OXYGEN CONSUMPTION AND TEMPERATURE IN THE AQUATIC ENVIRONMENT

BACKGROUND READING

ANIMALS & EQUIPMENT

**Living material**
Freshwater fish, probably goldfish.

**Equipment**
Macintosh computer; PowerLab virtual chart recorder; MacLab oxygen meter and the Cameron O₂ probe; BNC cables and other assorted cable connections; conditioned sea water; 100% N₂ gas; compressed air; large test tubes; beakers; tubing; respiration chamber; vacuum grease. *(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
This experiment is based on **closed system respirometry**.

**Calibration of the Oxygen Meter**
You will be recording the oxygen content of water within the respiration chamber. To make your data meaningful, however, you must first calibrate the oxygen meter. This is very similar to calibrating a pH meter except that the solutions used contain a known amount of O₂ instead of H+/OH-. In fact, the O₂ probe and meter work very much like the pH probe and meter with which you are all probably familiar.

1. Saturate one beaker of water with room air by swirling manually, and one flask with 100% N₂ by bubbling in N₂ gas through an air-stone for about 15 min. The pO₂ of these water samples will be approximately 21% and 0% O₂, respectively. Calculate the partial pressure of O₂ (in units of mmHg) in the 21% standard.

2. While recording the data with PowerLab, place the O₂ electrode in the 0% O₂ solution and wait until the reading stabilizes (about 3 min).

3. After the 0% reading has stabilized, transfer the electrode to the 21% O₂ solution while continuing to collect data, and wait until the reading stabilizes (about 3 min). Make sure that there are no air bubbles on the probe face.

4. Use the **UNITS CONVERSION** function on the PowerLab chart recorder to convert from voltage to mmHg.

5. Check the accuracy of your calibration by transferring the probe back to the 0% solution to check that the output is roughly 0 mmHg O₂. Repeat this step with the 21% O₂ solution. If the output values are substantially different than your initial calibration, repeat steps 2 - 4. You can do this even though you have already used
the UNITS CONVERSION function once .... just do it again and PowerLab will automatically over-write the first one.

**General Procedure Measuring Aquatic Oxygen Consumption:**

1. Fill a chamber with conditioned water and record the temperature of the water.

2. Gently place an animal in the chamber. Carefully place the lid on, making sure there are no air bubbles trapped inside.

3. Carefully introduce the O₂ probe into the chamber until it fits snugly. Screw in the pressure-compensating screw. You are now ready to begin measuring the oxygen consumption of the animal.

4. Monitor the decrease in pO₂ for 20 - 30 min. **Note the behavior of the animal during this time.** Be patient, not all animals will "rest" immediately (or at all, for that matter). **Do not let the pO₂ drop below 50 mmHg!**

5. Carefully remove the animal from the chamber and weigh it, in water, to the nearest 0.1 g on the top loading balance. Subtract the weight of the container + water.

6. Repeat steps 1 - 5 with another animal.

7. Repeat steps 1 - 5 with the same two animals but at a water temperature of room temperature ±5 °C such that you end up with a sample size of two for both temperatures. **How will you maintain the proper temperature throughout the experiment?**

**ADDITIONAL IMPORTANT POINTS**

- How do you know that you are measuring the oxygen consumption of the fish and not some goo clinging to the side of the chamber? What is the control for extraneous O₂ consumption? Run at least one control after testing the animals.

- Water will need to be bubbled with nitrogen for at least 20 min to achieve 0% O₂.

- 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.

- Use the pre-warmed water for the 25 °C temperature trials.

- Take chamber temps before and after each run and average. Use average temp for calculations.

- Ideally, you would want to run a control at of your experimental temperatures. For this lab, just make sure that you run a control at room temperature and assume that the system will behave similarly at the other two temperatures.
• Weighing stresses the animals. Therefore, **weigh each individual only after you have taken the VO2 measurements.**

**FLOW CHART OF BASIC PROCEDURE**

![Flow Chart](image)

**Calculations**

With MacLab's *Data Pad*, you can easily express O2 consumption as pO2/time (which will be in units of mmHg/min or mmHg/sec). Review the Help option on the main tool bar if necessary.

In published papers, oxygen consumption data is commonly represented in units of **ml O2/individual*h** or **ml O2/g*h**. Thus, you need to convert pO2 into ml O2. This can be a bit tricky since the amount of gas dissolved in a fluid is dependent upon more than just partial pressure. To convert pO2 into ml O2, you need to know the following:

- temperature
- salinity
- volume of the fluid in the respirometer.

You have measured the water temperature, and the salinity of sea water is roughly 32 ppt. Using these two factors, look up the solubility coefficient of O2 in your fluid sample from the table provided in lab.

When estimating the volume of the respiration chamber, remember to take into account the volume of the animal.

Now you can use Henry's law to calculate the O2 consumption of the fish in **ml O2/g*h**:

\[
\text{Oxygen Consumption} = \Delta \cdot \left( \frac{\Delta \text{pO}_2}{h} \right) \cdot V_{\text{H}_2\text{O}} \cdot \frac{1}{g}
\]

Where: \( \Delta \) = solubility coefficient in units of ml O2/L liquid; \( \Delta \text{pO}_2 \) = the change in partial pressure of O2; \( h \) = time; \( V_{\text{H}_2\text{O}} \) = volume of water in respirometer; \( g \) = weight of fish in grams. Be careful with units.
PRE-LAB
(Due at the start of the lab)

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? In today's experiment, how will you account for each factor listed when you analyze the data?

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 three biologically-based plausible reasons for this difference?

POST-LAB
(Due next week in lab)

1. Generate one graph that summarizes the main result of your study on how temperature influences fish oxygen consumption.
   - The graph must be computer generated and made as "pretty" as possible (i.e., don't just slap something together in StatView and print it out).
   - Don't forget to label the axes and include units.

2. Construct a complete figure legend to accompany the figure. Keep in mind the criteria outlined in the Paper Dissection lab and your writing handbook.

3. Calculate a Q_{10} value for oxygen consumption for each animal used in your study.

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.**

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 I want to emphasize?
- How should I deal with any outliers in my 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, how are you going to pitch your study to readers. For example, is this paper going to be about:

- General response of animals to changes in temperature (goldfish are simply a model system)?
- Specific responses of this species because they are of physiological importance (i.e., El Nino and global warming; in this case, you're really concerned with this particular animal).
- How metabolic rate changes with temperature (again, you are just using goldfish as a model system)?

Discussions should definitely include a comparison with some previous studies. Another element you could bring into the discussion concerns the experimental design. What do you think your results would look like if the animals were acclimated to or raised in the different temperatures rather than acutely exposed?

**OXYGEN SOLUBILITIES in ml O₂/(L*mmHg)**

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<th>Sea water (approx. 30ppt)</th>
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