Using Microrespirometers to Measure O_2 Consumption by Insects & Small Invertebrates

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How-To-Do-It

Using Microrespirometers To Measure O_2 Consumption by Insects & Small Invertebrates

Richard E. Lee, Jr.

A variety of physiological studies require the measurement of oxygen consumption. Unfortunately, the techniques for measuring respiration rate often require expensive equipment or difficult procedures that are not available or suitable for use in student laboratories. In this article I describe a sensitive, simple and inexpensive microrespirator that may be used readily by students. This constant pressure microrespirometer is similar to ones described by Engelmann (1963) and Conradi-Larsen (1974). It is useful for a range of invertebrates weighing 5–500 mg and for measurements at low, even subzero, temperatures (Lee & Baust 1982a,b; Bennett & Lee 1989). For smaller animals or at very low rates of oxygen consumption, several individuals may be run in a single microrespirometer. A general discussion of respirometric methods is available in Umbreit, Burris & Stauffer (1972).

For every molecule of oxygen that is taken up by an organism, one molecule of carbon dioxide is released when carbohydrate is the primary energy source. In a closed system, normal respiration causes no net change in atmospheric pressure because each molecule of oxygen that is removed is replaced by one of carbon dioxide. However, in this microrespirometry technique, when carbon dioxide molecules are released they are removed from the atmosphere and taken up by a potassium hydroxide solution. This results in a decrease in pressure within the closed environment and causes the potassium hydroxide solution to move down the micropipet. Since the movement of the potassium hydroxide is directly proportional to the amount of oxygen consumed, by knowing the volume of the micropipet and the measurement interval, one can easily determine the rate of oxygen consumption.

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Materials and Methods

Microrespirometers are made by gluing about one centimeter of a disposable micropipet into the tip of a disposable plastic syringe (Figure 1). Be careful not to plug the micropipet with glue. Hot melt glue works well and may be melted using heat from a candle. Since various factors, including the size of the animal and the temperature at which determinations are done, influence the rate of oxygen consumption, the size and combination of the syringe (1–5 cc) and micropipet (10 or 20 μl) must be determined empirically.

The syringe plunger is first put through a metal fender washer that holds the respirometer upright in a constant temperature bath (±0.2° C). The animal is placed in the barrel of the syringe; and the plunger is inserted and placed in the water bath so that only the micropipet extends above the surface of the water. A solution of approximately 10% potassium hydroxide is introduced into the top of the micropipet, serving both as an absorbent for carbon dioxide and a manometric indicating fluid. The linear length of the potassium hydroxide solution in the micropipet should be at least 5 mm. Food coloring or another dye may be added to the potassium hydroxide solution to make it easier to see.

Since it is important that the room temperature and barometric pressure remain constant during measurements, an empty microrespirometer, in addition to the ones containing organisms, serves as a thermobarometer to detect changes in the temperature of the water bath or the barometric pressure during the experiment. If the level of the potassium hydroxide in the thermobarometer changes during a run, the values for the respirometers containing animals should be adjusted accordingly. For example, if the thermobarometer decreased by 2 mm during an interval, that distance should be subtracted from the readings for the microrespirometers containing animals. However, if the temperature bath remains constant, this adjustment is usually not necessary.

Since the air pressure within the thermobarometer remains constant as long as temperature and room air pressure remain unchanged, it may be difficult to introduce the potassium hydroxide solution initially. If the bath temperature is less than room temperature, the respirometer may be slightly warmed by briefly lifting it out of the bath. Once it is returned to the bath, quickly apply a drop of the potassium hydroxide solution to the top of the micropipet (Filling a 1 cc syringe with this solution is a convenient way of applying the drop.) As the pressure within the closed volume of the respirometer is reduced, due to cooling, the potassium hydroxide solution will be pushed downward into the micropipet by the greater atmospheric pressure. If the water bath is at room temperature, simply lift the microrespirometer out of the bath and warm the outside of the chamber containing the invertebrate between one's fingers.

A period of about 10–15 minutes should be allowed for equilibration and to allow the potassium hydroxide solution to move away from the upper end of the micropipet before measurements start. The position of the potassium hydroxide solution should be recorded initially and at 5–10 minute intervals for 30–90 minutes. The rate of change should be calculated for each interval. Sometimes the initial one or two rates will be unusually low and should be ignored until the potassium hydroxide is moving at a relatively constant rate from one time interval to the next. The average rate of uptake over several intervals with similar values should be used to determine the

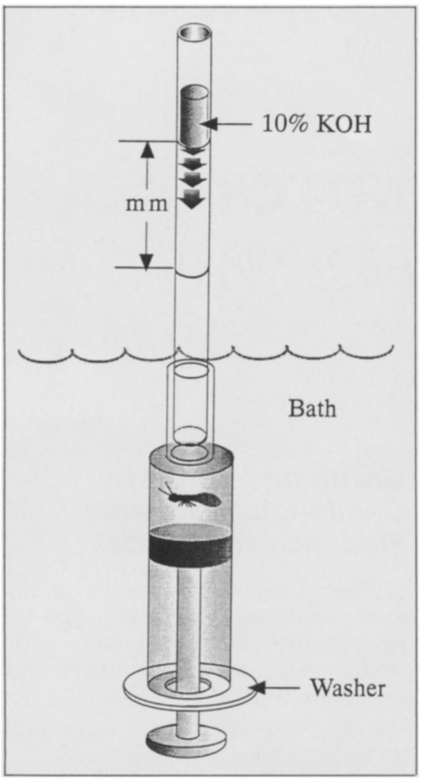

Figure 1. Diagram of microrespirometer positioned in a water bath.

rate of oxygen consumption for a given respirometer.

Oxygen consumption is calculated by multiplying the distance (mm) moved by the potassium hydroxide solution by the conversion factor (μl/mm) for the specific micropipet selected. For example, on a 20 μl-micropipet the distance from the end of the micropipet to a marked line near its middle indicates a volume of 20 μl. The conversion factor would be 20 μl divided by that distance in millimeters. The hourly rate of oxygen consumption is commonly reported on a live or dry weight basis, or per individual. For example, in overwintering adults of the convergent lady beetle, *Hippodamia convergens*, oxygen consumption was 1.1 μl/mg live weight/hr or 15.4 hr/individual at 20° C (Bennett & Lee 1989).

For subsequent experiments the syringes may be reused, but the micropipets are usually discarded because they are difficult to clean. Micropipets may be easily removed by gripping them with small pliers just above the junction with the syringe, and twisting.

Classroom Applications

Various experiments, including the effect of size, sex and age on oxygen consumption, may be done using this system. The effect of temperature on the rate of oxygen consumption may be studied using a temperature-controlled water bath. In invertebrates, oxygen consumption is often directly related to temperature over the range of 0–25° C (Figure 2). Alternatively and less expensively, stable water baths may be obtained by allowing one to equilibrate at room temperature (approximately 21–23° C) and a second 0° C bath to equilibrate in a container filled with a mixture of crushed ice and water. This approach may be used to determine the effect of temperature acclimation or other treatments on respiration rate (Lee & Baust 1982 a,b; 1985). Using these microrespirometers, acclimation of diapausing adults of the convergent lady beetle to warmer temperatures causes a 40% decrease in their respiration rate (Bennett & Lee 1989). This response is consistent with their exposed overwintering site and serves to conserve limited energy reserves during a 10-month diapause when beetles are unable to replenish these reserves (Lee 1980). Use of this technique would also be appropriate for science fair projects.

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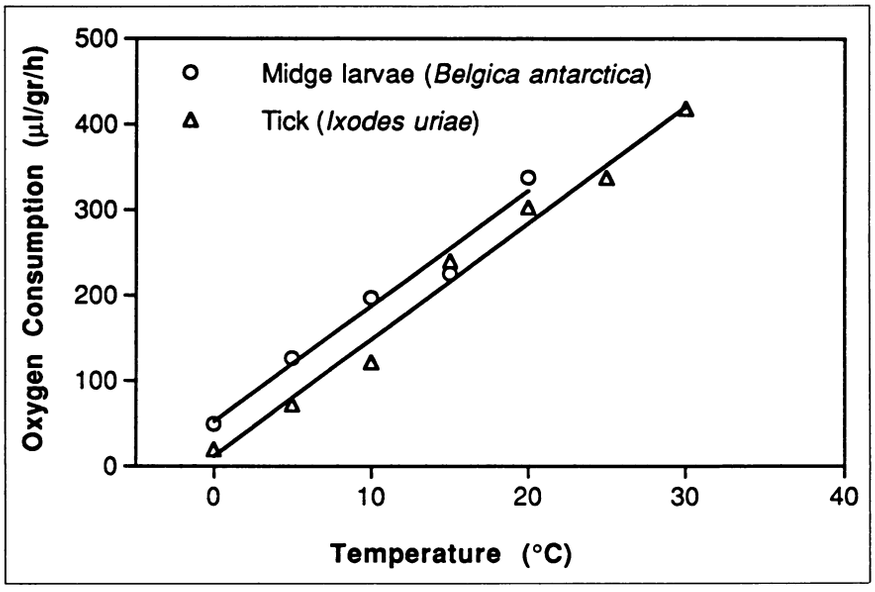

Figure 2. Effect of temperature on the rate of oxygen consumption determined using microrespirometers in two Antarctic terrestrial arthropods: adult females of the ixodid tick, *Ixodes uriae*, and chironomid larvae, *Belgica antarctica*. Data from Lee and Baust (1982 a,b).