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.

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).

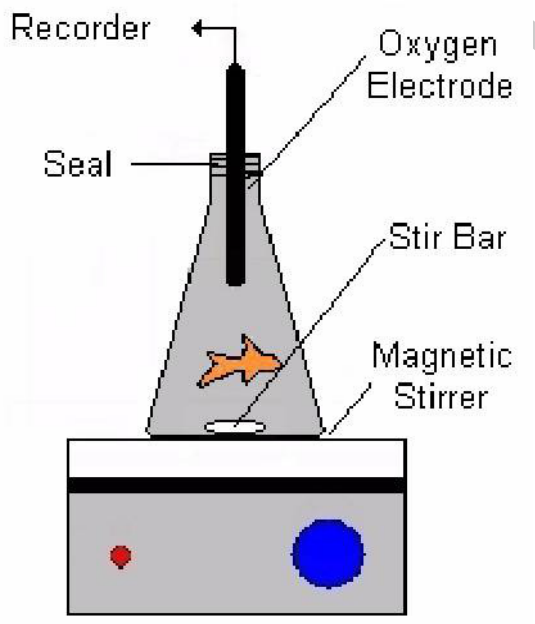

Figure CM-1-L1: Experimental setup for recording changes in the oxygen concentration in water containing a fish.

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 a 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 (Figure CM-1-L1).

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 Enter key on the keyboard 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 on Table CM-1-L1.
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.

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 CM-1-L2.
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?

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					

Recipe for Artificial Pond Water.

Concentration (mMolar)	Salt	Grams/Liter DI H_2O
64	Sodium Chloride	3.74
0.70	Potassium Chloride	0.0525
0.43	Magnesium Sulfate*$7H_2O$	0.105
2.50	Sodium Bicarbonate	0.21
0.71	Calcium Chloride*$2H_2O$	0.105
Dissolve all the salts, except $CaCl_2$*$2H_2O$, in 750 ml of deionized (DI) H_2O. Adding $CaCl_2$*$2H_2O$ to a solution with the other salts will cause precipitation. Dissolve $CaCl_2$*$2H_2O$ in 200 ml DI H_2O before adding it to the solution containing the other salts. Add DI H_2O to bring the final volume to 1 Liter.		

Zero-Percent Oxygen Calibration Solution

Concentration (mMolar)	Salt	Grams/Liter DI H_2O
15	Sodium Hydrosulfite	2.61