Part III

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Chapter I

Respiration of Petrelithes in relation to Environmental Conditions

All animals need oxygen to liberate energy for life processes and the quantity of oxygen and carbon dioxide involved in respiration depend on the metabolic substrate, its end products and the metabolic rate. The metabolic rate of any animal depends upon (1) species and tissues, (2) size of the animal, (3) activity, (4) temperature, (5) osmotic pressure and (6) oxygen concentration in the medium. The first three can be regarded as internal factors and the remaining as external factors.

Krogh (1916) studied the influence of temperature on the oxygen consumption of different animals. Further investigation on respiration in relation to temperature was done by Marshall, Michells and Orr (1935), Fox (1936), Edwards and Irving (1943) and Edwards (1946). Schloander et al (1953) have surveyed the oxygen consumption of a number of arctic and tropical cold-blooded animals at different temperatures. Gopalakrishnan (1957) studied the oxygen consumption in *Metapenaeus monoceros* in relation to environmental conditions. The respiratory metabolism of the crabs, *Hemigrapsus oregonensis* and *H. nudus* was studied.

The change in salinity of the external medium affects the oxygen consumption inversely. This was first shown by Schleper (1930) followed by Krog (1939) and Hopkins (1949). Kreps (1929) showed that the rate of oxygen consumption of *Balanus crenatus* remained unaffected between 12% to 30% salinity but decreased considerably after that. In hypotonic media, the rate of oxygen consumption of *Carcinus maenas* was increased (Schwabe, 1933). Similar changes were reported by Flemister and Flemister (1951) in *Ocydode albicans*.

Another external factor which affects the oxygen consumption is pH of the external medium. Little study has been made on the effects of pH on the oxygen consumption of crustaceans even though Powers (1922, 1930, 1932) studied this effect on the fishes. Helft (1928) recorded no relationship between pH and oxygen consumption in the crayfish, *Cambarus immunis*. Marshall et al (1935) observed that the effect of pH changes between 8.08 and
7.40 was negligible in *Galenus fimmaricus*. Gopalkrishnan (1957) showed the relationship of pH and oxygen consumption of *Metapenaeus monoceros*. Sarojini (1966) studied the same in *Diogenes bicristimanus*.

The effect of oxygen tension of the external medium on the respiration was variable in invertebrates according to Kregg (1916), Hiestand (1931), Marshall et al (1935). Wiens and Armitage (1961) studied the relationship between oxygen concentration and respiration in *Oropectes immunis* and *O. nasii*.

One of the internal factors affecting the oxygen consumption is the body size of the animal. The highest oxygen consumption and metabolic rate occurs in the small active forms. Zeuthen (1947) made the study of body size and metabolic rate in the animal kingdom. Ellenby (1951) investigated the relationship of oxygen consumption and body size in *Ligia oceanica*. Roberts (1957) studied the influence of body size on the metabolic rate in the crab, *Pachygrapsus crassipes*. Young (1963) recorded that the oxygen consumption decreased with size in *Pagurus*.

An increase in metabolic rate after the removal of eyestalk was recorded by Edwards (1950) in *Uca pugilator* and Bliss (1953) in *Gecarcmus lateralis*. But Scheer and
Scheer (1954) noticed no change in the rate of oxygen consumption after the ablation of the eyestalks from the prawn, *Leander serratus*. Fingerman (1955) recorded an increase in the metabolic rate after the removal of eyestalks.

The present study is aimed to investigate the effect of changes in temperature, salinity, pH, oxygen saturation, size and eyestalk ablation on the oxygen consumption of *Petrolisthes lamarckii*.

**MATERIAL AND METHODS**

*Petrolisthes lamarckii* were collected from Thana creek and were kept in the laboratory for two to three days so that they may adjust themselves to the new environment. The sea water was changed every day and the animals were not fed during the experimental period. The quantity of oxygen consumed was calculated in relation to unit wet weight of the animals and the values were expressed as rate of oxygen consumption in p.p.m. per gm. per hour. All the *Petrolisthes* were dried on the blotting paper to remove the excess of water and then weighed. The berried females were scrupulously excluded.

A constant flow apparatus similar to the one used by Gopalakrishnan (1957) was set up. The apparatus is
Plate I

Fig. 1 - General scheme of apparatus.

A, B = aspirator.

C = respiration chamber.

B = bath.

E = receiver jar.

F, K, I = thermometers.

H = overflow tube.

J, M = taps.

N = layer of liquid paraffin.
shown in Fig. 1. The test animals were kept in the respiration chamber and the water was circulated at a uniform rate through the respiration chamber. Tube 'H' does not allow the water in the second aspirator 'B' from rising above a certain level and as the first aspirator 'A' is at a higher level than 'B' and as the tube 'c' leading water from 'A' to 'B' is stouter than 'I', the water level in the aspirator 'B' is kept steady. This ensures a uniform speed for the flow of water through the respiration chamber as all the parts of the apparatus are clamped to fixed positions.

The rate of flow of water was calculated by measuring the volume of water collected in the receiver during a period of one hour. The experiment was run for four hours without interruption. Two samples were analysed after each hour by removing the receiver jar 'E' and substituting it by identical jar. A wide-mouthed thermos flask was used as the bath 'D' to maintain constant temperature of flowing water. The size of respiratory chamber was such that the test animals kept in it could do a minimum amount of movement.

Salinity was estimated by titrating with silver nitrate solution (Harvey, 1928). The pH of water samples
was measured by Heligö's comparator and estimation of dissolved oxygen by the standard Winkler's method. The pH of water was lowered by passing carbon dioxide into the water and increased by adding dilute sodium hydroxide solution (Marshall et al., 1935). The oxygen tension of the water was increased by oxygenating and lowered by passing successively washed hydrogen (Marshall et al., 1935).

**EXPERIMENTS AND RESULTS**

**Influence of temperature on oxygen consumption.**

Ten healthy animals were selected and were inserted into the respiratory chamber under sea water without causing any damage to the appendages. The sea water from the aspirators was allowed to run through the apparatus for fifteen minutes to remove any possible effects of handling the specimens and also to bring about the change in temperature of the running water. After taking the readings from the thermometers 'E' and 'T.' and adjusting the temperature of the bath the experiment was started with the collection of water in the receiver 'E'. The rate of flow of water through the apparatus was 50 ml/minute. Respiration was calculated at four different temperatures. After measuring the oxygen consumption at one temperature, the animals were
Fig. 2 - Respiration of *Petrolisthes* in relation to temperature.
Table 1

Oxygen consumption of *Petrolisthes* in relation to temperature.

Salinity of the medium = 30%

\[ \text{pH} = 7.2-7.5 \]

<table>
<thead>
<tr>
<th>Temperature of the experimental medium, °C</th>
<th>Oxygen consumption in p.p.m./gm. wet wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>0.125 ± 0.035</td>
</tr>
<tr>
<td>27</td>
<td>0.183 ± 0.024</td>
</tr>
<tr>
<td>33</td>
<td>0.246 ± 0.032</td>
</tr>
<tr>
<td>38</td>
<td>0.098 ± 0.014</td>
</tr>
</tbody>
</table>
transferred to the next temperature to study the respiratory rate.

The temperatures chosen for the study were 22°, 27°, 33° and 38° C. Petrolisthesia can tolerate the temperature from 22° C to 38° C without any thermal acclimation. The water in the bath was constantly watched and its temperature was adjusted within a narrow range. The pH of the inflow and outflow water did not vary much. Water samples were taken in duplicate to determine the oxygen contents. The results are presented in the Table 1.

The room temperature recorded at the time of the experiment was 27° C. The temperature of the outflowing water from the respiratory chamber was reduced or raised by adding ice cold water or hot water to the bath surrounding the respiratory chamber and allowing the temperature to vary through a range of 5° C. The experimental data, as presented in Table 1, show the average rate of oxygen consumption as 0.125 p.p.m. per gm. per hour at 22° C. At room temperature (27° C), the rate of oxygen consumption was raised to 0.183 p.p.m. per gm. per hour. While at 33° C it was raised to 0.246 p.p.m. per gm. per hour, as is shown in Fig. 2. But at 38° C there was an abrupt fall in oxygen consumption, being 0.098 p.p.m. per gm.
per hour.

The animals inside the respiration chamber did only slight movements and other factors like salinity, pH and oxygen content were all identical. Hence changes in the oxygen consumption were due to the effect of temperature.

The influence of temperature on the rate of oxygen consumption is represented graphically in Fig. 2.

Influence of salinity on oxygen consumption

The effect of salinity on the oxygen consumption of Petrolisthes was studied at five different salinities. Everytime ten healthy animals were put in the test salinity to measure the oxygen consumption, keeping the temperature and pH constant. After ascertaining the respiratory rate they were transferred to the next test salinity. The salinity of sea water was lowered by diluting it with distilled water.

The first experiment was done in a medium of 35% salinity, which is identical with the salinity of the environment. Then, the salinity was progressively reduced to 30%, 25%, 20% and 16% and the rate of oxygen consumption was recorded. The results are shown in Table 2. From the table it can be seen that the average rate of oxygen consumption of Petrolisthes was 0.070 p.p.m. per gm.
Table 2

Oxygen consumption of *Petrolisthes* in relation to salinity.

Temperature of the medium = 27° ± 1°C

pH = 7.2 - 7.6

<table>
<thead>
<tr>
<th>Salinity of the experimental medium %</th>
<th>Average oxygen consumption in p.p.m./gm/wet. wt/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>0.220 ± 0.006</td>
</tr>
<tr>
<td>30</td>
<td>0.160 ± 0.008</td>
</tr>
<tr>
<td>25</td>
<td>0.120 ± 0.006</td>
</tr>
<tr>
<td>20</td>
<td>0.075 ± 0.004</td>
</tr>
<tr>
<td>16</td>
<td>0.070 ± 0.002</td>
</tr>
</tbody>
</table>
Fig. 3 - Oxygen consumption of *Petrolisthes* in relation to salinity.
Fig. 4 - Respiration of *Petrolisthes* in relation to pH of the medium.
Table 3

Oxygen consumption of *Petrolisthes* in relation with pH of the medium

Salinity of the medium = 30%o

Temperature of the medium = 26° ± 1°C.

<table>
<thead>
<tr>
<th>pH of the experimental medium</th>
<th>Oxygen consumption in p.p.m./gm. wet wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>0.050 ± 0.002</td>
</tr>
<tr>
<td>6.0</td>
<td>0.099 ± 0.005</td>
</tr>
<tr>
<td>7.0</td>
<td>0.150 ± 0.003</td>
</tr>
<tr>
<td>8.0</td>
<td>0.070 ± 0.004</td>
</tr>
<tr>
<td>9.0</td>
<td>0.053 ± 0.007</td>
</tr>
</tbody>
</table>
per hour at 16%o salinity. Little change in the oxygen consumption occurred at 20%o salinity. As the salinity increased to 25%o the rate of oxygen consumption rose to 0.120 p.p.m. per gm. per hour. At 30%o and 35%o salinities the amount consumed was 0.162 and 0.220 p.p.m. per gm. per hour of oxygen respectively.

The relation between the rate of oxygen consumption and the salinity of the medium is shown in Fig. 3. A negligible change in the oxygen consumption occurred between the salinities ranging from 16%o to 20%o. But after 20%o salinity the rate of oxygen consumption increased with increase of the salinity percentage.

Influence of hydrogen-ion concentration on oxygen consumption

The influence of hydrogen-ion concentration on respiration was calculated at five different pH values of ranging from 5 to 9. The pH was increased or reduced as per the methods described under Material and Methods. After calculating the oxygen at one pH, the specimens were transferred to the next pH. The results are recorded in Table 3 and Fig. 4.

From the Table 3 and Fig. 4 it is seen that the rate of oxygen consumption was lowest at pH 5.0 and it increased
Fig. 5 - Influence of Oxygen tension on the respiratory rate of Petrolisthes.
Table 4

Oxygen consumption of Petrolisthes in relation with oxygen tension of the medium.

Salinity of the medium = 30%o

Temperature of the medium = 26°C ± 1°C

pH = 7.3 - 7.5

<table>
<thead>
<tr>
<th>Oxygen tension of the experimental medium in ml/L.</th>
<th>Oxygen consumption in p.p.m./gm. wet. wt/hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50</td>
<td>0.045 ± 0.002</td>
</tr>
<tr>
<td>3.00</td>
<td>0.083 ± 0.001</td>
</tr>
<tr>
<td>4.30</td>
<td>0.165 ± 0.002</td>
</tr>
<tr>
<td>6.50</td>
<td>0.185 ± 0.003</td>
</tr>
<tr>
<td>8.50</td>
<td>0.180 ± 0.0044</td>
</tr>
</tbody>
</table>
steadily up to pH 7.0. As the pH reached alkalinity the rate of oxygen consumption decreased, the lowest being at pH 9.0.

**Influence of oxygen tension on oxygen consumption**

Ten healthy *Petrolisthes* were subjected to waters of different oxygen concentrations and the effect on the oxygen consumption was recorded. A slight modification in the set up of the apparatus was done by pouring a thick layer of liquid paraffin in the first aspirator so that the air does not come in contact with the water. The oxygen content in sea water was either increased or decreased (vide Material and Methods). Filtered sea water of salinity 35‰ was used and after making the necessary changes in oxygen content, it was poured into the aspirator immediately.

The oxygen consumption was measured at different oxygen concentrations of 2.5, 3.0, 4.3, 6.5 and 8.0 ml/L. The results are presented in Table 4 and Fig. 5. Between 4.3 and 8.0 ml/L oxygen tension the rate of respiration did not change much but at very low oxygen concentration, the respiratory rate was considerably lowered.

**Effect of body weight on the rate of oxygen consumption**

To study the effect of body weight on respiratory
Fig. 6 - Relationship between body size and respiratory rate in *Petrolisthes*.
Table 5
Oxygen consumption of *Petrolisthes* in relation to the body weight.

Temperature = $27^\circ C \pm 2^\circ C$

Salinity = 30%o

$\text{pH} = 7.2 - 7.3$

<table>
<thead>
<tr>
<th>Weight of the animal</th>
<th>Oxygen consumption in p.p.m./gm./ wet wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.103</td>
<td>0.402 ± 0.021</td>
</tr>
<tr>
<td>0.292</td>
<td>0.210 ± 0.018</td>
</tr>
<tr>
<td>0.486</td>
<td>0.156 ± 0.016</td>
</tr>
<tr>
<td>0.812</td>
<td>0.103 ± 0.012</td>
</tr>
</tbody>
</table>
$O_2$ CONSUMPTION P.P. M$^3$/HR.

TIME AFTER E.S. REMOVAL

0 24 HRS 48
Fig. 7 - Effect of eyestalk removal on the respiratory rate of Petrolisthes.
rate, four sets of animals were chosen varying in their body weights. All other environmental conditions such as temperature, salinity, pH and oxygen tension were kept constant. Each experiment was repeated and the results are shown in Table 5 and Fig. 6.

The results indicate an inverse relationship between the body weight and the rate of oxygen consumption, the rate being highest in the smallest animals.

**Effect of eyestalk removal upon the rate of oxygen consumption**

Ten *Petrolisthes* were inserted into the respiratory chamber and their rate of oxygen consumption was determined. Then these animals were removed from the chamber and with a hot forceps the eyestalks were removed. Cauterisation was done to prevent the flow of the blood. After 15 minutes of the eyestalk ablation the rate of oxygen consumption was determined. The same experiment was repeated after 24 hours and 48 hours after the removal of the eyestalks, keeping the temperature, salinity, pH and oxygen concentration constant. The results are presented in the Table 6 and Fig. 7.

The rate of oxygen consumption fell immediately after eyestalk ablation. After 24 hours it increased to
Fig. 8 - Effect of desiccation on the oxygen consumption of *Petrolisthes*.
Table 6

Effect of eyestalk ablation on the oxygen consumption of *Petrolisthes*

Temperature = 27.5°C ± 2°C

Salinity = 30‰ ± 2‰

pH = 7.2 - 7.6

<table>
<thead>
<tr>
<th>Time of taking the respiratory reading</th>
<th>Oxygen consumption in p.p.m./gm. wet. wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before eyestalk removal</td>
<td>0.162 ± 0.001</td>
</tr>
<tr>
<td>15 minutes after removal</td>
<td>0.050 ± 0.001</td>
</tr>
<tr>
<td>24 hours after removal</td>
<td>0.159 ± 0.008</td>
</tr>
<tr>
<td>48 hours after removal</td>
<td>0.156 ± 0.009</td>
</tr>
</tbody>
</table>
Table 7

Effect of desiccation on the oxygen consumption of Petrolisthes.

Temperature of the medium = $27^\circ C \pm 1^\circ C$

Salinity = $30\% \pm 2\%$

pH = $7.2 - 7.6$

<table>
<thead>
<tr>
<th>Time of desiccation</th>
<th>Oxygen consumption in p.p.m./gm. wet wt./hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without desiccation</td>
<td>$0.162 \pm 0.002$</td>
</tr>
<tr>
<td>15 minutes</td>
<td>$0.130 \pm 0.004$</td>
</tr>
<tr>
<td>1 hour</td>
<td>$0.096 \pm 0.003$</td>
</tr>
<tr>
<td>2 hours</td>
<td>$0.050 \pm 0.003$</td>
</tr>
<tr>
<td>3 hours</td>
<td>$0.017 \pm 0.002$</td>
</tr>
</tbody>
</table>
the normal rate and remained steady for 48 hours.

Effect of desiccation on the oxygen consumption

As *Petrolisthes* live in the intertidal region, they are constantly exposed to the atmosphere during low tides. So the experiments were carried on to find out the effect of exposure to the atmosphere on the rate of oxygen consumption. Fifty animals were taken from aquarium and were divided into five sets of ten. The respiratory rate of the first set was determined immediately without exposing them to the atmosphere. The remaining four sets of animals were exposed to the atmosphere for 15 minutes, 1 hour, 2 hours and 3 hours respectively and they were inserted in the respiratory chamber and the rate of oxygen consumption was determined. The results are recorded in Table 7 and Fig. 8. It is seen that the rate of oxygen consumption was abruptly reduced even when the animals were exposed for 15 minutes. A steady decrease in the rate was recorded with the increase in the exposure time.

**DISCUSSION**

Brunow (1911) reported that the rate of oxygen consumption increased four times at 15°C than at 5°C during the period of six to ten hours in *Astacus*.
*C. finmarchicus* found that the rate of oxygen consumption increased directly with the rise in temperature. Identical results were obtained for *Carcinus maenas* (Capraro, 1939). Fox (1936) and Fox and Wingfield (1937) worked on the crustaceans collected from Plymouth and got a higher rate of oxygen consumption than the related species from the colder zone of Sweden. In *Metapenaeus monoceros* the rate of oxygen consumption increased in direct proportion with the rise in temperature (Gopalakrishnan, 1957). Dehnel (1960) found that the summer animals had higher oxygen consumption than winter forms of *Hemigrapsus oregonensis* and *H. nudus*. Sarojini (1966) worked on *Diogenes bicristimanus* and reported a decrease in oxygen consumption at lower temperature range but in the range of 27°C and 34°C, the rate did not vary much; at 38°C a sudden fall in oxygen consumption was noted.

In *Petrolisthes lamarcki* it was found that the rate of oxygen consumption increased from 22°C to 35°C and decreased at a temperature of 36°C. The low oxygen consumption at 22°C may be due to the fact that these tropical animals are acclimatized to live in a narrow
range of temperatures (Mayer, 1914, 1918). The fall in oxygen consumption at 38°C may be due to the animal's lethal temperature being near this temperature.

As the animals adapt themselves to the varying salinity conditions of external medium, energy has to be spent causing a variation in the oxygen consumption (Beadle, 1931; Lowenstein, 1935; Krogh, 1939; Hopkins, 1949). Lowenstein (1935) worked on *Gammarus chevreuxi* taken from 25% salinity and he found that the rate of oxygen consumption increased when the salinity was lowered. The same results were obtained by Marshall et al (1935) in *Galanus finmarchicus* and Schwabe (1933) and Krogh (1939) in *Garcinus maenas*. However, Schwabe (1933) recorded no change in the respiratory mechanism of *Eriocheir* in the sea water of salinities 15% and 32% and also fresh water. Sarojini (1966) found a slight change in oxygen consumption between the range 35% and 20% salinities, but below 20% salinity the rate decreased with the salinity. Kreps (1929) found the same results in *Balanus*. In *Petrolisthes* the rate of oxygen consumption decreased with the fall in the salinity of the medium.

The hydrogen-ion concentration in the external
medium is of great importance in animal life. Comparatively pH changes in sea water are less than those in fresh water. Powers (1930) gave great importance even to small changes of pH in sea water. But according to Helff (1928) the rate of oxygen consumption was not influenced by pH in Cambarus immunis. The same observations were made by Marshall et al (1935) in Calanus and by Gopalakrishnan (1957) in Metapenaeus monoceros. Powers (1922, 1930, 1932) reported that the fishes are affected directly by increase and decrease in the pH of the medium, and claimed that it is due to influence of pH of external medium on the alkali reserves of the blood. Sarojini (1966) observed no change in the respiratory rate between pH 6.0 and 7.0 and any more change in pH lowered the respiratory rate. In Petroliasthes the hydorgen-ion-concentration of the medium seemed to play a greater influence on the oxygen consumption. The highest rate of oxygen was seen at pH 7.0 and reduction in respiratory rate occurred with the change in the pH of the medium.

Henze (1910) observed that in several cold-blooded animals the rate of metabolism was independent
of the oxygen tension of the environment. These observations were based on the oxygen consumption in *Garcinus maenas* and *Scyllarus latus*. The prawn, *Palaemonetes*, was able to tolerate changes in oxygen concentration of the medium till the latter was lowered to about 50% oxygen saturation (Amberson et al, 1924). Similarly the crayfish, *Cambarus*, could maintain a constant rate of oxygen consumption till the saturation was reduced to about 25% (Helff, 1928; Hiestand, 1931). Marshall et al (1935) found that *Calanus* was unaffected by higher oxygen tension of the water, but in concentrations below 3 ml/L the respiratory rate was reduced greatly. In *Petrolisthes* little change in the rate of oxygen consumption was noted in the oxygen concentration range 8 ml/L to 4.3 ml/L. But below 4.3 ml/L oxygen tension a sharp fall in the respiratory rate was observed.

Many workers have emphasised the relationship between metabolism of the body and the body weight while making the comparison between the species and the genera of Crustacea (Edwards and Irving, 1943; Zeuthen, 1953; Roberta, 1957). The oxygen consumption varied
with the size and temperature levels in *Uca pugnax* and *Uca rapax* (Vernberg, 1959). Sarojini (1966) found the higher rate of metabolism in smaller animals of *Diogenes bicristimanus*. The *Petrolisthes* also showed the same trend. The smallest animals showed the highest rate of oxygen consumption, the fall in oxygen consumption being greater with the increase in body weight.

The endocrines play an important role in the metabolic rate has been demonstrated by numerous workers. Removal of sinus glands from the eyestalks of the crayfish, *Oreconectes immunis*, resulted in decrease in the rate of oxygen consumption and the extracts from the central nervous system increased the rate. Edwards (1950), working on *Uca pugilator* and Bliss (1953) using *Carcinus lateralis* found the increase in the metabolic rate after the ablation of the eyestalks. Similar opinion was expressed by Fingerman (1955). However, Scheer and Scheer (1954) recorded no change in the rate of oxygen consumption after the removal of eyestalks, in the prawn, *Leander serratus*. Teyan et al (1959) on *Uca pugnax* and *Uca*
pugilator noticed a decrease in the rate immediately after the removal of eyestalks. Sarojini (1966) working on *Diogenes bicristimanus*, found a decrease in the oxygen consumption of eyestalkless animals and slowly the rate of oxygen consumption became normal after 48 hours. In *Petrolisthes* a sudden fall in the respiratory rate occurred after the ablation of the eyestalks, even though enough care was taken by cauterisation to stop the flow of the blood but the normalcy was restored within 24 hours.

Many animals living in the intertidal zones seem to have the oxygen debt after being exposed to the low tide. This observation was made by Collip (1921) and Van Dam (1935) working on *Mya*, Nagabhushanam (1957) on *Martesia* and Gross (1955) on *Pachygrapsus crassipes*. Similar results were recorded by Sarojini (1966) on *Diogenes bicristimanus*. In *Petrolisthes* also, the rate of oxygen consumption decreased with the increase in time of desiccation. This may be due to the decrease in the metabolic rate.
1. The influence of environmental conditions like the temperature, salinity, pH, oxygen tension and desiccation as well as eyestalk ablation and body weight on the oxygen consumption was studied in *Petrolisthes lamarckii*.

2. The rate of oxygen consumption was increased with increasing temperature from 22\(^\circ\)C to 33\(^\circ\)C. The respiratory rate was considerably lowered at 38\(^\circ\)C.

3. The salinity of medium had a marked influence on the oxygen consumption, the respiratory rate being increased with the rise in salinity.

4. The oxygen consumption was maximum at pH 7.0 and was reduced on either side of this pH.

5. The rate of oxygen consumption was more or less steady between oxygen concentrations from 8 ml/L and 4.3 ml/L. But below 4.3 ml/L oxygen tension, the rate was reduced.

6. There appears to be an inverse relationship between the body weight of the animal and rate of oxygen consumption.
7. Immediately after the ablation of eyestalks a fall in the respiratory rate occurred, but normal rate was restored after 24 hours.

8. The longer the time of desiccation of animals, the lower was the rate of oxygen consumption.
<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Bibliography</th>
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<th>Title</th>
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Chapter II

Osmotic behaviour of the porcellanid crab, *Petrolisthes lamarckii*

The osmotic relationship between the body fluids of animals and the salinity of the external media is mainly based on their respective total concentrations. Under conditions of changing salinity the response of the animal may be a parallel change in the osmotic concentration of its body fluids or some degree of active osmoregulation. Prosser and Brown (1961), who reviewed the subject of osmotic balance, accordingly grouped animals as osmo-conformers and osmoregulators, although these are not necessarily rigid categories.

Among the decapod Crustacea osmoregulatory mechanisms have been extensively studied (Krogh, 1939; Jones, 1941; Panikkar, 1941; Gross, 1957; Parry, 1957; Dobkin and Manning, 1964). However in the anomurans very little work has been done on osmoregulation. Harms (1932) made a few freezing point determinations on the blood of anomuran, *Birgus* and Pearse (1934) did the same on *Coenobita clypeatus* but these workers were not explicit as to the environmental conditions under which the blood samples were taken from the animals. Gross (1955) demonstrated in
laboratory experiments that *Birgus latro* could control its blood concentration by selecting water of appropriate salinity. Gross and Holland (1960) demonstrated a similar behavioural mechanism in *Coenobita perlatus*. Sukumaran (1961) studied osmoregulation of *Clibanarius padavensis* under heterosmotic conditions.

In view of the fact that salinity is one of the important variables of the coastal waters, a study was undertaken of the effects of salinity changes on *Petrolisthes lamarckii*. These animals, being inhabitants of the intertidal region, exhibit in one way or other a high degree of tolerance to changes of salinity in order to survive. It was, therefore, felt that a study of the osmoregulatory mechanism of *P. lamarckii* would be of interest.

**Material and Methods**

*Petrolisthes* were collected from the Thana creek. They were brought to the laboratory and kept in large glass troughs filled with sea water to a depth of 1". Only those crabs were used in the experiments which showed active movements. Both males and females were used. All the specimens used in this investigation were mature and between moults. Solutions of different salinities were
made by diluting sea water with distilled water. The following salinities were used: 35, 30, 25, 20, 18, 15 and 10 parts per litre. In each experiment five specimens of healthy Petrolisthes were kept in a finger bowl having 500 ml. sea water of varying salinities. The bowls were loosely covered to prevent excessive evaporation and the water was changed daily. All the bowls were placed in a big tray of water on the laboratory table away from direct sunlight. The crabs were fed once a week on fish fry and the feeding was indicated by the discharge of faeces. The percentage of mortality in each salinity was observed for a period of 30 days. Water temperature varied from $25^\circ$ to $20^\circ$C. during the experiment.

The osmotic pressure of the body fluid was determined by the comparative melting point method of Jones (1941) and Gross (1954) as modified by Freeman and Rigler (1957). The procedure involves the comparison of the melting time of unknown samples of body fluid with that of a series of standard sodium chloride solutions. The body fluid was collected from the crab into a capillary tube of approximately 0.3 mm. inside diameter by making a puncture on the arthrodial membranes at the joints of the walking legs. Before this was done, the animal was dried between folds of filter paper. Both ends of the capillary
tube were immediately sealed with sealing wax after taking the sample. The samples of body fluid were taken from animals in each salinity after keeping them for two days in that medium.

Similarly, solutions of sodium chloride of known concentrations were sealed in capillary tubes. The lengths of the body fluid and sodium chloride columns were kept constant. The samples were stored in a freezer. Each of the samples was then allowed to melt in a pyrex vessel containing brine kept at a constant temperature of 2°C with the help of a cooling unit. The brine was held in a vertical position to get a better view and a magnifying glass was used to observe the frozen sample column. The time taken for the last crystal to disappear was noted. The entire experiment was performed in a cold room (18°C) so that the rate of warming of the brine was slowed down to 5 minutes to every degree of temperature. The osmotic concentration of the body fluid is expressed in terms of percentage sodium chloride and is calculated from the curve relating melting time to the strength of the standard sodium chloride solutions (Freeman and Rigler, 1957).
Results

*Petrolisthies lamarckii* was able to tolerate salinities from 18 to 35%. Between the salinities of 25 and 35% the survival rate was 100%. Between 18 and 25% salinities the survival rate of *Petrolisthies* increased with increase of the salt concentration. The lethal salinity appears to be 17.2%.

The osmotic pressure of the body fluid showed a significant difference from that of the external medium within very low salinities (Table 1). In 35% salinity the body fluid of *Petrolisthies* was slightly hypotonic to the medium, being equivalent to 1.50% sodium chloride. Between 30 and 20% salinities, however, the body fluid was more or less isotonic with the external medium. In 18% salinity and further below, there was a tendency for the body fluid to remain in a hypertonic concentration.

Discussion

The results of the present study indicate that *Petrolisthies lamarckii* appears to possess good osmoregulatory ability at low salinities. The animals maintained their body fluids hypertonic to the medium in 18% salinity and further dilutions. In salinities above 30% the body fluid was kept hypotonic to the medium.
*B. latro* controls its body fluid concentration by selecting water of appropriate salinity (Gross, 1955). On the other hand, *Pachygrapsus crassipes*, was shown to be capable of absorbing water against a gradient in order to maintain the body fluid hypotonic to sea water (Gross, 1957). Panikkar (1941) found that *Leander serratus, L. squilla* and *Palaemonetes varians*, maintained blood hypotonic to sea water and hypertonic to dilute sea water. Panikkar and Viswanathan (1948) found the same ability shown by *Metapenaeus monoceros*. To a lesser extent, *M. dobsoni*, *Penaeus indicus, P. carinatus* and *Eriocheir sinensis* also maintained their internal concentrations hypotonic in sea water and hypertonic in dilute sea water. Sukumaran (1961) found the same condition in *Clibanarius padavensis*. The behaviour of *Petrolisthes lamarckii* was found to be similar to the above described crustaceans.

The present investigation has shown that *Petrolisthes* is a hyper-osmoregulator in dilute sea water but where and how this mechanism works is not yet known. Gross (1957) has found a correlation between regulatory ability and exoskeleton permeability in certain decapod crustaceans. *Cambarus clarki* and *Pachygrapsus crassipes*, which are good
regulators, show the lowest permeability value. Differential permeability of the body surface may offer an explanation for the hyper-regulatory condition in *Petrolisthes*.
Summary

1. *Petrolisthes lamarckii* can tolerate salinity variations between 18 and 30%. 

2. Survival rate was maximum between 20 and 35% salinities. 

3. *Petrolisthes* maintained its body fluid hypotonic in sea water of salinities above 30% and hypertonic in 18% salinity and further dilutions. Isotonicity between the external medium and body fluid was established in the salinities from 20 to 30%.
Table 1

Freezing point depression of the body fluid of

*Petrolisthes lamarckii* in different salinities

(Average of five reading)

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<th>Medium (% salinity)</th>
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<td>10</td>
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Chapter III

Heat tolerance of Petrolisthes lamarckii in relation to temperature and salinity

Temperature tolerance as an experimental criterion for the demonstration of physiological change has found many uses. However, studies on temperature tolerance were mostly done on fish and relatively few experiments were performed on invertebrates. In several instances, the lethal point was determined by the temperature at which death occurred when the animal was subjected to slowly increasing temperatures (Huntsman and Sparks, 1924; Gowanloch and Hayes, 1926). Since different workers raised the temperature at different rates, the results could not be compared because the time over which the temperature was increased would be expected to have a marked effect on the final lethal point. Experiments in which animals were maintained at constant temperatures for a period of time provided a more accurate determination of the lethal temperatures.

Acclimation to low or high temperatures was demonstrated in many animals on the basis of laboratory acclimation. Edwards and Irving (1943) showed seasonal acclimation in Emerita talpoida, at all experimental
temperatures below 20° C. Oxygen consumption of animals in summer was less than that in winter. Another facet of acclimation is its effect on the temperature tolerance of a species. A previous temperature history at the upper levels of the physiological temperature range is known to raise the thermal resistance. Gain of heat tolerance was much more rapid than its loss. Brett (1946) showed that the goldfish, *Carassius auratus*, required a total of 30 days to acclimate to 29° C when brought from 4° C in 8° C steps. Loss of heat tolerance in the crayfish, *Orconectes rusticus*, was not completed by the end of the sixteenth day when the crayfish were transferred from 22-23° C to 4° C.

Studies on the effects of salinity change on temperature tolerance are rather few. Broekema (1941) found that *Crangon crangon*, could endure better low salinity when the temperature was high. The lobster, *Homarus americanus*, was shown to have higher lethal point when both salinity and temperature were high during the period of acclimation. A decrease in salinity with the acclimation temperature held constant resulted in a lowering of thermal resistance (McLeese, 1956). Todd and Dehnel (1960) studied in detail the effect of temperature
and salinity on heat tolerance in two grapsoid crabs, *Hemigrapsus nudus* and *H. oregonensis*.

The porcellanoid crab, *Petrolisthes lamarckii* is used in the present investigation to study the effect of temperature and salinity on the heat tolerance. Two sets of experiments were conducted with previously determined temperature and salinity combinations to determine if these environmental changes cause a resulting change in the upper temperature tolerance. Also, animals were acclimated to various combinations of temperature and salinity, to ascertain any resulting changes in the temperature tolerance.

**Material and Methods**

*Petrolisthes lamarckii* were collected from Thana creek. The lowest salinity recorded in this area was about 28‰ during the month of August/July and the highest salinity was about 36‰ in the month of November.

The temperature range was 24°C to 32°C. Both males and females were used in the temperature tolerance experiments when preliminary experiments showed no difference in the resistance of the sexes. Gravid females or individuals missing any appendages were discarded. All experiments were conducted in the months of January and February of the year 1968.
In this study, holding experiments refer to those in which the temperature tolerance of the *Petrolisthes* is determined without any previous laboratory acclimation. The animals were tested after keeping for 24 hours in the laboratory. This time period allowed the gut to be cleared partially so that at the high test tolerance temperatures, deposition of faeces and urine did not foul the water. On the other hand, in acclimation experiments, animals were held at previously determined acclimation conditions which differed from holding experimental conditions in one factor. In these experiments the crabs were acclimated for at least one week in the laboratory.

If the acclimation temperature differed from the holding temperature, *Petrolisthes* were gradually warmed or cooled until the desired temperature was reached; this usually took about 2 hours. Holding or acclimation temperatures were either 20° C or 33° C; the salinities were either 18% or 30%. Hence, four experimental combinations were used. Ten *Petrolisthes* were placed in each container. Animals were changed, usually once per 24 hours, to water of appropriate temperature and salinity
over the acclimation or holding period. The crabs were brought to the test temperature from the holding or acclimation temperatures over a period of 2-4 hours at which time the experiment was begun. The experiment was conducted for 24 hours. Animals were considered dead only when all movements ceased. Petrolisthes which moulted during the 24 hours of the experiment were discarded. Data were plotted on the lines of Todd and Dehnel (1960) after repeating all the experiments twice.

**Results**

**Experiment I.**

Average conditions of temperature and salinity taken into consideration for the first series of experiments were 33° C and 30% salinity. Survival values obtained from the animals which were kept at the above mentioned temperature with no laboratory acclimation (holding experiments) were considered as the base line curves. These curves were compared with heat tolerances of animals acclimated to (1) 33° C and 30%, (2) 33° C and 18%, (3) 20° C and 30%, and (4) 20° C and 18%. Temperature at which 50% survival occurred for 24 hours was used as the basis for comparison.
Fig. 1 - 50% survival values of animals kept at 33°C and 30% salinity without acclimation (holding experiment) – Base line experiment. Lethal temperature = 36°C.
**Fig. 2** - The influence of laboratory acclimation to 33°C and 30% salinity on 50% survival value for 24 hours.  
Lethal temperature = 42.3°C.
Fig. 3 - The influence of laboratory acclimation to 33°C and 15‰ salinity on 50% survival value for 24 hours.
Lethal temperature = 39.1°C.
Fig. 4 - The influence of laboratory acclimation to 20°C and 30‰ salinity on 50% survival value for 24 hours.
Lethal temperature = 33.7°C.
Fig. 5 - The influence of laboratory acclimation to 20°C and 18‰ salinity on 50% survival value for 24 hours.
Lethal temperature = 32.25°C.
Holding experiment: 33° C and 30% salinity.

Results of the base line experiments showed that the temperature at which 50% survival was found for Petrolisthes was 38.0° C for 24 hours (Fig. 1).

Acclimation experiments: (1) 33° C and 30% salinity.

Petrolisthes showed a marked increase in heat tolerance after acclimation to these temperature and salinity combination. The lethal temperature was found to be 42.3° C (Fig. 2).

(2) 33° C and 18% salinity.

This combination of temperature and salinity differed from the base line condition (Fig. 1) only in the salinity, having been changed from 30% to 18%. The 50% survival temperature was 39.1° C for 24 hours (Fig. 3).

(3) 20° C and 30% salinity.

Results showed that the tolerance was lowered compared with the above one. The 50% survival temperature was 33.7° C for 24 hours (Fig. 4).

(4) 20° C and 18% salinity.

Low temperature and low salinity combination appeared to be slightly less favourable for survival. The 50% survival temperature was 32.25° C for 24 hours (Fig. 5).
Fig. 6 - 50% survival value of animals kept at 20°C and 18% salinity without acclimation (holding experiment). Base line experiment. Lethal temperature = .29.5°C.
Fig. 7 - The influence of laboratory acclimation to 20°C and 18% salinity on 50% survival value for 24 hours.
Lethal temperature = 31.4°C.
Fig. 8 - The influence of laboratory acclimation to 20°C and 30‰ salinity on 50% survival value for 24 hours. Lethal temperature = 32.6°C.
Experiment II.

Average conditions of temperature and salinity taken into consideration for second series of experiments were 20° C and 18% salinity. The survival value of the animals kept at the above temperature and salinity combination without any laboratory acclimation (holding experiments) were considered as base line curves (Fig. 6). The animals were acclimated to four combinations: (1) 20° C and 18%, (2) 20° C and 30%, (3) 33° C and 30% and (4) 33° C and 18%.

Holding experiment: 20° C and 18% salinity.

The 50% survival for this experiment was 29.5° C for 24 hours (Fig. 6).

Acclimation experiments: (1) 20° C and 18% salinity.

Petrolisthes acclimated to this combination of temperature and salinity have better survival value compared to the holding experiment. The 50% survival temperature was 31.4° C (Fig. 7).

(2) 20° C and 30% salinity.

Animals acclimated to this low temperature and salinity combination showed the greatest difference from the counterpart of the first series of experiments. The 50% survival value for Petrolisthes was 32.6° C for 24 hours (Fig. 8).
Fig. 9 - The influence of laboratory acclimation to 33°C and 30‰ salinity on 50% survival value for 24 hours.
Lethal temperature = 38.0°C.
Fig. 10 - Influence of laboratory acclimation to 33°C and 18% salinity on 50% survival value for 24 hours.
Lethal temperature = 36.7°C.
(3) $33^\circ$ C and 30% salinity.

Acclimation to these conditions provided the most suitable environment to withstand the test tolerance temperatures; there was a marked increase in the temperature at which 50% survival occurred from the baseline. The 50% survival temperature was $38.0^\circ$ C for 24 hours (Fig. 9).

(4) $33^\circ$ C and 18% salinity.

The 50% survival temperature for Petrolisthes was $36.7^\circ$ C for 24 hours (Fig. 10).

Discussion

Acclimation to a high temperature, with resulting increase in the high temperature tolerance of the species has been demonstrated many times (Sumner and Doudoroff, 1938; Brett, 1946; Mellonby, 1954; Spoor, 1955 and McLeese, 1956). The porcellanid crab, Petrolisthes lamarckii, would seem to be no exception. Temperature tolerance in conjunction with salinity has been studied less extensively, and it is of interest to find that the salinity has a very marked effect on the temperature tolerance of the species.

It is evident from the present study that the porcellanid crab can regain its tolerance to high temperatures very rapidly after a low temperature history
by acclimation in the upper part of the physiological temperature range. This rapid gain of heat tolerance has been demonstrated many times. Mellanby (1954) reported that the heat coma point is shifted with experimental acclimation at the upper end of the tolerable range in the mealworm, Tenebrio molitor. Spoor (1955) found complete gain of heat tolerance in the crayfish in less than 24 hours with an approximate 18° C change in temperature (4° C to 22° C).

A rapid gain in heat tolerance would be of extreme advantage to most intertidal animals. For intertidal animals, the increase in temperature may be great over a period of few hours in the tidal rhythm. Probable increase in salinity aids the animals in resisting the high temperatures, as experimental results have indicated that high salinity provides the most favourable environment.

With the low salinity, low temperature combination, loss in heat tolerance is much greater. In nearly all instances low salinity resulted in a lower value for the 50% survival temperature than with high salinity, whether the temperature was 20° C or 33° C. Gross (1957) gives values for Hemigrapsus nudus and H. oregonensis which indicate the osmotic gradient maintained by these two species when placed in various concentrations of sea water.
The maintenance of this gradient presumably results in more work being done by the animals at lower salinity. It is probable that the lower 50% survival temperatures with the lower salinity as found in the present study with Petrolisthes is due to the increased metabolic work necessary at this salinity. The lethal or near lethal temperatures in the test tolerance experiments presumably alone are causing a marked strain on the metabolic activity of the crabs and the additional strain of maintaining a large gradient between the blood and the external medium in the low salinity results in the animal dying at a lower temperature.

A similar relation between salinity and temperature was found by Broekema (1941) in Grapsus grapsus and in Hemigrapsus by Todd and Dohnel (1960). The salinity optimum for length of life depended on the temperature. Kinne (1956) found that the hydroid, Cordylophora caspia, had slight osmoregulatory abilities and was found to withstand a high temperature better at high salinity than at lower ones.
Summary

1. The influence of laboratory acclimation to various temperature-salinity combinations on the heat tolerance was determined for the porcellanid crab, *Petrolisthes lamarckii*. There was a change in the 50% survival in the crabs when base lines from experiment I and II were compared.

2. Acclimation to a high temperature generally increased resistance to lethal temperatures whereas acclimation to low salinity generally decreased it. High temperature and high salinity was the most favourable combination to withstand the high test tolerance temperature. Gain in heat tolerance whether the salinity was low or high was rapid.
References


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