

AP[®] Biology Laboratory 12

Dissolved Oxygen and Aquatic Primary Productivity

Objectives

- Measure dissolved oxygen in a water sample using the Winkler Method
- Measure primary productivity
- Investigate some factors that can affect the primary productivity of a system

Background to Activity A

Because cellular respiration requires oxygen (see AP[®] Biology Lab 5, Cellular Respiration), the dissolved oxygen (DO) concentration in a body of water is critical to the water's ability to support most forms of aquatic life. Thus, DO is often used as an indicator of water quality. You have probably read or seen news reports of fish kills that have been linked to sewage spillage. Aquatic microorganisms metabolize the sewage, using up DO. As DO levels drop, fish cannot acquire the oxygen they need, and they die.

DO concentration is expressed in parts per million (ppm) or mg/L. One ppm DO is equivalent to 1 mg of oxygen per L of water, so the two are interchangeable. Desirable fish species such as trout and perch require a minimum of 8 mg/L dissolved oxygen to survive. Less-desirable fish such as carp can survive at dissolved oxygen levels as low as 2 mg/L. Below 2 mg/L, only invertebrates such as sludge worms and mosquito larvae can survive.

How does oxygen enter water? One way is by diffusion. Oxygen is more concentrated in air than in water; thus, oxygen diffuses from the atmosphere through the water's surface. (See AP[®] Lab 1, Diffusion and Osmosis.) If both the air and water are static (nonmoving), the concentration of DO falls rapidly with distance from the surface. However, if the water is in motion due to winds, currents, tides, etc., DO can be mixed throughout the water column, increasing the total amount of oxygen dissolved in the water. Other physical factors that might influence DO concentration in a water sample include temperature, pH, and the presence and concentration of solutes. In this activity you will investigate the effect, if any, of temperature on DO.

Activity A: Temperature and Dissolved Oxygen

Materials

3 BOD bottles, gloves, manganous sulfate, starch indicator, sulfamic acid and measuring spoon, alkaline potassium iodide azide, sodium thiosulfate, 2 titration syringes, 2 20-mL sampling vials, waterproof marker, thermometer, 60-mL syringe with tubing attached (optional; see Procedure, 2b).

Caution: Use extreme care when handling chemicals. Sulfamic acid and alkaline potassium iodide azide can irritate or burn the eyes, skin, and mouth. Avoid all skin contact with these and other chemicals. Do not put any chemical in or near your mouth. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.

Introduction

In Activity A, you will investigate the effect of temperature on the concentration of DO and on the ability of water to hold dissolved oxygen. You will begin with a single water sample, divide it into three portions, and let each portion equilibrate at a different temperature. Then you will use the Winkler Method to measure DO for each sample. The Winkler Method is a series of reactions that incorporates and removes from solution the oxygen dissolved in the water and releases free iodine. The amount of free iodine released is proportional to the amount of free dissolved oxygen in the original sample. The amount of free iodine in the sample is then determined by adding a starch indicator solution to the sample, which turns blue in the presence of free iodine, then titrating with sodium thiosulfate to a colorless endpoint. The amount of sodium thiosulfate needed to titrate the iodine is directly proportional to the concentration of dissolved oxygen in the original sample.

Procedure

1. Label 3 BOD bottles, one 4°C, one 25°C, and one 30°C.
2. Fill one BOD bottle with water of the matching temperature. It is important that you do not trap air in the bottle and avoid introducing any turbulence. Improper filling will mix air into the sample and increase the dissolved oxygen level. Consider the following methods:
 - (a) Fill the bottle by submerging it in the sample. Allow the bottle to fill, then cap it while it is still submerged.
 - (b) Use a 60-mL syringe with a piece of tubing attached. (This method works well if the sample container is deep or has a narrow mouth.) Place the end of the tubing at the bottom of an upright BOD bottle and introduce the sample gently. To ensure that there is no air trapped in the bottle to give elevated oxygen readings, fill the bottle until it overflows significantly. Cap the bottle tightly after filling.
3. Determine the DO of the sample by following the Winkler Method Protocol below. Record the DO for that temperature sample in Table 1.
4. Repeat this procedure for each of the other water temperatures.

Winkler Method Protocol

Step 1: Oxygen Fixation

- a. Uncap the BOD bottle.
- b. Add 8 drops of manganous sulfate solution to the bottle.
- c. Add 8 drops of alkaline potassium iodide azide to the bottle.
- d. Cap the bottle and mix. A precipitate will form. Allow the precipitate to settle to the shoulder of the bottle before proceeding.
- e. Use a 1-g spoon to add 1 gram (1 spoonful) of sulfamic acid powder to the bottle.
- f. Cap the bottle and mix until reagent and precipitate dissolve. The sample is now fixed.

Note: *This is an optional stopping point. Samples can be stored at room temperature until you are ready to continue.*

Step 2: Titration

- a. Uncap a BOD bottle and use it to fill the titration sampling vial to the 20 mL line. Be accurate; variations in filling from group to group and from bottle to bottle will result in inconsistent data.
- b. Fill the titration syringe to the top of the scale (1.0 mL) with sodium thiosulfate. Read the volume across the concave edge of the plunger.

- c. Add one drop of sodium thiosulfate at a time to the sample, swirling between each additional drop until the sample becomes a faint yellow color.
- d. Remove the titration syringe and the cap together, without disturbing the syringe. Add 8 drops of starch indicator solution.
- e. Replace the lid of the titration tube and swirl the sample. The solution should turn blue.
Note: If the solution does not turn blue, either there is not a measurable amount of oxygen present, or too much sodium thiosulfate was added in Step 2c. Pour out the sample, refill the titration tube from the BOD bottle, and start the titration again at Step 2b.
- f. Continue the titration with the sodium thiosulfate already in the syringe. Add one drop at a time, swirling the sample after the addition of each drop, until the blue color disappears. If the blue color does not disappear after the addition of the whole syringe of sodium thiosulfate, refill the syringe and continue. When the titration is complete, add the amount from the first syringe to the amount added from the second syringe to get the total amount of sodium thiosulfate used.
- g. Read the syringe at the bottom of the plunger. Each 0.1 mL of sodium thiosulfate used in the titration equals 1 ppm DO, or 1 mg DO per L of water. Record your data in Table 1.

Table 1: Temperature and Dissolved Oxygen

Temperature	Group DO	Class Average DO	Group % Saturation	Class Average % Saturation

Analysis of Results, Activity A: Temperature and Dissolved Oxygen

1. Plot the Class Averages for DO from Table 1. Title the graph and supply the following information:
 - a. The independent variable is _____.
 - b. The dependent variable is _____.

Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

2. Based on your class data, what is the relationship of temperature to DO?

3. Rarely is water saturated with oxygen. Usually, the amount of DO in a water sample is only part of what the water could hold. You can estimate the percent saturation of the water samples with oxygen using the Class Averages for DO from Table 1 and the nomograph shown in Figure 1. Use a pencil to mark the temperature of the water on the top scale of the nomograph and the amount of dissolved oxygen on the bottom scale, then use a straightedge to draw a line connecting the two marks. Read the percent saturation from the middle scale of the nomograph. Record this number in Table 1.

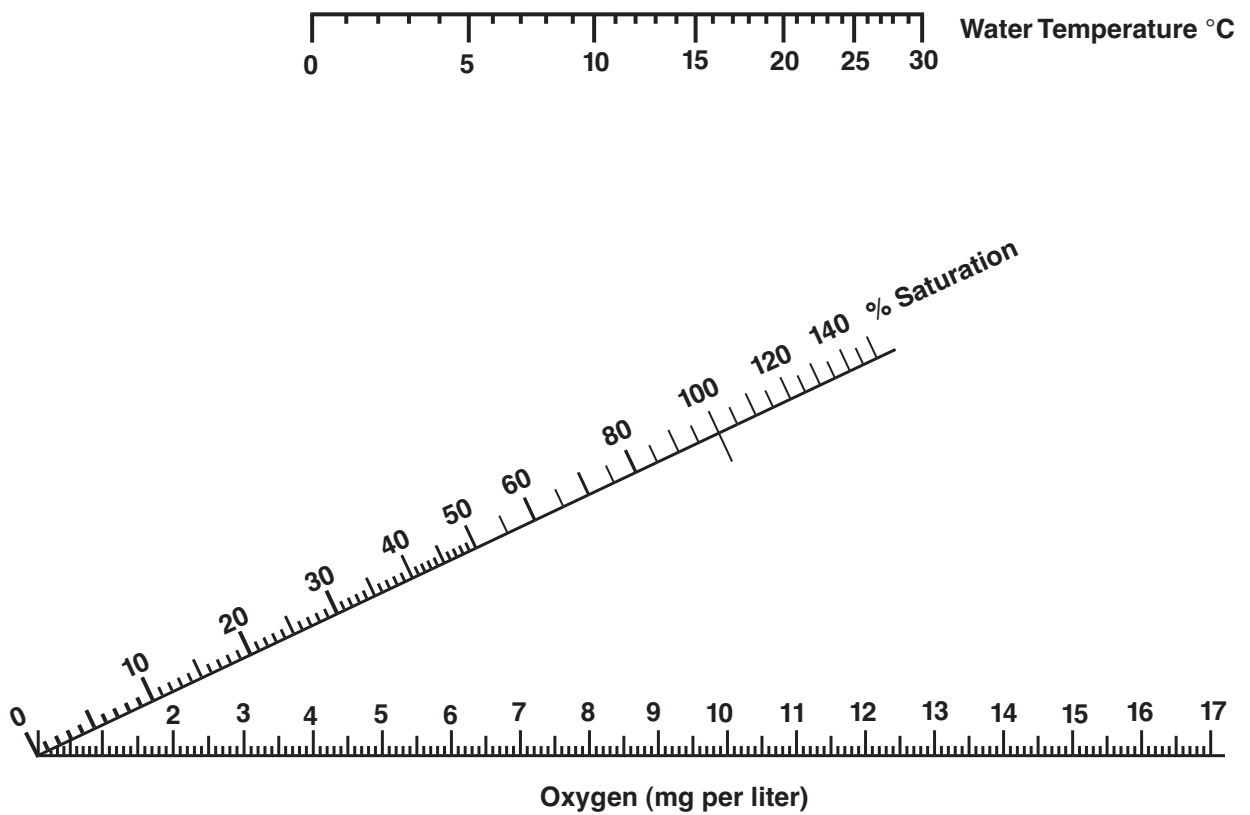


Figure 1: Nomograph of Oxygen Saturation

4. Plot the Class Averages for Percent Saturation from Table 1. Title the graph and supply the following information:

- The independent variable is _____.
- The dependent variable is _____.

Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

5. Based on your data and graphs, what is the relationship of temperature to percent saturation?

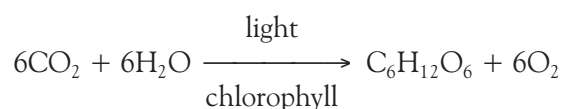
6. What inference can you draw from your answer to #5 that would help explain the relationship of temperature to DO given in your answer to #2?

Activity B: Primary Productivity

Background

Primary productivity is one of the key concepts of ecology. It refers to the rate at which autotrophs (producers) store organic materials. If the primary productivity of an ecosystem is high, the ecosystem can support a large biomass of autotrophs, which in turn will support a substantial (but smaller) biomass of heterotrophs (consumers and decomposers). If primary productivity falls, biomass at all levels must decrease as well. Thus, measuring the primary productivity of an ecosystem, especially if the measurement is repeated over time, can reveal a great deal about what is happening and what will happen in that ecosystem.

In most ecosystems, primary productivity is driven by the rate of photosynthesis in green plants and/or photosynthetic protists (collectively, phototrophs). Recall from AP[®] Biology Lab 4, Plant Pigments and Photosynthesis, the basic equation for photosynthesis:



From this equation, we see that primary productivity can be determined by measuring the rate of carbon dioxide consumption, the rate of formation of organic compounds, or the rate of oxygen production. In an aquatic ecosystem, the oxygen produced by photosynthesis commonly goes into solution, increasing the DO of the water. In this activity, you will use DO levels as a measure of primary productivity.

Determining productivity is complicated by the fact that phototrophs engage in both photosynthesis and cellular respiration. Phototrophs produce oxygen and glucose through photosynthesis, which requires light and carbon dioxide. Phototrophs also use some of the glucose they manufacture as an energy source through respiration, which requires oxygen. Phototrophs respire constantly, but photosynthesize only when light is available. It is therefore necessary to distinguish gross (total) primary productivity from net primary productivity.

You will use a light and dark bottle method to determine the primary productivity of a model pond ecosystem. In this method, two identical samples are incubated, one in light, the other in darkness. At the end of incubation, you will measure the DO of each sample. You will assume that the rate of respiration is the same in both samples. Thus, the sample in the light measures net primary productivity, and the sample in the dark measures the loss due to respiration. Adding the two will give the gross productivity. Most ponds vary in depth. You will simulate samples taken from differing depths of the model pond ecosystem by wrapping sample bottles with screens to block some of the light during incubation.

Materials

7 BOD bottles, *Chlorella* culture, gloves, manganous sulfate, starch indicator, sulfamic acid and measuring spoon, alkaline potassium iodide azide, sodium thiosulfate, 2 titration syringes, 2 20-mL sampling vials, 17 fiberglass screens, square of aluminum foil, waterproof marker, rubber bands, 60-mL syringe with tubing attached (optional; see Activity A Procedure).

Caution: Use extreme care when handling chemicals. Sulfamic acid and alkaline potassium iodide azide can irritate or burn the eyes, skin, and mouth. Avoid all skin contact with these and other chemicals. Do not put any chemical in or near your mouth. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.

Introduction

In this activity, you will measure oxygen production by the photosynthetic protist *Chlorella* under various light intensities, using the light and dark bottle method.

The first step involves measuring the DO of an initial sample of the simulated pond water. This will give you a baseline DO to which you can compare all other measurements of DO for the simulated pond. You will then prepare a light bottle sample and a dark bottle sample. These will be identical except that you will wrap the dark bottle in aluminum foil to block all light. You will simulate samples taken from different depths of a pond by preparing four additional samples, wrapping each bottle with one or more fiberglass screens.

Procedure

Day One

- 1. Determining the Initial (Baseline) DO.** Fill a BOD bottle with water from the model pond (*Chlorella* culture). Use the same procedure for filling that you used in Activity A, and cap the bottle. Determine the DO by following the Winkler Method Protocol. Record your data as the Baseline in Table 2. **Note:** If your sample contains a heavy algae load, the algae will form a black precipitate that will not go into solution. This will not affect your results.
- 2. 100% Light and Dark Bottle Preparation.** Fill two BOD bottles with water from the model pond and cap them. Wrap one bottle, which will be the dark bottle, in aluminum foil to exclude all light. The other bottle will be the 100% light bottle. Label the bottles with your group's name and the appropriate treatment. Lay the bottles on their sides under a fluorescent or grow light, seam side down, and leave them overnight.
- 3. Preparation of Simulated Depth Samples.** Prepare four additional BOD bottles with model pond water. Cover each one with one or more screens according to the table below. Secure the screens with rubber bands. Label the bottles with your group's name and the appropriate treatment. Lay the bottles on their sides under a fluorescent or grow light, seam side down, and leave them overnight. Notice that the light bottle prepared in Step 2 above serves as 100% light.

Number of Screens	Percent Light	Simulated Depth
0 (prepared in Step 2)	100%	0.0 m
1	65%	1.0 m
3	25%	2.0 m
5	10%	3.0 m
8	2%	4.0 m

Day Two

Determine the DO for each of your sample bottles by following the Winkler Method Protocol. Record your data in Table 2. **Note:** If your sample contains a heavy algae load, the algae will form a black precipitate that will not go into solution. This will not affect your results.

Table 2: Group Productivity Data

Bottle	DO	Net Productivity	Gross Productivity
Baseline (Initial)			
Dark			
Light (0 screens)			
1 screen			
3 screens			
5 screens			
8 screens			

Analysis of Results, Activity B: Primary Productivity

1. Calculate the loss of oxygen due to respiration and record here: _____ mg/L

$$R = I - D$$

where

R = loss due to respiration

I = DO baseline

D = DO dark bottle

2. Calculate net productivity of the other samples and record the data in Table 2.

$$P_n = L - I$$

where

P_n = net productivity

I = DO baseline

L = DO sample

3. Calculate gross productivity of each sample and record in Table 2.

$$P_g = P_n + R$$

where

P_g = gross productivity

P_n = net productivity

R = loss due to respiration

4. Determine class averages for net and gross productivities. Record the data in Table 3.

Table 3: Class Averages for Gross and Net Productivities

Bottle	Average Net Productivity	Average Gross Productivity
Light (0 screens)		
1 screen		
3 screens		
5 screens		
8 screens		

5. Graph the data for Average Gross and Average Net productivities from Table 3. Title the graph and supply the following information:

- a. The independent variable is _____.
- b. The dependent variables are _____.

Plot the independent variable on the x-axis, and the dependent variables on the y-axis.

6. From your graph:

- a. At approximately what light intensity does the rate of respiration equal the rate of photosynthesis?

- b. At approximately what depth in the simulated pond does this occur?

7. Two researchers, one at Toolik Field Station in northern Alaska and the other at La Selva Biological Station in Costa Rica, are studying populations of aquatic arthropods in freshwater pools during July. The researcher in Alaska determines an average of 280 arthropods per m³ of water in the pools she is studying. The researcher in Costa Rica determines an average of 125 arthropods per m³ at his study site. In terms of the current lab, how would you account for this difference?

8. You are the Water Quality Director for Derry County. In this capacity, you are asked to review reports of two fish kills in the county. Both involve artificial ponds with surface areas of approximately 0.8 hectares and maximum depths of 7 meters. Tests performed immediately after the fish kills detected no pesticides or other poisons. The dead fish showed no signs of fungal attacks or other disease. Case A involves a pond stocked with bass and used for recreational fishing. Meteorological records show that the kill occurred after 4 weeks of hot weather in which daytime temperatures reached 35–40°C. Case B involves a pond stocked with bluegill and used to irrigate pastureland. This kill occurred in the spring, before the heat wave and 9 days after a heavy rain. The file for Case B contains a photo showing dead fish floating in the pond. You also notice what appear to be mats of decaying algae floating on the surface of the water. A call to the farmer reveals that he applied ammonium nitrate to the pastureland the week before the rain. State your judgment as to the probable causes of these fish kills, and describe the chain of events that led to each.

Title: _____

