AP Biology

Lab # 12

Background Information

Dissolved Oxygen & Aquatic Primary Productivity

Oxygen plays a major role in the biochemical reactions used by organisms to produce energy for life. During oxidative phosphorylation and electron transport, it is generally the final electron acceptor. The greatest number of ATP's are produced per mole of glucose when oxygen is available. Its concentration in the environment can limit the maximal rates of metabolism. In air, there is ample concentration of oxygen available for utilization by living organisms. It represents nearly 20% of the total gases in the air. There is approximately 200ml of O_2 per liter of air, which is 9 mM O_2 /L. The situation in aquatic environments is quite different.

In aquatic environments, oxygen is not very soluble and its solubility is directly affected by the concentration of dissolved ions such as salt, the pH, and the temperature. Salinity is the content of dissolved salts in water. It is usually expressed in parts per thousand (ppt). The solubility of O_2 is inversely proportional to the concentration of salt and temperature. As the salt concentration and temperature increase, the solubility of O_2 decreases. Even at its maximum solubility, which is in fresh water at 0°C, its concentration is only 25% of the amount in air. In practical situations, the concentration of O_2 in aquatic environments usually does not exceed 3-4% of the concentration of O_2 in air. This is only 6.0ml O_2/L or 0.3 mM O_2 /L. In salt water, O_2 concentration would be less since oxygen is less soluble in solutions of increasing ionic strength.

Since the maximum concentration of dissolved O_2 in water is dependent upon other components dissolved in water, O_2 level is often used in water quality and pollution testing. The higher the level of **dissolved oxygen (DO)**, the better the water quality. Because of the low concentration of O_2 in aquatic environments, it has been suggested that O_2 is a major limiting nutrient for life. It is interesting to note that the largest aquatic animals, the whales, are air breathing mammals.

In addition to the chemical factors that influence dissolved oxygen, biological processes such as photosynthesis and respiration affect the maximum amount of aqueous oxygen concentrations. There are both biological demands and chemical demands placed on the oxygen available in water. These are often called the **Biological Oxygen Demand (BOD)**, and the **Chemical Oxygen Demand (COD)**. Photosynthesis in aquatic environments performed by plants, and small phytoplankton which are one-celled plants, will increase the concentration of DO in the water. Respiration will decrease the concentration of DO in an aquatic environment. Therefore during the day, when photosynthesis is at a maximum, the concentration of DO will increase. During the night, the concentration of DO will decrease.

Ecologists will often study an ecosystem by measuring or estimating the **"primary productivity"**. Energy to support the life requirements of

Dissolved Oxygen & Aquatic Primary Productivity

animals, plants, and bacteria generally enters an ecosystem as light energy, which is converted by photosynthetic organisms to chemical energy. **Primary productivity** is defined as the rate at which plants assimilate the energy of sunlight. This rate directly affects the growth of plants and other chlorophyll containing organisms. This in turn affects the growth of animals that feed on plants and others which are at higher **trophic** levels. Primary productivity therefore is at the base of the trophic structure (the organization of feeding relationships) in an ecosystem.

The equation for primary productivity, which is the utilization of carbon dioxide and water to produce glucose and oxygen during photosynthesis is;

$$6\,CO_2 + 6\,H_2O \rightarrow C_6H_{12}O_6 + 6\,O_2$$

From the above equation, the amount of oxygen produced can be directly related to the amount of carbon dioxide consumed. For each mole of O_2 produced, one mole of CO_2 is consumed. One milliliter of O_2 gas contains 1.432 mg of oxygen. Therefore, based on formula weights, one can calculate that for each milliliter of oxygen produced, approximately 0.536 mg of carbon has been consumed.

The accumulation of organic matter through the growth and reproduction of the plant is not the only productivity possible. A significant portion of the sugar produced by photosynthesis is used by the plant for other synthetic and maintenance reactions during **respiration**. Therefore, the **gross primary productivity (GPP)** is the sum of the organic material produced plus the respiration rate **(Rs)** of the plant or **GPP = NPP + Rs**. **Net primary productivity (NPP)** equals the gross productivity minus the respiration rate (Rs) or **NPP = GPP – Rs**. Net productivity is therefore a measurement of growth and reproduction. Gross productivity is a measurement of growth and reproduction plus the respiration rate.

In terrestrial environments, plant primary productivity is usually measured in terms of the increase in the amount of plant **Biomass**. In aquatic environments, we measure the plant primary productivity by gas exchange, since the concentration of dissolved oxygen in water is easily determined. The classical method for determining the productivity of an aquatic environment is the **light and dark bottle procedure**. In a natural setting, for example, sealed bottles containing samples of pond water would be suspended at different depths beneath the surface of the pond. A clear "light" bottle which allows sunlight to enter, and a "dark" bottle which excludes sunlight, would be suspended at each depth. In the laboratory, one can simulate the attenuation of sunlight, which increases as depth increases, by wrapping the light bottles with screens. We will assume that the respiration is equal at all depths. In the light bottles, the production of oxygen by photosynthesis and the consumption of oxygen occur simultaneously. Therefore the **change in oxygen levels in the light bottles is a direct measure** of **the "net productivity"**. In the dark bottles, oxygen is consumed since only respiration

AP Biology

Lab # 12

Background Information

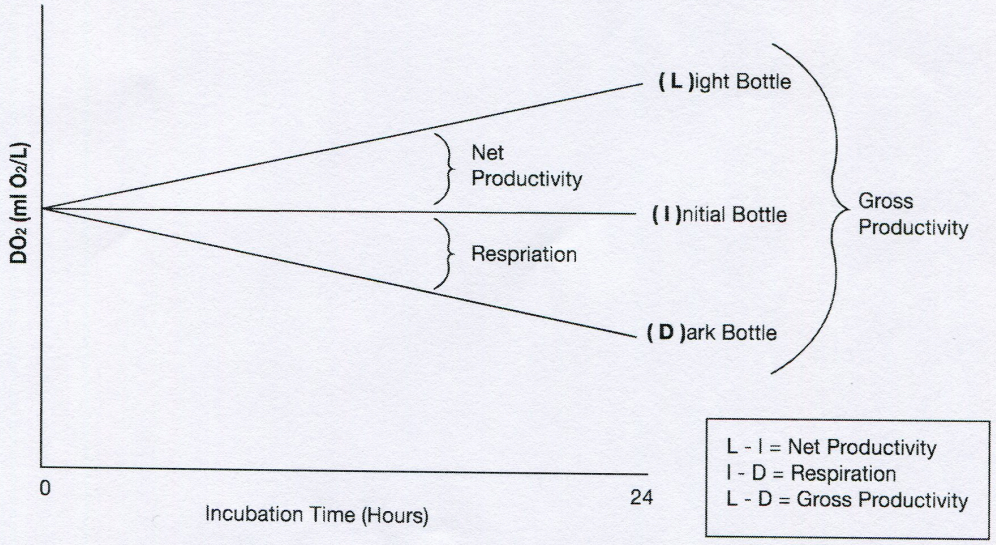
Dissolved Oxygen & Aquatic Primary Productivity

occurs. Therefore, **the change in oxygen levels in the dark bottles is a measure of respiration (Figure 1)**. Addition of the amount of oxygen consumed by respiration in the dark bottle and the oxygen produced in the light bottle, yields gross productivity. The respiration rate includes that of plants, animals, and bacteria. Therefore, only the gross productivity measurement is totally valid.

The **Winkler method** is used to determine dissolved oxygen. This procedure is an iodometric method. The iodide ion is an effective reducing agent which has been widely used for the quantitative analysis of many oxidants. Generally, sodium thiosulfate is used to titrate the iodine liberated by the chemical reaction. The endpoint is determined by the loss of color during titration. This procedure is explained in detail under Part A, Student Experimental Procedures.

The level of dissolved oxygen (DO) in water is a direct measurement of the quality of the water. This is affected by physical conditions such as temperature, salinity, and pH. In addition, it is dramatically affected by both the Biological Oxygen Demand (BOD), and Chemical Oxygen Demand (COD). Lakes loaded with decaying matter have low levels of oxygen, and a high BOD, since the oxygen is being consumed by bacteria and algae. This exercise demonstrates the methodology used for measuring dissolved oxygen and determining the primary productivity of a natural body of water.

Figure 1:
Gross Productivity Determined by Light/Dark Bottle Method



Experiment Overview

EXPERIMENT OBJECTIVES:

Students will:

1. Understand the factors that affect the solubility of dissolved gases in aquatic environments
2. Understand the effects of light intensity and nutrients on the rate of primary productivity
3. Understand the relationship between dissolved oxygen and the processes of photosynthesis and respiration and the affect on primary productivity
4. Be able to describe a method to measure dissolved oxygen, and define primary productivity and factors which influence it .

WORKING HYPOTHESIS

If light intensity and the addition of nutrients affect the rate of photosynthesis in aquatic environments, then primary productivity will also be affected.

LABORATORY SAFETY

Gloves and safety goggles should be worn routinely as good laboratory practice.

AP Biology

Lab # 12

Experiment Procedure

Part A: The Winkler Method for Dissolved Oxygen

THE WINKLER METHOD FOR DISSOLVED OXYGEN

The Winkler method will be used to determine the concentration of dissolved oxygen in water samples. The samples are first treated with an excess of manganese sulfate, sodium iodide, and sodium hydroxide. The white manganese (II) hydroxide precipitate that forms will rapidly react with the dissolved oxygen in the samples to form manganese (III) hydroxide. When the samples are acidified by the addition of sulfuric acid, the manganese (III) oxidizes iodide to iodine. The concentration of the liberated iodine, I_2 is titrated with sodium thiosulfate in the presence of a starch indicator solution.

It is important to note that the starch is added after the bulk of the iodine has been reduced. In acidic solutions, the starch would be decomposed by a large excess of iodine. For fine analysis, the starch is added after the titration has begun, near the point when the solution has become a faint, pale yellow color. For purposes of this laboratory, the starch can be added at the beginning of titration to facilitate end point detection.

The balanced equations for the series of chemical reactions used in the Winkler method are shown below.

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

When acidified by addition of sulfuric acid;

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

The liberated iodine is titrated to endpoint with sodium thiosulfate as shown below.

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

When the titration is nearly complete, the solution will begin to change from a purple/brown color to a colorless solution. As can be seen from the above reactions, four moles of sodium thiosulfate titrant, $Na_2S_2O_3$ are required per mole of dissolved oxygen, O_2. The concentration of the sodium thiosulfate titrant has been adjusted so one milliliter of sodium thiosulfate solution equals one milligram of dissolved oxygen per liter of sample, 1 mg DO/L.

AP Biology

Lab # 12

Experiment Procedure

Part A: The Winkler Method for Dissolved Oxygen

16. For the sensitivity required in this laboratory experiment, the total volume in milliliters of the sodium thiosulfate solution used to titrate the sample to the endpoint is exactly equivalent to the dissolved oxygen concentration in milligrams of dissolved oxygen (DO) per liter of sample solution, mg DO/L.

17. Record the temperature of your sample and the amount of dissolved oxygen in the chart. Use the nomogram of oxygen saturation graph on this page to determine the % saturation of DO in the sample. If your temperature lies between these values, estimate the % saturation of DO. Record the value in **Table 1**.

TABLE 1: Temperature/DO Data

Temperature	Your DO mg/L	%DO Saturation (from nomogram)	Class Mean DO mg/L	Class Mean DO Saturation (from nomogram)

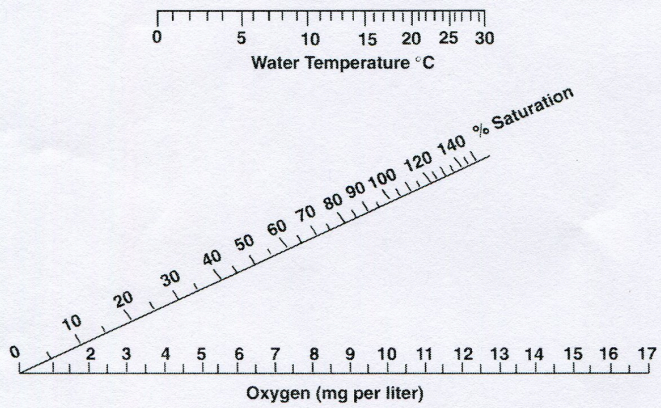


Figure 2:
Nomogram of Oxygen Saturation

18. Determine the class average data for the water samples at the three temperatures which were available. Record the values in **Table 1**.

19. Using linear graph paper or **Graph #1** provided on page 18, plot **Both** the lab group and class means percent saturation as function of temperature.

Part B: Primary Productivity

PRIMARY PRODUCTIVITY

For Part B., samples of natural 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. Review the Background section of this laboratory for a complete discussion of Primary Productivity.

Day One

1. Obtain 7 clean "BOD" (Biological Oxygen Demand) containers. Any container which holds approximately 250-300ml and which can be closed with an airtight seal is appropriate.
   - 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.
   - Stopper or close the container. Turn the container upside down and use a paper towel to remove any water which is around the outside of the stopper or lid.
2. Label the 7 bottles as follows:
   #1 - Initial
   #2 - Dark
   #3 - 100%
   #4 - 65%
   #5 - 25%
   #6 - 10%
   #7 - 5%
3. Bottle #1 is the initial starting bottle and serves as a baseline.
4. Wrap Bottle #2 in aluminum foil, for it serves as the dark (no light) control. Place in a dark place. No photosynthesis, only respiration, will occur here.
5. Bottles #3 –#7 will simulate the depth in a body of water that natural light will attenuate This is done by wrapping each bottle with a different number of screens. Bottle #3 will have no screens with 100% attenuation of light, Bottle #4 has 1 screen with 65% attenuation, Bottle #5 has 3 screens with 25% attenuation, Bottle #6 has 5 screens with 10% attenuation, and Bottle #7 has 7 screens with 5% attenuation. Cover the bottoms of the bottles so that no light can enter.

AP Biology

Lab # 12

Experiment Procedure

Part B: Primary Productivity

NOTE:
Each group will be assigned one of the following number of screens for Bottles 3, 4, & 5.

Percent Light	Number of Screens
100	0
65	1
25	3
10	5
2	8

6. Put bottles #3-7, labels down, on their sides exposed to a constant source of light. Your teacher will indicate where they should be placed. Leave these bottles overnight.

   Record the starting time here. _____.

7. You will fix the amount of dissolved oxygen in the Bottle labeled '#1 Initial'. This serves as the starting level of dissolved oxygen in the lake water sample. You will perform step 3 from the Winkler procedure used in Part A of this laboratory.

   - Open the container labeled "#1 Initial" and carefully pipet 2 ml of manganese sulfate into the container. Make sure the pipet tip is below the surface of the water in the container.
   - With a fresh pipet, add 2ml of the NaOH/NaI (alkaline-Iodide) solution into the sample in the container. Make sure the pipet tip is below the surface of the water in the container.
   - Stopper or seal your container. Firmly hold the stopper or lid of your container. Carefully invert the bottle to allow for complete mixing of the sample, manganese sulfate, and NaOH/NaI. A precipitate will form.
   - Let the bottle sit on the laboratory bench overnight. Finish processing it with the rest of the bottles tomorrow.

Optional Activity

If directed by the instructor, place several drops of the pond water on a microscope slide and cover with a coverslip. Observe the organisms found in the natural water sample. Draw what is observed and try to identify the organisms.

Part B: Primary Productivity

Day Two

1. Fix the dissolved oxygen in Bottles #2, 3, 4 , 5, 6 , 7. This is the beginning of the Winkler procedure.

   Record the time you begin here. _____

   - Open Bottle #2 and carefully pipet 2ml of manganese sulfate into the bottle. Make sure pipet tip is below surface of the water in the container.
   - With a fresh pipet, add 2ml of the NaOH /NaI (alkaline-iodide) solution to the sample in the bottle. Make sure pipet tip is below surface of water in the container.
   - Stopper or seal the bottle. Firmly hold the stopper or lid and carefully invert the bottle to allow for complete mixing of the sample, Manganese sulfate, and NaOH/NaI. A precipitate will form.

2. Repeat procedure in step 1 above with Bottles #3, 4, 5, 6, 7.

3. While precipitate is settling in Bottles #2, 3, 4, 5, 6 and 7, review the Winkler procedure used in Part A. Obtain Bottle #1.

4. You should clean and set up the burettes as outlined in Part A, steps 4-7.

5. Begin processing all of the Bottles, #1 - #7, using the Winkler procedure for the determination of dissolved oxygen from Part A. Part A Step 3, the fixing of the dissolved oxygen, has already been completed for all of the bottles. Now continue with Part A Step 8. Continue through steps 16. Record DO values in the appropriate table - **Table 2 or 3.**

TABLE 2: Respiration

	Individual Data	Class Mean
DO, Initial		
DO, Dark Bottle		
Respiration Rate (Initial - Dark)		

AP Biology

Lab # 12

Experiment Procedure

Part B: Primary Productivity

TABLE 3: Productivity of Screen-Wrappen Samples

# of Screens	% Light	DO	INDIVIDUAL DATA		CLASS MEAN PRODUCTIVITY		
			Gross Productivity	Net Productivity	DO	Gross Productivity	Net Productivity
0	100						
1	65						
3	25						
5	10						
7	5						

6. In reporting the data for productivity, the concentration of dissolved oxygen which has been determined, will be converted from mg/L to ml/L using the following conversion factor: 1 mg DO/L = 0.698ml DO/L. Therefore,

$$\frac{[0.698\text{ml DO/L}]}{1\text{mg DO/L}} \times (\#\text{mg DO/L}) = \#\text{ml DO/L}$$

7. Calculate and record the gross and net productivity for the natural water samples using the following equations. Productivity is a rate term, therefore, divide by the number of hours the experiment ran to arrive at a value of ml DO/L per hour.

   - 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. Pr #7) ml DO/L - Initial Bottle (#1) ml DO/L) /hours
   - Respiration rate = (Initial Bottle (#1) ml DO/L - Dark Bottle (#2) ml DO/L) /hours

Part B: Primary Productivity

8. Record the values for the individual experiment conditions here.
   - Respiration rate = _____ml DO/L/hour.
   - Bottle 3
     Gross Productivity rate = _____ ml DO/L/hour
     Net Productivity rate = _____ ml DO/L/hour
   - Bottle 4
     Gross Productivity rate = _____ ml DO/L/hour
     Net Productivity rate = _____ ml DO/L/hour
   - Bottle 5
     Gross Productivity rate = _____ ml DO/L/hour
     Net Productivity rate = _____ ml DO/L/hour
   - Bottle 6
     Gross Productivity rate = _____ ml DO/L/hour
     Net Productivity rate = _____ ml DO/L/hour
   - Bottle 7
     Gross Productivity rate = _____ ml DO/L/hour
     Net Productivity rate = _____ ml DO/L/hour

9. Record the individual and class average values for respiration in **Table 2** and for light of different intensities in the **Table 3**. The values should be reported in ml DO/L/hour.

10. Record the **average** class value for the Respiration rate here. _____.

11. Using linear graph paper or **Graph #2** provided on page 19, plot the average gross and net productivity on the Y-axis in ml DO/L/hr versus the percent of Light Intensity on the X-axis.

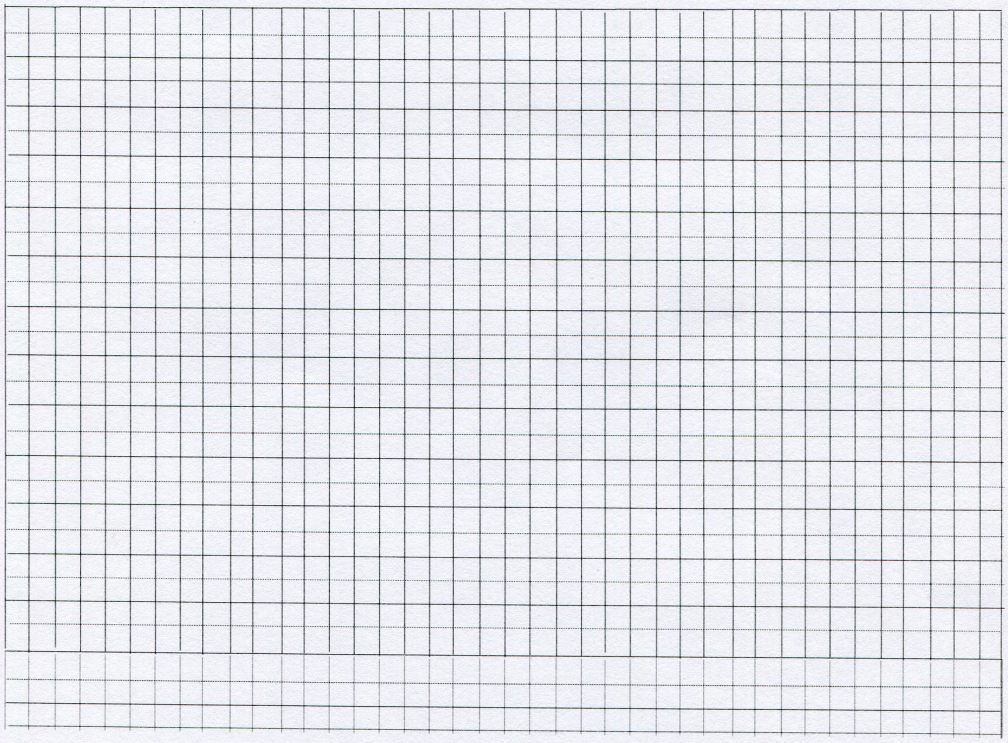
AP Biology

Lab # 12

Experiment Procedure

Analysis of Results - Graph #1

1. Graph both the lab group and class means percent saturation as function of temperature.
2. Title the Graph_____
3. Determine the *independent* variable (horizontal (X) axis). Label the graph.
4. Determine the *dependent* variable (vertical (Y) axis). Label the graph.
5. What hypothesis is being tested in this experiment?



Analysis of Results - Graph #2

1. Graph the average gross and net productivity values for samples as a function of light intensity (%).
2. Title the Graph_____
3. Determine the *independent* variable (horizontal (X) axis). Label the graph.
4. Determine the *dependent* variable (vertical (Y) axis). Label the graph.
5. What hypothesis is being tested in this experiment?