# Primary Productivity

Oxygen is vital to life. In the atmosphere, oxygen comprises over 20% of the available gases. In aquatic ecosystems, however, oxygen is scarce. To be useful to aquatic organisms, oxygen must be in the form of molecular oxygen, $O_2$. The concentration of oxygen in water can be affected by many physical and biological factors. Respiration by plants and animals reduces oxygen concentrations, while the photosynthetic activity of plants increases it. In photosynthesis, carbon is assimilated into the biosphere and oxygen is made available, as follows:

$$6\ H_2O + 6\ CO_2(g) + \text{energy} \longrightarrow C_6H_{12}O_6 + 6\ O_2(g)$$

The rate of assimilation of carbon in water depends on the type and quantity of plants within the water. Primary productivity is the measure of this rate of carbon assimilation. As the above equation indicates, the production of oxygen can be used to monitor the primary productivity of an aquatic ecosystem.

One method of measuring the production of oxygen is the *light and dark bottle* method. In this method, a sample of water is placed into two bottles. One bottle is stored in the dark and the other in a lighted area. Only respiration can occur in the bottle stored in the dark. The decrease in dissolved oxygen (DO) in the dark bottle over time is a measure of the rate of respiration. Both photosynthesis and respiration can occur in the bottle exposed to light, however. The difference between the amount of oxygen produced through photosynthesis and that consumed through aerobic respiration is the *net productivity*. The difference in dissolved oxygen over time between the bottles stored in the light and in the dark is a measure of the total amount of oxygen produced by photosynthesis. The total amount of oxygen produced is called the *gross productivity*.

The productivity of an aquatic environment varies seasonally throughout the year. During springtime, rains bring nitrogen and phosphates into the aquatic environment and increase the productivity. Human activities, such as fertilization of fields and the operation of sewage treatment facilities, can alter the natural balance of nitrogen and phosphates in water. In this lab, you will measure the primary productivity of a natural body of water and that of water enriched with nitrogen and phosphates.

## MATERIALS

- LabPro or CBL 2 interface
- TI Graphing Calculator
- DataMate program
- Vernier Dissolved Oxygen Probe
- two 1-mL pipettes
- aluminum foil
- six 25 x 150-mm screw top test tubes
- shallow pan
- nitrogen enrichment solution
- phosphate enrichment solution
- scissors
- siphon tube
- thermometer

## PRE-LAB PROCEDURE

**Important**: Prior to each use, the Dissolved Oxygen Probe must warm up for a period of 10 minutes as described below. If the probe is not warmed up properly, inaccurate readings will result. Perform the following steps to prepare the Dissolved Oxygen Probe.

1. Prepare the Dissolved Oxygen Probe for use.
   a. Unscrew the membrane cap from the tip of the probe.
   b. Using a pipet, fill the membrane cap with 1 mL of DO Electrode Filling Solution.
   c. Carefully thread the membrane cap back onto the electrode.
   d. Place the probe into a 250-mL beaker containing distilled water.


*Figure 1*

2. Plug the Dissolved Oxygen Probe into Channel 1 of the LabPro or CBL 2 interface. Use the link cable to connect the TI Graphing Calculator to the interface. Firmly press in the cable ends.

3. Turn on the calculator and start the DATAMATE program. Press CLEAR to reset the program.

4. Set up the calculator and interface for the Dissolved Oxygen Probe.
   a. If the calculator displays DO (MG/L) in CH 1, proceed to Step 5. If it does not, continue with this step to set up your sensor manually.
   b. Select SETUP from the main screen.
   c. Press ENTER to select CH1.
   d. Select D. OXYGEN (MG/L) from the SELECT SENSOR menu.
   e. Select OK to return to the main screen.

5. It is necessary to warm up the Dissolved Oxygen Probe for 10 minutes before taking readings. With the probe still in the distilled water beaker, wait 10 minutes while the probe warms up. The probe must stay connected at all times to keep it warmed up. If disconnected for a period longer than 5 minutes, it will be necessary to repeat this step. **Note:** The green light on the interface verifies that power is being provided to the probe.

6. Prepare the calculator and interface for calibration.
   a. Select SETUP from the main screen.
   b. Select CALIBRATE from the setup screen.

7. Calibrate the Dissolved Oxygen Probe.
   - If your instructor directs you to manually enter the calibration values, select MANUAL ENTRY from the CALIBRATION screen. Enter the slope and intercept values, select OK, then proceed to Step 1 of the procedure.
   - If your instructor directs you to perform a new calibration, select CALIBRATE NOW from the CALIBRATION screen and follow this procedure.

Zero-Oxygen Calibration Point

a. Remove the probe from the water bath and place the tip of the probe into the Sodium Sulfite Calibration Solution. **Important:** No air bubbles can be trapped below the tip of the probe or the probe will sense an inaccurate dissolved oxygen level. If the voltage does not rapidly decrease, tap the side of the bottle with the probe to dislodge the bubble. The readings should be in the 0.2- to 0.5-V range.
b. When the voltage stabilizes (~1 minute), press ENTER.
c. Enter "0" as the known value in mg/L.

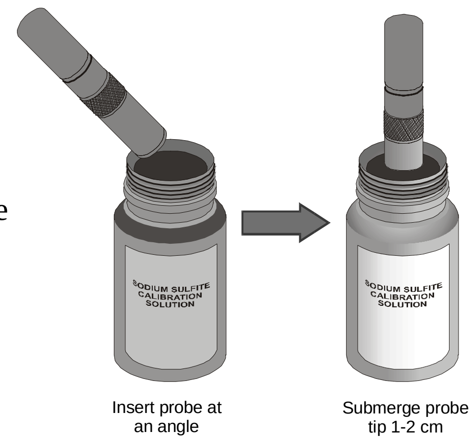


*Figure 2*

Saturated DO Calibration Point

d. Rinse the probe with distilled water and gently blot dry.
e. Unscrew the lid of the calibration bottle provided with the probe. Slide the lid and the grommet about 1/2 inch onto the probe body.
f. Add water to the bottle to a depth of about 1/4 inch and screw the bottle into the cap, as shown. **Important:** Do not touch the membrane or get it wet during this step.
g. Keep the probe in this position for about a minute. The readings should be above 2.0 V. When the voltage stabilizes, press ENTER.
h. Enter the correct saturated dissolved-oxygen value (in mg/L) from Table 6 (for example, "8.66") using the current barometric pressure and air temperature values. If you do not have the current air pressure, use Table 7 to estimate the air pressure at your altitude.
i. Select OK to return to the setup screen.
j. Select OK again to return to the main screen.
k. Return the Dissolved Oxygen Probe to the distilled water beaker.

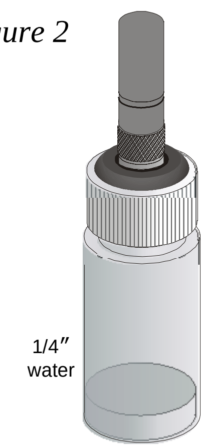


*Figure 3*

## PROCEDURE

**Day 1**

1. Obtain six water sampling test tubes and label them 1 – 6.
2. Fill each of the test tubes with the water sample. To fill a test tube:
   a. Obtain a siphon tube.
   b. Insert the tube into the water sample and fill the tube completely with water.
   c. Pinch the tube (or use a tube clamp) to close off the siphon tube.
   d. Place one end of the tube in the bottom of a test tube. Keep the other end in the water sample, well below the surface. Position the test tube lower than the water sample and above a shallow pan.

e. Siphon the water into the test tube. Fill the test tube until it overflows approximately 1/3 of the volume of the test tube. Fill the test tube completely to the top of the rim. Use the shallow pan to collect any water that spills over.

f. Tighten the cap on the test tube securely. Be sure no air is in the test tube.

3. Measure the temperature of the water sample in test tube 1 with a thermometer. Record the temperature in Table 2.

4. The treatment of each water sample is listed in Table 1 below:

| Table 1 | |
|---|---|
| Test Tube | Treatment |
| 1 | Initial, No Treatment |
| 2 | Light |
| 3 | Light and Nitrogen |
| 4 | Light and Phosphate |
| 5 | Light, Nitrogen, and Phosphate |
| 6 | Dark |

5. Place 1 mL of nitrogen enrichment solution into test tube 3. **CAUTION**: Handle the nitrogen enrichment solution with care. Do not allow it to contact your skin.

6. Place 1 mL of phosphate enrichment solution into test tube 4. **CAUTION**: Handle the phosphate enrichment solution with care. Do not allow it to contact your skin.

7. Place 1 mL of nitrogen enrichment solution and 1 mL of phosphate enrichment solution into test tube 5.

8. Wrap test tube 6 with aluminum foil so that it is light-proof. This water sample will remain in the dark.

9. Mix the contents of each tube. Be sure that no air pocket is apparent in any of the test tubes. Fill a tube with more sample water, if necessary.

10. If the calculator screen has gone blank, press the ON key to power up the calculator and select YES from the CONTINUE menu.

11. Place the probe into test tube 1 so that it is submerged half the depth of the water. Gently and continuously move the probe up and down a distance of about 1 cm in the tube. This allows water to move past the probe's tip. **Note**: Do not agitate the water, or oxygen from the atmosphere will mix into the water and cause erroneous readings.

12. After 90 seconds, or when the dissolved oxygen reading stabilizes, record the DO reading in Table 3. Discard the contents of test tube 1 and clean the test tube. Place the Dissolved Oxygen Probe back in the distilled water beaker.

13. Place test tubes 2–6 near the light source, as directed by your instructor.

**Day 2**

14. Repeat the pre-lab procedure to polarize the Dissolved Oxygen Probe.

15. Place the probe into test tube 2. Gently and continuously move the probe up and down a distance of about 1 cm in the tube. After 30 seconds, or when the dissolved oxygen reading stabilizes, record the DO reading in Table 3.

16. Repeat Step 15 for test tubes 3–6.
17. When all measurements have been made, select QUIT from the main screen.
18. Clean all of the glassware and probes. Place the Dissolved Oxygen Probe back in the beaker of distilled water.

## DATA

| Table 2 | |
|---|---|
| Temperature (°C) | |

| Table 3 | | |
|---|---|---|
| Test Tube | Treatment | DO (mg/L) |
| 1 | Initial | |
| 2 | Light Only | |
| 3 | Light + Nitrogen | |
| 4 | Light + Phosphate | |
| 5 | Light + Nitrogen + Phosphate | |
| 6 | Dark Only | |

## PROCESSING THE DATA

1. Determine the number of hours that have passed since the onset of this experiment. Subtract the DO value in test tube 1 (the initial DO value) from that of test tube 6 (the dark test tube's DO value). Divide the DO value by the time in hours. Record the resulting value as the respiration rate in Table 4.
2. Determine the gross productivity in each test tube. To do this, subtract the DO in test tubes 2–5 (the light test tube's DO value) from that of test tube 6 (the dark test tube's DO value). Divide each DO value by the length of the experiment in hours. Record each resulting value as the gross productivity in Table 5.
3. Determine the net productivity in each test tube. To do this, subtract the DO in test tubes 2–5 (the light test tube's DO value) from that of test tube 1 (the initial DO value). Divide the result by the length of the experiment in hours. Record each resulting value as the net productivity in Table 5.

| Table 4 | |
|---|---|
| Respiration (mg/L/hr) | |

| Table 5 | | | |
|---|---|---|---|
| Test Tube | Treatment | Gross Productivity (mg/L/hr) | Net Productivity (mg/L/hr) |
| 2 | Light Only | | |
| 3 | Light + Nitrogen | | |
| 4 | Light + Phosphate | | |
| 5 | Light + Nitrogen + Phosphate | | |

## QUESTIONS

1. Is there evidence that photosynthetic activity added oxygen to the water? Explain.
2. Is there evidence that aerobic respiration occurred in the water? If so, what kind of organisms might be responsible for this–autotrophs? Heterotrophs? Explain.
3. What effect did light have on the primary productivity? Explain.
4. What effect did the nitrogen ions have on the primary productivity? Explain.
5. What effect did phosphates have on the primary productivity? Both nitrogen and phosphates? Explain.
6. Was the primary productivity limited by either nitrogen or phosphate ions? If so, were both ions equally limiting? Explain.
7. Why would the presence of phosphates and nitrogen, in the form of nitrates and ammonium ions, be important to an aquatic ecosystem during the spring season? How do they accumulate in the watershed?
8. Why would phosphate and nitrogen ions be considered a pollutant in an aquatic ecosystem during the Fall season?
9. How would turbidity affect the primary productivity of a pond?

## EXTENSIONS

1. Vary the amount of nitrogen and phosphate ions to determine the optimal concentration of fertilizer for the water sample.
2. Vary the intensity of light to simulate different pond depths. How does light affect the primary productivity of a pond?
3. Measure the primary productivity of a pond at different temperatures. What is the effect of temperature on the primary productivity of a pond?

# CALIBRATION TABLES

| Table 6: 100% Dissolved Oxygen Capacity (mg/L) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **770 mm** | **760 mm** | **750 mm** | **740 mm** | **730 mm** | **720 mm** | **710 mm** | **700 mm** | **690 mm** | **680 mm** | **670 mm** | **660 mm** |
| **0°C** | 14.76 | 14.57 | 14.38 | 14.19 | 13.99 | 13.80 | 13.61 | 13.42 | 13.23 | 13.04 | 12.84 | 12.65 |
| **1°C** | 14.38 | 14.19 | 14.00 | 13.82 | 13.63 | 13.44 | 13.26 | 13.07 | 12.88 | 12.70 | 12.51 | 12.32 |
| **2°C** | 14.01 | 13.82 | 13.64 | 13.46 | 13.28 | 13.10 | 12.92 | 12.73 | 12.55 | 12.37 | 12.19 | 12.01 |
| **3°C** | 13.65 | 13.47 | 13.29 | 13.12 | 12.94 | 12.76 | 12.59 | 12.41 | 12.23 | 12.05 | 11.88 | 11.70 |
| **4°C** | 13.31 | 13.13 | 12.96 | 12.79 | 12.61 | 12.44 | 12.27 | 12.10 | 11.92 | 11.75 | 11.58 | 11.40 |
| **5°C** | 12.97 | 12.81 | 12.64 | 12.47 | 12.30 | 12.13 | 11.96 | 11.80 | 11.63 | 11.46 | 11.29 | 11.12 |
| **6°C** | 12.66 | 12.49 | 12.33 | 12.16 | 12.00 | 11.83 | 11.67 | 11.51 | 11.34 | 11.18 | 11.01 | 10.85 |
| **7°C** | 12.35 | 12.19 | 12.03 | 11.87 | 11.71 | 11.55 | 11.39 | 11.23 | 11.07 | 10.91 | 10.75 | 10.59 |
| **8°C** | 12.05 | 11.90 | 11.74 | 11.58 | 11.43 | 11.27 | 11.11 | 10.96 | 10.80 | 10.65 | 10.49 | 10.33 |
| **9°C** | 11.77 | 11.62 | 11.46 | 11.31 | 11.16 | 11.01 | 10.85 | 10.70 | 10.55 | 10.39 | 10.24 | 10.09 |
| **10°C** | 11.50 | 11.35 | 11.20 | 11.05 | 10.90 | 10.75 | 10.60 | 10.45 | 10.30 | 10.15 | 10.00 | 9.86 |
| **11°C** | 11.24 | 11.09 | 10.94 | 10.80 | 10.65 | 10.51 | 10.36 | 10.21 | 10.07 | 9.92 | 9.78 | 9.63 |
| **12°C** | 10.98 | 10.84 | 10.70 | 10.56 | 10.41 | 10.27 | 10.13 | 9.99 | 9.84 | 9.70 | 9.56 | 9.41 |
| **13°C** | 10.74 | 10.60 | 10.46 | 10.32 | 10.18 | 10.04 | 9.90 | 9.77 | 9.63 | 9.49 | 9.35 | 9.21 |
| **14°C** | 10.51 | 10.37 | 10.24 | 10.10 | 9.96 | 9.83 | 9.69 | 9.55 | 9.42 | 9.28 | 9.14 | 9.01 |
| **15°C** | 10.29 | 10.15 | 10.02 | 9.88 | 9.75 | 9.62 | 9.48 | 9.35 | 9.22 | 9.08 | 8.95 | 8.82 |
| **16°C** | 10.07 | 9.94 | 9.81 | 9.68 | 9.55 | 9.42 | 9.29 | 9.15 | 9.02 | 8.89 | 8.76 | 8.63 |
| **17°C** | 9.86 | 9.74 | 9.61 | 9.48 | 9.35 | 9.22 | 9.10 | 8.97 | 8.84 | 8.71 | 8.58 | 8.45 |
| **18°C** | 9.67 | 9.54 | 9.41 | 9.29 | 9.16 | 9.04 | 8.91 | 8.79 | 8.66 | 8.54 | 8.41 | 8.28 |
| **19°C** | 9.47 | 9.35 | 9.23 | 9.11 | 8.98 | 8.86 | 8.74 | 8.61 | 8.49 | 8.37 | 8.24 | 8.12 |
| **20°C** | 9.29 | 9.17 | 9.05 | 8.93 | 8.81 | 8.69 | 8.57 | 8.45 | 8.33 | 8.20 | 8.08 | 7.96 |
| **21°C** | 9.11 | 9.00 | 8.88 | 8.76 | 8.64 | 8.52 | 8.40 | 8.28 | 8.17 | 8.05 | 7.93 | 7.81 |
| **22°C** | 8.94 | 8.83 | 8.71 | 8.59 | 8.48 | 8.36 | 8.25 | 8.13 | 8.01 | 7.90 | 7.78 | 7.67 |
| **23°C** | 8.78 | 8.66 | 8.55 | 8.44 | 8.32 | 8.21 | 8.09 | 7.98 | 7.87 | 7.75 | 7.64 | 7.52 |
| **24°C** | 8.62 | 8.51 | 8.40 | 8.28 | 8.17 | 8.06 | 7.95 | 7.84 | 7.72 | 7.61 | 7.50 | 7.39 |
| **25°C** | 8.47 | 8.36 | 8.25 | 8.14 | 8.03 | 7.92 | 7.81 | 7.70 | 7.59 | 7.48 | 7.37 | 7.26 |
| **26°C** | 8.32 | 8.21 | 8.10 | 7.99 | 7.89 | 7.78 | 7.67 | 7.56 | 7.45 | 7.35 | 7.24 | 7.13 |
| **27°C** | 8.17 | 8.07 | 7.96 | 7.86 | 7.75 | 7.64 | 7.54 | 7.43 | 7.33 | 7.22 | 7.11 | 7.01 |
| **28°C** | 8.04 | 7.93 | 7.83 | 7.72 | 7.62 | 7.51 | 7.41 | 7.30 | 7.20 | 7.10 | 6.99 | 6.89 |
| **29°C** | 7.90 | 7.80 | 7.69 | 7.59 | 7.49 | 7.39 | 7.28 | 7.18 | 7.08 | 6.98 | 6.87 | 6.77 |
| **30°C** | 7.77 | 7.67 | 7.57 | 7.47 | 7.36 | 7.26 | 7.16 | 7.06 | 6.96 | 6.86 | 6.76 | 6.66 |

| Table 7: Approximate Barometric Pressure at Different Elevations | | | | | |
|---|---|---|---|---|---|
| **Elevation (feet)** | **Pressure (mm Hg)** | **Elevation (feet)** | **Pressure (mm Hg)** | **Elevation (feet)** | **Pressure (mm Hg)** |
| 0 | 760 | 2000 | 708 | 4000 | 659 |
| 250 | 753 | 2250 | 702 | 4250 | 653 |
| 500 | 746 | 2500 | 695 | 4500 | 647 |
| 750 | 739 | 2750 | 689 | 4750 | 641 |
| 1000 | 733 | 3000 | 683 | 5000 | 635 |
| 1250 | 727 | 3250 | 677 | 5250 | 629 |
| 1500 | 720 | 3500 | 671 | 5500 | 624 |
| 1750 | 714 | 3750 | 665 | 5750 | 618 |

teacher info **Primary Productivity**

1. The Dissolved Oxygen Probe must be calibrated the first day of use. Follow the pre-lab procedure to prepare and calibrate the Dissolved Oxygen Probe. To save time, you may wish to calibrate the probe and record the calibration values on paper. The students can then skip the pre-lab procedure and they will have the calibration values available for manual entry in case the values stored in the program are lost. Once the calculator has been set up for a Dissolved Oxygen Probe by the first group, all other groups can begin at Step 1 in the procedure.
2. At the end of class instruct the students to leave the DATAMATE program running. This will keep power going to the probes. The calculator screen may power down, but it will not interrupt power to the probes. When the next group of students come in, they only need to press the ON key on the calculator and begin at Step 1 of the procedure. They can skip the pre-lab procedure because the initial group of students has completed all of the setup. Have the last group of students for the day shut everything off and put things away.
3. During the course of this experiment, the calculator may shut off when not used for a period of time longer than 10 minutes. If the calculator has shut off, have the students examine the screen closely for any sign of activity. Press the ON key once to turn on the calculator if they are sure it has shut off. Pressing the ON key while the calculator is still on will automatically break out of the DATAMATE program.
4. Between classes store the Dissolved Oxygen Probes in a beaker of distilled water. At the end of the day be sure to empty out the electrode filling solution in the Dissolved Oxygen Probes and rinse the inside of the membrane caps with distilled water.
5. This experiment requires two 45-minute periods to complete. There will be time during the second period to discuss the experiment and to begin the follow-up activities.
6. Bottles or test tubes that can form an airtight seal must be used. Air should not be in any of the tubes while they are stored overnight.
7. Either pond water (or some other fresh water source) or a *Chlorella* culture should be used as a water source. You may want to place about 5 mL of *Chlorella* culture into a 1-L sample of water.
8. When setting up the Dissolved Oxygen Probe, be sure to remove the blue plastic cap from the end of the probe. The cap is made of a soft plastic material and easily slides off the probe end.
9. Plastic window screen is available from most hardware stores. The plastic screen is much easier to use than metal screen.
10. An excellent way to dispense the water sample is to use a carboy with a dispensing spout near its bottom. Attach a short length of tubing to the dispensing spout and place a shallow pan below the tube to catch any spill-over. Nalgene makes a variety of plastic carboys that work particularly well.
11. The test tubes should be water-tight when they are stored overnight. Lay the tubes horizontally on a table and lower a fluorescent light about 3 – 6 cm above the tubes. This provides ample light without heating the tubes. If the tubes are stored vertically, non-motile algae may sink to the bottom of the tube and be shielded from the light.

## SAMPLE RESULTS

The following data may be different from students' results. This water sample was taken over a 25-hour period.

Table 3

| Test Tube | % Light | DO (mg/L) |
|---|---|---|
| 1 | Initial | 7.5 |
| 2 | 100% | 7.8 |
| 3 | 65% | 7.6 |
| 4 | 25% | 6.8 |
| 5 | 10% | 6.7 |
| 6 | 2% | 6.5 |
| 7 | Dark | 6.5 |

Table 4

| | |
|---|---|
| Respiration (mg/L/hr) | -0.04 |

Table 5

| Test tube | % Light | Gross productivity (mg/L/hr) | Net productivity (mg/L/hr) |
|---|---|---|---|
| 2 | 100% | 0.052 | -0.012 |
| 3 | 65% | 0.044 | -0.004 |
| 4 | 25% | 0.012 | 0.028 |
| 5 | 10% | 0.008 | 0.032 |
| 6 | 2% | 0 | 0.04 |

## ANSWERS TO QUESTIONS

1. There is evidence that photosynthetic activity added oxygen to the water. The gross primary productivity was positive and greater than zero. The gross primary productivity is a measurement of the total amount of oxygen produced in the water sample, regardless of the amount of aerobic respiration that occurs. The net primary productivity may or may not be a positive value, depending on the BOD due to heterotrophs. If so, this does not mean that photosynthesis did not occur—just that the rate of photosynthesis was less than the rate of aerobic respiration.

2. There is evidence that aerobic respiration occurred in the water, as the respiration rate was negative and non-zero. The dark bottle had less oxygen after one day than the bottle had initially. Both autotrophs and heterotrophs may be responsible for removing oxygen, depending upon the types of organisms in the water sample.

3. Light was necessary for the assimilation of carbon into the ecosystem within the water sample, since light is necessary for photosynthesis.

4. Student answers will vary. According to the sample data, there would be no net productivity at 70% light and no gross productivity at 0% light.

5. The higher the turbidity of the water, the less clear it is. Less light reaches the plants going through photosynthesis, and less oxygen is produced. This results in a decrease in primary productivity.