Experiment CM-7: Oxygen Consumption and Aerobic Respiration in Goldfish

The lab has been written in conjunction with Lloyd Trueblood, Ph.D., La Sierra University, Riverside, CA

Equipment Required

PC or Mac Computer

IXTA, USB cable, power supply

500 ml Erlenmeyer flask/respirometer

Rubber stopper to fit the 500 ml Erlenmeyer flask

Parafilm or plastic wrap

Glass bottles with stoppers

100 and 250 ml beakers

1000 ml Graduated cylinder

Deionized Water in squirt bottle

ISE-730 Dissolved oxygen electrode

Top-loading balance

Container of fresh, aerated water (for entire class)

Aeration stone and aquarium pump (for each group)

0% Oxygen solution for calibration

Warning: The dissolved oxygen electrode has been prepared by the laboratory staff. When you receive your electrode: 1) Handle it carefully. The tip of the electrode is covered by a delicate Teflon (tm) membrane which can tear easily. 2) Do not tighten or loosen the plastic housing holding the Teflon (tm) membrane. Tightening the housing will stretch or tear the membrane; loosening the housing will cause the electrolyte to leak out of the electrode and affect its responsiveness.

Experimental Protocol: *This is to be done 3 days prior to lab*

1. A group of treatment goldfish has been maintained at room temperature (20^{o}C) without food (starved) for the past three days.
2. A second group of control organisms have been maintained at room temperature (20^{o}C) with food.
3. A third group has been kept at ~4^{o}C for three days.

You will test the difference in oxygen consumption rate between these three groups.

Dissolved Oxygen Electrode Setup

1. Locate the dissolved oxygen electrode and plug it into channel A5 on the front of the TA.

Figure CM-7-S1: Dissolved oxygen electrode connected to an IXTA.

Calibration of Dissolved Oxygen Electrode

Aim: To calibrate the dissolved oxygen electrode.

The standard used for calibrating the dissolved oxygen electrode is the known concentration of oxygen in air-saturated deionized water. The amount of oxygen that is dissolved in water is known as its solubility (S) and it is dependent upon the temperature, oxygen pressure in the air, and the concentrations of dissolved solutes in the water. Solubility (S) can be determined by using the following equation:

$$S = (\alpha/22.414)\ ((P-p)/P)\ (r\%/100).$$

In the equation, α is the absorption coefficient of O_2 at the temperature, p is the vapor pressure of water at the temperature, P is the barometric pressure, and r% is the percent oxygen in the air. For example, at 26°C and 760mmHg and a concentration of oxygen in air of 21%, S equals:

$$(0.02783/22.414\text{L/mole})(734.91\text{mmHg}/760\text{mmHg})(0.21) = 252\mu\text{MO}_2$$

Procedure

1. Air Saturation: Fill your oxygen respirometer (glass jar) almost all the way up with water. Place cork on top and shake jar vigorously for a moment. This is your air-saturated value (VA). This must be done for both the room temperature and chilled electrodes.
2. Place the oxygen electrode in the respirometer bottle. There needs to be enough water to submerge the tip of the oxygen electrode
3. Type **Saturation-DI Water Room Temp** in the Mark box.
4. Click Record on the Main window. The recording will eventually reach a stable level near the top of the recording channel. Click the mark button to mark the recording when the output of the electrode is constant. At this point in the recording, the output of the oxygen electrode is equal to the saturation concentration of oxygen in deionized water at room temperature.
5. This is your Saturated oxygen value (VA).
6. Zero Oxygen: Place the oxygen electrode in a beaker containing 0% oxygen calibration solution (or a just opened can of club soda). There needs to be enough to submerge the tip of the oxygen electrode and mark it as zero. This is your zero oxygen value (VZ).
7. Type **No Oxygen** in the Mark box.
8. The recording will eventually reach a stable level near the bottom of the recording channel. Click the mark button to mark the recording when the output of the electrode is constant. At this point in the recording, the output of the oxygen electrode is equal to no oxygen at room temperature (VZ).
9. These steps will be repeated at the beginning of Exercise 3 - using chilled electrodes and chilled solutions.
10. Click Stop to halt the recording.
11. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
12. Record your data on Table 1.
13. Rinse the electrode with deionized water from a wash bottle. Blot any drops of solution from the electrode and place it in a beaker of either room temperature or chilled deionized water, depending on your experiment.

Units Conversion

1. Measure the temperature (in ^{o}C) in the lab room. Assume the barometric pressure in the lab room is one atmosphere (760mmHg) and the concentration of oxygen in the air is 21%. From Table 2, find the dissolved oxygen concentration ([O_2]) in deionized water at room temperature. This concentration will be used in to calibrate the dissolved oxygen electrode.
2. Scroll to the beginning of the calibration data for the dissolved oxygen electrode.

3. Use the Display Time icons on the LabScribe toolbar to adjust the Display Time of the Main window to show the data collected at both the 100% (saturated) and 0% (unplugged) levels of oxygen in water on the Main window at the same time.
4. Click the Double Cursor icon so that two cursors appear on the Main window. Place one cursor on the flat section of data collected when the saturation of dissolved oxygen in water was 100% and the second cursor on the flat section of data collected when the saturation of oxygen was 0%.
5. To convert the output of the dissolved oxygen electrode from a voltage to the molarity of dissolved oxygen in a sample:
    - Click on the arrow next to the title of the [Oxygen] channel to open the channel menu.
    - Select Units from the channel menu and Simple from the Units submenu.
6. The Simple Units Calibration window will appear. On this window:
    - Select 2 point calibration from the pull-down menu in the upper-left corner of the window.
    - Put a check mark in the box next to Apply units to all blocks.
    - Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - From Table 2 find the concentration of dissolved oxygen in water at the room temperature that is 100% saturated. Enter this concentration in the corresponding box to the right of the voltage at 100% oxygen saturation. Enter zero in the corresponding box to the right of the voltage for 0% oxygen saturation.
7. Enter the name of the units, µMolar, in box below the concentration. Click on the OK button in the lower right corner of the window to activate the units conversion.

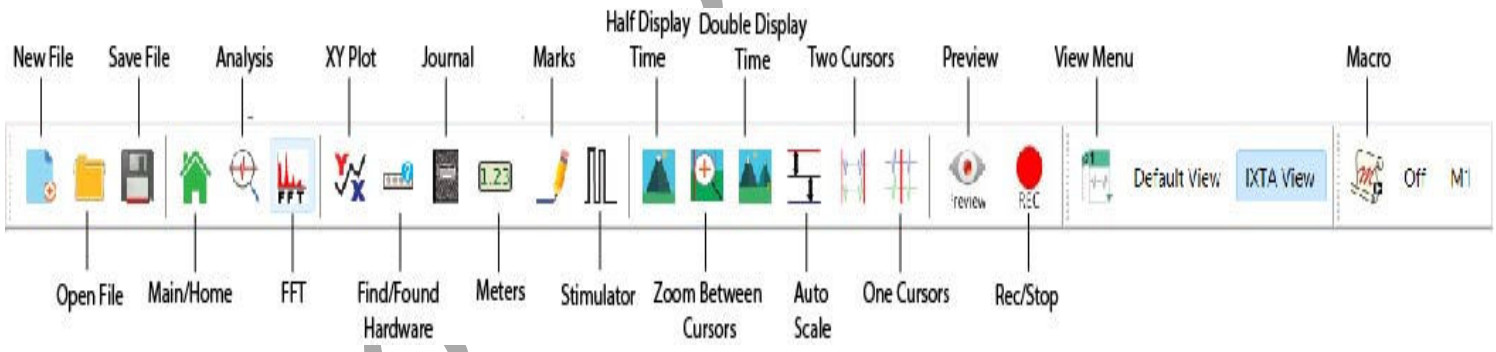

Figure CM-7-S2: The LabScribe toolbar.

Table CM-1-S2: Concentration of Oxygen [O2] in Air-Saturated Deionized Water at 1 Atmosphere.

Temp (°C)	O_2 Abs Coeff (a)	H_2O Vapor Press (p in mmHg)	[O2] (µM)
20	.03102	17.54	284
21	.03044	18.65	278
22	.02988	19.83	273
23	.02934	21.07	267
24	.02881	22.38	262
25	.02831	23.76	257
26	.02783	25.09	252
27	.02736	26.74	247
28	.02691	28.35	243
29	.02649	30.04	238
30	.02608	31.82	234

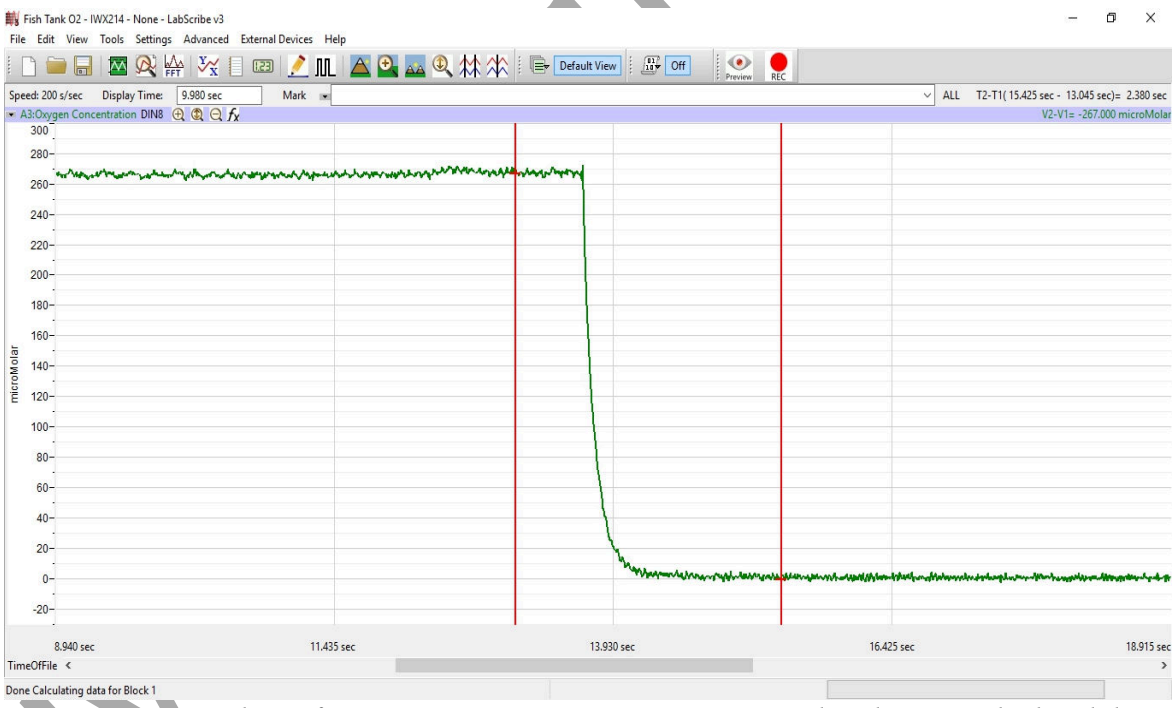

Figure CM-7-S3: Recording of oxygen concentrations in air saturated and oxygen depleted deionized waters used to convert the units of the Y-axis from voltage to O_2 concentration (µMolar).

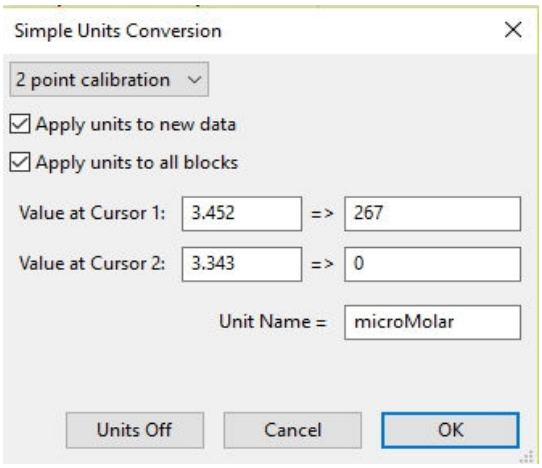

Figure CM-7-S4: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the dissolved oxygen concentrations used in calibration.

Experiment CM-7: Oxygen Consumption and Aerobic Respiration in Goldfish

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Exercise 1: Measure the Rate of Oxygen Consumption of the Goldfish at Room Temperature - Fed

Aim: To measure changes in dissolved oxygen concentration of water inhabited by fish over time.

Approximate Time: 60 minutes

Procedure

Note: *Each organism is placed in a respirometry chamber (jar or flask) with an appropriate volume of water. Allow the fish several minutes to calm down, and then seal jar with electrode and stopper. Mark your starting point (VB) and allow each experiment to run for 30 minutes. After at least 30 minutes, mark the end measurement VE. This needs to be done for all three fish groups: 4°C, 20°C, and 20°C fed.*

1. Half-fill the Erlenmeyer flask or respirometry chamber with fresh, aerated water.
2. Weigh the flask and its contents on the top-loading balance.
3. Catch a fish from the tank that housed them at room temperature - FED and place it in the same flask. Weigh the flask again.
4. Subtract the two weights of the flask. The difference is the weight of the fish.
5. Place a stir bar on the bottom of the flask and place the flask on the magnetic stirrer.
6. Fill the flask, which is holding the fish, close to the top with fresh, room temperature aerated water. Turn on the stirrer so that the bar rotates very slowly.

Note: *The stirrer should be rotating at a speed that does not agitate and stress the fish. Continue to aerate the water in this flask with an aeration stone connected to an aquarium pump.*

Warning: In this experiment you will measure the basal metabolic rate. Therefore you must keep the stress level of the fish to a minimum.

7. Cover the sides of the flask with paper towels to minimize disturbance from outside.
8. Let the fish equilibrate to the flask for about 10-15 minutes.
9. Remove the aeration line from the flask at the end of equilibration period.
10. Fill the flask to the brim with aerated water.
11. Tightly seal the top of the flask and around the cable of the oxygen electrode with plastic wrap or parafilm.

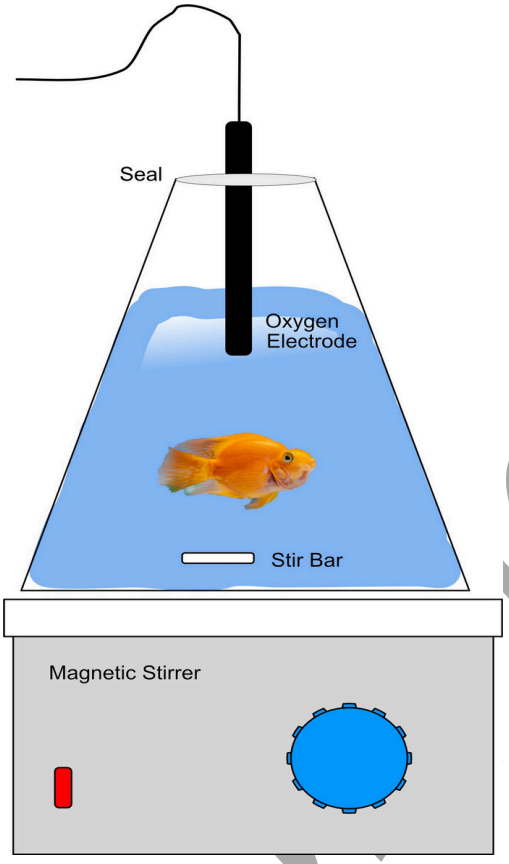

Figure CM-7-L1: Experimental setup for recording changes in the oxygen concentration in water containing a fish.

Note: *It is important that there are no air bubbles on the side of the flask.*

12. Type **VB (room)** in the Mark box.
13. Click Record and click the mark button to mark the recording. Record the output of the oxygen electrode for 30 minutes, or until the concentration of oxygen falls below 65% of the initial concentration at the beginning of the exercise. Mark the end of the measurement VE (room).

Note: *During this time you may elect to set up another fish in a second flask and allow it to equilibrate to its new surroundings.*

14. Click Stop to halt the recording.
15. Select Save in the File menu.

16. Open the container. Remove the dissolved oxygen electrode from the flask. Rinse the electrode with deionized water from the squirt bottle. Place the electrode in a beaker of deionized water.
17. Carefully pour all the water from the flask containing the fish into a graduated cylinder. Return the fish to the correct aquarium. Measure the volume of water in the cylinder. Return the water to the stock tank. Record the volume of water in the graduated cylinder.

Note: All data will be collected ahead of time. Data analysis will occur after data is collected for all three (3) fish.

Exercise 2: Measure the Rate of Oxygen Consumption of the Goldfish at Room Temperature - Starved

Aim: To measure changes in dissolved oxygen concentration of water inhabited by fish that have not been fed over time.

Approximate Time: 60 minutes

Procedure:

1. Repeat Exercise 1 in its entirety for a fish at room temperature that has not been fed.
2. Catch a fish from the tank that housed them at room temperature - STARVED and place it in the same flask used in Exercise 1. Weigh the flask again.
3. Mark the recording **VB (starved)** and **VE (starved)** as appropriate.
4. Continue with the experiment.

Exercise 3: Measure the Rate of Oxygen Consumption on the Goldfish at Cold Temperature ~ 4^{o}C

Aim: To measure changes in dissolved oxygen concentration of cold water inhabited by fish.

Approximate Time: 60 minutes

Note: *Repeat the Calibration of the Oxygen Electrode again at this point prior to doing this portion of the experiment. Use chilled DI-Water, chilled 0% oxygen solution, and a chilled electrode. This is part of the SetUp document.*

Procedure:

1. Repeat Exercise 1 in its entirety for a fish at cold temperature.
2. Catch a fish from the tank that housed them at cold temperature - 4^{o}C and place it in the same flask used in Exercises 1 and 2. Weigh the flask again.
3. Mark the recording **VB (cold)** and **VE (cold)** as appropriate.
4. Continue with the experiment using cold water in the flask/respirometer.

Note: *Use the chilled oxygen electrode for this experiment so that there is no temperature change between the water and the oxygen electrode.*

Data Analysis

1. Scroll through the data file and locate a section near the beginning of the recording for the fish at room temperature-FED. You should be very near the VB-room mark made during the recording.
2. Click the single cursor icon and measure and record the Value shown in the right hand margin of the Dissolved O_2 channel for VB-room.

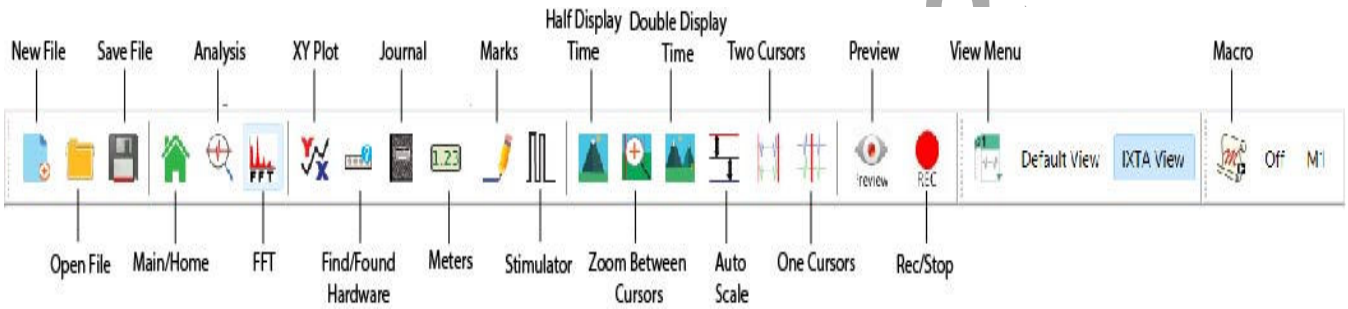

Figure CM-7-L2: The LabScribe toolbar.

3. Click the down arrow to the right of the Mark button and select VE-room. This will adjust your recording to the time at when you made this mark.
4. Click the single cursor icon and measure and record the Value shown in the right hand margin of the Dissolved O_2 channel for the fish at room temperature.
5. Repeat the above data analysis procedures for the fish at room temperature-STARVED and the fish at Cold (4^oC) temperatures.
6. Record all your data in Table 1.

Calculating a Metabolic Rate

In order to determine the amount of oxygen consumed you need to calculate the following:

1. Total oxygen consumed = VB - VE = O_2 Consumed
2. Individual MO_2 = (O_2 consumed/ time) * volume of water (liters)
3. Mass-specific MO_2 = O_2 consumed/(time * mass)

Note: *Assume a time of 30 minutes.*

Questions

1. How is the rate of oxygen consumption related to temperature?
2. How is the rate of oxygen consumption related to an organism being fed or not being fed?
3. How is the rate of oxygen consumption per gram of body weight related to the total weight of the animal? When you compare data from the different fish, is there a trend?
4. Explain the physiological parameters that affect metabolism - food consumption, temperature, etc...
5. Did you (or did you not) see any difference in the metabolism of the fish? Explain your answer.

Table CM-7-L1: Rate of Oxygen Consumption and Metabolism of Fish Under Different Conditions.

Values	Fish - Room Temperature FED	Fish - Room Temperature STARVED	Fish - Cold Temperature
VA - Air Saturated Value			
VZ - Zero % Value			
VB - Beginning			
VE - End			
MO_2/minute			
MO_2/minute/gram			