

WARD'S

AP Biology Lab 12

Dissolved Oxygen & Primary Productivity

Lab Activity

Student Study Guide

BACKGROUND



Eutrophic:

“well nourished”, an environment where high nutrient concentrations lead to high plant growth.

Oligotrophic:

An environment where low nutrient concentration leads to low plant growth.

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.

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. The only way to avoid complete anoxia is for oxygen to be replenished from the atmosphere and from the biological activity in the aquatic environment.

Untreated emissions from sewage treatment plants 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.

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 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.



DID YOU KNOW?

The most productive systems, in terms of primary production, in the world are: open oceans, tropical rainforests, savannas, and tropical seasonal forests.



DID YOU KNOW?

The primary production of the world's oceans has been measured and mapped in considerable detail using the oxygen method.

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 that 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 4 ppm (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 15 ppm.

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, or the total photosynthesis that occurs, 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 expended for production as well as for other biological functions such as maintenance and reproduction. The energy required for this is provided by a reverse of the photosynthetic process called 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 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. By determining the initial oxygen measurement taken from a control bottle, and subtracting it from the amount left at the end of the run, usually 24 hours, allows you to determine the quantity of oxygen utilized (respiration). The amount of oxygen in the light bottle added to the



Limnologists are scientists who study water movements (currents, waves, seiches), light, heat, sedimentation rates, and other physical phenomena in lakes and other inland waters.

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.

In a modified version of this method, the bottles can represent the whole aquatic ecosystem, 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. 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 milliliter 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.

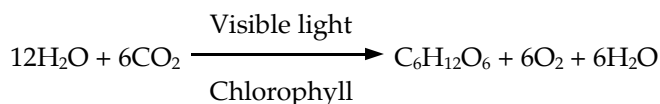


DID YOU KNOW?

Every time you open a bottle of soda pop, you see Henry's Law in action. In releasing the pressure in the bottle, you lower the solubility of CO₂, hence it comes out of solution as bubbles.

Photosynthesis

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



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 in free and 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

Effect of Altitude: With an increase in altitude to a more rarefied atmosphere, the value of p in the formula decreases. Therefore, solubility, expressed as the amount of gas dissolved at equilibrium with the air, lessens.

Effect of Temperature: With p constant and temperature altered, the solubility decreases as temperature rises. The inverse relationship demonstrates that cold water can hold more gas in solution than warm water.



DID YOU KNOW?

Domestic sewage refers to waste water containing impurities from households. When sewage enters a body of water, microorganisms begin to decompose the organic material. Oxygen is consumed as microorganisms use it in their metabolism, which can quickly deplete the available oxygen in the water.

Effect of Salinity: The occurrence of various minerals in solution lowers the solubility of the gas. The reduction of saturation values of gases in seawater, when compared with distilled water, is approximately 20%. Seawater is about 35% salinity; converted to parts per million or milligrams per liter, it is about 35,000. Inland waters are "pure", with 0% salinity in parts per thousand.

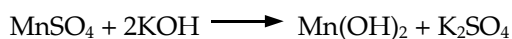
Relative Saturation: Gas saturation is quantified on the basis of equilibrium at the boundary of the surface of the water and the atmosphere. Gas solubility is the ratio of its concentration in the solution to its concentration above the solution.

Assaying Dissolved Oxygen in Aqueous Solutions

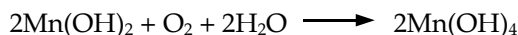
It is possible to determine the amount of oxygen in water with the Winkler titrametric method. The procedure involves the addition of alkaline iodide and manganous sulfate to a water sample. Manganous hydroxide is produced and, upon acidification, is converted to a manganese compound by the oxygen in 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 manganous hydroxide in the water sample to which manganous sulfate is introduced when KOH plus KI are added:



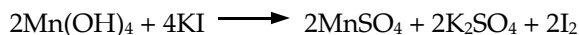
2. Oxidation of manganous hydroxide to manganic hydroxide by the dissolved oxygen in the sample:



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



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

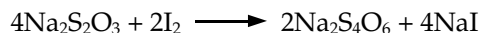




DID YOU KNOW?

Fish in waters containing excessive dissolved gases may suffer from "gas bubble disease". The bubbles block the flow of blood through blood vessels causing death.

5. Titration of the iodine solution with sodium thiosulfate until all free iodine combines into sodium iodide. Often, starch indicator is added to the sample to make it easier to see the titration endpoint. The endpoint is marked by the disappearance of the purple color:



Procedure Notes



All steps involving sulfamic acid should be performed by the instructor. Wear appropriate personal protection equipment, including chemical splash goggles, face shield, nitrile rubber gloves, and acid-resistant apron, and perform the procedure under a ventilated fume hood within a 15-second walking distance of an eyewash station. Read the MSDS before performing this procedure.

- Do not leave water samples in open air for extended periods of time; the dissolved oxygen concentration will approach 100% saturation.
- Normally encountered levels of iron, copper, chromate, nitrite, nitrate, sulfite, sulfide, chlorine, hypochlorite, hardness, salinity, dissolved gases, acids, or alkalis will not interfere with the dissolved oxygen determination in fresh water, seawater, or municipal wastewater.
- No correction is required for temperature, barometric pressure, or salinity.
- Excessive alkalinity (pH more than 10), occurring in certain industrial wastes, can cause erroneous results. If you observe this, have your instructor add 10% hydrochloric acid to the sample to neutralize it, and repeat the test procedure.
- Excessive acidity will also cause erroneous results. If the pH of the sample is less than 2, neutralize with 2% sodium hydroxide solution and repeat the test procedure.
- Your instructor may have collected water samples for the class to use, or you may have to collect your own samples. Collect samples carefully. Do not leave the sample in open air or allow it to become agitated; a change in gaseous content will develop. Collect surface water samples in narrow-mouthed bottles with caps. Avoid entraining or dissolving atmospheric oxygen. Use of a



DID YOU KNOW?

The factors that limit primary productivity in an aquatic system are usually some combination of the availability of nutrients, light, and/or grazing by zooplankton.

Kemmerer-type sampler for streams and ponds of moderate depth is recommended. Bleed the sample from the bottom of the sampler through a tube extending to the bottom of the sample bottle. Fill the bottle to overflowing (overflow approximately 10 seconds) and prevent turbulence and formation of bubbles while filling.

- Samples should be analyzed for dissolved oxygen immediately after sampling. Samples may be stored for a few hours after manganous sulfate solution, alkali-iodide solution, and H_2SO_4 are added and the samples mixed. Keep samples out of strong sunlight.

OBJECTIVES

- Measure dissolved oxygen concentration.
- Calculate gross productivity, net productivity, and respiration rate.
- Demonstrate the importance of carbon and oxygen cycles in an ecosystem.
- Identify physical and biological factors affecting solubility of dissolved gases in aquatic ecosystems.
- Measure primary productivity in an aquatic ecosystem.
- Demonstrate the effect of light and nutrients on photosynthesis.

MATERIALS

MATERIALS NEEDED PER GROUP

- Chlorella culture or water sample
- Pipet
- Microscope slide
- Coverslip
- Light
- Compound microscope
- 17 Cloth squares
- Aluminum foil
- 7 BOD bottles
- Direct-reading titrator
- Titration vial

SHARED MATERIALS

- Manganous sulfate solution
- Alkaline potassium iodide azide
- Sulfuric acid
- Starch indicator solution
- Sodium thiosulfate

PROCEDURE



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



Your instructor may have collected the water sample in advance. If this is the case, begin with Step 2.



DID YOU KNOW?

Chlorella, known as the “Jewel of the Orient,” has a high concentration of vitamins, minerals, amino acids, nutrients, and enzymes.



DID YOU KNOW?

In water, dissolved oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills.

A. Measurement of Dissolved Oxygen

1. Thoroughly rinse out a sampling bottle. Remove the cap and slowly submerge the bottle in the water. Allow the bottle to fill and remove any air bubbles from the side of the bottle by tapping on the side. Cap the bottle while it is 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.
2. 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.
3. Place your bottles in a shallow pan or similar container to catch the overflow. Add eight drops of manganous sulfate to the sample bottle with a dropping bottle or pipet. Be sure no air is added.
4. Add eight drops of alkaline iodide to the sample bottle. 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. Your instructor will 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.
8. Carefully measure out 20 ml of the sample into a titration vial. Place the cup on top of a white sheet of paper to better see the color of the sample.
9. Add eight drops of starch indicator to the 20 ml sample. The starch solution will change the liquid's color from yellow to purple. Place the cap on the vial and swirl gently to mix.
10. Carefully fill the titration syringe with sodium thiosulfate working solution. Insert the tip of the titration syringe into the hole in the vial cap.
11. While continually swirling the sample, titrate the 20 ml sample with sodium thiosulfate working solution. Titrate one drop at a time until the color changes from purple to a pale yellow/colorless solution; this is the titration endpoint where all free iodine has been converted to sodium iodide by the addition of sodium thiosulfate.



DID YOU KNOW?

Dissolved oxygen concentrations may steadily decline during the night, when photosynthesis cannot counterbalance the loss of oxygen through respiration and decomposition. It is lowest right before dawn, when photosynthesis resumes.

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

$$\text{mg/L Dissolved Oxygen} = \text{ml Titrant Used}$$

Record this value in Table 1 in the Analysis section of the lab.

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

$$\text{mg O}_2/\text{L} \times 0.698 = \text{ml O}_2/\text{L}$$

14. Using the nomograph in the Analysis section and a straightedge or ruler, estimate the percent saturation of dissolved oxygen in your sample. Record this value in Table 1 in the Analysis section.
15. 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 in the Analysis section.

B. Measurement of Primary Productivity

The productivity per square meter of a water column in an aquatic environment can be measured using the light-dark bottle oxygen method. In this experiment, the reduction of natural light levels at various depths will be simulated with screen filters. Temperature is constant so only a single variable, light intensity, is assessed.

1. Obtain seven water sample bottles. One bottle will serve as the initial sample. With a permanent marker, label the bottle "#1 — Initial". A second bottle will serve as the dark bottle; label it "#2 — Dark". Label the other bottles according to light intensity: "#3 — 100%", "#4 — 65%", "#5 — 25%", "#6 — 10%", and "#7 — 2%".
2. Carefully fill each bottle with the water sample: Remove the cap and slowly submerge the bottle in the water. Allow the bottle to fill and 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.



Titration:

A technique common in chemistry laboratories, used to find the concentration of a substance by slowly adding a known amount of another substance.

3. Wrap bottle #2 with aluminum foil. Refer to the chart below to determine the number of screens required to create each light intensity level. Stack that number of screens together and wrap them around the appropriate bottle. Keep the screens in place with tape, rubber bands, or similar.

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

4. Cap bottles #2 through #7 tightly and lie them down on their sides under a light source. Keep the overlapping portion of the screens to the bottom and leave overnight.
5. Fix the sample in bottle #1 by performing Steps 3 through 7 as described in part A. Keep the #1 bottle at room temperature until you process the other samples.
6. Prepare a wet mount of the water sample and observe it under a compound microscope. Use the *General Guide to Aquatic Organisms*, included, to identify the organisms on the wet mounts. On a separate sheet of paper, illustrate and identify the organisms you observed.
7. The next day, fix bottles #2 through #7 by performing Steps 3 through 7 as described in part A.
8. After all bottles have been fixed, determine the dissolved oxygen of all samples by performing Steps 8 through 13 from part A. Record your results in Table 3 in the Analysis section of the lab.



For productivity and respiration studies, dissolved oxygen is usually reported in milliliters.

9. Calculate the gross and net productivities and respiration rate of your samples using the following formulas. Enter your results in Table 3 in the Analysis section.

Gross Productivity = Light Bottle (ml O₂/L) – Dark Bottle (ml O₂/L)

Net Productivity = Light Bottle (ml O₂/L) – Initial Bottle (ml O₂/L)

Respiration Rate = Initial Bottle (ml O₂/L) – Dark Bottle (ml O₂/L)

10. Collect the class data and determine both the mean gross productivity and mean net productivity. Enter the values in Table 3 in the Analysis section.
11. Convert your mean gross productivity data (ml O₂) for your samples to carbon productivity (mg C/m³) using the following formulas:

$$\text{ml O}_2/\text{L} = 0.698 \times \text{mg O}_2/\text{L}$$

$$\text{mg C/L} = 0.536 \times \text{ml O}_2/\text{L}$$
12. To convert liters to meters cubed, divide liters by 0.001. Enter values in Table 3 in the analysis section.



Actual answers will vary with each water sample.

C. Productivity Simulation



DID YOU KNOW?

By identifying the organisms present in a water source, the water quality can be predicted.

Productivity of a body of water can be estimated by measuring the productivity of water samples and 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 (ZSD) 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



DID YOU KNOW?

A dissolved oxygen probe may be used to determine the level of dissolved oxygen in a water sample.

1. Using the Gross Productivity data (mg C/m^3) from Table 3 in the Analysis section, simulate primary productivity for the two data sets above.
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.

WARD'S
AP Biology Lab 12
Dissolved Oxygen & Primary Productivity
Lab Activity

Name: _____
Group: _____
Date: _____

ANALYSIS

Nomograph

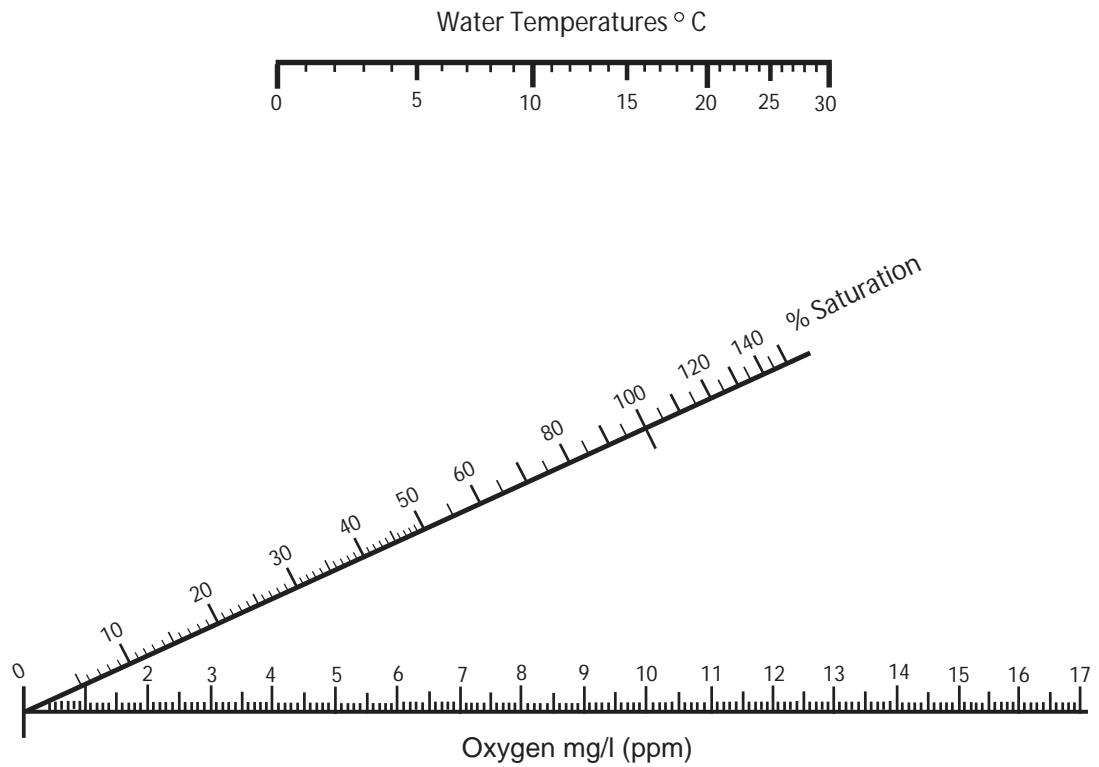


Table 1
Dissolved Oxygen Concentration
Lab Group Sample

Temperature	Dissolved Oxygen (mg/L)	% Saturation

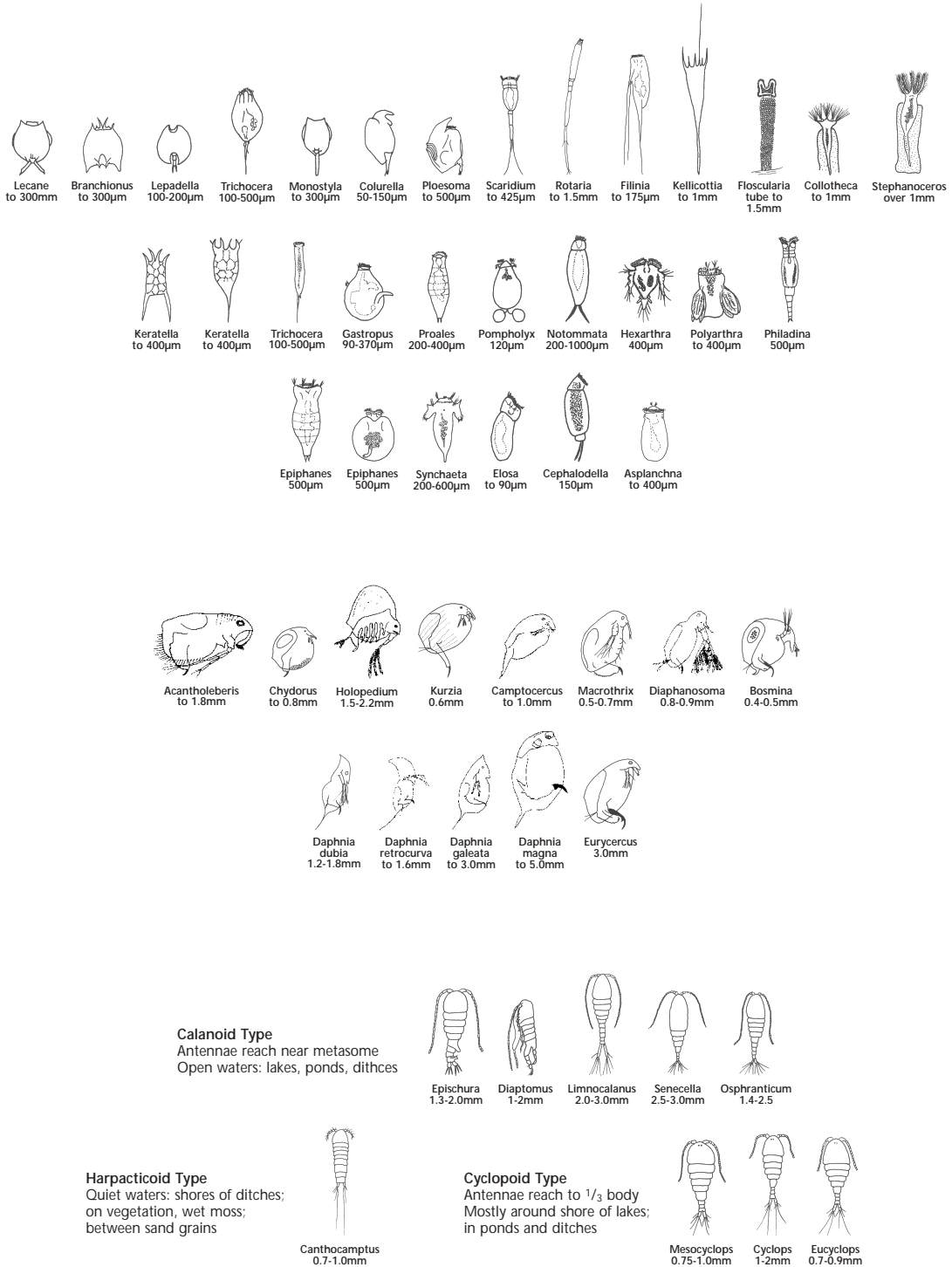
Table 2
Dissolved Oxygen Concentration
Class Sample

Temperature	Dissolved Oxygen (mg/L)	% Saturation
0		
20		
30		

Table 3
Gross and Net Productivity/Respiration Rate
Class Sample

Percent Light	Dissolved Oxygen (mg/L)	Gross Productivity	Net Productivity	Gross Productivity (mg C/m ³)
Initial		—	—	—
Dark		—	—	—
100%				
65%				
25%				
10%				
2%				

General Guide To Aquatic Organisms



Calanoid Type
Antennae reach near metasome
Open waters: lakes, ponds, ditches

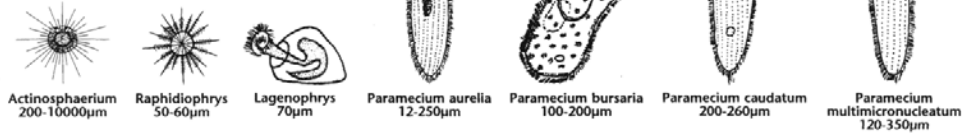
Harpacticoid Type
Quiet waters: shores of ditches;
on vegetation, wet moss;
between sand grains

Cyclopoid Type
Antennae reach to 1/3 body
Mostly around shore of lakes;
in ponds and ditches

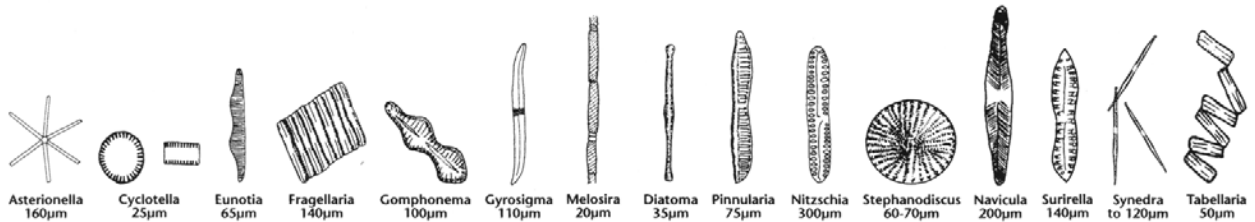
General Guide To Aquatic Organisms

Ciliates

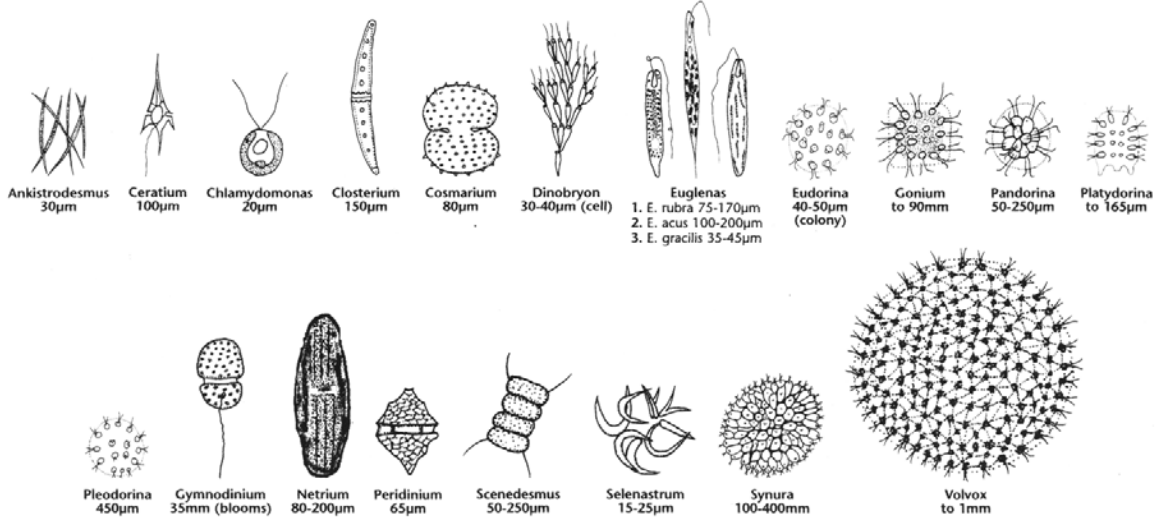
Actinopods



Diatoms



Green Protists



ASSESSMENT

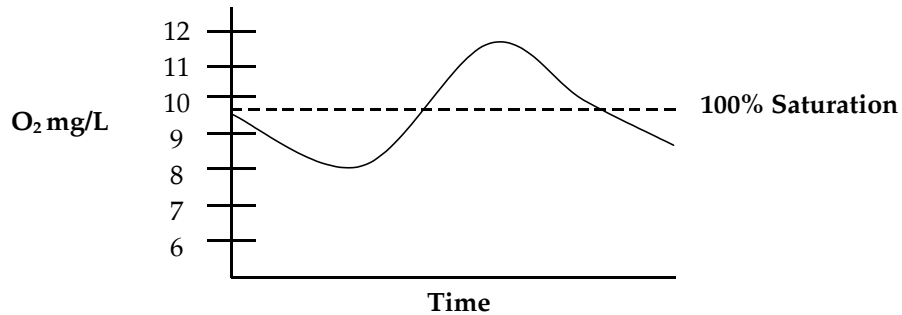
1. How does temperature affect the solubility of oxygen in water?
2. How does salinity affect the solubility of oxygen in water?
3. Would you expect to find higher dissolved oxygen content in a body of water in winter or summer?
4. Discuss how each of the following factors could influence the dissolved oxygen concentration of a body of water.

Wind:

Temperature:

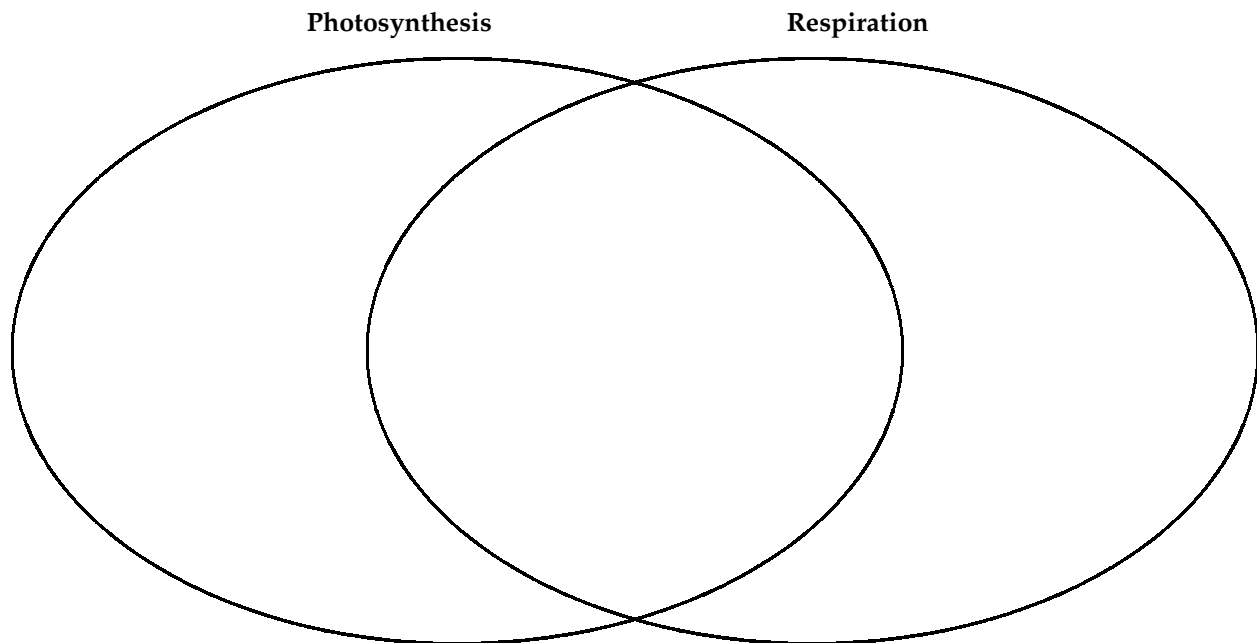
Altitude:
5. Do you think it would be wise to stock a pond with game fish if it had a dissolved oxygen content of 3 ppm? Why or why not?

6. Below is a graph of dissolved oxygen levels in a body of water. Represented on the Y-axis is the amount of dissolved oxygen in mg/liter. The X-axis is unlabeled but represents a period of time. Examine the graph and place time values on the X-axis. Explain why you chose the time values and the event or events that occurred over that period.



7. In part B of the experiment, were any of the samples light-limited? Why?
8. Based on your analysis of the lakes presented in part C of the lab, which lake is more productive?
9. What is used as the basis for measuring primary productivity?

10. In the Venn diagram below, list at least three similarities and three differences between photosynthesis and respiration.



11. Below is a list of steps involved in the Winkler method. First, put the steps in their proper order for the determination of dissolved oxygen (assume a water sample has already been collected) and then describe what each step does in the procedure.

Addition of alkaline iodide, titration with sodium thiosulfate, addition of manganous sulfate, addition of sulfamic acid, addition of starch indicator

