The Effect of Temperature on the Aerobic Respiration of *Tenebrio Molitor* (Coleoptera: Tenebrionidae) Measured Using a Simple Microrespirometer

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Extended Abstract

Microrespirometers make a handy instrument for indirectly measuring respiration of small organisms such as plants or insects. This experiment involves the use of microrespirometers to indirectly measure the oxygen consumption of mealworms *Tenebrio molitor* (Order Coleoptera, Family Tenebrionidae). The respirometer adapted from Lee (1995) is made from a 1-ml syringe that has a capillary tube glued in the place of the needle. Additionally, there are two wads of cotton inside the syringe near the capillary tube one of which is standard absorbent cotton containing approximately 10 μ l of a 15% KOH (potassium hydroxide) solution. The other is a portion of non-absorbent cotton that protects the test organism from the corrosive KOH. The capillary tube is sealed with a small amount of liquid that is added to the tube. Respirometers work by enclosing organisms or a sample of an organism in a chamber. The KOH, a highly corrosive basic liquid, can take CO₂ (gas) out of the air and convert it to a solid. During cellular respiration CO₂ (gas) is released as O₂ is consumed. Since liquids and solids take up far less space than gases, the net result of the O₂ loss and CO₂ conversion from gas to solid is that the pressure decreases in the chamber. This pressure decrease is observed by the movement of liquid in the capillary tube. This liquid, referred to as manometer fluid, moves away from the capillary's opening and is measured using a millimeter ruler attached to the capillary tube.

The study organisms, meal worms, are not in fact worms, but rather beetles that are grain pests. These beetles make excellent organisms for animal food, bait for fishing, and subjects for experiments. Because they are grain pests, they are very easy to raise and take little effort to culture. In this study the effect of temperature on respiration is observed. The two temperatures are room temperature (ca. 20°C) and cold (an ice water bath at 0°C). As insects and their arthropod kin are ectothermic their respiration should decrease with a decrease in temperature.

Procedure

- 1. Determine which experimental group to test cold or at room temp and note the location of the proper water bath. Record its temperature.
- 2. Record the mass of the larva.
- 3. Place the larva in one of the microrespirometers tail-first. Place the plunger directly on the 1 ml mark of the syringe. The microrespirometer with the beetle will be the experimental group.
- 4. Place the microrespirometers in the proper water bath such that the capillary tubes are above the water but the rest of the device is under water.
- 5. Wait 10 minutes for the temperature of the respirometers and water to equilibrate.
- 6. *For the control group: CAREFULLY* place a small drop of manometer fluid (red food coloring mixed with water and dish soap) in the control microrespirometer with a capillary tube. This can be done by taking a capillary tube and dipping it into the manometer fluid. A small drop of the fluid should move into the tube. Carefully place the tips of the capillary tube and the open end of the respirometer's capillary tube together. The fluid in the capillary tube will move into the tube of the respirometer.
- 7. *CAREFULLY* adjust the fluid to move to the 50 mm mark. This is very tricky with the cold water and may take trial and error. Place the respirometer back into the water bath and wait for the manometer fluid to stop moving before proceeding. Be careful not to touch the body of the syringe as any change in temperature will affect the respirometer.

- 8. *For the experimental group: CAREFULLY* place a small drop of manometer fluid into the tube with the larva using the capillary tube as in step 9. Again be careful not to touch the body of the syringe.
- 9. When the manometer fluid in the experimental group has reached 0 mm on the ruler, begin keeping track of time.
- 10. Record the total distance the manometer fluid has moved at 5, 10, 15, and 20 min. for both the experimental and the control.
- 11. Remove the larva from the respirometer when finished and return it to the supply area.
- 12. Convert mm of fluid movement to volume and then divide the volume by the mass of the larva to get volume/mass. Data can then be shared with the other groups and graphed.

Notes for the Instructor

- -Capillary tubes used in the respirometer were 50 µl calibrated micropipettes purchased from Drummond Scientific Company. 1 mm of manometer movement in the micropipette is equivalent to ca. 0.685 µl of volume.
- -1-ml disposable syringes were purchased from Carolina Biological Supply.
- -The control respirometer is quite tricky, so you may want to do it yourself instead of having the students work with it directly. You can also try to adjust the position of the manometer fluid while the device is under water. This may help to avoid temperature fluctuations when the device is removed from the water bath.
- -Data can be graphed by hand or computer. (Use a scatterplot and add a best fit line for both variables). Comparisons can be made using the slope of the best fit line or by T-test of the 20 min. data.
- -To make this lab more of an inquiry lab, you may want to eliminate the assumption that temperature and respiration are linked with ectothermic organisms. They can make this discovery based on their data.

Literature Cited

Lee, R. L. 1995. Using microrespirometers to measure O₂ consumption by insects and small invertebrates. *The American Biology Teacher*, 5: 284-285.

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