THE EFFECT OF SALINITY CHANGES ON THE WATER CONTENT AND RESPIRATION OF MARINE INVERTEBRATES

BY L. C. BEADLE

(From the Zoological Dept., Birmingham University.)

(Received 23rd August, 1930.)

(With Six Text-figures.)

INTRODUCTION.

The majority of marine invertebrates are incapable of withstanding more than very small changes in salinity of the sea water. A number of workers have found that, as the salinity and consequently the osmotic pressure of the water is reduced by dilution, these animals progressively gain in weight; and freezing-point determinations have shown that the osmotic pressure of the body fluids follows very closely that of the surrounding medium. These so-called "poikilosmotic" animals are therefore incapable of resisting the osmotic inflow of water and so of maintaining a constant internal osmotic pressure under these conditions, any large departure from the normal value of which will result in death (Frédéricq, 1904; Henri and Lalou, 1904; Höber, 1926, pp. 336-342). A few, however, are partially "homoiosmotic"; they are to a greater or less extent capable of maintaining the osmotic pressure of their body fluids higher than that of the surrounding water when this is diluted (Duval, 1925; Schlieper, 1929 a, b). The latter, therefore, possess some mechanism which the former do not, whereby this difference of osmotic pressure is maintained. The effectiveness of this mechanism varies with different animals. It is, for instance, more effective in Carcinus moenas than in Nereis diversicolor, both of which are partially homoiosmotic (Schlieper, 1929 a).

Of the nature of this mechanism very little is known but, since marine animals could never have invaded brackish and fresh waters without some means of maintaining their body fluids hypertonic to the surrounding water, its elucidation is of great importance for the study both of the distribution of marine animals and of the evolution of the fresh water from marine forms. These homoiosmotic marine invertebrates do not necessarily represent (physiologically) a stage in the evolution of fresh-water invertebrates, but they are obviously worth investigating from this point of view.

To maintain a difference of osmotic pressure between the body fluids and the surrounding medium, energy must be expended. This energy must originate in some chemical reaction, and it might be expected that oxygen would be concerned.
The main object of the following experiments was to bring more evidence to bear upon the question of the effect of low salinity on the respiration of these two classes of marine invertebrates.

Upon evidence which, for reasons stated below, is not quite conclusive, Schlieper (1929 a) has formulated the theory that the homoiosmotic forms respire more rapidly in dilute than in normal sea water, while this is not the case with the poikilosmotic forms. From this he suggested that the energy expended by the former in resisting the osmotic inflow of water is derived from some oxidative mechanism. By freezing-point determinations Schlieper showed that *C. moenas* and *N. diversicolor* are partially homoiosmotic, and that *Mya arenaria* is poikilosmotic. He then measured their respiration rate in sea water and in dilute water by placing them in closed bottles of water and estimating, by Winkler's method, the amount of oxygen consumed in a given time. Now, although he took certain precautions to ensure that the animals were kept motionless, such as darkening the bottles and leaving the animals in them some time before starting the experiments in order to accustom them to their new surroundings, it does not seem that he eliminated all possibility of increased movement in the more dilute water. He admits that very small stimulation of *Carcinus* will cause a rise in respiratory rate of anything from 10 per cent. to 50 per cent. Yet the greatest rise which he ever found with *Carcinus* was 50 per cent. and with *Nereis* 17-6 per cent. on transference from water of one salinity to that of a lower salinity. There were also large individual differences in the amount by which the respiration of *C. moenas* and *N. diversicolor* was increased in dilute water; in one or two cases it was even decreased. It would obviously be important to determine whether these individual differences were correlated with any differences in the water uptake.

To surmount these objections the Barcroft manometer was used in the present investigation for measuring respiration rate. This gives a continuous record over a given period, and it is possible to make parallel measurements of water uptake with the same animal. Single animals were placed in the manometer bottle at intervals and the weight, as an index of the amount of water taken up, was determined immediately after each measurement of respiratory rate. While in the bottles they were immersed in water of the required salinity, which contained a standard strength of narcotic to eliminate all possibility of increased movement.

The animals used in these experiments were two species of *Nereis*, *N. diversicolor* and *N. cultrifera*. As will be shown later, in water of a given salinity, the former remains active and takes up much less water after 24 hours than the latter, which becomes much swollen and sluggish. These two forms, therefore, should afford a profitable comparison.

The other animal investigated was *Gunda ultvae*, the marine triclad which is found under stones in places where fresh-water streams flow into the sea, and where, owing to the tides, it is every day subjected to very great changes of salinity (Pantin, 1931 a), from that of sea water to that of the stream water. It has previously been shown that it can live indefinitely in a mixture of 10 per cent. sea water and 90 per cent. distilled water (Jordan Lloyd, 1914). In this case, owing to
the small size of the animal, the weight cannot be used as an index of the water uptake, but the changes of volume were measured.

The problem was also attacked from another standpoint. If oxygen consumption is necessary for the maintenance of this resistance to the inflow of water at low salinities, then the resistance should break down if the respiration of the animal is paralysed by cyanide or if it is deprived of oxygen. Both these possibilities were tested.

METHODS.

(1) Narcotic. The standard narcotic used in the manometer bottles was 1 part by volume of an aqueous solution of chloretone saturated at room temperature (it has a very low solubility) in 4 parts of the water of the required salinity, i.e. water of one-quarter the salinity of sea water would be made up as follows:

1 part saturated solution chloretone,
2 parts distilled water,
1 part sea water.

For narcotisation in sea water a mixture of 1 part saturated solution chloretone in sea water and 3 parts sea water was used.

(2) Weighing. Before each weighing of Nereis the animal was placed on blotting paper for a few moments to remove water from the surface. Weighing was done to the nearest 0.01 gm.

(3) Volume measurements. With Gunda these were done by a modification of the method used by Weil and Pantin (1931). The animal was compressed between two microscope slides, kept at a constant distance apart by two coverslips fastened by a layer of wax to the lower slide. It was then drawn in outline by means of a microscope with a 1-inch objective and a camera lucida. The area enclosed by this outline was measured by means of a planimeter. The volume of the Gunda is therefore proportional to the area of the drawing, and thus changes of volume can be estimated. It was necessary to narcotise the animals for this purpose and the same standard chloretone used for the respiration measurements was employed.

(4) Cyanide. In all cases M/1000 KCN was used. In a mixture of 25 per cent. sea water and 75 per cent. distilled water this was very alkaline (pH over 9·5). The pH was adjusted to 8·2 by addition of HCl. The animals subjected to cyanide were placed in glass covered dishes containing about 100 c.c. of liquid in the case of Nereis and about 10 c.c. in the case of Gunda.

(5) Oxygen-free water. This was prepared by the method used by Pantin for Amoeba, in which the last traces of oxygen are removed from boiled water by treatment with alkaline sodium hydrosulphite, using methylene blue as indicator (Pantin, 1930).
EXPERIMENTS WITH _N. DIVERSICOLOR_ AND _N. CULTRIFERA._

(a) _Weight changes and respiratory rate._

In the first series of experiments a mixture of 25 per cent. sea water and 75 per cent. distilled water was used. The rate of respiration was determined first in sea water. In Fig. 1 the weight and the respiratory rate (expressed as cubic mm. of oxygen per gm. original weight per hour) are represented at 0 hours. The animals were then transferred to dilute water immediately after recovery from the narcotic, and the weight and respiratory rate were determined at intervals. The times given in Fig. 1 indicate the number of hours after transfer to dilute sea water. Many experiments had to be abandoned owing to the fact that the worms began to shed eggs after a certain time.

The results of a typical experiment on one _N. diversicolor_ are given in Fig. 1, A. The weight rose rapidly and reached a maximum (1.75 times the original) after about 24 hours and then gradually fell towards a value about 1.4 times the original. In all cases the animals became slightly less active when the weight was at the maximum, but regained their normal activity towards the end of the experiment. The water was changed every 12 hours. The respiratory rate increased on the average to a value about twice that in sea water, after which the rate on the whole tended to fall gradually towards the original value (Fig. 1, B).

In one case out of four the respiratory rate rose only very slightly, and after 60 hours was actually lower than the original. This was never found with any other _N. diversicolor_. This worm, however, was very transparent and contained no eggs in the body cavity, the weight of living matter must therefore have been considerably less (proportionally) than in the other worms, the body cavities of which were full of eggs or sperm. It would consequently be expected that there would be a smaller rise of respiratory rate.

It was found that, on the whole, the longer the weight takes to settle down to the final comparatively steady value, the longer it is before the respiratory rate begins to fall.

There are also in Fig. 1 (C, D) curves for weight and respiratory rate from a worm kept constantly in sea water. Here there is comparative constancy of weight and respiratory rate, although both show a slight steady rise.

Fig. 1, E, F, shows the effect of the same conditions on _N. cultrifera_. The figures are again taken from one experiment; two others gave similar results. The weight here increased more rapidly and to a higher value than in the case of _N. diversicolor_ (2.3–2.4 times the original), and there was no subsequent decrease, in fact the weight continued to rise slightly to the end of the experiment. After the first 24 hours the worms appeared very expanded and were incapable of any but very slight movements, but death did not occur even after about 94 hours. Blood could always be seen moving through the dorsal blood vessel, and such worms recovered in normal sea water. In two of the three cases the respiratory rate was increased in the first 24 hours, but the higher of the two maxima was about equal to the lowest
maximum in the experiments on *N. diversicolor*. In a third case the respiratory rate remained constant for the first 30 hours and then rose slightly.

It appears, therefore, that in water of this salinity the respiratory rate of both species is raised, but to a greater extent on the average in *N. diversicolor* than in *N. cultrifera*; and from weight measurements it is obvious that the former is resisting the inflow of water more than the latter.

![Graph showing respiratory rate and weight changes over time for *Nereis diversicolor* and *N. cultrifera*.](image)

Now, since *N. cultrifera* does not actually die under these conditions, it is possible that the rise recorded in its respiratory rate indicates that it also possesses a similar mechanism by which a difference of osmotic pressure is maintained but which is less effective than that of *N. diversicolor*, although sufficient to prevent death in 25 per cent. sea water. Thus it is conceivable that subjection to a still lower salinity might furnish conditions in which *N. cultrifera* could not survive but
which *N. diversicolor* could still withstand, and in this case there should be a greater disparity between the respiration curves of the two.

To test this the experiments were repeated, using a mixture of 16.6 per cent. sea water in distilled water. The results are recorded in Tables III and IV. Unfortunately, of a number of *N. diversicolor*, all except one shed eggs soon after transference to the dilute water. In this one case (Fig. 2, *A*, *B*) the weight rose in 20 hours to nearly 2.5 times the original, and subsequently settled down towards a value over twice the original. Both these values are considerably higher than those of *N. diversicolor* in the higher salinity. Here again the animal had regained its normal activity at the end. The changes of respiratory rate are of a similar type to those found with the higher salinity. The maximum is about equal to the highest value obtained in any of the former experiments.

A second case (Fig. 2, *C*, *D*) is an animal which shed eggs after 70 hours, before which the weight rose to the abnormally high value of 2.75, a phenomenon which
is perhaps associated with the shedding of eggs. The respiratory rate rose to an even higher value than in the previous case (Fig. 2, B) after 24 hours and then began slowly to decrease.

The results of one of three experiments on *N. cultrifera* are shown in Fig. 2, E, F. All died after about 50 hours in this salinity. The weights of these three animals rose to between 2.5 and 3 in this time, values much higher than those reached in the higher salinity. The respiratory rate shows an initial rise which is very small compared with that obtained with *N. diversicolor*, and there is a tendency to settle down to about the original value at the end of the experiment (48 hours).

![Graph](image_url)

**Fig. 3.** *N. diversicolor* in 25 per cent. sea water.

In this last experiment, therefore, a certain low salinity has been found in which *N. cultrifera* does not live more than 50 hours, while *N. diversicolor* may survive indefinitely. In addition to this there is a marked difference between the respiration curves of the two, that of *N. diversicolor* showing a far greater rise than that of *N. cultrifera*.

(b) *Cyanide and oxygen lack.*

The effect of these on the weight curve of *N. diversicolor* in 25 per cent. sea water in distilled water is shown in Fig. 3. The continuous lines indicate the length of time of treatment with cyanide or oxygen-free water and the dotted line the
recovery period in aerated water. Both treatments had a depressing effect on the activity of the animals, and it seemed inadvisable to continue the treatment longer. The animals, however, subsequently regained some of their former activity. If the cyanide and oxygen lack curves are compared with the normal weight curve in the same salinity (also Fig. 3), it will be seen that the weight rises to a considerably higher value in 45 hours in the treated than in the untreated animals. These curves, in fact, show an approach to the condition of the normal weight curves of *N. cultrifera* (Fig. 1). After removal of the cyanide or on introducing oxygen there is a tendency for the weight to decrease. The depressing effect of the cyanide upon the respiration of one of the animals is also shown in Fig. 3. After removal of the cyanide the rate rises, as would be expected, to a value above the original. Three experiments were done both with cyanide and oxygen-free water, and the curves in Fig. 3 are typical cases.

From a study of sections of *N. diversicolor* it appears that the water, on entering the animal, does not cause any visible swelling of the tissues, but merely increases the volume of the fluid in the body cavity.

**EXPERIMENTS WITH GUNDA ULVAE.**

*(a) Low salinity and respiratory rate.*

It was impossible to weigh these animals; the respiratory rate was therefore expressed as centimetres on the manometer scale per hour at N.T.P. For each experiment roughly the same number of worms were used, so that the initial rate was about the same in each case. Table I *(a)* and *(b)* represent two batches of animals which were subjected to progressive dilution of the sea water. The respiratory rate was determined 20 minutes after transfer to the water of the given salinity, after which time it had been found from previous experience that the maximum rate was attained.

<table>
<thead>
<tr>
<th>Distilled water (%)</th>
<th>Respiratory rate cm. per hour (manometer)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>(a)</em></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.83</td>
</tr>
<tr>
<td>16.6</td>
<td>3.15</td>
</tr>
<tr>
<td>33.3</td>
<td>3.35</td>
</tr>
<tr>
<td>50.0</td>
<td>4.0</td>
</tr>
<tr>
<td>66.6</td>
<td>5.20</td>
</tr>
<tr>
<td><em>(b)</em></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.8</td>
</tr>
<tr>
<td>50.0</td>
<td>4.0</td>
</tr>
<tr>
<td>90.0</td>
<td>6.6</td>
</tr>
<tr>
<td>95.0</td>
<td>6.65</td>
</tr>
<tr>
<td><em>(c)</em></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.95</td>
</tr>
<tr>
<td>75.0</td>
<td>5.30</td>
</tr>
<tr>
<td><em>(c)</em></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.46*</td>
</tr>
<tr>
<td>75.0</td>
<td>3.66*</td>
</tr>
</tbody>
</table>

* Narcotised with chloretone.
With decreasing salinity the respiratory rate is progressively increased until in a mixture containing 90 per cent. distilled water the rate is more than twice that in normal sea water.

This higher rate of respiration in water of a given low salinity was maintained for at least 17 hours, provided that the animals were put into a dish, and not left during the entire period in the manometer bottle, in which case the rate fell again after 15 hours to about the original value.

In the above experiments no narcotic was used, therefore the possibility of increased movement was not entirely eliminated, although there were no apparent differences in the amount of movement in waters of different salinities. A control experiment was performed by measuring the rates of respiration of a batch of Gunda in sea water and in water containing 75 per cent. distilled water (Table I (c)).

The worms were then returned to sea water, and after an interval of about 2 hours the same experiment was repeated, but with standard narcotic in the water (Table I (c')). The narcotic, therefore, depresses the respiration, but they still respire more rapidly in the dilute than in the normal sea water. It was also noteworthy that the depressing effect of the narcotic was greater in the water of lower salinity.

The above results are illustrated graphically in Fig. 4. In this figure the curve of ($p_1 - p_0$) is also shown, where $p_1$ is the initial osmotic pressure of the body fluids in atmospheres (assumed to be equal to that of natural sea water) and $p_0$ is that of the surrounding medium according to its salinity (calculated from tables given by Krümmel, 1907). The respiration curves are very roughly parallel to this, i.e. the increase in respiratory rate is roughly proportional to the initial difference of osmotic pressure inside and outside the animals. This might be expected if the energy obtained from the extra oxygen consumed in the dilute water were employed in doing work against this osmotic difference.
The effect of this osmotic work is to retard the natural accumulation of water within the animal which tends to take place in a medium of lowered osmotic pressure. In this the mechanism is far from being completely effective, for though it reduces the extent of the swelling it by no means abolishes it. Weil and Pantin (1931) have shown that the worms swell in dilute water, the extent of the swelling being the greater the lower the salinity.

Sections of *Gunda* are shown as sketches in Fig. 5. *A* is that of a normal worm and *B* that of a worm treated with dilute sea water containing 75 per cent. distilled water. It appears that none of the tissues (including the ectoderm) are visibly affected by the dilute water except the cells lining the gut, which become enormously swollen and vacuolated. This phenomenon was also noted by Jordan Lloyd (1914) in another connection. She describes how these vacuoles are increased in size by hypotonic and decreased by hypertonic sea water. Therefore, when *Gunda* swells in dilute sea water, the mechanism is probably seated in the gut cells.

That this swelling is probably due solely to the lowering of osmotic pressure on dilution, and not to any other possible effect resulting from the lowering of the concentration of some specific ion, was shown by the fact that when the animals were subjected to a solution of 50 per cent. sea water and 50 per cent. of a nonelectrolyte (glycerol) isotonic with sea water, the volume was practically unaltered. Thus in one case the relative volume when normal was 1, after half an hour in the solution 0.86, after 1½ hours 1, and after 2 hours 0.95.

Thus, far from any appreciable increase in volume, there is in some a tendency for the volume to decrease slightly, while experiments communicated to me by Mr Pantin have shown that in water containing 50 per cent. sea water and 50 per
cent. distilled water (and thus containing the same concentration of electrolytes as the above mixture, but of a lower osmotic pressure) *Gunda* swells in a little over an hour to a volume about 2.75 times the original.

**b) Oxygen lack and cyanide.**

If the increased oxygen consumption of *Gunda* in dilute sea water allows work to be done which retards the uptake of water, it would be expected that deprivation of oxygen would cause a breakdown of the mechanism by which the osmotic difference is maintained, and that animals in dilute sea water would increase in volume when respiration was inhibited.

Owing to the variation found in the maximum volume reached by different worms in water of the same salinity, it seemed necessary to devise experiments in such a way that the volumes attained by the same worm in normal dilute sea water and in the same water containing cyanide or free of oxygen could be compared. This was done by leaving the animals for 12 hours in water of the required salinity, in order that a steady volume should have been reached. (The maximum volume is probably reached in about 1 hour (Weil and Pantin, 1931).) The volume was then measured by the method described above, and after a period of treatment with cyanide or anaerobic conditions in the same dilute water, the volume was measured again. All volumes were expressed relative to the steady volume after 12 hours.

**Table II. Gunda ulvae.**

*A* and *B* represent two sets of experiments.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Time in hours</th>
<th>Relative volume</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>A</em></td>
<td><em>B</em></td>
</tr>
<tr>
<td>25 % sea water</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 % sea water</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>1.02</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>10 % sea water</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.15</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.08</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.18</td>
<td>1.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 % sea water</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.21</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.38</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>1.16</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>1.02</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Sea water</td>
<td>0</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.06</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.90</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>
The effect of oxygen lack is shown in Table II. The initial volume is represented at 0 hours. The effect of anaerobic conditions was investigated in three media: 10 per cent. sea water, 25 per cent. sea water, and undiluted sea water. It was first found that exposure to half an hour oxygen lack produced no appreciable effect upon the volume in any medium, but after an hour’s treatment there was a marked increase in volumes in the dilute waters.

The figures show, firstly, that the maximum volume reached after a given period of oxygen lack was greater in the more than in the less dilute water; secondly, that in the same water the longer the treatment the greater was the maximum volume; and thirdly, that after return to normal conditions the volume fell again to about the original value. In 10 per cent. sea water after return to aerobic conditions the volume at first continued to rise and did not begin to fall immediately, as was the case in 25 per cent. sea water.

In normal sea water after 1 hour’s anaerobic conditions, one animal increased slightly in volume and the other remained practically constant. Two worms, treated for 1½ hours, both decreased appreciably. The activity of the worms did not suffer very greatly from this treatment, though after anaerobic conditions for 1½ hours in dilute water they were slightly less active.

In order to show that the above volume changes were the result of the anaerobic conditions only, and were not due to any other possible effects of the chemicals introduced to produce those conditions, the experiments were repeated, but the oxygen-free water was first shaken with air so that the colour of the methylene blue returned. The relative volume was found to remain within 6 per cent. of the normal
Water Content of Marine Invertebrates

before treatment. It would appear then that the increase of volume in dilute water shown in Table II is due solely to lack of oxygen.

The results of the experiments given in Table II are what would be expected on the assumption that, in dilute water, *Gunda* is maintaining an osmotic difference between the body fluids and the surrounding water, for which oxygen is necessary, and which is broken down (reversibly) by a certain period of oxygen lack. It would be expected that in the more dilute water the volume would increase more than in the less dilute, and that the maximum would be greater the longer the treatment with oxygen-free water. It would also be expected that in normal sea water the volume would not be increased by lack of oxygen. The decrease in volume after 1½ hours' treatment in undiluted sea water is certainly unexpected, but perhaps it may indicate that the osmotic pressure of the body fluids of *Gunda* is normally slightly higher than that of the sea water—a fact which has been noted in a number of marine invertebrates (Monti, 1914; Botazzi, 1925, p. 518).

The occurrence of an initial lag period of half an hour, in which no appreciable effect is produced upon the volume, perhaps indicates that the worm during this period is setting up an oxygen debt, such as has been shown by Slater (1928) to be possible in some invertebrates.

The results of experiments, conducted in a similar manner, on the effect of *M*/1000 KCN are shown in Table III. The dilute water used here contained 10 per cent. of sea water, and the effect of cyanide in this and in normal sea water was investigated.

Table III. *Gunda ulvae.*

<table>
<thead>
<tr>
<th>Medium</th>
<th>Time in hours</th>
<th>Relative volume</th>
<th>M/1000 KCN present</th>
<th>KCN absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) 10 % sea water</td>
<td>0</td>
<td>1.00</td>
<td>1.00 KCN present</td>
<td>KCN absent</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>1.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.75</td>
<td>1.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.75</td>
<td>1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.75</td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.5</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) 10 % sea water</td>
<td>0</td>
<td>1.00</td>
<td>1.03 KCN present</td>
<td>KCN absent</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>1.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>1.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Sea water</td>
<td>0</td>
<td>1.00</td>
<td>1.00 KCN present</td>
<td>KCN absent</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>0.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>1.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There was again a lag period (20 minutes) during which no appreciable change of volume occurred, after which there were large increases in most of the animals in the dilute water: (a) is typical of five experiments. But in sea water, although the volume fluctuated considerably (in some cases decreasing), it approximated far more closely to the original value (c). There is here no such clear relation between

---

*Water Content of Marine Invertebrates*
the length of time of the cyanide treatment and the maximum volume reached as was found in the experiments on the effect of oxygen lack. But there is again a tendency towards recovery of the original volume on discontinuing the treatment. One animal (b), subjected to a longer period of cyanide than the others (1½ hours), continued to increase in volume after removal of the cyanide, until it began to disintegrate after reaching the high value of 1·6 times the original.

Experiments to determine the effect of M/1000 KCN upon the respiration of *Gunda* in 10 per cent. sea water and 90 per cent. distilled water, were done with a Barcroft manometer. It was found that about 30 minutes pass after addition of the KCN before the animals entirely cease absorbing oxygen. When it is remembered that there was a period of 20 minutes during which the cyanide did not effect the volume, it would seem that here we have evidence that the cyanide causes the increase of volume by virtue of its depressing effect on the respiration.

**DISCUSSION.**

Other work bearing on the effect of salinity changes upon respiration appears to be scarce. Tarussov (1927) found by carbon dioxide measurement with a modified Osterhout apparatus that the respiratory rate of *N. diversicolor* is increased in hypotonic and decreased in hypertonic sea water. He apparently attributed these results to the hydration and dehydration of the cell colloids. It is, however, difficult to see how hydration of these should cause an increase of respiration and vice versa, and it is also probable, as stated above, that the water does not enter the cells to any appreciable extent. Skujin (1927) showed, by estimation of chlorides, that mammalian blood corpuscles can, within certain limits, maintain a higher internal chloride content than that of the surrounding salt solution. He concluded that this was a case of "osmotic resistance," and showed that in presence of carbon dioxide or narcotics, or if the corpuscles were allowed to stand for some time, this resistance was to a great extent broken down. This he attributed to the depressing effect of these conditions on the uptake of oxygen which is necessary for maintenance of the resistance.

Straub (1929) has found that the freezing-point of the yolk of a living egg is 0·15° C. lower than that of the white. This corresponds to a difference in osmotic pressure of 1·8 atmospheres. He concludes that the membrane surrounding the yolk, being freely permeable to water, could not possibly support this pressure, and suggests that this difference of pressure on the two sides is maintained by an active process of oxidation occurring at the membrane surface. It would be interesting to try the effect of conditions depressing the oxygen uptake upon the power of maintaining this difference of pressure.

Jordan Lloyd (1915) found that the frog's sternocutaneous muscle initially gained weight when placed in hypotonic solutions of various substances, and that this could be suppressed by previous exposure to oxygen. It was suggested that this gain in weight was due to the production within the cells of substances of low
molecular weight under anaerobic conditions, which were removed on exposure to oxygen.

The question of the water uptake of muscle stimulated under anaerobic conditions has recently been investigated by Hill and Kupalov (1930), who have established beyond doubt by vapour pressure measurements that a great rise of internal osmotic pressure occurs under these conditions. It does not appear, however, that this would explain the increase of volume of *Gunda* in oxygen-free water, since, if the internal osmotic pressure were increased under these conditions, an increase of volume in normal sea water would be expected, which in fact does not occur.

The experiments described above afford reasonably strong support to Schlieper's hypothesis. There seems no doubt that the respiratory rate of *N. diversicolor* and *G. ulvae* is increased in dilute water progressively with lowering of the salinity, and that this effect is not as marked in *N. cultrifera* which cannot prevent the osmotic inflow of water at low salinities to the same extent as can the two former. The action of cyanide and of anaerobic conditions on the water content of *N. diversicolor* and *Gunda* would seem to support Schlieper's second postulate that it is by virtue of this extra oxygen uptake that the difference of osmotic pressure between the water and the body fluids is maintained. It must be remembered, however, that, if this is true, the mechanism whereby the oxygen is used for this purpose in *N. diversicolor* must be of a slightly different nature from that in *Gunda*. With *N. diversicolor* the respiratory rate rose comparatively rapidly to a maximum on transference to dilute water and immediately began to fall again, while with *Gunda* the respiratory rate increased very rapidly to the maximum and then remained constant for some time (perhaps indefinitely). In addition to this the water taken up by the former apparently mainly enters the body cavity and does not cause increase of volume of the cells, while in the latter an increase of volume occurs only in the gut cells.

The subsequent fall in both weight and respiratory rate after reaching a maximum in *N. diversicolor* may possibly be explained on the assumption that the surfaces separating the water from the body fluids, previously permeable to water only, after a given period of exposure to dilute water become to a certain extent permeable to salts, which therefore leak out. Thus the osmotic pressure of the body fluids would be reduced and some of the water would pass out again. The result would be a loss of weight and a decrease of the respiratory rate due to the fact that there would now be a smaller difference between the internal and external osmotic pressures. Whether salt leakage actually occurs, and if so, whether it starts when the weight begins to fall, could be determined. Pantin (1931 b) has shown by measurement of the conductivity of the water that, in distilled, tap and river water there is a continuous leakage of electrolytes from *Gunda*, and that this occurs to a lesser extent in waters containing a higher proportion of calcium. Whether sufficient salt leakage could occur from *N. diversicolor* in water containing 25 per cent. sea water (a very high salinity compared with that used by Pantin in his experiments) to account for the observed great decrease in weight after 24 hours seems questionable. If leakage does occur, it is in no way harmful to the animals, in fact they appear to
be more active after they have passed the maximum weight and least active when
they weigh most. This decrease of activity may, of course, be merely the mechanical
effect resulting from a swollen body cavity. Why should there then be no subsequent
decrease of weight in _N. cultrifera_? There seems no obvious reason why there
should be less salt leakage in the latter, in fact it might almost be expected that
there would be more. Experiments to decide these points should be done next.

Schlieper (1929 a) adopts a rather rigid classification of marine invertebrates
into “homoiosmotic” and “poikilosmotic” forms. But the above experiments
show that no such definite distinction can be made. _N. cultrifera_, an animal which
in its normal environment never encounters appreciable changes of salinity, ap-
parently possesses the same mechanism, though in a lesser degree, by which _N. di-
versicolor_ is able to withstand a great lowering in salinity of the sea water. It would
appear more probable, then, that this mechanism is a fundamental property of
living cells, which has become more highly developed in certain forms. These are
thereby enabled to survive the great salinity changes which occur where fresh
waters join the sea.

**SUMMARY.**

1. Schlieper’s theory of the function of increased oxygen intake by “homo-
iosmotic” marine invertebrates in dilute sea water in maintaining their body fluids
hypertonic to the surrounding water is discussed, and objections are brought
forward to the methods used in the experiments on which his conclusions were
based.

2. By periodic weighings, and measurements of respiratory rate (under narcotic)
by Barcroft manometers, it was found that the weight of _N. diversicolor_, on trans-
ferral to water of low salinity, at first increases and then falls, and that the
respiratory rate is at first increased and later tends to decrease.

3. With _N. cultrifera_ the weight increases to a higher value and does not sub-
sequently fall, and the respiratory rate is also increased but to a lesser extent than
with _N. diversicolor_.

4. These differences in the amount of increase in respiratory rate are more
marked in water containing only 16·6 per cent. sea water than in water containing
25 per cent. sea water.

5. _N. diversicolor_ maintains its activity while _N. cultifera_ becomes practically
inert in dilute water. The latter does not actually die in 25 per cent. sea water after
100 hours, but dies in 16·6 per cent. sea water after about 50 hours.

6. Exposure to _M/1000_ KCN or to anaerobic conditions in dilute water tends
to break down the mechanism by which the free osmotic inflow of water in _N. di-
versicolor_ is prevented, and the weight curves under these conditions approach the
_N. cultrifera_ form.

7. The respiratory rate of _G. ulvae_ increases progressively with dilution of the
sea water, and is roughly proportional to the initial difference of osmotic pressure
inside and outside the animal.
Water Content of Marine Invertebrates

8. The swelling of Gunda in dilute water is due to swelling of the gut cells, which become much vacuolated. The other tissues appear unaltered.

9. M/1000 KCN or anaerobic conditions cause a greater amount of swelling in Gunda in a given salinity than normally occurs.

10. These experiments seem to give reasonably good support to Schlieper's hypothesis.

11. The mechanism responsible for this "osmotic resistance" in N. diversicolor must be of a somewhat different nature from that in G. ulvae.

12. A rigid distinction between "homoiosmotic" and "poikilosmotic" marine animals cannot be supported.

I am much indebted to Prof. H. Munro Fox and to Mr C. F. A. Pantin for continual help and suggestions.

REFERENCES.


