AN AUTOMATIC RESPIROMETER FOR DETERMINING OXYGEN UPTAKE IN CRAYFISH (AUSTROPOTAMOBIIUS PALLIPES (LEREOUBULET)) OVER PERIODS OF 3–4 DAYS

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SUMMARY

1. An automatic respirometer has been developed for continuous measurements over 3–4 days on 1–15 g crayfish. The sensor is a modified Mackereth oxygen electrode. Respiration is recorded on a millivolt potentiometric pen recorder during closed periods when the O₂ concentration in the medium falls to a predetermined level. A solenoid-operated valve is then opened via a relay circuit energized by a reed switch mounted on the recorder. Medium flows through the respirometer until the O₂ concentration is raised back to another predetermined level. Artificial media containing little or no nutrient salts are used to restrict the growth of microbes.

2. Respiration was determined chiefly on crayfish (Austropotamobius pallipes) with body wet weights of 7.0–12.5 g at 10.0 °C. In unrestrained but relatively quiescent animals, standard metabolism is described by the regression equation, ln O₂ uptake = 3.3037 + 1.002 ln body wt. In restless crayfish active metabolism is described by the equation, ln O₂ uptake = 4.4412 + 0.861 ln body wt.

INTRODUCTION

Continuous measurements of O₂ uptake for periods of 3–4 days were required to determine the energetic cost of osmoregulation in crayfish kept in Na-free media and in artificial lakewater media (Sutcliffe & Carrick, 1975). Ideally this should be done with animals held at a constant level of activity, so that one may determine either standard metabolism characteristic of 'resting' animals or active metabolism characteristic of some fixed level of locomotor activity. This approach is now well-established in work on various aspects of fish respiration (e.g. Brett, 1964; Stevens & Randall, 1967; Dickson & Kramer, 1971), and the technique of measuring active metabolism was used to estimate the energetic cost of osmoregulation in Salmo gairdneri (Rao, 1968). For our studies we decided to estimate standard metabolism, as it was thought that crayfish might become exhausted if subjected to continuous and prolonged high levels of activity induced, for example, by applying repetitive electrical shocks. To measure standard metabolism it was therefore important not to disturb the animals once they were placed in the respirometer, and the crayfish were not fettered or restrained in any way. This paper describes a respirometer used with crayfish weighing
5–15 g. By changing the size or volume of the animal chamber, the respirometer can be used for estimating $O_2$ uptake in small fish and in groups of small invertebrates, e.g. gammarid crustaceans (D. W. Sutcliffe & T. R. Carrick, MS in preparation). A principal feature of the respirometer is that the $O_2$ tension in the medium is automatically held between any two selected levels in the range 0–100% air-saturation. The difference between two selected levels may be as little as 15%. $O_2$ uptake is estimated from the rate of fall in $O_2$ concentration between the two selected levels. Fresh medium is then run through the respirometer both to raise the $O_2$ concentration back to the required level and to remove metabolic waste products.

Also presented are some results and observations of a general nature concerning standard metabolism in the crayfish *Austropotamobius pallipes* (Lereboullet). Until recently (Bott, 1950; Karaman, 1962; Holthuis, 1967) there was some confusion on the identity and nomenclature of European freshwater crayfish. Hence one cannot always be certain of the identity of animals used in the older studies on respiration in crayfish, especially with respect to the edible crayfish *Astacus astacus* (L.). A brief synopsis of the nomenclature and a key to crayfish in western Europe are given in Gledhill, Sutcliffe & Williams (1976). *Austropotamobius pallipes* is confined to Britain, Ireland and France. There appear to be no published studies on respiration in this species.

**DESCRIPTION OF RESPIROMETER**

The respirometer is constructed out of Perspex, comprising two circular chambers joined together by a short tube (Fig. 1). The total volume was 305.0 ± 0.5 ml. One chamber holds a Mackereth oxygen electrode (Lakes Instrument Company, Ltd). This was an improved version of the silver-lead galvanic cell described by Mackereth (1964), excluding the thermistor originally used for measuring temperature. The instrument was further modified by reducing the silver electrode to a thin ring of silver, ca. 35 mm diameter and 1.6 mm wide. This reduced the $O_2$ consumption of the electrode to $7.5 \pm 1.0 \mu g$ $O_2/h$ in water saturated with air at 10.0 °C.
Neoprene O-rings provide gas-tight seals between the Mackereth electrode and the tubular wall of the chamber. By rotating a PTFE-coated magnetic follower at the base of the chamber, ca. 85 ml water is circulated vigorously in an annular space around the oxygen electrode. The magnetic follower is driven by a magnet mounted externally on a 1440 rpm 1/2 h.p. single-phase electric motor. A constant speed of stirring in the electrode chamber ensures that the electrical output of the Mackereth electrode remains constant at any particular O₂ tension. The electrode chamber is connected to the animal chamber by a sleeve of Neoprene tubing (12 mm bore). Inside this tube a rectangular piece of thin celluloid sheet, dimensions 12 x 57 mm, is held in the vertical plane with one end extending into the annular space between the Mackereth electrode and the wall of the electrode chamber (Fig. 1). The piece of celluloid effectively divides the tube into two half-sections. When the magnetic follower is turning, water is forced along one half-section of the tube and passes into the animal chamber, whilst a return current in the other half-section moves water back into the electrode chamber. In this manner the medium in the two chambers is kept constantly in motion and thoroughly mixed, but the animals are not exposed to the highly turbulent current which is necessary to saturate completely the Mackereth electrode.

The animal chamber is made from a Perspex tube, diameter 70 mm and wall height 58 mm, providing sufficient room to accommodate a crayfish with a body wet weight of up to 15 g. A 'shelf' of Perspex is sited 25-30 mm above the floor and extending across about one third of the chamber (Fig. 1). This forms the roof of a shelter or retreat in which a crayfish can sit. When placed in the chamber, crayfish first vigorously explore it, but within 10 min some move into the shelter and immediately settle down. Others remain active for an hour or two, and a few individuals repeatedly remain active throughout long periods in the respirometer. Illumination is controlled as a further inducement to sit quietly in the retreat. The outer wall of the animal chamber is covered with black tape so that light enters it only from above, through the Perspex lid. Continuous illumination (in a windowless constant temperature room) is provided from a 60 W tungsten bulb, but the light intensity is less than 10 Lux.
immediately above the respirometer. In addition, a shadow is cast on to the animal chamber, further reducing the illumination inside the respirometer to a very low level. The combination of constant illumination at low intensity outside the respirometer, shading inside the respirometer, and the provision of a retreat, greatly reduced the frequency of active movements (walking, and attempts to escape) to an acceptable level in the majority of experiments with crayfish.

The respirometer is housed in a constant temperature water bath at 10.0 ± 0.02 °C. Two 2 l glass reservoirs (B and C, Fig. 2) are also placed in the water bath. They are connected in series to the respirometer and to a third 10 l reservoir (A) outside the water bath as shown in Fig. 2. Reservoir A is at 9.0 ± 1.0 °C, in a constant temperature room. Reservoirs B and C ensure that medium is brought to the temperature of the water bath before it enters the respirometer. Accurate temperature control is necessary because the electrical output from the Mackereth electrode is temperature-dependent. (A temperature-compensated version of the Mackereth electrode is now available from the Lakes Instrument Company). The reservoirs are interconnected with 2 mm bore Nylon tubing, and this tubing is connected to the inlet and outlet spigots of the respirometer by two 6 cm lengths of Neoprene tubing, 2.5 mm bore, 5 mm wall thickness. When reservoirs B and C are completely filled, negative pressure is applied at the outlet side of the respirometer and water siphons through the system. The flow is stopped by closing a solenoid-operated Skinner water control valve (type V 52 DA 2022, 240 V, 50 Hz) fitted on the outside of the water bath and below the level of the reservoirs (Fig. 2). With the Skinner water valve closed, only a negligible amount of O₂ diffuses into the respirometer from water held in the inlet and outlet tubing.

The Skinner water valve is opened and closed via the relay control circuit shown in Fig. 3, where the relays are depicted in the unenergized position. The relays are energized by two reed switch units (LHS and RHS, Fig. 4) mounted on top of
Labograph Recorder, type E 478. Each switch unit is made from a block of PVC plastic, dimensions 22 × 18 × 10 mm. A hexagonal hole is made through the 10 mm side face of the block. The hole is a good sliding fit to a piece of hexagonal brass rod, 28.5 mm long and 6 mm across flats. The switch units can be locked into position on the brass rod by turning a screw at the rear of each unit. At the front end a 3 mm diameter hole is drilled to hold a reed switch (MRR-5 (20–40 Amp Turns) from Flight Refuelling Ltd). Two copper pins or spills (18 SWG) are fitted into the block on either side of the reed switch to act as fixed terminations for the leads from the reed switch (see Fig. 4C). From these pins two insulated flexible leads are taken to a three-way terminal block fixed to the centre of the metal cover on top of the recorder. One of the leads from each switch unit is connected to a common terminal.

The hexagonal brass rod holding the switch units is fitted between angle brackets attached to the top of the recorder. The brass rod is mounted so that the switch units can be moved freely along it; their positions can thus be varied to set the required levels of O₂ tension within the respirometer. In addition the brass rod is set back from the front of the recorder to allow an operating magnet to pass directly in front of the switch units. The magnet (Mullard reed switch magnet type TS 1318) is fixed to an aluminium rod bolted to the pen holder of the recorder (see Fig. 4). This magnet will energize a reed switch at a distance of less than 10 mm.

The control system is set up so that when LHS is energized the Skinner water...
control valve is opened; it is closed when RHS is energized. A double coil latching relay \((\text{D/1 E/1}}\) Fig. 3) is used to keep the Skinner valve in correct phase with the switch units. The latching relay was used because the recorder was occasionally affected by transient voltage signals. Some of these transients produce 'kicks' on the recorder, and may cause the magnet on the pen carriage to pass in front of the same reed switch more than once in succession. To reduce the possibility of contact-bounce in \(a_1\) and \(b_1\) affecting relay \(C/1\) (Fig. 3) the voltage to operate \(C/1\) is supplied by a capacitor; the charging circuit has a time constant of 2.5 s. Relay \(C/1\) is a stepping relay with alternate 'make'/‘break’ contacts. The switch contacts \(a_x\) and \(b_x\) stay in one position or the other until the next switching sequence is initiated at the recorder, when the appropriate reed switch is closed as the pen and magnet move up to RHS or down to LHS on the recorder.

CALIBRATION

The two leads from the Mackereth oxygen electrode are connected across a 100 \(\Omega\) resistance and the potential difference is fed to the high impedance millivolt potentiometric pen recorder. The sensitivity of the recorder is adjusted to provide ca. 95 % full-scale deflexion when the oxygen electrode is immersed in water saturated with air at 10.0 °C and 760 Torr. A zero base line is obtained by placing the oxygen electrode in water deoxygenated by adding sodium sulphite. For calibrations, reservoir \(B\) is disconnected from reservoir \(C\) (Fig. 2). The latter is filled with deionized water and thoroughly aerated. The respirometer is then completely filled from reservoir \(C\) and a slow drip through the system is maintained while recording the saturation \(O_2\) value. At 10.0 °C and 760 Torr, air-saturated pure water contains 11.26–11.28 mg \(O_2/l\) (Montgomery, Thom & Cockburn, 1964; Carpenter, 1966; Murray & Riley, 1969). Hence it was calculated that the respirimeter, with a total volume of 305.0 ± 0.5 ml, contains 3437 ± 6 \(\mu g\) \(O_2\) at 760 Torr. Corrections are made for changes in local barometric pressure. The respirometer is always calibrated before the start of an experiment. In a series of 32 experiments, calibration errors were less than ±2 % of the mean value. Deviations from the mean are mainly due to combined read-out errors on the pen-chart-recorder system (< ±1 %) plus deviations from 100.0 % air saturation that result when the barometric pressure is either rising or falling. (With a Mackereth electrode it is possible to measure changes of less than 1 % in the partial pressure of dissolved \(O_2\) following small changes in barometric pressure). Daily variation in the electrical output of the Mackereth electrode is normally negligible.

EXPERIMENTAL ERRORS

A background component has to be subtracted from the \(O_2\) uptake of the animal. This background consists of \(O_2\) consumed by \((a)\) the oxygen electrode and \((b)\) by microbial populations in the respirometer. Errors under \((a)\) can be minimized by maintaining the same relatively small change in \(O_2\) tension during repetitive measurements of \(O_2\) uptake by an animal. \(O_2\) consumption by the electrode can then be regarded as approximately constant. In fact the rate of \(O_2\) uptake by the electrode falls from 6.75 to 5.25 \(\mu g\) \(O_2/l\) over the range 90–70 % saturation at 10.0 °C. But the error involved in assuming a constant rate of uptake is less than 1 % of the \(O_2\) uptak
by a 10 g crayfish. In practice the errors under (a) are combined with much larger errors under (b). The microbial O₂ demand is variable, but it may represent as much as 13% of the standard metabolic rate of a 10 g crayfish, even when artificial media are used instead of natural water. Prior sterilization of the media does not prevent microbial growth in the respirometer, as microbes are introduced along with the experimental animal. In the case of crayfish and Gammarus, the introduced microbes may belong to both the intestinal fauna and the fauna sited on the external body surfaces. Most of the microbial growth occurs on the internal surfaces of the respirometer, rather than in the water column; the internal surfaces are made less suitable by coating with Silicone 'Repelcote' (Hopkin & Williams Ltd). The addition of antibiotics to experimental media (Marshall & Orr, 1958) was only partially successful in depressing the growth of microbes. Much more effective is the use of media containing little or no nutrient salts, made by adding Analytical Grade salts to deionized water. These artificial media resemble natural fresh waters with respect to the concentrations of the major ions (including potassium but excluding nitrate) and with most freshwater animals they are suitable for experiments lasting for at least 7 days.

Nevertheless the background component due to microbes must be assessed for each experiment. This is best done by measuring the initial background O₂ uptake by the clean respirometer immediately before the start of an experiment. A period of ca. 4–6 h is necessary to obtain a reasonable estimate of the rate of O₂ uptake before introducing the experimental animal. When the latter is removed at the end of the experiment, the respirometer is 'topped up' with the experimental medium and the final background O₂ uptake is measured again for some 4–6 h.

It may be noted here that the classical blank bottle used as a control for estimating microbial respiration in the experimental medium may not be, in fact, a suitable control, as it does not include microbes introduced with the experimental animal. In our experience these are a major source of the background component that develops in relatively long-term measurements of respiration. Microbial growth on the internal surfaces of the respirometer probably also accounts for the curious remark concerning 'the considerable oxygen removal by the plastics' in a closed respirometer described by Eriksen & Feldmeth (1967).

In 32 experiments on crayfish (Sutcliffe & Carrick, 1975) the initial background O₂ uptake rates were very similar to that of the oxygen electrode alone when this is determined in sterilized or freshly prepared deionized water at 10–15 °C. After experiments lasting for 51–98 h, the final background rates (including the oxygen electrode) had a mean value of 25.1 ± 7.2 (s.D.) µg O₂/h. Tests showed that, as might be expected, the microbial O₂ demand does tend to increase with time, but one cannot predict the rate of O₂ uptake at any selected point during the course of an experiment. Thus it was not possible to predict how the microbial demand might increase, or vary, in two or more experimental media of differing concentrations when measuring the respiration rate of crayfish in, for example, deionized water for 24 h followed by a medium containing 0.6 mM/l NaCl for 24–48 h. As an approximation, the mean background rate for each experiment (i.e. initial + final background ÷ 2) was subtracted from each O₂ uptake rate determined on a crayfish. The metabolic rate of crayfish therefore may be slightly underestimated in the first stages and slightly overestimated in the later stages of an experiment. In a 10 g crayfish with a standard metabolic rate of 273 µg O₂/h the mean
background error, excluding the oxygen electrode (6 μg O₂/h), is expected to be usually less than 25·1 - 6·0 = (19·1/273) x 100 = 7·0%. Background errors in some individual experiments might, however, occasionally be as high as ±10%.

ESTIMATION OF RATES OF OXYGEN UPTAKE IN CRAYFISH

Between experiments, crayfish were held in Windermere lakewater and fed on live earthworms and Gammarus pulex. Before each experiment a crayfish was transferred to artificial lakewater media or to deionized water and held at 9·0 °C without food for 72 h. In the respirometer, animals were exposed to two or three different experimental media for varying periods of time. Further details are given by Sutcliffe & Carrick (1975).

For measurements of O₂ uptake the O₂ concentration in the medium was allowed to fall to 7·9 mg/l (70% saturation). Fresh medium was then run through the respirometer until the O₂ concentration was raised back to 10·1 mg/l (90% saturation). Over this range the rate of O₂ uptake is independent of the O₂ concentration in the medium. Each flushing or open period lasted for ca. 10 min due to a slow rate of siphoning from reservoir A through B and C to D (Fig. 2). The volume of medium flushed during each open period was approximately equal to the volume of the respirometer. The relatively large capacity of the reservoir system (14 l) ensured that the respirometer could be left unattended for periods of 16–24 h. When flushing was stopped by closure of the Skinner water valve, the media in the respirometer became thoroughly mixed during the first 5 min of the next closed period. Each closed period usually lasted for some 2–4 h.

From the decrease in O₂ concentration during each closed period the rate of O₂ uptake by a crayfish was estimated in two ways. A series of hourly rates was obtained from the measured decrease in O₂ during each successive hour (e.g. HR₁, HR₂, Fig. 5) in a closed period. In addition a single averaged rate/h was obtained from the total decrease in O₂ during each timed closed period (excluding the first 5 min) e.g. C₁–C₂ and d₁–d₂ in Fig. 5.

RESULTS AND DISCUSSION

Figs. 6, 7 show examples of experiments in which crayfish remained relatively quiescent for periods of 71–98 h. The hourly rate of O₂ uptake was never constant, although it was sometimes nearly so in successive periods of up to 6 h, e.g. crayfish A₁. Occasionally there was a temporary large increase in the O₂ uptake rate when it increased by a factor of ca. 1·5–2·0. These high values may have been associated with short bouts of locomotor activity, as similar high values were often recorded during the first hour or two after placing crayfish in the respirometer. Otherwise the range of variation in the standard metabolic rate represents some 77–123% of the mean rate in crayfish A₁, 70–139% of the mean rate in the same crayfish on another occasion (A₁, Fig. 6), and 57–172% of the mean rate in crayfish D₁ (Fig. 7). Similar fluctuations, measured at 10 min intervals, were found in Pacifastacus leniusculus (Moshiri et al. 1971). Despite this variability, which was even greater in some of the other experiments in the present study, the level of metabolism in each crayfish generally remained unaltered for long periods. In the three examples shown in Fig. 6 the standard
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Fig. 5. A typical recorder trace at the start of an experiment. a is the calibration trace; b is the point where a crayfish was placed in the respirometer (the electrode output falls when the respirometer is removed from the water bath); c1-c3 is the first closed period, flushing starts at c3; d1-d4 is the second closed period. HR = hourly rate of O₂ uptake. AR = averaged rate of O₂ uptake/h for the period c₁-c₄.

metabolic rate apparently declined slightly during the course of the experiments. But in others the standard metabolic rate either showed a slight increase or else it remained at the same level, and it was concluded that there was no consistent fall in the metabolic rate like that found, for example, during the first week of starvation in the shore crab Carcinus maenas (Marsden, Newell & Ahsanullah, 1973; Wallace, 1973). In addition there was no sign of a 24 h endogenous rhythm in the metabolic rate of crayfish exposed to constant illumination.

In Fig. 7 hourly rates of O₂ uptake are compared with averaged rates of O₂ uptake. As expected, the averaged rates smooth out some of the variability within the hourly rates; thus the mean standard deviation is normally smaller in the averaged rates (Table 1). But since the number of observations (N) is also smaller, the values for the
Fig. 6. Hourly O₂ uptake rates for standard metabolism in crayfish D₄, and in crayfish A (two separate experiments.) Experimental media are depicted as follows: •, deionized water; ○, artificial lakewater containing 0.5 or 0.6 mM Na; and ×, 0.20 or 0.25 mM Na.

95% confidence limits are generally increased. Confidence limits were calculated from the standard error of the mean multiplied by $t_{0.05}$; the latter is the appropriate value for $t$ (with $N - 2$ degrees of freedom) in Student's $t$-distribution. When $N > 20$ the value for $t_{0.05}$ approaches 2.0, and thus the 95% confidence limits are generally smaller for averaged rates than for hourly rates. But when $N < 20$, and especially when $N < 10$, the increasing values for $t_{0.05}$ result in larger 95% confidence limits for averaged rates compared with hourly rates estimated on the same animal. This is also shown in Table 1 where the 95% confidence limits are expressed as percentages of the mean O₂ uptake rates. Note that, during at least one series of measurements in three out of four experiments, the 95% confidence limits exceed 10% of the mean rates calculated from both averaged and hourly O₂ uptake rates. With variability of this magnitude even in relatively quiescent animals, an observed change in metabolic rate equivalent to less than 20% of the standard rate will rarely be statistically significant (Table 1).

Fig. 8 is a log-log plot of standard metabolism against body wet weight. Each point is the mean value for averaged O₂ uptake rates obtained from crayfish held...
deionized water, Na-free media or artificial lakewater media (Sutcliffe & Carrick, 1975). The line drawn through forty-five points represents the regression \( \ln Y = a + b \ln X \), where \( Y \) and \( X \) respectively are \( O_2 \) uptake (\( \mu g/h \)) and body wet weight (g); \( a = 3.3037 \), and \( b = 1.002 \pm 0.262 \), 95% confidence limits. The correlation coefficient \( r \) is highly significant (\( r = 0.762, P < 0.001 \)). Hence the mean standard metabolic rate in a 10-g crayfish was 273.4 \( \mu g \) \( O_2/h \), and the rate is directly proportional to body wet weight. In other crustaceans the metabolic rate often decreases relative to an increase in body weight; values for \( b \) generally range from 0.6 to 1.0 (Wolvekamp & Waterman, 1960; Bulnheim, 1972). In the present study the weight range was restricted to medium-sized animals weighing 7.0–12.5 g (Fig. 8). Possibly crayfish with a body weight of only 1.0 g may have a standard metabolic rate greater than the predicted mean rate of 272 \( \mu g \) \( O_2/h \), although two out of three measurements obtained so far were within the 95% confidence limits of the values predicted from the above regression equation (Table 2).

In Fig. 8 a distinction is made between \( O_2 \) uptake measured in the summer months, June–September, and measurements obtained during October–May. The crayfish moulted in the summer months, and in some decapods the metabolism increases during the premoult and moult stages, declining thereafter in the intermoult stage (Scudamore, 1947; Rice & Armitage, 1974). In this study most of the experiments were carried out on intermoult animals, and there was no obvious increase in standard metabolism during the summer months (Fig. 8). But on one occasion crayfish D8 began to moult when held in the respirometer. In the preceding 86 h mean values for
Table 1. A comparison of the mean values for hourly and averaged $O_2$ uptake rates in crayfish exposed to deionized water (DW) or artificial lakewater media (ALW) containing 0.2–0.6 mM Na

<table>
<thead>
<tr>
<th>Crayfish</th>
<th>Medium</th>
<th>Hourly $O_2$ uptake rate (µg/h)*</th>
<th>Difference between means† (µg/h)</th>
<th>95% confidence limits (% mean rate)</th>
<th>Averaged $O_2$ uptake rate (µg/h)*</th>
<th>Difference between means† (µg/h)</th>
<th>95% confidence limits (% mean rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1$</td>
<td>DW</td>
<td>313.8 (17) ± 72.09 µg/h</td>
<td>19.2</td>
<td>11.8</td>
<td>284.9 (7) ± 39.38 µg/h</td>
<td>5.9</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>0.25 ALW</td>
<td>294.6 (30) ± 30.73 µg/h</td>
<td>19.3</td>
<td>7.0</td>
<td>279.0 (15) ± 23.05 µg/h</td>
<td>20.8</td>
<td>4.6</td>
</tr>
<tr>
<td>$A_7$</td>
<td>DW</td>
<td>275.3 (17) ± 37.90 µg/h</td>
<td>6.1</td>
<td>5.4</td>
<td>242.6 (12) ± 24.12 µg/h</td>
<td>3.7</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>0.2 ALW</td>
<td>243.1 (31) ± 36.15 µg/h</td>
<td>6.1</td>
<td>5.7</td>
<td>246.3 (9) ± 30.94 µg/h</td>
<td>6.3</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td>0.6 ALW</td>
<td>237.0 (24) ± 31.82 µg/h</td>
<td>6.1</td>
<td>5.7</td>
<td>246.3 (9) ± 28.94 µg/h</td>
<td>6.3</td>
<td>9.3</td>
</tr>
<tr>
<td>$D_1$</td>
<td>DW</td>
<td>236.3 (19) ± 55.12 µg/h</td>
<td>11.8</td>
<td>11.2</td>
<td>205.0 (7) ± 32.90 µg/h</td>
<td>13.5</td>
<td>14.8</td>
</tr>
<tr>
<td></td>
<td>0.5 ALW</td>
<td>224.5 (21) ± 54.40 µg/h</td>
<td>11.8</td>
<td>11.2</td>
<td>218.5 (19) ± 36.56 µg/h</td>
<td>13.5</td>
<td>14.8</td>
</tr>
<tr>
<td>$D_4$</td>
<td>0.6 ALW</td>
<td>184.3 (18) ± 18.74 µg/h</td>
<td>25.9†</td>
<td>5.0</td>
<td>176.5 (6) ± 44.95 µg/h</td>
<td>22.8†</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>0.2 ALW</td>
<td>158.4 (19) ± 21.86 µg/h</td>
<td>16.9</td>
<td>6.6</td>
<td>153.7 (6) ± 10.19 µg/h</td>
<td>7.0</td>
<td>27.8</td>
</tr>
<tr>
<td></td>
<td>0.6 ALW</td>
<td>188.3 (19) ± 66.28 µg/h</td>
<td>16.9</td>
<td>6.6</td>
<td>197.5 (6) ± 52.34 µg/h</td>
<td>43.8</td>
<td>27.8</td>
</tr>
</tbody>
</table>

* Mean (number of observations) ± standard deviation.
† These differences were not significant at the 5% level, except those marked (†) where $P < 0.001$. 
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Fig. 8. Log-log plot of standard metabolism (Y) against body wet weight (X). The solid line is drawn from the regression In Y = 3.3037 + 1.002 In X (see the text). Measurements made during \( \bullet \), October–May; during \( \times \), June–September; \( \Delta \), excluded from the regression.

Table 2. Hourly rates of \( O_2 \) uptake measured in four small crayfish at 10.0 °C
(The predicted rates were calculated from the regressions drawn in Fig. 8 (standard metabolism) and Fig. 10 (active metabolism).)

<table>
<thead>
<tr>
<th>Wet weight (g)</th>
<th>Observed ( O_2 ) uptake (µg/animal.h)*</th>
<th>Predicted ( O_2 ) uptake (µg/animal.h)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.63</td>
<td>35.0 (43) ± 3.4</td>
<td>17.1 (7.9–37.3)</td>
</tr>
<tr>
<td>1.03</td>
<td>67.0 (32) ± 9.3</td>
<td>28.0 (14.4–54.5)</td>
</tr>
<tr>
<td>2.35</td>
<td>69.9 (42) ± 6.5</td>
<td>64.1 (39.3–104.5)</td>
</tr>
<tr>
<td>2.35</td>
<td>214.4 (32) ± 25.5</td>
<td>177.2 (99.3–347.4)</td>
</tr>
<tr>
<td>1.75</td>
<td>167.7 (37) ± 20.1</td>
<td>137.4 (63.4–298.0)</td>
</tr>
</tbody>
</table>

* Mean (N) ± 95% confidence limits.
† Mean (95% confidence limits for a single predicted value).

averaged \( O_2 \) uptake rates in this animal were 452.0 ± 35.54 S.D. \((N = 13)\) during 23 h in deionized water and 452.4 ± 28.85 S.D. \((N = 24)\) during 58 h in artificial lakewater containing 0.6 mM sodium. The wet weight of crayfish D8 was 7.2 g prior to ecdysis, with a predicted standard metabolism of about 200 µg \( O_2 \)/h (Fig. 8). Its \( O_2 \) uptake rate during ecdysis was more than double the predicted value. \( O_2 \) uptake was also doubled during ecdysis in Cambarus (Scudamore, 1947).

Fig. 9 illustrates the increased rate of \( O_2 \) uptake associated with locomotor activity in a crayfish that previously had been inactive for 36 h. Some crayfish were almost continuously active in the respirometer, exploring the walls of the animal chamber and occasionally rearing up on the walking legs to push with the large chelipeds against the lid of the chamber. In these cases the averaged \( O_2 \) uptake rates were similar to those shown in Fig. 9 during the second part of the experiment.
Fig. 9. Averaged O₂ uptake rates in crayfish C. showing standard metabolism in deionized water (●) and active metabolism in artificial lakewater containing 0.5 mM Na (○).

Fig. 10 is a log-log plot of active metabolism against body wet weight. Each point is the mean of a series of averaged O₂ uptake rates recorded for periods ranging from 21 to 66 h. These results were obtained from experiments where locomotor activity was either observed or inferred from the elevated and highly variable hourly rates of O₂ uptake. The solid line drawn through sixteen points in Fig. 10 again represents the regression In Y = a + b In X. For active metabolism, a = 4.4412, b = 0.861 ± 0.392, 95% confidence limits; r = 0.783 (P < 0.001). In a 100 g crayfish the mean active metabolic rate was 616.6 μg O₂/h, more than double the standard rate (dotted line in Fig. 10). In two crayfish respectively weighing only 1.75 g and 2.35 g (Table 2) the active metabolic rates were similar to the mean values predicted from the regression for active metabolism in 7–14 g animals. These results on A. pallipes may be compared with O₂ uptake rates in other European crayfish (Wolvekamp & Waterman, 1960), i.e. 43–77 μg O₂/g.h at 15 °C in A. astacus, 100 μg O₂/g.h at 20 °C in A. leptodactylus and 143 μg O₂/g.h at 20 °C in A. torrentium. When allowance is made for differences in temperature (assuming Q₁₀ = 2.0) these O₂ uptake rates are very similar to the active metabolic rates found in A. pallipes.
Oxygen uptake in A. pallipes

Fig. 10. Log-log plot of active metabolism (Y) against body wet weight (X). The solid line is drawn from the regression \( \ln Y = 4.4412 + 0.861 \ln X \) (see the text). Symbols as in Fig. 8. The dotted line represents the regression of standard metabolism shown in Fig. 8.

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REFERENCES


