

# LABORATORY 6

## **Aerobic Metabolism in Multicellular Eukaryotes**

As you sit reading this, you are also breathing. At least, we hope so. And if you are, have you ever really thought about...why? As a biology major, you are no doubt aware that your body is taking in oxygen, dissolving it into your blood, and then transporting it to mitochondria where mobilized sugars are broken down to release their stored energy. This energy makes it possible for you to read this page, among other amazing feats. You also are aware that your own oxygen requirements can change, depending on your activity levels and other environmental factors.

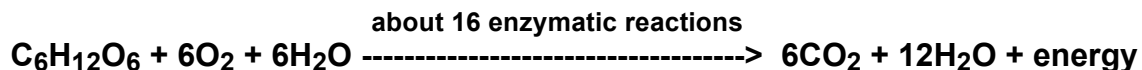
Other eukaryotic species' need for oxygen is no different. Different species vary in the rate at which they consume oxygen (Table 6-1), and members of the same species' oxygen requirement may vary with environmental conditions.

The individual cells of plants, animals, and fungi obtain the energy stored in sugars by breaking sugar molecules apart via the enzyme-mediated reactions of the Krebs Cycle. Energy is extracted most efficiently in the presence of oxygen via the process known as **aerobic respiration**. Under conditions where oxygen is scarce or absent, some cells are still able to split glucose to obtain energy via the process of **fermentation**--but far less energy per glucose is extracted, since glucose cannot be fully broken down without oxygen.

In today's lab, you will work in teams of four to examine aerobic respiration rate in multicellular eukaryotes. You will pose a hypothesis about a respiration problem, make predictions about what you expect to see under selected experimental conditions, and then design and execute an experiment to test your hypothesis. This time, your team will collaborate to produce a written scientific paper, the proper format of which is outlined in Appendix 3 of your online lab manual. Check your syllabus for the date this paper is due.

### **I. Overview of Aerobic Metabolism**

Almost all eukaryotic and many prokaryotic organisms are dependent upon a continuous supply of oxygen in order to sustain life. Ultimately, molecular oxygen taken into the organism is used as the terminal electron acceptor in the electron transport chain and appears as part of a water molecule at the end of the reaction. At the same time, carbon dioxide is removed via decarboxylation reactions of the Krebs Cycle. Most of this carbon dioxide is released as a waste product, ultimately to be expelled by the organism into its environment. The breakdown of carbohydrates to produce ATP requires a series of enzyme-mediated reactions. The *overall* process of aerobic metabolism, however, may be expressed as follows.



Rate of aerobic metabolism differs among various organisms. Body size, the amount of metabolic energy used to regulate body temperature, life stage (*e.g.*, growing juvenile vs. mature adult), physiological state (*e.g.* dormant vs. actively growing), and activity level are just some of the factors that may influence the rate of respiration. When your team confers to discuss a respiration-related question/problem, consider these things. Choose a problem that is scientifically relevant and has implications for

further study. In your paper, you will be expected to discuss these. The following sections will give a bit of background on a few factors your team might wish to consider when posing a hypothesis.

## **A. Temperature and Respiration**

You may have heard the term "warm-blooded" used to describe mammals and birds, and "cold-blooded" to describe just about everything else. These colloquial terms are inaccurate at best, and should be avoided in scientific discussion. Body temperature and metabolic rate are complex phenomena. It is more accurate to consider the *source* and *regulation mode* of body heat with respect to a given organism's metabolism.

Living organisms may be either ectothermic or endothermic. An **ectotherm** obtains its body heat from the external sources, such as the sun or a warm substrate, whereas an **endotherm** obtains its body heat from its own metabolic reactions.

Living organisms also may be either poikilothermic or homeothermic. The body temperature of a **poikilotherm** is regulated by its external environment, whereas a **homeotherm** regulates its own body temperature metabolically. Homeothermy and endothermy are generally more energy-expensive than poikilothermy or ectothermy, and this is reflected in the relative oxygen consumption rates of each type of organism.

Consider how environmental changes in temperature might affect oxygen consumption in each of these types of organisms. You may also wish to consider current information on global climate change. What might this mean in terms of the respiration rate of various organisms in the categories discussed here? Does this bring any testable hypotheses to mind?

## **B. Life Cycle Stage and Other Physiological Factors**

Do juvenile and adult organisms of the same species have the same energy requirements? Consider what the life cycle stage of an organism means to its current energy budget and consider potential problems to solve.

Do different body parts utilize energy at different rates? You might consider posing a hypothesis regarding this question. You will not be able to dismember animals to test this hypothesis, as the various parts of the animal generally do not respire once the animal has been taken apart. (And besides, it's not nice.) However, different parts of a plant may have different functions (e.g., storage vs. energy acquisition), and their metabolic rates might differ accordingly.

## **C. Ecological Communities and Energy Consumption**

As you have learned from your Biodiversity Safari, soil (terrestrial and aquatic) and water contain complex assemblages of organisms. In bodies of water, the concentrations of nitrogen, phosphorus, and other nutrients can be used as indices of the water's **trophic state index (TSI)**. Because nitrogen and phosphorus are necessary for plant (and hence, animal) growth, they can be limiting factors in water where they are scarce. A body of water is said to be **oligotrophic** if it is low in nutrients (and plant life) and high in oxygen. Oligotrophic systems are quite rare in southern Florida, where nutrient runoff from human activity provides copious nitrogen and phosphorus. Bodies of water rich in nitrogen, phosphorus and plant life are said to be **eutrophic**. The process by which a body of water changes from a nutrient-poor one to a nutrient-rich one is known as **eutrophication**. **Cultural eutrophication** is the

process of eutrophication enhanced or sped up by human activity.

The trophic index of any body of water can be used as a rough indicator of its biological condition. Why is trophic level a concern? Might communities at different trophic indices differ in their oxygen consumption or production? Why might this be relevant and interesting?

Soil, too, contains a living community of organisms. Might soil samples from different habitat types have different energy needs? Consider this, and pose a testable hypothesis.

## **D. Complex Systems, Complex Interactions**

Your team will be provided with different types of respiring organisms (see below) and various tools for manipulating your system. Check the availability of the items in lab, and then confer to decide whether your team will perform a (statistical) **pilot study**, or to attempt to pose **multiple, competing hypotheses** about a particular observable phenomenon you already know is true. If you choose a pilot study, then you will be expected to pose competing hypotheses in your paper, and make predictions about outcomes of possible experiments you could do to test each one.

Remember that multicellular organisms and ecosystems are complex, with many factors at work. By now, you should be familiar with the proper way to pose falsifiable hypotheses, and to collect sufficient data to be able to determine whether your results are significant. But don't forget that in a multifactorial system such as a living, multicellular organism or an ecosystem, there may be more than one possible explanation for results. When you analyze and present your results, include discussion of alternative hypotheses, as appropriate, and suggest ways to test them.

## **II. Measuring Respiration Rate**

To find the rate of respiration, one could theoretically measure an organism's intake of any of the reactants, or its output of any of the products (including energy), over time. In practice, however, some measurements are easier to perform than others. Often, researchers measure either O<sub>2</sub> consumption or CO<sub>2</sub> production over time. You will use an electrochemical O<sub>2</sub> gas sensor (Vernier Software, [www.vernier.com](http://www.vernier.com)) to measure the consumption of O<sub>2</sub> gas in a closed chamber that will serve as a **respirometer**.

### **A. Materials: Organisms and Tools**

There are several types of small, respiring organisms available, which may include:

- Animals such as insects
- Germinating and dormant seeds
- Potatoes or other plant storage organs; fruits, etc.
- Organic soil or compost
- Living plant parts or invertebrate animals obtainable outside by your team

Some of the tools available include:

- Ice, hotplates
- thermometers
- large beakers for making waterbaths of various temperatures
- foil for darkening the reaction chamber

Your team may wish to provide its own study organisms. If you choose this option (in advance), please note that **NO VERTEBRATES ARE ALLOWED IN THE LAB.** Insects, mollusks, worms, and other invertebrates are permitted. These can be collected in the biome area of the Gifford Arboretum directly behind the Cox Building. Lifting rocks is a good way to find interesting species, and it also gives you some insight into their natural habitat and behavior. But remember that you must have sufficient replicates of your experiment in order to get meaningful results.

**If you are using animals in the laboratory, you must treat them humanely, no matter what species they are. Inhumane treatment of any type of animal in the laboratory is grounds for dismissal from the lab and a failing grade.**

**You may abuse the beans all you want, however. We are nothing if not speciesist.**

Examine the variety of specimens and potential environmental conditions. Consider whether the organisms are immature (undergoing relatively rapid mitosis), mature, or dormant. What is each organism using for a nutrient/energy source? How might this affect its rate of oxygen consumption? Might the presence or absence of light affect the rate of respiration, by causing a metabolic or behavioral change?

Consider metabolic modes. Is the organism endothermic or ectothermic? Is it homeothermic or poikilothermic? Is the organism undergoing any other metabolic reactions that might affect the amount of oxygen in your experimental system? Consider these questions carefully, but don't *assume* anything. If you're not sure, ask your TA or use published sources of sound information. Google Scholar ([www.google.com](http://www.google.com)) is a good place to start. Rates of respiration for some representative species are shown in table 6-4.

### **1. If your team is performing a pilot study:**

What is your question? \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

What is your  $H_0$ ? \_\_\_\_\_  
\_\_\_\_\_

What is your  $H_a$ ? \_\_\_\_\_  
\_\_\_\_\_

What is your rationale for the alternative hypothesis? Why is this relevant or interesting? \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

*For teams doing a pilot study, be sure to complete the following section in addition to the pilot study section above. The results of your experiment are your observable phenomenon. You should include a prose discussion of the following in your paper.*

**2. If your team is trying to explain an observable phenomenon:**

What is your hypothesis? \_\_\_\_\_

\_\_\_\_\_

What is your prediction?: \_\_\_\_\_

\_\_\_\_\_

What are your possible outcomes, and an explanation of each?

Outcome #1: \_\_\_\_\_

Explanation: \_\_\_\_\_

Outcome #2: \_\_\_\_\_

Explanation: \_\_\_\_\_

Outcome #3: \_\_\_\_\_

Explanation: \_\_\_\_\_

**B. Methods: Measuring Oxygen Levels**

Before designing experiments, you should first become familiar with how the O<sub>2</sub> sensor works, and then with the basic procedure for measuring the rate of respiration (oxygen consumption) by a living organism. Each team member should participate in all aspects of all activities, and should be able to explain the rationale for each step of the methods.

**1. Setting up the O<sub>2</sub> sensor and data logger software**

One team member with a PC or Mac laptop computer will kindly volunteer it for data collection.

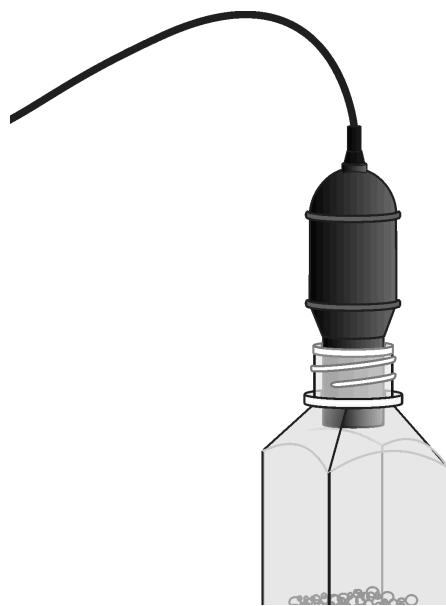
1. Holding it only by the edges, place the Logger Lite CD in the CD drive and follow the installation directions that appear.

2. *Only after you have installed the software*, connect the O<sub>2</sub> gas sensor and interface to the USB port on your computer. **Keep the O<sub>2</sub> sensor upright at all times! Treat it with extreme care or you may damage it and will be penalized!**
3. Start the Logger Lite 1.4 software by clicking on the icon on the desktop.
4. The software will detect the sensor and load a data table and graph. You are now ready to collect data!

**WHEN YOU HAVE FINISHED DOWNLOADING THE SOFTWARE, PLEASE EJECT THE DISK AND RETURN IT TO YOUR TA IMMEDIATELY! We have limited numbers of disks, and the copies don't work on Macs. So we need the originals returned!**

## **2. Collecting data with the O<sub>2</sub> sensor**

1. **Carefully and gently**, place the O<sub>2</sub> gas sensor into the plastic reaction chamber as shown in figure 6-1. Gently push the sensor down until it stops. The sensor is designed to seal the chamber without unnecessary force.



**Figure 6-1. O<sub>2</sub> gas sensor and reaction chamber ready to collect data.**

2. Click **“Collect”** on the toolbar at the top of the Logger Lite window. The sensor will now start measuring, 1x per second, the O<sub>2</sub> concentration (as %O<sub>2</sub>) of the air in the chamber. Note that the current %O<sub>2</sub> is displayed in the lower left corner of the window, while the readings over time are displayed on the data table and graph.
3. When the %O<sub>2</sub> value has stabilized, click **“Stop”** on the toolbar.
4. Record the %O<sub>2</sub> value.
5. Click **“Store”** on the toolbar to store this data run, and to prepare the software for collecting new data.

6. Gently remove the O<sub>2</sub> sensor and place it upright in its dry 250 mL beaker.
7. Breathe several times into the reaction chamber. Try to replace the air in the chamber with your exhaled breath.
8. Quickly, but still carefully and gently, place the O<sub>2</sub> sensor into the chamber as in step #1 above.
9. Collect data as in steps 2 - 6 above.
10. Click "**Save**" to save the results of this exercise.
11. Gently remove the O<sub>2</sub> sensor from the chamber and place it upright in its beaker.

Results:

What is the % concentration of O<sub>2</sub> in the atmosphere? \_\_\_\_\_

What is the % concentration of O<sub>2</sub> in your exhaled breath? \_\_\_\_\_

How long did it take the O<sub>2</sub> sensor to detect fully the %O<sub>2</sub>? \_\_\_\_\_

### **3. Customizing data collection and graphical display**

To make your subsequent work easier, let's now get familiar with some of the features of the Logger Lite software.

1. Click "Open" on the toolbar at the top of the window.
2. Click "Tutorials" in the "Experiments" folder that opens.
3. Click on "Tutorial 3 – Customizing." The first page will tell you that a Go Temp probe should be attached. Ignore this; you will still be able to use the information in the tutorial. Click on "Pages" on the toolbar to go to other pages.
4. Using the information in this tutorial, be able to do the following on your saved graph (your instructor may check to be sure that you can do this):
  - Change the rate and duration of data collection
  - Give the graph a title
  - Un-connect the points of the graph
5. Now open "Tutorial 4 – Working with graphs." Be able to:
  - Change the scale of the x and y axes
  - Stretch the x and y axes
  - Autoscale the axes

### **4. Collecting Data on Oxygen Consumption**

Your precise methods will depend on the type of sample you are measuring. Follow the appropriate directions carefully.

**If you are using macroscopic organisms, follow these steps:**

1. Decide how many individuals to test. Do a trial run with 10-20 individuals and modify if necessary (e.g. if little or no O<sub>2</sub> consumption is measured).
2. Weigh the organism(s) you will test. If they are wet, gently pat them dry with a tissue before weighing.

3. Keep organisms in a covered beaker while they are awaiting testing.
4. Carefully place the weighed organism(s) in the respirometer chamber (Figure 6-3) and measure their rate of respiration as described below.

**If you are using a soil sample follow these steps:**

1. Decide how much soil to test. Do a trial run with about 1” of soil in the chamber and modify if necessary (e.g. if little or no O<sub>2</sub> consumption is measured). **Do not allow the soil or any moisture to touch the O<sub>2</sub> sensor.**
2. Weigh the soil sample you will test.
3. Place the soil in the respirometer chamber (Figure 6-3) using a wide neck (or paper) funnel, and measure the rate of respiration as described below.

**Helpful Hints:**

- If testing the effects of temperature, incubate each organism for 10 minutes beforehand in an open container, at the desired temperature. Maintain the respirometer chamber at that temperature as well. **Do not exceed 38°C.**
- If testing the effects of light, incubate the organisms for 10 minutes beforehand under the appropriate conditions.
- Because each group has only one respirometer, you will have to perform your treatments sequentially. Plan your incubations accordingly! Complete one set of treatments before starting any replicate trials.
- Keep the organisms as unstressed as possible while they are waiting to be tested.

**5. Data Analysis**

You can use the software to calculate a “raw” rate of respiration for each treatment. Examine the curves on your graph. Each curve shows the time course of O<sub>2</sub> consumption. At first there may be several minutes of inconsistent readings, as the organism adjusts to the conditions and the system equilibrates. Then, there should be a steadily falling linear portion of the curve that represents the rate of respiration. After finding this “raw” rate of respiration, you must correct it for:

- Weight of the organisms
- Volume of O<sub>2</sub> (instead of %O<sub>2</sub>) consumed
- Standard temperature and pressure (optional)

Follow the steps below for each curve.

1. Highlight the (mostly) linear portion of the curve using your mouse.
2. Click “**Analyze**” on the top menu, then “**Linear Fit.**” In the dialog box that appears, be sure to check the curve you’ve just highlighted. A **best fit linear regression line** will now appear for your highlighted points, along with a floating box containing the equation of the line. The *correlation* statistic (*r*) shows how

well your actual data points fit the line. (For example, a correlation of 1.0 shows a perfect fit.) Using the mouse, you can grab and move the brackets to change the highlighted points, to see if there is a better fit. The line, correlation, and equation will automatically update.

3. Look at the equation of the best fit line. The slope, **m**, shows the change in %O<sub>2</sub> over the change in time. In other words, this gives you the raw (uncorrected) rate of respiration as %O<sub>2</sub>/minute.
4. Convert %O<sub>2</sub>/minute to %O<sub>2</sub>/hour.
5. Now divide the raw rate of respiration by the actual weight of the organism(s) used in this trial to get %O<sub>2</sub>/g/hour.
6. Repeat steps 1 - 5 for all the replicate trials for this same treatment.
7. Average the raw rates of respiration for all replicate trials of this treatment to get a **mean** %O<sub>2</sub>/g/hour.
8. The working volume of the respiration chamber, with the O<sub>2</sub> gas sensor inserted, is approximately 280 mL. Now you can calculate the mean mL O<sub>2</sub>/g/hour respired by the organism as a percentage of the total working volume. Calculate the standard deviation also, if you ran three or more trials for a given treatment.
9. *Optional:* If your TA requires it, correct for standard temperature and pressure using the formula:
10. In order to calculate a standardized figure for the average amount of oxygen consumed by the experimental organisms per unit time, you must convert the volumes of oxygen they used to standard temperature and pressure values. Use the following formula to calculate V<sub>2</sub>, the corrected value of oxygen consumed by your organisms over the period of the experiment.

$$V_2 = \frac{P_1 V_1 T_2}{P_2 T_1}$$

Where V<sub>2</sub> is the corrected volume of oxygen consumed under STP and

P<sub>1</sub> = atmospheric pressure of the lab (in mm Hg, from the **barometer** at the front of the lab)

V<sub>1</sub> = oxygen consumed under experimental conditions; just use the mL O<sub>2</sub>/g/hour

T<sub>1</sub> = temperature of the respirometer (in degrees Kelvin; K = °C + 273)

P<sub>2</sub> = 760 mm Hg (standard pressure)

T<sub>2</sub> = 273 °K (standard temperature)

Note that the units of V<sub>2</sub> will be expressed as mL O<sub>2</sub>. Once you obtain this value you can simply use it as the corrected mean mL O<sub>2</sub>/g/hour.

1. Repeat steps 1 - 9 for each treatment.



**Table 6-1.** Oxygen consumption of various species at rest (in ml O<sub>2</sub>/g/hour). Data from Florey: *An Introduction to General and Comparative Physiology*, W.B. Saunders Company. Philadelphia, 1967.

Phylum	common name	genus	ml O <sub>2</sub> /g/hour
Ciliophora	paramecium	<i>Paramecium</i>	1.00
Rhizopoda	amoeba	<i>Amoeba</i>	0.20
Porifera	sponge	<i>Suberites</i>	0.24
Cnidaria	sea anemone	<i>Anemonia</i>	0.013
	jellyfish	<i>Aurelia</i>	0.0034
Nematoda	hookworm	<i>Ascaris</i>	0.50
	roundworm	<i>Rotylenchus</i>	0.66
Mollusca	clam	<i>Anodonta</i>	0.002
	mussel	<i>Mytilus</i>	0.055
	octopus	<i>Octopus</i>	0.09
Annelida	lugworm	<i>Arenicola</i>	0.031
	tube worm	<i>Chaetopterus</i>	0.008
Arthropoda	crab	<i>Uca</i>	0.005
	lobster	<i>Homarus</i>	0.50
	crayfish	<i>Cambarus</i>	0.10
	butterfly	<i>Vanessa</i>	0.60
Echinodermata	sea cucumber	<i>Holothuria</i>	0.01
	sea star	<i>Asterias</i>	0.03
Chordata	goldfish	<i>Carrasius auratus</i>	0.07
	trout	<i>Salmo trutta</i>	0.22
	rat	<i>Rattus norvegicus</i>	0.95
	cat	<i>Felis cattus</i>	0.44
	human	<i>Homo sapiens</i>	0.20

**PLANTAE, Anthophyta (flowering plants)**

Plant name	type of structure	condition	ml O <sub>2</sub> /g/hour
alfalfa	seed	dormant	0.38
	seed	germinating	1.06
barley	seed	germinating	0.715
	endosperm	nutrient supply	0.076
carrot	root	mature	0.025
	leaf	mature	0.440
spinach	leaf	mature	0.515
sweet potato	root	mature	0.96
ash tree	cambium	mitotic	0.233
	phloem	mature	0.154
	sapwood	conducting	0.047
red maple	cambium	mitotic	0.220
	phloem	mature	0.170
apple	flesh	ripe	0.030
	skin	ripe	0.095