10 - -0.20 power of the body weight. Such a correlation suggests that the rate of influx per unit body weight increases with size. Smith (1967) hypothesized that the permeable area increased with body weight, but Gray (1957) has demonstrated the opposite correlation for the gill surface area of several crabs. The limited amount of information available from this study of water balance in Cancer magister indicates that a water influx for animals in both isosmotic and hypoosmotic media is dependent upon the 0.45 power of the body weight (Fig. 6a). The flux per unit weight decreases with an increase in size. The rate constant, \( K_1 \), was also shown to vary inversely with body weight. This assessment of the relationship between body weight and water influx may be misleading, however, because of the limited range of weights examined.

Cannulation of the urinary bladder is the method of preference for recording urine production rates in fishes. Because of the ventro-anterior position of the excretory openings of decapod crustaceans, direct collection of urine is nearly impossible. In decapods of the macruric form, cannulation has been attempted with some success (Parry, 1955; Kamemoto and Ono, 1968), but the brachyuric form of Cancer magister and many other decapods creates an additional problem: the proximity of
the thoracic appendages to the excretory openings. The classical technique of micturition assessment involves occlusion of the nephridiopores with dental cement or another suitable substance, and either monitoring the weight gain through time, or collecting the urine after a prescribed period (e.g. Nagel, 1934; Parry, 1955). The primary criticisms of this procedure are: (1) the possible inhibition of urine formation due to back pressure; and (2) the adverse physiological reactions that may ensue. Dyes have been employed (e.g. Parry, 1955), but the problems incurred in accurately quantifying the results and selecting a substance that is not reabsorbed or actively secreted are not easily overcome. Shaw (1961a), employing $^{35}$S-labeled sulphate ions, measured urine production indirectly by assaying the rate of sulfate penetration. A similar assay, involving the measurement of magnesium accumulation in the external milieu, has been developed (Gross and Marshall, 1960). The obvious objection to both these methods is the assumption that the antennary gland is the sole pathway for the efflux of magnesium and sulfate ions. Recently, Binns (1969b), and Lockwood and Inman (1973), have used labeled compounds that are believed to be discharged only via the excretory system. Because these tracers are radioactive substances, quantification of such a technique is simplified.

Upon equilibration, the U/S of $^{125}$I-Glofil is comparable to the observed ratio of $^{14}$C-inulin (Lockwood and Riegel, 1969; Binns, 1969a). The urine-serum ratios for both tracers are highest for animals in seawater and hypertonic seawater, indicating some degree of water reabsorption or secretion of the tracers. $^{131}$I-sodium diatrizoate has been
used to determine micturition rates in the amphipod, *Gammarus duebeni* (Lockwood and Inman, 1973). The reported U/S for seawater-adapted animals was 1.05; it was higher for amphipods in dilute media, averaging 1.25. The three isotopic tracers are probably treated similarly by the malacostracan antennary gland. The time for equilibration of these compounds throughout the circulatory and excretory systems is extremely variable. For *Carcinus maenas* in seawater, a steady \(^{14}\)C-inulin U/B was attained after 115 hr. The time is much shorter for \(^{131}\)I-sodium diatrizoate equilibration in amphipods and \(^{125}\)I-sodium iothalamate distribution in *Cancer magister*, being 36 hours for the latter (Table 6), and approximately six hours for the former (Lockwood and Inman, 1973). These times are probably dependent upon the mobility of the compound in the animal, which is a function of the molecular weight. The time required for attainment of a steady state in dilute seawater media is reduced for all three substances, due to the increased rate of flow through the excretory system. Because of the presence of free iodine, significant errors have been encountered with the use of \(^{131}\)I-sodium diatrizoate (Sigman, et al., 1965). Using Glofil, only 2% free radioactive iodine was present up to three weeks after labeling (Sigman et al., 1965).

The calculations for micturition rate in the experiments of Binns (1969b) and Lockwood and Inman (1973) were based upon the time constant computed from the clearance of tracer from the blood. A simpler analysis, dependent upon the increase in activity in the medium, was used in this examination. The time required for completion of an exper-
iment in this study was comparatively short, an important consideration in sustaining large animals in systems of restricted volume. Binns (1969b) also utilized a procedure directed at measuring the appearance of $^{14}$C-inulin in the medium as an assay for urine production. The results of his two methods were indistinguishable. It seems, therefore, that the simplified mathematical treatment used here is justified.

Additionally, the continuous recording of micturition in Cancer magister (Fig. 7) provided another tool for the study of the physiology of the antennary gland of crustaceans.

One application of this technique is an analysis of the hypothesis that magnesium accumulation in the bladder is a time-dependent secretory process (Gross and Capen, 1966). The discharge and filling of the bladder are events that can be predicted from the chart recording (Fig. 7). Sampling of the urine before and after discharge demonstrated no apparent ionic concentration differences. It is probable, however, that the pulse-like urinations do not represent complete evacuation of fluid from the excretory organ.

The rates of urine production recorded for Cancer magister (Table 7) in isosmotic and hypoosmotic environments can be compared with those determined for another brackish water crab, Carcinus maenas (Binns, 1969b), as both sets of experiments were performed at 10°C. For animals in 100%, 75%, 50%, and 40% seawater, micturition rates of 4.4%, 10.8%, 19.9%, and 21.1% of the body weight per day, respectively, were reported. The higher rates for Carcinus maenas in hypoosmotic media must result from a higher sustained osmotic gradient (Nagel, 1934), as
the diffusional influx constants for the two animals at 10°C. are equal (Rudy, 1967; this study Table 5b). Shaw's (1961a) data for osmo-regulating Carcinus maenas indicate that the increment of difference between the external and internal osmotic concentrations does not increase in media less concentrated than 60-50% seawater. It has been demonstrated in this study that the rate of micturition is a function of the solvent mole fraction difference (Fig. 9), and it can be readily observed that a species may inhabit media of varying osmotic concentration and yet exhibit equivalent urination rates (Table 7). The observed increase in urine production of Carcinus maenas in 40% seawater is somewhat puzzling.

The difference in micturition rate for seawater-adapted Cancer magister and Carcinus maenas is also perplexing, because both animals are essentially isotonic in the marine environment (Webb, 1940; this study, Table 1). In fact, micturition in seawater itself is a disconcerting process when the two principal effectors, $K_1$, and the solvent mole fraction difference are considered. Some other element may indirectly influence the excretory rate. The necessity for some excretion of waste products in seawater is unquestionable, but the source of this 1.4 to 4% of the body weight per day is not well understood. Lockwood and Inman (1973) have suggested that the net movement of water into seawater-exposed Gammarus duebeni results from an association of water and ion transport. Through treatment with thionine, an inhibitor of active sodium transport in crustaceans (Koch and Evans, 1956), the urine production rate in seawater markedly declined.
Exposure to an osmotically equivalent solution of mannitol, from which very little transport of solute is presumed to occur, did not verify the hypothesis, however.

In Cancer magister, the serum osmotic concentration is slightly hyperosmotic to seawater (Tables 1, 7), and this osmotic gradient will result in a net water influx of approximately 0.3% of the body weight per day. Assuming a \( P_{os}/P_d \) membrane characteristic of 2 for animals in 100% seawater (Table 8), the disparity between the observed and predicted values still exists (0.6% vs. 1.4%). The transport of water is apparently not altogether osmotically engineered, and therefore some interaction between the movements of water and salt is a distinct possibility.

A difference in the osmotic and diffusional permeabilities (Table 8) is not uncommon. Combining Rudy's (1967) water permeability data for Carcinus maenas with the results from urine production experiments executed at an equivalent temperature (Binns, 1969b), the \( P_{os}/P_d \) for crabs in 40% seawater is calculated to be 3. In 40% seawater Gammarus duebeni exhibits a ratio of 1.4; this value approaches unity in very dilute media (Lockwood and Inman, 1973). The phenomenon in teleosts has been extensively studied. Marine fishes generally display a ratio of 1 (Motais et al., 1969; Evans, 1967, 1969b), especially when rectal loss of water and drinking are considered (Evans, 1969a). The ratio for many fishes inhabiting freshwater is greater than one, and usually approaches 3 (Motais et al., 1969; Evans, 1969a). Several euryhaline fish are exceptional, however, as the osmotic-diffusional permeability
ratio is approximately 1 for both freshwater and seawater inhabitants (Potts et al., 1967; Evans, 1967, 1969b).

The force or mechanism underlying this difference has not been satisfactorily characterized. Theories dependent upon the presence of unstirred layers (Dainty and House, 1966), and the bulk flow of water through pores (Koefoed-Johnsen and Ussing, 1953), have been advanced. But a differential manipulation of the water and heavy water molecules by membranes, which is seemingly implied, is questionable when the DHO-THO studies of King (1969) and Chinard and Enns (1954) are recalled. These workers found little variation in the rates of penetration for these two isotopes. Smith and Rudy (1972) have predicted that a 5-10% discrepancy between observed and theoretical net water influx rates is the maximum that can be attributed to an "isotope effect."

An accurate comparison of the osmotic and diffusional permeabilities may be impaired by the exclusion of several minor pathways of water movement. Evans (1969a) has stressed the importance of recording drinking rates and rectal losses in fishes. When these pathways were considered, the disparity between the $P_{os}$ and the $P_d$ was lessened. Drinking by Uca pugilator accounts for 3% of the total water influx in seawater, and 1.3% in brackish water (Hannan and Evans, 1973), apparently an insignificant contribution. Whatever amount the crab drinks, although not treated in the calculation of the diffusional permeability, contributes to the net influx of water, however. The

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1It is assumed that drinking is not necessarily dependent upon an osmotic differential.
net water influx through the gills of *Cancer magister* in 30% seawater accounts for 0.5% of the total water influx; drinking should therefore be considered in any comparison of the measured osmotic to the predicted diffusional permeability. This does not, however, explain the large $P_{os} - P_{d}$ ratios observed in membrane preparations (Koefoed-Johnsen and Ussing, 1953).

An alternative explanation dependent upon differential rates of water influx and efflux has not been fully explored. This could conceivably produce a $P_{os} - P_{d}$ greater or less than unity. Investigations that have included the measurement of both total water influx and efflux were performed upon animals exhibiting an osmotic–diffusional permeability ratio of approximately 1 (Potts *et al.*, 1967; Lockwood and Inman, 1973).

Hannan and Evans (1973) have elegantly partitioned the water influx of *Uca pugilator* into its separate pathways. The gills are responsible for 85% of the total flux, the exoskeleton contributes 11%, and the animal drinks the remaining 3%. In dilute media, the total flux does not decline, and the only change is a reduction in the drinking component to 1.5% of the total. Although these particular pathways were not defined in *Cancer magister*, a comprehensive understanding of the achievement of water balance in isosmotic and hypoosmotic media was elucidated. For a 100 g crab in seawater, the animal transports approximately 1300 g of water between the internal and external pools.

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1 The mole fraction difference in 30% seawater, Table 7, is 0.5% of the mole fraction of solvent in the external milieu.
per day (500-600 μg/g animal/hr.). A measured 1.4 g of water effluxes via the excretory system, and the remainder is partitioned between the gills and the exoskeleton. The source of the net uptake of water could be drinking, a slight solvent mole fraction difference, or solute-associated movement. Under certain circumstances, possibly activity, the total flux may increase by 2.5 times.

In dilute media, the net water influx increases in response to the onset of osmoregulatory processes. The net influx is both a function of the sustained osmotic gradient and the influx constant, $K_i$. The urinary output for a 100 g Dungeness crab in 30% seawater is about 15 g per day, or 1% of the total flux; a very small but yet important component. Observed urinary efflux is actually greater than a theoretical calculation would indicate. The antennary gland of *Cancer magister* nevertheless is capable of performing the necessary osmoregulatory function. The water influx constant, $K_i$, is low when compared to the constant calculated for stenohaline crustaceans (Rudy, 1967); this is presumably an adaptation to life in brackish water. The influx constants for Dungeness crabs in 30% and 100% seawater are apparently equivalent, although some response of the animal in 100% seawater, presumably activity, was observed to trigger an increase in $K_i$. Pressure changes in the gill chamber and ventilatory or circulatory modifications should be investigated in this regard.
CHAPTER 5

IONIC REGULATION

Methods

Determination of the Percent Water of Seawater, Urine and Serum

Because of differences in the water content of seawater, serum, and urine samples, all ionic concentrations measured in this investigation are expressed as molal solutions (meq/kg H₂O). To correct the measured values to molality, the percentage of water in the individual samples was determined. Samples were initially weighed in small aluminum receptacles, dried at 110°C. for 24 hours, stored in a desiccator for 36-48 hours, and weighed again.

Sodium, Potassium, and Calcium Ion Determinations

A Coleman Model 21 Flame Photometer burning a mixture of propane and oxygen was used in these analyses. The Coleman Model 6/20 Junior II Spectrophotometer was wired for use as a galvanometer, and served as a readout device. A 100 μl aliquot was required for the measurement of all three ionic concentrations.

In an effort to eliminate ion interference in these analyses, standards with an ionic composition approximating that of seawater were prepared. This procedure was particularly necessary in the calcium...
analysis. Five analyses of standard solutions yielded the following standard deviations: Na\(^+\), 400 meq, ± 1.4 meq; K\(^+\), 12 meq, ± 0.7 meq; Ca\(^{++}\), 24 meq, ± 1.3 meq.

Determination of Chloride Ion Concentration

Chloride content of the body fluid and seawater samples was measured through amperometric titration with a Buchler-Cotlove Chloridometer. The sample size required was 20 μl. Triplicate measurements of a 200 meq standard solution produced a standard deviation of ± 5.5 meq.

Determination of the Magnesium Ion Concentration

A modification of the method described by Sky-Peck (1964) was employed in this study. It is a colorimetric analysis based on red color formation produced by a Clayton yellow-Mg(OH)\(_2\) complex in alkaline solution. Serum samples were deproteinized by treatment with a 5% solution of trichloracetic acid (TCA). The principal alteration involved the manipulation of reagent volumes in the development of a microtechnique for magnesium ion analysis. This procedure demanded only a 20 μl sample of fluid. The absorbance was measured with a Coleman Jr. II Spectrophotometer, Model 6/20, set at a wavelength of 540 nanometers. Standards with a seawater-simulated constitution were utilized. The standard deviation from 5 analyses of a 100 meq standard was 1.1 meq.

Sulfate Ion Determination

The turbidimetric method of Berglund and Sorbo (1960) provided the
basis for this analysis. A barium chloride-gelatin reagent was used to elicit barium sulfate precipitation. Serum samples were deproteinized by treatment with a 5% TCA solution. Reagent volumes were modified to limit the required sample size to 50 μl. Absorbance was measured with the Coleman Jr. II Spectrophotometer at a wavelength of 360 nanometers. Artificial seawater standards were employed. Four determinations with a 72 meq standard solution yielded a standard deviation of ± 4.0 meq. Berglund and Sorbo (1960) reported a recovery of added sulfate from human serum samples ranging from 99-104%. Unfortunately, recovery was not determined in this study.

Results

Figures 10-15 illustrate the patterns of ionic regulation for several ions of the serum and urine of Cancer magister. Points of equivalence between medium and body fluid concentrations are signified by the dashed diagonal lines. These patterns are the result of the static assessment of Na⁺, Cl⁻, K⁺, Ca⁴⁺, Mg²⁺, and SO₄⁻ ion concentrations in the body fluids of the Dungeness crab from isosmotic and hypoosmotic experimental media. A detailed account of this examination has been assembled in Table 10. Ionic concentrations of the several seawater media used are indicated in Table 10; these values represent actual measurements of the ionic constituents in meq/1. The final expressions in meq/kg H₂O were obtained with a correction factor which was computed from the table of Potts and Parry (1963, p. 90). The procedure employed in deriving the meq/kg H₂O expression for serum and
Table 10. Serum and urine ionic concentrations for animals in isosmotic and hypoosmotic media.

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>100% SW x ± s.d. n</th>
<th>75% SW x ± s.d. n</th>
<th>50% SW x ± s.d. n</th>
<th>30% SW x ± s.d. n</th>
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<tr>
<td>Na⁺ as meq/kg H₂O</td>
<td>Medium</td>
<td>455 339</td>
<td>230 142</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>502 22 86</td>
<td>443 30 40</td>
<td>373 39 26</td>
<td>253 38 16</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>422 35 77</td>
<td>400 47 39</td>
<td>318 61 26</td>
<td>252 30 12</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>0.85 .08 77</td>
<td>0.90 .09 39</td>
<td>0.86 .14 26</td>
<td>1.01 .16 11</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.001 &lt;.001</td>
<td>&lt;.001 &lt;.05</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td>Cl⁻ as meq/kg H₂O</td>
<td>Medium</td>
<td>521 390</td>
<td>261 157</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>531 24 87</td>
<td>432 31 40</td>
<td>375 47 26</td>
<td>285 45 16</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>545 25 77</td>
<td>432 42 38</td>
<td>361 51 27</td>
<td>308 40 14</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>1.03 .06 76</td>
<td>1.01 .05 37</td>
<td>1.04 .08 27</td>
<td>1.02 .10 14</td>
</tr>
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<td>P</td>
<td>&lt;.001 &gt;.05</td>
<td>&gt;.05 &gt;.05</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td>K⁺ as meq/kg H₂O</td>
<td>Medium</td>
<td>9.9 7.3</td>
<td>5.1 3.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>11.5 1.2 86</td>
<td>9.4 1.5 40</td>
<td>7.9 1.3 26</td>
<td>5.7 1.0 16</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>8.2 2.7 77</td>
<td>7.5 2.0 39</td>
<td>6.8 1.8 26</td>
<td>6.3 1.4 12</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>0.73 .26 77</td>
<td>0.82 .21 39</td>
<td>0.86 .26 26</td>
<td>1.10 .43 11</td>
</tr>
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<td>P</td>
<td>&lt;.001 &gt;.05</td>
<td>&gt;.05 &gt;.05</td>
<td>&gt;.05</td>
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</tr>
<tr>
<td>Ca⁺⁺ as meq/kg H₂O</td>
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<td>22.2 16.2</td>
<td>10.8 7.0</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>26.9 2.2 85</td>
<td>24.5 1.8 39</td>
<td>20.2 2.7 26</td>
<td>11.9 2.3 16</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>24.8 2.7 77</td>
<td>23.8 2.8 39</td>
<td>20.4 3.0 26</td>
<td>10.0 2.4 10</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>0.93 .12 75</td>
<td>0.97 .10 39</td>
<td>1.02 .14 26</td>
<td>0.92 .28 10</td>
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<td>&gt;.05 &gt;.05</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td>Mg⁺⁺ as meq/kg H₂O</td>
<td>Medium</td>
<td>103 76</td>
<td>52 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>42 12 75</td>
<td>29 12 38</td>
<td>17 9 26</td>
<td>20 5 17</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>156 29 69</td>
<td>94 44 35</td>
<td>87 61 28</td>
<td>23 6 16</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>3.91 1.11 67</td>
<td>3.59 2.32 34</td>
<td>5.03 2.85 21</td>
<td>1.21 .35 16</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.001 &lt;.001</td>
<td>&lt;.001 &lt;.05</td>
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<td></td>
</tr>
<tr>
<td>SO₄²⁻ as meq/kg H₂O</td>
<td>Medium</td>
<td>55 41</td>
<td>25 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>47 10 74</td>
<td>27 9 38</td>
<td>19 7 26</td>
<td>21 4 16</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>75 17 65</td>
<td>48 18 35</td>
<td>40 21 26</td>
<td>25 5 16</td>
</tr>
<tr>
<td></td>
<td>U/S</td>
<td>1.58 .32 64</td>
<td>1.99 1.28 34</td>
<td>1.78 .57 22</td>
<td>1.26 .35 16</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>&lt;.001 &lt;.001</td>
<td>&lt;.001 &lt;.01</td>
<td>&gt;.05</td>
<td></td>
</tr>
</tbody>
</table>

U/S = urine-serum ratio; SW = Coos Bay seawater
P = significance of student's t test
urine samples and the rationale for such a representation has been dis-
cussed (p. 70). The urine-serum ionic ratios (U/S) are also presented
in Table 10. A U/S less than one may indicate some degree of re-
absorption through the antennary gland. A ratio greater than one sug-
gests the promotion of preferential excretory processes for certain
ions.¹

**Sodium Regulation (Fig. 10)**

After a 24 hour experimental exposure to 100% Coos Bay seawater,
and a 72-96 hour immersion in 75%, 50%, and 30% seawater solutions, the
Dungeness crab maintains the serum sodium concentration² well above that
of the medium. This pattern of regulation is similar to that described
for the osmotic concentration (Fig. 1) with the serum sodium gradually
decreasing with a decrease in the external sodium. The sustained
gradient between the external and internal sodium pools progressively
increases with decreasing salinity, and appears to be expressed
maximally in 50% seawater. The decline in serum sodium exhibited from
50% to 30% seawater approximately parallels the sodium reduction in the
external milieu.

In the three hypoosmotic external environments, 75%, 50%, and 30%
Coos Bay seawater respectively, the $[\text{Na}^+]_s$ is reduced to 97%, 82% and

¹¹.2 is a more reasonable figure when reabsorption of water is
considered (Riegel and Lockwood, 1961).

²Ionic concentrations can be expressed as $[\text{Ion}]$. The subscript
denotes serum (s), urine (u), or seawater (sw).
Fig. 10. Sodium ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.

BODY FLUID (Na$^+$) as meq Na$^+$/Kg H$_2$O

MEDIUM (Na$^+$) as meq Na$^+$/Kg H$_2$O

$\Delta$ = serum
$\bullet$ = urine
56% of the 100% seawater sodium concentration (Table 11). The serum osmotic concentration in dilute media was observed to be 88%, 75%, and 56% of the serum osmotic concentration in 100% seawater. This difference is probably due in part to the high [Na⁺]s achieved by Cancer magister in 100% Coos Bay seawater. When expressed as a percentage of the serum sodium concentration in 100% seawater, the serum Na⁺ is 88%, 75% and 50% of the original seawater concentration (Table 11).

Table 11. Ionic reductions for animals exposed to hypoosmotic media, expressed as % seawater (SW) and isosmotic serum (IS) ionic concentrations.

<table>
<thead>
<tr>
<th>Seawater (mOsm)</th>
<th>1000</th>
<th>750</th>
<th>500</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>% SW</td>
<td>% SW</td>
<td>% S</td>
<td>% SW</td>
<td>% S</td>
</tr>
<tr>
<td>Na⁺</td>
<td>110.3</td>
<td>97.4</td>
<td>88.2</td>
<td>81.8</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>101.9</td>
<td>82.9</td>
<td>81.4</td>
<td>71.8</td>
</tr>
<tr>
<td>K⁺</td>
<td>116.2</td>
<td>94.9</td>
<td>81.7</td>
<td>79.8</td>
</tr>
<tr>
<td>Ca⁺⁺</td>
<td>121.2</td>
<td>110.4</td>
<td>91.1</td>
<td>90.5</td>
</tr>
</tbody>
</table>

% SW = percentage of 1000 mOsm seawater ionic concentration
% S = percentage of 1000 mOsm serum ionic concentration

The mean values for the [Na⁺]u are significantly lower than those of the serum when the crabs are bathed in 100%, 75%, and 50% seawater; the serum and urine concentrations are essentially equivalent in 30% seawater (Fig. 10, Table 10). In only one medium, 100% seawater, is the urine sodium less concentrated than that of the external milieu, however.

The standard deviations for the mean [Na⁺]u are of comparatively
greater magnitude than those of the serum in all seawater media employed, except perhaps 30% seawater.

Chloride Regulation (Fig. 11)

The pattern of chloride regulation resembles the sodium mechanism, as the serum concentration is greater than the medium concentration in hypoosmotic environments. There is a decline in internal chloride as the chloride in the external medium is decreased, accompanied by an increasing internal-external chloride gradient. The increase in the gradient ceases when the external concentration approximates 167 meq Cl⁻/kg water (30% seawater).

Several distinct differences between the two regulatory patterns do exist, however. The slope of the regulation curve indicates that this ion may not be regulated as strongly as sodium; when the [Cl⁻]₀ is expressed as a percentage of the concentration in the isosmotic condition, it is seen that this may be a correct interpretation (Table 11). There is no distinction between the [Cl⁻]₀ and that of the medium when the animals are in 100% seawater. In this environment Cancer magister does produce a urine that has a significantly higher concentration than the serum. In 75%, 50%, and 30% seawater the [Cl⁻]ᵤ is approximately equivalent to the [Cl⁻]₀.

Potassium Regulation (Fig. 12)

The mean value for the [K⁺]₀ of the Dungeness crab in Coos Bay seawater containing 9.9 meq K⁺/kg water, is 11.5 meq K⁺/kg H₂O (Table 10).
Fig. 11. Chloride ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.

BODY FLUID (Cl⁻)

as meq Cl⁻/Kg H₂O

△ = serum
〇 = urine

MEDIUM (Cl⁻) as meq Cl⁻/Kg H₂O
Fig. 12. Potassium ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.

BODY FLUID (K⁺)
as meq K⁺/Kg H₂O

MEDIUM (K⁺) as meq K⁺/Kg H₂O

△ = serum
○ = urine
This established hyperionic regulation is exhibited in the brackish water media with a progressively increasing internal-external $K^+$ ion differential.

The $[K^+]_u$ is regulated at a significantly lower concentration than that of the serum in 100%, 75%, and 50% seawater. In only one environment, the isosmotic medium, is $[K^+]_u$ maintained effectively below that of the medium. As indicated by Fig. 12 and the $K^+$ urine-serum ratios from Table 10, the magnitude of this difference declines in the 75% and 50% seawater media. The two extracellular fluids have essentially equivalent $K^+$ concentrations in 30% seawater.

The exceptionally large standard deviations of the mean $[K^+]_u$ warranted an examination of the data for some possible explanation. Upon first observation, it was discovered that male animals generally exhibited a lowered $[K^+]_u$ and the females commonly possessed an equivalent $[K^+]_u$, when compared with the serum concentrations. A statistical analysis of this hypothesis is presented in Table 12. In 100% seawater the difference between male and female urine $K^+$ was highly significant: a urine widely variable but usually low in potassium ions was characteristic of male crabs; the female urine potassium was equivalent to that of the serum. In 75% seawater this difference is significant, but there was no appreciable variation between male and female mean urine $K^+$ values in 50% seawater. The data were insufficient to test the hypothesis for 30% seawater animals.
Table 12. Comparative analysis of potassium ion regulation in male and female Cancer magister.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Males</th>
<th></th>
<th></th>
<th>Females</th>
<th></th>
<th></th>
<th>Pt</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% SW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>29</td>
<td>11.1</td>
<td>1.2</td>
<td>29</td>
<td>11.6</td>
<td>1.2</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Urine</td>
<td>32</td>
<td>6.1</td>
<td>1.7</td>
<td>33</td>
<td>10.3</td>
<td>2.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Medium</td>
<td>9.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75% SW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>14</td>
<td>9.6</td>
<td>1.2</td>
<td>15</td>
<td>10.0</td>
<td>1.8</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Urine</td>
<td>14</td>
<td>6.9</td>
<td>1.5</td>
<td>14</td>
<td>8.8</td>
<td>2.2</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>Medium</td>
<td>7.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% SW</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>16</td>
<td>7.4</td>
<td>1.2</td>
<td>10</td>
<td>8.7</td>
<td>1.1</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Urine</td>
<td>17</td>
<td>6.5</td>
<td>1.5</td>
<td>9</td>
<td>7.3</td>
<td>2.2</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Medium</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ionic concentrations expressed as meq K⁺/kg. H₂O.

Pt = significance of Student's t test.

Calcium Regulation (Fig. 13)

The pattern of regulation for calcium ions is somewhat analogous to that of sodium, chloride, and potassium ions. Serum levels of calcium for crabs in Coos Bay seawater and the diluted seawater milieux are supported well above the [Ca⁺⁺]sw. In isosmotic seawater, both serum and urine calcium are regulated at a higher concentration than that of the medium, but the [Ca⁺⁺]u is significantly lower than that of the serum (Table 10). In 75%, 50%, and 30% seawater, the mean expressions for these two components are statistically indistinguishable. Standard deviations for serum and urine measurements, possibly due to the lack of precision encountered with use of flame photometry for calcium analyses, are moderately large.
Fig. 13. Calcium ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.
The change in slope of the calcium regulatory curve at the 30% seawater level is much more pronounced than that exhibited by the sodium, chloride, and potassium curves. *Cancer magister* may abandon calcium regulation in this medium.

**Magnesium Regulation (Fig. 14)**

It is readily observed that *Cancer magister* employs an entirely different mode of ionic regulation for magnesium and sulfate ions, when this mechanism is compared to the regulatory processes described for sodium, chloride, potassium, and calcium ions. In 100%, 75%, and 50% seawater, the serum and urine magnesium concentrations are significantly different (Table 10). The urine-serum ratio for the magnesium ion is extremely high, being 3.9 for animals inhabiting Coos Bay seawater. In this medium, the serum magnesium is sustained at a concentration far below that of the medium. The \([Mg^{++}]_u\) averages approximately twice that of the medium. Although the effective pattern is the same in 75% and 50% Coos Bay seawater, the urine and serum magnesium concentrations are observed to decline with a decrease in the \([Mg^{++}]_{sw}\). The U/S in 75% seawater is nevertheless maintained at 4. In 50% seawater this ratio is higher (5), being generated primarily by the radically low \([Mg^{++}]_s\). In 30% seawater, the magnesium concentration of both extracellular fluids is less than the medium concentration. There is no significant difference in the mean concentrations of these two components. The standard deviations for the mean urine magnesium concentrations in 100%, 75%, and 50% Coos Bay seawater are extraordinarily large.
BODY FLUID (Mg++)

as meq Mg++/Kg H₂O

Fig. 14. Magnesium ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.
Sulfate Regulation (Fig. 15)

The ionic regulatory curves for magnesium and sulfate ions are congruous, although the urine and serum sulfate concentrations are not so extremely different. In 100% seawater the U/S for sulfate is 1.6. In 75%, 50%, and 30% seawater this ratio is greater than 1, although the difference is not significant in the 30% seawater medium. In contrast to the serum magnesium concentrations, serum sulfate concentrations are only moderately low when compared to the medium concentrations of 100%, 75%, and 50% seawater. Additionally, sulfate concentrations of the serum and urine are greater than the medium concentration for crabs in 30% seawater.

Ionic Interactions (Table 13)

To obtain information pertaining to interionic relationships, particularly those concerned with the physiology of the antennary gland, linear regression analyses for paired urine Mg$^{++}$-SO$_4^{2-}$ and urine Mg$^{++}$-Na$^+$ data sets were performed. The results are assembled in Table 13. Highly significant correlations exist between sodium and magnesium urine ionic concentrations for animals in 75% and 50% seawater. The Mg$^{++}$-Na$^+$ relationship is significant in 100% seawater, but not in 30% seawater. Although no such relationship was detected between urine magnesium and sulfate ions for crabs in isosmotic and 30% seawater media, it was observed in animals inhabiting 75% and 50% seawater.
Fig. 15. Sulfate ion concentrations in the body fluids of the Dungeness crab, as a function of salinity.

**BODY FLUID \( (SO_4^{--}) \)**

as meq \( SO_4^{--}/Kg \ H_2O \)

\( \triangle = \text{serum} \)
\( \bullet = \text{urine} \)

MEDIUM \( (SO_4^{--}) \) as meq \( SO_4^{--}/Kg \ H_2O \)
Table 13. Linear regression analyses of ionic interactions in the antennary gland of Cancer magister.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Medium</th>
<th>d.f.</th>
<th>( P_r )</th>
<th>Linear Regression Analysis Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Na}^+ (x) ) and ( \text{Mg}^{++} (y) )</td>
<td>1000</td>
<td>67</td>
<td>&lt;.01</td>
<td>( y = -0.33x + 295.51 )</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>33</td>
<td>&lt;.001</td>
<td>( y = -0.51x + 296.98 )</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>24</td>
<td>&lt;.001</td>
<td>( y = -0.67x + 295.96 )</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>9</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td>( \text{Mg}^{++} (x) ) and ( \text{SO}_4^{--} (y) )</td>
<td>1000</td>
<td>64</td>
<td>&gt;.05</td>
<td>( y = 0.22x + 27.54 )</td>
</tr>
<tr>
<td></td>
<td>750</td>
<td>30</td>
<td>&lt;.01</td>
<td>( y = 0.31x + 15.45 )</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>24</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>11</td>
<td>&gt;.05</td>
<td></td>
</tr>
</tbody>
</table>

Medium osmotic concentrations expressed as mOsm/kg H_2O.

d.f. = degrees of freedom.

\( P_r \) = significance of correlation coefficient.

Calculation of Urinary Solute Loss

By combining the urine production rate data (Table 7), and the urine ionic concentrations (Table 10), the rates of urinary ion efflux for the Dungeness crab in different osmotic environments can be calculated. The rates of ion loss, expressed as \( \mu \text{eq per g animal per day} \), are listed in Table 14. These measurements are useful in quantitatively assessing salt balance.
Table 14. Urinary effluxes of the ionic constituents of Cancer magister Dana exposed to isosmotic and hypoosmotic media.

<table>
<thead>
<tr>
<th>ION</th>
<th>Treatment (% Seawater)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>Na (^{++}) ((\mu\text{eq/g/da}))</td>
<td>5.82</td>
</tr>
<tr>
<td>Cl (^{-}) ((\mu\text{eq/g/da}))</td>
<td>7.52</td>
</tr>
<tr>
<td>K (^{+}) ((\mu\text{eq/g/da}))</td>
<td>.11</td>
</tr>
<tr>
<td>Ca (^{++}) ((\mu\text{eq/g/da}))</td>
<td>.34</td>
</tr>
<tr>
<td>Mg (^{++}) ((\mu\text{eq/g/da}))</td>
<td>2.15</td>
</tr>
<tr>
<td>SO(_4^{--}) ((\mu\text{eq/g/da}))</td>
<td>1.04</td>
</tr>
</tbody>
</table>

Discussion

Ionic Regulation by Cancer magister in Seawater

Even though marine invertebrates are generally thought to be isosmotic with seawater, the concentrations of their body fluid ionic constituents are not necessarily equivalent to those of the medium (Potts and Parry, 1963). Cancer magister is not exceptional in this regard. The concentrations of sodium, potassium calcium, magnesium, and sulfate ions in the body fluids may vary considerably from seawater. It is conceivable that the ionic concentration differences between seawater and the serum of marine crustaceans are not wholly generated by ionic
regulatory processes. It has been calculated that a Donnan distribution and indiffusible "bound" ions can account for a 3% variation with monovalent ions, and a 6% difference for divalent ions. In addition, the latitude of analytical errors must also be considered (Robertson, 1949).

In any event, a number of seawater-serum ionic concentration differences lie outside the realm of this inherent variation. There have been several hypotheses expounded for the physiological basis of this variability. Pantin (1931) suggested that the differences between seawater and serum ionic composition could be explained through the heterogeneous mobilities of the several ionic constituents--an untenable proposal when the ionic concentration gradients are considered. Evidence for differential ion permeabilities dependent upon the direction of the flux has been presented (Yonge, 1936), but a subsequent performance of the experiments by another investigator produced dissimilar results (Webb, 1940). The proposition that ionic regulation in crustaceans inhabiting seawater can be attributed to the differential excretion and reabsorption of the ions by the antennary gland is more acceptable (Scholles, 1933). Whereas low magnesium and sulfate concentrations in the serum have been explicitly demonstrated to result from the preferential excretion of these ions by the antennary gland (Webb, 1940), the contribution of this organ's reabsorptive mechanisms to the increased sodium, calcium, and potassium concentrations in the serum is not well understood. The combined achievements of the antennary gland and the absorptive capacity of the gills is a more generally accepted explanation for the ionic constitution of the body
fluids of marine crustaceans (Webb, 1940; Robertson, 1939, 1949; Potts and Parry, 1963).

The pattern of ionic regulation for *Cancer magister* in seawater that was elucidated through this investigation can be briefly summarized. The \([\text{Na}^+]_s\) and \([\text{K}^+]_s\) are greater than the corresponding seawater concentrations, and some reabsorption of these ions by the antennary glands is indicated by the lowered urine concentrations. Low serum concentrations of magnesium and sulfate, coupled with the high concentrations in the urine, reveal a preferential excretory mechanism for these constituents. The concentration of chloride ions in the serum is approximately equal to the seawater concentration; the urine chloride is slightly but significantly higher. The measured serum and urine calcium concentrations are greater than the seawater concentration. The \(U/S\) is less than unity, and the antennary gland may possibly, although somewhat ineffectively, provide a pathway for the conservation of calcium ions.

Alspach (1972) presented a pattern of ionic regulation for the Dungeness crab in the marine environment that closely parallels the results of this examination. His study included analyses of \(\text{Na}^+, \text{K}^+, \text{Cl}^-, \text{and Ca}^{++}\) concentrations. The one perceptible difference in the two investigations is the quantitative assessment of calcium regulation. Alspach's (1972) measurements for the serum and urine calcium concentrations averaged approximately 10 meq/l. more than the corresponding serum and urine values expressed in this study. Some minor variation may be attributed to the modes of expression of the ionic concentrations.
meq/l vs. meq/kg H$_2$O. Although postmolt and premolt animals were pre-
ferentially excluded from both studies, premolt crabs are difficult to
identify. The serum calcium concentration of premolt marine brachyurans
is higher than that of intermolt animals (Robertson, 1960). An unavoid-
able inclusion of premolt individuals could have biased the results.

Sodium and Chloride Regulation

The model for sodium regulation in seawater that is presented for
Cancer magister in Table 10 and Fig. 10 has been demonstrated for a
number of other decapods (Webb, 1940; Robertson, 1939; Prosser et al.,
1955; Gross, 1959, 1964; Gross et al., 1966). The [Na$^+$]$_s$ is somewhat
variable among the species, being either hyperionic or isoionic to the
sodium concentration of the external environment. Generally, urine
sodium is lower than serum sodium (U/S ≈ 0.80), indicating sodium re-
absorption in the antennary gland. This pattern of sodium regulation is
not without exception, however. Robertson (1949, 1953) and Gross (1964)
have studied an assortment of littoral and sub-littoral species of
decapods. These studies indicate that stenohaline crustaceans usually
possess extracellular body fluids with a sodium composition approaching
the seawater concentration. The littoral grapsids, Hemigrapsus nudus
and H. oregonensis, offer another exception to the sodium regulatory
mechanism demonstrated by many euryhaline crustaceans. A peculiarity of
wintering individuals of these two species is the production of urine
with a significantly greater sodium concentration than that of the
serum. In the summer, these two shore crabs display a sodium U/S in
seawater equal to or slightly greater than 1 (Dehnel and Carefoot, 1965). No explanation for this puzzling phenomenon has been proposed.

Because it has been ascertained that the antennary gland of Cancer magister is capable of reabsorbing sodium ions, the intriguing possibility that this action may be responsible for the increased concentration of sodium in the serum is worthy of exploration. Robertson (1939) and Webb (1940) have suggested that resorption of sodium ions from the excretory system and sodium uptake from the external milieu by extrarenal mechanisms are both involved. The specific contributions for the two separate pathways were not revealed. An analysis of Shaw's (1961a) results for sodium movements in Carcinus maenas, as presented by Potts and Parry (1963), indicated that an extrarenal active uptake element is principally accountable for the maintenance of sodium balance in seawater. Although the exact relationship of the serum sodium concentration to that of seawater is not distinct in Pachygrapsus crassipes (comparing the data of Prosser et al., 1955, and Gross, 1959), passive forces are the only extrarenal contributions involved in sodium balance for animals in seawater (Rudy, 1967). It is possible to assess the role of the antennary gland in sodium regulation if the blood volume, the rate of urine discharge, and an estimate of the total sodium turnover are realized. For the Dungeness crab in isosmotic and hypoosmotic environments, the blood volume (Alspach, 1972), rate of urine production (Table 7), and sodium turnover rate (Table 15, Chapter 6), have been determined.

The blood volume of the Dungeness crab in seawater is approximately
35% of the body weight. It can be calculated from Alspach's (1972) equation for the regression of the blood volume, \( y \), upon the body weight, \( x \): \( y = 0.28x + 9.1 \). This volume is compatible with the estimates of other workers for marine and brackish water brachyurans (Webb, 1940; Nagel, 1934; Krogh, 1938; Prosser et al., 1955). The contribution of the antennary gland to sodium balance is approximately equal to the following expression: \( Q_r / Q_b \).

\[
Q_r (\text{da}^{-1}) = U (\text{da}^{-1}) \times (C_s - C_u).
\]

\[
Q_b (\text{da}^{-1}) = BV \times W \times (C_s - C_{sw}) \times 24 / T_t.
\]

\( Q_r \) is the quantity of sodium reabsorbed in a day; \( Q_b \) is the quantity of sodium required to maintain sodium balance in a day; \( BV \) is blood volume; \( W \) is body weight; \( U \) is the amount of urine excreted per day; \( C_s, C_u, \) and \( C_{sw} \) are the sodium concentrations of the serum, urine, and seawater; and \( T_t \) is the turnover time of the sodium pool (Table 15, Chapter 6). The contribution of antennary gland reabsorption to sodium balance in 100% seawater was calculated to be only 2-3% of the load required. As this amount is relatively insignificant, the possibility of an active uptake component or an inside-negative directed potential difference providing the route of accumulation will be explored in Chapter 6.

Because the antennary gland of *Cancer magister* does not contribute significantly to the serum sodium accumulation, the low concentration of sodium in the urine of seawater-adapted animals may result from another stimulus. Prosser et al. (1955) demonstrated that when *Pachygrapsus crassipes* is immersed in media more concentrated than
seawater, the sodium U/S surprisingly decreases, accompanied by a drastic increase in the magnesium concentration of the urine. These investigators postulated that this high concentration of magnesium suppresses the urinary excretion of sodium through competition for transport across the membranes of the antennary gland. This phenomenon has since been recognized in many marine and brackish water decapods (Gross, 1959, 1964; Gross and Marshall, 1960; Riegel and Lockwood, 1961), but the causal relationship between the two ionic concentrations is not well-defined. The linear regression analysis of sodium and magnesium concentrations in the urine of Cancer magister in seawater yielded a significant correlation coefficient (Table 13), thus suggesting an absolute relationship in accordance with the regression formula. De Leersnyder (1967b) has established an analogous interaction for Eriocheir sinensis, by plotting the change in the sodium U/S with the variation in the magnesium U/S. Whether this mechanism involves a direct sodium-magnesium exchange has not yet been convincingly demonstrated. Green, Prosser, and Chow (1959) and Riegel and Lockwood (1961) have rejected the possibility of a direct exchange, as the observed ratio of the sodium concentration change to the magnesium concentration change (from the seawater isoionic quantities) is less than an expected value of 2. Gross and Capen (1961) have lucidly demonstrated a very distinct correlation between the sodium and magnesium concentrations in the urine of Pachygrapsus crassipes. They have presented evidence which suggests that a concentration difference ratio of two sodium ions reabsorbed for each magnesium ion secreted may not be
an accurate evaluation of a direct exchange in this system.

One must recall that it is the activity and not the concentration of the ionic constituent that is important in a consideration of ion movements (Potts and Parry, 1963, p. 24). The activity coefficient for sodium and chloride ions in seawater is approximately 0.6, but that of magnesium and sulfate ions is less than 0.2 (Weast and Selby, 1966). For the Dungeness crab in seawater, 80 and 55 mM/kg H₂O are the serum-urine concentration differences of sodium and magnesium, respectively, that result from either reabsorptive (sodium) or secretory (magnesium) processes (Table 10). Application of the activity coefficients for these ions produces values of 48 mM of Na⁺, and 11 mM of Mg²⁺ that could be involved in a functional exchange. The magnitude of the net sodium efflux from the excretory fluid may be too large instead of too small, as supposed by Green, Prosser, and Chow (1959), Riegel and Lockwood (1961), and Lockwood and Riegel (1969), to affect a direct 2:1 exchange.

The maintenance of electrochemical neutrality in the urine and the reabsorption of a limited amount of water complicate an analysis of the sodium-magnesium exchange process (Riegel and Lockwood, 1961; Gross and Capen, 1966). This achievement of electrochemical balance provides a feasible explanation for the observation that the chloride concentration in the urine of Cancer magister is significantly higher than that of the serum (Table 10). Movements of water and salt directed at providing electrochemical neutrality and urine-serum isosmoticity in the production of the final excretory product, may disguise the true character of an active exchange pathway for magnesium ions.
Magnesium and Sulfate Regulation

Gross and Capen (1966) have conclusively demonstrated that magnesium ions are secreted into the urine of *Pachygrapsus crassipes* by the bladder membrane; the concentration of magnesium ions in excreted urine depends directly upon the period of retention of the excretory fluid in the bladder. Water removal could increase the magnesium concentration, but it is believed to be minimal (Gross and Capen, 1966; Riegel and Lockwood, 1961). The final concentration of magnesium in the urine depends upon the salinity of the medium (Gross and Marshall, 1960; Lockwood and Riegel, 1969). In *Carcinus maenas* this concentration is also controlled by the medium magnesium concentration (Lockwood and Riegel, 1969), but the existence of such a regulatory process in *Pachygrapsus crassipes* is disputed (Gross and Marshall, 1960; Prosser et al., 1955).

The low serum and high urine concentrations of magnesium ions exhibited by *Cancer magister* in seawater (Table 10, Fig. 14) are well-established phenomena for marine, brackish water, and terrestrial decapods (Bialasewicz, 1933; Webb, 1940; Robertson, 1939, 1949, 1953; Gross, 1957, 1964; Prosser et al., 1955; Gross et al., 1966). In seawater, the Dungeness crab exhibits a U/S for magnesium that is well within the range of ratios expressed by other decapods. Higher U/S ratios and higher urinary concentrations of magnesium are more characteristic of the terrestrial species (Gross, 1964).

The physiological advantages associated with the maintenance of an internal milieu low in magnesium ions has been a speculative subject.
It is now apparent that artificially imposed high concentrations of magnesium in the blood of vertebrates affect the peripheral motor innervation of muscles, causing a partial paralysis (Engbaek, 1952). The specific action seems to be a decrease of acetylcholine release at the neuromuscular junction, an inhibitory process that is reversible by increasing the calcium ion concentration (Del Costillo and Engbaek, 1954). Even though neuromuscular transmission in crustaceans may not be principally affected by acetylcholine (Prosser and Brown, 1962, p. 611), an increase of the serum magnesium concentration to a level 2.5 times higher than the normal blood concentration in Carcinus maenas causes a cessation of muscular activity in this animal (Katz, 1936).

Robertson (1953) has attempted to interpret the variation in serum magnesium concentration seen in marine invertebrates, by correlating the ionic concentration with the natural activity of a particular species. His hypothesis has merit as a causal explanation, but his assay for activity involved questionable qualitative criteria.

The accumulation of sulfate in the urine of marine and brackish water decapods is not well understood. An interaction between the urinary magnesium and sulfate concentrations is a possible explanation, but linear regression analysis, at least for animals in seawater, suggests independence (Table 13). The U/S ratio for sulfate is 1.6, whereas the U/S for magnesium ions is 4.0 (Table 10). Additionally, the sulfate concentration of the serum of the Dungeness crab in seawater is not as impressively disparate from the medium concentration as is the serum magnesium concentration. Few studies of ionic regulation have
considered surfate, possibly because of the absence of dependable analytical procedures. Nevertheless, the serum and urine sulfate concentrations of related astacurans (Robertson, 1949) and *Carcinus maenas* (Webb, 1940) are similar to those of *Cancer magister*. Because the activities for magnesium and sulfate ions are equivalent (Morris, 1968), the pattern of sulfate regulation displayed by *Cancer magister* in seawater suggests that the mechanism of magnesium secretion in the antennary gland does not involve a direct association with the sulfate ion. More correctly, the involvement of sulfate with magnesium secretion in the antennary gland is quite possibly a passive event created by the achievement of electrochemical neutrality.

**Potassium and Calcium Regulation**

The patterns of potassium and calcium regulation for decapod crustaceans in seawater appear to be very susceptible to both interspecific and intraspecific variation. Because of the relatively low concentration of potassium and calcium ions in the body fluids and seawater, the functional impact of any manipulation of these ions may be directed toward systems other than those directly involved in osmotic regulation.

Three different patterns of potassium regulation have been described for marine decapod crustaceans. Several species reabsorb potassium ions from the urine (Webb, 1940; Robertson, 1939, 1949; Gross, 1964; Bialasewicz, 1933). The serum and urine potassium concentrations are equal in the few grapsid crabs that have been studied, although intra-
specific variations are common (Dehnel and Carefoot, 1965; Prosser et al., 1955; Gross, 1959, 1964). A few animals, primarily terrestrial and semi-terrestrial species, maintain a U/S for potassium ions greater than 1, thus actively discharging this component via the urine (Gross, 1959, 1964; Gifford, 1962).

*Cancer magister* reabsorbs potassium ions when in seawater (Alspach, 1972; this study), but as this investigation has revealed, apparently only the male crabs exhibit this adjustment. The serum of both male and female animals is slightly higher in potassium than the medium. It is possible that the reabsorptive processes in the antennary gland may be responsible for this difference in male crabs. It is more likely that a Donnan equilibrium (estimated as a 3% difference for the serum of crabs by Robertson, 1949) or an inside-negative electrical potential difference evokes this increase in serum potassium concentration for both male and female animals.

The problem that is most outstanding regarding potassium regulation in crustaceans, is the vast array of intraspecific and interspecific differences in the body fluid potassium concentrations. Both Robertson (1960) and Gross (1964) have addressed themselves to this heterogeneity, and Robertson has suggested that a dietary correlation is possible. Gross and Holland (1960) recorded the lowest U/S for potassium ions in starved hermit crabs, *Coenobita perlatus*. The source of food may also be significant. The marine alga, *Ulva lactuca*, contains potassium ions at a concentration of 350 mM/kg H₂O (Scott and Hayward, 1954), which is approximately 3.5 times greater than the observed potassium concentra-
tion of muscle fibers from *Carcinus maenas* (Shaw, 1955).

It is difficult to conceptualize any significant dietary influence upon potassium regulation in male and female *Cancer magister*, as both sexes are believed to possess similar feeding habits (MacKay, 1942). However, it should be noted that in the collections of crabs for this investigation, females were uncommon, thus suggesting some difference in the habits of male and female Dungeness crabs. Any crucial involvement of potassium ions in the developmental processes of male and female crabs is unknown to me; therefore the stimulation of this sex-related potassium regulatory phenomenon remains a mystery.

Diverse patterns of ionic regulation for male and female *Eriocheir sinensis* have been demonstrated for magnesium and calcium ions (De Leersnyder, 1967a). The females may accumulate greater serum concentrations of these ions, and a connection with vitellogenesis was suggested, but later rejected (De Leersnyder, 1967b). Gilbert (1959b) has observed lowered anionic concentrations in the serum of female *Carcinus maenas*, but the significance is not known.

Intraspecific variation is a common aspect of calcium regulation for marine crustaceans, and the relative concentrations of calcium in the several stages of the molting cycle has been extensively studied (Robertson, 1960; Glynn, 1968; Sather, 1967; Travis, 1955). Inter-specific variations in the patterns of calcium regulation are also

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1 According to MacKay (1942), the sex ratio for the Dungeness crab is approximately 1. He has speculated that the disproportionate catches recorded in some areas result from a segregation of the sexes due to physical factors in the external environment.
prevalent in the marine Decapoda. Several members of the brachyuran family Grapsidae have a U/S for calcium ions that is greater than one (Gross, 1959, 1964; Dehnel and Carefoot, 1965). Conversely, Carcinus maenas (Webb, 1940), Cancer magister (Alspach, 1972; this study), and a number of terrestrial and semi-terrestrial species (Gross, 1964; Gross et al., 1966), display a calcium U/S that is less than one. Gross (1964) has noted that the serum calcium concentration is higher in semi-terrestrial and terrestrial crabs than in the more aquatic species. His thesis is supported in this study by the relatively low serum calcium concentration exhibited by Cancer magister. Both Webb (1940) and Robertson (1939, 1949, 1960) have stressed the need for dialysis measurements, as a detectable amount of calcium in the serum of crustaceans is protein-bound and consequently indiffusible. The low calcium U/S displayed by Cancer magister and other decapods may not be significant in a consideration of reabsorptive or secretory processes, as it is the ion activity and not the concentration that is basic to any ionic movements between pools (Morris, 1968).

Ionic Regulation in Hypoosmotic Media

In hypoosmotic media, the Dungeness crab stabilizes the serum concentrations of sodium, chloride, potassium, and calcium above the external concentrations of these ions. Magnesium and sulfate concentrations in the serum remain less concentrated than the outside concentrations. The serum regulatory patterns displayed for sodium and chloride ions (Figs. 10, 11) closely parallel osmotic regulation in
this species (Fig. 1), a predictable observation when Pantin's (1931) statement, that osmotic regulation is a direct consequence of ionic regulation, is considered. As is the case with most euryhaline animals (Potts and Parry, 1963), these two ions provide the bulk of the solute in the serum and urine of the Dungeness crab.

Sodium and Chloride Regulation

Based upon the data presented in Table 11, and the difference in slope between the sodium and chloride regulatory curves (Figs. 10, 11), it can be stated the mechanism for the accumulation of chloride ions is visibly less effective than that for sodium. Alspach (1972) has reported similar findings for this same species. Because sodium, but not chloride, is reabsorbed by the antennary glands of crabs in 75% and 50% seawater (U/S data, Table 10), one might speculate that it is this subtle difference between the sodium and chloride balance mechanisms that provokes the observed superior efficiency of the sodium system. A calculation designed to estimate the contribution of reabsorptive processes in the antennary gland to the increased serum concentration of a particular ionic constituent was outlined in the preceding discussion. Using this procedure, the estimated conservation of sodium through antennary gland retention by an animal in 75% and 50% seawater accounts for approximately 5% of the input required to maintain a steady state. If the observed reduction in the serum sodium and chloride concentrations for animals in 75% and 50% seawater is expressed as a percentage of the 100% seawater concentrations, there is a 10-15% difference in
the concentrating capacity of the two mechanisms (Table 11). However, if it is assumed that the process responsible for the enhancement of the serum sodium for animals in Coos Bay seawater is operable in dilute media (most probably an inside-negative electrical potential difference), then an expression for the ion reductions in terms of 100% seawater serum concentrations provides a more graphic analysis. Through this expression, the diminution of chloride ions is observed to be only 5-6% greater than that sustained by the sodium ion concentration (Table 11). Hence a 5% increase in the serum sodium concentration due to reabsorption in the antennary gland furnishes a possible explanation for the presumed inferior performance of the chloride accumulating mechanism. In 30% seawater, Cancer magister was not observed to reabsorb sodium ions (Table 10). At this experimental seawater concentration there is no apparent distinction between the serum-concentrating capacity of the sodium and chloride balance mechanisms, as evidenced by the data in Table 11. These two observations, when consolidated, tend to imply that the sodium reabsorptive machinery in the excretory organ assists in maintaining the high [Na⁺] for animals in 75% and 50% seawater.

Wintering Hemigrapsus nudus and H. oregonensis inhabiting brackish water exhibit a sodium U/B that approaches 1.5 (Dehnel and Carefoot, 1965). The U/B of sodium for summer animals is equal to or slightly greater than 1. This seems unusual, as Dehnel and Stone (1965) have demonstrated that the urine osmotic concentration is sustained effectively below the serum concentration by wintering individuals in
hypotonic media. Renal reabsorptive mechanisms of some sort are functionally important in the achievement of osmoregulation. Prosser et al. (1955) have obtained a U/B for sodium greater than one for another grapsid in dilute media, Pachygrapsus crassipes, whereas Gross (1959) reported a ratio slightly less than one for this animal. The fiddler crab, Uca crenulata, provides a point of contrast to many brachyurans. In 50% seawater, the U/B for sodium is 0.75. This extensive renal reabsorption of sodium could effectively augment the serum sodium concentration (Gross, 1964).

In discussing the sodium concentration of the urine for crabs in dilute media, magnesium regulation must again be considered. The unusually low level of sodium in the urine of Uca crenulata is accompanied by a high concentration of magnesium ions (Gross, 1964). Cancer magister also displays this reciprocity (Table 10), although the relative concentration differences between serum and urine are smaller. Interestingly, both sodium reabsorption and the active excretion of magnesium are terminated by the Dungeness crab in 30% seawater (U/S approaching 1, Table 10). The dependent relationship between urine sodium and magnesium concentrations for Pachygrapsus crassipes in both isosmotic and hypotonic environments has been developed (Gross and Capen, 1966). It is probable, then, that the limited sodium reabsorption seen in Cancer magister and a few other decapod crustaceans can be attributed to its functional association with the magnesium ion; the reduced $[Na^+]_u$ is merely a reflection of the increased $[Mg^{++}]_u$ and is not an indispensable contributor to sodium balance.
The deflection of the osmoregulatory curve for the Dungeness crab in environments less concentrated than 50% seawater (Fig. 1) is analogous to the patterns of sodium and chloride regulation in these media (Figs. 10, 11). Shaw (1961a) has extensively studied sodium balance in Carcinus maenas, and has recorded a similar sodium regulatory pattern. Below an external sodium concentration of about 250 mM/l, a decrease in the external concentration is paralleled by an equivalent decline in the serum sodium. Shaw (1961a) proposed that this change in slope of the sodium regulatory curve represented the external sodium concentration at which the sodium uptake mechanism is fully activated. His hypothesis provides a suitable explanation for the observed pattern of sodium regulation in Cancer magister. The external sodium level at which full activation occurs has not been specifically characterized, but it is likely that it lies within the range 200–250 meq Na+/kg H2O. Why the animal does not fully activate the mechanism and maintain a more rigid degree of ionic homeostasis with the onset of the osmoregulatory response, is not clear. Perhaps tolerance to ionic fluctuations and or acclimation are phenomena that are intricately important.

Calcium and Potassium Regulation

Potassium and calcium ions, although imparting little to the maintenance of the osmotic integrity of the extracellular fluids of the Dungeness crab in hypoosmotic media, are regulated in the serum at greater concentrations than the external medium. Although passive movements initiated by the onset of sodium and chloride regulatory pro-
cesses are probably real, the significance of potassium and calcium regulation may pervade other physiological and biochemical realms. Calcium regulation is of course essential for the proper operation of the molting process in crustaceans (Travis, 1955; Robertson, 1960). Even though intermolt crabs were used in this study, the serum concentration of calcium is greater than that of the medium for animals exposed to dilute media. In 75% and 50% seawater, calcium is regulated with greater precision than that exhibited for the osmotically important ions, sodium and chloride (compare Figs. 10 and 13). This strict regulation is abandoned in very dilute media. A similar constancy for blood calcium during osmotic stress has been demonstrated for a number of euryhaline decapods (Gross, 1959; Prosser et al., 1955; Dehnel and Carefoot, 1965; Gifford, 1962). Gross (1964) particularly emphasized this circumstance, but did not speculate upon its possible functional significance. The calcium regulation curve illustrated by AlspaCh (1972) for Cancer magister is similar in pattern to that presented in this study, although his measurements were somewhat higher.

In dilute environments the urine calcium concentration of Cancer magister is isoionic with the blood. In reviewing several other studies of ionic regulation, it can be observed that the disposition of the urine calcium concentration relative to the serum concentration for hyperosmoregulating decapods is isoionicity, moderate hyperionicity, or moderate hypoionicity (Dehnel and Carefoot, 1965; Gross, 1964, 1959; Gifford, 1962; De Leersnyder, 1967b). Therefore the pattern of calcium regulation that emerges in the Decapoda indicates that calcium
reabsorption by the antennary glands is not a primary factor in the rather extraordinary preservation of calcium homeostasis in hypoosmotic environments. To provide for such a great accumulation of calcium ions during a period of osmotic stress, at least three possible alternatives are available. (1) The calcium uptake mechanism may be more sensitive to fluctuations than the sodium or chloride balance machinery, thereby operating at maximal activation in 75% and 50% seawater. (2) The absorption system could have a greater transport capacity than either the sodium or chloride balance mechanisms, providing an increased number of sites for ion movement. (3) Extravascular salt pools might supply an ample number of calcium ions to sustain a high calcium concentration, if the required load is temporary. Gross (1958, 1959) has postulated that extravascular salt pools are important in enabling Pachygrapsus crassipes to achieve a steady state for sodium, calcium, and particularly potassium ions, as this animal is susceptible to intermittent osmotic stresses in its natural habitat. In this study, high serum calcium concentrations were maintained by the Dungeness crab after a four day exposure to dilute seawater. A calculation of the amount of calcium required for the observed homeostasis indicated that it was impossible for the soft tissues to supply a substantial portion of the load. It seems unlikely that the mobilization of calcium from the carapace would be practicable. It is possible that exposure to a hypoosmotic medium initiates the molt, in which case reabsorption of calcium from the carapace is inevitable, and the high degree of calcium regulation is secondarily induced.
Although calcium balance was not extensively studied in *Cancer magister*, an explanation for the increased accumulation based upon maximal activation of the uptake mechanism in 50% and 75% seawater is questionable. Fig. 13 illustrates an increase in the ionic differential between the external and internal compartments in 50% seawater. Information that would support or reject the third alternative for an uptake mechanism with an increased capacity is not available. If the absorptive pathway for calcium influx associated with the molt is morphologically and physiologically distinct, a difference in carrying capacity from familiar uptake systems is not difficult to visualize. A distinct mechanism for calcium uptake is implicated.

The failure of the calcium uptake mechanism in 30% seawater (Fig. 13) may signify saturation or full activation of the system, or it may represent a channeling of energy to salt pumps more directly involved in maintaining some semblance of osmotic integrity. Because the sustained calcium ion differential between seawater and serum in 30% seawater is actually less than that attained in 50% seawater, the second possibility should be seriously entertained. This medium may be an encroachment upon the range of physiological tolerance to hypoosmotic media for this species. Although temporarily habitable, permanent colonization of this environment may not be feasible.

Webb (1940) suggested that the ion uptake mechanism in crustaceans could not discriminate between potassium and sodium ions, and that their accumulation is proportional to the concentration in the external milieu. The similarity in the regulatory pattern of sodium, chloride,
and potassium ions, and the absence of any functional input of potassium ions to the achieved serum osmotic concentration of hyperosmoregulating crabs, certainly indicates that potassium influx may be associated with sodium or chloride movements. Shaw (1960a) has shown that sodium transport in the freshwater crayfish, Astacus pallipes, occurs independently of the external potassium concentration. The external potassium concentration has little effect upon chloride movements in this animal (Shaw, 1960b). The effects of sodium and chloride concentrations in seawater upon potassium fluxes is not well understood, however. Arthropod membranes are capable of transporting potassium ions independently, and so the possibility of an operable potassium pump cannot be ignored (Harvey and Nedergaard, 1964).

The urine potassium of many decapods inhabiting dilute seawater is more concentrated than the serum potassium, particularly for semi-terrestrial and terrestrial species (Gross, 1959, 1964; Prosser et al., 1955; Gifford, 1962). A U/S less than one has been demonstrated for Cancer magister (Alspach, 1972; this study). The apparent dependency of the potassium U/S upon gender is prevalent in 75% seawater, but not in the 50% nor the 30% seawater environments. As the osmotic stress is increased, phenotypic variability in the physical tolerance of the species to hypoosmotic media may conceal any sex-related differences in potassium regulation. Any apparent cause for the difference in the urinary potassium levels between male and female crabs has eluded me. More field information concerning the ecology of female animals may be helpful.
Magnesium and Sulfate Regulation

The U/S of the magnesium concentrations in *Cancer magister* is not altered in moderately hyposaline waters (Table 10). Sulfate regulation follows a similar pattern. Quantitatively, the U/S for both these ions increases in 50% seawater, indicative of the increased standard deviations in dilute media and of the numerically diminutive values for the serum concentrations (Figs. 14, 15). In 30% seawater, however, the urine-serum ratios for both ions approach unity, hence the excretion of magnesium and sulfate is curtailed.

Because of a susceptibility for the passive loss of solute when in dilute media, conservation of magnesium and sulfate could be advantageous in the Dungeness crab, as the seawater concentrations of these ions are large when compared to potassium and calcium. Such an adaptation is non-existent in 75% and 50% seawater, as the urine-serum ratios for magnesium and sulfate ions (Table 10), and the micturition rates (Table 7) in these media are substantial. The control mechanism for an accessory function must predominate in these environments. Presumably the factors promoting magnesium and sulfate excretion outlined in the preceding discussion of ionic regulation in seawater are more demanding. Results from other studies (Gross, 1959, 1964; Gross and Marshall, 1960; Prosser et al., 1955; Parry, 1954) substantiate this conclusion.

*Carcinus maenas* in 50% seawater (Lockwood and Riegel, 1969), and decapods in freshwater (Gross et al., 1966; De Leersnyder, 1967a), exhibit equivalent serum and urine magnesium concentrations. The low
concentration of magnesium and sulfate ions in freshwater (Potts and Parry, 1963, p. 165) sufficiently explains the regulation of magnesium and sulfate by the freshwater animals, but Carcinus maenas provides a rather curious contrast to the phenomenon observed in the Dungeness crab. From inulin clearance and urine concentration data, Lockwood and Riegel (1969) calculated the quantity of magnesium excreted in 150%, 100%, and 50% seawater. With decreasing salinity, the excretion rate of magnesium was progressively less, and these workers concluded that the conservation of magnesium ions in a hypoosmotic environment was a response directed at alleviating the osmotic stress. Gross and Marshall (1960) had demonstrated previously that Pachygrapsus crassipes, an animal naturally inured to both hypoosmotic and hyperosmotic stresses, excretes magnesium when in dilute seawater at a highly accelerated rate. Lockwood and Riegel (1969) subsequently postulated that the difference in the magnesium excretory pattern for these two species could be ecologically correlated. These writers observed that Carcinus appeared to be more tolerant of low salinities, whereas Pachygrapsus was assumed to be better adapted for continual intermittent exposures to hyper-osmotic and moderate hypoosmotic environments.

The ionic excretory efflux calculations of Table 14 clearly illustrate a highly expanded excretory load of magnesium and sulfate ions when the Dungeness crab occupies dilute media. The urinary loss of magnesium is maximal in 50% seawater, with an efflux almost five times that exhibited in Coos Bay seawater. It has been established that Cancer magister, although a sub-littoral marine animal, is a common
estuarine resident, occasionally subjected to hypoosmotic waters approaching 40% seawater (Tom Wayne, personal communication). Its native environment, then, although perhaps not as frequently hypoosmotic, appears to be closely aligned to that of Carcinus maenas. Magnesium excretion in Hemigrapsus oregonensis, another crab ranging into brackish water, is patterned after the type discussed for Pachygrapsus and Cancer magister (Gross, 1964). The conclusions of Lockwood and Riegel (1969) concerning a magnesium-conserving adaptation in dilute media may not be applicable to all brackish water inhabitants.

Because the urine concentrations are substantially lower, the magnesium and sulfate excretory losses in 30% seawater are lower than the rates seen in 50% seawater (Table 14), even though the micturition rate in this medium is slightly higher (Table 7). A low observed urinary magnesium and sulfate concentration would be expected when a decapod is discharging its antennary glands frequently, as magnesium, and probably sulfate, accumulation in the urine is dependent upon a time-delayed secretory process (Gross and Capen, 1966). The calculated excretory rates of magnesium and sulfate in 30% seawater are not, however, consistent with the rates exhibited by animals in 50% and 75% seawater—a change in the magnesium and sulfate excretory patterns is observed for crabs exposed to 30% seawater. Conservation of these divalent ions is apparently important in this environment. It has not been determined whether the onset of magnesium and sulfate conservation represents a mechanism with a lower threshold than that outlined for Carcinus maenas (Lockwood and Riegel, 1969).
From the preceding discussion of ionic regulation by the Dungeness crab in hypoosmotic media, 50% and 75% seawater environments seem eminently more compatible than the 30% seawater medium. In the more moderate hypoosmotic environments, ionic regulation mediated by the antennary gland is directed toward the excretion of magnesium ions, and sodium and sulfate concentrations in the urine are dependent upon this process. In 30% seawater, this operation is abandoned, and the conservation of magnesium and sulfate ions is seemingly of critical importance for the functional integrity of the osmoregulatory apparatus. Potassium and calcium regulatory patterns are also altered in 30% seawater, and the sodium and chloride absorption mechanisms attain maximal activation. This very dilute medium may be an osmotically intolerable situation for extended periods, even though an eight day exposure in this investigation was not uncommon. The osmoregulatory capacity of the Dungeness crab does, however, allow access to hypoosmotic environments that prove to be intolerable circumstances for many other marine invertebrates.
CHAPTER 6

SODIUM BALANCE

Methods

Determination of Total Sodium Efflux

The method of Rudy (1966) provided a guideline for these measurements. Following the preliminary preparation of the animals, the crabs were maintained at 10 ± .05°C. in a constant-temperature refrigerator. Animals were exposed to one liter baths of varied seawater concentration containing approximately 0.025-0.05 μc/ml of the radioisotope, $^{22}$Na (Amersham-Searle Corp.). The time required for equilibration of the external and internal radiosodium was determined to be approximately 96 hours.

Following the loading interval, crabs were rinsed thoroughly in non-radioactive seawater to remove the tracer from the external surface and from the gill chamber. "Unloading" was initiated in one liter beakers containing 500 ml of aerated seawater. 25 ml samples were removed periodically and counted in the Picker scintillation apparatus described in Chapter 4. The following time sequence was utilized: 3, 6, 9, 12, 24, 48, 72, and 96 hours. Samples were replaced immediately after each counting procedure. 25 ml aliquots of the loading solution
were counted before and after each experimental sequence. The sodium concentrations of the loading solution and the bathing medium were measured by flame photometry, and the specific activity of the samples (expressed as cpm/meq Na⁺) was determined.

The mathematical description of the efflux was formulated by Rudy (1966). The turnover rate \( (T_x) \), an expression of the fraction of exchangeable body sodium exchanged hourly, and the turnover time \( (T_t) \), the time required for total turnover of the internal sodium pool, were determined from the exchange rate \( (m) \) and the amount of exchangeable sodium within the Crab \( (A) \). \( T_x = m/A \), and \( T_t = A/m \). Total body sodium was determined for a comparison with the calculated total exchangeable sodium. Crabs were dissolved in concentrated nitric acid, and the solution was diluted volumetrically and analyzed for sodium by flame photometry.

**Electrical Potential Difference (p.d.)**

These measurements were performed in a constant temperature chamber at 10 ± 0.5°C. The apparatus consisted of a two liter aquarium containing the appropriate seawater medium, a contrivance including several ring stands and clamps from which the animal and the electrodes were suspended in the bathing medium, a Bausch and Lomb recording voltmeter (10 mV, VOM 5), and two miniature calomel reference electrodes with thirty inch leads. Electrode adapters were prepared in accordance with a method designed by Tom Wayne (personal communication). Eppendorff pipette tips (Brinkmann Instruments Inc.) filled with a 2 molar KCl-3%
agar mixture were connected to the electrodes with an adjoining saturated KCl collar (1 inch length of rubber tubing). The adapters were checked for fluid continuity; those with air bubbles were discarded.

The electrodes were tested for electrical balance by immersing the adapters in the bathing medium and recording the potential difference between the electrodes. Electrodes with a p.d. of more than ±1 mV were rejected. For measurements of p.d. between the internal and external medium, a small hole was drilled through the carapace latero-posteriorly in the pericardial region. The tip of the electrode adapter was positioned and sealed with an acetone-ethyl cellulose mixture to prevent leakage. The electrode was rigidly clamped to the apparatus cradling the crab. The p.d. was recorded until a steady reading was obtained.

Assessment of the Role of Exchange Diffusion

For the analysis of exchange diffusion, crabs adapted to 100% seawater were exposed to the $^{22}$Na loading solution (0.025-0.050 μc/ml) for two hours. These animals were removed from the loading environment, rinsed in seawater, and monitored for radioactivity. They were then immersed for one-half hour in seawater with an osmotic concentration of approximately 1000 mOsm, and transferred for a one-half hour exposure to a 1000 mOsm solution of mannitol. Seawater concentrations of $K^+$, $Ca^{++}$, $Mg^{++}$, and $SO_4^{--}$ were added to the mannitol environment. For several crabs, a lithium chloride (1000 mOsm) immersion followed the mannitol exposure. After each exposure, the crabs were rinsed and the total cpm
determined with the Picker scintillation system. The sodium efflux constant was calculated using the following formula: 

\[ K_e = \frac{1}{T} \ln \left( \frac{A_o}{A_t} \right) \]  

(Potts et al., 1967). \( K_e \) is the fraction of exchangeable sodium in the crab effluxing per hour; \( A_o \) represents the total counts per minute in the crab prior to the exposure to seawater, mannitol, or LiCl; \( A_t \) is equal to the cpm in the crab following an exposure to 100% seawater, mannitol, or LiCl; and \( T \) is 1/2 hour.

Results

The analysis of total sodium efflux for crabs in varied concentrations of seawater is presented in Table 15. The range of body weights used in 100%, 75%, and 30% seawater environments was consistent, averaging approximately 60 g. The crabs employed in the efflux experiments conducted in 50% seawater were larger, however, averaging 80 g. The sodium exchange rate, \( m \), is expressed as \( \mu \)g sodium exchange per g animal per hour. This rate decreases with the salinity of the external environment. More accurately, it is a function of the concentration of the internal sodium pool, and because the serum concentration declines with the sodium concentration in the external environment, (Fig. 10), the variability in the exchange rate is predictable. \( T_x \), the turnover rate constant, is the fraction or percentage of the internal sodium pool that is exchanged per hour. Apparently not all of the sodium in the crab is exchangeable; the ratio of the calculated exchangeable sodium to the measured total sodium is
Table 15. Sodium exchanges of Cancer magister exposed to isosmotic and hypoosmotic environments.

<table>
<thead>
<tr>
<th>Weight Range (g)</th>
<th>Average Weight</th>
<th>Exch. Na(^+)/Total Na(^+)</th>
<th>Turnover Rate*</th>
<th>Exchange Constant (T(_x))**</th>
<th>Turnover Time (T(_t))</th>
</tr>
</thead>
<tbody>
<tr>
<td>41-89</td>
<td>66</td>
<td>0.800</td>
<td>21.59</td>
<td>0.114</td>
<td>9.44</td>
</tr>
<tr>
<td>37-66</td>
<td>56</td>
<td>0.031</td>
<td>6.40</td>
<td>0.032</td>
<td>2.24</td>
</tr>
<tr>
<td>74-85</td>
<td>81</td>
<td>--</td>
<td>12.86</td>
<td>0.081</td>
<td>13.27</td>
</tr>
<tr>
<td>38-83</td>
<td>61</td>
<td>--</td>
<td>2.85</td>
<td>0.020</td>
<td>3.78</td>
</tr>
<tr>
<td>41-89</td>
<td>66</td>
<td>0.806</td>
<td>9.50</td>
<td>0.069</td>
<td>14.80</td>
</tr>
<tr>
<td>37-66</td>
<td>56</td>
<td>0.164</td>
<td>4.92</td>
<td>0.008</td>
<td>13.13</td>
</tr>
<tr>
<td>74-85</td>
<td>81</td>
<td>0.031</td>
<td>0.92</td>
<td>0.008</td>
<td>13.13</td>
</tr>
<tr>
<td>38-83</td>
<td>61</td>
<td>0.164</td>
<td>4.78</td>
<td>0.024</td>
<td>3.72</td>
</tr>
</tbody>
</table>

Exch. Na\(^+\) = Exchangeable sodium

* \(\mu\)eq Na\(^+\)/g/animal/hr.

** Fraction of exchangeable Na\(^+\) exchanging per hour

0.8\(^1\) Alternatively, a portion of the internal sodium is exchanging at an extremely slow rate, being undetectable with the applied technique.

When the turnover rate constants and time constants for crabs in isosmotic and hypoosmotic media are compared, the expressions appear to

---

\(^1\) The exchangeable sodium is quantity A (Rudy, 1966).
be lower for crabs adapted to hypoosmotic environments. The $T_r$ and $T_t$ functions are independent of a decrease in the internal sodium concentration, as they are indicative of a fraction of the internal sodium pool. In only one case, however, is the difference significant. The $t$ test for 100\% and 50\% seawater-adapted populations demonstrated a significant difference ($P < .02$). The inverse body weight-sodium exchange relationship observed in *Pachygrapsus crassipes* provides an explanation for this discrepancy (Rudy, 1966). A larger weight range utilized in the 50\% seawater experiments may have caused the lower rate constant. Nevertheless, the rate constants for animals in dilute environments approached 0.080, whereas that observed for animals in 100\% seawater was 0.114. Although the differences may not be statistically significant, some decrease in the sodium exchange constant, elicited by hyperosmotic regulation, is suggested.

The expression, $a - b/a_o$, is plotted versus time in Figures 16a-d. $a - b/a_o$ is equal to the specific activity in the crab at a given time minus the activity in the external bath, divided by the original specific activity of the crab. Each point represents the mean value for all animals tested in a particular environment. If the sodium in the animal were being exchanged as one pool governed by a single rate constant, the plot would yield a straight line. It is apparent, however, that in both isosmotic and hypoosmotic media, the sodium within *Cancer magister* is exchanged as a multi-pool system.

In the process of loading the 30\% seawater-adapted crabs for the assessment of sodium efflux, the influx constant was determined for
Fig. 16. Relationship between the specific activity of the crab and the time from initiation of $^{22}$Na efflux.
comparative purposes. Blood samples were taken after a two hour exposure to the loading solution, and again after 96 hours, when equilibrium was attained. $K_i$, the sodium influx constant, is equal to $\frac{1}{T} \ln \left( \frac{A_e}{A_{\infty}} - A_t \right)$. $A_{\infty}$ is the specific activity at equilibrium (cpm/μeq Na$^+$); $A_t$ is the specific activity after two hours; and $T$ is time (Potts et al., 1967). For animals tested in 30% seawater, the influx constants ($K_i$) are compared with the efflux constants ($T_r$) in Table 16. Although the two expressions are not equivalent in each animal, the mean expressions are indistinguishable. Such an observation would be expected for animals achieving a steady state sodium balance. The comparison establishes reliability in employing different calculations designed to analyze one function.

The results from experiments designed to assess the role of exchange diffusion in sodium turnover are displayed in Table 17. The sodium efflux constant, $K_e$, was apparently reduced when crabs were

Table 16. A comparison of the total sodium influx ($K_i$) with the total sodium outflux ($T_r$) for nine crabs in 30% seawater.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Influx Constant ($K_i$)</th>
<th>Efflux Constant ($T_r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.086</td>
<td>0.060</td>
</tr>
<tr>
<td>2.</td>
<td>0.175</td>
<td>0.128</td>
</tr>
<tr>
<td>3.</td>
<td>0.075</td>
<td>0.113</td>
</tr>
<tr>
<td>4.</td>
<td>0.068</td>
<td>0.066</td>
</tr>
<tr>
<td>5.</td>
<td>0.068</td>
<td>0.061</td>
</tr>
<tr>
<td>6.</td>
<td>0.073</td>
<td>0.102</td>
</tr>
<tr>
<td>7.</td>
<td>0.065</td>
<td>0.055</td>
</tr>
<tr>
<td>8.</td>
<td>0.106</td>
<td>0.082</td>
</tr>
<tr>
<td>9.</td>
<td>0.090</td>
<td>--</td>
</tr>
<tr>
<td>X</td>
<td>0.089</td>
<td>0.083</td>
</tr>
<tr>
<td>s.d.</td>
<td>0.031</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Table 17. Sodium efflux constants ($K_e$) for animals exposed to 1000 mOsm solutions of seawater, mannitol, and lithium chloride.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>$K_e$ in S.W.</th>
<th>$K_e$ in Mannitol</th>
<th>% S.W. Mannitol Flux</th>
<th>$K_e$ in LiCl Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>.116</td>
<td>.000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>.215</td>
<td>.129</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>.084</td>
<td>.056</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>.135</td>
<td>.127</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>.073</td>
<td>.000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>.099</td>
<td>.008</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>.146</td>
<td>.060</td>
<td>41</td>
<td>.115</td>
</tr>
<tr>
<td>8.</td>
<td>.124</td>
<td>.047</td>
<td>38</td>
<td>.109</td>
</tr>
<tr>
<td>9.</td>
<td>.090</td>
<td>.061</td>
<td>68</td>
<td>.008</td>
</tr>
<tr>
<td>Average</td>
<td>.120</td>
<td>.054</td>
<td>45</td>
<td>.077</td>
</tr>
</tbody>
</table>

immersed in sodium-free solutions. Because of the large variance in the measurements, it is difficult to estimate quantitatively the fraction of the total efflux that is attributable to exchange diffusion. The mannitol and lithium chloride solutions were extremely discomforting to the animals, often evoking violent initial reactions. Death ensues soon after a one hour exposure. Although the results tend to suggest the presence of a functional exchange diffusion component, the information is inconclusive.

The measured electrical potential differences between the interior and exterior compartments are presented in Table 18. For animals in 100% and 30% seawater, the potential differences were inside-negative, averaging approximately -4 mV. The mean values for both populations of crabs were not statistically different. The range of the measurements is also included, because of the variability encountered.

The electrical potential differences necessary to establish the
Table 18. Measured electrical potential differences (millivolts) for crabs in 100% and 30% seawater.

<table>
<thead>
<tr>
<th></th>
<th>100% Seawater</th>
<th>30% Seawater</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>x</td>
<td>-3.5</td>
<td>-4.6</td>
<td></td>
</tr>
<tr>
<td>s.d.</td>
<td>±2.1</td>
<td>±4.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>-0.5 to -6.0</td>
<td>-0.7 to -12.5</td>
<td>P &gt; .05</td>
</tr>
</tbody>
</table>

external–internal concentration differences observed for several ionic constituents (data from Table 10) are demonstrated in Table 19. These potentials were calculated by application of the Nernst equation:

\[ E = \frac{RT}{zF} \times \ln\left(\frac{\text{Concentration outside}}{\text{Concentration inside}}\right) \]

where \( E \) is the equilibrium potential, \( R \) the gas constant, \( T \) is the absolute temperature, \( z \) is valency, and \( F \) is the Faraday. By comparing Tables 18 and 19, it is readily observed that the measured potential difference is sufficient to account for the existent concentration differences of \( \text{Na}^+ \) and \( \text{K}^+ \) ions for animals in 100% seawater.

**Discussion**

In seawater, *Cancer magister* exchanges its internal sodium with the sodium in the external milieu at a rate that is comparable with that determined for other brachyurans. The turnover rate constant \( (\text{hr.}^{-1}) \) for *Pachygrapsus crassipes* in seawater is 0.100 (Rudy, 1966), that of *Carcinus maenas* is 0.145 (Shaw, 1961a), and \( T_x \) for the Dungeness crab is 0.114 (Table 15). Seawater-adapted fishes exhibit a sodium exchange...
Table 19. Calculated equilibrium potentials* for the serum ionic constituents of Cancer magister.

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Cl⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100% Seawater</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meq/kg H₂O outside</td>
<td>455</td>
<td>9.9</td>
<td>521</td>
</tr>
<tr>
<td>meq/kg H₂O inside</td>
<td>502</td>
<td>11.5</td>
<td>531</td>
</tr>
<tr>
<td>Equilibrium Potential (mV)</td>
<td>-2.4</td>
<td>-3.7</td>
<td>+0.5</td>
</tr>
<tr>
<td><strong>75% Seawater</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meq/kg H₂O outside</td>
<td>339</td>
<td>7.3</td>
<td>390</td>
</tr>
<tr>
<td>meq/kg H₂O inside</td>
<td>443</td>
<td>9.4</td>
<td>432</td>
</tr>
<tr>
<td>Equilibrium Potential (mV)</td>
<td>-6.5</td>
<td>-6.2</td>
<td>+2.5</td>
</tr>
<tr>
<td><strong>50% Seawater</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meq/kg H₂O outside</td>
<td>230</td>
<td>5.1</td>
<td>261</td>
</tr>
<tr>
<td>meq/kg H₂O inside</td>
<td>373</td>
<td>7.9</td>
<td>375</td>
</tr>
<tr>
<td>Equilibrium Potential (mV)</td>
<td>-11.8</td>
<td>-10.7</td>
<td>+8.8</td>
</tr>
<tr>
<td><strong>30% Seawater</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>meq/kg H₂O outside</td>
<td>142</td>
<td>3.0</td>
<td>157</td>
</tr>
<tr>
<td>meq/kg H₂O inside</td>
<td>253</td>
<td>5.7</td>
<td>235</td>
</tr>
<tr>
<td>Equilibrium Potential (mV)</td>
<td>-14.1</td>
<td>-15.7</td>
<td>+14.5</td>
</tr>
</tbody>
</table>

*These calculations are based upon ionic concentrations rather than the preferred measurement, ionic activity.

rate that is high when compared to that of brachyurans (Potts et al., 1967; Potts and Evans, 1967; Motais et al., 1966), but a considerable portion of the sodium flux in these fishes has been attributed to a phenomenon known as exchange diffusion (Motais et al., 1966; Evans, 1967). In an external milieu with an osmotic concentration equivalent to 100% seawater, but lacking sodium ions, a 50-90% decrease from the original sodium efflux measurement has been observed. This has indicated that sodium efflux, for example, may be partially dependent upon the concentration of this ion in the external medium. The existence of an exchange diffusion component could have a great effect upon the
quantitative analysis of sodium balance in the Dungeness crab. The results from experiments designed to test the hypothesis were inconclusive, however (Table 17). There was some indication that sodium efflux declined when crabs were exposed to mannitol or LiCl, but the behavior of the crabs in these environments was too abnormal to justify any confidence in the data. An isolated gill preparation could provide a means of resolving this problem.

Exchange diffusion of sodium ions has not been conclusively demonstrated in any other crustacean. Rudy (1966) found no difference in the sodium loss rates determined by flame photometry and $^{22}$Na flux studies when Pachygrapsus was exposed to distilled water. He concluded that an exchange diffusion component was absent. Exchange diffusion was reported for Artemia salina immersed in Na$^+$-substituted seawater solutions (Thuet et al., 1968), but the observed decreases in sodium efflux were in all probability due to changes in electrical potential difference or diffusional permeability (Smith, 1969b). Although exchange diffusion of sodium evidently does not occur in the brine shrimp, 70% of the chloride flux can be attributed to such a phenomenon (Smith, 1969b).

When immersed in a hypoosmotic environment, Cancer magister maintains a serum sodium concentration that is greater than that of the medium (Fig. 10). The animal is obviously permeable to sodium ions (Table 15), and the sodium diffusional gradient thus created dictates a passive loss of ions to the external milieu. Retardation of the passive loss of sodium could provide an effective means of conserving
ions, thereby reducing the load that must be acquired by presumably active processes. A comparison of the turnover rate constants for crabs in isosmotic and hypoosmotic media demonstrates a tendency for a reduction in total sodium movements by animals exposed to hypoosmotic media (Table 15). The euryhaline prawn, *Palaemonetes varians*, exhibits a turnover rate (hr$^{-1}$) of 1.17 in seawater, but the exchange is drastically reduced to 0.20 when the animal inhabits 2% seawater (Potts and Parry, 1964). Freshwater crustaceans typically exhibit a reduced permeability to sodium movements when compared to the brackish water species *Carcinus maenas* and *Cancer magister*. The exchange constant (hr$^{-1}$) for *Astacus fluviatilis*, for example, is 0.004 (Bryan, 1960). Shaw (1961b) has examined sodium balance in crustaceans from a variety of habitats. The freshwater crabs *Potamon niloticus* and *Eriocheir sinensis* exhibit a turnover rate that is markedly reduced when compared to *Cancer magister* and *Carcinus maenas* in brackish water. The crayfish, *Astacus pallipes*, is even less permeable to sodium movements. Shaw (1961b) has concluded that the reduction in the permeability of the body surfaces to salt movements was a primary factor in the adaptation of the Crustacea to freshwater. The inability of *Cancer magister* to lower effectively its permeability to sodium probably restricts the species to brackish water.

A better understanding of the functional sodium balance mechanism enabling *Cancer magister* to inhabit brackish water can be gained by partitioning the total sodium flux (Table 15) into its several pathways. Sodium efflux contains two elements, passive exchange and
urinary efflux. The urinary ion fluxes have been tabulated in Table 14. Passive sodium efflux is the difference between the total and the urinary outfluxes. As passive efflux and the sodium concentrations of the external and internal environments (Table 10) are known quantities, passive sodium influx can be calculated from the following relationship (Potts and Parry, 1964):

\[
\frac{\text{Inward diffusion}}{\text{Outward diffusion}} = \frac{\text{External Na}^+}{\text{Internal Na}^+}
\]

For an animal in sodium balance, total outflux and influx are equivalent (Table 16); therefore, active influx is equal to the difference between total sodium flux and passive influx. The results of the partitioning of the fluxes are illustrated in Fig. 17. The dashed diagonal line represents the values for passive sodium influx in various salinities that would be expected if this component were dependent solely upon the external sodium concentration. Passive sodium influx in 100% seawater served as the reference point for the line. The solid line is the linear regression line. The linear regression correlation coefficient for the relationship between the external concentration and passive sodium influx is highly significant \((p < .001)\). Variations of the passive influx rates from this line may be due to heterogeneity among the means that is unrelated to the magnitude of the external concentration, or residual heterogeneity around the regression line.

Interestingly, the passive influx components for crabs in 30% and 50% seawater are equivalent, suggesting an increase in the diffusional permeability to sodium in 30% seawater. Such an increase could indicate a weakening of the balance mechanism in this medium.
Fig. 17. Sodium exchanges in Cancer magister, as a function of salinity.

Diagram showing sodium exchange rates in Cancer magister at different salinity levels. The x-axis represents percent seawater, ranging from 30 to 100, and the y-axis represents sodium exchange (meq/g animal/hr) ranging from 0 to 20. The bars indicate active and passive exchanges, while the lines represent influx and efflux.
Alternatively, the difference could be a product of the experimental procedure. If sodium exchange is inversely related to body weight in this species (see Rudy, 1966), the larger weight range utilized in 50% seawater might possibly yield lower exchange constants (Table 15).

In the analysis presented in Fig. 17 two major assumptions were made: exchange diffusion and an electrical potential difference are inconsequential. The measured inside negative potential difference (Table 18) is of sufficient magnitude to account for the high serum sodium concentration of animals in 100% seawater (Table 19), and active transport is probably not a functional pathway for sodium influx in this environment. By considering the electrical potential difference as a pathway through which similar quantities of sodium are transported in each medium, the partitioning can be more accurately presented (Fig. 18). The flux components are expressed as a percentage of the total sodium turnover. The source of the developed p.d. between the internal and external environments has not been characterized. The p.d. could be created by the active transport of chloride ions from the outside to the inside. Alternatively, as Smith and Linton (1971) have indicated from studies with gill tissue treated with formaldehyde, the p.d. could be an inherent physical property of crustacean membranes.

The maximum urinary efflux of sodium that was observed represented 16% of the total flux in 50% seawater (Fig. 18). Urinary loss in *Carcinus maenas* in 40% seawater also approximates 16% of the total

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1. The measured electrical potential of *Cancer magister* is independent of salinity (Table 18).
Fig. 18. Schematic representation of sodium balance in Cancer magister. Fluxes are expressed as percentages of the total turnover.
(Shaw, 1961a). Because urinary loss is a small fraction of the total, any reduction produced by salt conservation in the antennary gland would not be significant. Energetically, the production of a dilute urine is feasible only when the internal-external concentration gradient is extremely large (Potts, 1954), and when the body surfaces are relatively impermeable to solute (Shaw, 1959b). Active influx comprises approximately 25-30% of the total sodium influx for crabs exposed to 50% and 30% seawater (Fig. 18). Active uptake accounts for 50% of the flux in *Astacus pallipes* (Shaw, 1959a), and 30-40% of the total sodium influx of *Gammarus duebeni* (Shaw and Sutcliffe, 1961). Figures 1 and 10, and Table 2, indicate that the osmoregulatory machinery is operating maximally in 30%-50% seawater. If an active component contributing from 30-50% of the total flux represents maximal achievement, it appears that *Cancer magister* is not capable of inhabiting waters much more dilute than 30% seawater because it cannot effectively reduce its permeability to sodium ions. Any further reduction in the medium sodium concentration is paralleled by a reduction in the internal concentration (Fig. 10). Sustained activity in media less concentrated than 30% seawater is dependent upon the tolerance of the tissues to a reduction in sodium concentration.

Table 19 demonstrates the effect of an electrical potential difference upon sodium balance. An inside-negative potential difference of 14 mV would create the observed sodium concentration gradient in 30% seawater. If the range of measured potential differences is applied instead of the mean values, due to the encountered variability of
potential recordings (see Potts and Parry, 1964), active transport of sodium by *Cancer magister* in hypoosmotic media need not be postulated. Even with application of the mean potential differences, electrically-enhanced sodium influx is of the same magnitude as the proposed active transport quantity in all of the hypoosmotic media (Fig. 18). The importance of active sodium transport in this brackish water species is questionable. Smith (1969a) has demonstrated that the sodium movements in seawater-adapted *Artemia* can be attributed to the existent electrical potential difference; only chloride is being actively transported. Active transport of sodium by the isolated perfused gills of *Callinectes sapidus* apparently occurred when the gills were exposed to very dilute media, but in 25% and 50% seawater the electrical potential difference was sufficient to maintain the concentration difference (Smith and Linton, 1971). Although active sodium transport is unquestionably important to freshwater crustaceans, its impact upon sodium balance in brackish water crustaceans may be of dubious significance; especially when compared to the energy requirement necessary for achieving chloride regulation.
CHAPTER 7

CONCLUSION

The mechanisms by which Cancer magister Dana regulates the osmotic and ionic concentrations of its extracellular fluids when in isosmotic and hypoosmotic environments has been examined. In seawater, this species is in osmotic equilibrium with the external milieu, but the ionic concentrations of the serum and urine vary from concentrations in seawater. Potassium, calcium, and sodium concentrations are maintained at a higher level than the seawater concentrations; these increases are principally effected by an inside-negative electrical potential difference, although reabsorption through the antennary gland occurs. Magnesium and sulfate are preferentially excreted. It appears that sufficient sodium is reabsorbed in the antennary gland to postulate a direct sodium-magnesium exchange, when the ionic activities are considered. The urine production rate in seawater is low; a slight osmotic differential, drinking, or solute-accompanying water movements are possible sources.

In hypoosmotic media, the serum concentrations of sodium, potassium, calcium, and chloride are maintained at a higher concentration than the seawater concentrations. The osmotic differential thus created yields a net inward movement of water that is discharged effectively by
the excretory organ. The apparent permeability to water is not altered in dilute media. The animals in 100% seawater displayed an increased water flux under certain conditions, however. It was suggested that different levels of activity could produce a permeability change.

It is possible that the permeability to sodium ions is reduced in hypoosmotic media. The reduction is not of sufficient magnitude to enable the animal to penetrate environments less concentrated than approximately 30% seawater. Uptake of sodium ions to compensate for the diffusional loss takes place via an inside-negative electrical potential difference, and probably some active uptake. The sodium uptake mechanism is apparently maximally activated in the 50%-30% seawater range. Urinary loss of sodium is of minor consequence. It was therefore concluded that the inability to reduce effectively the permeability of the body surfaces to sodium ions restricts this species to brackish water.

Magnesium and sulfate are preferentially excreted in 75% and 50% seawater; however, in 30% seawater these ions are conserved. The pattern of calcium regulation is also altered in 30% seawater. Curiously, the relationship between body weight and osmoregulatory capability is apparent only in this medium. These observations suggest that the animal is exerting a maximum effort to alleviate the problems imposed by the extreme osmotic stress of 30% seawater. This medium probably represents the maximum hypoosmotic stress which the species is capable of tolerating.
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Krogh, A. (1938). The active absorption of ions in some freshwater animals. Z. vergl. Physiol. 25, 235-250.


