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Catalog Nos. FB1853, FB1854

Publication No. 10804

Dissolved Oxygen Laboratory Kit

Advanced Placement Biology Lab 12

Introduction

Aerobic organisms, including bacteria, algae, plants, and animals, all require oxygen to survive. If the environment surrounding an aerobic organism becomes oxygen-deprived, the organism must relocate to an oxygen-rich environment or eventually it will die. How much oxygen does an organism need and what type of oxygen is necessary for survival?

Biological Concepts

- Biological (Biochemical) Oxygen Demand (BOD)
- Dissolved Oxygen (DO)
- Gross Primary Productivity (GPP)
- Net Primary Productivity (NPP)
- Respiration rate

Objectives

After doing this laboratory, you should be able to:

- Measure gross primary productivity based on changes in dissolved oxygen in a controlled experiment.
- Investigate the effects of changing light intensity on gross primary productivity in a controlled experiment.

Background

Dissolved Oxygen

Oxygen in its free state (O_2), where it is not bound in compounds, is required for respiration in all aerobic organisms. The atmosphere contains an abundance of oxygen in its free state. The concentration of O_2 in air is about 200 milligrams (mg) of oxygen for every liter (L) of air, which is equivalent to 200 parts per million (ppm) of oxygen. In the special case of aquatic organisms, the free-state oxygen must be dissolved in the water in order for the oxygen to either diffuse through the external cells or through the gill cells of the organism. In a healthy body of water, there is typically 5 to 10 mg of free-state oxygen dissolved in each liter of water, or 5–10 ppm *dissolved oxygen* (DO). If the body of water has less than 2 ppm dissolved oxygen, it is considered "dead" since no fish can survive. The amount of dissolved oxygen in a body of water is an indicator of the health or quality of the body of water.

The relationship between the amount of dissolved oxygen in water and water quality can also be expressed in terms of percent oxygen saturation. At any given temperature, water can hold a certain maximum amount of oxygen. The percent saturation refers to what percent of the maximum amount possible is contained within the water. Turbulent water often contains greater than 100% oxygen saturation. Rivers that have oxygen saturation levels between 90% and 110% are considered healthy. Water less than 90% saturated may contain large amounts of oxygen-demanding organic material. The graph shown in Figure 1 is called a *nomogram*. It is used

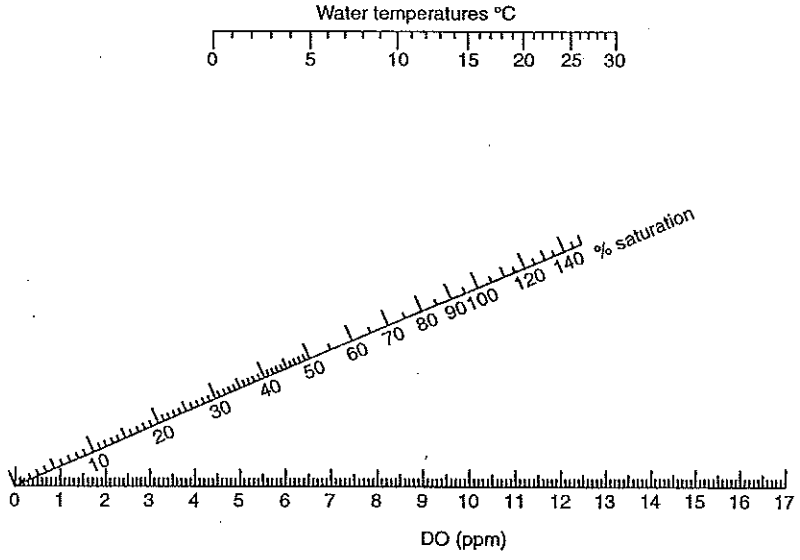


Figure 1.

to determine percent oxygen saturation in the water at sea level based on the concentration of dissolved oxygen in the water at a specific temperature. *Example:* Water containing 9.0 ppm DO at a temperature of 12 °C is about 80% saturated.

The quantity and distribution of dissolved oxygen in water depends on several biochemical and physical factors. The major biochemical factor that adds oxygen to water is photosynthesis. Oxygen is a by-product of photosynthesis, thus photosynthesis is a significant source of dissolved oxygen in water. The amount of oxygen is highest in the afternoon after a full day of photosynthesis. One physical factor that adds oxygen to water is diffusion. Oxygen diffuses from the oxygen-rich air into the water. As described above, turbulence in the water will also cause the oxygen level of the water to increase—this is called *aeration.* Slow moving or stagnant water has lower oxygen levels due to a lack of aeration. Turbulence, tides, currents, and winds are physical factors that contribute to the mixing or distribution of oxygen in water.

Several biochemical and physical factors also cause the amount of dissolved oxygen to vary. Temperature inversely affects dissolved oxygen—as the water temperature increases, the amount of oxygen that can dissolve decreases (see Figure 2). In the summer, extremely warm water temperatures may result in very low dissolved oxygen. The *partial pressure* of oxygen in the air above the water affects the amount of DO in the water. Less oxygen is present at higher elevations since the air itself is less dense and therefore, water at higher elevations contains less oxygen. At 4,000 meters in elevation (about 13,000 feet), the amount of dissolved oxygen in water is less than two-thirds what it is at sea level.

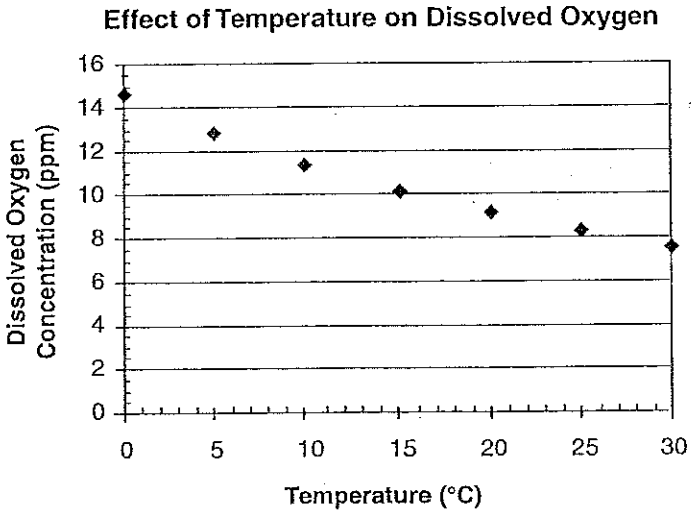


Figure 2.

Salinity also inversely affects dissolved oxygen—as the amount of salinity increases, the amount of dissolved oxygen decreases. *Salinity* is the amount of salts dissolved in the water. Salinity is usually expressed as total grams of dissolved salts in one kilogram of water or parts per thousand (ppt). On the average, salinity in freshwater is 0.5 ppt, in brackish water is 0.5 to 17 ppt, in seawater is 18 to 37 ppt, and in hypersaline water is 100 to 666 ppt. For example, the upper level of the Dead Sea has a salinity of 210 to 300 ppt. The high salinity and the low dissolved oxygen in the Dead Sea have resulted in a body of water that is only inhabited by some bacteria and fungi.

Saprophytes are microorganisms such as bacteria and fungi, that decompose organic waste. When organic matter originating from dead plants, sewage, and dead animals are present in water, saprophytes derive energy and nutrients by breaking down the organic matter into usable forms of carbon, nitrogen and sulfur. The amount of organic waste present in water is directly proportional to the amount of decomposers in the water. Aerobic decomposers use cellular respiration to break the waste material apart. Cellular respiration uses the dissolved oxygen present in the water. Consequently, large amounts of decomposers *decrease* the amount of dissolved oxygen in the water.

Primary Productivity

The amount of light energy converted to chemical energy (organic compounds) by autotrophs during a given time period is the ecosystem's *gross primary productivity* (GPP). Only about 1% of visible light that reaches the Earth's surface is converted to chemical energy by photosynthesis, the rest is absorbed or reflected by bare ground or bodies of water. Of that 1%, autotrophs use some of that energy for their own cellular respiration needs, so the GPP is not a true measure of the amount of organic material stored by autotrophs and therefore available to heterotrophs. The amount of chemical energy stored by autotrophs as new organic compounds, and therefore available for heterotrophs to consume, in a given time is the *net primary productivity* (NPP). The estimated GPP for land autotrophs is 50 to 70 billion tonnes (metric tons) of carbon annually. The estimated GPP for marine ecosystems is 35 to 50 billion tonnes of carbon annually. Since scientists are usually interested in the primary productivity within a specific ecosystem, terrestrial GPP and NPP are generally reported in units of grams of carbon per square meter of area per day or $gC/m^2/day$. Aquatic GPP and NPP are generally reported in grams of carbon per liter of water per day or gC/L/day.

The amount of new organic compounds produced is restricted by limiting factors. *Limiting factors* are defined as physical, chemical or biological factors that are either too abundant or to scarce to support more life. In some areas the amount of sunlight that reaches the area is the limiting factor. In the *photic zone* (upper layer) of water, the amount of soluble nutrients such as nitrogen, phosphorus or iron is often the limiting factor. In the ocean, single-celled autotrophs called phytoplankton produce between

92 and 96% of the chemical energy in the ocean. The primary productivity of phytoplankton is restricted by limited nutrients, not by limited amounts of carbon dioxide, water or sunlight. Each phytoplankton captures soluble nutrients to create new usable organic material (called biomass) but when it dies it sinks. The dead phytoplankton takes vital nutrients to the bottom of the ocean, leaving the photic zone nutrient-deprived.

If mixing occurs, so that water from the bottom, nutrient-rich zone mixes into the upper, nutrient-depleted zone, a harmful algae bloom (HAB) will occur. This mixing occurs each spring and fall in lakes and ponds when the change in weather causes "turnover" and a thick bright green layer of algae (the algal bloom) covers the body of water. Harmful algae blooms also occur when fertilizer "run off" from fields or chemicals discharged from water treatment facilities add excess nitrogen and phosphorus to the water. The excess nutrients permit explosive growth to occur in algae which continues until either the nutrients or the dissolved oxygen are used up. If the dissolved oxygen becomes depleted, the heterotrophs will also die. A similar process also occurs in the ocean, where an increase in the growth of phytoplankton gives rise to HABs called "red tides."

Winkler Test

The *Winkler test* is one type of test that can be used to determine the level of dissolved oxygen in *freshwater* samples. The method was first developed by Lajos Winkler (1863–1939) while working on his doctoral dissertation in 1888, and it is still considered to be the most sensitive and accurate method available. The Winkler method involves three basic steps:

1. Manganese sulfate and a basic potassium iodide solution are added to convert the dissolved oxygen to an insoluble manganese–oxygen complex, which then precipitates out of the solution. This step "fixes" the dissolved oxygen and prevents the oxygen from being consumed or reacting with other substances. Both the manganese sulfate and iodide solutions are added in excess to ensure that all of the oxygen has been sequestered. These solutions should be added as soon as possible, preferably in the field, immediately after a water sample is collected.
2. Concentrated sulfuric acid is added to lower the pH of the solution and to dissolve the manganese–oxygen complex. The dissolved manganese–oxygen complex then reacts with iodide ions to generate iodine, which turns the solution a golden yellow color.
3. The iodine released in this reaction is titrated using a standard sodium thiosulfate solution with starch as an indicator, which turns the solution a dark blue (to make the end point more visible). The endpoint will be colorless.

The *respiration rate* is the amount of cellular respiration that occurs in a liter of freshwater within a specified amount of time such as one hour, one day or one week. To calculate the respiration rate, the dissolved oxygen test is conducted twice and the results are subtracted (see Equation 1). The first DO test is conducted immediately after the sample is collected, while the second DO test is conducted after a specific amount of time. The second water sample is incubated in the dark for the specified amount of time. Because photosynthesis is inhibited in the dark in the second sample, the autotrophs are not able to add any new dissolved oxygen to the water sample. Respiration occurs, however, as bacteria decompose any organic waste in the water. The difference between the initial DO level and the level remaining in the second sample is the respiration rate—the amount of oxygen consumed in a given time period as a result of biological activity (respiration). Respiration rate is usually reported in parts per million (ppm) which is equivalent to number of milligrams of dissolved oxygen per liter of water (mg O_2/L H_2O).

$$\text{One Day Respiration Rate (ppm)} = DO_{Initial} - DO_{Dark} \qquad \textit{Equation 1}$$

After determining the respiration rate of a sample the amount of carbon fixed in the sample may be calculated using the following equations. If the samples were collected and analyzed under standard pressure (1 atm) at room temperature (25 °C), the number of milliliters of dissolved oxygen per liter of water can be calculated using Equation 2. Finally, the amount of carbon fixed in photosynthesis can be calculated using Equation 3.

$$\text{Volume of oxygen per liter of water } \frac{\text{mL } O_2}{\text{L } H_2O} = \text{ppm} \times 0.764 \frac{\text{mL } O_2}{\text{mg } O_2} \qquad \textit{Equation 2}$$

$$\text{Amount of carbon fixed per liter of water } \frac{\text{mg } C_{fixed}}{\text{L } H_2O} = \frac{\text{mL } O_2}{\text{L } H_2O} \times 0.536 \frac{\text{mg } C_{fixed}}{\text{mL } O_2} \qquad \textit{Equation 3}$$

The *biological (biochemical) oxygen demand* (BOD) is a measure of the oxygen used by microorganisms to decompose the organic waste in a sample of water kept at 20 °C over a 5-day period. The BOD test is similar to the respiration rate except that the BOD is defined as a five-day test. BOD levels *increase* as water becomes more polluted and the dissolved oxygen levels decrease. BOD levels of 1–2 ppm are considered very good and are typical of clear water. BOD levels of 3–5 ppm are indicative of moderately clean water quality. BOD levels of 6–9 ppm are considered poor and somewhat polluted. BOD levels greater than 100 ppm are indicative of very poor, polluted water. For reference, the influent coming into most wastewater treatment plants is about 200 ppm.

Poor water quality is due to an abundance of saprophytes that decompose an overabundance of organic waste in the water and use up the dissolved oxygen in the water in the process. A lack of dissolved oxygen will lead to the death of many organisms, especially those that are very sensitive to oxygen levels in the water. Sensitive organisms include trout, salmon, and many species of macroinvertebrates. Some species are able to survive in the oxygen-depleted water and they may even thrive if their competitors or predators are no longer present. Tolerant organisms include carp, midge larvae, leeches, and sludge worms. Biologists survey the aquatic species present in lakes, rivers, and streams as a measure of water quality.

Experiment Overview

In Activity 1 the amount of dissolved oxygen is determined for water samples at three separate temperatures. In Activity 2, field water samples are collected and the respiration rate, gross primary productivity, and net primary productivity values are determined using the Winkler method.

Activity 1. Temperature Effect on DO

Materials

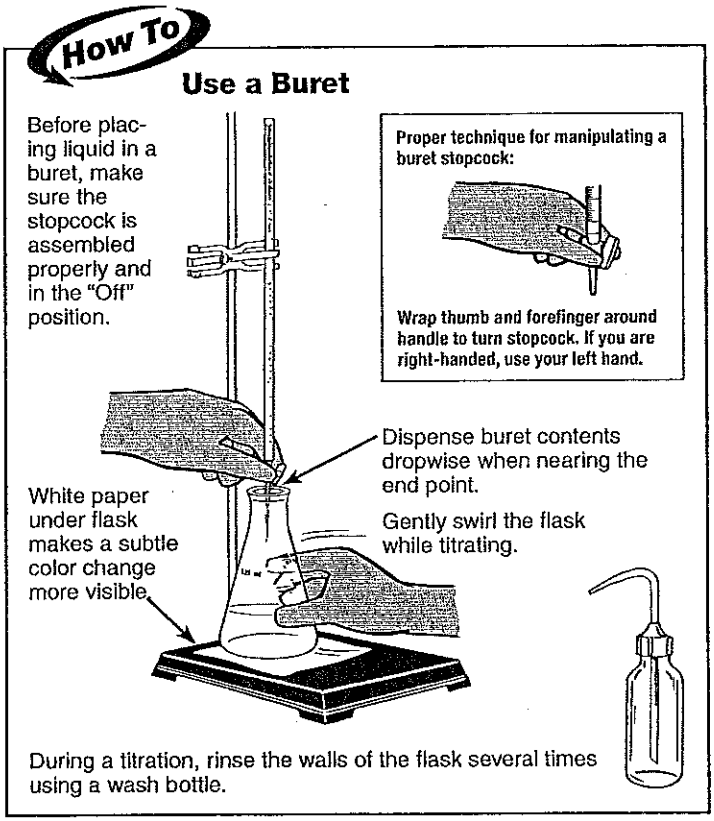
Buret
Clamp, test tube
Cups, clear plastic, 2
Graduated cylinder, 25-mL
Marker or wax pencil
Paper, white
Pipet, graduated, disposable
Sodium thiosulfate solution, 0.0025 M, 30 mL
Starch solution, 5%, 1 mL
Stoppers, solid rubber, #2, 3
Sulfuric acid, concentrated, 1 mL
Support stand
Test tube rack
Test tubes, large (20 mm × 150 mm), 3
Water sample, cold (≈5 °C), 40 mL
Water sample, room temperature (≈20 °C), 40 mL
Water sample, warm (≈30 °C), 40 mL
Winkler solution #1, 1 mL
Winkler solution #2, 1 mL

Safety Precautions

Sulfuric acid is extremely corrosive to skin, eyes and other tissues. Winkler solution #2 contains sodium hydroxide and potassium iodide—it is a concentrated base solution and is caustic and severely corrosive. Concentrated sodium hydroxide solutions are especially dangerous to the eyes. Sodium thiosulfate is a body tissue irritant. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all normal laboratory guidelines.

Procedure

1. Use a marker or wax pencil to label each of the three test tubes with your group name and one of the following sample labels: cold, room, and warm.
2. Record the temperature (°C) of the cold water sample in Table 1 on the Temperature Worksheet.
3. Carefully immerse a large test tube in the cold water sample, taking care not to agitate the water (which would add oxygen to the sample). Ensure there are no air bubbles in the test tube. *Note:* Air bubbles would add oxygen to the sample causing inaccurate results.



4. Dip a rubber stopper into the cold water sample to wet it. While the test tube is still under water, stopper it. Ensure there are no air bubbles in the test tube.
5. Remove the stoppered test tube from the cold water sample and place it into a test tube rack
6. Remove the stopper and, using the tip of the dropper bottle, quickly add 6 drops of Winkler solution #1 directly to the water in the test tube, holding the tip of the dropper bottle as close to the water surface as possible.
7. Carefully add 6 drops of Winkler solution #2 directly to the water in the test tube, again holding the tip of the dropper bottle as close to the water surface as possible. *Caution:* Winkler solution #2 is a concentrated base solution and is caustic and corrosive.

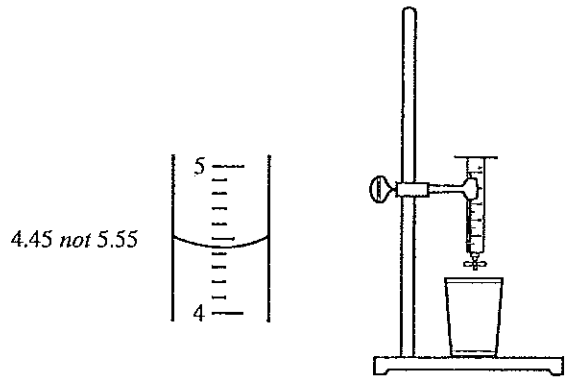


Figure 3.

8. Stopper the test tube (some liquid will overflow) being careful not to add any air bubbles to the sample. *Note:* Air bubbles will add oxygen to the test sample.
9. Invert the stoppered test tube several times to mix the contents—a brown precipitate will quickly form. This step fixes the dissolved oxygen in the water. Fixed samples can be held up to one week. Allow the brown precipitate to settle to at least one-half the volume of the test tube (about 10–15 minutes).
10. Repeat steps 1 to 9 two more times using the room temperature and the warm water samples.
11. At a central location, remove the rubber stopper and carefully add 6 drops of concentrated sulfuric acid using a glass medicine dropper to each test tube. *Caution:* Concentrated sulfuric acid is extremely corrosive. In the event of a spill, notify the teacher immediately.
12. Replace the stopper in the test tube and invert it several times to mix. The acid should cause the precipitate to dissolve, giving a clear amber (yellow-gold) solution.
13. Titrate the fixed water sample as follows:
    a. Use a test tube clamp to clamp the buret to a support stand (see Figure 3). Place a waste cup beneath the buret.
    b. Adjust the clamp so the buret tip is about 1 cm above the top of the waste cup.
    c. Fill the buret with 10 mL of 0.0025 M sodium thiosulfate solution using a clean, disposable pipet.
    d. Slowly allow a few drops of sodium thiosulfate solution to drip into the waste cup below the buret. This removes any air bubbles in the buret tip.
    e. Record the initial volume of sodium thiosulfate solution in the buret in Table 2 of the Temperature Worksheet. Read from the bottom of the meniscus each time (see Figure 3).
    f. Using a 25-mL graduated cylinder, pour 20.0 mL of the fixed water sample from the cold water test tube into the clear plastic cup. Transfer the top solution only—do not transfer any precipitate that remains in the test tube.
    g. Place the cup containing the water sample on a piece of white paper under the buret on the support stand.
    h. Use the buret to add sodium thiosulfate one drop at a time to the sample.
    i. Gently swirl the sample in the cup after adding each drop.
    j. Continue to add one drop at a time until the water fades to a pale yellow color.
    k. Use a graduated pipet to add 6 drops of starch solution to the treated water in the cup and swirl to mix. The sample will turn dark blue.
    l. Continue adding sodium thiosulfate solution dropwise from the buret, swirling the sample cup after adding each drop, until the blue color fades completely. This is the colorless end point.
    m. Record the final buret reading in Table 2 on the Dissolved Oxygen Worksheet.
    n. Calculate the volume of sodium thiosulfate added by subtracting the final volume of sodium thiosulfate from the initial volume of sodium thiosulfate. Record the value in Table 2 on the Temperature Worksheet.

o. Record the dissolved oxygen concentration in Table 2 on the Temperature Worksheet. *Note:* For 20.0 mL of water, the milliliters (mL) of 0.0025 M sodium thiosulfate added equals the dissolved oxygen concentration in parts per million (ppm).

p. Discard the titrated sample into a waste bottle as directed by your instructor.

q. Rinse the 25-mL graduated cylinder and the clear plastic cup thoroughly with distilled water.

r. Repeat steps *a–q* twice more for the room temperature water sample and the warm water sample.

14. On the nomogram of oxygen saturation (see Figure 1), use a ruler to estimate the percent saturation of DO in each water sample. Line up the edge of the ruler with the temperature of the water on the top scale and the measured DO on the bottom scale, and then read the percent saturation from the middle scale.
15. Record the percent saturation for each water sample in Table 2 on the Temperature Worksheet.
16. Answer the questions on the Temperature Worksheet.

Activity 2. Primary Productivity

Materials

Aluminum foil
Beakers, borosilicate, 100-mL, 4
Buret
Clamp, test tube
Cups, clear plastic, 2
Light source
Marker or wax pencil
Meter stick
Paper, white
Sodium thiosulfate solution, 0.0025 M, 70 mL
Starch solution, 5%, 2–3 mL
Stoppers, solid rubber #2, 6
Sulfuric acid, 18 M, 2 mL
Support stand
Test tube rack
Test tubes, large (20 mm × 150 mm), 6
Field water sample, 240 mL
Winkler solution #1, 2–3 mL
Winkler solution #2, 2–3 mL

Safety Precautions

Sulfuric acid is extremely corrosive to skin and eyes and other tissues. Winkler solution #2 contains sodium hydroxide and potassium iodide—it is a concentrated base solution and is caustic and severely corrosive. Concentrated sodium hydroxide solutions are especially dangerous to the eyes. Sodium thiosulfate is a body-tissue irritant. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Follow all normal laboratory guidelines.

Procedure

1. Use a marker or wax pencil to label along the top edge of six large test tubes with your group name and one of the following sample labels: initial, dark, 50 cm, 75 cm, 100 cm, and 125 cm.
2. Fill the four 100-mL borosilicate beakers ¾ full with tap water. *Note:* The water acts as a heat sink to make certain the samples remain at a moderate temperature overnight.
3. Carefully immerse the "initial" test tube in the field water sample container. Ensure there are no air bubbles in the test tube.
4. Dip a rubber stopper into the field water sample to wet it. While the test tube is still under water, stopper it.
5. Remove the stoppered test tube from the field water sample container and place it into a test tube rack.
6. Remove the stopper and, using the tip of the dropper bottle, quickly add 6 drops of Winkler solution #1 directly to the water in the test tube, holding the tip of the dropper bottle as close to the water surface as possible.
7. Carefully add 6 drops of Winkler solution #2 directly to the water in the test tube, again holding the tip of the dropper bottle as close to the water surface as possible.
8. Stopper the test tube (some liquid will overflow) being careful not to add any air bubbles to the sample.

9. Invert the stoppered test tube several times to mix the contents—a brown precipitate will form. This step fixes the dissolved oxygen in the water. Fixed samples can be held up to one week.
10. Repeat steps 3 to 5 for the remaining five test tubes. *Note:* Do not "fix" the remaining samples yet.
11. Cover the "dark" test tube with aluminum foil so that no light can enter. Immediately place the "dark" test tube in a dark location such as a cupboard or drawer as directed by the teacher. No photosynthesis can occur in the "dark" test tube, so the only thing that will change the amount of dissolved oxygen will be the process of respiration by all of the organisms present. It will remain in the dark overnight.
12. The reduction in the amount of natural light that occurs due to depth in a body of water will be simulated by placing the samples at specific distances from the light source (see Figure 4).
    a. Use the meter stick to measure and place one of the four beakers 50 cm from the light source and to one side from "center" of the light.
    b. Place the second beaker 75 cm from the light source and just to one side of the first beaker.
    c. Place the third beaker 100 cm from the light source and just to one side of the second beaker.
    d. Place the fourth beaker 125 cm from the light source and just to one side of the third beaker.

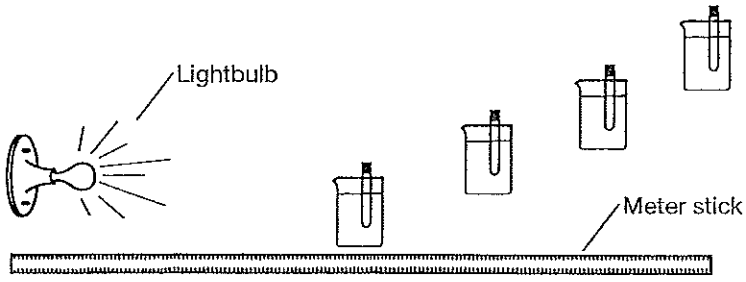


Figure 4.

13. Place one test tube into each beaker. Be sure that no test tube is in the "shadow" of another beaker by viewing the test tubes while standing behind the light. Adjust the beakers' positions as needed.
14. Turn off *all* other lights, leaving the test tubes in the path of the light overnight.
15. On the following day, gather the dark test tube and the simulated depth test tubes and use steps 6 through 9 to fix the samples. Allow the brown precipitate to settle to at least one-half the volume of the test tube.
17. Place the initial test tube with the dark test tube and the simulated depth test tubes.
18. At a central location, remove the rubber stopper and carefully add 6 drops of concentrated sulfuric acid, using a glass medicine dropper, to each test tube.
19. Replace the stopper in the test tube and invert it several times to mix. The acid should cause the precipitate to dissolve, giving a clear amber (yellow-gold) solution.
20. Titrate the fixed water samples using the procedure outlined in step 13 of Activity 1.
21. Calculate the respiration rate for one day using Equation 1. Record the one-day respiration rate as question 1 on the Primary Productivity Worksheet.
22. Calculate the gross primary productivity for each simulated depth sample using Equation 4:

$$\text{Gross Primary Productivity} = DO_{depth} - DO_{dark} \qquad \textit{Equation 4}$$

23. Record the gross primary productivity value for each simulated depth sample in Table 2 on the Primary Productivity Worksheet.
24. Calculate the net primary productivity for each simulated depth sample using Equation 5:

$$\text{Net Primary Productivity} = DO_{depth} - DO_{initial} \qquad \textit{Equation 5}$$

25. Record the net primary productivity value for each simulated depth sample in Table 2 on the Primary Productivity Worksheet.
26. Use Equation 2 to calculate the volume of oxygen per liter of water for each simulated depth sample. Record the results in Table 2 on the Primary Productivity Worksheet.
27. Use Equation 3 to calculate the amount of carbon fixed per liter of water for each simulated depth sample. Record the result in Table 2 on the Primary Productivity Worksheet.
28. Answer the questions on the Primary Productivity Worksheet.

Disposal

Consult your instructor for appropriate disposal procedures.

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Name: _____

Temperature Worksheet

Observations and Analysis

Table 1.	Cold Water	Room Temperature Water	Warm Water
Temperature (°C)			

Table 2.	Cold Water	Room Temperature Water	Warm Water
Initial Buret Volume (mL)			
Final Buret Volume (mL)			
Volume of Sodium Thiosulfate (mL)			
Concentration of DO (ppm)			
Oxygen Saturation (%)			

Questions

1. Use graph paper to plot the dissolved oxygen concentration as a function of temperature for the cold, room temperature, and warm water samples.
   a. The independent variable.
   b. The dependent variable.
2. For water that is 100% saturated, describe the relationship between water temperature and the amount of dissolved oxygen.

3. On a sunny day, at what time of day is the concentration of dissolved oxygen the highest? Explain.

4. Would the DO of water taken from a stream where it enters a lake be higher or lower than the DO of water taken from mid-depth of the lake? Explain.

Name: _____

Primary Productivity Worksheet

Observations and Analysis

Table 1.

	Initial Water Sample	Dark Water Sample	50 cm Depth	75 cm Depth	100 cm Depth	125 cm Depth
Initial Buret Volume (mL)						
Final Buret Volume (mL)						
Volume of Sodium Thiosulfate (mL)						
Concentration of DO (ppm)						

Table 2.

	50 cm Depth	75 cm Depth	100 cm Depth	125 cm Depth
Gross Primary Productivity (Equation 4)				
Net Primary Productivity (Equation 5)				
Volume of Oxygen per L H_2O (Equation 2)				
Amount of carbon fixed per L H_2O (Equation 3)				

Questions

1. What is the one-day respiration rate for the field samples? (Equation 1)

2. Use graph paper to plot the net primary productivity versus simulated depth and the gross primary productivity versus simulated depth.

   a. The independent variable.

   b. The dependent variable.

3. What is the relationship between oxygen production and assimilation of carbon?

4. Refer to the productivity graph. At what simulated depth did respiration approximately equal productivity? (*Hint:* The point at which there is no net productivity.)

5. A mammal uses only 1 to 2 percent of its energy to breathe, while a fish uses about 15% of its energy to move water over its gills. Explain why a fish must expend more energy than a mammal to breathe.