

OXYGEN CONSUMPTION AND TEMPERATURE IN THE TERRESTRIAL ENVIRONMENT

BACKGROUND READING

Animal Physiology by Hill, Wyse & Anderson, 2004: pp. 130–139 & 214–221.

PRE-LAB

(Due at the start of the lab)

- ** In your lab notebook, draw a flow chart of the basic procedure for the experimental procedure you will be using in today's lab, including stabilization & calibration of the instrument, O₂ consumption measurements, and weighing the animals.
- ** On a separate piece of paper to be handed in, please answer the following questions. These must be word-processed
 1. Why can oxygen consumption be used as a measure of metabolic rate?
 2. When a physiologist equates oxygen consumption with metabolic rate, what one big assumption is the physiologist making? Is this a valid assumption for the experiment you are doing today?
 3. What are three factors other than temperature that can influence an animal's metabolic rate? For each of factors listed, cite where in your book (page number) or in your lecture notes (date of lecture) you obtained this information.
 4. When comparing your results to literature values, you discover that the oxygen consumption that you measured was, on average, twice as high as that reported in the literature for the same animal. What are two biologically-based plausible reasons for this difference? Cite where in your book (page number) or in your lecture notes (date of lecture) you obtained this information.

EXPERIMENT

ANIMALS & EQUIPMENT

Living material

You will be measuring oxygen consumption of a mouse ... a typical small endotherm.

Equipment

You will measure oxygen consumption with open flow respirometry. Specifically, you will use an electrochemical oxygen analyzer (S3-A Ametek Applied Electrochemistry) to measure the number of moles of oxygen entering and leaving a metabolic chamber over a known period of time. Air will be drawn through A metabolic chamber by a flow controller air pump at a known flow rate. Additional equipment and material include: a humidified respirometer, a balance, and filters containing Dririte and soda lime (see below). (*Include a sketch of the equipment set up in your lab book that is of sufficient quality that you could use it to set up the same experiment at a later date*)

STANDARD EXPERIMENTAL PROCEDURE

Preparation of the Analyzer

b. Make Filters

Filters must be made so that water vapor and carbon dioxide can be removed from the gas stream. Both gases (water vapor and carbon dioxide) will cause a decrease in the partial pressure of oxygen. If these gases were not removed the oxygen partial pressure decrease would not be solely from the animal consuming oxygen! Filters should have glass wool or cotton at both ends. 2/3 of the filter should be filled with the drying compound (Dririte). When Dririte becomes saturated with water it turns from blue to pink. The color should be checked before each measurement and changed if necessary. Next, a piece of a glass wool should be added on top of the Dririte. The carbon dioxide remover (soda lime) should be added on top of the glass wool and the plastic connector added. Soda lime turns from whitish to pinkish when saturated. Don't allow the soda lime to touch your hands or come in contact with water! Put the filters in the system as demonstrated by the instructor.

Never turn off the oxygen analyzer!

If turned off, it will take a day for the sensor to stabilize at the appropriate temperature.

Never allow moisture or any particulate matter to enter the oxygen sensors (always keep a filter on)!

This will ruin the instrument permanently. Note: This happened in the fall of 2002 and your professors were not happy.

5. Connect tubing, filters, and close chambers

Connect the tubing to the filters and to the chambers as directed in the pre-lab lecture. Make sure that the chambers are closed tight so that no air will enter through the lid. Set the flow rate to the level appropriate for your animal.

- **Record the following in your notebook** (these values are necessary for calculating oxygen consumption):
 - a. Chamber temperature
 - b. Barometric pressure
 - c. Flow rate

Analyzer Stabilization

After setting up the analyzer as described above, start collecting data on the PowerLab. While collecting data, allow the analyzer to sit undisturbed for at least 15 min. or until the readings stabilize around an average value.

Calibration of the Analyzer (do only after the instrument has stabilized; if you calibrate before the analyzer has stabilized, you'll be asked to go through the entire process again).

The oxygen concentration entering and leaving the chamber will be measured by two cells or sensors. One channel (#1) will act as a reference channel. Gas will flow through this channel, but will not enter the animal chamber. This will be equivalent to measuring the oxygen concentration of the air entering the chamber. The gas flowing in channel #2 will first pass through the metabolic chamber. When the analyzer is set in the ΔO_2 mode, the value that is present in the digital read-out represents the % difference in oxygen between channels #1 and #2

To perform the calibration:

- 1) Close the metabolic chamber and attach to the tubing leading to channel 2.
- 2) Turn the cell selector knob to cell #1. Use the cell zero 1 dial to set the concentration in the digital read-out for dry air, 20.95% O₂.
- 3) Turn the cell selector knob back to the Δ O₂ mode. With the cell zero 2 dial set the concentration difference to zero (0.000% O₂).
- 4) Wait for the reading to stabilize. The reading will drift during this period. After 10-15 min or when the reading has stabilized, repeat steps 2 and 3.

General Procedure for Measuring Terrestrial Oxygen Consumption

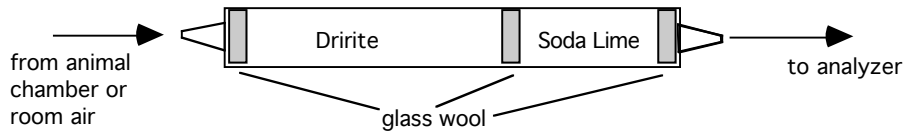
- * Because we do not need to worry about wasting chart recorder paper, you can continue to collect data as you put the animal in the chamber, as the reading reaches a steady state, and as the animal rests.
- 1) Open the chamber and put in the animal. Close the chamber quickly, being careful not to breathe into the chamber.
 - 2) The Δ O₂ % concentration will increase. Because of mixing in the chamber and tubing a steady-state will not be attained until 5-10 min.
 - 3) Let the animal sit undisturbed for at least 20 min.
 - 4) Leveling out of the trace means that the animal's O₂ consumption has stabilized. Look at the trace on the computer. Has it leveled out? If so, you can proceed to step 5. If not, wait another 5 minutes and then re-evaluate the situation. You must record long enough to get at least 5 minutes of relatively constant oxygen consumption. This may take 20 min, 30 min, 40 min, whatever. Every animal is different so patience and understanding is needed. Be sure to take copious notes about what the animal is doing while in the chamber so you know what sort of oxygen consumption you are measuring (e.g., standard, resting, basal, routine, etc.).
 - 5) Remove the animal and weight it to the nearest 0.1 g.
 - 6) **IMPORTANT → Check the filters and re-fresh if necessary. If the filters become exhausted during a measurement, that measurement will need to be re-done.**
 - 7) **IMPORTANT → After checking the filters and refreshing if necessary, repeat the instrument stabilization and re-zero prior to putting in another animal.** If you forget to do this, your values will be meaningless. Thus, your instructor will ask you to stop the measurement and complete this step before proceeding.
 - 8) Repeat steps 1 - 5 with another animal.

Temperature Effects

Perform two measurements on each individual, one at room temperature and one at 15° C. How will you maintain the chamber temperature lower than room temperature during these measurements? When will you record the chamber temperature?

TERRESTRIAL O₂ CONSUMPTION: IMPORTANT POINTS

- How do you know that you are measuring the oxygen consumption of frog and not some pesky microorganisms on the side of the chamber? What is the control that would answer this question? If you don't think that you have performed this control, FEAR NOT, 'cause I bet you have!
- Attach filters to the set up in the following orientation.



* The goal is to dry the air BEFORE it hits the soda lime;
soda lime + H₂O = toxic fumes

- Keep a close eye on the mice. There is not much room for error in these small ectotherms, especially in the cold. If you are at all concerned with the health of your animal during the experiment, ask your professor. Always error on the side of caution.
- You may want to cover the respirometer to minimize light. Why might this help the experiment? If you choose to do this, don't forget to note this in your lab notebook.
- The flow rate you use will, in part, determine the size of change you see. In general, the lower the metabolic rate, the lower the flow rate should be. I have set the instruments at a flow rate of 300 ml/min for these mice, although we might adjust it.
- Weighing stresses the animals. Therefore, **weigh each individual only after you have taken all of the the VO₂ measurements.**

Calculations

Oxygen consumption can be calculated as follows (units are italicized below equation):

$$V_{O_2} = \text{Flow Rate} * (\Delta O_2 \% / 100) / \text{Body mass}$$

*(ml/hr/g = ml/min * 60min/hr * dimensionless / g)*

Many organismal physiologists express V_{O_2} in terms of the volume of O_2 consumed (ml or l). However, recall that the quantity of gas held by a given volume will vary as a function of temperature, pressure and the amount of water vapor. Fortunately we can use a standard condition (called Standard Temperature Pressure Dry, STPD) so that the quantities of gas measured in different experiments can be compared.

To correct a volume: $PV = nRT$ gas law

-but-

$$nR [\text{STPD}] = nR [\text{uncorrected}]$$

-so-

$$V_2 P_2 / T_2 [\text{STPD}] = V_1 P_1 / T_1 [\text{uncorrected}] \text{ (where } V_1 = V_{O_2} \text{ that you measured)}$$

-thus-

$$V_2 [\text{STPD}] = P_1 / P_2 * T_2 / T_1 * V_1$$

P_1 = barometric pressure (torr), T_1 = chamber temperature °C + 273.15°K, T_2 = 273.15°K, P_2 = 760 torr, no water vapor (dry) ... (*Why can we use the value for dry air???*)

POST-LAB

(Due next week in lab)

1. Write a two short paragraphs that verbally describe your results. Do not include graphs or figures of any sort; the purpose of this post-lab is to practice writing informative and descriptive results text without the crutch of a figure.
 - Since oxygen consumption will partly depend on the behavior of the animal, describe the behavior before you write about the oxygen consumption. In this way, your reader will have an idea of how to interpret the numbers. Without knowing what the animals were doing, a reader will not be able to really evaluate the numbers.
 - The paragraph should enable your reader to visualize the results. Thus, don't just say that oxygen consumption differed between the two temperatures, indicate the direction oxygen consumption changed in response to changing temperature.
 - Longer is NOT better; the paragraphs should not be excessively wordy. Unnecessarily wordy paragraphs will be marked down.
 - Don't forget to include units.
 - Include your calculations of oxygen consumption as an appendix with the post-lab.

RESEARCH PAPER

****NOTE: If you are going to write this paper up for credit, you need to incorporate data from additional lab groups. This will give you a larger sample size and make your analysis much more meaningful and fun. Your professor will facilitate data exchange among lab groups.**

You have just performed a classic experimental study in animal physiology ==> you've taken one species and subjected it to different environmental conditions and monitored its response.

As with previous studies, one of the best ways to begin writing this study up into a paper is to determine what your results are saying and how to present them. Some questions to consider when thinking about how to present your results:

- What type of metabolic rate have you measured (e.g., standard, resting, basal, routine, etc.)?
- Is it important to show raw data? Why or why not?
- What is the best way to show the trends that you want to emphasize?
- How should you deal with any outliers in your data set?
- You should have enough data points to analyze your data statistically. What aspects of your data would it be logical to compare? What test(s) will you use? How will you present the statistical results?

One of the keys to writing this study up into a paper will also be determining what you want this study to be about. In other words, what angle are you going to take to pitch your study to readers. For example, is this paper going to be about:

- General response of small endotherms to changes in temperature (mice were simply a model system)?
- Specific responses of this species because they are of physiological importance (i.e., Climate changes and the possible link to declining rodent populations. In this case, you're really concerned with this particular animal).
- How metabolic rate changes with temperature in endotherms (again, you are just using mice as a model system)?

Discussions should definitely include a comparison with previous studies. Another element you could bring into the discussion concerns the experimental design. For example, what do you think your results would look like if the mice were *acclimated* to the different temperatures rather than acutely exposed?