Experiment GB-4: Ecological Balance

Equipment Required

PC or Mac Computer

IXTA, USB cable, power supply

ISE-730 Dissolved oxygen electrode

ISE-100 combination pH electrode

Magnetic stirrer, Stir bar

Ringstand and Utility clamps (2) for holding electrodes

Lamp with 75 watt bulb

100ml beakers (2)

250 ml beakers (3)

400 ml beaker and 1000 ml beaker

Roll of plastic wrap

pH 4 and pH 7 buffer solutions

250 ml graduated cylinder

Thermometer

Pond water and Deionized water

Zero-percent oxygen calibration solution

Goldfish

6" branch of *Elodea* or similar aquatic plant

Dissolved Oxygen Electrode

Warning: The ISE-730 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.

The ISE-730 dissolved oxygen electrode used in this experiment has a Teflon(tm) membrane that permits a limited amount of oxygen to diffuse from the solution being measured to the electrolyte solution inside the removable membrane housing of the electrode. The number of electrons flowing between the cathode and the anode is proportional to the concentration of oxygen that has diffused across the Teflon(tm) membrane into the electrolyte that surrounds the cathode and the anode.

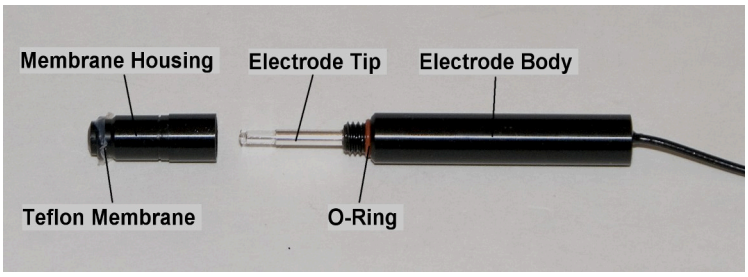

Figure GB-4-S1: The ISE-730 dissolved oxygen electrode, shown with its membrane housing removed. The end of the housing is covered with a Teflon(tm) membrane secured in place by an O-ring. The platinum wire inside the glass tube is the cathode and the silver sleeve that surrounds the glass tube is the anode. Electrons are conducted between the two electrodes by an electrolyte solution that fills the housing.

Dissolved Oxygen and pH Electrodes Setup

1. Locate the ISE-730 dissolved oxygen electrode and plug the BNC connector into the channel labeled DO2.
2. Locate the ISE-100 pH electrode and plug the DIN8 connector of the ISE-100 pH electrode into the Channel A5.

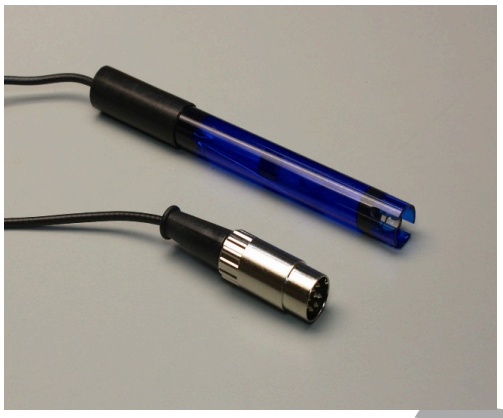

Figure GB-4-S3: The ISE-100 pH electrode.

Figure GB-4-S4: The ISE-730 dissolved oxygen probe and pH probe connected to the IXTA.

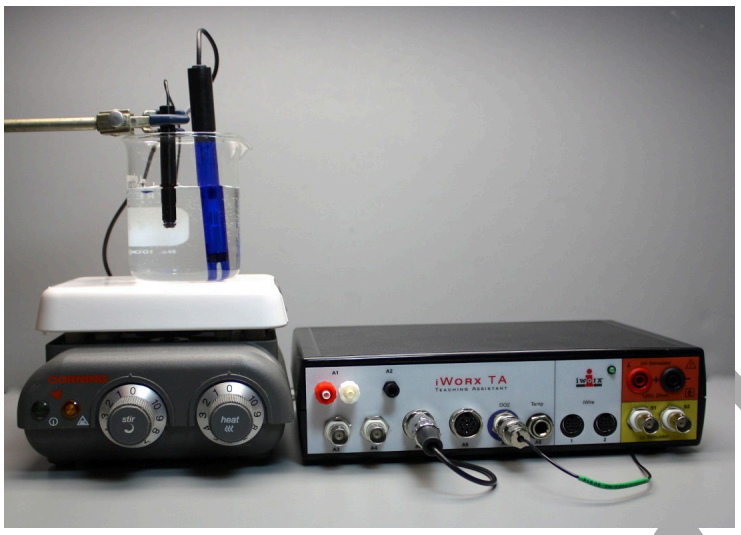

Figure GB-4-S5: The setup for recording oxygen concentration levels and pH in a small closed ecosystem using the IXTA.

Calibration of the 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. 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. 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. Click Stop to halt the recording.
5. 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.
6. 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.
7. 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.
8. Type **No Oxygen** in the Mark box.
9. Click Record. 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.
10. Click Stop to halt the recording.
11. Select Save As in the File menu, type a name for the file. Choose a destination on the computer in which to save the file (like your lab group folder). Designate the file type as *.iwxdata. Click on the Save button to save the data file.
12. 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-Dissolved Oxygen Probe

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 ISE-730 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.
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 voltages at the positions of the cursors to % Oxygen values, use the Simple Units Conversion dialogue window. To access this dialogue window, click on the arrow to the left of the channel title, O2 Concentration, to open the channel menu. Select Units from the channel menu, and select Simple from the Units submenu.
6. On the units conversion window, make sure 2 point calibration is selected in 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.

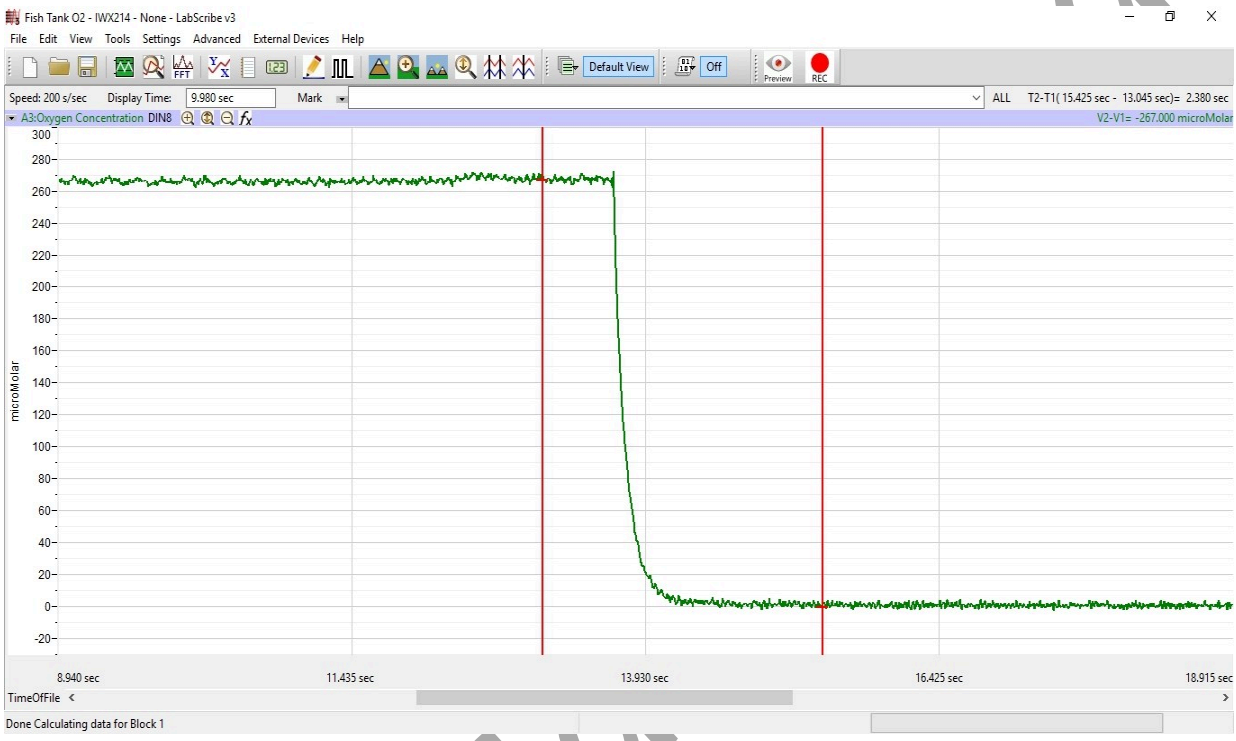

Figure GB-4-S7: Recording of oxygen concentrations in air saturated and oxygen depleted deionized waters used to convert the units of the Y-axis from voltage to O_2 concentration (µMolar).

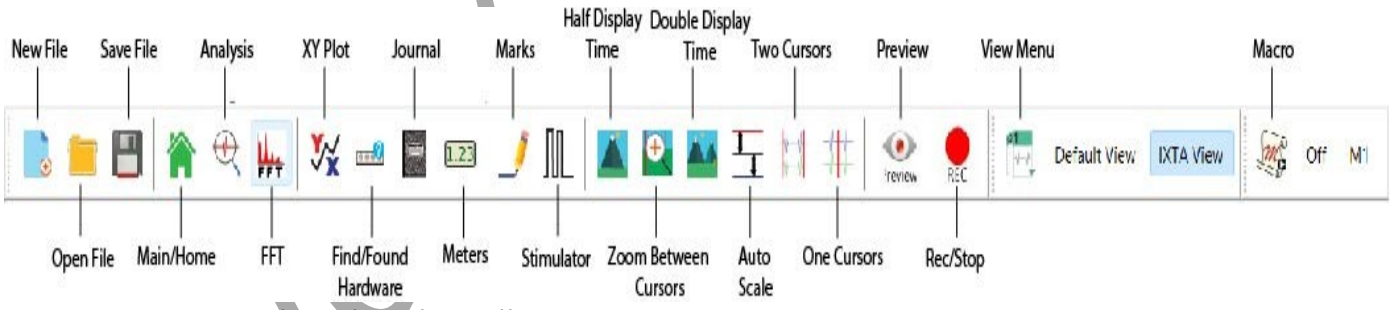

Figure GB-4-S6: The LabScribe toolbar.

Table GB-4-S1: Concentration of Oxygen [O2] in Air-Saturated Deionized Water at 1 Atmosphere.

Temp (^oC)	O_2 Abs Coeff (a)	H_2O Vapor Click (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

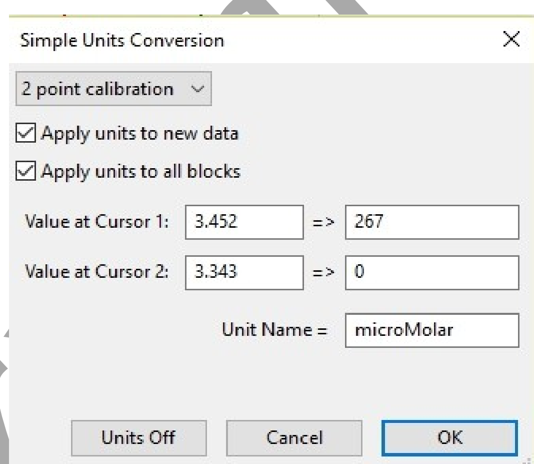

Figure GB-4-S8: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the dissolved oxygen concentrations used in calibration.

7. From Table 1, 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.

8. 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.
9. Select Save in the File menu, type a name for the file. Designate the file type as *.iwxdata. Click on the Save button to save the data file.

Calibration of the pH Electrode

1. If the ISE-100 pH electrode is still stored in its bottle of buffer, remove the electrode from the bottle. Rinse the electrode with deionized water while holding the electrode over a 1000 ml beaker used for the collection of waste liquids.
2. Place the tip of the ISE-100 pH electrode in a 100 ml beaker containing enough room temperature deionized water to submerge the tip. Keep the electrode in deionized water for at least ten minutes.
3. Prepare two 100 ml beakers, each filled with 50 ml of the pH buffers used for calibrating the pH electrode. The buffers should be at room temperature. Fill one beaker with pH 7 buffer; and the other with pH 4 buffer. Each beaker should be filled with enough buffer to cover the tip of the ISE-100 pH electrode, and also allow the stir bar in the beaker to spin without touching the electrode.
4. Place the beaker containing the pH 7 buffer on the magnetic stirrer. Carefully place a stir bar in the beaker. Remove the electrode from the deionized water and blot any extra drops of water. Position the tip of the electrode in the beaker of pH 7 buffer so that the tip is away from the stir bar. Adjust the speed of the stirrer so the stir bar is rotating evenly at a slow speed.
5. Click Record on the LabScribe Main window to begin recording. After a few seconds, the trace will reach a stable baseline toward the top of the recording channel. Type the words **Calibration - pH 7** in the Mark box. Click the mark button to mark the stable baseline of the recording. This will mark the output of the ISE-100 pH electrode in pH 7 buffer. Continue recording while changing the beakers of buffers.
6. Turn off the stirrer and remove the ISE-100 pH electrode from the beaker of pH 7 buffer. Hold the electrode over the beaker used for collecting waste liquid and rinse it with deionized water. Blot any extra drops of water.
7. Place the beaker of pH 4 buffer on the stirrer. Carefully place a stir bar in the beaker. Position the tip of the electrode in the beaker of pH 4 buffer so that it is away from the stir bar. Adjust the speed of the stirrer so the stir bar is rotating evenly at a slow speed.
8. As you continue to record, the trace will reach a stable baseline toward the bottom of the recording channel. Type the words **Calibration - pH 4** in the Mark box. Click the mark button to mark the stable baseline of the recording. This marks the output of the ISE-100 pH electrode in pH 4 buffer.
9. Click Stop to halt the recording.
10. Click on the Save button to add this information to your data file.

11. Turn off the stirrer. Remove the electrode from the beaker of pH 4 buffer. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any extra drops of water and place the electrode in a beaker of deionized water.

Units Conversion-pH

1. Scroll to the beginning of the calibration data for the ISE-100 pH electrode.
2. Use the Display Time icons to adjust the Display Time of the Main window to show the data collected at pH 7 and pH 4 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 and,
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window.
3. Click the 2-Cursor icon so that two blue cursors appear on the Main window. Place one cursor on the flat section of data collected when the ISE-100 pH electrode was in the pH 7 buffer and the second cursor on the flat section of data collected when the electrode was in the pH 4 buffer.
4. To convert the voltages at the positions of the cursors to pH values, use the Simple Units Conversion dialogue window. To access this dialogue window, click on the arrow to the left of the channel title, pH, to open the channel menu. Select Units from the channel menu, and select Simple from the Units submenu.
5. On the units conversion window, make sure 2 point calibration is selected in 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. Enter the two buffers used in the calibration recording in the corresponding boxes on the right side of the conversion equations. Enter the name of the units, pH, in box below the buffer values. Click on the OK button in the lower right corner of the window to activate the units conversion.
6. Select Save in the File menu.
7. Turn off the stirrer. Remove the pH electrode from the beaker of pH 4 buffer. Hold the electrode over the beaker used for collecting waste liquid, and rinse it with deionized water from a wash bottle. Blot any drops of water from the electrode and place it in a beaker of deionized water.

Experiment GB-4: Ecological Balance

Exercise 1: Dissolved Oxygen Concentration and pH in an Aquatic Environment with an Animal

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

Approximate Time: 45 minutes

Procedure

1. Place a magnetic stirrer on or next to the base of a ringstand. Place 200 mL of fresh pond water, at room temperature, in a 400 ml beaker. Add a stir bar to the beaker and place the beaker on a magnetic stirrer. Turn on the stirrer and position the stir bar to one side of the beaker bottom.
2. Adjust the position of the clamps that will hold the dissolved oxygen and the pH electrodes in the beaker of pond water.
3. Remove the dissolved oxygen and the pH electrodes from the beakers of deionized water. Blot the drops of deionized water from each device. Mount the electrodes on the ringstand using the clamps, and position the tips of the devices in the pond water. Cover the top of the beaker with plastic wrap to prevent the exchange of gases over the pond water with those in the environment.
4. Turn on the stirrer so that the stir bar rotates slowly and evenly. Wait two minutes before recording the dissolved oxygen concentration and pH level of the pond water.
5. Click Record. When the recordings on both channels reach a stable baseline, type the words **Pond Water Alone** in the Mark box. Click the mark button to mark the stable baseline of the recording.
6. After recording at least fifteen seconds of stable baseline on each channel, type the words **Goldfish in Pond Water** in the Mark box. Peel back the plastic wrap that covers the top of the beaker and place a goldfish in the pond water. Cover the top of the beaker with the plastic wrap, and click the mark button to mark the recording. Lower the speed of the stirrer, if the goldfish seems stressed.
7. Record the dissolved oxygen concentration and pH of the pond water for thirty minutes. At the end of thirty minutes, click Stop to halt the recording.
8. Select Save in the File menu. Before analyzing the data from this exercise, complete Step 9 and then proceed directly to Exercise 2.
9. Remove the dissolved oxygen and the pH electrodes from the beaker of pond water. Remove the goldfish from the beaker and place it in the fish tank. Discard the pond water and refill the beaker with 200 ml of fresh pond water. Reposition the electrodes and the stir bar in the beaker of fresh pond water. Cover the beaker with plastic wrap.
10. Continue directly to Exercise 2.

Exercise 2: Dissolved Oxygen Concentration and pH in an Aquatic Environment with a Plant

Aim: To measure changes in dissolved oxygen concentration and pH of water in the presence of an aquatic plant.

Approximate Time: 30 minutes

Procedure

1. Using the setup completed at the end of Exercise 1, make sure the stir bar rotates slowly and evenly. Wait two minutes before recording the dissolved oxygen concentration and pH level of the pond water.
2. Click Record. When the recordings on both channels reach a stable baseline, type the words **Pond Water Alone** in the Mark box. Click the mark button to mark the stable baseline of the recording.
3. Record at least fifteen seconds of stable baseline on each channel, type the words **Plant in Pond Water** in the Mark box. Peel back the plastic wrap that covers the top of the beaker and place a piece of aquatic plant, like Elodea, in the beaker. Aim a lamp toward the beaker of pond water containing the plant. Turn on the light to illuminate the plant material in the beaker.

Warning: It is important to prevent the temperature of the pond water from rising while the light is on. You may need to place a large beaker with water, that will act as a heat reservoir, between the light and the beaker of pond water.

4. Cover the top of the beaker with the plastic wrap, and click the mark button to mark the recording.
5. Record the dissolved oxygen concentration and pH of the pond water containing the aquatic plant for ten minutes.
6. At the end of ten minutes, click Stop to halt the recording. Select Save in the File menu; then immediately, click Start to begin recording again. Do not turn off the light at any time.
7. Proceed directly to Exercise 3.

Exercise 3: Dissolved Oxygen Concentration and pH in an Aquatic Environment with a Plant and an Animal

Aim: To measure changes in dissolved oxygen concentration and pH of water inhabited by a fish in the presence of an aquatic plant.

Approximate Time: 45 minutes

Procedure

1. Type the words **Goldfish & Plant in Pond Water** in the Mark box. Peel back the plastic wrap that covers the top of the beaker with the plant. Place a goldfish in the same beaker.
2. Cover the top of the beaker with the plastic wrap, and click the mark button to mark the recording. Lower the speed of the stirrer, if the goldfish seems stressed.

3. Record the dissolved oxygen concentration and pH of the pond water containing the plant and the goldfish for thirty minutes. At the end of thirty minutes, click Stop to halt the recording.
4. Select Save in the File menu.
5. Turn off the light and carefully move it away from the beaker. Remove the dissolved oxygen and the pH electrodes from the beaker of pond water. Remove the goldfish and the plant material from the beaker and place them in the fish tank. Discard the pond water. Place the electrodes in beakers of deionized water.

Data Analysis

Exercise 1: Environment with Animal

1. Scroll to the section of data recorded during Exercise 1 where the goldfish was introduced to the ecosystem.
2. Use the Display Time icons on the LabScribe toolbar to position the complete recording on the Main window. The required data can also be selected by:
    - Placing the cursors on either side of the section of data needed. Place one cursor on the stable dissolved O2 and pH levels recorded from pond water alone. Place the second cursor on the stable dissolved O2 and pH levels recorded after a total of 30 minutes with the presence of the goldfish in the water
    - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the segment of data to the width of the Main window
3. Click on the Analysis window icon.
4. Look at the Function Table that is above the uppermost channel displayed in the Analysis window. The mathematical functions that are listed should include Title, Value1, Value2, V2-V1 and T2-T1. The values for these parameters from each channel are seen in the table across the top margin of each channel.
5. Place a cursor at the point in the recording when the goldfish was placed in the beaker of pond water (Time = 0). Place the second cursor at the point in the recording that is thirty minutes after the goldfish was placed in the beaker (Time = 30).
6. Once the cursors are placed in the correct positions for determining the dissolved oxygen concentration and pH levels, the values for both can be recorded in the on-line notebook of LabScribe by typing the names and values directly into the Journal.
7. The functions in the channel pull-down menus 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 dissolved oxygen from the O2 Concentration channel.
    - Transfer the name of the mathematical function used to determine the dissolved O2 to the Journal using the Add Title to Journal function in the O2 Concentration channel pull-down menu.

- Transfer the value for the dissolved oxygen to the Journal using the Add Ch. Data to Journal function in the O2 Concentration channel pull-down menu.

8. Place a cursor on the stable baseline recorded just before the goldfish was placed into the beaker of pond water. Place the second cursor at the point in the recording that is 30 minutes after the goldfish was placed in the water.
9. Measure the values for the following parameters from the O2 Concentration channel for the region of data selected:
    - O2 Conc - Time 0, which is Value1 on the O2 Concentration channel.
    - O2 Conc - Time 30, which is Value2 on the O2 Concentration channel.
10. Record the values for these parameters in the Journal using one of the procedures described in Step 6, and in Table 1.
11. Measure the Overall Change in O2 Concentration using the parameter, V2-V1, from the Function Table in the Analysis window.
12. Divide the Overall Change in O2 Concentration by the initial O2 Concentration of the pond water, and multiply by 100 to determine the percent change in dissolved oxygen.
13. Repeat Steps 7 through 12 for pH, using the pH channel.
14. Click Save in the File menu
15. Click on the Main icon in the LabScribe toolbar to return to the Main window.

Exercise 2: Environment with Plant

1. Scroll to the section of data recorded during Exercise 2. Display the complete recording from Exercise 2 on the Main window.
2. Use the same techniques used in Exercise 1 to measure the dissolved oxygen concentration and pH levels of the pond water after the plant was immersed in the pond water.
3. Use the same techniques explained in Exercise 1 to record the values of both the O2 concentration and pH levels in the Journal, and in Table 1.
4. Click Save in the File menu.
5. Click on the Main icon in the LabScribe toolbar to return to the Main window.

Exercise 3: Environment with Plant and Animal

1. Scroll to the section of data recorded during Exercise 3. Display the complete recording from exercise 3 on the Main window.
2. Use the same techniques used in Exercise 1 to measure the dissolved oxygen concentration and pH levels of the pond water after both the plant and the goldfish were immersed in the pond water.

3. Use the same techniques explained in Exercise 1 to record the values of both the O2 concentration and pH levels in the Journal, and in the data table.
4. Click Save in the File menu.
5. Click on the Main icon in the LabScribe toolbar to return to the Main window.

Table GB-4-L1: Changes in Dissolved Oxygen Concentrations and pH While Organisms Are in a Closed Ecosystem.

Organisms in Pond Water	pH Level			Dissolved Oxygen Concentration (µMolar O_2)			
	Initial	Ending	Change (Δ)	Initial	Ending	Change (Δ)	Rate (µMO_2/min)
Animal, 30 Mins							
Plant, 10 Mins							
Plant & Animal, 30 Mins							

Questions

Exercise 1: Environment with Animal

1. Do any changes in the dissolved oxygen concentration and pH of the pond water take place while the fish is in the beaker?
2. What could be the causes of the changes or the lack of changes?

Exercise 2: Environment with Plant

1. Do any changes in the dissolved oxygen concentration and pH of the pond water take place while the plant is in the beaker?
2. What could be the causes of the changes or the lack of changes?

Exercise 3: Environment with Plant and Animal

1. Do any changes in the dissolved oxygen concentration and pH of the pond water take place while the fish and the plant are in the beaker together?
2. What could be the causes of the changes or the lack of changes?

3. If increases or decreases in the oxygen concentration and/or pH of large bodies of water (lakes, seas, oceans) took place, what would be some of the causes of these changes? Make sure you indicate whether the cause would create an increase or decrease in pH or dissolved oxygen concentration.