Experiment CM-1: Oxygen Consumption and Size

Equipment Required

PC or Mac Computer

IXTA, USB cable, power supply

500 ml Erlenmeyer flask

Rubber stopper to fit the 500 ml Erlenmeyer flask

Parafilm or plastic wrap

100 ml beakers (2)

250 ml beaker

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)

Zero-percent O_2 calibration solution (see the Lab Exercise)

Magnetic stirrer and stirring bar

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.

Dissolved Oxygen Electrode Setup

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

Figure CM-1-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^{o}C and 760mmHg and a concentration of oxygen in air of 21%, S equals:

$$(0.02783/22.414\text{L/mole})(734.91\text{mmHg}/760\text{mmHg})(0.21) = 252\mu MO_2$$

Procedure

1. Place the oxygen electrode in a 100 ml beaker containing room temperature deionized water. There needs to be enough water in the beaker to submerge the tip of the oxygen electrode, and keep its tip away from the stir bar in the beaker. Place the beaker on a magnetic stirrer. Adjust the speed of the stirrer so the stir bar is rotating quickly and evenly.
2. Type **Saturation-DI Water** in the Mark box.
3. 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.
4. Obtain a 100 ml beaker containing zero-percent oxygen calibration solution at room temperature. Make sure there is enough solution in the beaker to keep the tip of the electrode clear of the stir bar.
5. Turn off the magnetic stirrer. Remove the oxygen electrode from the beaker of deionized water and remove the beaker of deionized water from the stirrer.
6. Place the beaker containing zero-percent oxygen calibration solution on the stirrer. Turn on the stirrer and adjust the speed of the stirrer so the stir bar is rotating quickly and evenly. Place the oxygen electrode in the beaker with the zero-percent oxygen calibration solution.
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 being dissolved in deionized water at room temperature. Click Stop to halt the recording.
9. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.
10. Turn off the stirrer. Remove the electrode from the beaker of calibration solution. Hold the electrode over the beaker used for collecting waste liquid, and 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 deionized water.

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 1 find the dissolved oxygen concentration ([O_2]) in deionized water at room temperature. This concentration will be used in Step 6 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% and 0% saturation levels of oxygen in water on the Main window at the same time. The required data can also be selected by:

- Placing the cursors on either side of data required.
- Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment with both the 100% and 0% saturation levels of oxygen in water to the width of the Main window.

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 dissolved oxygen in water 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.

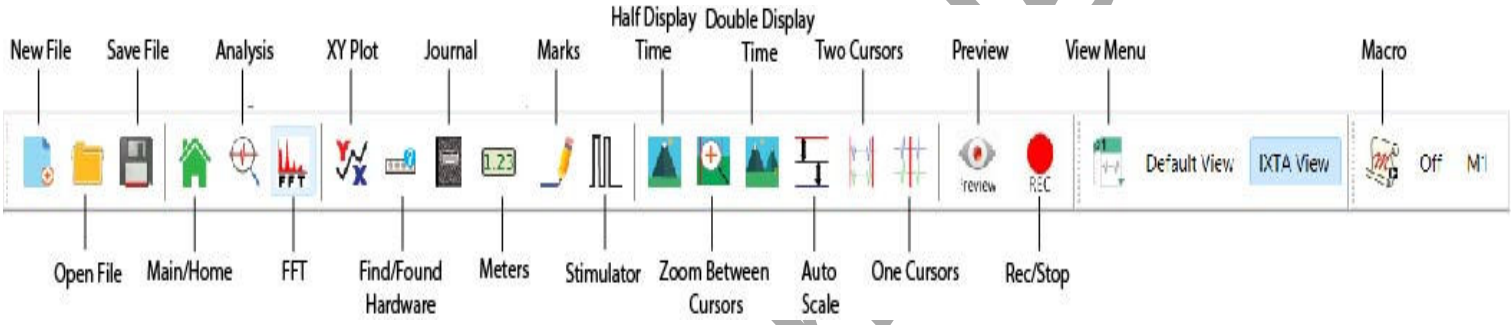

Figure CM-1-S2: The LabScribe toolbar.

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 the table 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.

Table CM-1-L1: 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

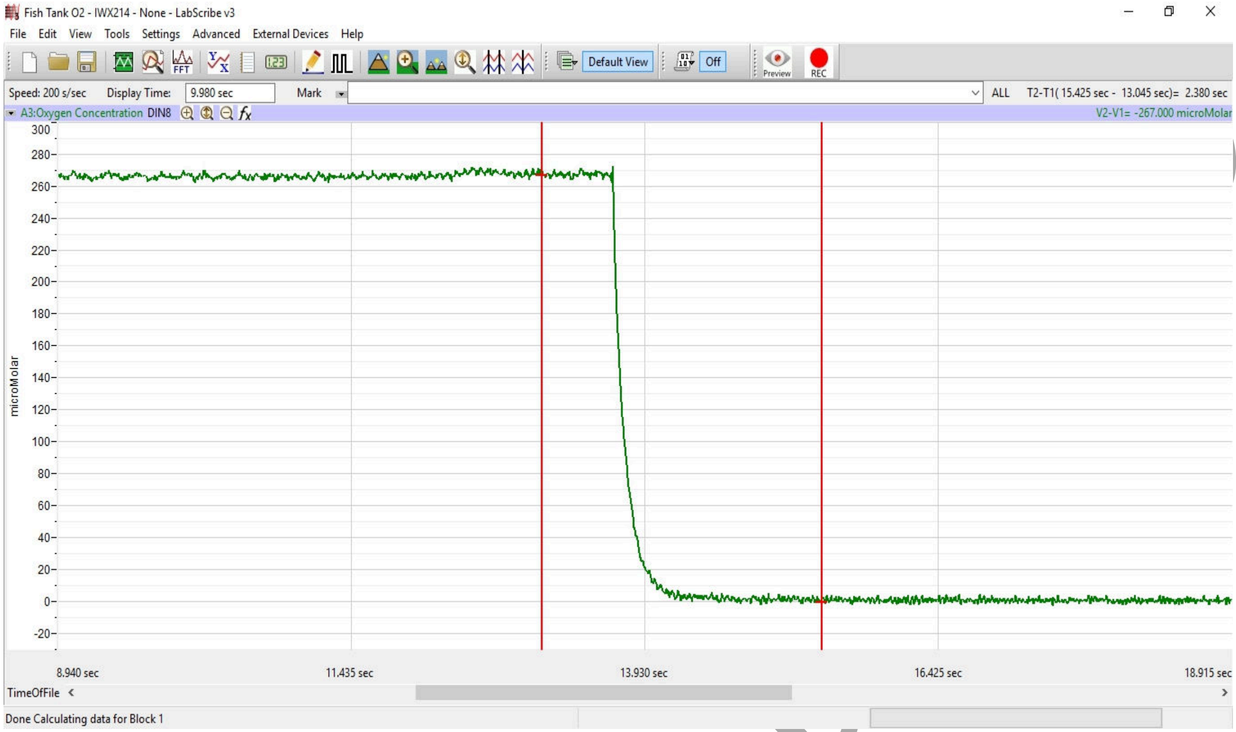

Figure CM-1-S3: Recording of oxygen concentrations in air saturated and oxygen depleted deionized waters used to convert the units of the Y-axis from voltage to O_2 concentration (µMolar).

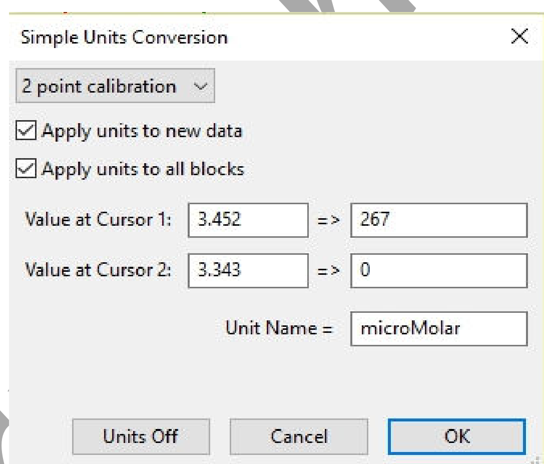

Figure CM-1-S4: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the dissolved oxygen concentrations used in calibration.

Experiment CM-1: Oxygen Consumption and Size

Exercise 1: Measure the Rate of Oxygen Consumption

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

Approximate Time: 60 minutes

Procedure

1. Half-fill the Erlenmeyer flask with fresh, aerated water.
2. Weigh the flask and its contents on the top-loading balance.
3. Catch a fish 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. **5** Place a stir bar on the bottom of the flask and place the flask on the magnetic stirrer.

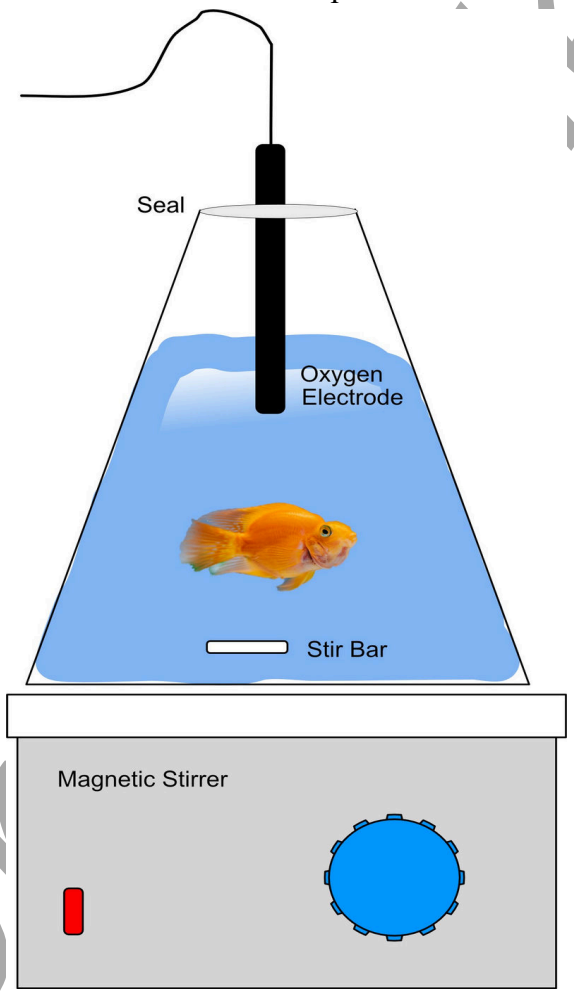

Figure CM-1-L1: Experimental setup for recording changes in the oxygen concentration in water containing a fish.

6. Fill the flask, which is holding the fish, close to the top with fresh, aerated water. Turn on the stirrer so that the bar rotates slowly. 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.

Note: *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.

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

12. Type Baseline Oxygen Consumption in the Mark box to the right of the Mark button.
13. Click Record and press 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.

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 aquarium holding the fish. Measure the volume of water in the cylinder. Return the water to the stock tank. Record the volume of water in the graduated cylinder in Table CM-1-L1.

Data Analysis

1. Scroll through the data file and locate a section near the beginning of the recording where the slope is consistent.
2. Use the Display Time icons to adjust the Display Time of the Main window to display a seventy-second section of recording with a consistent slope on the Main window.

This section of data can also be selected by:

- Placing the cursors on either side of the seventy-second section of the recording, and
- Clicking the Zoom between Cursors button on the LabScribe toolbar to expand or contract the seventy-second recording to the width of the Main window.

3. Click on the Analysis window icon in the toolbar or select Analysis from the Windows menu to transfer the data displayed in the Main window to the Analysis window.
4. Look at the Function Table that is above the [Oxygen] channel in the Analysis window. The mathematical functions, V2-V1 and T2-T1, should appear in this table. The values for these parameters are displayed in the table across the top margin of the [Oxygen] channel.
5. Once the cursors are placed in the correct positions for determining the change in oxygen concentration (V2-V1) in the chamber in a one minute (T2-T1)section of the recording, the values for these parameters can be recorded in the on-line notebook of LabScribe by typing the names and values of the parameters directly into the Journal.
6. The functions in the channel menu of the Analysis window can also be used to enter the names and values of the parameters from the recording to the Journal. To use these functions:
   - Place the cursors at the locations used to measure the oxygen concentration in a minute.
   - Transfer the names of the parameters to the Journal using the Add Title to Journal function in the [Oxygen] channel menu.
   - Transfer the values for the parameters to the Journal using the Add Ch. Data to Journal function in the [Oxygen] channel menu.
7. On the [Oxygen] channel, use the mouse to click on and drag a cursor close to the left margin of the data displayed on the Analysis window. Drag the other cursor to the right of the left cursor until the value for T2-T1 equals 60 seconds.
8. Record the values for the change in the oxygen concentration (V2-V1) in one minute (T2-T1) in the Journal using the one of the techniques described in Steps 5 or 6, and in Table 1.
9. Calculate the oxygen consumption rate (μmoles/liter/minute) by dividing the value for V2-V1, expressed as μmoles/liter, by the value for T2-T1, expressed in minutes. Enter the value for the rate of oxygen consumption in the data table.
10. Move to a section of data in the middle of the recording and repeat Steps 2 through 9 to determine the change in oxygen concentration per minute for a section of data in the middle of the recording. Repeat Steps 2 through 9 on a section of data at the end of the recording.
11. Calculate the mean rate of oxygen consumption by averaging the rates from the beginning, middle, and end of the recording. Enter the value in the data table.

Table CM-1-L1: Rate of Oxygen Consumption During Different Segments of the Recording.

	V2-V1 [O2] Change (µmoles/liter)	T2-T1 Time Period (min)	Rate of O_2 Consumption (µmoles/liter/min)
Beginning			
Middle			
End			
Mean			

Exercise 2: Size and the Rate of Oxygen Consumption

Aim: To measure the rate of oxygen consumption in fish of different weights.

Approximate Time: 60 minutes

Procedure

Follow the procedures explained in Exercise 1 to record the changes in oxygen concentration in flasks of fresh, aerated water containing fish of different weights.

Data Analysis

1. Use the same techniques explained in the data analysis section of Exercise 1 to measure and record the change in oxygen concentration and rate of oxygen consumption for each fish at the beginning, middle, and end of the recording.
2. Calculate the mean rate of oxygen consumption (µmoles/liter/minute) from each fish by averaging the rates from the beginning, middle, and end of its recording. Enter the value for each fish in Table 2.
3. Calculate the amount of oxygen consumed per minute (µmoles/minute) by each fish by multiplying its mean rate of oxygen consumption (µmoles/liter/minute) by the volume (liters) of water in its flask. Enter the value for each fish in the data table.
4. Use the data in the table to graph the relationship between the oxygen consumed/minute by the fish as a function of their weights.
5. Calculate the amount of oxygen consumed per minute per gram of body weight (µmoles/minute/gram) by each fish by dividing the oxygen consumed per minute (µmoles/minute) by each fish by its own body weight (grams). Enter the value for each fish in the data table.
6. Use the data in the table to graph the relationship between the oxygen consumed/minute/gram by the fish as a function of their weights.

Questions

1. How is the rate of oxygen consumption related to weight?
2. 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 fish of different weights, is there a trend?

   Note: This is easily done by making two graphs - one graphing the log of mass (x) against the log of whole-animal O2 Consumption. The other graphs the log of mass against the log of mass-specific O2 consumption. The first "should" have a slope of about .75, the other a slope of -.25. This is a good test of "Kleiber's Rule".

Table CM-1-L2: Rate of Oxygen Consumption of Fish of Different Weights.

Fish	Mean Rate of O_2 Consumption (µmoles/liter/min)	Volume of Water in Flask (liters)	O_2 Consumed per Minute (µmoles/min)	Weight of Fish (grams)	O_2 Consumed per Minute per Gram Body Weight (µmoles/min/gram)
1					
2					
3					
4					
5					