EDVOTEK®

THE **BIOTECHNOLOGY EDUCATION** COMPANY®

AP ENVIRONMENTAL

Edvo-Kit # 292

Edvo-Kit #292

Dissolved Oxygen and Aquatic Primary Productivity

Experiment Objective:

Dissolved oxygen levels are used to monitor the health and productivity of aquatic ecosystems. In this kit, students familiarize themselves with the Winkler method by examining the relationship between temperature and dissolved oxygen concentrations. They then use local water samples to examine primary productivity at different water depths. Finally, they will observe the beginning stages of eutrophication in their water samples.

See page 3 for storage instructions.

Table of Contents

Safety Data Sheets can be found on our website: **www.edvotek.com/safety-data-sheets**

The Advanced Placement (AP) Program is a registered trademark of the College Entrance Examination Board.
These laboratory materials have been prepared by EDVOTEK, Inc. which bears sole responsibility for their contents.

Experiment Components

Component	Check (✓)
Store all components below at room temperature.	
A Sodium iodide	❑
B Sodium hydroxide	❑
C Manganese sulfate	❑
D Starch solution	❑
E Sodium thiosulfate	❑
F Nitrogen enrichment solution	❑
G Phosphorous enrichment reagent	❑

Experiment #292 contains reagents for 10 groups.

All experiment components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

REAGENTS & SUPPLIES

Store all components below at room temperature.

- Transfer pipets ❑
- Microcentrifuge tubes ❑
- 15 ml conical tubes ❑

Requirements *(not included with this kit)*

- Tap water
- Local water samples or *Chlorella* spiked water
- Light source
- BOD bottles
- Burettes
- Flasks or beakers (at least 500 ml)
- Graduated cylinder (at least 200 ml)
- Distilled water
- Thermometers
- Screens
- Rubber bands
- Goggles and gloves
- Sulfuric acid, concentrated (98%+ or 18 mol/L+)
- Acid spill clean up material
- Glass pipet
- Pipet pump
- Filter paper (#1 Whatman)
- Aluminum foil
- Waterproof markers
- Linear graph paper or computer with spreadsheet program
- Microscopes, microscope slides, and cover slips (optional)

Background Information

A skillful limnologist can probably learn more about the nature of a lake from a series of oxygen determinations than from any other kind of chemical data.

— George Evelyn Hutchinson (1957)

DISSOLVED OXYGEN

If you've ever been high above sea level, you've experienced the detrimental effects of low oxygen absorption. Altitude sickness can cause headaches, dizziness, nausea, fatigue, and most seriously pulmonary and cerebral edema. This is because humans — as well as most other life forms — use oxygen as electron receptors when converting nutrients (such as sugar and amino acids) into ATP (the energy currency of cells). And while certain sulfates and nitrates can also act as electron receptors during respiration this anaerobic process is far less efficient and often produces toxic by-products. Luckily for us, O_2 makes up 20.9% of the air we breathe (although we can have trouble absorbing it into our blood above 5,000 ft). In contrast, O_2 concentrations in water environments rarely exceed 4% and can easily drop to near zero levels. This means that for many aquatic organisms oxygen plays a fundamental role in determining where they can and cannot live. Consequently the dissolved oxygen level can be a telling measure of a water system's health.

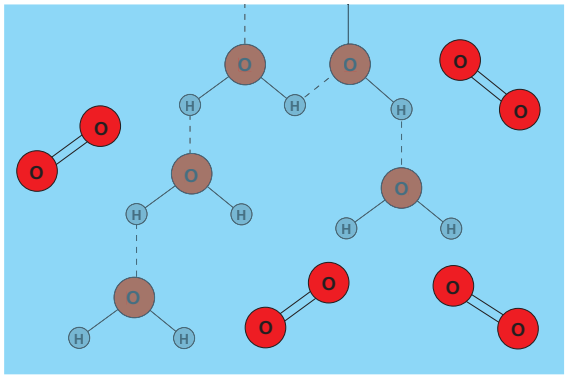


Figure 1: Dissolved oxygen molecules in water.

Although water contains an oxygen atom, it is inaccessible to aquatic life — aquatic organisms can only use molecular oxygen dissolved in the water (Figure 1). Most dissolved molecular oxygen (DO) enters the aquatic system through two processes: atmospheric O_2 diffuses into the water at the surface, and aquatic plants and phytoplankton release O_2 as a by-product of photosynthesis. DO is lost from water when it is consumed during respiration or decomposition. In addition, chemical processes like nitrification and sulfide oxidation also consume small amounts of molecular oxygen. Finally, water can only hold small amounts of DO and additional molecules of oxygen will eventually diffuse back into the atmosphere. The maximum DO concentration that water can hold varies depending on several abiotic factors including temperature, atmospheric pressure, hydrostatic pressure, and salinity. At 20° C and at sea level the maximum amount of dissolved oxygen in fresh water is 9.03 mg/L.

Because DO concentrations are driven by constantly changing biotic and abiotic factors (photosynthesis and respiration, water currents, surface winds, temperature, etc.), they fluctuate over time. For example, DO levels increase during the daylight because of higher photosynthesis rates and then decline overnight when only respiration is occurring. Another temporal pattern is known as winterkill, which occurs when snow and ice cover a lake to block oxygen exchange with the air. This lack of aeration combined with lower photosynthesis rates lead to a decline in dissolved oxygen levels and death of fish with high oxygen requirements such as trout. DO levels also vary by geographic location and by depth. In lakes, DO levels are high at the surface but can drop to zero at their deepest parts due to declining light and temperature — although internal mixing can counteract this process. Fast moving streams often have higher DO levels than their slower moving down-stream counterparts due to higher rates of aeration and less organic material. Large rivers carrying organic material and excess nutrients also cause sharp spatial variations in ocean DO level (Figure 2.)

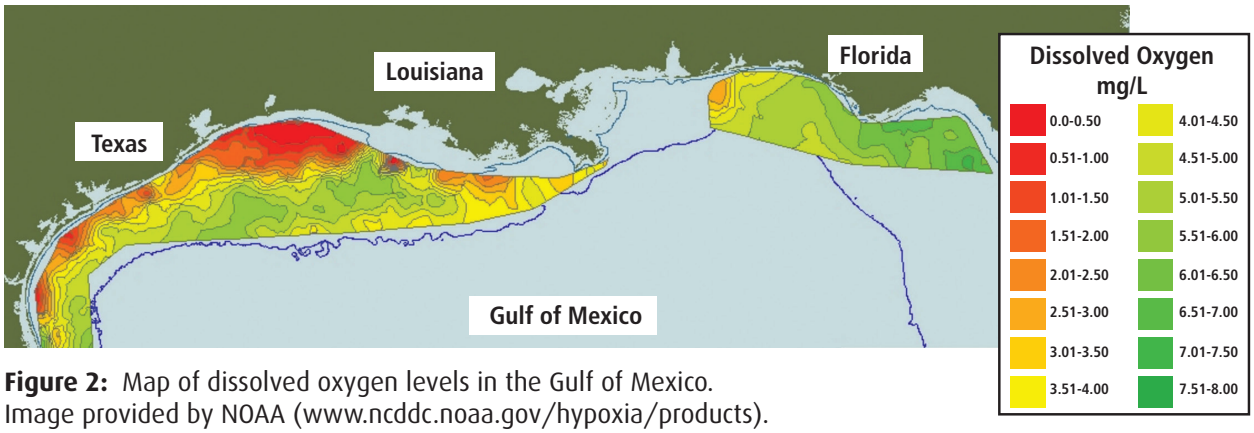


Figure 2: Map of dissolved oxygen levels in the Gulf of Mexico. Image provided by NOAA (www.ncddc.noaa.gov/hypoxia/products).

Local land use and pollution also influence the dissolved oxygen levels in nearby water bodies. Surprisingly, two of the most problematic pollutants are nutrients — nitrogen and phosphorus. When extra nutrients enter a system they can cause a temporary explosion in primary producers. However, when these producers die (in part due to more limited sunlight transmission and toxic photosynthesis by-products) decomposition rates increase leading to an eventual and significant decrease in DO. This process is known as eutrophication. Low oxygen conditions lead to fish kill, decreased biodiversity, and dead zones. In addition, some algal blooms produce toxins that are dangerous to humans and other consumers.

Scientists and trained volunteers monitor dissolved oxygen levels in rivers, lakes, estuaries, and wetlands across the country. These measurements are used to detect organic pollution and to monitor overall aquatic health and diversity (Figure 3). Water quality criteria for public water supplies are general 4 mg DO/L or more while the ideal levels in certain aquatic ecosystems are 5 mg DO/L or higher.

MEASURING PRIMARY PRODUCTIVITY IN AN ECOSYSTEM

Dissolved oxygen measurements represent one way to describe the primary productivity of aquatic ecosystems. Primary productivity is the rate at which plants and other autotrophs synthesize organic compounds from carbon dioxide and water (Equation 1). The energy for this conversion primarily comes from the sun although a few organisms also use the oxidation or reduction of inorganic chemicals. In both cases primary productivity represents the starting point for measuring energy flow within an ecosystem. For this experiment, we will focus on primary productivity through photosynthesis.

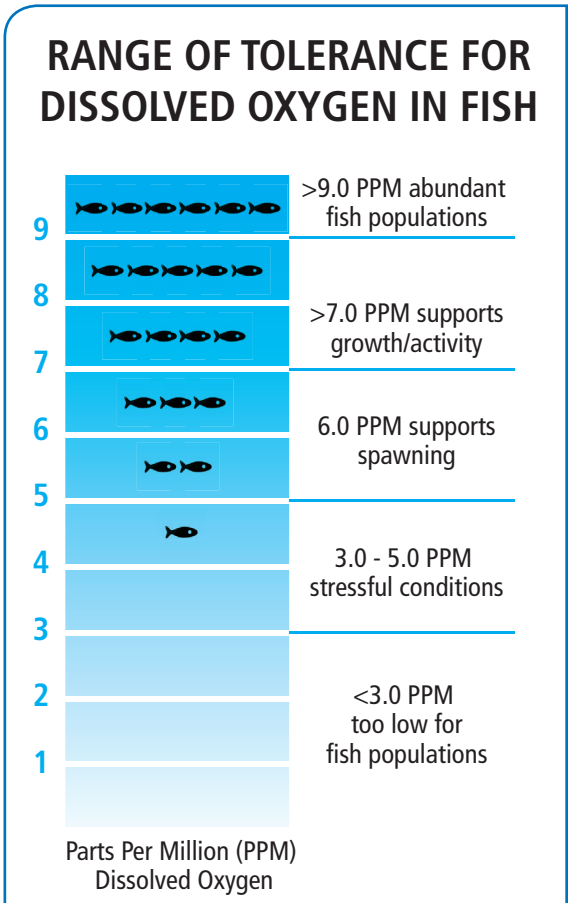


Figure 3: Low dissolved oxygen concentrations can impact the structure and function of aquatic ecosystems.

Primary productivity can be further described as either gross or net. Gross primary productivity (GPP) is the amount of chemical energy produced by autotrophs in an ecosystem over a specific amount of time. In contrast, net primary productivity (NPP) is the amount of chemical energy produced by autotrophs in an ecosystem over a specific amount of time that is available to other organisms. The difference in these values arises because some of the organic compounds generated by photosynthesis are used for energy by the autotroph. This is described by the Equation 2. Ecologists refer to an ecosystem's net primary productivity as its "energy budget" because it represents the amount of energy that is available for use by consumers.

Equation 1: Photosynthesis

$$6CO_2 + 6H_20 \longrightarrow C_6H_{12}O_6 + 6O_2$$

Equation 2: Net Primary Productivity

$$NPP = GPP - R$$

NPP is Net Primary Productivity
GPP is Gross Primary Productivity
R is Rate of Autotroph Respiration

In terrestrial ecosystems, NPP is measured as an increase in the amount of carbon per square meter per year. These numbers are obtained by weighing plant biomass in sample plots or by extrapolating from recorded changes in percentage reflection. In aquatic ecosystems, NPP is calculated by monitoring the production of oxygen by a body of water. An initial DO measurement of a water sample is taken and the sample is then incubated for a period of time. During this time autotrophs in the sample are simultaneously producing oxygen by photosynthesis and consuming it by respiration. Following incubation a second DO measurement is taken. Oxygen levels will increase if the rate of photosynthesis outpaces respiration and decrease if the rate of respiration is higher. Based on Equation 1 and the molecular weights of the molecules involved, one milliliter of oxygen produced or lost per liter sample of water is equal to 0.536 mg of carbon produced or lost per liter sample.

Dissolved oxygen can also be used to calculate GPP in aquatic systems. In this case an additional sample of water must be collected, covered with a light impenetrable layer (such as tin foil), and incubated along side the NPP sample. Without light access autotrophs in this bottle do not produced oxygen through photosynthesis, instead the only process occurring is the decrease in oxygen due to respiration. Based on Equation 2, the gross productivity of a water sample can be calculated by adding the amount of oxygen lost in the respiration bottle to the oxygen change in the NPP bottle. This method is sometimes referred to as the dark and light bottle method and is illustrated in Figure 4.

THE WINKLER METHOD

How do you measure a dissolved gas that is colorless, odorless, and tasteless? In 1889, Ludwig Winkler created a technique to determine the concentration of dissolved oxygen using a series of chemical reactions. During these reactions, free iodine is first released — two molecules of I_2 for each O_2 molecule originally present in the water sample. Iodine is more stable than oxygen in biologically active samples. However, it does react with sodium thiosulfate in a color changing redox reaction. This reaction allows the concentration of iodine to be calculated by titration.

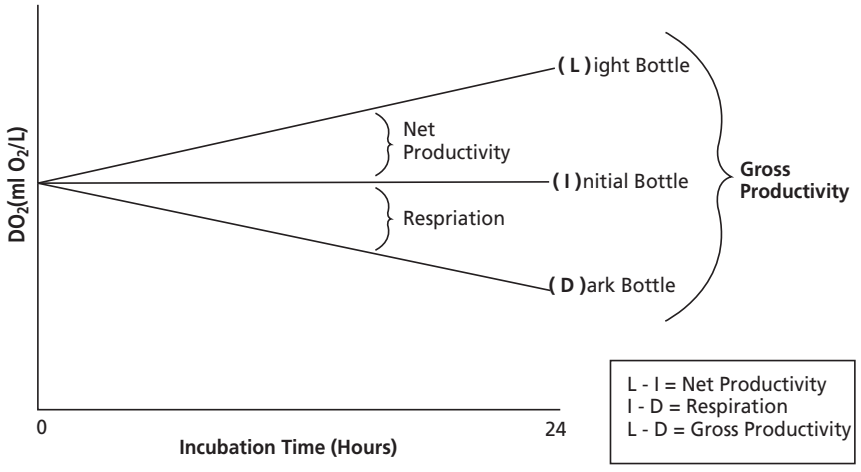


Figure 4: Gross productivity can be determined by light and dark bottle method.

Care and speed are needed when handling water samples slated for dissolved oxygen analysis. This is because dissolved oxygen levels can change after sampling due to continued photosynthesis/respiration. In addition, unintentional aeration when moving the sample can also artificially increase DO levels. In the Winkler method both problems are addressed by "fixing" the oxygen immediately after collection. A solution containing manganese sulfate and sodium iodide is added to the water samples. The manganese sulfate interacts with oxygen to create manganese (III) hydroxide (Equation 3). Next, sulfuric acid is added. In the new low pH environment sodium iodide reacts with manganese (III) hydroxide to produce free iodine molecules (Equation 4). After this "fixation" process the samples will turn pale yellow and can be stored for several hours or transported.

Equation 3: Winkler Method's Fixation Step

$$4Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 4Mn(OH)_3$$

Equation 4: Winkler Method's Fixation Step, continued.

$$2Mn(OH)_3 + 2I^- + 6H^+ \longrightarrow I_2 + 6H_2O + 2Mn^{2+}$$

Equation 5: Winkler Method Titration Step

$$2(S_2O_3)^{-2} + I_2 \longrightarrow S_4O_6^{-2} + 2I^-$$

Once back in the lab, the fixed water sample is titrated with a solution of sodium thiosulfate. This chemical neutralizes the iodine in the water sample (Equation 5) and causes the solution to change from pale yellow to clear when the two reactants are balanced. During titration, the sodium thiosulfate (the titrant) solution is slowly added until the exact moment that a complete loss of color occurs. It is then possible to calculate the original concentration of dissolved oxygen based on molar ratios, the volume of sodium thiosulfate added, the concentration of the thiosulfate, and the volume of the water sample. Before titration, starch may be added to the fixed water sample. In the presence of iodine, starch turns a deep purplish blue color that is easier to visualize than the straw yellow color of the fixed water samples. This addition makes it easier to observe the endpoint of sodium thiosulfate titration.

In this experiment students will explore the Winkler method by examining the relationship between temperature, dissolved oxygen concentration, and dissolved oxygen saturation. They will then use local water samples to examine the relationship between primary productivity and water depth. Resources are also provided for students to explore how additional nutrient pollution could effect dissolved oxygen levels in sampled water.

Citations

Hutchinson, G.E. 1957. A Treatise on Limnology. Vol. 1: Geography, Physics, and Chemistry. John Wiley and Sons.

Experiment Overview

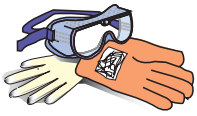
EXPERIMENT OBJECTIVE

Dissolved oxygen levels are used to monitor the health and productivity of aquatic ecosystems. In this kit, students familiarize themselves with the Winkler method by examining the relationship between temperature and dissolved oxygen concentrations. They then use local water samples to examine primary productivity at different water depths. Finally, they will observe the beginning stages of eutrophication in their water samples.

LABORATORY SAFETY

Be sure to READ and UNDERSTAND the instructions completely BEFORE starting the experiment. If you are unsure of something, ASK YOUR INSTRUCTOR!

- Gloves and goggles should be worn routinely as good laboratory practice.
- DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
- Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.
- Wear protective equipment and exercise extreme caution when handling or when near acids.



Wear gloves and safety goggles

LABORATORY NOTEBOOKS:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be documenting your experiment in a laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Carefully read the introduction and the protocol. Use this information to form a hypothesis for this experiment.
- Predict the results of your experiment.

During the Experiment:

- Record your observations.

After the Experiment:

- Interpret the results – does your data support or contradict your hypothesis?
- If you repeated this experiment, what would you change? Revise your hypothesis to reflect this change.

Module I
1.5 hours + 0.5 hours for analysis

↓

Module II
Day 1 – 1.5 hours Day 2 – 1.5 hours + 0.5 hours for analysis

↓

Module III
Day 1 – 1 hour Day 2 – 1.5 hours + 0.5 hours for analysis

NOTE: Experimental times are approximate.

Module I: Practicing the Winkler Method

NOTE: During this procedure, samples may overflow when additional solutions are added. To keep your work area clean, place containers in the center of a small tray. At the end of this experiment, any liquid in this tray can be disposed down the sink.

Forming the Manganese (III) Hydroxide Precipitate

1. **OBTAIN** a water sample from the instructor. Samples will be at either 5° C, 20° C, or 30° C. Different groups will use water at different temperatures. **MEASURE** and **RECORD** the temperature of the water with a thermometer.

*Record the temperature of your water here:*_____

NOTE: In steps 2, 3, and 4 it is critical to handle samples carefully so that no oxygen is introduced into or lost from the water. Make sure to completely fill bottles so that no air is trapped at the top. Also move and invert BOD bottles gently.

2. **FILL** the Biological Oxygen Demand (BOD) bottle with the sample water by either:
   (a) submerging the BOD bottle in the larger class sample and capping the bottle while still submerged or
   (b) slowly pouring the sample water into the BOD bottle until the bottle is completely filled and begins to overflow, then cap.

3. **INVERT** the container and use a paper towel to remove any water around the outside of the stopper or lid.

NOTE: Chemicals in Step 4 are strongly alkaline and corrosive. Wear chemical splash goggles, gloves, and an apron or lab coat.

4. **CREATE** manganese hydroxide precipitate in your water sample:
   a. Open the container and carefully **ADD** 2 ml of manganese sulfate. Make sure the pipet tip is below the water surface in the container.
   b. With a fresh pipet, **ADD** 2 ml of the alkaline-iodide solution into the sample in the container. Make sure the pipet tip is below the surface of the solution in the container.
   c. **SEAL** the container. Carefully **INVERT** the bottle 6 to 8 times to allow for complete mixing of the water sample, manganese sulfate, and alkaline-iodide.
   d. **LOOK** for precipitate (the precipitate will make the jar appear cloudy and should occupy approximately 50% of the volume of the container). It may take 10-15 minutes for the precipitate to fully form and settle. While waiting for the manganese hydroxide precipitate to settle, set up your burette (Appendix A).

Module I: Practicing the Winkler Method, continued

Addition of the Concentrated Sulfuric Acid

NOTE: Sulfuric Acid is extremely corrosive to eyes, skin, and other tissues. Wear chemical splash goggles, gloves, and an apron or lab coat.

5. **ADD** concentrated sulfuric acid to your sample.
   a. **BRING** your container to the station set up by the instructor for pipetting the concentrated sulfuric acid.
   b. **OPEN** your container and place it on the lab bench.
   c. Standing away from the container, have the instructor **ADD** 2 ml of the concentrated sulfuric acid directly into your container. The tip of the pipet should be below the surface of the solution in your container.
   d. Stopper the container and **MIX** by carefully inverting the container several times.

6. Continue to **MIX** occasionally. You should observe that the precipitate begins to dissolve and the sample will become yellowish as free iodine (I_2) is formed in the container. Remember that the quantity of free I_2 released in this step is directly equivalent to the concentration of dissolved oxygen in your sample.

OPTIONAL STOPPING POINT:
Once the sulfuric acid has been added water samples are considered "fixed" and can be stored up to one week in a dark and cool location.

Module I: Practicing the Winkler Method, continued

Titration of the Free I_2

7. **REMOVE** exactly 200 ml of solution from the container with a graduated cylinder and **TRANSFER** to a 500 ml flask.

8. **ADD** 1 ml of starch solution to the pale yellow solution in the 500 ml flask. The solution will immediately become purple. **SWIRL** the flask gently to completely mix.

9. **PLACE** the 500 ml sample flask under your burette tip. Adjust the burette if necessary. **RECORD** the initial starting volume of the sodium thiosulfate in the burette.

*Record the starting burette volume here:*_____

10. Slowly and carefully **OPEN** the stopcock of the burette until the sodium thiosulfate begins to drip out of the burette tip. Continue to **SWIRL** the 500 ml flask to insure thorough mixing of the sodium thiosulfate with the sample. **CLOSE** the stopcock when the solution in the flask is a pale purple color.

11. **RE-OPEN** the stopcock carefully so that the sodium thiosulfate solution drips out slowly. Continue to gently **SWIRL** the flask to ensure complete mixing. The solution should continue to lighten in color and go to completely clear. This should only take another 2-3 drops of titrant. Try not to miss the endpoint. Once the solution is clear immediately **CLOSE** the stopcock.

12. **RECORD** the final position of the meniscus of sodium thiosulfate in the burette.

*Record the ending volume here:*_____

13. **DETERMINE** the total volume of sodium thiosulfate which was used to titrate your sample to the endpoint by calculating the difference between the starting volume recorded in step 9 and the ending volume recorded in step 12. The total volume in milliliters of the sodium thiosulfate solution used to titrate the sample is equivalent to the dissolved oxygen concentration in milligrams of dissolved oxygen per liter of sample solution.

Record the total volume of sodium thiosulfate used here: _____
(This is the same as water's DO concentration in mg/L.)

14. **ESTIMATE** the percentage saturation of DO in your sample by using a nonogram (Figure 5). Place a ruler over the nomogram so that the ruler intersects the top line at your measured water temperature. Next, pivot the flat edge so that it simultaneously intersects the bottom line at your measured dissolved oxygen concentration. Finally, observe the point at which your straight edge intersects the % saturation line. Figure 6 shows an example of this using a starting temperature of 15° C (red square) and a dissolved oxygen measurement of 6.9 mg/L (red triangle) which results in a 65% saturation measurement (red circle).

Record the percentage saturation of your water sample here: _____

15. **DETERMINE** the class average data for the water samples at the three temperatures. **RECORD** the values in Table 1.

21. Using linear graph paper, **PLOT** both the lab group and class DO concentration as a function of temperature.

Module I: Practicing the Winkler Method, continued

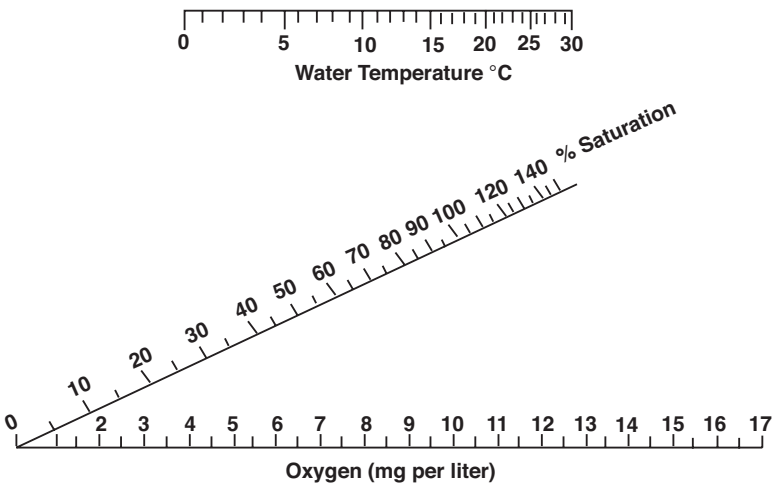


Figure 5: Nomogram of Oxygen Saturation.

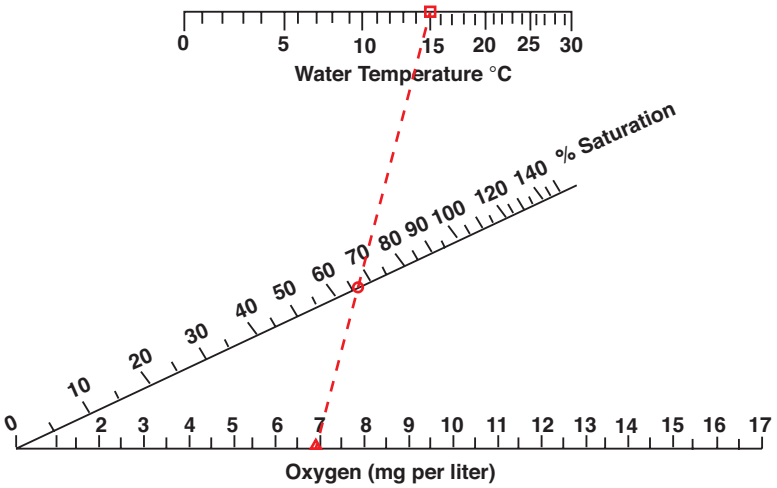


Figure 6: Using a Nomogram.

Temperature (° C)	Your DO (mg/L)	%DO Saturation (from nonogram)	Class Mean DO (mg/L)	Class Mean %DO Saturation (from nonogram)

Table 1: Temperature and dissolved oxygen data.

Module II: Primary Productivity and Light Attenuation

For Module II, samples of local water will be used to determine gross productivity, net productivity, and respiration rates. To mimic the light attenuation found as one goes deeper beneath the surface of a body of water, screens will be used to reduce the light intensity.

DAY ONE

1. **OBTAIN** seven clean "BOD" (Biological Oxygen Demand) containers.

2. **LABEL** the bottles as follows: 1 – Initial, 2 – Dark, 3 - 100%, 4 - 65% , 5 - 25%, 6 - 10%, and 7 - 5%.

3. **PREPARE** bottles.
   a. Bottle 1 is the initial starting bottle and serves as a baseline so no modification is needed. This bottle will be fixed on day one.
   b. **WRAP** bottle 2 in aluminum foil. This bottle serves as the dark (no light) control.
   c. **WRAP** bottles 3 through 7 in a different number of screens to simulate different depth in a body of water. Bottle 3 will have no screens with 100% attenuation of light. Bottle 4 will have 1 screen with 65% attenuation. Bottle 5 will have 3 screens with 25% attenuation. Bottle 6 will have 5 screens with 10% attenuation. Bottle 7 will have 7 screens with 5% attenuation. Cover the bottoms of the bottles with aluminum foil so that no light can enter.

NOTE: In steps 4 though 7 it is critical to handle samples carefully so that no oxygen is introduced into or lost from the water. Make sure to completely fill bottles so that no air is trapped at the top. Also move and invert BOD bottles gently.

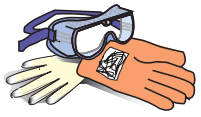
4. Completely **FILL** each bottle with a water sample from the lake or pond. Make sure that no air is trapped at the top of any bottle. Handle bottles gently.

5. **CLOSE** the containers. Turn the containers upside down and use a paper towels to remove any water around the outside of the stoppers or lids.

6. **PLACE** bottles 2-7 on their sides and expose to a constant source of light. Leave these bottles overnight. These bottles will be fixed tomorrow.

Record the starting time here: _____

NOTE: Chemicals in step 7 are strongly alkaline and corrosive. Wear chemical splash goggles, gloves, and an apron or lab coat.



7. **CREATE** manganese hydroxide precipitate in your water sample:
   a. Open the container and carefully **ADD** 2 ml of manganese sulfate. Make sure the pipet tip is below the water surface in the container.
   b. With a fresh pipet, **ADD** 2 ml of the alkaline-iodide solution. Make sure the pipet tip is below the surface of the solution in the container.

Module II: Primary Productivity and Light Attenuation, continued

c. **SEAL** the container. Carefully **INVERT** the bottle 6 to 8 times to allow for complete mixing of the water sample, manganese sulfate, and alkaline-iodide.
d. **LOOK** for precipitate (the precipitate will make the jar appear cloudy and should occupy approximately 50% of the volume of the container). It may take 10-15 minutes for the precipitate to fully form and settle.

NOTE: Sulfuric Acid is extremely corrosive to eyes, skin, and other tissues. Wear chemical splash goggles, gloves, and an apron or lab coat.

8. **ADD** concentrated sulfuric acid to your sample.
   a. **BRING** your container to the station set up by the instructor for pipetting the concentrated sulfuric acid.
   b. **OPEN** your container and place it on the lab bench.
   c. Standing away from the container, have the instructor **ADD** 2 ml of the concentrated sulfuric acid directly into your container. The tip of the pipet should be below the surface of the solution in your container.
   d. Stopper the container and **MIX** by carefully inverting the container several times.

9. Continue to **MIX** occasionally. You should observe that the precipitate begins to dissolve and the sample will become yellowish as free iodine (I_2) is formed in the container. Remember that the quantity of free I_2 released in this step is directly equivalent to the concentration of dissolved oxygen in your sample.

10. **STORE** bottle 1 in a dark and cool location.

OPTIONAL STOPPING POINT:
If needed, photosynthesis/respiration can continue for up to 5 days.

OPTIONAL ACTIVITY:

If directed by the instructor, **PLACE** several drops of the pond water on a microscope slide and **COVER** with a cover-slip. **OBSERVE** the organisms found in the natural water sample. **DRAW** or photograph what is observed and try to identify the organisms.

DAY TWO

11. **FIX** the dissolved oxygen in bottles 2 through 7. To do this, repeat steps 7 though 9 on pages 14-15. Record the time and **CALCULATE** the total light/dark experiment time.

Record the end time here: _____

Total experiment time: _____

12. While precipitate is settling in bottles 2-7, **RETRIEVE** bottle 1 and **SET UP**/clean your burette (Appendix A).

13. **REMOVE** exactly 200 ml of solution from each container with a graduated cylinder and **TRANSFER** to labeled 500 ml flasks.

14. **ADD** 1 ml of starch solution to the pale yellow solutions in the 500 ml flasks. The solutions will immediately become purple. **SWIRL** the flasks gently to completely mix.

Module II: Primary Productivity and Light Attenuation, continued

15. **PLACE** the 500 ml sample flask for bottle 1 under your burette tip. Adjust the burette if necessary. **RECORD** the initial starting volume of the sodium thiosulfate in the burette. **RECORD** the starting burette volume in Table 2.

16. Slowly and carefully **OPEN** the stopcock of the burette until the sodium thiosulfate begins to drip out of the burette tip. Continue to **SWIRL** the 500 ml flask to insure thorough mixing of the sodium thiosulfate with the sample. **CLOSE** the stopcock when the solution in the flask is a pale purple color.

17. **RE-OPEN** the stopcock carefully so that the sodium thiosulfate solution drips out slowly. Continue to gently SWIRL the flask to insure complete mixing. The solution should continue to lighten in color and go to completely clear. This should only take another 2-3 drops of titrant. Try not to miss the endpoint. Once the solution is clear immediately **CLOSE** the stopcock.

18. **RECORD** the final position of the meniscus of sodium thiosulfate in the burette in Table 2.

19. **DETERMINE** the total volume of sodium thiosulfate which was used to titrate your sample to the endpoint by calculate the difference between the starting volume recorded and the ending volume recorded in Table 2. The total volume in milliliters of the sodium thiosulfate solution used to titrate the sample is equivalent to the dissolved oxygen concentration in milligrams of dissolved oxygen per liter of sample solution. **RECORD** the total volume of sodium thiosulfate used in Table 2.

20. **REPEAT** steps 15 through 19 for the remaining six bottles.

	Starting Volume	Ending Volume	TOTAL Volume of Sodium Thiosulfate
Bottle 1 Initial			
Bottle 2 Dark			
Bottle 3 100% Light			
Bottle 4 65% Light			
Bottle 5 25% Light			
Bottle 6 10% Light			
Bottle 7 5% Light			

Table 2: Module II Titration Volumes

Module II: Primary Productivity and Light Attenuation, continued

21. **RECORD** DO (mg/L) values in the first column of Tables 3 and 4. Remember that this experiment has been set up so that the concentration of dissolved oxygen (in mg/L) is equal to the total volume of sodium thiosulfate used during titration (in ml).

22. **CONVERT** the concentration of dissolved oxygen from mg/L to ml/L using the following conversion factor: 1 mg DO/L = 0.698 ml DO/L. **RECORD** in Tables 3 and 4.

23. **CALCULATE** the respiration rate and the gross and net productivity for the natural water samples using the following equations:

    Respiration rate = (Initial bottle (1) ml DO/L - Dark bottle (2) ml DO/L) /hours*

    Gross Productivity = (Light bottle (3, 4, 5, 6, or 7) ml DO/L - Dark bottle (2) ml DO/L)/hours*

    Net Productivity = (Light bottle (3, 4, 5, 6, or 7) ml DO/L - Initial bottle (1) ml DO/L) /hours*

* Productivity is a rate term so divide the final amount of dissolved oxygen produced or consumed by the number of hours that the experiment ran (step 11) to arrive at a value of ml DO/L per hour.

Record the values for the individual experiment conditions here:

Respiration rate = _____ml DO/L/hour.

- Bottle 3 (100% light attenuation)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 4 (65% light attenuation)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 5 (25% light attenuation)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 6 (10% light attenuation)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 7 (5% light attenuation)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

24. **DETERMINE** the class average data for the water samples. **RECORD** the individual and class average values for respiration in Table 3 and for gross and net productivity in the Table 4. The values should be reported in ml DO/L/hour.

25. Using linear graph paper or a spreadsheet program, **PLOT** the average gross and net productivity on the Y-axis versus the percent of light intensity on the X-axis.

Module II: Primary Productivity and Light Attenuation, continued

	INDIVIDUAL DATA		CLASS MEAN	
	DO (mg/L)	DO (ml/L)	DO (mg/L)	DO (ml/L)
DO, Initial				
DO, Dark Bottle				
Respiration Rate (Initial-Dark)/experimental hrs.				

Table 3: Respiration rates during Module II.

	INDIVIDUAL DATA				CLASS DATA			
Sample Treatment	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)
100% Light (0 screens)								
65% Light (1 screen)								
25% Light (3 screens)								
10% Light (5 screens)								
5% Light (7 screens)								

Table 4: Productivity of Screen-Wrapped Samples.

Module III: (Optional) Primary Productivity and Nutrient Pollution

In Module III, you will observe the initial stage of eutrophication by adding two common nutrient pollutants to local water samples. The light and dark bottle method will be used again to determine gross productivity, net productivity, and respiration rates.

DAY ONE

1. **OBTAIN** five clean "BOD" (Biological Oxygen Demand) containers.

2. **LABEL** the bottles as follows: 1 – Initial, 2 – Dark, 3 – Light and Nitrogen Enriched, 4 – Light and Phosphorous Enriched, 5 – Light and Unenriched.

NOTE: In steps 3 through 7 it is critical to handle samples carefully so that no oxygen is introduced into or lost from the water. Make sure to completely fill bottles so that no air is trapped at the top. Also move and invert BOD bottles gently.

3. Completely **FILL** each bottle with water sample from the lake or pond. Allow the sample to overflow the container so that it will be completely filled. Make sure that no air is trapped at the top of any bottle. Handle bottles gently.

4. **CLOSE** the containers. Turn the containers upside down and use a paper towel to remove any water around the outside of the stopper or lid.

5. **PREPARE** bottles:
   a. Bottle 1 is the initial starting bottle and serves as a baseline so no modification is needed. This bottle will be fixed on day one.
   b. **WRAP** bottle 2 in aluminum foil. This bottle serves as the dark (no light) control.
   c. **ADD** 1 ml of nitrogen enrichment solution to bottle 3.
   d. **ADD** 1 ml of phosphorous enrichment solution to bottle 4.
   e. Bottle 5 is a control and will receive no nutrient supplementation. However, it will be exposed to light.

6. **PLACE** bottles 2 through 5 on their sides and expose to a constant source of light. Leave these bottles overnight.

*Record the starting time here:*_____

NOTE: Chemicals in step 7 are strongly alkaline and corrosive. Wear chemical splash goggles, gloves, and an apron or lab coat.

7. **CREATE** manganese hydroxide precipitate in your water sample:
   a. Open the container and carefully **ADD** 2 ml of manganese sulfate. Make sure the pipet tip is below the water surface in the container.
   b. With a fresh pipet, **ADD** 2 ml of the alkaline-iodide solution into the sample in the container. Make sure the pipet tip is below the surface of the water in the container.
   c. **SEAL** the container. Carefully **INVERT** the bottle 6 to 8 times to allow for complete mixing of the water sample, manganese sulfate, and alkaline-iodide.
   d. **LOOK** for precipitate (the precipitate will make the jar appear cloudy and should occupy approximately 50% of the volume of the container). It may take 10-15 minutes for the precipitate to fully form and settle.

Module III: Primary Productivity and Nutrient Pollution, continued

NOTE: Sulfuric Acid is extremely corrosive to eyes, skin, and other tissues. Wear chemical splash goggles, gloves, and an apron or lab coat.

8. **ADD** concentrated sulfuric acid to your sample.
   a. **BRING** your container to the station set up by the instructor for pipetting the concentrated sulfuric acid.
   b. **OPEN** your container and place it on the lab bench.
   c. Standing away from the container, have the instructor **ADD** 2 ml of the concentrated sulfuric acid directly into your container. The tip of the pipet should be below the surface of the solution in your container.
   d. Stopper the container and **MIX** by carefully inverting the container several times.

9. Continue to **MIX** occasionally. You should observe that the precipitate begins to dissolve and the sample will become yellowish as free iodine, I_2, is formed in the container. Remember that the quantity of free I_2 released in this step is directly equivalent to the concentration of dissolved oxygen in your sample.

10. **STORE** bottle 1 in a dark and cool location.

DAY TWO

11. **FIX** the dissolved oxygen in bottles 2 through 5. To do this, repeat steps 7 though 9 on pages 19-20. **RECORD** the experimental stop time and then **CALCULATE** the total experiment time.

Record the end time here: _____

Total experiment time: _____

12. While precipitate is settling in bottles 2 through 5, **RETRIEVE** bottle 1 and **SET UP** / clean your burette (Appendix A).

13. **REMOVE** exactly 200 ml of solution from the containers with a graduated cylinder and **TRANSFER** to labeled 500 ml flasks.

14. **ADD** 1 ml of starch solution to the pale yellow solutions in the 500 ml flasks. The solutions will immediately become purple. **SWIRL** the flasks gently to completely mix.

15. **PLACE** the 500 ml sample flask of bottle 1 under your burette tip. Adjust the burette if necessary. **RECORD** the initial starting volume of the sodium thiosulfate in the burette in Table 5.

16. Slowly and carefully **OPEN** the stopcock of the burette until the sodium thiosulfate begins to drip out of the burette tip. Continue to **SWIRL** the 500 ml flask to insure thorough mixing of the sodium thiosulfate with the sample. **CLOSE** the stopcock when the solution in the flask is a pale purple color.

17. **RE-OPEN** the stopcock carefully so that the sodium thiosulfate solution drips out slowly. Continue to gently **SWIRL** the flask to insure complete mixing. The solution should continue to lighten in color and go to completely clear. This should only take another 2-3 drops of titrant. Try not to miss the endpoint. Once the solution is clear immediately **CLOSE** the stopcock.

18. **RECORD** the final position of the meniscus of sodium thiosulfate in the burette in Table 5.

19. **DETERMINE** the total volume of sodium thiosulfate which was used to titrate your sample to the endpoint by calculate the difference between the starting volume recorded and the ending volume recorded in Table 5.

Module III: Primary Productivity and Nutrient Pollution, continued

The total volume in milliliters of the sodium thiosulfate solution used to titrate the sample is equivalent to the dissolved oxygen concentration in milligrams of dissolved oxygen per liter of sample solution. **RECORD** the total volume of sodium thiosulfate used Table 5.

20. **RECORD** DO (mg/L) values in the first columns of Tables 6 and 7.

21. **CONVERT** the concentration of dissolved oxygen from mg/L to ml/L using the following conversion factor: 1 mg DO/L = 0.698 ml DO/L.

22. **REPEAT** steps 15 through 21 for the remaining bottles.

23. **CALCULATE** the respiration rate and the gross and net productivity for the natural water samples using the following equations:

    Respiration rate = (Initial bottle (1) ml DO/L - Dark bottle (2) ml DO/L) /hours*****

    Gross Productivity = (Light bottle (3, 4, and 5) ml DO/L - Dark bottle (2) ml DO/L)/hours*****

    Net Productivity = (Light bottle (3, 4, or 5) ml DO/L - Initial bottle (1) ml DO/L) /hours*****

***** Productivity is a rate term so divide the final amount of dissolved oxygen produced or consumed by the number of hours that the experiment ran (step 11) to arrive at a value of ml DO/L per hour.

Record the values for the individual experiment conditions here:

Respiration rate = _____ml DO/L/hour.

- Bottle 3 (Nitrogen enriched)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 4 (Phosphorous enriched)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

- Bottle 5 (Unenriched)
  Gross Productivity rate = _____ ml DO/L/hour
  Net Productivity rate = _____ ml DO/L/hour

24. **DETERMINE** the class average data for the water samples. **RECORD** the class average values for Respiration Rate in Table 6 and for gross and net productivity in Table 7. The values should be reported in ml DO/L/hour.

25. Using linear graph paper or a spreadsheet program, **PLOT** the average gross and net productivity on the Y-axis versus nitrogen enriched, phosphorous enriched, and unenriched samples on the X- axis.

Module III: Primary Productivity and Nutrient Pollution, continued

	Starting Volume	Ending Volume	TOTAL Volume of Sodium Thiosulfate
Bottle 1 Initial			
Bottle 2 Dark			
Bottle 3 Nitrogen			
Bottle 4 Phosphorous			
Bottle 5 Unenriched			

Table 5: Module III Titration Volumes

	INDIVIDUAL DATA		CLASS MEAN	
	DO (mg/L)	DO (ml/L)	DO (mg/L)	DO (ml/L)
DO, Initial				
DO, Dark Bottle				
Respiration Rate (Initial - Dark)				

Table 6: Respiration rates during Module III.

	INDIVIDUAL DATA				CLASS DATA			
Sample Treatment	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)
Nitrogen added								
Phosphorus added								
Unenriched								

Table 7: Productivity of Nitrogen or Phosphorous Enriched Samples.

Study Questions

1. Name at least two ways for dissolved oxygen to enter an aquatic ecosystem and at least two ways for it to be lost from an aquatic ecosystem.

2. Reorganize the steps to show the process of eutrophication:
   (a) decomposition rates increase
   (b) excess nutrients enter a system
   (c) dissolved oxygen levels decrease
   (d) rapid growth of primary producer populations
   (e) fish and other aquatic life die
   (f) water becomes cloudy and has a high concentration of toxic by products

3. How are NPP and GPP similar measurements? How are they different measurements?

4. Use chemical equations 3, 4, and 5 from the background section to calculate how many moles of sodium thiosulfate titrant ($Na_2S_2O_3$) are required to neutralize one mole of dissolved oxygen.

5. Why would you record the collection time for a water sample intended for a dissolved oxygen reading? What other details might you record?

Instructor's Guide

OVERVIEW OF INSTRUCTORS PRE-LAB PREPARATION

This section outlines the recommended pre-lab preparations and approximate time requirement to complete each pre-lab activity.

Preparation for:	What to do:	When:	Time Required:
Module I	Gather burettes, BOD bottles, glassware etc.	Day before Module I or earlier	15 minutes
	Prepare the sulfuric acid station	Day before Module I or earlier	10 - 20 minutes
	Prepare temperature water samples	Day before Module I	10 minutes
	Prepare solutions	Day before Module I	30 minutes
	Aliquot manganese sulfate, sodium iodide/sodium hydroxide, and starch solution	Day of Module I	20 minutes
	Dilute and aliquot sodium thiosulfate	During Module I	10 minutes
Module II	Collect local water sample (for Modules II and III)	1 or 2 weeks before Module II	Variable
	Set up light source	Day before Module II or ealier	Variable
	Prepare screen covers	Day before Module II or earlier	Variable
	Gather burettes, BOD bottles, glassware etc.	Day before Module II or earlier	10 minutes
	(Optional) Set up microscopes	Day before Module II	Variable
	Aliquot manganese sulfate, sodium iodide/sodium hydroxide, and starch solution	Day of Module II	20 minutes
	Dilute and aliquot sodium thiosulfate	During Module II	10 minutes
Module III	Gather burettes, BOD bottles, glassware etc.	Day before Module III or earlier	10 minutes
	Prepare and aliquot nutrient solutions	Day of Module III	15 minutes
	Aliquot manganese sulfate, sodium iodide/sodium hydroxide, and starch solution	Day of Module III	20 minutes
	Dilute and aliquot sodium thiosulfate	During Module III	10 minutes

Pre-Lab Preparation

SPECIAL REQUIREMENTS

Local Water Sample:
A natural source of water will be needed for Module II and III. Alternatively, an algal culture of *Chlorella* can be prepared. Depending on the size of the BOD bottles up to 7.2 liters of natural source water per group will be needed. For natural water we suggest finding water sources with a stable ecosystem that includes aquatic producers and consumers. Still pond water or water from slow moving steams are ideal. Avoid collecting water after heavy rains as the sediment skews the aquatic population towards consumers. Both collected water samples and *Chlorella* cultures benefit from being stored under light for several days before being used in Modules II and III.

BOD Bottles:
Biological oxygen demand (BOD) bottles have watertight seals. This seal is a precaution against drawing air into the bottle and also ensures that no leakage occurs. Module I, II, and III of this kit require 10, 70, and 50 BOD bottles, respectively (250 or 300 ml). A container that can be closed with an airtight seal is a good alternative for a true BOD bottle. If you are going to reuse BOD bottles we recommend washing, triple rinsing, and DI rinsing each bottle.

Light Source:
Aquatic producers will photosynthesize under a variety of light conditions. For best results keep bottles close (<20 cm) to the light source. Check that the light source does not let off excessive heat.

Screens:
Rolls of plastic window or patio screens can be purchased from most large hardware stores. Test before hand the amount needed to wrap a single BOD bottle. Each group will need 17 square screens of this size.

MODULE I: PRACTICING THE WINKLER METHOD

Most solutions can be prepared 1 or 2 days before the laboratory. The concentrated sodium thiosulfate solution should be prepared the day before and stored in the refrigerator and then diluted on the day of the laboratories.

Preparing the Water Samples:

1. Module I requires up to 1.5 liters each of fresh tap water at three distinct temperatures. We suggest 5° C, 20° C, and 30° C. The water should be held covered at these temperatures the day before the laboratory to allow for oxygen equilibration.

Preparing the Manganese Sulfate Solution:

1. Add all of the manganese sulfate (Component C) to a total volume of 300 ml of distilled water.
2. Filter with #1 Whatman filter paper.
3. Store covered at room temperature.
4. Label ten 15 ml tubes* "manganese sulfate" and dispense 2.2 ml for each group. The remaining solution will be used in Modules II and III.

* These tubes will be refilled and reused for Modules II and III. Collect or have students return the "manganese sulfate", "alkaline-iodide", and "starch" tubes at the end of the module.

Pre-Lab Preparation, continued

Preparing the Alkaline-iodide Solution:

1. Put on gloves and goggles.
2. Add all of the NaOH pellets (Component B) to a large beaker.
3. Add distilled water to a volume of 150 - 175 ml. Stir or swirl to dissolve. ***CAUTION: The solution will get hot.***
4. Add all of the NaI (Component A) and adjust to a final volume of 300 ml with distilled water. Mix.
5. Store covered at room temperature.
6. Label ten 15 ml tubes* "alkaline-iodide" and dispense 2.2 ml for each group. The remaining solution will be used in Modules II and III.

* These tubes will be refilled and reused for Modules II and III. Collect or have students return the "manganese sulfate", "alkaline-iodide", and "starch" tubes at the end of the module.

Preparing the Starch Solution:

1. Label ten 15 ml tubes* "starch" and dispense 1.2 ml of Component D to each group. The remaining solution will be used in Modules II and III.

* These tubes will be refilled and reused for Modules II and III. Collect or have students return the "manganese sulfate", "alkaline-iodide", and "starch" tubes at the end of the module.

Preparing the Sulfuric Acid Station

NOTE: Sulfuric Acid is extremely corrosive to eyes, skin, and other tissues. Wear chemical splash goggles, gloves, and an apron or lab coat.

1. Read the SDS for sulfuric acid.
2. Check your supply of sulfuric acid. You will need 20 ml of concentrate sulfuric acid for this module. The acid should be concentrated (98%+ or 18mol/L+) and should ideally be in a PVC coated glass bottle. Because sulfuric acid is a strong dehydrating agent also check for contamination. If the solution has turned brown replace it.
3. Check eye wash station.
4. Choose a central dispensing location.
5. Set up a distribution station. Gather the acid, pipet pump, and glass pipets. Also have acid spill clean up material readily available (sand, absorbent, and neutralizer). A stabilized test tube to store the dispensing pipet is also useful. Finally, use lab mats and or plastic trays to contain potential spills or drips.

For Module I
Each Group should have:

- Tap water (300-350 ml)
- Manganese sulfate (2.2 ml)
- Alkaline-iodide (2.2 ml)
- Sodium thiosulfate (14 ml)
- Starch (1.2 ml)
- BOD bottle with cap
- Small tray to contain water overflow
- Pipet and pipet tips
- Ring stand and clamp
- Burette
- 500 ml flask
- 500 ml beaker
- 200 ml+ graduated cylinder

Preparing the Sodium Thiosulfate Solution:

1. Boil 4 liters of distilled water for 10-15 minutes. Cool to room temperature, seal, and finally store in the refrigerator. (Boiling depletes the oxygen in the water so that it will not interfere with the analysis.)
2. Before Module I, add all of the sodium thiosulfate (Component E) to boiled and cooled distilled water to make 2 liters final volume. This is your "working stock" solution. Seal and store in the refrigerator.
3. During Module I, while students are setting up their burettes, add 37.5 ml of the working stock solution to 112.5 ml of boiled and cooled distilled water.
4. Label ten 15 ml conical tubes "sodium thiosulfate" and dispense 14 ml for each group. The remaining solution will be used in Modules II and III.

NOTE: For these experiments it is not necessary to standardize the thiosulfate solution.

Pre-Lab Preparation, continued

MODULE II: PRIMARY PRODUCTIVITY AND LIGHT ATTENUATION

1. Distribute locally sampled water to each group and 17 sheets of window screen.
2. Refill "manganese sulfate " tubes with 15 ml for each group.
3. Refill "alkaline-iodide" with 15 ml for each group.
4. Just prior to the titration step add 250 ml of the working sodium thiosulfate stock solution to 750 ml of boiled and cooled distilled water.
5. Label ten beakers "sodium thiosulfate" and dispense 98 ml for each group.
6. Refill "starch" tubes with 8.4 ml of Component D for each group.
7. Set up the concentrated sulfuric acid station (page 26).
8. Set up microscopes if the optional activity is planned.
9. Set up growth lights or under counter fluorescent lights.

FOR MODULE II
Each Group should have:
- Locally sampled water
- Manganese sulfate (15 ml)
- Alkaline-iodide (15 ml)
- Sodium thiosulfate (98 ml) in 100 ml+ beaker
- Starch (8.4 ml)
- 7 BOD bottle with cap
- Aluminum foil
- 17 screens
- Pipet and pipet tips
- Ring stand and clamp
- Burette
- 500 ml flasks (7)
- 500 ml beaker (for burette waste)
- 200 ml+ graduated cylinder
- Microscope slide and coverslip (optional)

MODULE III: (OPTIONAL) PRIMARY PRODUCTIVITY AND NUTRIENT POLLUTION

Preparing the Nitrogen Enrichment Solution:

1. Add 10 ml of the nitrogen enrichment solution (Component F) to 500 ml of distilled water.
2. Store covered in the refrigerator.
3. Label ten microcentrifuge conical tubes "nitrogen" and dispense 1.2 ml for each group.

Preparing the Phosphorous Enrichment Solution:

1. Add all the phosphorous enrichment reagents (Component G) to 100 ml distilled water. Mix.
2. Store covered in the refrigerator.
3. On the day of the lab, add 10 ml of stock solution to 500 ml of distilled water.
4. Label ten microcentrifuge conical tubes "phosphorous" and dispense 1.2 ml for each group.

Additional Preparations:

1. Distribute locally sampled water to each group.
2. Refill "manganese sulfate " tubes with 11 ml for each group.
3. Refill "alkaline-iodide" tubes and dispense 11 ml for each group.
4. Just prior to the titration step, add 200 ml of the working sodium thiosulfate stock solution to 600 ml of boiled and cooled distilled water.
5. Refill "sodium thiosulfate" beakers with 70 ml for each group.
6. Refill "starch" tubes with 6 ml for each group.
7. Set up the concentrated sulfuric acid station (page 26).
8. Set up growth lights or under counter fluorescent lights.

FOR MODULE III
Each Group should have:
- Pond water (1,750 ml)
- Manganese sulfate (11 ml)
- Alkaline-iodide (11 ml)
- Sodium thiosulfate (70 ml) in 100 ml+ beaker
- Starch 6 ml)
- Nitrogen enrichment solution (1.2 ml)
- Phosphorous enrichment solution (1.2 ml)
- 5 BOD bottle with cap
- Aluminum foil
- Pipet and pipet tips
- Ring stand and clamp
- Burette
- 500 ml flasks (5)
- 500 ml beaker (for burette waste)
- 200 ml+ graduated cylinder

Experiment Results and Analysis

MODULE I

These are representative results. Your results will vary.

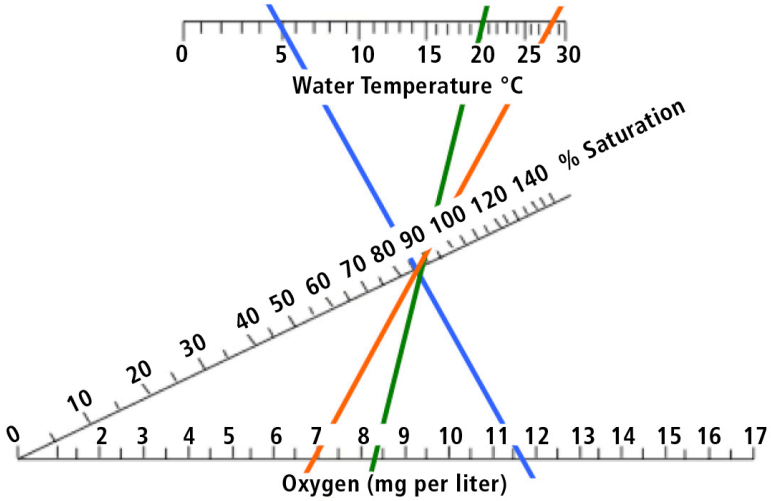
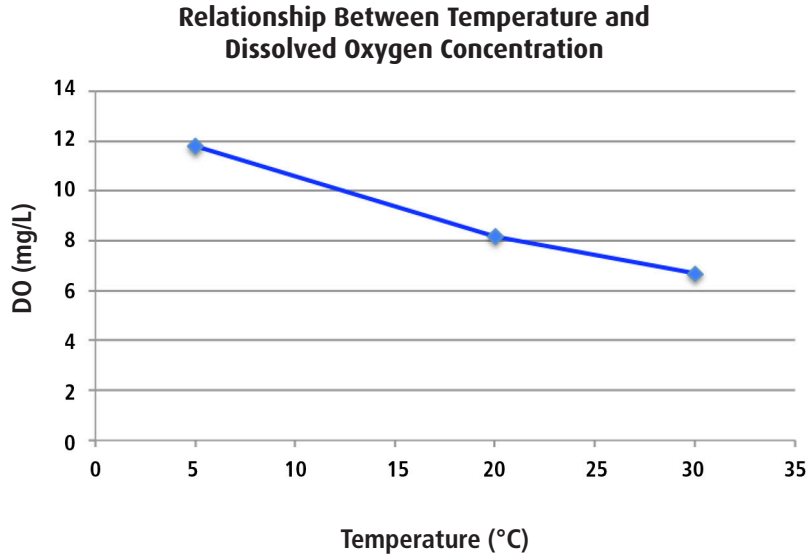


Table 1: Temperature and dissolved oxygen data.

Temperature	Your DO mg/L	%DO Saturation (from nonogram)
Temp 1 - 5°C	11.8	87%
Temp 2 - 20°C	8.2	87%
Temp 3 - 30°C	6.7	87%



Experiment Results and Analysis, continued

MODULE II

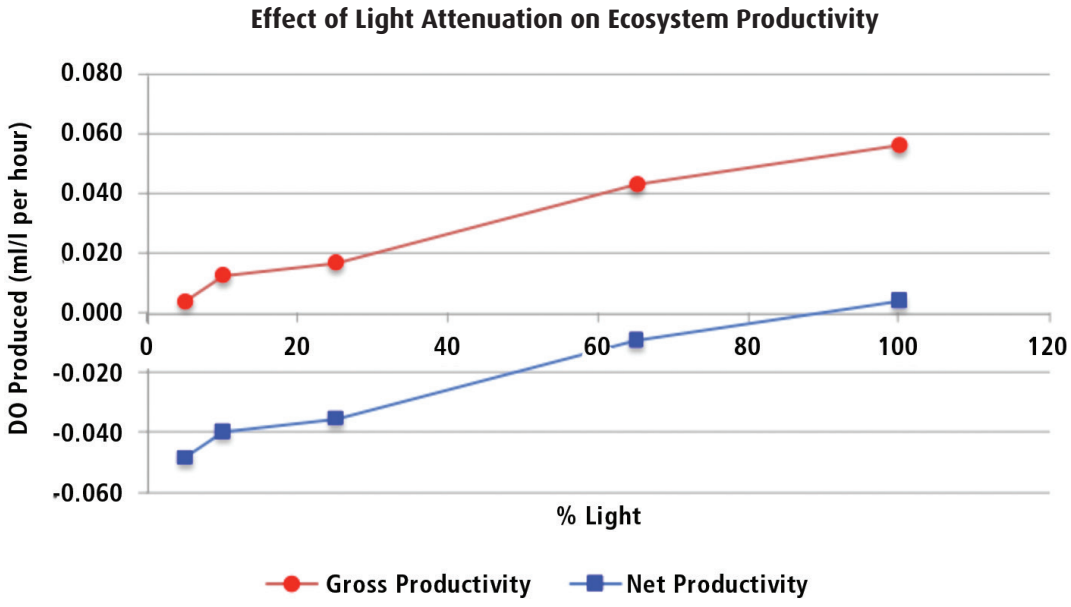
These are representative results. Your results will vary.

	Individual Data	
	DO (mg/ml)	DO (ml/L)
DO, Initial	7.1	4.956
DO, Dark Bottle	5.9	4.118
Respiration Rate (Initial - Dark)		0.838

Table 3: Respiration.

	INDIVIDUAL DATA			
Sample Treatment	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)
100% Light (0 screens)	7.2	5.026	0.057	0.004
65% Light (1 screen)	6.9	4.816	0.044	-0.009
25% Light (3 screens)	6.3	4.397	0.017	-0.035
10% Light (5 screens)	6.2	4.328	0.013	-0.039
5% Light (7 screens)	6.0	4.188	0.004	-0.048

Table 4: Productivity of Screen-Wrapped Samples.



Experiment Results and Analysis, continued

MODULE III

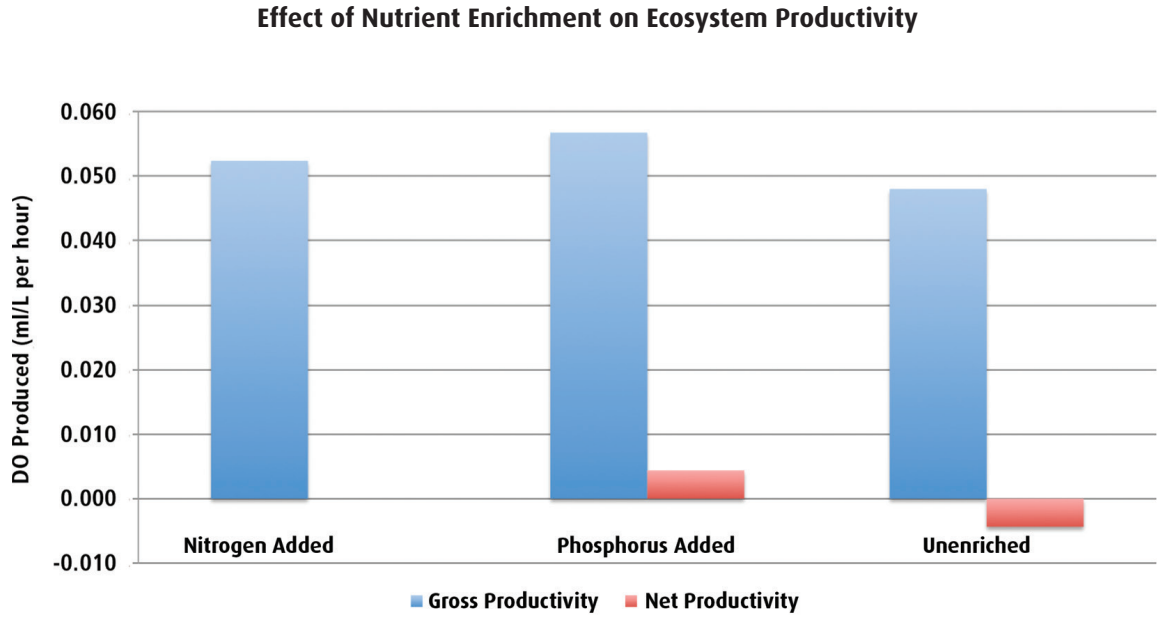
These are representative results. Your results will vary.

	Individual Data	
	DO (mg/ml)	DO (ml/L)
DO, Initial	7.3	5.095
DO, Dark Bottle	6.1	4.258
Respiration Rate (Initial - Dark)		0.838

Table 6: Respiration.

	INDIVIDUAL DATA			
Sample Treatment	DO (mg/L)	DO (ml/L)	Gross Productivity (ml/L per hr.)	Net Productivity (ml/L per hr.)
Nitrogen added	7.3	5.095	0.052	0
Phosphorus added	7.4	5.165	0.057	0.004
Unenriched	7.2	5.026	0.048	-0.004

Table 7: Productivity of Nitrogen or Phosphorous Enriched Samples.



Please refer to the kit insert for the Answers to Study Questions

Appendix A
Setting Up the Burette

Burettes are used during titration to distribute a variable but precise volume of liquid. Before titration, burettes should be checked for air bubble and for a constant rate of flow in order to ensure accurate volume measurements.

SETTING UP THE BURETTE

1. **ATTACH** a burette to a ring stand with a clamp. The burette's volume gradations should be easily visible and there should be enough space under the burette for a 500 ml flask.

2. **LABEL** a 500 ml beaker 'Burette Waste Solution' and **PLACE** it under the tip of the burette.

3. **TEST** the proper functioning and seal of the stopcock at the bottom of the burette.
   a. **CLOSE** the stopcock and **FILL** the burette with distilled water.
   b. **OPEN** and **CLOSE** the stopcock. When open, the water should flow out. When closed, the water should not leak out of the burette tip or around the stopcock assembly. Ask your instructor for help if the burette leaks.
   c. **PRACTICE** slightly opening the stopcock so that the water drips out slowly.
   d. Completely **DRAIN** the burette. **CLOSE** the stopcock.

4. **FILL** the burette with 3 ml of the sodium thiosulfate solution. **OPEN** the stopcock and allow the sodium thiosulfate solution to drain from the burette. Again, practice opening the stopcock so that the sodium thiosulfate solution drips out slowly. When the burette is completely empty **CLOSE** the stopcock.

5. Completely **FILL** the burette with the sodium thiosulfate solution.