FURTHER STUDIES ON THE OSMOTIC AND IONIC REGULATION IN THE CRAB, CANCER BOREALIS, STIMPSON

GINO ANGELO TREVISANI

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FURTHER STUDIES ON THE OSMOTIC AND IONIC REGULATION IN THE CRAB,
CANCER BOREALIS STIMPSON

BY
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A.B., Utica College of Syracuse University, 1954
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A DISSERTATION
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This dissertation has been examined and approved.

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Sept 12, 1936
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INTRODUCTION

The manner in which a decapod crustacean maintains a relatively constant body fluid concentration, with respect to changes in the external environment, has interested many previous investigators. Interest centers not only on the marked ability of the organism to adapt physiologically to a particular habitat but also on the philosophical implications, that is, the possible evolutionary changes which might have occurred in the animal as it attempted to explore new habitats and emancipate itself from the shackles of its environment.

An organism such as a decapod crustacean, which leaves its normal habitat, is confronted with problems of salt and water balance. This necessitates mechanisms for osmotic and ionic regulation in order that tissues may have the proper concentrations of salts to carry on normal metabolic processes. In order, therefore, for other environments to be successfully colonized, the total concentration of the salts of the internal body fluids and consequently their ionic concentrations necessarily must be maintained within a relatively narrow range.

It was found from preliminary work (Trevisani, 1956) that limited powers of osmoregulation and considerable powers of ionic regulation existed for at least a two hour period in
Cancer borealis Stimpson, a rock crab found along the New England coast. Although it was suspected that at least three mechanisms were involved in osmotic and ionic regulation of Cancer borealis, namely, its low permeability to salts and water at low dilutions; the active absorption of salts, probably through the gill surface; and the differential excretion of ions and water by the antennary gland, further studies to obtain additional evidence for some of these mechanisms seemed desirable.

Although it was discovered that Cancer borealis did have osmotic and ionic regulatory powers in dilute sea water for at least two hours, a study to determine whether these regulatory processes existed for longer periods of time was not pursued.

It was also suggested by the author that these crabs were relatively impermeable to salt and water because osmotic measurements on the blood of these animals showed that for at least two hours they could maintain a relatively constant internal environment when subjected to various dilutions of sea water. However, no direct evidence was obtained in these preliminary studies.

A peculiar problem arose during the process of obtaining urine for osmotic and ionic studies on these animals in this previous investigation. When the crabs were subjected to normal sea water, the urine flowed copiously and was easily collected. However, in the lower dilutions of sea water it was noted that the urine was scarce and obtainable
only after great effort. This suggested two possibilities: (1) Either the animals became less permeable to water at lower dilutions with a concomitant decrease in urine output or (2) The crabs were equally permeable, as when in normal sea water, but in dilute media more water moved into their bodies, followed by a consequent increase in urine formation, a rapid release from the antennary glands, and possibly excretion of water through an extraneous route such as the gut. Urine in these instances would be extremely difficult to collect. These possibilities were left unexplored.

In using sea water dilutions in previous studies, natural sea water was collected at Portsmouth, New Hampshire, and this was assumed, on the basis of Coast and Geodetic Survey Salinity Tables, to be approximately 75% sea water. Dilutions were made from this varying stock rather than from a stock of 100% or sea water containing 35 parts of chloride per thousand. Because of the fact that normally collected shore sea water varies in salinity on different tides and because of organic and other substances interfering with ion analyses, it appeared that a stock of 100% artificial sea water, prepared by standard formulae, would give more accurate dilutions and would enable ion analyses to be carried out avoiding the problem of the variability of natural sea water. Furthermore, it was felt that by refining these techniques and methods of ion analysis, more accurate results would be obtained in further studies.
Present interest in the physiological mechanisms which enable this animal to penetrate more dilute salinities and the possible evolutionary significance of such regulatory mechanisms has, therefore, grown out of the author's earlier thesis for a master's degree.

The present investigation was initiated to explore further the problems left unsolved in the preliminary studies. *Cancer borealis* was subjected to various dilutions of sea water for longer periods of time to determine whether osmotic and ionic regulatory powers existed in the animal for at least twenty-four hours. Weight determinations were made throughout a twenty-four hour period in order to determine the permeability of the animals to water and thus to elucidate the role of the antennary glands and gut in osmotic regulation. A further study of the role of the antennary glands in ionic regulation was pursued.

Generally, then, the investigation reported herein was designed to elucidate the physiological mechanisms by which *Cancer borealis* is able to survive in media outside its normal habitat by osmotic and ionic regulatory abilities and to clarify the reasons for the inability of these mechanisms to function properly in very dilute salinities.
MATERIALS AND METHODS

Animals

Crabs used in these experiments were obtained during the winter months from the Marine Biological Supply House, Woods Hole, Massachusetts. The stock of Cancer borealis was collected in the late fall and maintained at the Marine Biological Supply House in live cars. These crabs were shipped to the Zoology Department, University of New Hampshire, at various intervals throughout the entire winter. Animals were acclimated to the sea water tanks at the University of New Hampshire for at least 24 hours before being used for experimentation.

Sea Water

In all dilutions in which the animals were placed, artificial sea water was used. The Marine Biological formula taken from Formulae and Methods IV (1956) was used to make all dilutions of sea water. A salinity of 35 parts per thousand chloride (p.p.t.) was taken as 100% sea water and dilutions of 87% (31 p.p.t.), 75% (26 p.p.t.), 50% (18 p.p.t.), and 40% (14 p.p.t.) sea water were made from this 100% stock.

Weight Determinations of Normal Animals

In order to determine whether or not Cancer borealis took up water osmotically when placed in 100%, 87%, 75%, 50%, and 40% sea water, weight determinations were made at
various intervals throughout a twenty-four hour period. Before a weight determination was made the animals were wrapped in cheesecloth to remove as much sea water from the surface as possible. The greatest possible care was taken to shake out most of the water in the gill chamber.

**Weight Determinations on Animals with Plugged Antennary Glands**

In order to reveal the role of the antennary glands in adapting the animals osmotically to sea water dilutions, the glands were plugged and weight determinations made as above. The weights of these animals were compared to those animals with unplugged antennary glands. Plugging of the antennary glands was done by drying the area around the operculum of the gland and covering it with jeweler's cement. The cement was made soft and pliable by warming and was then applied to the operculum of the antennary gland where upon standing it became hard and made a very good seal. *Cancer borealis* with glands plugged were kept in the experimental media for no longer than eight hours because it was felt that longer intervals might cause adverse effects to the animals.

**Weight Determinations on Animals with Plugged Guts**

To reveal the role of the gut in ridding the animal of excess osmotic water as the animal penetrates more dilute waters, crabs with guts plugged were subjected to 100%, 87%, 75%, 50%, and 40% sea water. In order to close the oral opening without injuring the animal, a roll of cotton was
placed in the esophagus. The cavity between the mouth opening and the mandible was then filled with dry cotton and made waterproof by pouring warm paraffin over it. More warm paraffin was poured over this first layer, which made a thick closure without injuring the animals for as long as twenty-four hours. The hind gut was closed by tying a tight ligature around the intestine. Animals were kept in the experimental media no longer than eight hours. Weight determinations were made throughout the eight hour period.

Osmotic Determinations

Blood and urine were collected by the same method previously described by the author (1956). Osmotic pressure determinations were made on 100%, 87%, 75%, 50%, and 40% sea water and on the blood and urine of animals kept in these dilutions of sea water for twenty-four hours. The melting point method of Gross (1952) was used to measure osmotic pressures. Pieces of capillary tubing (1 mm. bore) containing 0.05 mls. of fluid were sealed at both ends with clay or vaseline and mounted in a grooved plastic rack and quickly frozen on dry ice. This plastic rack containing both the unknown solutions (blood, urine, or sea water) and usually four or five standard NaCl solutions, was then placed in a cold brine solution (Fig. 1) below the temperature of the unknowns and standards. The cold brine was cooled by dry ice to -5°C. A stirrer was used to agitate the brine, which warms slowly depending on the amount of insulation in the box
Figure 1. Apparatus used to determine osmotic pressures of sea water, blood, and urine.
supporting the dish of brine (Fig. 1). A light source
(Fig. 1) from below passes through a sheet of polaroid then
through the dish containing the capillaries, and through a
plastic cover on which a second piece of polaroid is placed.
The frozen crystals when viewed by polarized light glow
brightly. As the brine solution warms slowly, the transition
of the glowing crystals from the solid to the liquid state
is abrupt and sharp. The time of complete melting of the
fluid in each capillary is recorded on a kymograph. The
time of melting of each tube is then plotted against the
known concentrations and the values of the unknowns are
obtained by interpolation. The results are expressed as
equivalent normality of NaCl.

The standard NaCl solutions used in this investigation
were 0.89N, 0.75N, 0.59N, 0.45N, and 0.20N.

Ionic Determinations

Although freezing point determinations indicate the
degree to which animals are able to maintain, osmotically,
their body fluids above that of the external medium, they
do not indicate how this is done on the ionic level. To
determine the degree of ionic regulation in Cancer borealis,
animals were placed for a period of twenty-four hours in
100%, 87%, 75%, 50%, and 40% sea water. Then the sea water,
blood, and urine were analyzed for total chloride, sodium,
magnesium, calcium, and potassium.
Potassium, sodium, and calcium were measured with a Perkin-Elmer Flame Photometer, using an acetylene flame. The urine samples were read directly after appropriate dilutions. The blood samples were allowed to clot and after dilution they were read as above. The sodium samples were diluted 1:1000 and the potassium and calcium samples were diluted 1:500.

A Bausch and Lomb Spectrophotometer (Spectronic "20") was used for the chloride and magnesium determinations. Analyses were made after the chloride samples were diluted 1:1000 and the magnesium samples diluted 1:50.

The principle behind the chloride determination was adapted from Sendroy (1937). The sample is shaken with silver iodate and the iodate is determined photometrically.

\[
\text{NaCl} + \text{AgIO}_3 \rightarrow \text{AgCl} + \text{NaIO}_3
\]

The silver chloride is insoluble and comes out of solution. Sodium iodate is soluble and goes into solution thus serving as an accurate measure of the chloride present.

The magnesium determination was done by a modification of the method of Denis (1922). After the calcium is precipitated as calcium oxalate in an acid medium, the magnesium is then precipitated as magnesium ammonium phosphate and the latter estimated by a colorimetric phosphate determination.
RESULTS

Weight Changes in Normal Animals

As *Cancer borealis* is subjected to lowered salinities of sea water (Table 1) a gain in weight of the normal animals is noted.

In 100% sea water (Table 1 and Figure 2) the animals lost an average of 1.5% of their body weight after two hours. At the end of four and twenty-four hour periods an equilibrium apparently had been established, for constant weight decreases of 0.9% and 0.8% were evident.

When the animals were subjected to 87% sea water (Table 1 and Figure 2) a decrease in weight is still noticed but there is a tendency for the weight change to be less than it was in 100% sea water. An average percent weight loss of 0.4%, 0.9%, 0.5%, 0.7%, and 0.8% was revealed after two, four, six, twelve, and twenty-four hours respectively.

In 75% sea water the weights of the experimental animals showed little change (Table 1 and Figure 2). After two hours a 0.2% increase in body weight is noted. After six hours a 0.4% increase is evident. When the animals were weighed at the eight hour interval a 0.4% increase was found. Following both the eighteen and twenty-four hour periods a 0.1% weight decrease was recorded.
## Table 1

Average Percent Weight Changes of Normal Animals Compared to Animals with Plugged Antennary Glands and Guts.

Average Percent Weight Changes * Standard Error of Mean

<table>
<thead>
<tr>
<th>Time</th>
<th>100% Sea Water</th>
<th>87% Sea Water</th>
<th>75% Sea Water</th>
<th>50% Sea Water</th>
<th>30% Sea Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 h</td>
<td>-1.5 ± 0.6</td>
<td>-0.1 ± 0.2</td>
<td>-0.2 ± 0.2</td>
<td>-0.4 ± 0.3</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>4 h</td>
<td>-0.9 ± 0.2</td>
<td>-0.4 ± 0.3</td>
<td>-0.8 ± 0.3</td>
<td>-0.9 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>6 h</td>
<td>-0.4 ± 0.5</td>
<td>-0.5 ± 0.1</td>
<td>-0.8 ± 0.2</td>
<td>0.7 ± 0.8</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>8 h</td>
<td>+0.4 ± 0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>12 h</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>14 h</td>
<td></td>
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<tr>
<td>16 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>20 h</td>
<td>-0.3 ± 0.3</td>
<td>-0.8 ± 0.4</td>
<td>-0.8 ± 0.4</td>
<td>-0.8 ± 0.4</td>
<td>-0.8 ± 0.4</td>
</tr>
</tbody>
</table>

[Note: The table continues with similar entries for each condition.]
Fig. 2. Percent weight changes of normal animals subjected to various salinities of sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
It would seem that animals in 75% sea water show little change in body weight over their initial weight. This is possibly due to the fact that this salinity closely approaches that of the normal environment of the animal and the slight changes noted are perhaps due to the normal loss or retention of urine at any one time when the animals are weighed. However, it must be borne in mind that the salinity of the stock aquarium tanks in which these crabs were placed fluctuated from 75% to 100% sea water at different times throughout the year. Because of this fluctuation, any group of animals placed in the stock tanks containing a concentration of 75% sea water would have approached equilibrium with the environmental sea water and would show minor fluctuations in weight around the initial weight. By the same token, if stock animals happened to be placed in aquaria with a stock salinity of 100% sea water, before initial weights were taken one would expect such animals to come to equilibrium with this environment and similarly show minor fluctuations around their initial weight.

Animals subjected to 50% sea water (Table 1 and Fig. 2) showed an average increase in weight at every weight measurement interval. After two and four hours a 1.9% increase was noted. A 0.9%, 1.0%, 1.1%, and 1.9% increase was evident after six, eight, ten, twenty, and twenty-four hours respectively.
The greatest increase in weight was revealed when *Cancer borealis* was placed in 40% sea water (Table 1 and Fig. 2). At the end of two hours a 2.1% increase was recorded. At the end of four hours a 3.0% increase was evident. The six hour weighing showed a 2.5% increase and at the end of eight and twenty-four hours a 3.0% increase was maintained.

Although some of the weight changes of animals in the various dilutions of sea water do not seem to be statistically significant when compared (Fig. 2) a trend towards increase in weight as the salinity is lowered seems to be present.

**Weight Changes of Animals with Plugged Antennary Glands**

The weight differences of animals with their antennary glands plugged (Table 1) show in general that they gain more weight than normal crabs in the same concentration of sea water.

After two hours the average percent decrease in weight of animals placed in 100% sea water was only 0.4% (Table 1 and Fig. 3). Normal animals lost an average of 1.5% of their body weight after this period. After four hours the decrease in weight of antennary gland-plugged animals was only 0.1% and after six hours an increase of 0.4% is indicated. When these weight losses are compared with normal animals it is evident that the latter lose more weight than the former.
Fig. 3. Percent weight changes of normal animals as compared to antennary gland and gut-plugged animals in 100% sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
□ Normal Animals
□ Cut-Plugged Animals
■ Antennary T and-Plugged Animals

Time in Hours

Percent Weight Change

2 4 6 8 24
Table 1 and Figure 4 give the values obtained when *Cancer borealis* is subjected to 87% sea water. Two hours after the animals were placed in this dilution of sea water with their glands plugged an average increase in weight of 0.3% is evident. After four and six hours a 0.8% increase was recorded. Normal animals in the same dilution all showed a decrease in weight at every weight measurement.

Gland-plugged animals in 75% sea water (Table 1 and Figure 5) show after two, four, and six hours an average percent increase in weight of 1.6%, 2.5%, and 2.7% respectively. Normal animals show little change in weight when placed in this dilution (Table 1 and Figure 5).

The results with the 50% dilution (Table 1 and Figure 6) again show a marked increase in weight of gland-plugged animals with respect to normal animals. At two hours a 3.9% increase in weight is noted. At four hours a 4.9% increase is evident and at six hours a 6.2% increase is clearly seen.

When *Cancer borealis* is subjected to 40% sea water (Table 1 and Figure 7) with its glands plugged 4.2%, 4.1% and 4.8% weight gains are noted after two, four, and six hours.
Fig. 4. Percent weight changes of normal animals as compared to antennary gland and gut-plugged animals in 87% sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
□ Normal Animals

■ Out-Plugged Animals

■ Antennary Gland-Plugged Animals

Percent Weight Change

Time in Hours

2 4 6 12 24
Fig. 5. Percent weight changes of normal animals as compared to antennary gland and gut-plugged animals in 75% sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
Percent Weight Change

- Normal Animals
- Out-Plugged Animals
- Antennary Gland-Plugged Animals

Time in Hours
Fig. 6. Percent weight changes of normal animals as compared to antennary gland and gut-plugged animals in 50% sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
Fig. 7. Percent weight changes of normal animals as compared to antennary gland and gut-plugged animals in 40% sea water. Horizontal line at zero percent weight change represents the initial weights of the animals. Squares indicate the arithmetical mean of the percent weight changes of the number of animals used (Table 1) at each specific point. Vertical lines at each square indicate plus or minus standard error of the mean percent weight change. Time in hours is indicated along the bottom abscissa. The percent weight change is indicated along the ordinate.
The results of plugging the antennary glands clearly indicate that there is an increase in urine production as the external medium is lowered. From 100% sea water to 50% sea water the increase in production of urine would seem to be responsible for the increases in weight above that of normal animals. For example, at the end of eight hours in 100% sea water and six hours in 87% sea water, gland plugged animals showed an increase in weight of 1.3% above normal animals in the same dilution (Fig. 3 and 4). In 75% sea water a 2.3% increase above normal animals was noted and in 50% sea water the increase rose to 5.3% after six hours (Fig. 5 and 6).

A discrepancy is noted when one compares gland-plugged animals in the 50% dilution with animals in the 40% dilution (Fig. 6 and 7). Crabs gain more weight in 50% sea water than in 40% sea water. Since 40% sea water is the limit in dilution of the external medium which Cancer borealis is able to tolerate, the mechanism for increased urine production in all probability breaks down in this dilution. Because of this failure, it is conceivable that urine production is hindered and that, as a result, the weights of the gland-plugged animals in 40% sea water are less than animals in 50% sea water.
Weight Changes in Animals with Plugged Guts

Weight changes of gut-plugged animals subjected to 100%, 87%, 75%, 50%, and 40% sea water are depicted in Table 1 and Figures 3, 4, 5, 6, and 7.

In 100% sea water (Table 1 and Fig. 3) a decrease in weight of 0.2% after two hours, 0.1% after four hours, and 0.4% after eight hours was noted. This is a smaller decrease in weight than is observed in normal animals but similar to the gland-plugged animals.

A 0.1% increase in weight is evident after the animals were subjected to 87% sea water for two hours (Table 1 and Fig. 4). At the end of four and six hours an increase in weight of 1.0% and 0.7% were noted respectively. Again the changes in weight differ considerably from normal animals but show the same trends as in gland-plugged animals.

In 75% sea water (Table 1 and Fig. 5) an increase of 0.6% was noted after two hours, 2.5%, and 2.4% at the end of four and six hours respectively.

Animals placed in 50% sea water with their guts plugged show an average increase in weight of 3.6%, 4.2%, and 4.0% after two, four, and six hours respectively (Table 1 and Fig. 6). The weights of these animals differ markedly from the normal animals but are very similar to those of gland-plugged animals.

It is evident that gut-plugged animals gain more weight
than normal animals in the same concentrations of sea water, thus suggesting another route by which excess water is eliminated from the crabs. After eight hours in 100% sea water, gut-plugged animals showed an increase in weight of 1.3% as compared to normal animals in the same dilution (Fig. 3). In 87% sea water (Fig. 4) after six hours the increase above normal animals was 1.2%. Animals in 75% sea water (Fig. 5) show an increase of 2.5% over normals while crabs in 50% sea water show an increase in weight above normal animals of 3.0% after six hours (Fig. 6). In 40% sea water, however, an increase of 2.3% is evident at the end of six hours (Fig. 7).

The weights of gut-plugged and normal animals seem to overlap in 40% sea water. Again this suggests that cellular mechanisms that normally secrete water into the gut for elimination break down in this dilution.

Osmotic Regulation

Osmotic concentrations (Table 2), expressed as equivalents of normal NaCl, show that in 100% sea water the blood is slightly hypotonic and the urine almost isotonic to the sea water.

In 87% sea water the hypotonicity of the blood with respect to the external medium is still present. Urine measurements again follow close to the sea water value (Table 2).
Animals in 75% sea water have an internal environment which is essentially isotonic to the external medium (Table 2) but the trend seems to be towards a slight hypotonicity. The urine in this case seems to vary, for it is isotonic or hypertonic to the blood and hypotonic, isotonic, or hypertonic to the external medium.

The blood of the animals in 50% sea water (Table 2) displays a slight hypertonicity to the external medium. Urine values show a hypertonicity, an isotonicity or hypotonicity to the sea water.

In 40% sea water again, a hypertonic internal environment, with respect to the sea water, is evident. The urine values are similar to those of the external medium. Although the blood is hypertonic to the external medium in this dilution, survival is poor and death occurs before an isotonicity is attained.

A graph showing the external environment concentration plotted against the internal environment is depicted in Fig. 8. It will be observed from this figure that the crabs have an hypotonic or isotonic internal environment with respect to the sea water in 100%, 87%, and 75% sea water. The body fluids become hypertonic in 50% and 40% sea water but survival is poor in the latter. The limited powers of Cancer borealis to regulate its internal environment osmotically appears to be drastically impaired in 40% sea water and death ensues before an equilibrium is reached.
Table 2. Osmotic Concentrations of the Blood and Urine of Cancer borealis in Varying Salinities of Sea Water. All values are expressed in equivalents of normal NaCl.

<table>
<thead>
<tr>
<th>Percent Sea Water</th>
<th>No. of Animals Used</th>
<th>Conc. of Sea Water (1) ± Standard error of the mean</th>
<th>Mean Conc. of Blood of Urine (1) ± Standard error of the mean</th>
<th>Mean Ratio of Blood to Sea Water (1) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%</td>
<td>4</td>
<td>0.57±0.0 0.54±0.01</td>
<td>0.58±0.01</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>37%</td>
<td>3</td>
<td>0.49±0.0 0.43±0.02</td>
<td>0.48±0.01</td>
<td>0.89±0.04</td>
</tr>
<tr>
<td>75%</td>
<td>4</td>
<td>0.43±0.0 0.40±0.03</td>
<td>0.43±0.04</td>
<td>0.93±0.09</td>
</tr>
<tr>
<td>50%</td>
<td>4</td>
<td>0.28±0.0 0.33±0.01</td>
<td>0.29±0.04</td>
<td>1.20±0.01</td>
</tr>
<tr>
<td>40%</td>
<td>3</td>
<td>0.20±0.0 0.26±0.02</td>
<td>0.21±0.01</td>
<td>1.30±0.01</td>
</tr>
</tbody>
</table>

* A ratio (1) of less than one designates an hypotonic internal environment with respect to the external medium; of one indicates isotonicity; and of more than one indicates an hypertonic internal environment.
Although the ratio of the blood to the external environment in this salinity is 1.30 (Table 2), indicating that the blood is hypertonic to the sea water, animals cannot survive more than twenty-four hours in this medium.

** Ionic Regulation **

The results of the chemical analyses of the sea water, blood, and urine appear in Tables 3, 4, 5, 6, and 7 and represent analyses of pooled samples from four animals.

In 100% sea water (Table 3) potassium and sodium are the only ions which appear to be absorbed against a concentration gradient, for blood concentrations are slightly higher than those of the sea water. Calcium, magnesium, and chloride, on the other hand, enter the blood by simple diffusion for they are slightly lower than the sea water concentrations. In the urine, potassium, sodium, and chloride are removed passively from the body fluids while calcium and magnesium are removed actively.

When the animals are subjected to 87% sea water (Table 4) all the ions seem to enter passively and active excretion of every ion by the antennary glands is noted. The urine values are all higher than the blood concentrations.

Analyses of animals in 75% sea water (Table 5) indicate that potassium again is the only ion to be actively absorbed. Calcium, chloride, sodium, and magnesium enter the blood passively. Chloride and magnesium are excreted actively, whereas potassium and calcium diffuse out passively. Sodium,
Fig. 8. Curve showing the freezing point depression of the blood of *Cancer borealis* plotted as a function of the freezing point depression of the external environment. Numbers along ordinate and abscissa are expressed as Normal NaCl. △1 indicates internal body fluids while △° represents external medium.

\[
\begin{align*}
\text{.57} & = 100\% \text{ sea water} \\
\text{.49} & = 87\% \ " \ " \\
\text{.43} & = 75\% \ " \ " \\
\text{.28} & = 50\% \ " \ " \\
\text{.20} & = 40\% \ " \ " 
\end{align*}
\]
However, shows an indication of being conserved. Its value in the urine is lower than the blood concentration.

Animals subjected to 50% sea water (Table 6) show again an active absorption of potassium and of sodium as well. Calcium, chloride, and magnesium all enter the body fluids by passive forces. Potassium and sodium seem to be lower in the urine, indicating selective retention of these ions by the antennary glands. Calcium, chloride, and magnesium are removed passively.

*Cancer borealis* when placed in 40% sea water (Table 7) still maintains a potassium concentration of the body fluids above that of the external medium. Likewise, calcium and sodium seem to be actively concentrated by the animals in this salinity. The amounts of these ions concentrated by the crabs are apparently not sufficiently high to maintain conditions compatible with life since survival is poor in this medium. All other ions seem to enter the body fluids passively. The urine concentration of potassium is lower than the blood value, indicating a selective retention of this ion. All other ions are actively forced out of the body fluids by the antennary gland.

When one looks at the results of the chemical analysis as a whole (Fig. 9, 10, 11, 12, and 13) several aspects stand out. The first thing that is noticeable is that in every dilution (Fig. 9) the potassium concentration of the blood is maintained above that of the external medium in which the crabs are placed except in 87% sea water where it is isotonic.
Table 3. Total Potassium, Calcium, Chloride, Sodium, and Magnesium Concentrations of 100% Sea Water as Compared to the Blood and Urine of Cancer borealis

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sea Water</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.74</td>
<td>2.00</td>
<td>1.96</td>
</tr>
<tr>
<td>Ca</td>
<td>1.90</td>
<td>1.52</td>
<td>2.16</td>
</tr>
<tr>
<td>Na</td>
<td>52.44</td>
<td>54.62</td>
<td>53.05</td>
</tr>
<tr>
<td>Mg</td>
<td>4.7</td>
<td>2.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Cl</td>
<td>70.0</td>
<td>67.0</td>
<td>63.0</td>
</tr>
</tbody>
</table>
Table 4. Total Potassium, Calcium, Chloride, Sodium, and Magnesium Concentrations of 87% Sea Water as Compared to the Blood and Urine of Cancer borealis

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sea Water</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.61</td>
<td>1.61</td>
<td>1.74</td>
</tr>
<tr>
<td>Ca</td>
<td>1.71</td>
<td>1.54</td>
<td>1.80</td>
</tr>
<tr>
<td>Na</td>
<td>46.79</td>
<td>41.74</td>
<td>51.75</td>
</tr>
<tr>
<td>Mg</td>
<td>4.0</td>
<td>2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Cl</td>
<td>61.6</td>
<td>46.0</td>
<td>57.0</td>
</tr>
</tbody>
</table>
Table 5. Total Potassium, Calcium, Chloride, Sodium, and Magnesium Concentrations of 75% Sea Water as Compared to the Blood and Urine of Cancer borealis

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sea Water</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.48</td>
<td>1.57</td>
<td>1.56</td>
</tr>
<tr>
<td>Ca</td>
<td>1.52</td>
<td>1.55</td>
<td>1.54</td>
</tr>
<tr>
<td>Na</td>
<td>41.57</td>
<td>41.74</td>
<td>40.88</td>
</tr>
<tr>
<td>Mg</td>
<td>3.5</td>
<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Cl</td>
<td>46.0</td>
<td>35.6</td>
<td>44.0</td>
</tr>
</tbody>
</table>
Table 6. Total Potassium, Calcium, Chloride, Sodium, and Magnesium Concentrations of 50% Sea Water as Compared to the Blood and Urine of Cancer borealis

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sea Water</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.03</td>
<td>1.36</td>
<td>1.15</td>
</tr>
<tr>
<td>Ca</td>
<td>.98</td>
<td>.99</td>
<td>.96</td>
</tr>
<tr>
<td>Na</td>
<td>27.00</td>
<td>31.31</td>
<td>29.22</td>
</tr>
<tr>
<td>Mg</td>
<td>2.3</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Cl</td>
<td>34.0</td>
<td>33.0</td>
<td>37.0</td>
</tr>
</tbody>
</table>
# Table 7. Total Potassium, Calcium, Chloride, Sodium and Magnesium Concentrations of 40% Sea Water as Compared to the Blood and Urine of Cancer borealis

<table>
<thead>
<tr>
<th>Ion</th>
<th>Sea Water</th>
<th>Blood</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>.77</td>
<td>1.06</td>
<td>.99</td>
</tr>
<tr>
<td>Ca</td>
<td>.76</td>
<td>.88</td>
<td>.95</td>
</tr>
<tr>
<td>Na</td>
<td>22.05</td>
<td>22.96</td>
<td>29.40</td>
</tr>
<tr>
<td>Mg</td>
<td>2.0</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Cl</td>
<td>27.0</td>
<td>23.0</td>
<td>31.5</td>
</tr>
</tbody>
</table>
Similarly, it will be noted that the potassium concentrations of the urine are in general hypotonic to the blood and hypertonic to the sea water. Secondly, the chloride concentrations (Fig. 10), although deviating slightly from the sea water, are maintained below the external medium in every dilution. The urine concentrations of this ion are, in general, hypotonic to the external medium with the exception of the concentration in 50% and 40% sea water, in which the urine level is hypertonic to the sea water. Thirdly (Fig. 11), the magnesium content of the blood is always lower in every dilution. Similarly, the urine concentration of magnesium is in all dilutions, except 50% sea water, hypertonic to the blood and in all sea water dilutions it is hypotonic to the external medium. Fourthly (Fig. 12), a remarkable feature observed is that the calcium concentration in the blood remains relatively constant, in 100%, 87%, and 75% sea water, and does not deviate more than 0.03 mEq/100 ml. in each concentration. However, a decrease in the blood calcium concentration occurs when the animals are placed in 50% and 40% sea water. The urine concentrations of calcium in the higher salinities (100% and 87%) are hypertonic to the blood and sea water and in 75%, 50% and 40% dilutions the urine concentrations are isotonic to the blood and sea water. The fifth factor which one notices is that the sodium concentrations (Fig. 13) of the blood are practically isotonic to 100%, 75%, and 40% sea water when the crabs are placed in these dilutions. On the other hand, in 87% sea water the
Fig. 9. Potassium Concentrations of Blood, Urine and Sea Water. Abscissa: concentration of medium expressed as percent sea water. Ordinate: concentration of potassium expressed as mEq/L. Results represent duplicate analyses from pooled samples of four animals.
Fig. 10. Chloride Concentrations of Blood, Urine and Sea Water. Abscissa: concentration of medium expressed as percent sea water. Ordinate: concentration of chloride expressed as mEq/L. Results represent duplicate analyses from pooled samples of four animals.
Sea Water

Blood

Urine

Percent Concentration of Sea Water

m^2/l
Fig. 11. Magnesium Concentrations of Blood, Urine and Sea Water. Abscissa: concentration of medium expressed as percent sea water. Ordinate: concentration of magnesium expressed as mEq/L. Results represent duplicate analyses from pooled samples of four animals.
Fig. 12. Calcium Concentrations of Blood, Urine and Sea Water. Abscissa: concentration of medium expressed as percent sea water. Ordinate: concentration of calcium expressed as mEq/L. Results represent duplicate analyses from pooled samples of four animals.
Fig. 13. Sodium Concentrations of the Blood, Urine, and Sea Water. Abscissa: concentration of medium expressed as percent sea water. Ordinate: concentration of sodium expressed as mEq/L. Results represent duplicate analyses from pooled samples of four animals.
Percent Concentration of Sea Water
blood value is hypotonic to the external medium and in 50% sea water a slight hypertonicity is evident. In 100% and 75% sea water the urine values for sodium are isotonic to the external medium, whereas in 50% sea water the concentration is hypotonic to the blood and hypertonic to the external medium. In 40% sea water the urine concentration for sodium is hypertonic to both blood and sea water. The sixth factor which is evident from these results, is the high concentrations of ions in the urine of Cancer when the animals are placed in 40% sea water. This is especially evident for potassium, chloride, magnesium, and sodium.

The results of the ionic determinations indicate that considerable powers of ionic regulation exist in Cancer borealis in 100%, 87%, 75%, and 50% sea water. Although regulation of certain ions is observed in 40% sea water, the cellular mechanisms responsible for this regulatory ability are ostensibly inadequate for continued survival of these crabs in this medium.
DISCUSSION

One of the most salient features of physiological evolution in animals has been the development of a special internal body fluid, which is relatively well controlled. This feature has enabled many organisms to become quite independent of their surroundings. A brief review, therefore, of the mechanisms which are involved in the maintenance of a constant internal environment is necessary to the discussion of the problems investigated in this thesis.

The maintenance of the constancy of body fluids is a problem which is directly related to the remarkable properties of membranes because the selective permeability of these membranes has been one of the most important factors contributing to the maintenance of life (Pantin, 1931). As representative animals in question, the decapod crustaceans, with their tough exoskeletons, are structurally adapted to penetrate diluted waters. The relative impermeability of their exoskeleton limits the greater part of salt and water exchanges, which may go on between the internal and external environment, to places such as the gills and other soft parts of the body. Thus, if the animal penetrated diluted waters the rate at which water would enter the body fluids osmotically would be slow as far as the exoskeleton is concerned. However, this does not mean that the exoskeleton is completely impermeable to salts or water for Nagel (1934)
found that the exoskeleton of *Carcinus maenas* was permeable to sodium iodide. Gross (1957) compared the water permeability of exoskeletons of *Cambarus, Pachygrapsus, Hemigrapsus nudus, H. oregonensis, Cancer gracilis, C. antennarius,* and Pugettia. It was found, therefore, that *Cambarus, Pachygrapsus, Hemigrapsus nudus,* and *H. oregonensis,* which are all true "regulators," -- animals which can maintain a relatively constant internal environment with varying salinities -- had less permeable exoskeletons than *Cancer gracilis,* *Cancer antennarius* and Pugettia, the "non-regulators" (i.e. those animals not able to maintain a constant internal environment in the varying salinities). In this case, one sees that in the "regulators" the exoskeleton shows a passive permeability to water much more evident than in "non-regulators." The property of increased impermeability of membranes to water may aid an animal in penetrating other habitats. Margaria (1931) suggests that an active mechanism might be involved in reducing the permeability of animals to water as it penetrates dilute media. He claims that the impermeability in *Carcinus maenas* (and to some degree *Portunus puber*) is not a passive property of the membrane, for initially these animals appear to be quite permeable to water and salts when the external medium is altered. However, it is not inconceivable that both a passive property of increased impermeability of the exoskeletons of some crustacea to water, along with the property of active impermeability,
possibly by the gills, has enabled these animals to explore other habitats. It could be hypothesized that animals with less permeable exoskeletons would have a better chance of surviving in dilute media for the active processes of gill impermeability and the active excretion of water by the antennary glands and gut would be lessened under these conditions.

As an animal penetrates brackish waters from more concentrated sea water it is confronted with the problem of having its internal body fluids diluted by the external medium. The majority of marine organisms, on the other hand, do not attempt this migration and possess an internal osmotic pressure that is close to the external medium in which they live (Pantin, 1931). However, some marine animals show a divergence in the ionic composition of their blood. One sees a greater increase of potassium in the body fluids and a decrease of magnesium and sulfate. Cole (1940) reports that the lobster, Homarus americanus, shows a higher calcium content in the serum than in the surroundings in which it lives. The spider crab Maia squinado, similarly, shows an accumulation of potassium, calcium, and chloride against concentration gradients (Robertson, 1939). These animals although unable to survive in dilute sea water, apparently have mechanisms to absorb salts against concentration gradients. The work of Webb (1940) on Carcinus maenas, a true regulator, shows that in normal sea water the animal
concentrates sodium, potassium, calcium, and chloride. This crab must, as it penetrates dilute media, intensify the process of actively transporting salts if it is to survive in brackish water for, here, the salts are much less concentrated than they are in pure sea water. The phenomenon of active ion transport goes on in both regulators and non-regulators. In regulators the process of active transport is intensified whereas in non-regulators it breaks down in dilute media. The intensification of the process of actively transporting ions against a concentration gradient is, therefore, another mechanism by which animals are able to penetrate other environments.

The gills have been implicated as the site of this active transport, by eliminating other structures in these animals (Margaria, 1931; Nagel, 1934; Krogh, 1938). Gross (1957) produced direct evidence that the gills can maintain an osmotic gradient. This investigator found the mean salinity of the gill chambers of _Pachygrapsus crassipes_, which had been immersed in tap water, to be equivalent to 14% sea water while the blood was equivalent to 75% sea water. The mean salinity of the gill chambers of animals in 25% sea water was 64% sea water while the blood concentrations were estimated to be greater than 85% sea water. Because of the dynamic flux of salt and water exchange in the gill chamber of _Pachygrapsus_, there is further evidence that the gills could be osmoregulatory organs concerned in the transport of both salts and water.
The antennary glands of the decapod Crustacea have been assumed to have an osmoregulatory capacity. By excreting an hypotonic urine, an animal could maintain a blood concentration above that of the external medium. That this is the case in the crayfish, Potomobius, was demonstrated by Peters (1935). He showed that in the long nephridial canal, interposed between the labyrinth and the bladder, salts could be reabsorbed into the blood stream. By excreting an hypotonic urine with respect to the blood, the crayfish is well adapted for a fresh water existence. Urine excretion in Carcinus maenas, a crab with well marked osmoregulatory abilities, increases with a lowering of the salinity of the external medium. By plugging the excretory pores, Nagel (1934) found that these animals, when placed in dilute sea water, gain weight rapidly. This suggests that the antennary glands are very important in ridding the animal of water in dilute media.

When the antennary glands and the guts of Carcinus maenas were plugged it was found that the animal gained more weight in dilute sea water than when the excretory pores alone were plugged (Nagel, 1934). This would suggest that excess water in dilute media is also eliminated by the gut. Therefore, the guts of Crustacea, by eliminating excess water from the organism as it penetrates dilute sea water, would aid the animal in an osmoregulatory capacity.
Another means by which an animal could invade other environments is one in which no mechanism exists for the maintenance of a constant body fluid concentration within very narrow limits but one in which altered concentration of salts within the internal fluids apparently causes no harm to the organism. This can be called a phenomenon of tissue adaptation. In fact, Beadle (1940) claims that fresh water adaptation of marine animals could have evolved by a lowering of the blood concentration of salts with a consequent lessening of the gradient between the internal and external environment. The lower total concentration of salts in the body fluids of fresh water animals when compared to salt water forms, seems to support this view.

Shaw (1955), working with the electrolyte composition of the carpopodite and flexor muscles of Carcinus maenas when the animals were placed in dilutions of sea water, states that the extent to which the muscle cells can withstand blood dilution depends on the degree to which they can resist swelling. By resisting swelling, the muscle is actually resisting an inflow of water. In so doing, the muscle tissue can become slowly acclimated to dilute solutions and survive. One more example of this phenomenon is worthy of mention. Wells and Ladingham (1940) have shown with isolated muscle preparations of the polychaete worms Perinereis cultivata and Arenicola marina, that if these animals are exposed to dilutions of the external medium slowly, their muscle tissue
apparently functions normally. On the other hand, if the rate of the dilution of the external medium is rapid, the muscle preparations lose the ability to contract. Thus, with a gradual dilution of the external medium, the tissues of these worms can be acclimated slowly to dilute sea water. Both these animals are normally strictly marine without any known osmoregulatory mechanisms but can withstand considerable dilutions of the external medium.

Here one sees another means by which animals can continue to invade other environments, namely, by the tissue tolerance to various salinities of sea water and tissue adaptation to such changes.

Attention will now be focused on the osmotic and ionic regulatory mechanisms which might enable Cancer borealis, the crab used in this investigation, to explore other habitats, other than open sea water.

It was suggested previously that Cancer borealis was relatively impermeable to water (Trevisani, 1956). The results of the weight changes of normal animals placed in 100%, 87%, 75%, 50%, and 40% sea water indicate that the crabs are permeable to water and possibly salts, for a trend exists in which one sees an increase in weight as the salinity of the external medium is lowered (Fig. 2). However, if the animal were only permeable to water one would expect much greater increases in weight as the salinity is lowered. Although Cancer borealis is permeable to water to
a certain degree, relatively small changes in weight of normal animals are revealed in the various dilutions of sea water. These changes in weight indicate that Cancer is not freely permeable to water and this restricted permeability to water would assist the animal, in an osmoregulatory manner, to penetrate other habitats.

When the weights of the antennary gland-plugged animals and normal animals are compared, it is seen, as was mentioned previously, that antennary gland-plugged animals gain more weight than normal animals in the same concentration of sea water. In fact, it is shown (Figs. 3, 4, 5, 6, and 7) that as the salinity is lowered to 50% sea water the difference in weight between antennary gland-plugged animals and normal animals increases. This suggests that urine production in Cancer borealis is increased as the external medium is lowered. Thus, the antennary gland plays an important role in ridding the animal of excess water which may enter the body fluids as the external environment becomes more dilute. This is also an explanation of the problem that arose in previous work when urine was being collected from the crabs that were placed in low dilutions of sea water. The difficulty of collecting urine in low salinities was probably due to the fact that these crabs were excreting it at such a rapid rate in low salinities that the bladder never became completely filled. Cytological evidence that
the antennary gland of *Cancer borealis* functions to rid the animal of excess water in dilute sea water has been reported by Beers (1958). His studies reveal that apical vacuoles in the labyrinth cells of the antennary glands of the animal increase in size and number as the salinity of the external medium is lowered. Furthermore, these apical vacuoles are extruded in a merocrine fashion. Weight determinations of the antennary gland-plugged animals in this investigation (Figs. 3, 4, 5, 6, and 7) coupled with Beers' (1958) findings give substantial evidence that the antennary glands of *Cancer borealis* definitely aid in ridding the animal of excess water and thus play an important osmoregulatory role in the animal.

Results of this investigation revealed that *Cancer* with its glands plugged gained more weight in 50% sea water than in 4.0% sea water. It was suggested that normal urine production was hindered in the 4.0% dilution because cellular mechanisms responsible for water elimination are drastically impaired. That this actually happens was shown by Beers (1958). When he subjected the same animal to 4.0% sea water it was observed that the pore border of the labyrinth epithelial cells remained partially broken down in this dilution, whereas the pore border is normally reconstituted in other salinities. He suggests that there is so much excess water to be eliminated in this dilution that there is no chance for the labyrinth border to be reconstituted. Because of this, there
is a high mortality rate among animals subjected to 40% sea water.

The gut is also important in ridding Cancer borealis of excess water (Figs. 3, 4, 5, 6, and 6). An increased tendency of water to be eliminated by the gut exists in the animal as the sea water is diluted to 50%. This suggests that cellular mechanisms in the gut of these crabs are also involved in ridding the latter of excess water, thus denoting another main route through which an osmoregulatory function is performed. Again in 40% sea water one observes that the weights of normal animals and gut-plugged animals overlap. This suggests that cellular mechanisms that are normally involved in water secretion are impaired in this dilution, as has been shown to be the case in the pore border of the labyrinth cells of the antennary gland (Beers, 1958).

Osmotic determinations on the body fluids of Cancer borealis show that in practically every dilution of sea water some osmo-regulatory powers exist for at least twenty-four hours. It is evident that this animal maintains an hypotonic internal environment in 100% and 87% sea water and an hypotonic or isotonic internal environment in 75% sea water. However, in 50% and 40% sea water one observes that the body fluids are hypertonic to the external medium. The degree of hyperosmotic regulation of the internal environment of Cancer in 50% and 40% sea water is not as high as that of good regulators such as Carcinus maenas. However, a low degree of
hyperosmotic regulation is not unknown for other Crustacea for Jones (1941) showed that *Rithropanobius harrisi*, another crab, had a low degree of hyperosmotic regulation. *Rithropanobius* differs from *Cancer* in that it survives in very dilute media with slight osmoregulation. *Cancer*, on the other hand, cannot tolerate much more than a 40% dilution of the external medium. Apparently its limited powers of osmoregulation in this dilution of sea water are insufficient for survival of the animal. The tissues, as has been pointed out previously, cannot tolerate this dilution.

Osmotic determinations on the urine of *Cancer borealis* (Table 2) reveals that the animal excretes a slightly hypertonic urine with respect to the blood in 100%, 87%, and 75% sea water. Although there are trends indicating that the antennary gland excretes an hypertonic urine in concentrated sea water and a hypotonic urine in the lower salinities, it does not seem as though these trends would play a significant role in the osmotic regulation of this animal. It was noted, for example, that the antennary glands increase urine production in the lower salinities of sea water. But along with increased urine production, the urine concentration of salts is increased (Table 2). Thus, *Cancer* has a mechanism for getting rid of excess water but no efficient mechanism apparently exists for conserving salts in the lower dilutions. This may be due to the fact that the pore border of the labyrinth cells fail to undergo reconstitution, thus remaining partially broken down (Beers, 1958).
Under such conditions membrane mechanisms which normally function in retention of salts may be so drastically impaired that salt loss, primarily, could account for the death of the animals at low salinities. These results are similar to those of Botazzi (1897) for *Carcinus maenas* and to Prosser and Chow (1957) for *Pachygrapsus crassipes*. Unlike the kidney in man, which tends to conserve salts as its initial mode of action as the body fluids tend to become diluted, the poor regulators of the decapod Crustacea increase urine production but along with this, an increase in salt loss is an inevitable result.

Although *Cancer borealis* is able actively to concentrate and conserve certain ions for at least twenty-four hours, the concentration of some of these ions is maintained only slightly above that of the external environment in all salinities.

Active concentration, possibly by the gills, to conserve potassium, and to a slight extent sodium, exist in 100% sea water (Table 3). Calcium, magnesium, and chloride enter the body fluids passively. Active excretion of calcium, and magnesium by the antennary gland occurs in this salinity while potassium, sodium and chloride are passively excreted.

All the ions are passively absorbed in 87% sea water (Table 4) and active excretion by the antennary gland of every ion seems to exist.
In 75% sea water (Table 5) active absorption of potassium and to some extent calcium occurs. Chloride, sodium and magnesium are passively brought into the body fluids. Potassium, calcium, and sodium seem to be excreted passively by the antennary gland while chloride and magnesium are actively excreted.

The ionic analyses of animals placed in 50% sea water (Table 6) reveal that potassium and sodium are actively absorbed against concentration gradients while calcium, chloride, and magnesium probably enter the body fluids passively. Active excretion of chloride by the antennary gland is present but all other ions seem to be eliminated passively.

In 40% sea water again potassium is actively absorbed along with calcium and sodium (Table 7). Magnesium and chloride enter the body fluids passively. It is of interest to note that the antennary gland in this dilution actively excretes calcium, sodium, magnesium and chloride while potassium is passively excreted.

Several aspects of ionic regulation in *Cancer borealis* are worthy of further explanation. It was observed that in 100%, 87%, and 75% sea water the calcium concentration of the internal medium did not deviate more than 0.03 mEq/100 ml. However, in 50% and 40% sea water the value dropped considerably. One may hypothesize that since calcium has long been known to be an essential ion in maintaining the integrity of
cell membranes, the lack of sufficient quantities of this ion in the lower dilutions of sea water could result in the death of Cancer borealis, due to the breakdown of certain cell membranes. In 40% sea water, for instance, ionic analyses suggest an active excretion of calcium by the antennary gland. In this dilution, however, four out of the five ions analyzed would appear to be actively excreted. It is possible that this apparent active excretion is a result of the lack of a sufficient concentration of calcium for the maintenance of the cell membranes. As a result, therefore, a "wholesale" loss of ions by cells occurs in the antennary gland and possibly cells of the gut and other tissues over the body. As evidence to support at least part of this view, the work of Beers (1958) indicates that the apical border of the labyrinth cells of the antennary gland remains partially broken down in this dilution.

The chloride concentration of the blood is maintained below that of the external medium in every concentration of sea water (Fig. 10). The urine concentrations of these ions in 100%, 87%, and 75% sea water would indicate that the antennary glands are not the sole mechanism involved in maintaining the chloride concentration of the blood below the external medium. Urine analyses reveal that the concentration of chloride in the urine is also below that of the external medium. This suggests that some mechanism other
than the antennary gland might be involved in this phenomenon. Since the animal is permeable to chloride, some sort of "active pump" mechanism could be involved in maintaining a chloride gradient outside the animal which is greater than that found in the body fluids. This mechanism, which possibly occurs in the cells of the gills, would actively rid the animals of the chloride so that less of the anion would be located in the internal body fluids than outside the animal. That such mechanisms occur in the gills of fish and other tissues has been suggested by many investigators. The "sodium pump" mechanism is well substantiated for muscle and nerve. Here one sees that the cells are actually permeable to sodium but the cell membrane helps maintain a concentration gradient whereby sodium is actually pumped out of the cell faster than it can enter. Although the true manner in which the pump phenomenon actually works is still highly theoretical, the possibilities that such mechanisms also exist in the epithelial tissues of the gills and perhaps other tissues of Cancer borealis should not be excluded.

One more fascinating observation about the chloride ion is evident when one looks at the concentration of the anion in the urine of Cancer placed in 50% and 40% sea water. Here the urine concentration of this ion is hypertonic to the blood and sea water. Since the crabs have the problem of salt retention in dilute sea water, it is possible that this
active pump mechanism for pumping chloride out of the body ceases to function at low dilutions so that the chloride ion would enter the blood freely. When so doing, the burden of the chloride excretion rests on the antennary gland. The results of this investigation indicate that this might be the case because the concentration of chloride found in the urine of this animal in both 50% and 40% sea water is hypertonic to the sea water and the blood.

Another factor which should be mentioned concerning the ion analyses is the high concentration of ions found in the urine of *Cancer* in 40% sea water. It is highly probable that the breakdown of the labyrinth cells of the antennary gland in this dilution, due to the tremendous amounts of water it must excrete (Beers, 1958), causes an outpouring of ions from the blood into the urine resulting in the animal's death.

It is evident that the ionic regulatory abilities of *Cancer borealis* are sufficient to enable the animal to survive in dilute solutions of sea water down to 50% for 24 hours. Apparently these mechanisms are insufficient or break down in 40% sea water, resulting in the death of the animal.

Another aspect of the ability of *Cancer borealis* to tolerate fluctuations of the external medium is one of tissue adaptation. This animal shows limited powers of
osmotic regulation (Table 2 and Figure 8) but yet it survives well in 100%, 87%, 75%, and 50% sea water. Fluctuations of the various ions in the body fluids of this animal also exist (Tables 3, 4, 5, 6, and 7). This probably means that the tissues of these animals can be adapted to changes in the external medium because of the slow penetration of water through the thick exoskeleton and thus tissue adaptation to the dilutions would enable the animal to survive in various media. However, in 40% sea water the rate of penetration might be so rapid that death occurs before the animals' tissues can become adapted to the changes.

The results of this investigation seem to explain some of the problems which arose from previous work on the osmotic and ionic regulatory phenomena that exist in the crab, *Cancer borealis*. It seems that: the relative impermeability of this animal to water, the action of the antennary gland and gut in ridding the animal of excess water in dilute media, the active absorption of salts (possibly through the gills) from the external medium for at least twenty-four hours, and the adaptation of the tissues of the animal to changes in the body fluids without any observable injury, all aid in enabling this decapod crustacean to penetrate other environments. The impairment or breakdown of some of these mechanisms, however, limits the type of habitat to which *Cancer* can be exposed.
SUMMARY

1. _Cancer borealis_ shows a trend to gain weight slightly as the salinity of the external environment is decreased from 100% to 40% sea water.

2. Antennary gland-plugged animals gain more weight than normal animals subjected to the same salinity of sea water for the same number of hours.

3. Gut-plugged crabs gain more weight than normal animals in the same dilution of sea water but this gain is less than that of the antennary gland-plugged animals.

4. _Cancer borealis_ shows limited powers of osmotic regulation in 100%, 87%, 75%, 50%, and 40% sea water for at least twenty-four hours. It maintains a hypotonic internal environment in 100% and 87% sea water and a hypotonic or isotonic internal environment in 75% sea water. In 50% and 40% sea water the body fluids are hypertonic to the sea water.

5. Ionic regulation also exists in _Cancer_ for at least twenty-four hours in 100%, 87%, 75%, 50% and 40% sea water.

6. Active concentration, possibly by the gills to conserve potassium and to a slight extent sodium, exists in 100% sea water. Calcium, magnesium, and chloride enter the body fluids passively. Active excretion of calcium and magnesium by the antennary gland occurs in this salinity while potassium, sodium, and chloride are passively excreted.
7. Potassium, sodium, calcium, magnesium and chloride are passively absorbed in 87% sea water and active excretion by the antennary gland of every ion exists.

8. In 75% sea water active absorption of potassium and to some extent calcium occurs. Chloride, sodium and magnesium are passively brought into the body fluids. Potassium, calcium, and sodium seem to be excreted passively by the antennary gland while chloride and magnesium are actively absorbed.

9. When the animals are placed in 50% sea water potassium and sodium are actively absorbed against concentration gradients while calcium, chloride and magnesium enter the body fluids passively. Active excretion of chloride by the antennary gland is present but all other ions seem to be eliminated passively.

10. In 40% sea water potassium is actively absorbed along with calcium and sodium. Magnesium and chloride enter the body fluids passively. The antennary gland in this dilution actively excretes calcium, sodium, magnesium, and chloride while potassium is passively excreted.

11. There seems to be at least five physiological mechanisms involved in the osmotic and ionic regulation of Cancer borealis. These are:

   a) The relative impermeability of this animal to water.
   
   b) Increased water excretion by the antennary gland to rid the animal of excess water as the external medium is lowered.
c) The action of the gut to rid the animal of excess water as the external environment becomes more dilute.

d) Active absorption of salts, probably across the gill surface.

e) The adaptation of the tissues of Cancer to changes in body fluids without any observable injury.

12. The impairment or breakdown of some of these mechanisms in low dilutions of sea water which causes the death of this animal is also discussed.


