# LAB TWELVE

# DISSOLVED OXYGEN AND AQUATIC PRIMARY PRODUCTIVITY

**OVERVIEW**

1. In Exercise 12A you will measure and analyze the dissolved oxygen (DO) concentration in water samples at varying temperatures.

2. In Exercise 12B you will measure and analyze the primary productivity of natural waters or lab cultures using screens to simulate the attenuation (decrease) of light with increasing depth.

**OBJECTIVES**

**Before doing this lab you should understand:**
- The biological importance of carbon and oxygen cycling in ecosystems,
- How primary productivity relates to the metabolism of organisms in an ecosystem,
- The physical and biological factors that affect the solubility of gases in aquatic ecosystems, and
- The relationship between dissolved oxygen and the processes of photosynthesis and respiration and how these processes affect primary productivity.

**After doing this lab you should be able to:**
- Measure primary productivity based on changes in dissolved oxygen in a controlled experiment, and
- Investigate the effects of changing light intensity on primary productivity in a controlled experiment.

**INTRODUCTION**

In the aquatic environment, oxygen must be in solution in a free state ($0_2$ ) before it is available for use by organisms. Its concentration and distribution in the aquatic environment are directly dependent on chemical and physical factors and are greatly affected by biological processes. In the atmosphere there is an abundance of oxygen, with about 200 milliliters of oxygen for every liter of air. Conversely, in the aquatic environment there are only about 5 to 10 milliliters of dissolved oxygen in a liter of water.

Oxygen, found in both aquatic and terrestrial environments, is necessary to the metabolic processes of virtually all life-forms. Dissolved oxygen, therefore, is an important indicator of water quality.

Look at the data with respect to milliliters of oxygen given in the first paragraph. Aquatic and terrestrial environments do not have the same ability to hold oxygen. If an equal volume of air and very cold water were compared it would be found that the air contained over 95% more oxygen than the water. In addition, water's ability to hold dissolved oxygen rapidly decreases as the temperature of the water increases. Because water does not hold oxygen as efficiently as air, respiration and organic degradation easily deplete its dissolved oxygen concentration. Take the example of untreated emissions from sewage treatment plants. These emissions are often responsible for oxygen depletion. The organic composition of this waste requires a great deal of oxygen as it decomposes; areas near municipal treatment facilities are often monitored for their dissolved oxygen content because of this. The only way to avoid complete anoxia (lack of oxygen) is for oxygen to be replenished from the atmosphere and from the biological activity in the aquatic environment.

**Dissolved Gases**

Surface waters in contact with a mixture of gases and water vapor absorb some of its components. There are five important gases dissolved in aquatic environments; all have biological and physiochemical functions, but they differ from one another in behavior and origin. Nitrogen, oxygen, and carbon dioxide are especially important; nitrogen and oxygen are the most abundant constituents of the atmosphere, about 78% and 21 % respectively, at sea level. Water vapor is present in varying amounts up to 3% by volume.

Some oxygen goes into solution if the water is undersaturated. It is about one-fourth as abundant in the air as nitrogen but is more than twice as soluble. The amount of oxygen absorbed depends on temperature, salinity, and pressure. Cold water absorbs more oxygen than does warm water, salinity decreases solubility, and pressure increases it

Most gases obey Henry's Law, which states that at a constant temperature, the amount of gas absorbed by a given volume of liquid is proportional to the pressure in atmospheres that the gas exerts. Carbon dioxide, however, may combine with various cations upon

entering natural waters to become more abundant than the precepts of Henry's Law dictate. It is found both free and in combined states. With the following formula, the amount of an atmospheric component found dissolved in an aquatic environment can be predicted.

$$c = K \times p$$

c = Concentration of the gas that is absorbed K = Solubility factor (differs from gas to gas) p = Partial pressure of the gas

## Other factors also affect the dissolved oxygen content of a body of water:

**Temperature-As** the temperature of the water increases, the concentration of the dissolved oxygen decreases. As a result, there is a seasonal fluctuation in dissolved oxygen concentration in a body of water.

**Wind-**oxygen is mixed into the water as wind blows across the surface. On windless nights, oxygen depletion can be so severe that it can cause substantial fish kills.

**Turbulence-**As water runs its course in a stream or riverbed, oxygen is mixed in at the water flow and is agitated by various obstructions such as rocks, fallen trees, and is waterfalls. A great deal of variation in dissolved oxygen concentration can be observed through dissolved oxygen measurements taken along a course of a stream or river

**Trophic State**-The amount of nutrients, such as calcium or nitrates, in the water determines how much life can be sustained in the aquatic environment, which affects the amount of oxygen used or released in the water. There are two classifications: eutrophic or oligotrophic. An eutrophic body of water is one which has a fluctuating dissolved oxygen content from varying amounts of activity of the life in the body of water, and is always rich in nutrients. An oligotrophic body of water is always rich in oxygen content but is poor in plant nutrients. The oxygen content is constant because there isn't much variation in life activity that could cause serious depletion.

Although standards for dissolved oxygen vary, it has been found that a concentration of dissolved oxygen less than 4ppm (parts per million) is stressful to most forms of aquatic life. The ideal range for an adequate game fish population of bass, pike, or walleye, for example, is about 8 to 15ppm.

**Primary Production**

Energy accumulated by plants is termed production or, more specifically, primary production, since it is the first and basic form of energy storage. All production in an ecosystem stems from the energy in organic substances that autotrophs, or primary producers, create from inorganic raw materials. The flow of energy through a community starts with the fixation of sunlight by plants (photosynthesis), which in itself demands the expenditure of energy. All of the sun's energy that is assimilated in photosynthesis is termed gross primary production. Since plants, like other organisms, must overcome the tendency of energy to disperse (entropy), free energy (available to do work) must be expanded for production as well as for other biological functions such as maintenance and reproduction. The energy required for this is provided by the reverse of the photosynthetic process-respiration. The energy remaining after respiration and stored as organic matter is termed net primary production, or plant growth.

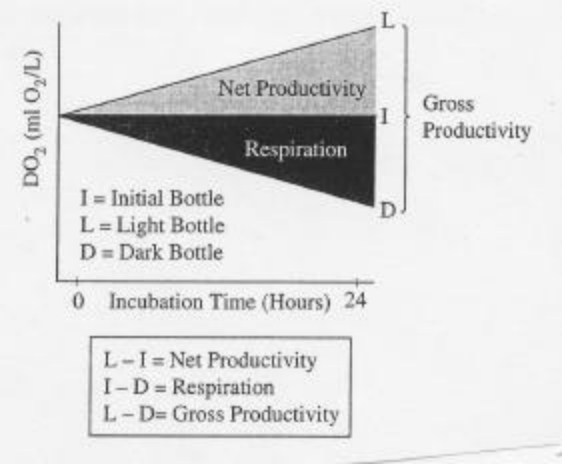
**Primary Production Measured by the Oxygen Method**

Two bottles with a given concentration of phytoplankton (small aquatic organisms) are suspended at the depth from which the samples were obtained. The "dark" bottle is wrapped in electrical tape, aluminum foil, etc., to exclude light; the "light" bottle is clear. A quantity of oxygen proportional to the total organic matter fixed (gross production) is produced by photosynthesis in the light bottle. At the same time, some of the oxygen is being utilized in respiration. The amount of oxygen left is proportional to the amount of fixed organic matter remaining after respiration (net production). The quantity of oxygen in the light bottle indicates the net photosynthesis, or net primary production in the dark bottle, oxygen is utilized but not produced. Subtracting the quantity of oxygen at the start, determined by the oxygen measurement taken from a control (initial bottle), from the amount left at the end of the run-usually 24 hours, determines the quantity of oxygen utilized (respiration). The amount of oxygen in the light bottle added to the amount used in the dark bottle provides an estimate of total photosynthesis , or gross production.

Respiration = Initial Bottle - Dark Bottle
Net Primary Production = Light Bottle - Initial Bottle
Gross Production = (Light Bottle - Initial Bottle) + (Initial Bottle - Dark Bottle)

This equation can be shortened to read Gross Production = Light Bottle - Dark Bottle, but without the Initial Bottle test neither Respiration or Net Primary Production can be learned. See Figure 12.3 for a pictorial representation

Figure 12.3




In a modified version of this method, the whole aquatic ecosystem can be represented by the bottles, with the light bottle representing the daytime and the dark bottle representing the night. The oxygen content of the water is measured every two to three hours over a 24-hour period, providing the rise and fall of oxygen during the day and night that can be plotted on a diurnal curve.

Still another modification of the light and dark bottle method suited for terrestrial communities involves measuring the amount of carbon dioxide produced. A transparent plastic bag is placed over a sample. Air is drawn through the enclosure and passed over carbon dioxide-absorbent materials. The same procedure is conducted with a dark plastic bag. The amount of carbon dioxide produced under the dark bag is a measure of respiration; under the transparent bag is the quantity of carbon dioxide equivalent to the amount of photosynthesis minus the amount of respiration. The two results added together indicate gross production.

**Conversion of Oxygen Data to Carbon**

Limnologists prefer to express primary production in terms of carbon fixed rather than oxygen evolved. Oxygen values, therefore, are often converted to carbon. One method assumes that 1 mole of oxygen is released for each mole of carbon dioxide that is fixed, as implied in the simple photosynthetic formula below. The molecular weights, 44 for carbon dioxide and 32 for oxygen, are used to convert oxygen evolved to carbon dioxide consumed: 44/32 = 1.375.

A measure of oxygen production over time provides a means of calculating the amount of carbon that has been found in organic compounds over a period of time. For each millimeter of oxygen produced, approximately 0.536 milligrams of carbon has been fixed.

**Oxygen Cycle**

Oxygen, free in the atmosphere and dissolved in water, is a byproduct of photosynthesis. Life-forms (microorganisms, plants, animals) use oxygen in respiration and return it to the air and water in the form of raw carbon dioxide. The carbon dioxide is utilized by various microbes and green plants as an essential raw material for carbohydrate synthesis.

**Photosynthesis**

Microorganisms such as anaerobic phototrophic bacteria, cyanobacteria, green protists, etc., and plants sustain all life on earth by transforming the energy of sunlight and carbon dioxide into food and oxygen:

Visible Light (Photosynthesis)

$$12H_20 + 6CO_2 => C_6H_{12}O_6 + 60_2 + 6H_2O$$

**Assaying Dissolved Oxygen in Aqueous Solutions**

It is possible to determine the amount of oxygen in water with the Winkler titrametric method. The procedure involves the addition of alkaline iodide and manganous sulfate to a water sample. Manganous hydroxide is produced and, upon acidification, is converted to a manganese compound by the oxygen in the water sample. The compound immediately reacts with the iodide to release iodine, which colors the water a dark yellow. The quantity of free iodine is equivalent to the amount of oxygen in the sample. The amount of iodine is quantified by titration with sodium thiosulfate until an endpoint is reached, signified by the sample losing its color. The Winkler method's precision range is 0.1 to 0.6%.

**Steps in Winkler Method**

1. Production of a manganous hydroxide in the water sample to which manganous sulfate is introduced when KOH plus KI are added:

$$MnS0_4 + 2KOH => Mn(OH)_2 + K_2S0_4$$

2. Oxidation of manganous hydroxide to manganic hydroxide by the dissolved oxygen in the sample:

$$2Mn(OH)_2 + 0_2 + 2H_20 => 2Mn(OH)_4$$

3. Conversion of manganic hydroxide to manganic sulfate when concentrated sulfuric acid is added:

$$2Mn(OH)_4 + 4H_2S0_4 => 2Mn(SO4)_2 + 8H_20$$

4. Replacement of iodine in an iodide (KI) by sulfate, releasing free iodine:

$$2Mn(OH)_4 + 4KI => 2MnSO_4 + 2K_2SO_4 + 2I_2$$

5. Titration of the iodine solution with sodium thiosulfate until all free iodine combines into sodium iodide. The endpoint, marked by the disappearance of the yellow color:

$$4Na_2S_2O_3 + 2I_2 => 2Na_2S_40_6 + 4NaI$$

**Materials Needed**

Chlorella Culture or Water Sample
Pipet
Microscope Slide
Coverslip
Light Compound Microscope
Cloth Squares
Aluminum Foil
BOD Bottle.

**Shared Materials**

Direct-Reading Titrator
Titration Tube
Manganous Sulfate Solution
Alkaline Potassium Iodide Azide
Sulfuric Acid
Starch Indicator Solution
Sodium Thiosulfate

## A. Measurement of Dissolved Oxygen

**Procedure**

**Safety:** *Wear personal protection equipment: nitrile rubber gloves, apron, chemical safety goggles.*

**Note:** *Your instructor may have collected the water sample in advance. If this is the case, begin with step 2.*

1. Your instructor will assign one or more water temperatures for your sample: 0°, 20°, or 30°C. You may want to verify the temperature of your sample to ensure that it has reached, and remains at, the desired temperature

2. Thoroughly rinse out the sampling bottle. Remove the cap and slowly submerge the bottle in the water. Allow the bottle to fill. Remove any air bubbles from the side of the bottle by tapping on the side. Cap the bottle while still submerged. If using a water sampler, siphon or drain the tube on the sampler to fill a BOD bottle. Place the siphon or drain hose at the bottom of the bottle, filling the bottle to overflowing by approximately one-third its volume, Seal the bottle with a cap so that no air pockets are created and excess water is removed.

3. Add eight drops of manganous sulfate to the sample bottle with a pipet. Be sure no air is added.

4. Add eight drops of alkaline iodide to the sample bottle with a pipet. Be sure no air is added. Note that the precipitate manganous hydroxide is produced immediately.

5. Cap the bottle and mix by inverting it several times.

6. Allow the manganous hydroxide precipitate to settle until it is below the shoulder of the bottle.

7. While the precipitate is settling, carefully fill the titration syringe with thiosulfate working solution.

8. Carefully add one scoop of acid to the sample bottle, then will mix by inverting the bottle several times. Note that the precipitate dissolves. The sample should turn a clear yellow as free iodine is formed.

9. Carefully measure out 20ml of the sample into a sample cup. Place the cup on top of a white sheet of paper to better see the color of the sample.

10. Add eight drops of starch solution to the 20ml sample. The starch solution will change the liquid's color from yellow to purple.

11. While continually swirling the sample, titrate the 20ml sample with sodium thiosulfate working solution. Titrate one drop at a time until the color changes from purple to a pale yellow color; this is the titration endpoint-all free iodine has been converted to sodium iodide by the addition of sodium thiosulfate.

**Analysis**

1. The volume of sodium thiosulfate (in ml) used to titrate the 20ml sample is approximately equivalent to the concentration of dissolved oxygen (mg/1) in the original sample. Convert the mg/l of dissolved oxygen to ml/I using the following formula:

$$Mg\ 0_2/L\ X\ 0.698 = ml\ 0_2/L$$

2. Determine the concentration of dissolved oxygen in the sample by observing how much sodium thiosulfate working solution was required to convert free iodine.

mg/L Dissolved Oxygen = ml Titrant Used Record this value in Table 1.

3. Using the nomo graph (figure 12.2) and a straight-edge ruler, estimate the percent saturation of dissolved oxygen in your sample. Record this value in Table 1.

Table 1
Dissolved Oxygen Concentration
Lab Group Sample

| Temperature | Dissolved Oxygen (mg/l) | % Dissolved Oxygen |
|---|---|---|
| | | |

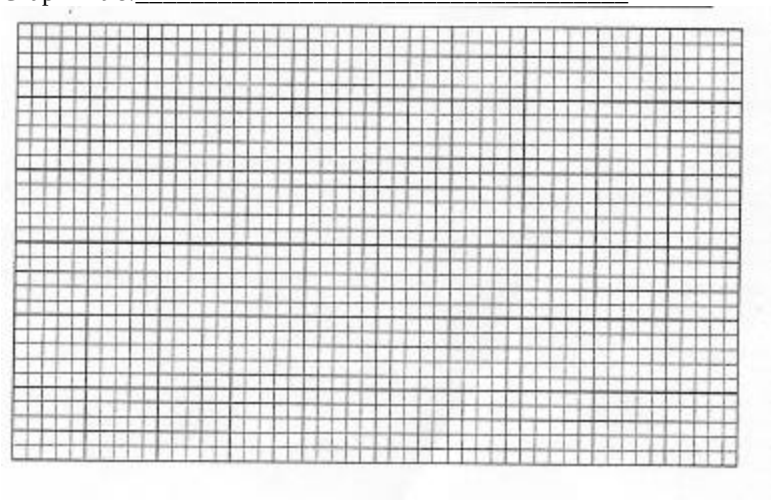
4. Collect class data for all tested samples. Calculate the mean concentration of dissolved oxygen and use the nomograph to estimate the percent oxygen saturation at each of the three temperatures used in the experiment. Record this data in Table 2.

Table 2
Dissolved Oxygen Concentration
Class Sample

| Temperature | Mean Dissolved Oxygen (mg/l) | % Dissolved Oxygen |
|---|---|---|
| | | |
| | | |
| | | |

5. Graph the class means percent saturation as a function of temperature.
   For this graph you will need to determine the following:
   a. The independent variable:_____
      Use this to label the horizontal (x) axis
   b. The dependent variable:_____

Use this to label the vertical (y) axis
Graph Title:_____

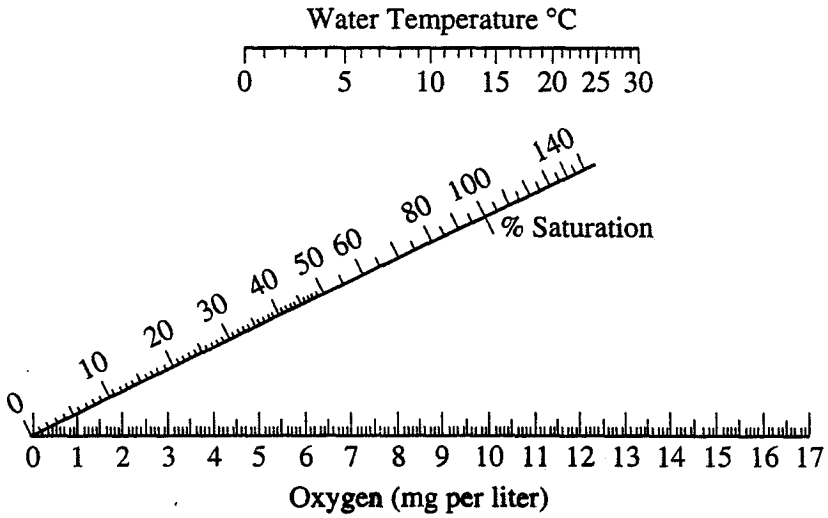


**Questions**

1. How does temperature affect the solubility of oxygen in water?

2. List and discuss three factors that could influence the dissolved oxygen concentration of a body of water.

**Figure 12.2: Nomogram of Oxygen Saturation**




**Productivity**
The **primary productivity** of an ecosystem is defined as the rate at which organic materials (carbon-containing compounds) are stored. Only those Organisms possessing photosynthetic pigments can utilize sunlight to create new organic compounds from simple

inorganic substances. Green plants obtain carbon for carbohydrate synthesis from the carbon dioxide in the water or the air according to the basic equation for photosynthesis:

$$6CO_2 + 6H_20 \text{ -+ } C_6H_{12}O_6 + 60_2$$

The rate of carbon dioxide utilization, the rate of formation of organic compounds, or the rate of oxygen production can be used as a basis for measuring primary productivity. A measure of oxygen production over time provides a means of calculating the amount of carbon that has been bound in organic compounds over a period of time. For each milliliter of oxygen produced, approximately 0.536 milligrams of carbon has been assimilated.

One method of measuring the rate of oxygen production is the **light and dark bottle method.** In this method, the DO concentrations of samples of ocean, lake, or river water, or samples of laboratory algal cultures, are measured and compared before and after incubation in light and darkness. The difference between the measurements of DO in the initial and dark bottles is an indication of the amount of oxygen that is being consumed in respiration by the organisms in the bottle. In the bottles exposed to light, the biological processes of photosynthesis and respiration are occurring; therefore, the change over time in DO concentration from the initial concentrations is a measure of **net productivity.**

The difference over time between the DO concentrations in the light bottle and the dark bottle is the total oxygen production and therefore an estimate of gross productivity.

**EXERCISE 12B: A Model of Productivity as a Function of Depth In a Lake**

**Day One**

1. In your group obtain 7 water sampling bottles (these are also called BOD bottles, for "biological oxygen demand"). Fill all the bottles with the lake water or algal sample provided. (You may be asked to add a specific weight of aquatic plants to each bottle.) Be careful not to leave any air bubbles at the tops of the bottles.

2. Use masking tape to label the cap of each bottle. Mark the labels as follows: I (for "initial"), D (for "dark"), 100%, 65%, 25 %, 10%, and 2%.

3. Determine the DO for the "Initial" bottle now. Record this DO value in Table 2 (on next page) and in the data table on the blackboard. Record the class "Initial" bottle mean in Table 2. This is the amount of DO that the water has to start with (a base line).

4. Cover the "Dark" bottle with aluminum foil so that no light can enter. In this bottle no photosynthesis can occur, so the only thing that will change DO will be the process of respiration by all of the organisms present.

5. The attenuation of natural light that occurs due to depth in a body of water will be simulated by using plastic window screens. Wrap screen layers around the bottles in the following pattern: 100% light - no screens; 65% light - 1 screen layer; 25% light - 3 screen layers; 10% light - 5 screen layers; and 2% light - 8 screen layers. The bottles will lie on their sides under the lights, so remember to cover the bottoms of the bottles to prevent light from entering there. Use rubber bands or clothespins to keep the screens in place.

6. Place the bottles on their sides under the bank of lights in the classroom. Be sure to turn the bottles so that their labels are down and do not prevent the light from getting to the contents. Leave overnight under constant illumination.

7. (Optional Exercise.) If time permits, make a wet mount slide of a sample of the lake water used for this experiment and draw some of the organisms you observe. Can you identify them?

Table 2
Respiration

| | Individual Data | Class Mean |
|---|---|---|

| | | |
|---|---|---|
| Initial DO | | |
| Dark DO | | |
| Respiration Rate (Initial-Dark) | | |

**Day Two**

8. Determine the DO in all the bottles that have been under the lights. Record the "Dark" bottle DO in Table 12.2. Calculate the respiration rate using the formula in the table. Record the values for the other bottles in Table 12.3. Complete the calculations in Table 12.4 to determine the gross and net productivity in each bottle. The calculations will be based on a time period of 1day. Enter your respiration rate and gross and net productivities in the data table on the class blackboard. Determine the class means. Enter these means in Table 12.2 and Table 12.4.

Table 3
Individual Data-Productivity of Screen-Wrapped Samples

| # Of Screens | % Light | DO | Gross Productivity (light Bottle - Dark Bottle] | Not Productivity [light Bottle - Initial Bottle] |
|---|---|---|---|---|
| 0 | 100% | | | |
| 1 | 65% | | | |
| 3 | 25% | | | |
| 5 | 10% | | | |
| 8 | 0% | | | |

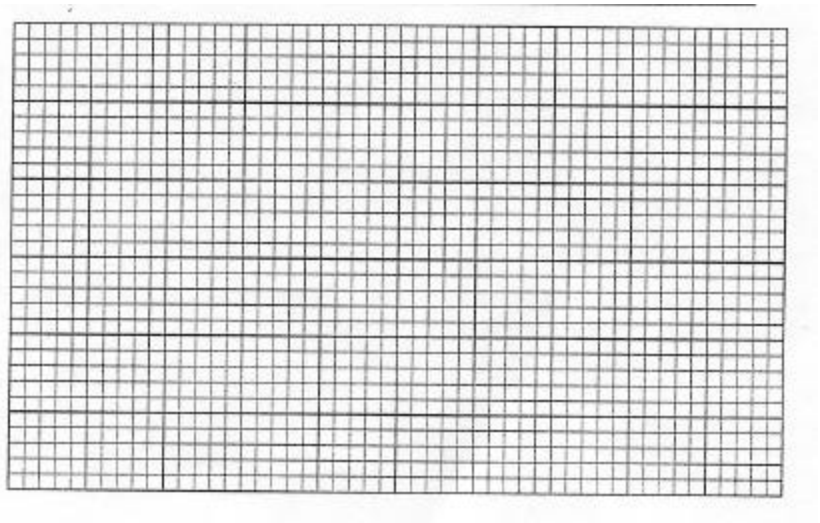
Table 4
Class Data-Mean Productivity

| # Of Screens | % light | Gross Productivity | Net Productivity |
|---|---|---|---|
| 0 | 100% | | |
| 1 | 65% | | |
| 3 | 25% | | |
| 5 | 10% | | |
| 8 | 2% | | |

9. Graph both net and gross productivities as a function of light intensity (class means). The two kinds of productivity may be plotted on the same graph. For this graph you will need to determine the following:

a. The *independent* variable:_____-
Use this to label the horizontal (x) axis.

b. The *dependent* variable:_____
Use this to label the vertical (y) axis.



**Questions**

1. What are three ways primary productivity can be measured?

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

3. From your graph of the temperature data, what is the effect of temperature on the amount of oxygen that water at different temperatures can hold?

4. Refer to your graph of productivity and light intensity. At what light intensity do you expect there to be:

No gross productivity? _____ No net productivity?_____

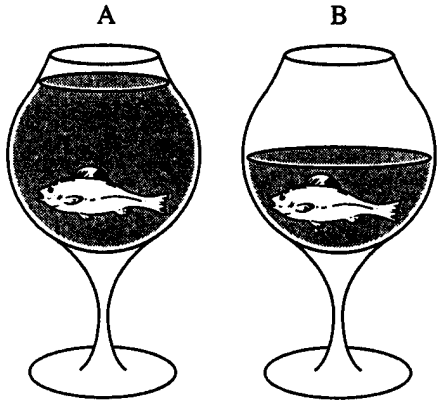
5. A mammal uses only I to 2 percent of its energy in ventilation (breathing air in and out) while a fish must spend about 15 percent of its energy to move water over its gills. Explain this huge difference in their efforts to collect oxygen.

6. Would you expect the DO in water taken from a stream entering a lake to be higher or lower than the DO taken from the lake itself? Explain.

7. Would you expect the DO concentration of water samples taken from a lake at 7:00 a.m. to be higher or lower than samples taken at 5:00 p.m.? Explain.

8. In the following drawings of identical containers with identical fish but with different volumes of water, which one, A or B, would have more oxygen available to the fish? Explain.

9. What is eutrophication? Research and explain why allowing nitrogen or phosphorous fertilizers to run into a body of water can negatively affect life in it.



Part C Productivity Simulation (This analysis is to be completed in groups of two. Find a partner and make sure both names are on this sheet)

**Procedure**

Productivity of a body of water can be estimated by measuring the productivity of water samples and then plotting the rates on a depth profile graph. To create a depth profile, the degree to which the body of water attenuates (reduces) light must be known. Usually these data are generated by photometer readings. An estimate of the compensation level ($Z_{sd}$) is done using a Secchi disk. Data was collected from two lakes:

**Lake 1**

| Percent of Incident Light | Depth (meters) |
|---|---|
| 100% | 0.0 |
| 65% | 0.5 |
| 25% | 1.5 |
| 10% | 2.5 |
| 2% | 4.0 |

**Lake 2**

| Percent of Incident Light | Depth (meters) |
|---|---|
| 100% | 0.0 |
| 65% | 1.5 |
| 25% | 4.0 |
| 10% | 7.0 |
| 2% | 11.0 |

## Analysis

1. Simulate primary productivity for the two data sets, above, by converting your Gross Productivity data for the samples to carbon productivity (mg C/$M^3$). Convert your respiration data to carbon in Mg C/M3 using the following formula. Enter this data in the last column of Table 3.

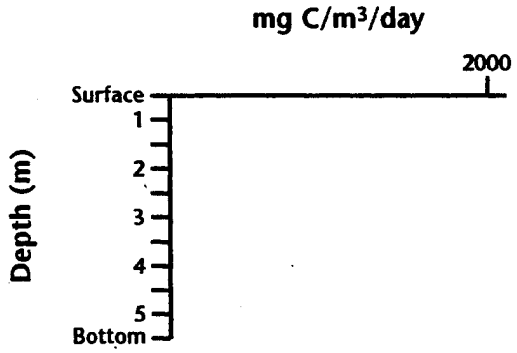
   Ml 02/1 = 0.698 x Mg 02/1

   For each ml of oxygen produced, 0.536mg of carbon is assimilated into organic compounds.

   Gross Productivity = Light Bottle (02/1) - Dark Bottle (02/1)

   To convert liters into meters cubed, divide the liters by 0.001.

2. On graph paper, plot this converted data at the depth at which they could occur in each of these lakes. Assume that the respiration rate is the same for all depths.




## Questions

1. Based on your analysis, which lake is more productive?
2. What is used as the basis for measuring primary productivity?