

## A DIFFERENTIAL VOLUMETRIC MICRO-RESPIROMETER FOR USE WITH SMALL AQUATIC ORGANISMS

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A micro-respirometer is described for use with small aquatic organisms whose rates of oxygen consumption fall within the range  $0.1-5 \mu\text{l O}_2 \text{ h}^{-1}$ . The major design improvement which this apparatus has over existing models (e.g. Fenn, 1927) is a relatively large capillary bore. Kok, Veltkamp & Gelderman (1953) having shown a proportional decrease in the sensitivity of volumetric respirometers with capillaries smaller than 1 mm diameter. The most recent treatment of the theoretical aspects of differential respirometry is by Umbreit, Burris & Stauffer (1972).

The apparatus (Fig. 1 A) comprises a respiration vessel with an alkali well, valve and graduated capillary containing an index drop of Apiezon oil C (Edwards High Vacuum, Crawley), connected by a short length of thin-walled polythene tubing to a compensation system. The respirometer is mounted on a perspex holder and supported by a frame in a water bath.

The wide-mouthed respiration and compensation vessels, internal capacities of about 5.5 ml (1 dram vials), were supplied by F. B. G. Trident Ltd., London. The capillary tubing is manufactured by Clay Adams, U.S.A., as 100  $\mu\text{l}$  micro-pipettes (Yankee, Micropet). A satisfactory scale was constructed from lacquered, 1 mm graph paper threaded onto the horizontal arm of the measuring capillary by means of two transverse slits at either end. A finer scale has been produced by photographic reduction.

No. 17 rubber bungs were drilled to accommodate a glass well and capillary tubing (Fig. 1 B, C). The wells comprise 10 mm lengths of glass tubing (internal diameter ca. 5 mm), sealed at one end. The micropipettes were bent into a right-angle approximately 30 mm from one end, in a cool flame. Valves were constructed from 40 mm lengths of glass capillary tubing inserted through the bungs, with 20 mm lengths of polythene tubing (internal diameter 2 mm) pushed over their free ends so that 10 mm extended beyond each capillary. The holder consists of a strip of 3 mm perspex drilled to take four 25 mm long pegs which hold the bungs in position. A slotted rack (Fig. 1 D) was constructed to accommodate 6 respirometers (5 experimental and 1 control) approximately 50 mm apart in a water bath. This rack was supported in the water bath by four S-shaped hooks.

For the successful operation of the apparatus it is essential that the gas volumes in the respiration and compensation sections of the respirometer are identical. Since the combined internal volumes of the capillary and valve are the same in each section, it remains only to determine the internal capacities of the two vessels when attached

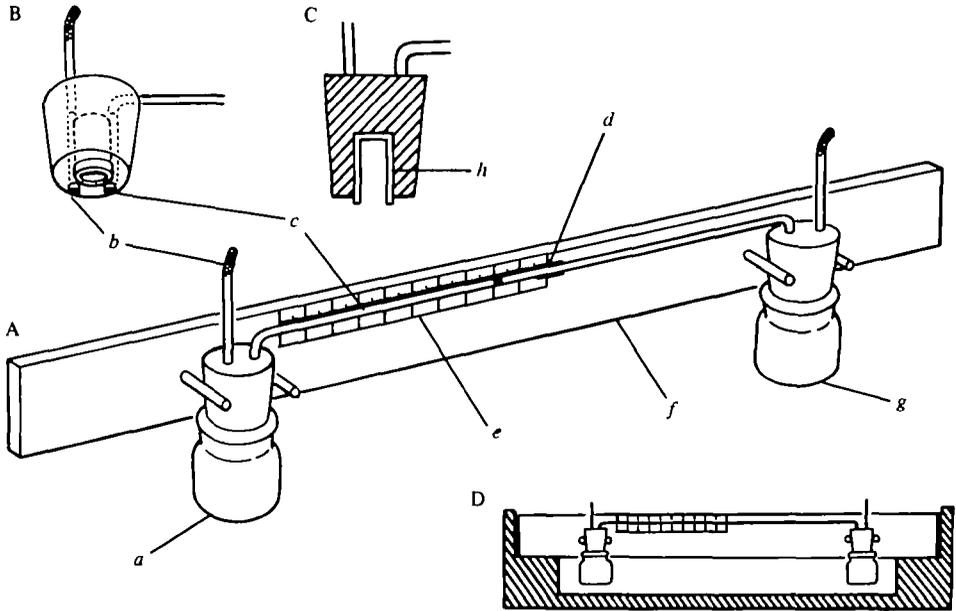


Fig. 1. (A) Volumetric micro-respirometer. (B), (C) The bung assembly. (D) Vertical section through the slotted rack showing a single respirometer unit in position. a, respiration vessel; b, valve; c, capillary; d, connecting tube; e, graduated scale; f, perspex holder; g, compensation vessel; h, alkali well. As an indication of scale, the perspex holder is about 30 cm long. (Drawing by A. J. Lee).

to the bungs. This was achieved using a gravimetric method, in which mercury is run into the vessel up to the level of the bung. The mercury is then weighed after its temperature has been measured. The weight of the mercury in grammes, divided by its density at that temperature (see Dixon, 1951, p. 141) gives the required volume in millilitres. It is, of course, essential that the bung be inserted to the same depth in the vessel each time, and this is facilitated by the presence of a thickened rim in the vessels described above, the lower edge of which acts as a convenient reference line.

To ensure that the index fluid moves freely, the tube wall must be ideally wetted with Apiezon oil, and this is achieved by running some of the oil through the measuring capillary under gravity. The capillary should then be stood upright on some paper tissue until the excess oil has completely drained through. A 3 mm long index droplet of the oil is then introduced into the long arm of the capillary which should be tilted to allow the droplet to travel down into the graduated section. The respirometer can then be assembled and all the joints greased with silicone high vacuum grease (supplied by Edwards High Vacuum Ltd., Sussex, England).

It is essential that the vessels are kept scrupulously clean and free from all traces of algal or bacterial contamination. This is achieved by washing the vessels thoroughly after use, and autoclaving (15 p.s.i. for 20 min) prior to each experiment. The bases of the bungs should be wiped clean with paper tissue wetted with a dilute solution of antibiotics (streptomycin sulphate (50 mg l<sup>-1</sup>) and benzylpenicillin (30 mg l<sup>-1</sup>). As a further precaution against contamination, water used in the respirometer was filtered through a sterile 0.2  $\mu$ m membrane filter and treated with antibiotics (Marshall Orr, 1955).

A sufficient volume of water is added to the respiration vessel to cover the animal completely. In the case of *Ficopomatus*, 1.5 ml was used. To ensure a rapid exchange of gases between the gaseous and liquid phases in the vessel it is important that the surface to volume ratio of the liquid is as large as possible. Under the above conditions, where the surface area : volume ratio is about 2, shaking of the apparatus was found to be unnecessary. Sufficient sterile water should be added to the compensation vessel to balance exactly the gas volumes on the two sides of the system. After the lip of the glass well in the respiration chamber has been lightly greased to prevent alkali creep, a fluted wick cut from Whatman no. 42 (starch-free) filter paper, wetted with a 5% solution of potassium hydroxide, is inserted into the well, taking care to blot off excess alkali. The respirometers should be arranged in the water bath so that the water level is the same as the perspex pegs.

After sufficient time has passed for temperature equilibration, usually 30–60 min, the valves should be closed with silicone grease and after a minute the initial reading is made. The graduated scale should be slid along until a convenient mark is aligned with the meniscus on the leading edge of the index fluid droplet. A lens will prove useful when measuring very small changes in gas volume, and care must be taken when reading the position of the lowest point on the meniscus to avoid errors resulting from parallax.

The readings in mm of oxygen are converted to  $\mu\text{l}$  at S.T.P. using the equation:

$$x = d \left[ 2A \cdot \frac{P}{P_0} \cdot \frac{273}{T} \right],$$

where  $x$  = the amount of gas absorbed at S.T.P.,  $d$  = the distance in mm through which the oil moves in the capillary,  $A = 1.32 \text{ mm}^2$ , the cross-sectional area of the capillary,  $P$  = the barometric pressure at the time the valves were closed (in mm of Hg),  $P_0$  = standard pressure (760 mmHg),  $T$  = the absolute temperature of the water bath.

It should be noted that this is a simplification of the equation given by Dixon (1951, p. 47). Omission of the volume, solubility, and additional pressure terms has an insignificant effect on the constant when the apparatus is used in the present context. Readers are referred to Dixon's excellent book (pp. 24–33 and 46–47) should they decide to use the respirometer for other purposes. At the end of a run the valves should be opened, by snipping off the blocked sections, before dismantling the apparatus.

The average respiration run has been 6 h but respirometers have been left for 12 h without a detectable reduction in the measured rate of oxygen consumption. It is advisable to recharge the wick with fresh alkali if longer periods are intended, and the build up of waste products should be taken into consideration. The index droplet can be re-set without dismantling the respirometer by opening the valves and attaching a hypodermic syringe with a wide bore needle to the compensation valve. The position of the index droplet in the capillary is then adjusted by manipulating the plunger.

For its successful operation the respirometer should be maintained at a constant temperature. Satisfactory thermal stability of  $\pm 0.1^\circ\text{C}$  was achieved by opposing the cooling effect of a dip cooler (Cambridge Instruments Ltd.) by the more powerful,

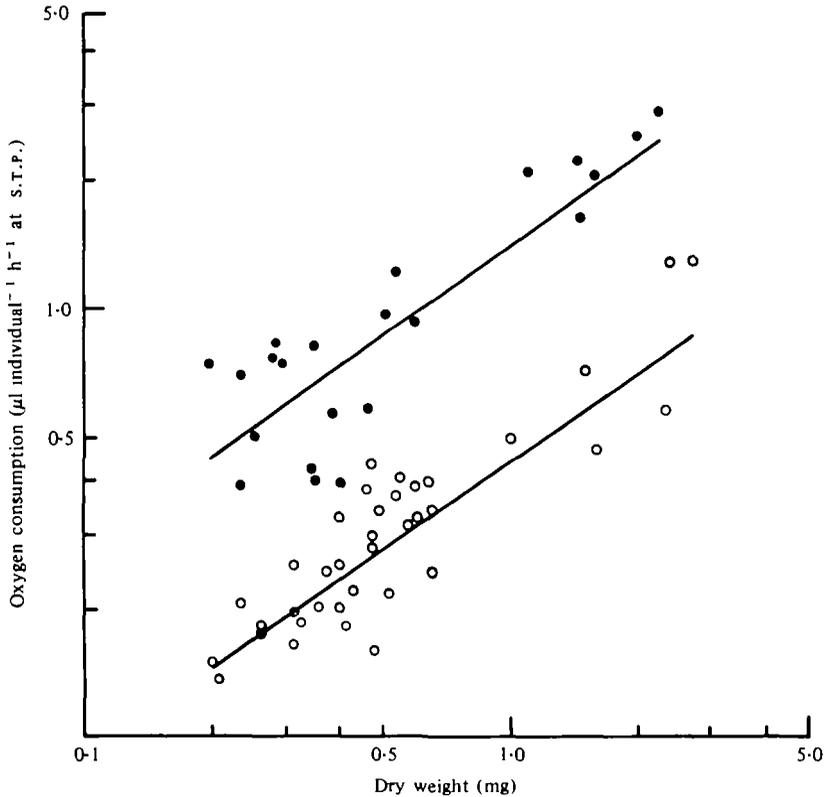


Fig. 2. Individual oxygen consumption rates of *Ficopomatus enigmaticus* acclimated to 10 °C (open circles) and 20 °C (closed circles) plotted as a function of dry weight. The regression lines were fitted by the method of least squares, assuming linear relationships after logarithmic transformation of the data.

thermostat-controlled heater of a large Grant Instruments water bath. Excellent results have also been obtained with a water bath and cooler manufactured by Heto Birkerød, Denmark, for controlled temperature oscillations, when used at a fixed temperature setting.

Oxygen consumption values for the serpulid polychaete *Ficopomatus* are shown in Fig. 2 for two experimental temperatures.

The figure shows oxygen consumption rates of worms acclimated to 10 °C, and 20 °C, expressed as a function of dry weight. Rates for individual worms were derived by calculating the slopes of regression lines fitted to individual data when expressed as incremental oxygen consumption, measured at hourly intervals over a 6 h period, plotted against time (Dixon, 1977). The regression coefficient ( $b$ ) for the 10 °C relationship is 0.74, and for the 20 °C relationship,  $b = 0.71$ . Good correlations exist between the two parameters at both acclimation temperatures,  $r = 0.8$  at 10 °C, and 0.75 at 20 °C. Both values considerably exceed the tabulated figures at the  $P = 0.05$  level of significance.

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