Chapter 10. The Measurement of Respiration

WINFRIED LAMPERT

1 Introduction

The aim of estimating secondary production cannot only be to obtain a number for the productivity of a certain lake or river. In order to understand the functioning of a system, one must know which factors limit secondary production and how production changes with changing environmental conditions. The production of different species may respond differently to the change of one factor. Production is the visible result of many physiological processes; it is, therefore, worthwhile to examine closely the processes which contribute to it and the effect of environmental changes. Losses of energy or matter during metabolism are one of the processes important to production.

When production is considered as the sum of accumulated matter in somatic growth and reproduction, it can be determined in long-term experiments without measuring any metabolic losses. However, when the energy or material budget is the center of interest, exact knowledge of the losses and the way in which they vary is essential. It should be mentioned that there is no coupling between the ‘metabolic activity’ of an animal and its production, *per se* even though animals that exhibit a high metabolic turnover also have a high potential rate of production under optimal conditions, this production rate may not always be realized. Large-scale interspecific comparisons resulted in linear correlations between \( \log R \) and \( \log P \) (McNeill & Lawton 1970, Humphreys 1979, Lavigne 1982). Different regression lines could be calculated when metabolically similar species were grouped. For a group of short-lived poikilotherms including several freshwater animals, McNeill & Lawton (1970) established the regression line:

\[
\log P = 0.8262 \log R - 0.0948
\]  

(10.1)

(both expressed as kcal m\(^{-2}\) yr\(^{-1}\)). If reliable measurements of respiration during the season are available, accumulated production can be estimated in this way, even if this can only be a rough approximation due to the considerable scatter of the points obtained. Predictions for individual species under certain environmental conditions should not be made (Lavigne 1982).
When environmental conditions change, the parameters of the balance equation:

\[ \text{Production} = \text{Assimilation} - \text{Respiration} - \text{Excretion} \quad (10.2) \]

may vary independently and may affect production considerably. Availability of food, for example, is a factor which influences assimilation rate and respiration rate differently. Their relationship is illustrated in Fig. 10.1. The assimilation rate is much more dependent on food availability than is the respiration rate. Without any food the assimilation rate is zero, whereas the respiration rate is positive. Because production is the difference between assimilation and metabolic loss, it may be positive or negative, depending on whether assimilation is greater than the losses, or \textit{vice versa}. Negative production may occur under food limitation.

Starving animals lose a considerable amount of body weight per day (Lemcke & Lampert 1975). Of course, this cannot last very long, but how long...
a starving animal will survive depends, besides other factors, on its specific respiration rate. Species-specific or size-specific differences in the respiration rate may, therefore, have consequences for the structure of a community under conditions where food is limited (Threlkeld 1976). Intensity of respiration also influences the food concentration at which production is zero. This is an environmental threshold (Lampert 1977), defining the minimum food availability that allows positive production. In Fig. 10.1, for example, a certain threshold of food availability where positive production is attained can be determined. Since assimilation and respiration rate may respond differently to other environmental changes, this threshold may also vary—for example, when temperature increases above some optimum, the respiration rate will further increase, whereas the assimilation rate will decrease. This results in a dramatic shift of the 'food threshold' to some higher value. From these ideas it becomes clear that under food limiting conditions the production rate is very sensitive to environmental changes. It is evident, therefore, that laboratory measured parameters which are to be applied in field situations must be determined in conditions as close as possible to those in nature.

One more important fact can be seen from Fig. 10.1. The small vertical bars indicate the effect of a 10% error of the measurement of respiration. When the food is limited, the effect of this error on production estimates is much more pronounced. In our case, the 10% respiration measurement error, which is easily introduced by applying only the respiration rates of starving animals to a budget of feeding ones, results in a 50% error in the calculated production. As a consequence, one should measure respiration as exactly as possible. For animals with unlimited food under optimal conditions, this may not be so important. However, we are interested in estimating production in the field where the animals rarely, if ever, live under optimal conditions.

2 Measures of Metabolism

2.1 Choice of the principle method

Because metabolic losses must be measured as exactly as possible, the conditions during the measurement should be as similar to natural conditions as possible. Animals that usually burrow in the sediment will not show the same respiration rates under bright light without any shelter; running water insects need current; a freely swimming zooplankter should not be confined in a very small volume of water. In addition, the energy budget of a feeding animal is different from that of a starving one, so respiration should be measured using fed animals. These conditions will help to ensure that measured rates are similar to natural rates.

Unlike mammals, a 'basal metabolic rate' cannot be defined for
poikilotherms, because metabolism is temperature dependent. Therefore, the term 'standard metabolic rate' is used for the minimal respiration rate of a fasting animal at a certain temperature. Total metabolism comprises 'standard metabolism', plus energy costs of motion and increased activity, costs of food gathering (e.g. filtering), and costs of digestion, absorption and transportation of the absorbed food (SDA). For ecologically relevant studies it is not sufficient to know the 'standard metabolic rate' of an animal. A motionless fasting animal will not be able to have a positive production rate. No distinction has to be made between 'resting' and 'active' metabolism, because it should be the aim of the investigator to study respiration at the normal level of activity related to the given environmental conditions.

There are different approaches to measuring metabolic losses. The method chosen depends on the facilities of the researcher and the aim of the study. One might be interested in the flow of energy, or in the flow of matter (e.g. of carbon). Most investigators try to analyze the flow of energy but because energy flow is difficult to measure directly, some uncertain conversions are nearly always included in the calculations. Thus, it might sometimes be better to restrict the conclusions to the mass flow.

True energy output is measured by direct calorimetry. Measuring the heat production is the only method of direct determination of energy losses, and although this method is being developed rapidly it is not yet frequently used.

Measurement of oxygen consumption has a long tradition. Under certain conditions it reflects metabolism and can, therefore, be used as a measure of respiration. However, for use in a budget, oxygen consumption rates must always be converted into some other units and this conversion introduces uncertainties. Nevertheless, it is the mode used most frequently in tackling the problem of respiration.

CO₂ excretion is a true measure of carbon flux. In a budget based on carbon it produces correct figures for the output along with the excretion of organic carbon, but conversion to energy units is still complicated.

A biochemical measure of the potential metabolic activity is provided by a relatively recent method of measuring the activity of the respiratory electron transport system (ETS). It yields integrated measures of respiration which reflect true in situ rates, but cannot be used in short-term experiments.

2.2 Direct calorimetry

In metabolism, energy is lost as heat. The best method of measuring metabolic losses would be the measurement of heat production by direct calorimetry. During recent years great progress has been made in developing direct calorimetry techniques, even in flow-through systems (see Gnaiger 1979, 1982). With new electronic developments, calorimeters have become very
sensitive and stable, so that long-term monitoring of small aquatic animals is possible when a flow-through system is used.

Direct calorimetry is the only method which can be used when anoxic fermentative reactions contribute to the total metabolism. These are not reflected by oxygen consumption, but may sometimes be very important especially for the various aquatic animals, especially benthic organisms, which are able to live, at least partly, under reduced oxygen conditions (Prosser 1973). Direct measurement of heat production is highly recommended, but unfortunately, calorimeters are still expensive and complicated and the method needs some special skill. When the animals are placed in the calorimeter the instrument will be thermally disturbed and re-equilibration requires some hours. Thus, it is not possible to begin the measurements immediately. This might be an advantage because the animals can acclimatize to experimental conditions. To avoid oxygen depletion and accumulation of metabolic products in the chamber, a flow-through system should be used. The flow rate must be low (e.g. 3·3 ml h\(^{-1}\); Gnaiger 1979). As the time constant of the system is dependent on the size of the chamber, the animals must be confined to a small volume of water (e.g. 0·5 ml). Larger chambers may be used, but in this case the time resolution of the system is low, and complicated calculations must be applied. Confinement to a small volume may be a disadvantage for freely swimming animals.

Direct calorimetry is a standard method in biochemistry and physiology but there have been few examples of its application to ecological problems. Nevertheless, this technique is a very valuable tool in ecology, especially when combined with measurements of oxygen consumption.

2.3 Oxygen consumption

2.3.1 General

Measurement of oxygen consumption is the most frequently used method in studies of metabolism. Numerous methods have been developed for a broad range of animal sizes. There are three different modes of experiments in use:

1) In a closed system the concentration of oxygen or \(\text{pO}_2\) is measured at the beginning and at the end of the experiment, or is monitored continuously. Respiration rate is calculated from decreases of oxygen and the volume of the vessel.

2) Animals are kept in an open-flow system, where water passes slowly through a chamber containing the animals. The concentration of oxygen in the water is measured before and after the chamber. The respiration rate is calculated from the reduction of oxygen concentration and the flow rate of the water.
Animals are kept in water which is equilibrated and in contact with a known volume of air. During the experiment the dissolved oxygen consumed by the animals is replaced by oxygen from the air and the carbon dioxide produced is removed by an absorbant. The volume of air, therefore, decreases and this change in volume can be measured gasometrically.

The first two methods involve determination of the oxygen in water, which can be done by measuring the amount of dissolved oxygen by chemical methods or the partial pressure with a polarographic electrode. The third mode requires the measurement of the change in gaseous oxygen. This can be done by holding the pressure constant and measuring the difference in volume, or by monitoring the pressure changes necessary to keep the volume constant. The type of experiment selected and the method of oxygen detection used depends on the sensitivity needed, the size of the animals, and the environmental conditions to be simulated. The size of the animals is an especially important factor to be considered when an experiment is designed. Some literature data, utilizing these methods for animals varying in size by 5 orders of magnitude are compiled in Fig. 10.2. For the smallest animals (< 1 μg) only the Cartesian diver principle is used, whereas all the other methods cover a broad range of sizes. Few measurements have been made using single animals, however. In the medium size range, most determinations have been made with many animals per sample. The use of numerous animals in one experiment may be an advantage, because individual variation is averaged. On the other hand, a lot of information on individual performance is lost in this way.

Experiments have to be designed so that the oxygen reduction needed to measure respiration can be determined with good precision. When dissolved oxygen is measured with a good system, a 5% reduction may be sufficient. The difference should not be greater than absolutely necessary, since the respiration rate can be proportional to the concentration of oxygen. Reduction of oxygen in a closed system depends on the total weight of the animals, the volume of water, the duration of the experiment, and the ambient temperature. In an open-flow system the difference between the influent and effluent oxygen concentrations depends mainly on the animals' weight, the flow rate, and the temperature. The size of the respiration chamber has no influence on the final concentration but on the response time of the system. If the ratio of chamber-volume to flow-rate is high, then the final concentration will be reached very slowly and the response time will be slow. This ratio cannot be lowered by increasing the flow-rate because in this case, the reduction of the oxygen concentration might be too small to detect. Thus, small chambers should be used to improve precision.

A standard procedure cannot be recommended. Preliminary experiments
The Measurement of Respiration

Fig. 10.2 Respiratory rate of different sized aquatic animals at 20 °C. The regression equation for all animals weighing <0.8 mg (solid line) is \( \log R = 0.794 \log W + 0.4828 \) \( r = 0.986; n = 21; 95\% \text{ conf. lim. of } b: 0.736-0.852 \). The slope of the dashed line is 1-0. Symbols indicate different methods of measuring respiration:

○, Cartesian diver; ◇, gasometric methods; □, 'closed bottle', Winkler determination; ○, closed system, membrane electrode; ▽ open-flow system; △ CO\(_2\)/IR. Numbers represent different species: 1, *Chaoborus trivittatus* (Swift 1976); 2, *Daphnia pulex* (Goss & Bunting 1980); 3, *Daphnia magna* (Goss & Bunting 1980); 4, *Cloéon dipterum* (Kamler 1969); 5, *Pisidium annicum* (Holopainen & Ranta 1977); 6, *Daphnia pulex* (Richman 1958); 7, *Brachionus calyciflorus* (Leimeroth 1980); 8, *Diaptomus siciloides* (Comita 1968); 9, *Diaptomus leptopus* (Comita 1968); 10, *Brachionus plicatilis* (Doohan 1973); 11, *Cyclops leuckarti* ♦ (Scherbakoff 1935); 12, *Cyclops leuckarti* ♣ (Scherbakoff 1935); 13, *Cyclops strenuus* (Shcherbakoff 1935); 14, 15, *Daphnia magna* (Schindler 1968); 16, *Stenonema pulchellum* (Trama 1972); 17, *Boeckella delicata* (Green & Chapman 1977); 18, *Calamoecia lucasi* (Green & Chapman 1977); 19, *Brachionus rubens* (Pilarska 1977); 20, *Eudiaptomus gracilis* (Kibby 1971); 21, *Chaoborus trivittatus* (Giguère 1980); 22, *Daphnia magna* (Lampert, unpublished); 23, *Daphnia magna* (Kersting & v.d. Leeuw-Leegwater 1976); 24, *Gammarnus fossarum* (Franke 1977).
must be performed in all cases. For a rough overview of the literature the conditions in selected experiments of both open and closed types are assembled in Table 10.1.

Methods involving a gas phase are limited by the rate of diffusion of oxygen from air into water; therefore, the volume of water must be small. If a larger volume is needed, the dissolved oxygen concentration must be determined. There is some discussion in the literature comparing the 'closed bottle' to the 'open-flow' method (Kamler 1969; Dries et al. 1979; Gnaiger 1982). Kamler (1969) found that the rates of oxygen consumption varied considerably with time, depending on the length of the experimental period. Due to handling of the animals or increased activity, the respiration rates were higher during the first period of an experiment. In an open-flow respirometer, these first measurements can be rejected, but this is impossible in a 'closed bottle' system and the results are, therefore, always too high. This overestimation will increase with decreasing experiment length. Similar results are reported by other authors. Directly after the transfer of Gammarus fossarum into a closed chamber with flowing water, the respiration rate was twice as high as it was 4 h later (Franke 1977). Increased output of CO₂ by the mollusc Pisidium amnicum during the first hours was observed by Holopainen & Ranta (1977). Larvae of the stonefly Isoperla buresi put into a closed bottle showed increased searching and respiratory movements (Kamler 1969).

Rejection of the initial values is possible if the oxygen tension in a closed bottle is continuously recorded by an electrode. In gasometric methods, reading of oxygen consumption may begin some time after the introduction of the animals.

In fact, the interpretation of the initial elevation of the respiratory rate is not easy. Part of the decrease with time may be due to the experimental conditions—the normal activity of the animals may be reduced by the confinement, or the change could be induced by starvation. It is, therefore, not clear whether the initial or the reduced respiratory rate reflects 'normal' conditions. A comparison of the features of both experimental modes adds more arguments to the discussion (Table 10.2). Open-flow respirometry has many advantages and should be used if possible. It is especially useful for continuous long-term monitoring of metabolism and studies on the response of the respiratory rates to changes in environmental factors.

Because respiration is measured with different methods, the results are given in different units, the most frequently used unit being μl O₂ at Standard Temperature and Pressure conditions (STP), i.e. 0°C and 760 mg Hg. Chemical determinations yield differences in mg O₂. Conversion from microlitres to micrograms can be made by use of the molar volume (1 mol O₂ = 22.3931). Since these units are not usually used in other fields such as physiology, biochemistry or chemistry there are good reasons for unifying
Table 10.1 Technical features of some published experiments on respiration of aquatic animals of different size.

<table>
<thead>
<tr>
<th>Species</th>
<th>Ind. weight (mg)</th>
<th>Animals per ml</th>
<th>Approx. weight per ml (mg)</th>
<th>Volume of water (ml)</th>
<th>Period (hrs)</th>
<th>Temp. °C</th>
<th>Method</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brachionus calyciflorus</em></td>
<td>0.00032</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>2–3</td>
<td>20</td>
<td>Cart. diver</td>
<td>Leimeroth, 1980</td>
</tr>
<tr>
<td><em>Brachionus plicatilis</em></td>
<td>0.00018</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>max. 5</td>
<td>20</td>
<td>Cart. diver</td>
<td>Doohan, 1973</td>
</tr>
<tr>
<td><em>Brachionus plicatilis</em></td>
<td>0.0002</td>
<td>3000</td>
<td>0.09–0.585</td>
<td>0.0003</td>
<td>2–3</td>
<td>24</td>
<td>Cart. diver</td>
<td>Epp &amp; Lewis, 1979a; personal communication</td>
</tr>
<tr>
<td><em>Chaoborus trivittatus</em></td>
<td>0.8</td>
<td>0.33</td>
<td>0.26</td>
<td>3</td>
<td>3–30</td>
<td>5–25</td>
<td>volumetric</td>
<td>Swift, 1976</td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>0.025–0.097</td>
<td>1.25</td>
<td>0.031–0.121</td>
<td>4</td>
<td>8</td>
<td>5–30</td>
<td>volumetric</td>
<td>Goss &amp; Bunting, 1980</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0.141–0.365</td>
<td>1.0</td>
<td>0.141–0.365</td>
<td>4</td>
<td>8</td>
<td>5–25</td>
<td>volumetric</td>
<td>Goss &amp; Bunting, 1980</td>
</tr>
<tr>
<td><em>Simocephalus exspinosus</em></td>
<td>0.0018–0.112</td>
<td>0.22–2.2</td>
<td>0.004–0.025</td>
<td>9</td>
<td>1</td>
<td>25</td>
<td>volumetric</td>
<td>Obreshkove, 1930</td>
</tr>
<tr>
<td><em>Asellus aquaticus</em></td>
<td>0.34–4.78</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>4</td>
<td>23</td>
<td>volumetric</td>
<td>Prus, 1972</td>
</tr>
<tr>
<td><em>Mysis relicta</em></td>
<td>0.067–13.4</td>
<td>0.04–0.017</td>
<td>0.003–0.022</td>
<td>25–60</td>
<td>20–48</td>
<td>2.4–6.8</td>
<td>Winkler</td>
<td>Ranta &amp; Hakala, 1978</td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>0.006–0.028</td>
<td>0.37–0.74</td>
<td>0.004–0.01</td>
<td>135</td>
<td>24</td>
<td>20</td>
<td>Winkler</td>
<td>Richman, 1958</td>
</tr>
<tr>
<td><em>Diaptomus siciloides</em></td>
<td>0.0032</td>
<td>1.8</td>
<td>0.006</td>
<td>2.2</td>
<td>2.5–3.4</td>
<td>20</td>
<td>Winkler</td>
<td>Comita, 1968</td>
</tr>
<tr>
<td><em>Diaptomus leptopus</em></td>
<td>0.0222</td>
<td>0.9</td>
<td>0.02</td>
<td>2.2</td>
<td>6.2–7.4</td>
<td>20</td>
<td>Winkler</td>
<td>Comita, 1968</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0.003–0.3</td>
<td>0.5–0.1</td>
<td>0.0015–0.03</td>
<td>250</td>
<td>6–12</td>
<td>20</td>
<td>Winkler</td>
<td>Schindler, 1968</td>
</tr>
</tbody>
</table>

(continued)
Table 10.1—(contd)

<table>
<thead>
<tr>
<th>Species</th>
<th>Ind. weight (mg)</th>
<th>Animals per ml</th>
<th>Approx. weight per ml (mg)</th>
<th>Volume of water (ml)</th>
<th>Period (hrs)</th>
<th>Temp. °C</th>
<th>Method</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Stenonema pulchellum</em></td>
<td>0-5-2</td>
<td>0-04</td>
<td>0-02-0-08</td>
<td>250</td>
<td>24</td>
<td>20</td>
<td>Winkler</td>
<td>Trama, 1972</td>
</tr>
<tr>
<td><em>Boeckella dilatata</em></td>
<td>0-0045</td>
<td>1-3-3</td>
<td>0-005-0-015</td>
<td>3</td>
<td>4-10</td>
<td>10-25</td>
<td>Winkler</td>
<td>Green &amp; Chapman, 1977</td>
</tr>
<tr>
<td><em>Gammarus pulex</em></td>
<td>1-14</td>
<td>0-07-0-44</td>
<td>0-44-0-93</td>
<td>45</td>
<td>2-10</td>
<td>2-15</td>
<td>Winkler</td>
<td>Nilsson, 1974</td>
</tr>
<tr>
<td><em>Mysis relicta</em></td>
<td>0-12-0-7</td>
<td>0-016-0-07</td>
<td>0-008-0-01</td>
<td>150-250</td>
<td>5-8</td>
<td>0-13</td>
<td>Winkler</td>
<td>Lasenby &amp; Langford, 1972</td>
</tr>
<tr>
<td><em>Diaptomus gracilis</em></td>
<td>0-008</td>
<td>6-7</td>
<td>0-05</td>
<td>100</td>
<td>2-24</td>
<td>5-20</td>
<td>Winkler</td>
<td>Kibby, 1971</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0-138</td>
<td>0-27</td>
<td>0-038</td>
<td>110</td>
<td>0-5</td>
<td>18</td>
<td>Electrode</td>
<td>Kersting &amp; v.d Leeuw-Leegwater, 1976</td>
</tr>
<tr>
<td><em>Limnocalanus macrurus</em></td>
<td>0-0001-0-034</td>
<td>0-5-12</td>
<td>0-0012-0-015</td>
<td>6-30</td>
<td>1-8-60</td>
<td>0-2-15</td>
<td>Electrode</td>
<td>Roff, 1973</td>
</tr>
<tr>
<td><em>Gammarus fossarum</em></td>
<td>0-3-5</td>
<td>1-6</td>
<td>0-5-8</td>
<td>80</td>
<td>0-17</td>
<td>5-23</td>
<td>Electrode</td>
<td>Franke, 1977</td>
</tr>
<tr>
<td><em>Mysis relicta</em></td>
<td>2-10</td>
<td>0-015</td>
<td>0-03-0-15</td>
<td>667</td>
<td>3</td>
<td>4</td>
<td>Electrode</td>
<td>Foulds &amp; Roff, 1976</td>
</tr>
<tr>
<td><em>Pisidium amnicum</em></td>
<td>0-2-9-0</td>
<td>1-4-2-9</td>
<td>0-57-12-9</td>
<td>0-35-0-7</td>
<td>10-30</td>
<td>3-20</td>
<td>CO²/IR</td>
<td>Holopainen &amp; Ranta, 1977</td>
</tr>
<tr>
<td>Blackfly larvae</td>
<td>0-025-1-5</td>
<td>0-5-1-1</td>
<td>0-28-0-75</td>
<td>10</td>
<td>0-5-1</td>
<td>15-5</td>
<td>CO²/IR</td>
<td>Wotton, 1978</td>
</tr>
<tr>
<td>Species</td>
<td>Ind. weight (mg)</td>
<td>N</td>
<td>Total weight (mg)</td>
<td>Chamber volume (ml)</td>
<td>Flow rate (ml h⁻¹)</td>
<td>Temp. °C</td>
<td>Sensor</td>
<td>Author</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----</td>
<td>-------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>----------</td>
<td>-------------------------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Open-flow systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Isoperla buresi</em></td>
<td>5.34</td>
<td>2</td>
<td>10.68</td>
<td>1.2</td>
<td>19.4</td>
<td>8</td>
<td>Dropping mercury electrode</td>
<td>Kamler, 1969</td>
</tr>
<tr>
<td><em>Cloëon dipterus</em></td>
<td>0.214</td>
<td>30–40</td>
<td>0.63–0.85</td>
<td>7.3</td>
<td>38</td>
<td>20</td>
<td>Dropping mercury electrode</td>
<td>Kamler, 1969</td>
</tr>
<tr>
<td><em>Tubifex barbatus</em></td>
<td>2</td>
<td>20–40</td>
<td>40.80</td>
<td>?</td>
<td>50–500</td>
<td>16</td>
<td>Dropping mercury electrode</td>
<td>Berg &amp; Jonasson, 1965</td>
</tr>
<tr>
<td>Mayfly larvae</td>
<td>?</td>
<td>10–100</td>
<td>?</td>
<td>?</td>
<td>60</td>
<td>8</td>
<td>Dropping mercury electrode</td>
<td>Nagell, 1973</td>
</tr>
<tr>
<td><em>Eurytemora hirundoïdes</em></td>
<td>?</td>
<td>11</td>
<td>?</td>
<td>1</td>
<td>1</td>
<td>5–20</td>
<td>Membrane electrode</td>
<td>Gyllenberg, 1973</td>
</tr>
<tr>
<td><em>Cyclops abyssorum</em></td>
<td>0.036</td>
<td>40</td>
<td>1.42</td>
<td>0.5</td>
<td>5.65</td>
<td>6</td>
<td>Twin flow respirometer</td>
<td>Gnaiger, 1982</td>
</tr>
</tbody>
</table>
Table 10.2 Comparison of 'closed bottle' and 'open-flow' experiments.

<table>
<thead>
<tr>
<th>'Closed bottle'</th>
<th>'Open-flow'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disturbance of animals by transfer results in increased respiration rates. Initial values can be rejected, if oxygen is monitored continuously.</td>
<td>Increased rates at the beginning can be ignored.</td>
</tr>
<tr>
<td>Oxygen is gradually depleted which can affect animals with oxygen concentration dependent metabolism.</td>
<td>pO₂ constant</td>
</tr>
<tr>
<td>Excretory products accumulate. Water stagnant, especially harmful for lotic animals. No possibility of measurement at constant reduced oxygen tensions. Volume may be large. Calculation simple.</td>
<td>Excretory products washed out. Water exchange at the body surface of the animal. Low pO₂ conditions can be simulated. Volume of the chamber must be small, otherwise calculations can be complicated (see Section 2.3.3.).</td>
</tr>
</tbody>
</table>

units and giving all results in terms of molecular volumes. One mole is a relatively large unit so that for freshwater animals μmol (10^{-6} mol) and nmol (10^{-9} mol) are usually the appropriate units. The use of the mole not only makes results more comparable but also allows an easier conversion to energy or carbon units (cf. Table 10.4).

2.3.2 'Closed bottle' methods

This type of technique includes all systems which enclose animals in a fixed volume of water. Ground glass stoppered bottles are often used, but there are other designs which keep the animals in a very small chamber fixed to an electrode tip or in a respiration chamber where water current is produced. The container may be of variable volume (see Table 10.1). The common feature of all these systems is that the oxygen concentration decreases during the experimental period and it is this reduction which is then measured.

A typical example of an experiment using chemical determination of oxygen would be as follows: seven glass bottles of known volume are filled with water from the same well-mixed container. Three bottles receive animals, while two bottles serve as final controls. All bottles are then stoppered and kept under the desired conditions. The remaining 2 bottles are titrated immediately to determine the initial oxygen concentration. The experiment is
terminated after some hours. Subsamples are taken from all bottles, avoiding air contamination, and the concentration of oxygen is determined. Animals are removed from the bottles and weighed. Oxygen consumption rates in the bottles with animals have to be corrected for the change in oxygen in the bottles without animals. The true oxygen consumption of the animals is:

\[
\text{Oxygen consumption (mg h}^{-1}\text{)} = \left( \frac{C_i - C_a}{t_a} - \frac{C_i - C_e}{t_c} \right) \times \frac{V}{1000} \quad (10.3)
\]

\(C_i\), \(C_a\), \(C_e\) are the oxygen concentrations (mg l\(^{-1}\)) of the initial samples, the bottles with animals, and the controls, respectively; \(t_a\) and \(t_c\) are the incubation periods of the animal bottles and the controls (h), and \(V\) is the volume of the bottles containing the animals (ml). If the incubation time is identical for all bottles the formula is reduced to:

\[
\text{Oxygen consumption (mg h}^{-1}\text{)} = \frac{(C_e - C_a) \cdot V}{t \cdot 1000} \quad (10.4)
\]

Multiplication by 31.25 converts mg to \(\mu\)mol. Oxygen consumption may be divided by the number of animals to yield the individual respiration rate (\(\mu\)mol ind\(^{-1}\) h\(^{-1}\)) or by the total weight of the animals to yield the specific respiration rate (\(\mu\)mol mg\(^{-1}\) h\(^{-1}\)).

No separate bottles are needed for the initial samples if oxygen is measured by a micro-technique using only a very small amount of water or by an electrode since the initial concentration in each bottle can be measured before it is closed. Alternatively, an electrode may be fixed in the bottle, allowing the oxygen concentration to be recorded continuously. The problem is that the water at the surface of the electrode must be moved. Teal & Halcrow (1962) used a magnetic stirrer, and in order to avoid disturbance of the animals by this stirring, they built a respiration chamber divided into 2 parts by a mesh screen. This mesh allowed mixing of the whole chamber but prevented the animals from coming into contact with the stirrer. Kersting & v.d. Leeuw-Leegwater (1976) used a self-stirring BOD oxygen probe in a similar bottle. Stirring can be omitted when the respiration chamber is very small; Davenport (1976) fixed a very small vial (0.8 ml) at the electrode tip and the water was stirred by the animals themselves.

The results obtained in replicate measurements are always variable. It is, therefore, absolutely necessary to have sufficient replicates. Some of this variability might be introduced by bacterial respiration and some investigators have used antibiotics to reduce the respiration of bacteria incidentally introduced into the vessel (e.g. Kamler 1969; Gyllenberg 1973). They reported that there was no effect on the respiration rate of the animals. Even if this is reasonable in extreme cases, when the oxygen consumption of
the controls is very high, one should avoid the additional stress probably introduced by the chemical if possible.

2.3.3 Open-flow systems

Some advantages and disadvantages of open-flow systems have already been discussed. Open-flow apparatus is usually more complicated than closed systems, and this may account for its infrequent use.

In principle, the concentration of oxygen is measured at the inflow and at the outflow of the animal chamber. Respiring animals reduce the oxygen content of the chamber water until a steady state is reached. Under steady state conditions the oxygen consumption is:

\[ \text{Oxygen consumption (mg h}^{-1}) = (\text{Concentration at inflow} - \text{Concentration at outflow}) \times \text{Flow per hour} \]  

(10.5)

Thus, the final difference in oxygen concentration is dependent on the metabolic rate of the animals and the flow rate of water but not on the size of the animal chamber. Flow rate and biomass of the animals have to be adjusted so that a sufficient difference in oxygen concentrations is obtained. The required size of the difference depends on the precision of the oxygen detection system.

The time needed for approaching the steady state depends on the 'system-flushing characteristic time':

\[ J(h) = \frac{\omega}{U} \]  

(10.6)

where \( \omega \) is the chamber volume (ml) and \( U \) is the velocity of flow (ml h\(^{-1}\)). A steady state will be established at a time greater than six times \( J \) (Propp et al. 1982). Animal size, flow rate, and chamber volume should, therefore, be in an optimal relation. Decreasing the flow rate increases the difference of oxygen concentrations before and after the chamber and the biomass can, therefore, be smaller. At the same time, however, it increases \( J \) so that the chamber must be small to prevent too long a 'response time' for the system. This is especially important if the response of the animals to sudden environmental changes shall be measured.

To avoid waiting for the establishment of a steady state, and to allow monitoring of changes of the metabolic activity with sufficient time resolution, equations have been developed which describe the time course of the oxygen concentrations in the chamber (Nimi 1978; Northby 1976; Propp et al. 1982). These equations allow the calculation of respiratory rates in a flow-through system for any time after the start of the experiment, but they are, however,
much more complicated than equation 10.5, which requires a system in the steady state.

A very simple open-flow system was described by Feldmeth (1971). The respiration chamber is a piece of thick glass tube closed at both ends with rubber stoppers. Glass tubes of smaller diameter pass through the stoppers, so that the chamber is open at both sides. At one side (outflow) the glass tube is formed into a siphon. The animal chamber is submerged in a large tank filled with water, so that the water can run out of the tank and through the animal chamber by means of the siphon. The siphon must be long enough and the tank large enough to assure a constant flow of water for some time. The flow is regulated by a clamp. The end of the siphon is placed at the bottom of a Winkler type oxygen analysis bottle, so the water runs into the bottle. Before oxygen is determined chemically, the bottle should have been flushed with at least twice its volume of water. Overflowing water is collected in a measuring cylinder to determine the flow rate.

Polarographic oxygen sensors allow some improvements to this system. The principle design of such a system is presented in Fig. 10.3a. Water is pumped very slowly from a reservoir through the experimental chamber to the electrodes, where pO$_2$ is measured. A second path leads directly to the electrode, avoiding the respiration chamber. The electrode can be supplied either with water from the chamber or directly from the reservoir by the use of two 3-way valves. When the system is switched into reference position, water flows through the chamber and the flow rate can be measured at the outlet. Oxygen consumption is calculated as the difference between the oxygen readings in the reservoir and the chamber multiplied by the flow rate.

The first systems of this kind used dropping mercury electrodes for the detection of oxygen (Mann 1958). A very detailed description is given by Klekowski & Kamler (1968) and a condensed description by the same authors (1971) is also available. The system was used successfully by Nagell (1973). Handling of dropping mercury electrodes is not easy, and some authors have replaced them with membrane electrodes. Gyllenberg (1973) used an electrode chamber which has a very small dead volume and does not need to be stirred. Similar systems have been used by Bulnheim (1972), and Scharf et al. (1981).

Open-flow systems with one electrode do not provide continuous measurements because recording is necessarily interrupted when the oxygen concentration of the reference water is measured. This disadvantage can be overcome by using two membrane electrodes, one upstream and one downstream from the animal chamber. Dropping mercury cannot be used for this purpose. A system with two electrodes, described by Dries et al. (1979), incorporated a valve which enabled the experimental medium to flow through the respiration chamber or to bypass it. Oxygen consumption was calculated automatically from the difference in the oxygen concentrations.
A further improvement is the principle of the twin-flow respirometer (Gnaiger 1982), presented in Fig. 10.3b. This system also contains two membrane electrodes, but each electrode can be used alternately before or after the animal chamber, allowing calibration of the electrodes during the experiment. The flow of water is regulated by two 4-way valves which can be switched simultaneously. In the position drawn in Fig. 10.3b, the electrode $E_1$ is the reference and electrode $E_2$ measures the oxygen content of the water which passed through the chamber. When the valves are turned by $90^\circ$, $E_2$ becomes the reference electrode and $E_1$ measures post-chamber oxygen concentrations, while the direction and magnitude of the water flow through the chamber does not change. Interruptions are reduced to the time taken for new equilibration of the electrode. The valves may be driven by a motor and a timer, so that long-term monitoring of oxygen consumption can be done automatically. The precision of the method is high since the electrodes are frequently recalibrated. To avoid dead space, all of the connecting tubes in a flow-through system should be small in diameter. This often causes problems
because tubings are permeable to oxygen, especially when water with low oxygen content is used. Therefore, gold capillaries are used in the twin-flow respirometer. Comparisons of the open-flow system with other methods have been made by Kamler (1969), Gyllenberg (1973), and Dries et al. (1979). Ultimately, however, the sensitivity of open-flow and closed bottle methods depend upon the precision of the oxygen determination method.

2.3.4 Chemical determination of dissolved oxygen

The most wide-spread method of measuring dissolved oxygen is the chemical determination described by Winkler (1888). The procedure is well known, and is described in all collections of water chemistry methods (e.g. Standard Methods 1976; Wetzel & Likens 1979). The reaction is based upon the oxidation of manganous hydroxide by the oxygen dissolved in the water, resulting in the formation of a tetravalent compound. In a second step, the solution is acidified and potassium iodide is oxidized, resulting in the liberation of free iodine. The number of moles of dissolved oxygen is equivalent to the number of moles of iodine liberated. Thus, when the iodine is titrated with a standard solution of sodium thiosulphate, this yields a measure of the oxygen present in the sample.

Carrit & Carpenter (1966) discussed the basic chemistry of the Winkler procedure and carried out intercalibration experiments which showed that there was a great variation in the results of participants from different institutions. The errors resulted mainly from photochemical oxidation of iodide and from loss of iodine through volatilization, but several other possible sources of errors have been pointed out by Carpenter (1965a), e.g. oxygen contributed by reagent solution and a difference between titration end-point and equivalence point.

The Winkler method has been the subject of many modifications which aim to reduce the errors, improve the accuracy by better titration, and to adapt it to very small volumes of water. Fox & Wingfield (1938) carried out reactions in a syringe pipette and used only 1.5 ml of water while obtaining 2% accuracy even at low concentrations. Several authors have used the micro-Winkler technique as described in Barnes (1959). The method applied by Ranta & Hakala (1978) is similar. By analyzing 3 ml and using an automatic burette (0.005 ml accuracy), they obtained an absolute accuracy of 0.0001 mg O₂ and a coefficient of variation of 2%.

The precision of the Winkler method is usually considered to be 0.02 mg O₂ l⁻¹ (Strickland, 1960), i.e. the standard deviation resulting from parallel experiments. There have been some attempts to improve this precision by using different titration techniques: Carpenter (1965b) provides a detailed description of a modification that results in 0.1% accuracy. Talling (1973)
reported a reproducibility of 0.02–0.04 mg O_2 l^{-1} with amperometric end-point titration. Bryan et al. (1976) achieved a precision of 0.01 mg O_2 l^{-1} using photometric end-point detection without starch. Tschumi et al. (1977) designed a 'phototitrator' consisting of a sensitive photometer and a motor-driven piston burette. After strictly standardizing all manipulations they found a standard deviation of 0.002–0.004 mg O_2 l^{-1} under laboratory conditions. With samples exposed in situ, this value was 0.005 mg O_2 l^{-1}. A similar photometric system was described in detail by Vargo & Force (1981).

Variation is always greater in natural waters than in pure laboratory experiments, and it is not always clear how the precision has been determined. The density of phytoplankton present is important, since some iodine may be adsorbed to the algae. In addition, oxidizing and reducing substances may interfere with the reactions.

The error attributed to organic substances is avoided by the iodine difference method developed by Ohle (1953), but this cannot always be used because two subsamples are needed for comparison. Some problems (e.g. volatilization of iodine, interference of dissolved organic carbon) may be overcome by using the ceriometric method (Golterman & Wisselo 1981), which is similar to the Winkler procedure, but is based on the oxidation of Ce(OH)_3. Its precision is said to be 1% but this may be improved. It has not yet been used in respiration studies.

2.3.5 Polarographic sensors

When a platinum electrode in water is held at a potential of about −1 volt to a silver-silver oxide reference electrode, molecular oxygen is reduced at the platinum surface, causing a current to flow. This current is proportional to the amount of oxygen that comes into contact with the platinum and these electrodes can, therefore, be used to measure the concentration of oxygen present. The current will not be stable for long in a medium which is not completely pure, however, because the platinum surface becomes covered by metals plating out or by other materials that reduce the access of oxygen to the electrode.

There are two ways of overcoming this problem. A dropping mercury electrode (Briggs et al. 1956) may be used in which the surface of the electrode is frequently renewed. The second possibility is to cover the platinum electrode with a thin membrane (Clark et al. 1953) which is permeable to oxygen but impermeable to impurities. Both systems have advantages and disadvantages. Klekowski (1971a) discusses these and favors the dropping mercury electrode, but 10 years of experience and improved equipment have rendered many of his arguments against the membrane electrode unimportant. Membrane electrodes are much more convenient than the dropping mercury electrode,
since safe handling of mercury is not easy, especially when it is distilled or highly purified. Moreover, measurements should be made outside of the animal container in order to avoid possible mercury toxicity. Handling inconvenience may be the reason that the dropping mercury electrode is not used more frequently, whereas a membrane oxygen sensor can be found in most laboratories.

Recently, many good commercial sensors have become available so that there is no need to produce them by hand (see Teal 1971) for routine purposes. The cathode is usually made of platinum, or sometimes of gold, and the anode is silver. Membranes are usually made of polyethylene, teflon or polypropylene. A very small amount of electrolyte solution (e.g. KOH, KHCO₃, phosphate-NaCl buffer) is placed between the membrane and the electrode head. In the electrode reaction, oxygen from the buffer is consumed and replaced by diffusion through the membrane. After some period of exposure, a steady-state is reached between consumption and replacement. A strong current of water must be generated to avoid depletion of oxygen in the boundary layer at the surface of the membrane, although this is not necessary if a specially designed electrode chamber with a 70 µl sample volume is used. For more technical comments on the functioning of electrodes refer to Carey & Teal (1965), Fatt (1976), Hitchman (1978), or Gnaiger & Forstner (1982).

An electrode for use in respiration experiments should have three special properties:

1. The oxygen consumption of the electrode should be low. By using very small cathodes, modern sensors have a very low consumption—it can be<br>\[ \text{<0.1 µg O}_2 \text{ h}^{-1} \text{ p.p.m. oxygen}^{-1} \].
2. The response time should be rapid. This is dependent on the material, the thickness of the membrane and the temperature. When the oxygen concentration is changed, 99% of the new value should usually be reached within 1–2 min.
3. Precision of measurements should be high. Sensitivity of the respiration measurement depends mainly on the precision of the electrode, i.e. the error obtained by repeatedly measuring the same sample.

As the quantity to be estimated is the difference between oxygen concentrations, the 'accuracy', i.e. the closeness of a measured value to the 'real' oxygen consumption is not as important as the precision. With some skill a precision of 0.01–0.02 mg O₂ 1⁻¹ may be reached. One factor determining the precision of measurement is the stability of the system—a good electrode shows a drift of \( \leq 1\% \) per day. How long an electrode can be used without renewing the membrane depends on the material and thickness. Precise electrodes have thin membranes (about 20 µm), which have to be
replaced more often. Nevertheless, an electrode should maintain its quality for some weeks.

Polarographic oxygen sensors measure partial pressure of oxygen in water, thus they have to be calibrated frequently. This is usually done by a two-point calibration. For adjustment of the zero-point, solutions of sodium sulfite, sodium dithionate or a commercially available ‘zero-solution’ can be used. Saturated water is measured for the second reference point. The water must be equilibrated carefully. The partial pressure of oxygen is then calculated according to the barometric pressure.

\[ pO_2 = (p_a - p_w) \cdot 0.2093 \]  

(10.7)

where \( pO_2 \) is the desired partial pressure (mmHg), \( p_a \) is the barometric pressure (mmHg), and \( p_w \) is the partial pressure of steam at the given temperature, which may be taken from tables (e.g. Opitz & Bartels 1955; Chemical Rubber Company 1976). The sensor is then immersed into the saturated water and the display is set to the calculated partial pressure. Calibration of the ‘slope’ by saturated water has to be done more frequently than zero-point calibration. In experiments at very low oxygen tensions, this type of calibration may not be sufficient. Even at zero oxygen, a sensor shows a small residual current which may be unstable, and under extreme conditions, calibration by a series of measurements on different levels of oxygen is recommended (Gnaiger 1982). Oxygen content can be calculated according to:

\[ O_2 (mg l^{-1}) = \frac{S \cdot pO_2}{760} \]  

(10.8)

where \( S \) is the solubility of oxygen at a certain temperature under standard pressure, and \( pO_2 \) is the measured partial pressure (mmHg). Values for the oxygen content of air-saturated water can be taken from commonly used tables (e.g. Hitchman 1978; Wetzel & Likens 1979; Mortimer 1981). The preparation of saturated water often needs more time than expected and may be unsatisfactory. A check of the calibration by Winkler titration can, therefore, be useful.

### 2.3.6 Volumetric methods

When oxygen is consumed from a given gas volume by respiring animals, and when the CO\(_2\) evolved is trapped simultaneously, the resulting change in gas volume is a direct measure of oxygen consumption. The best known system of this type is the Warburg apparatus, but because shaking of the respirometer flasks may disturb the animals (Wightman 1977), it cannot be recommended. Richman (1958) found the Warburg apparatus unsuitable for *Daphnia*
because the animals were caught in the surface film. When the volume of water is small, shaking can be omitted since gas exchange between water and air by diffusion is sufficiently rapid. Two basic types of gasometric respirometers exist: those in which pressure in the system may change and those in which pressure is kept constant.

In the simplest system (Fenn 1927), which has been used for aquatic animals by Obreshkove (1930) and Shcherbakoff (1935), pressure is allowed to change. Two vessels, one containing water with animals, the second containing only water, are connected by a capillary (Fig. 10.4a) in which a

Fig. 10.4 Schematic drawings of gasometric devices for measurement of respiration: (a) Constant volume, changing pressure (Obreshkove, 1930). (b) Constant pressure, changing volume (Klekowski 1975). AC, animal chamber; TB, thermobarometer; A, CO₂ absorbant; ID, indicating drop; P, threaded piston; M, mercury.
drop of kerosene is allowed to move. Both vessels contain a spoonlike device to hold a piece of filter paper soaked with NaOH for trapping the CO₂. The system is immersed in a water bath to maintain constant temperature. Two valves allow connection of the chambers to the open air and equilibration. When the valves are closed and the oxygen in one bottle is consumed, the kerosene indicator drop begins to move, and its movement per unit time is recorded. The capillary must be calibrated for the calculation of the volume of oxygen consumed. As the reduction of pressure is shared equally between the two vessels, the volume of oxygen is twice the volume calculated from the distance the drop moved and the cross-sectional area of the capillary.

This system has been improved by holding the pressure constant and changing the volume of the system (Fig. 10.4b) in a micro-respirometer described by Scholander et al. (1952). Klekowski (1975) used the same principle. His respirometer, originally designed for terrestrial animals but also used for aquatic ones (e.g. Swift 1976), also consists of two vessels, one containing the animals, the second serving as a thermobarometer. Both vessels contain filter paper saturated with KOH or NaOH, and there are two connections between them, one allowing equilibration, the other, a capillary containing a kerosene drop. The vessel containing the animals is connected to a capillary filled with mercury which is attached to a threaded piston. By turning the piston, the level of mercury in the capillary is moved. At the beginning of an experiment the valve in the equilibration tube is closed. The indicator drop then starts to move and is kept at the original position by gently turning the piston. Thus, the reduction of the volume is compensated by movement of the mercury. The mercury capillary can be calibrated so that the volume of oxygen consumed can be read from the revolutions of the piston.

Scholander et al. 1952 demonstrated how CO₂ evolution can be estimated from the difference between experiments with and without absorbant. The absorbant can be removed between two trials or the container holding the filter paper may be closed by a lid without opening the system. However, if the medium is highly buffered the evolution of CO₂ can be confounded, because the gas is directly absorbed into the water. For the same reason an absorbant is not usually necessary if the water is buffered well.

The volumetric respirometers described above are made for oxygen uptake rates of the order of 1 μmol O₂ h⁻¹. A much more sensitive apparatus, described by Klekowski (1977), uses containers in the shape of a Cartesian diver (see Section 2.3.7) but is modified for shipboard work where a freely floating diver cannot be used. The animal is contained in a drop of water separated from a drop of NaOH solution by a small volume of air. As oxygen from the air is consumed and CO₂ is absorbed, the air volume becomes smaller. The respiration chamber is connected to a thin capillary tube with an indicator bubble of air which moves when oxygen is consumed. The bubble is
kept at a fixed position by changing the pressure in the system. This respirometer can be used for measurement of oxygen consumption in the range from 0.005 nmol to 0.02 μmol O₂ h⁻¹, i.e. from single small nauplii to adult copepods.

2.3.7 Cartesian and gradient divers

'Divers' are the most sensitive tool for respiration measurement and most studies with very small animals, such as rotifers, have used this type of technique (see Fig. 10.2 and Table 10.1). Even though the use of divers requires some skill, the relatively simple equipment can usually be made by the investigator. The Cartesian diver is a constant volume, variable pressure system in which the animal is enclosed in a small drop of water in a specially designed container (see Fig. 10.5). In addition to water, the container holds an air bubble. The entire 'diver' floats freely in a medium (0.1 NaOH) and is enclosed in a floating vessel which is connected to a manometer filled with Brodie's fluid (Fig. 10.6). The pressure in this closed vessel can be regulated by means of coarse and fine adjusting screws. The diver is equilibrated in the floating medium by adjusting the pressure. Because the bubble enclosed in a

![Fig. 10.5 Stoppered diver. 1, diver chamber; 2, chamber head; 3, gas bubble; 4, 0.1 N NaOH solution; 5, stopper. (From Klekowski, 1971b).](image-url)
'stoppered diver' is connected to the surrounding medium, an increase in pressure results in a decrease in the volume of the bubble. Thus, the specific weight of the suspended diver becomes larger and it sinks. If the pressure is decreased, the bubble volume is enlarged and the diver moves upwards. By carefully adjusting the pressure, the diver will stay at its position as long as the volume of the gas is not changed. When an animal inside the diver respires, it consumes oxygen and produces CO₂ which is absorbed by the NaOH solution. The diver sinks and the pressure must be lowered to bring it back to its original position.

Between two measurements the pressure is held at its original level to prevent oxygen moving from the water phase to gas phase, and during this time the diver remains at the bottom of the vessel. From time to time the pressure is lowered until the diver reaches its original position and the changes in pressure are recorded. Eight to ten pressure vessels can be used
simultaneously. To calculate the volume of oxygen consumed one must know the volume of the air bubble, which is the so-called 'diver constant'. The oxygen consumption \( (\text{VO}_2; \mu l \text{ O}_2 \text{ h}^{-1}) \) is calculated according to the formula:

\[
\text{VO}_2 = \frac{V_g \cdot \Delta P \cdot 273}{P_0 \cdot \Delta t \cdot T}
\]

where \( V_g \) = diver constant in \( \mu l \), \( \Delta P \) = change of equilibrium pressure (mm Brodie solution), \( P_0 \) = normal pressure (10000 mm Brodie solution), \( T \) = water temperature (degrees Kelvin), \( \Delta t \) = time between readings.

Klekowski (1971b) gives a very detailed and useful description of the system, including the preparation, filling, handling, and calibration of the divers, the experimental protocol, and also includes a literature review.

When the divers are operated in a density gradient instead of a homogeneous flotation solution, the system can be open to the atmosphere and need not even be temperature controlled. The theoretical basis and a test of suitability of 'gradient divers', along with practical recommendations are given by Nexo et al. (1972). Both closed divers (ampulla) and stoppered divers are in use. The ampulla diver is more sensitive but the stoppered diver can be used repeatedly. A 'control' diver is put into the gradient and finds a steady equilibrium position. If an animal is enclosed, which reduces the gas volume, the diver will sink according to its specific gravity. Control and experimental divers both perform migrations due to changes in barometric pressure and temperature. The oxygen consumption rate is calculated by measuring the difference in position change between control and experimental divers.

Linear gradients are made of \( \text{H}_2\text{O} \) and \( \text{Na}_2\text{SO}_4 \) solutions (density \( \sim 1.06 \text{g ml}^{-1} \)) and their preparation takes 2–3 h. Density standards (calibrated glass beads) are needed to determine the steepness of the gradient. The sensitivity of the method depends on the precision with which the diver's migration is measured and on the determination of the diver constant. Reading accuracy of the diver's position is \( \pm 0.5 \text{ mm} \) with binocular magnification, \( \pm 0.02 \text{ mm} \) with photographic recording, and \( \pm 0.005 \text{ mm} \) when a cathedometer is used (Nexo et al. 1972). Total error in the determination of the diver constant is in the order of 5% (Hamburger 1981). For animals with a respiratory rate of \( < 10^{-3} \mu l \text{ O}_2 \text{ h}^{-1} \), ampulla divers are recommended (Hamburger 1981). For these divers, the reading accuracy of the oxygen consumption is in the order of \( 10^{-4}–10^{-6} \mu l \) (Nexo et al. 1972). Technical advice for making ampulla divers, density gradients and glass beads for calibration is given by Klekowski et al. (1980).

Reading of the diver's position is tedious, so that there have been several attempts to develop automatic recording techniques. One method involves photographic documentation in a density gradient. Another possibility is the
use of an 'electromagnetic' diver which is held in position by the magnetic force generated by a coil located beneath the vessel. This coil starts working when the sinking diver interrupts a light beam, as described by Løvtrup (1973).

2.3.8 Oxycaloric equivalents

When an energy budget is constructed, the energy loss is rarely measured directly as heat production but is usually calculated from oxygen consumption. This is done by use of an energy equivalent, $Q_{ox}$ which, for a given amount of oxygen consumed, depends on the substrate burned, i.e. on the proportion of carbohydrate, fat, and protein. Numerous different values for $Q_{ox}$ can be found in the literature, arising from different substrates. A critical revision of published values and new calculations were made by Elliot & Davison (1975).

Published values of $Q_{ox}$ for carbohydrate agree well with each other, and only a small amount of variation is found among $Q_{ox}$ values determined for fats. In the catabolism of protein, however, oxidation is incomplete and the oxycaloric equivalents will not only depend upon the amino acid composition of the protein, but also upon the nitrogenous compounds excreted. This is not very complicated for aquatic animals because, with rare exceptions, they all excrete ammonia. According to Elliot & Davison (1975), the energy lost in excreta when ammonia is the end product is $82.9 \text{kJ mol}^{-1} \text{O}_2$. These authors recalculated $Q_{ox}$ values for a 'standard protein'. Their energy equivalents for ammoniotelic animals are assembled in Table 10.3.

There is still some discussion about the correct oxycaloric equivalents for protein, generally due to the questions which surround the amino acid composition of the proteins. Gnaiger (personal communication) assumes that the $Q_{ox}$ for protein should be about 5% higher than the reported value. The main source of error, however, results from the fact that one does not know which substrate is being burned. Table 10.3 shows that the difference

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Oxycaloric equivalent ($Q_{ox}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cal mg$^{-1}$</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>3.53</td>
</tr>
<tr>
<td>Fat</td>
<td>3.28</td>
</tr>
<tr>
<td>Protein</td>
<td>3.20</td>
</tr>
</tbody>
</table>
between the $Q_{ox}$ for pure carbohydrate and pure protein is of the order of 10%. The true $Q_{ox}$ will be between the value for pure protein and that for pure carbohydrate. Some evidence about the substrate can be gained from the RQ (see Section 2.4.5), but a conversion will always include some uncertainty, and this is an argument for the use of direct determination of heat production.

2.4 Excretion of carbon dioxide

2.4.1 General

Very often, parameters of the energy budget are estimated by completely different methods; e.g. energy intake is estimated using $^{14}$C-labeled material, growth by increases in dry weight, and metabolic losses by oxygen consumption. All of these measurements must be converted to the same units. To express all parameters of the above example in units of energy one has to know the specific activity and the caloric value of the food, the energy content per dry weight of the animals at the beginning and at the end of the experiment, and the proper oxycaloric equivalent. Since all conversions may introduce specific errors into the budget, it would be better to measure all components of the budget directly in the same units. For example, if $^{14}$C is used to measure intake, then it would be logical to measure everything else in terms of carbon, without any conversion factors, i.e. growth as increase of body carbon and respiration as CO$_2$ losses. There is, therefore, a strong argument for measuring respiration as CO$_2$ excretion. Moreover, the ratio of CO$_2$ output to oxygen consumption can provide some insight into the metabolic substrate.

Unfortunately, methods of measuring CO$_2$ release in water are not as well developed as measurements for oxygen. This is due to the fact that CO$_2$ is not only dissolved in the water, like oxygen, but is partly bound to the carbonate buffer system. The pool of inorganic carbon is usually rather large, except in waters with very low alkalinity, so that the small difference produced by respiring animals is difficult to measure. Only part of the CO$_2$ produced by the animals remains free; the pH determines the proportion of the CO$_2$ that becomes bound chemically (see e.g. Stumm & Morgan 1981).

CO$_2$ electrodes are available (Jensen et al. 1966) but because they measure pCO$_2$ they cannot be used as a single measure in a buffered system; the pH must be measured simultaneously to calculate the amount of CO$_2$ that has been converted into bicarbonate. Moreover, pCO$_2$ electrodes are not as easy to handle as oxygen sensors, are relatively slow, and must be calibrated more frequently. They have, therefore, rarely been used for studies of the respiration of aquatic animals (Gyllenberg 1973). Good precision may be attained using
the volumetric methods mentioned above (Section 2.3.6), but CO₂ is only a by-product of measuring the oxygen consumption. Calculation of the respiratory quotient (see Section 2.4.5) is the main aim of CO₂ measurement in most studies; only a few studies use carbon dioxide production as the only measure of animal metabolism. More effort should be put into the measurement of CO₂ excretion.

2.4.2 Change in pH

In a closed system, differences of pCO₂ can be estimated from changes of pH (Verduin 1951; Beyers 1963): the pH of a given water depends on the dissolved CO₂ concentration, so that changes in the CO₂ content result in changes in the pH. The relationship between CO₂ concentration and pH is not linear, however, and a calibration curve must be constructed for each particular water by stepwise titration (Beyers et al. 1963). Measurement of pH must be carried out very carefully: a pH meter with a resolution of 0.001 pH should be used (Raymont & Krisnaswamy 1968). This technique has been applied by Richman (1958) and Kibby (1971).

2.4.3 Infra-red analysis

Direct determination of CO₂ is based on extraction and measurement of CO₂ in the water. This has been done by trapping the CO₂ from a stream of air recirculating through the water sample in a closed system (Aldrich 1975), but more often the absorption of infra-red by CO₂ is measured. Holopainen & Ranta (1977) incubated their animals in small cuvettes (0.35–0.7 ml) with sterilized, aerated water for 10–30 h. After the incubation, subsamples of the water were acidified below pH 4 and the CO₂ was extracted by bubbling a carrier gas through the water. CO₂ concentration in the gas was then measured by an infra-red analyser. This method measures total CO₂, and small differences are difficult to detect if the carbonate content of the water is high. In some experiments the authors removed carbonate before the experiment in order to improve the sensitivity: the water was titrated with HCl to pH 5.1, aerated to eliminate CO₂, and titrated back to neutral pH with NaOH. The same system has been used by Wotton (1978) and Ranta & Hakala (1978). The latter authors report an accuracy of 0.01 μg CO₂ and a coefficient of variation of 1%.

Difficulties in making precise measurements of CO₂ concentration in buffered water may perhaps be overcome by improvements in the method of Teal & Kanwisher (1968). Air is continuously circulated through the water in a closed system; thus an equilibrium of CO₂ is established between water and air. CO₂ concentration in the gas phase is determined by infra-red absorption. If animals in the water produce CO₂ the equilibrium concentration in the air
will change, and small differences can be detected since it is not the large quantity of bound CO₂ which is measured but only the change in the gas phase. CO₂ is calculated from the change of equilibrium CO₂ and a previously determined relationship between pCO₂ and total CO₂, unique for each particular water.

2.4.4 Isotope radiotracer method

Respiratory carbon losses can be measured very sensitively by measuring the ¹⁴CO₂ output of animals whose tissues have been homogeneously labeled with ¹⁴C (Sorokin 1968). In order to do this, animals are grown for some time with labeled food, which can be prepared by providing algae with ¹⁴C-bicarbonate or bacteria with ¹⁴C-glucose. The period of feeding with radioactive food must be long enough to ensure that the animals are homogeneously labeled—ideally, they are raised from birth with food of the same specific activity.

To measure the CO₂ release, the animals are rinsed and transferred into clean water. This can be done in an open vessel if the pH of the water is above 8 (Sorokin 1968). After some time, the pH of an aliquot is raised and it is passed through a membrane filter to remove labeled particles produced by the animals. The ¹⁴CO₂ in the water is then measured and the specific activity of the animals is determined. The ¹⁴CO₂ may be precipitated as Ba¹⁴CO₂ but a more convenient method is to measure the ¹⁴C activity directly in a liquid scintillation counter (LSC), using a fluor for aqueous samples.¹ Because excretion of labeled dissolved organic carbon may occur, a second subsample also has to be acidified, bubbled and measured in the LSC. The excreted ¹⁴CO₂ is calculated from the difference in activity between these two types of samples.

The sensitivity of the method depends mainly on the specific activity of the animals. When the water is put directly into the scintillation vial, up to 7 ml can be counted per sample. The sensitivity can be improved if a larger amount of water is acidified, the CO₂ being extracted by bubbling with nitrogen and then caught in a small amount of absorbant (e.g. Phenethylamine). Carbon losses can be calculated:

\[ \text{L}_c = \frac{\text{DPM}_w \cdot V \cdot 100}{\text{DPM}_a \cdot \Delta t} \]  

(10.10)

¹ Note: Some of the liquid scintillation cocktails for aqueous samples are acidic; therefore, the CO₂ will escape from the fluor into the overlying air or through the wall of the plastic vial. Significant losses result from this effect. This problem can be eliminated by adding to the sample a small amount of absorbant, which forms a carbamate with the CO₂.
where \( L_c \) = carbon loss in % of body carbon \( h^{-1} \), \( DPM_w \) = radioactivity of \( CO_2 \) ml\(^{-1}\) water, \( DPM_a \) = radioactivity of the animals, \( V \) = container volume, \( \Delta t \) = experimental time period (h).

Carbon dioxide production can be calculated:

\[
CO_2 (\text{mmol} \ h^{-1}) = \frac{DPM_w \cdot C \cdot V \cdot 0.0833}{DPM_a \cdot \Delta t}
\]  

(10.11)

where \( C \) = carbon content of the animals (mg).

The radiotracer method is more sensitive than all of the other methods mentioned and can be recommended, but some comments are necessary, because Sorokin's (1968) description contains errors. After removing the animals from labeled food, he fed them unlabeled food for 3-4 h. He also states that the animals may be fed unlabeled food during the experiment. However, with regard to the \( CO_2 \) output, an animal must be considered to be at least a two compartment system (Lampert 1975). These compartments include a 'metabolic pool' that turns over rapidly and a 'structural pool' that turns over more slowly. If a labeled animal eats unlabeled food, its structural pool retains a high specific activity, but the specific activity of the metabolic pool decreases due to dilution by unlabeled food. Thus, the released \( CO_2 \) has a much lower specific activity than the animal, and calculated respiration rates are underestimates. For this reason, animals must have no access to unlabeled food. If the gut must be emptied to avoid interference from the gut content, this must be done using inorganic material as a 'food' source rather than with unlabeled metabolizable food. The animal can be fed during an experiment (e.g. to measure the influence of the food on the respiration rate), but the food must have the same specific activity as the animal. Controls must be performed to account for \( CO_2 \) release by the food and these controls must be corrected for the amount of food eaten by the animals.

2.4.5 Respiratory quotient

The respiratory quotient (RQ) is needed if oxygen consumption values are to be converted to \( CO_2 \) excretion or vice versa. It is also needed if one wishes to convert respiration rates to energy equivalents. RQ is defined as the ratio:

\[
\frac{\text{vol. } CO_2 \text{ evolved}}{\text{vol. } O_2 \text{ consumed}}
\]

Because both oxygen consumption and \( CO_2 \) excretion must be measured to calculate RQ, there are not very many measurements available in the literature.

The respiratory quotient depends on the substrate metabolized. When carbohydrate is metabolized, the RQ is 1.0, but because fat oxidation requires
more oxygen than carbohydrate, the RQ of fat is lower (about 0.7). Many textbooks give the RQ for protein metabolism as about 0.8 but Elliot & Davison (1975) point out that this is only true for ureotelic animals. Because ammonia is the major excretory product of nearly all aquatic animals, the RQ is 0.949. It may even be a little higher than 1 (Gnaiger personal communication), because the calculation depends on the composition of the 'standard' protein considered. When carbohydrates are transformed into fat, values of RQ > 1.0 can be found. Normally, no pure substrate will be burned, so that the RQ will be somewhere in the range mentioned.

RQ values reported in the literature cover this entire range. Variations within species are very large—for Mysis relicta, Ranta & Hakala (1978) found respiratory quotients ranging from 0.61 to 1.17 (mean 0.96 ± 0.13). Moshiri et al. (1969) found that the RQ increased from 0.61 to 0.95 with temperature in Leptodora kindtii. Part of this variation may be due to the relatively large errors inherent in the CO2 analysis. Another factor involved may be the nutritional state of the animals. Richman (1958) studied the effect of starvation on the RQ of Daphnia pulex. Animals were fed and then starved for 6 days. During this time they showed considerable weight loss. Initially the RQ was 0.92–1.24 (mean 1.03); this dropped to 0.71 after 6 days of starvation. The decrease was due to a reduction of CO2 excretion, whereas the oxygen consumption on a weight basis did not change.

Little is known about the RQ of actively feeding aquatic animals. From experiments of Bohrer & Lampert (in preparation) it seems that low food concentrations have the same effect as starvation in Daphnia magna. RQ increases from 0.7 at very low concentrations of algae to 1.1 at high concentrations.

Differences in the nutritional state may also be responsible for the great seasonal variations in RQ detected by Kibby (1971). During winter, Diaptomus gracilis exhibited a respiratory quotient of 0.74 which shifted to 1.25 during summer. Temperature may also contribute to this effect: Grainger (1956) showed that sudden temperature changes caused strong fluctuations in the RQ.

The great variability of reported respiratory quotients may be a problem when comparisons are to be made. Energy budgets are usually constructed for fed animals, however, and all reported values for animals in good nutritional state are rather high. Thus, it may be reasonable to use an RQ near 1 in this case. However, any error inherent to the RQ affects the result of the calculation linearly. If, e.g., carbon or weight losses of an animal under poor food conditions are calculated from oxygen consumption and an RQ of 1 is erroneously applied instead of 0.7, the losses will be overestimated by 42%. This may have serious consequences for the budget (see Introduction and Fig. 10.1).
2.5 ETS activity

Recently, a completely different method of estimating the metabolic activity of aquatic organisms has been developed (Packard 1971), based on a biochemical determination of the activity of the respiratory electron transport system. Owens & King (1975) have introduced a modification of Packard's method, designed especially for zooplankton.

The idea is that there should be a correlation between the rate of oxygen consumption and the activity of the biochemical structures in the cells which consume the oxygen. These structures are the mitochondrial and microsomal respiratory electron transport system (ETS), a complex chain of cytochromes, flavoproteins, and metallic ions that transports the electrons from catabolized substrate to oxygen. Because the system is complex, the activity of the key enzymes controlling oxygen utilization must be measured in the step that limits the rate of electron 'transport. This is the oxidation of the coenzyme Q–cytochrome B complex. It can be measured using the artificial electron acceptor 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT), together with a suitable substrate. The animals are collected from the field, homogenized immediately, centrifuged at 0–4°C and incubated with

Table 10.4 Conversion factors for use in studies of respiration.

<table>
<thead>
<tr>
<th>Conversion Factor</th>
<th>Conversion Factor</th>
<th>Conversion Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mol O₂ = 32 g = 22.393 litres</td>
<td>1 mg O₂ = 31.25 μmol</td>
<td>1 ml O₂ = 44.66 μmol</td>
</tr>
<tr>
<td>1 mg O₂ = 0.7 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mol CO₂ = 44.01 g = 22.262 litres</td>
<td>1 mg CO₂ = 22.72 μmol</td>
<td>1 ml CO₂ = 44.92 μmol</td>
</tr>
<tr>
<td>1 mg CO₂ = 0.506 ml</td>
<td>1 mg CO₂ = 0.272 mg C</td>
<td>1 ml CO₂ = 0.5395 mg C</td>
</tr>
<tr>
<td>1 μmol O₂ = 1 μmol C = 0.012 mg C*</td>
<td>1 ml O₂ = 0.5363 mg C*</td>
<td>1 mg O₂ = 0.3753 mg C*</td>
</tr>
</tbody>
</table>

* to be multiplied by RQ

Conversion of a measured gas volume to S.T.P. conditions:

\[ V_{(S.T.P.)} = V_a \cdot \frac{P_a \cdot 273}{760 \cdot T} \]

\( V_a = \) actual volume, \( P_a = \) actual barometric pressure in mmHg, \( T = \) temperature, degrees Kelvin.)
INT, NADH, NADPH, buffer and some other chemicals (for details see Packard 1971; Owens & King 1975). The sample is measured photometrically. A refrigerated centrifuge is needed for sample preparation.

ETS activity must be calibrated against some other measure of respiration rate—ratios of ETS activity to oxygen consumption are given by King & Packard (1975). They range from 0.54 to 2.16, depending on the species considered. The ratio is not influenced by temperature and is only slightly affected by the weight of the organisms.

The ETS activity shows a slow response to environmental changes; a new equilibrium of ETS activity is reached 3-4 h after a change in environmental conditions (Båmstedt 1980). It cannot, therefore, be used for the detection of rapid changes in the respiratory rate, but gives an estimate of the upper limit of oxygen consumption under given conditions. When used to measure the in situ respiration of a population in vitro, this slow response is an advantage because the animals are not handled prior to the experiment, and the results are not affected by short-term changes in factors associated with the sampling procedure. To measure the ETS activity, the animals should be processed immediately and held in the laboratory for as short a time as possible.

2.6 Conversions

As respiration is measured by so many different methods, conversions are necessary for comparisons. Table 10.4 gives the most important conversion factors, and others may be derived from these.

3 Factors Affecting the Respiratory Rate

3.1 Endogenous factors

3.1.1 Body size

Over a wide range of body size, there is a general trend in the respiration rate, both within and among species. Larger individuals consume more oxygen than smaller ones, but the increase of the respiration rate is slower than the increase of the weight. Thus, on a weight basis, the respiration rate (oxygen consumption mg⁻¹ weight) decreases with increasing size of the animals (Zeuthen 1953, 1970).

The most common way of expressing the functional relationship between oxygen consumption and body weight is a power function \( y = ax^b \) (or in logarithmic form: \( \log y = \log a + b \log x \)). The coefficients (a) and (b) are easily obtained by plotting the data on a double logarithmic scale and by calculating the appropriate linear regression. Log (a) is given by the intercept of the
regression line with the ordinate and (b) by the slope of the regression line (cf. Fig. 9.2). However, some inaccuracy is inherent to this procedure (see Chapter 7) and an iterative method (Glass 1969) may give more precise results. Despite this, the measured relationships for aquatic animals are usually presented as power functions \(R = aW^b\); \(\log R = \log a + b \log W\), giving the individual respiration rate, or in the weight specific form:

\[
\frac{R}{W} = a \cdot W^{(b-1)}; \quad \log \frac{R}{W} = \log a + (b - 1) \log W \tag{10.12}
\]

where the respiratory rate is given per unit weight.

Ever since Zeuthen (1953) showed that interspecific comparisons of rates of oxygen consumption yield similar slopes over a wide range of sizes, the exponent \(b\) has been in the centre of interest. He found \(b = 0.95\) for metazoa containing <1 mg N. Sushchenya (1970) obtained a value of 0.75 for a number of crustaceans. The slope of the regression line calculated for the literature data presented in Fig. 10.1 is about 0.8 and not significantly different from 0.75. A value of 0.75 is generally accepted for large scale interspecific comparisons of 'basal' or 'standard metabolic rate' (Hemmingsen 1960; Lavigne 1982), but the variability of the exponent is greater when a single genus or species is considered. Environmental factors like temperature may affect small and large animals differently and thus change \(b\) (Newell & Roy 1973; Holopainen & Ranta 1977). To show the possible range, some examples are listed in Table 10.5:

A linear relationship between weight and respiratory rate was found by Trama (1972) for the ephemeropteran *Stenonema pulchellum*.

**Table 10.5 Values of \(b\) from the literature.**

<table>
<thead>
<tr>
<th>Species</th>
<th>(b)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Daphnia pulex</em></td>
<td>0.882</td>
<td>Richman (1958)</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0.816</td>
<td>Kersting &amp; v.L.L. (1976)</td>
</tr>
<tr>
<td><em>Diaptomus</em> (5 species)</td>
<td>0.626</td>
<td>Comita (1968)</td>
</tr>
<tr>
<td><em>Diaptomus</em> (3 species)</td>
<td>0.517</td>
<td>Siefken &amp; Armitage (1968)</td>
</tr>
<tr>
<td><em>Diaptomus gracilis</em> (average)</td>
<td>0.615</td>
<td>Kibby (1971)</td>
</tr>
<tr>
<td><em>Limnocalanus macrurus</em></td>
<td>0.698</td>
<td>Roff (1963)</td>
</tr>
<tr>
<td><em>Chaoborus trivittatus</em></td>
<td>0.598-0.885</td>
<td>Swift (1976)</td>
</tr>
<tr>
<td><em>Mysis relicta</em></td>
<td>0.957</td>
<td>Ranta &amp; Hakala (1978)</td>
</tr>
<tr>
<td><em>Pisidium amnicum</em></td>
<td>0.795-0.881</td>
<td>Holopainen &amp; Ranta (1977)</td>
</tr>
<tr>
<td>3-10°C</td>
<td>0.579</td>
<td></td>
</tr>
<tr>
<td>20°C</td>
<td>0.71-0.81</td>
<td>Klekowski et al. (1979)</td>
</tr>
</tbody>
</table>
Considerable changes may occur in the relationship between size and metabolism during the development of an animal. Epp & Lewis (1980a) found a power function with an exponent \( b \) near 1 in nauplii of cyclopoid and calanoid copepods. A sudden increase in metabolic rate appeared between the last naupliar stage and the first copepodite stage. For copepodites and adults, \( b < 1 \). The slope of the regression line for all stages of *Macrocyclops albidus* was 0.84, but differences appeared when single stages were considered separately (Klekowski & Shushkina 1966).

Many of the \( b \) values listed in Table 10.5 are probably not significantly different but the slope of the regression line is not the only way in which species may differ with respect to their metabolic rate. Schiemer & Duncan (1974) demonstrated that \( b \) calculated for the nematode *Tobiulus gracilis* was not significantly different from values of other nematode species, but the elevation of the regression line, \( a \), was. Thus the elevation may be an indication of the species' metabolic intensity.

These few examples show that, when an average 'exponent' is applied to the data instead of measuring animals of different sizes, considerable errors can result. One should keep in mind that the logarithmic relationship obscures the absolute errors on an untransformed scale. For example, the small deviation of point (2) from the regression line in Fig. 10.2 represents a difference of about 40%.

### 3.1.2 Activity, diel rhythms

As stated earlier, respiration should be measured at 'normal' rates of activity; however, this 'normal' activity may vary considerably with time. Increased activity means higher energy expenditures, and, therefore, a higher respiration rate. This has been documented by some studies—Berg *et al.* (1962) found a linear increase of oxygen consumption with rate of activity of *Chaoborus flavicans*, but only about 2% of the metabolic energy was spent in prey attack by *Chaoborus trivittatus* (Giguère 1980). Even the costs of swimming are not easy to evaluate. The cladoceran *Simocephalus vetulus*, which is normally attached to plants, exhibited a 30% increase in the respiratory rate when swimming (Ivanova & Klekowski 1972), but Vlymen (1970) calculated that the additional costs of vertical migration of continuously swimming copepods were very small.

'Activity' is a complex phenomenon and its components are difficult to separate. It is, therefore, almost impossible to determine the reasons for diurnal rhythms in the metabolic rate; there are a few experiments where these rhythms have been studied. The mayfly nymph, *Isonychia* sp., has been found to exhibit rhythmic diurnal fluctuations with significantly higher night time oxygen utilization. These rates were controlled by both endogenous and
exogenous (light) factors (Ulanoski & McDiffett 1972). Duval & Geen (1976) have reported endogenous diurnal rhythms in respiration of mixed zooplankton. The curve of the respiratory rate was bimodal with maxima at dawn and dusk and night respiration rates 2-3 times higher than daytime values. The respiratory rate of Chaoborus punctipennis also varied (Sigmon et al. 1978), but no predictable diurnal cycles were found. More long-term experiments, best done in flow-through systems, must be performed to find out how frequently aquatic animals exhibit diurnal cycles in metabolism. Using such a flow-through system, Gyllenberg (1981) demonstrated an increase in the respiratory rate of Eudiaptomus gracilis after midnight, corresponding to the migratory activity of the animals in the field.

3.2 Exogenous factors

3.2.1 Temperature

Temperature has a striking influence on the rate of metabolism of poikilotherms. Therefore, numerous studies deal with this subject (see review by Ivleva 1980). Response curves are usually similar and the respiratory rate increases more rapidly at higher temperatures (Fig. 10.7). There are some exceptions, however. A pronounced peak was observed at 8°C for Cyclops bicuspidatus (Laybourn-Parry & Strachan 1980). Respiration rate is frequently stable over a portion of the normally encountered thermal range (Obreshkove & Abramowitz 1932; Ranta & Hakala 1977; Epp & Lewis 1980a). This may indicate the ability to maintain a 'preferred' level of metabolism (Epp & Lewis 1980b).

The magnitude of the acceleration of the metabolic rate is generally characterized by the ratio of rates resulting from a temperature increase of 10°C (Q_{10}). A Q_{10} value of 2 would mean that a 10°C increase in temperature caused a doubling of the respiration rate. For the calculation of Q_{10}, it is not necessary to determine two rates exactly 10°C apart; any two temperatures sufficiently far apart to give reliable information on the temperature effect can be used. Then:

$$Q_{10} = \left( \frac{R_2}{R_1} \right)^{\frac{T_2-T_1}{10}}$$

(10.13)

where $R_1$ and $R_2$ are the measured respiratory rates and $T_1$ and $T_2$ the corresponding temperatures. With a known $Q_{10}$, the respiratory rate at a different temperature can be estimated by:

$$R_2 = R_1 \cdot Q_{10}^{\frac{T_2-T_1}{10}}$$

(10.14)
Q_{10} values reported in literature vary remarkably within the same species. For different size classes of *Pisidium amnicum* and over different parts of the temperature range, Holopainen & Ranta (1977) obtained values between 1.5 and 5.6. The same range (1.83–5.3) was measured for *Diaptomus* by Siefken & Armitage (1968). There is evidence that the Q_{10} of a given species varies with the habitat temperature to which it is adapted (Rao & Bullock 1954): in the snail, *Littorina littorina*, the Q_{10} was lower during summer than during winter. Temperature increases elevate the slope of the regression lines relating log metabolism to log dry weight; thus, the Q_{10} differs for animals of different size (Newell & Roy 1973). Distinct changes in Q_{10} during development were also reported for the tropical freshwater copepod *Mesocyclops brasilianus* (Epp & Lewis 1979b).

Conover (1978) reviews the relationship between metabolic rate, activity level and temperature for some marine invertebrates. Different Q_{10} values can be found for 'standard' and 'active' metabolism. The standard respiratory rate is often relatively independent of temperature and shows no acclimatization. The active rate is more temperature dependent and exhibits partial acclimatization to temperature. At very high temperatures, the 'active' rate of oxygen consumption may decrease, because the animals become inactive. Therefore, 'standard' and 'active' metabolism are similar at low and high temperatures, and a great 'scope for activity' (the difference between 'standard' and 'active' rates) is found at some intermediate, and presumably at optimum, temperature conditions.

Acclimatization is important when the temperature dependency of respiration is measured (Ivleva 1980). After a rise of temperature from 16° to 20°C *Gammarus fossarum* initially showed an overshoot of the acclimatized respiratory rate. A lower final plateau was attained after about two days (Franke 1977). Vollenweider & Ravera (1958) transferred *Daphnia obtusa* from 6.5°C to 24°C and vice versa. There were significant differences according to whether or not the animals had been adapted to the new temperature for 24 h.

Even though the Q_{10} is often near 2 (e.g. Shcherbakoff 1935; Comita 1968; Trama 1972; Roff 1973), the actual value is dependent on the section of the temperature range over which it is calculated. In order to obtain a single numerical value which describes the whole temperature dependency, a 'temperature characteristic' (μ) can be used. This is analogous to the Van't Hoff and Arrhenius formula describing the influence of temperature upon the velocity of chemical reactions. The metabolic rate is assumed to follow the equation:

\[ R = R_0 \cdot e^{-\mu r \cdot T} \]  

(10.15)

where R = respiration rate at the given temperature, \( r = \text{gas constant} \) (8.3 J
mol\(^{-1}\) degree\(^{-1}\), \(T\) = absolute temperature (°K), \(R_o\) = constant reflecting the metabolism when \(T\) approaches 0, \(\mu\) = activation energy (J mol\(^{-1}\)).

The numerical value of \(\mu\) is, therefore, a 'temperature characteristic' which describes the accelerating influence of temperature on the metabolic rate, provided that it agrees with the Vant-Hoff and Arrhenius law. In fact, this has been found to be true in many studies (Obreshkove & Abramovitz 1932; Ivleva 1973). If a graph is plotted with the logarithm of the respiratory rate on the ordinate and the inverse of the absolute temperature on the abscissa, the points very often form a straight line, the slope giving \(\mu\). The 'temperature characteristic' can be calculated (Ivleva 1970) from measured values of the respiration rate \((R_1, R_2)\) and the corresponding absolute temperatures \((T_1, T_2)\):

\[
\mu = \frac{\log R_2 - \log R_1}{1/T_1 - 1/T_2} \quad (10.16)
\]

It is usually in the range of 54–67 kJ mol\(^{-1}\). The \(Q_{10}\) can be calculated from \(\mu\) by the equation:

\[
\log Q_{10} = 2.187 \cdot \frac{\mu}{T_1 \cdot T_2} \quad (10.17)
\]

It is evident from this formula that \(Q_{10}\) will change depending on the absolute temperature considered in the calculation.

When measurements of the respiratory rate have been made at only one temperature, and \(\mu\) and \(Q_{10}\) are unknown, an estimate of the metabolic activity at a different temperature can be obtained by using Krogh’s empirical curve. As can be seen from Fig. 10.7, this curve approximates many measured relationships and it can, therefore, be used for temperature corrections. Factors for correction are tabulated in Winberg (1956 and 1971).

3.2.2 Oxygen tension

Generally speaking, there are two principle ways in which animals can respond to unfavourable oxygen conditions. If oxygen is reduced, some animals (regulators) maintain the consumption rate until a critical pressure is reached; further reduction in oxygen causes a rapid decline. Such animals exhibit a wide range over which their respiration is independent of changes in oxygen pressure. The consumption rate by other animals (conformers) is proportional to the ambient concentration of oxygen (Prosser 1973) and their possibilities of regulating the rate of oxygen consumption are limited. At low oxygen tensions animals have to switch to anaerobic metabolism—many animals do this before their supply of oxygen is exhausted (Mangum & Van Winkle 1973). Oxygen is especially important for profundal and bottom
Fig. 10.7 Effect of temperature on the respiratory rate of aquatic animals. 1, Leptodora kindtii (Moshiri et al. 1969); 2, Stenonema pulchellum (Trama 1972); 3, Chaoborus punctipennis (Sigmon et al. 1978); 4, Limnocalanus macrurus (Roff 1973); 5, Cyclops bicuspidatus (Laybourn-Parry & Strachan 1980); 6, Daphnia magna (Obreshkove & Abramowitz 1932); K. Krogh's normal curve (Winberg 1971).

dwelling animals. Berg et al. (1962) described three types of reactions of benthic animals to decreasing oxygen tensions:

1. A nearly constant oxygen consumption is maintained from saturation to some critical point (7–25 % saturation), below which a marked decrease occurs (Tubifex barbatus, Ilyodrilus hammonensis, Chironomus anthracinus).

2. Oxygen consumption decreases at a lower rate down to 20–30 % saturation and then more markedly (Lumbriculus rivalis, Procladius, Pisidium casertanum).
(3) Other animals are not able to maintain a constant level. Oxygen consumption is proportional to oxygen concentration over a wide range (Chaoborus flavicans).

There are many examples of animals which behave intermediately between true regulators and true conformers (cf. Fig. 10.8). For example, in Gammarus fossarum (Franke 1977) the respiratory rate is influenced by temperature and current, but the shape of the dependence of oxygen consumption on oxygen concentration remains similar. Konstantinov (1971) found some chironomid larvae that showed reaction types (2) and (3) to varied oxygen tension. Leptodora kindtii maintained the same respiration rate between 14 and 8 mg O₂ l⁻¹ (Moshiri et al. 1969).

Differences may occur in closely related species. Heisey & Porter (1977) found Daphnia galeata to be a conformer, whereas Daphnia magna regulated its oxygen consumption rate. They explain this by the different habitats colonized by animals. Daphnia magna lives in ponds where it is often exposed to low oxygen tensions whereas the pelagic Daphnia galeata does not. Some daphnids contain visibly more haemoglobin when living under reduced oxygen conditions (Fox & Phear 1953); it would be interesting to see whether

![Graph showing respiratory rate vs. oxygen concentration for different species](image)

**Table 10.8** Alternate response of some aquatic animals to changing oxygen concentrations. A, Ancylus fluviatilis (Berg 1952); D.m., Daphnia magna, D.g., Daphnia galeata mendotae (Heisey & Porter 1977); G, Gammarus fossarum (Franke 1977); S, Simocephalus vetulus (Hoshi 1957).
the type of response curve is subject to acclimatization in the same species living under different conditions of available oxygen.

Experiments on oxygen utilization at different oxygen concentrations have been made in different ways. Animals have either been put into a container so that the oxygen was reduced by the animals themselves or they have been provided with oxygen deficient water in an open-flow system. The first method is not recommended because there is the possibility that oxygen consumption is influenced by the accumulation of excretory products (e.g. ammonia). This error is avoided in the open-flow method.

3.2.3 Food

The effect of food on the respiratory rate has not yet been studied very extensively. The animals are usually fed or starved prior to the experiment, and only a few studies deal with animals that are actually feeding.

Food may influence the respiration rate in different ways. Activity may change because the searching behavior is changed. For the predaceous cladoceran Polyphemus pediculus, visual perception of prey crustacea is enough to increase the respiration rate (Butorina 1979). In filter-feeders, the rate of movement of filtering appendages may decrease with increasing food concentration. When food is ingested, metabolic costs of digestion and biochemical processing of the food (specific dynamic action; SDA) occur. Giguère (1980) estimated the SDA of Chaoborus trivittatus to cause an increase of 37% in oxygen consumption compared to unfed animals.

When the animals are fed before oxygen consumption is measured, respiration rates are usually increased when compared to starved animals. This was found by Comita (1968) for several species of Diaptomus. La Row et al. (1975) pre-fed Diaptomus copepodites and Daphnia at different food concentrations and reported an increase of oxygen consumption of about 80% from the lowest to the highest food level. A 10-fold increase in density of bacterial food resulted in a 14% increase in the respiration rate of the nematode Plectrus palustris (Klekowskij et al. 1979); on the other hand, Richman (1958) obtained no reduction in the rate of oxygen consumption per unit weight due to starvation of Daphnia pulex.

Experiments with feeding animals give controversial results. The presence or absence of natural densities of nannoplankton had no effect on the respiration of Diaptomus oregonensis (Richman 1964). Kersting & Van der Leeuw-Leegwater (1976) presented interesting results for Daphnia magna fed different concentrations of Chlorella—respiration rate was maximum at the incipient limiting level (ILL; see Chapter 8) and decreased at higher concentrations. At a Chlorella density of about 6 times the ILL, it amounted only to 46% of the maximum. These experiments have been repeated with
Daphnia magna with different concentrations of Scenedesmus as food (Bohrer & Lampert in preparation) and the outcome of the experiments was completely different. Oxygen consumption increased by 40–50% from zero-food to the ILL (approximately 0–3 mg C l\(^{-1}\)) where a plateau was reached. No reduction was found at high concentrations (up to 3.5 mg C l\(^{-1}\)).

With the exception of the findings of Kersting & Van der Leeuwr Leegwater (1976), feeding seems to increase the metabolic rate. Because energy budgets are usually made for growing animals (i.e. those that are fed), while respiration is usually measured using starved animals, respiratory losses are probably underestimated in many budgets, clearly suggesting the need for more reliable studies on the respiration of feeding aquatic animals.

3.2.4 Light

The effect of intensity and quality of light on respiration has been studied for some animals, but the results are again controversial and too scarce. No significant effect of light intensity was found by Bishop (1968) for mixed zooplankton or by Schindler (1968) for Daphnia magna. On the other hand, light intensity affected the exponent of the length:respiration regression of Daphnia pulex, whereas variation in wavelength produced no significant effect (Buikema 1972). Chironomid larvae also increased their respiratory rates in response to light (Konstantinov 1971); this may have been a result of increased mobility. Because many measurements are performed in the dark, the results of Moshiri et al. (1969) are very important. They discovered that the respiration rate of Leptodora kindtii in the light was up to twice as high as that in the dark. The effect of light level requires more research, especially since light very often controls activity, and interactions of increased motion and feeding may significantly affect the respiratory rate.

3.2.5 Current

It is obvious that water current is a very important environmental factor to the animals found in running water; they are adapted to their habitat and often have special respiratory organs to utilize the water flow. Ambühl (1959) studied the response of the respiratory rate of stream insect larvae to current and oxygen content of the water. He described different types of dependencies:

1. Ephemerella ignita responded to oxygen content but not to current.
2. Ecdyonurus venosus was sensitive to current only at low oxygen concentrations.
3. The respiration rates of some species (caseless and case-bearing caddisflies, Baetis vernus, Rhitrogena semicolorata) were positively dependent on both oxygen content and water velocity.
A good example showing the interacting influence of water flow, temperature, and oxygen partial pressure on the respiratory rate of *Gammarus fossarum* is given by Franke (1977).

When the effect of water velocity is to be studied, a current of several cm sec\(^{-1}\) must be produced. The flow rate in an open-flow system (see Section 2.3.3) is much too low to fulfill these requirements, and measurements must, therefore, be performed in a closed system. Recirculating systems containing an animal chamber have usually been used. The animals are prevented from leaving the chamber by mesh screens and the bottom must be roughened to allow the animals to keep their position in the current. A strong current is produced by a propeller (Ambühl 1959), a magnetic stirrer (Zahner 1959; Wotton 1978) or a magnetically impelled centrifugal pump (Eriksen & Feldmeth 1967; Franke 1977).

### 3.2.6 Crowding and container size

In measurements of respiration, the animals are confined to more or less artificial conditions. Many animals are held in a relatively small volume of water, or single animals are kept in small containers. Several attempts have been made to test the influence of these conditions on the respiratory rate.

No significant crowding effect was detected for *Daphnia magna* and *Daphnia pulex* (0.5–2.0 ind ml\(^{-1}\); Goss & Bunting 1980), or *Limnocalanus macrurus* (0.1–1.8 ind ml\(^{-1}\); Roff 1973). Crowding does not seem to be important at the densities normally used, even though these densities are higher than in nature. Very high densities, on the other hand, necessitate correction (Duval & Geen 1976). The effect should be scrutinized more carefully because the respiration rates of marine zooplankton may increase linearly with density (Satomi & Pomeroy 1965).

Roff (1973) kept *Limnocalanus macrurus* in containers of different sizes (30–330 ml) but at the same density (1 ind ml\(^{-1}\)) and discovered no effect on the metabolic activity. Extreme conditions preventing the animals from moving freely should be prevented, however. When Zeiss (1973) enclosed several *Daphnia magna* singly in small mesh covered glass tubes (0.12–0.24 ml) and placed them in a larger vessel, the respiration rate was more than doubled, compared to the same number of animals in a vessel but not confined to the small space.

### 3.2.7 Other factors

Animals in the field may be exposed to many other factors which may affect the metabolic rate but which have not yet been studied. Such effects may arise from chemical, osmotic or other physical stresses. For example, Ivanova & Klekowski (1972) showed that the pH of the water influenced the respiratory
456 Chapter 10

rate of *Simocephalus vetulus*. Hydrostatic pressure was found to suppress the respiration rate of mixed freshwater zooplankton (Bishop 1968) but marine zooplankton were not affected in the range of 16–50 atm (Pearcy & Small 1968).

4 In Situ Studies

Many attempts have been made to measure the respiration rates of aquatic animals or communities under *in situ* conditions (Straskraba 1967; Bishop 1968; Cremer & Duncan 1969; Duncan *et al.* 1970; Roff 1973; Nowak 1975; Ranta & Hakala 1978; Makarewicz & Likens 1979). Except where measurements of the ETS activity are made, however, the methods involved do not measure real *in situ* respiration rates; most are merely laboratory experiments carried out in the field, put into a closed container, and then incubated *in situ*. Usually, animals must be concentrated because no measurement of oxygen consumption can be made at natural densities. This is dangerous because concentration requires that the animals be handled. In addition, the animals are often without sufficient food because they are put into filtered water or the available natural food is exhausted quickly due to the abnormal animal densities.

Glass bottles or disposable syringes (Ranta & Hakala 1978) have been used and experiments have been carried out according to the 'closed bottle' method (Section 2.3.2). Most *in situ* studies have been made with zooplankton, because the animals can be concentrated easily using a net. Nowak (1975) tried to minimize handling of the animals before the experiment by attaching a bottle filled with filtered water to the end of a plankton net. After towing the net, he lifted it upwards, leaving the lower part with the bottle in the water. The animals then migrated downwards into the bottle, which could be closed.

When different groups or species must be measured separately, the animals must be sorted by hand, by sieving, or by another technique. Straskraba (1967) developed a narcotization technique to separate rotifers, cladocerans, and copepods. Although this procedure may stress the animals, he still obtained measurements of the respiration rate similar to the results of other investigators. At a temperature of 10–13°C, in April, when copepods dominated the community, he found the respiration rate to be 0.295 μmol O₂ mg⁻¹ h⁻¹, which is in the same range as laboratory data (cf. Table 9.4). The average oxygen consumption of mixed zooplankton at 18–20°C, determined by Makarewicz & Likens (1979), was 0.666 μmol mg⁻¹ h⁻¹, which is also in the range to be expected, provided the average size of the zooplankters was relatively small.

There are considerable seasonal changes in the metabolic rate. Nowak
(1975), for example, measured respiration rates for mixed zooplankton of 0.08 μmol O₂ mg⁻¹ h⁻¹ at 2°C in January and 0.67 μmol O₂ mg⁻¹ h⁻¹ at 20°C in August. A large part of this variation, but not all of it, is explained by the differences in experimental temperature (Cremer & Duncan, 1969).

A comparison of 'field' measurements using the bottle method and laboratory determination of oxygen consumption with the Cartesian diver was carried out by Duncan et al. (1970) in London reservoirs. In this case 'field' values were considerably higher, but the reason was not clear. Such comparisons lead one to question whether it is worthwhile to perform field experiments which are not really in situ measurements. It might be better to calculate field respiration from pure laboratory results, including the effects of abundances, size structure, and temperature on the populations. Phillipson (1970) is in favour of the latter method proposing a 'best estimate' of respiratory metabolism which is calculated from laboratory data of respiration of all life stages or size classes of a species studied, measured at constant temperature. This yields an average oxygen consumption per unit weight for the species. By correction for the average temperature of the habitat and multiplication by the mean annual biomass, the method provides an estimate of the annual respiration of the population. The author states that the application of the 'best estimate' method gives results with 10% deviation from more detailed studies.

5 Similarities and Dissimilarities

A collection of measured respiration rates is presented in Table 10.6. This compilation is selective since not all of the available information could be included but it tries to cover a broad range of freshwater invertebrates. In many cases, values for different temperatures and body sizes are contained in the original papers. The reader may refer to them for details. Data are presented as oxygen consumption rates and rates of body carbon loss, because the latter are more relevant to secondary production.

There is a clear partition between planktonic and benthic animals, but this is due to the larger body size of the second group. Despite all of the problems mentioned in the foregoing paragraphs, the values agree astonishingly well in some groups, even though they have been produced by different authors. The three numbers for *Mysis relicta*, for example, are very close to each other. Respiration rates within the calanoid copepods and the daphnids are also very similar. Temperature and body weight seem to produce the main differences.

More variability can be seen in the estimates for large and very small animals. The reason for the greater homogeneity of values for medium sized zooplankton species may be that they have been obtained under more adequate conditions. In addition to body size and temperature, food is the
Table 10.6 Examples of published respiratory rates.

<table>
<thead>
<tr>
<th>Species</th>
<th>Individual dry weight (mg)</th>
<th>Temp. (°C)</th>
<th>Individual respiration rate (µl O₂ ind⁻¹ h⁻¹)</th>
<th>Specific respiration rate (µmol O₂ mg⁻¹ h⁻¹)</th>
<th>Loss of body carbon (% per day)*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rotatoria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brachionus rubens</em></td>
<td>0.13 × 10⁻³</td>
<td>20</td>
<td>2.69 × 10⁻³</td>
<td>0.924</td>
<td>60.0</td>
<td>Pilarska, 1977</td>
</tr>
<tr>
<td><em>Brachionus plicatilis</em></td>
<td>0.16 × 10⁻³</td>
<td>20</td>
<td>2.66 × 10⁻³</td>
<td>0.752</td>
<td>38.9</td>
<td>Doohan, 1973</td>
</tr>
<tr>
<td><em>Brachionus calyciflorus</em></td>
<td>0.32 × 10⁻³</td>
<td>20</td>
<td>3.04 × 10⁻³</td>
<td>0.425</td>
<td>27.6</td>
<td>Leimeroth, 1980</td>
</tr>
<tr>
<td><strong>Copepoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Calamoecia lucasi</em></td>
<td>1.23 × 10⁻³</td>
<td>15</td>
<td>0.0098</td>
<td>0.356</td>
<td>23.0</td>
<td>Green &amp; Chapman, 1977</td>
</tr>
<tr>
<td><em>Diaptomus siciloides</em> **</td>
<td>0.0032</td>
<td>20</td>
<td>0.075</td>
<td>1.045</td>
<td>67.7</td>
<td>Comita, 1968</td>
</tr>
<tr>
<td><em>Diaptomus gracilis</em></td>
<td>0.008</td>
<td>20</td>
<td>0.061</td>
<td>0.341</td>
<td>22.1</td>
<td>Kibby, 1971</td>
</tr>
<tr>
<td><em>Diaptomus oregonensis</em></td>
<td>0.011</td>
<td>22</td>
<td>0.090</td>
<td>0.365</td>
<td>23.5</td>
<td>Richman, 1964</td>
</tr>
<tr>
<td><em>Diaptomus graciloides</em> §</td>
<td>0.0065</td>
<td>20</td>
<td>0.0668</td>
<td>0.459</td>
<td>29.8</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><em>Diaptomus graciloides</em> ♀</td>
<td>0.0123</td>
<td>20</td>
<td>0.0828</td>
<td>0.301</td>
<td>19.4</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><em>Diaptomus leptopus</em> **</td>
<td>0.0222</td>
<td>20</td>
<td>0.178</td>
<td>0.358</td>
<td>23.2</td>
<td>Comita, 1968</td>
</tr>
<tr>
<td><em>Boeckella delicata</em></td>
<td>0.0101</td>
<td>15</td>
<td>0.050</td>
<td>0.219</td>
<td>14.2</td>
<td>Green &amp; Chapman, 1977</td>
</tr>
<tr>
<td><em>Limnocalanus macrurus</em></td>
<td>0.0342</td>
<td>15</td>
<td>0.2286</td>
<td>0.298</td>
<td>15.4</td>
<td>Roff, 1973</td>
</tr>
<tr>
<td><em>Cyclops leuckarti</em> ♀</td>
<td>0.0035</td>
<td>20</td>
<td>0.0432</td>
<td>0.551</td>
<td>35.8</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><em>Cyclops leuckarti</em> §</td>
<td>0.9 × 10⁻³</td>
<td>20</td>
<td>0.012</td>
<td>0.595</td>
<td>38.4</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><em>Cyclops bicuspidatus</em></td>
<td>0.008</td>
<td>8</td>
<td>0.0336</td>
<td>0.188</td>
<td>12.2</td>
<td>Laybourn-Parry &amp;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Strachan, 1980</td>
</tr>
<tr>
<td><em>Cyclops strenuus</em></td>
<td>0.0434</td>
<td>20</td>
<td>0.203</td>
<td>0.209</td>
<td>13.4</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><strong>Cladocera</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ceriodaphnia reticulata</em></td>
<td>0.0041</td>
<td>22</td>
<td>0.050</td>
<td>0.541</td>
<td>35.0</td>
<td>Gophen, 1976</td>
</tr>
<tr>
<td><em>Daphnia longispina</em></td>
<td>0.0222</td>
<td>20</td>
<td>0.156</td>
<td>0.314</td>
<td>20.4</td>
<td>Shcherbakoff, 1935</td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>0.0346</td>
<td>20</td>
<td>0.2887</td>
<td>0.373</td>
<td>24.0</td>
<td>Goss &amp; Bunting, 1980</td>
</tr>
<tr>
<td><em>Daphnia pulex</em></td>
<td>0.028</td>
<td>20</td>
<td>0.194</td>
<td>0.309</td>
<td>19.9</td>
<td>Richman, 1958</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0.1524</td>
<td>20</td>
<td>0.7021</td>
<td>0.206</td>
<td>13.2</td>
<td>Goss &amp; Bunting, 1980</td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>0.15</td>
<td>18</td>
<td>0.882</td>
<td>0.263</td>
<td>17.0</td>
<td>Kersting &amp; v.d. Leeuw</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Leegwater, 1976</td>
</tr>
<tr>
<td>Taxon</td>
<td>RQ 1</td>
<td>RQ 2</td>
<td>RQ 3</td>
<td>Carbon (%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------------</td>
<td>-----------------------------</td>
<td></td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>0.15</td>
<td>20</td>
<td>0.915</td>
<td>0.272</td>
<td>17.6 Schindler, 1968</td>
<td></td>
</tr>
<tr>
<td>Simocephalus exspinosus</td>
<td>0.059</td>
<td>25</td>
<td>0.73</td>
<td>0.552</td>
<td>35.8 Obreshkove, 1930</td>
<td></td>
</tr>
<tr>
<td>Simocephalus vetulus</td>
<td>0.070</td>
<td>22</td>
<td>0.36</td>
<td>0.230</td>
<td>16.7 Ivanova &amp; Klekowski, 1972</td>
<td></td>
</tr>
<tr>
<td>Leptodora kindti</td>
<td>?</td>
<td>15</td>
<td>-</td>
<td>0.749</td>
<td>52.3 Moshiri et al., 1969</td>
<td></td>
</tr>
<tr>
<td>Amphipoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gammarus fossarum</td>
<td>3.0</td>
<td>12</td>
<td>1.92</td>
<td>0.029</td>
<td>1.9 Franke, 1977</td>
<td></td>
</tr>
<tr>
<td>Gammarus pulex</td>
<td>8.5</td>
<td>15</td>
<td>1.9</td>
<td>0.078</td>
<td>5.28 Nilsson, 1974</td>
<td></td>
</tr>
<tr>
<td>Isopoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asellus aquaticus</td>
<td>3.0</td>
<td>23</td>
<td>4.47</td>
<td>0.066</td>
<td>4.3 Prus, 1972</td>
<td></td>
</tr>
<tr>
<td>Mysidacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mysis relicta</td>
<td>5.0</td>
<td>4.6</td>
<td>3.85</td>
<td>0.034</td>
<td>1.9 Ranta &amp; Hakala, 1978</td>
<td></td>
</tr>
<tr>
<td>Mysis relicta</td>
<td>5.0</td>
<td>4</td>
<td>3.70</td>
<td>0.033</td>
<td>2.3 Foulds &amp; Roff, 1976</td>
<td></td>
</tr>
<tr>
<td>Mysis relicta</td>
<td>5.0</td>
<td>4</td>
<td>4.2</td>
<td>0.0375</td>
<td>2.64 Lasenby &amp; Langford, 1972</td>
<td></td>
</tr>
<tr>
<td>Mollusca</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pisidium amnicum</td>
<td>5.0</td>
<td>20</td>
<td>0.916</td>
<td>0.0082</td>
<td>0.48 Holopainen &amp; Ranta, 1977</td>
<td></td>
</tr>
<tr>
<td>Plecoptera†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoperla buresi</td>
<td>5.34</td>
<td>8</td>
<td>1.506</td>
<td>0.0126</td>
<td>0.82 Kamler, 1969</td>
<td></td>
</tr>
<tr>
<td>Ephemeroptera†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloeon dipterum</td>
<td>0.214</td>
<td>20</td>
<td>0.568</td>
<td>0.119</td>
<td>7.7 Kamler, 1969</td>
<td></td>
</tr>
<tr>
<td>Stenonema puckellum</td>
<td>2.0</td>
<td>20</td>
<td>3.48</td>
<td>0.078</td>
<td>5.0 Trama, 1972</td>
<td></td>
</tr>
<tr>
<td>Rhithrogena sp.</td>
<td>1.94</td>
<td>18.5</td>
<td>21.86</td>
<td>0.503</td>
<td>31.2 Ambühler, 1959</td>
<td></td>
</tr>
<tr>
<td>Ecdyonurus venosus</td>
<td>5.5</td>
<td>18.5</td>
<td>17.3</td>
<td>0.141</td>
<td>9.1 Ambühler, 1959</td>
<td></td>
</tr>
<tr>
<td>Diptera†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simuliidae**</td>
<td>0.139</td>
<td>15.5</td>
<td>1.04</td>
<td>0.335</td>
<td>24.0 Wotton, 1978</td>
<td></td>
</tr>
<tr>
<td>Chaoborus trivittatus</td>
<td>0.8</td>
<td>20</td>
<td>0.751</td>
<td>0.042</td>
<td>2.7 Swift, 1976</td>
<td></td>
</tr>
<tr>
<td>Chaoborus flavicans</td>
<td>5.0</td>
<td>18</td>
<td>3.2</td>
<td>0.029</td>
<td>1.9 Berg et al., 1962</td>
<td></td>
</tr>
<tr>
<td>Chironomus anthracinus</td>
<td>8.0</td>
<td>15</td>
<td>1.74</td>
<td>0.0097</td>
<td>0.6 Berg et al., 1962</td>
<td></td>
</tr>
</tbody>
</table>

# If no other values were given by the author, an RQ of 0.9 and 40% carbon of dry weight was assumed.
* ash free.
** fed.
† larvae.
main factor influencing the respiration rate of these animals, but this factor is excluded from Table 10.6 because, with two exceptions, all of the respiration rates were measured using starved animals. Both Diaptomus species, which were fed, in fact showed fairly high values. For larger crustaceans and insect larvae, adequate measurements cannot be made so easily because water movement and substrate may be very important. Thus, greater variations can be expected.

An increasing number of papers on respiration of freshwater animals have been published during recent years, and there are many additional studies on marine animals that have not been considered here. We now seem to have a general overview of the effects of body size and temperature, but information on other topics is missing. Therefore, more effort should be put into simulating environmental conditions as exactly as possible with regard to these other factors. With respect to secondary production, the effect of food on respiration rate seems to be the most important, severely neglected factor. As stated in the introduction of this chapter, respiration losses must be measured with the greatest possible accuracy, when a budget is to be constructed.

When carbon loss values for all non-fed crustacean zooplankters in Table 10.6 are corrected to 20°C by Krogh's normal curve (see Section 3.2.1) and to 0.04 mg body weight by use of the exponent \( b = -0.25 \) (see Section 3.1.1), the average is 19.1% ± 4.8% per day \( (n = 20) \). This is a rather low standard deviation. Variations due to different feeding conditions seem to be much higher than the species-specific differences. Therefore, new techniques for measuring respiration of feeding animals must be developed. Investigators performing experiments with small, starved animals must be aware that their subject may lose considerable weight during the experimental period. For the zooplankters mentioned above, this was 19% per day (at 20°C). Until 'field' experiments allow animals to eat their natural foods, good laboratory experiments may yield results closer to nature than poor field experiments. To estimate the total respiration in the field, it might be better to extrapolate from laboratory respiration rates of feeding animals, while emphasizing improvements in estimates of animal abundance in the field.

6 References


The Measurement of Respiration


Bohrer R.N. & Lampert W. (In preparation) Simultaneous measurement of the effect of food concentration on assimilation and respiration in *Daphnia magna*.


Chapter 10


Nagell B. (1973) The oxygen consumption of mayfly (Ephemeroptera) and stonefly (Plecoptera) larvae at different oxygen concentrations. *Hydrobiologia*, 42, 461-489.


The Measurement of Respiration


