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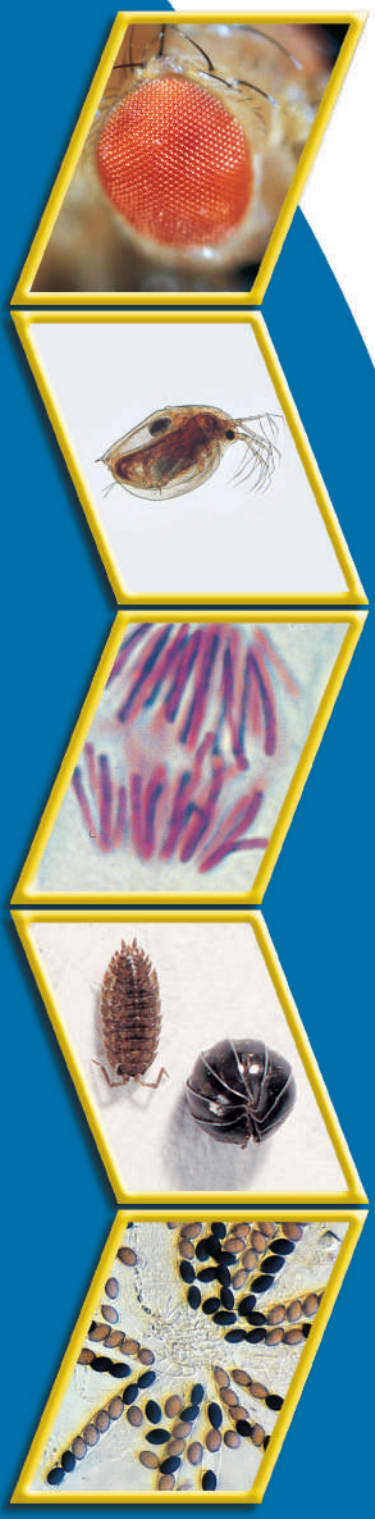
AP® BIOLOGY



LABORATORY 12

Dissolved Oxygen and Aquatic Primary Productivity

TEACHER'S MANUAL WITH STUDENT GUIDE

74-6630	8-Station Kit
74-6631	1-Station Kit
74-6635	Replacement Set

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Units of Measure Useful in AP® Biology

Property Measured	Unit	Symbol	Description
Length	*meter	m	100 cm = 10^2 cm
	centimeter	cm	0.01 m = 10^{-2} m
	millimeter	mm	0.001 m = 10^{-3} m
	micrometer	μm	10^{-6} m = 10^{-3} mm
	nanometer	nm	10^{-9} m = 10^{-3} μm
Mass	*kilogram	kg	1000 g
	gram	g	1000 mg
	milligram	mg	0.001 g = 10^{-3} g
	microgram	μg	10^{-6} g
Amount of Substance	*mole	mol	6.02 x 10^{23} particles (atoms, ions, or molecules)
Concentration of a Solution	mass percentage	%	Mass % = mass of solute/total mass of soln. × 100
	parts per million	ppm	ppm of solute = mass of solute/total mass of soln. × 10^6 or 1 ppm = 1 mg solute/L soln.
	molarity	M	Molarity = moles solute/L soln.
Volume (gases and liquids)	kiloliter	kL	1000 L
	liter	L	1000 mL = 1 dm^3 = 10^{-3} m^3
	milliliter	mL	mL = cm^3 = 10^{-3} L
	microliter	μL	10^{-6} L = 10^{-3} mL
Temperature (thermodynamic)	*kelvin	K	K = °C + 273
Temperature (common)	Celsius	°C	0 K = –273°C
Force	newton	N	kg•m/s^2
Heat or Energy	joule	J	N•m
	**calorie	cal	4.184 J
	**Calorie (food)	Cal	1000 calories = 1 kcal
Time	*second	s	60 s = 1 min
	millisecond	ms	10^{-3} s
Pressure	pascal	Pa	N/m^2 = kg/m•s^2
	**atmosphere	atm	101,325 Pa = 101.325 kPa = 760 torr = 14.7 lb/in^2
	Bar	bar	10^5 Pa
	**Torr	torr	mm Hg = 133.3 Pa

* SI Base Unit

**Non-metric

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For complete listings of Carolina™ AP® Science materials, including the *Advanced Placement® Biology Laboratory Manual for Teachers* (RN-74-6681) and the *Advanced Placement® Biology Laboratory Manual for Students* (RN-72-6682), log on to www.carolina.com/ or refer to the current *Carolina™ Science* catalog or the current *Carolina™ Biotechnology & AP® Biology* catalog.

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Laboratory 12. Dissolved Oxygen and Aquatic Primary Productivity

Overview

This lab consists of two parts:

Activity A (Temperature and Dissolved Oxygen): measurement of the amount of oxygen dissolved in water and the relationship of water temperature to the amount of dissolved oxygen

Activity B (Primary Productivity): students use the measurements of dissolved oxygen to determine the primary productivity of an aquatic system

Objectives

- Measure dissolved oxygen in a water sample using the Winkler Method
- Measure primary productivity
- Investigate some factors that can affect the primary productivity of a system

Content Standards

This kit is appropriate for Advanced Placement® high school students and addresses the following National Science Education Standards:

Unifying Concepts and Processes

- Systems, order, and organization
- Evidence, models, and explanation
- Constancy, change, and measurement

Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

Life Science

- The interdependence of organisms
- Matter, energy, and organization in living systems

Time Requirements

This activity requires a minimum of three 45-minute lab periods. Two periods must be on consecutive days, because the samples for Activity B must be incubated overnight. If you have a 90-minute laboratory period, Activity A and the Day 1 portion of Activity B can be performed on the same day. The Data Analysis sections can be done as homework.

Activity A (45 minutes)

Students perform dissolved oxygen determinations on the samples at different temperatures. Note that there is an optional stopping point after the oxygen is

fixed. Refer to the Winkler Method Protocol in the Student Guide for details. This gives you the option of spreading Activity A over two periods.

Activity B, Day 1 (45 minutes)

Students determine a baseline DO and set up the *Chlorella* cultures, in bottles with screens, for incubation overnight.

Activity B, Day 2 (45 minutes)

Students perform dissolved oxygen determinations on the *Chlorella* cultures.

Safety

Use this kit only in accordance with prudent laboratory safety precautions, including approved safety goggles, lab aprons or coats, and gloves. Know and follow all school district guidelines for lab safety and for disposal of laboratory wastes.

Preparation and Presentation

Photocopy the blackline master Student Guide for each student or group of students. Photocopy the graph template at the end of the Student Guide as needed.

This lab is a fitting climax to the series of 12 AP®-recommended biology labs. It applies knowledge gained in several of the other labs, especially Lab 4 on photosynthesis and Lab 5 on cellular respiration. You may wish to review with students those labs and food/energy pyramids before beginning Lab 12. (Notice that secondary productivity, the rate at which organic materials are stored at heterotrophic levels, is not covered in Lab 12.) If students will be taking AP® Environmental Science, this lab can be used as a link between the two courses.

Our instructions describe the process of determining DO by the Winkler Method. However, Lab 12 can also be done with dissolved oxygen probes. The use of probes eliminates most of the problems associated with the use and disposal of chemicals. If using probes, be aware that some require a warm-up period of as much as 10 minutes, so refer to the instructions that come with the probe. If the instructions are lost, they can probably be downloaded from the manufacturer's Web site. There can be variation in readings from probe-to-probe; therefore, we recommend that groups use the same probe for all measurements.

Activity A: Temperature and Dissolved Oxygen

The *Advanced Placement® Biology Laboratory Manual* recommends that each student group determine DO of water samples at three different temperatures: 0–5°C, 20°C, and 30°C. You can save time by assigning each group one temperature of water for DO determination and taking class averages. If you do this and have temperature-controlled water baths, consider adding one or two more temperatures for sampling, perhaps 15°C and 25°C.

It is easiest to set up the water samples on the day before the lab, but at least 30 minutes prior to the lab, fill three 500-mL beakers with tap water. There must be time for the samples to reach equilibrium for dissolved gasses at the given temperature. Place one beaker in an ice bath or refrigerator, place one beaker at room temperature, and place one beaker in a 30°C water bath or

incubator. (Containers other than beakers can be used, but remember that the larger the amount of water, the longer the time required for temperature and dissolved gasses to reach equilibrium.) Remove the containers when it is time for students to get their samples. Have thermometers available so students can record actual water temperatures. Notice that once the water samples are fixed, you have the option of stopping the exercise for the day. The fixed water samples can be stored and titrated the next day if necessary.

Activity B: Primary Productivity

For this lab to give satisfactory results, the *Chlorella* culture must be vigorous. Return the card included with this kit to receive the *Chlorella* in time to prepare for the lab.

Three or more days before lab, dilute the *Chlorella* culture in 4 L (or 1 gallon) of springwater and add the Alga-Gro® medium. You can also use dechlorinated tap water or distilled water[1]. Keep the culture under continuous moderate light (florescent light is ideal) for 72 hours or more at 25°C. By the day of the lab, a green mass of algae 1–2 inches deep should be present in the bottom of the container. If you will need the culture early in the week, you may grow the algae for three days during the week before the experiment, then remove it from continuous lighting (put it in a window) over the weekend and use it the following week. **Note:** If you cannot grow the culture under continuous light, start it a week before the lab and let it grow in a window. The culture will continue to grow for several weeks; however, the longer you keep it, the greater the chance that other organisms, both photosynthetic and nonphotosynthetic, will invade the culture. This can impact the culture's productivity.

The *Advanced Placement® Biology Laboratory Manual* recommends that each student group determine DO of water samples of all the test conditions; however, you can save time by having each team determine DO for just one or two of the test conditions. Thus, one group would do the light and dark bottles, one group would do the 1- and 3-screen bottles, and another would do the 5- and 8-screen bottles. It is best if at least two groups perform measurements on each test condition. In this way, if one group's results are questionable, there will be a backup. We strongly recommend this approach.

Prior to filling the BOD bottles, the algae should be uniformly resuspended in the total volume by swirling (avoid turbulence) or by stirring on a stirplate. If the concentration of algae is different from sample to sample, variations in the amount of photosynthesis and respiration will occur. As before, once the samples are fixed, you have the option of stopping and doing the titration the next day.

[1]Some water treatment plants disinfect water using a process that creates chloramines in the water. This water cannot be dechlorinated by traditional aging methods. Chloramines can only be removed by use of a chemical water conditioner.

Station Setup

Following is a list of the materials needed for one group of students to perform the activities in this lab. Prepare as many setups as needed for your class.

	Activity A	Activity B, Day 1	Activity B, Day 2
BOD bottles	3	7	
manganous sulfate	1 bottle	1 bottle	1 bottle
starch indicator	1 bottle		1 bottle
sulfamic acid	1 vial with spoon	1 vial with spoon	1 vial with spoon
gloves	5 pairs	5 pairs	5 pairs
60-mL syringe with tubing (optional)	1	1	
alkaline potassium iodide azide	1 bottle	1 bottle	1 bottle
titration syringes	2		2
20-mL sampling vials	2		2
sodium thiosulfate	1 bottle		1 bottle
fiberglass screens		17	
Chlorella culture		500 mL	
incubated *Chlorella* cultures			from 12B, Part 1
fixed initial samples			from 12B, Part 1
*5½" square of aluminum foil		1	
*paper towels	as needed	as needed	as needed
*waterproof marker	1	1	
*rubber bands		as needed	
*thermometer	1		

*Not supplied.

Chemistry of the Winkler Method

1. The first step in the Winkler Method is the addition of manganous sulfate and alkaline potassium iodide azide to the sample. The alkaline potassium iodide azide solution contains both potassium iodide and potassium hydroxide. Manganous sulfate and potassium hydroxide react to form manganous hydroxide, which appears as a white precipitate.

   $MnSO_4 + 2KOH \rightarrow Mn(OH)_2 + K_2SO_4$

2. The oxygen in the water sample immediately oxidizes manganous hydroxide to manganic hydroxide, which is brown.

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

3. The next step is the addition of sulfamic acid, which does two important things. First, it reacts with the manganic hydroxide to form manganic sulfamate.

   $Mn(OH)_3 + 3NH_2SO_3H \rightarrow Mn(H_2NSO_3)_3 + 3H_2O$

4. Second, the addition of sulfamic acid neutralizes any remaining potassium hydroxide and manganous hydroxide in the solution. This fixes the reaction by preventing further formation of manganic hydroxide by free oxygen. Once the reaction is fixed, introduction of additional oxygen does not affect the results of the titration. The manganic sulfamate oxidizes the iodide from the alkaline potassium iodide azide solution to free iodine, manganous sulfamate, and potassium sulfamate. The free iodine turns the solution yellow-gold.

   $2Mn(H_2NSO_3)_3 + 2KI \rightarrow 2Mn(H_2NSO_3)_2 + 2K(H_2NSO_3) + I_2$

5. The final step in the Winkler Method is the titration of free iodine with sodium thiosulfate. The conversion of free iodine to sodium iodide is indicated by starch, which is blue in the presence of free iodine but becomes colorless when the iodine is converted to iodide. The amount of free iodine in the sample is proportional to the initial dissolved oxygen level, so the amount of sodium thiosulfate needed to titrate it reveals the original dissolved oxygen concentration.

   $2Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2NaI$

Troubleshooting

Problems with this lab can usually be traced to mixing oxygen from the air into the water sample that is tested. This most often occurs during the filling the BOD bottle with the sample. If the water is simply poured into the bottle and allowed to splash, the amount of DO will change. The correct method of pouring a sample is to tilt the BOD bottle to a 45° angle and allow the water to run down the inside of the bottle. This must be done slowly, so the water does not swirl around the bottom of the bottle. It is probably best if students do not pour the samples, but if they do, demonstrate the correct method and let them practice before attempting it themselves. Whatever method is used to fill the BOD bottles, turbulence must be avoided. Failure to tightly cap or completely fill the BOD bottle can also cause problems by trapping air bubbles inside.

All BOD bottles should be labeled with the test conditions represented: 1 screen, 3 screens, 5 screens, etc.; otherwise, students can easily misidentify the bottles.

In Step 1f of the Winkler Method protocol, students may find that not all of the precipitate dissolves after mixing. If this occurs, allow the remaining precipitate to sink to the bottom of the bottle before continuing.

Sample Answers to Questions in the Student Guide

Activity A: Temperature and Dissolved Oxygen

Procedure

Sample Table 1: Temperature and Dissolved Oxygen

Temperature	Class Average DO	Class Average % Saturation
5°C	6.50 mg/L	53%
20°C	5.60 mg/L	60%
30°C	4.80 mg/L	63%

Actual results should reflect the data collected.

Analysis of Results

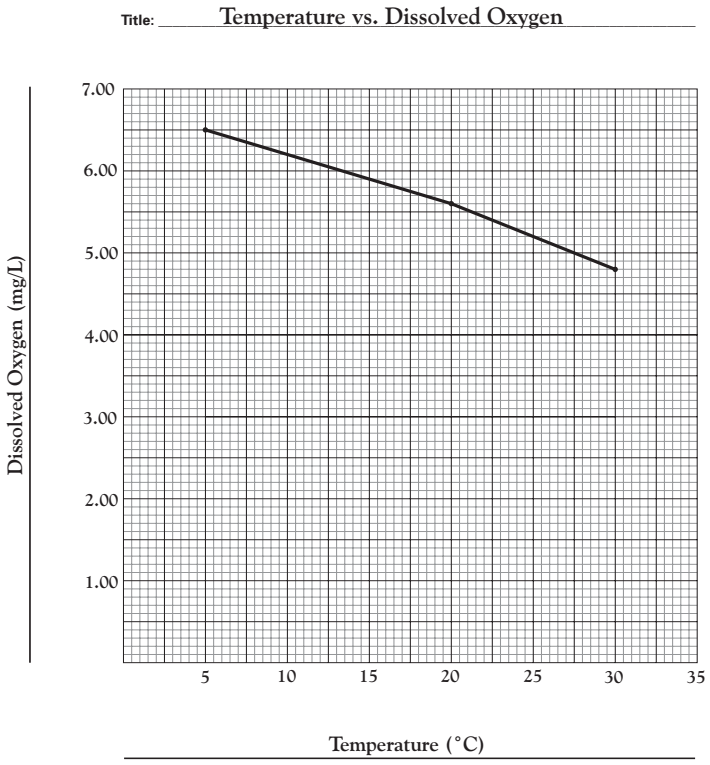
1. Plot the Class Averages for DO from Table 1. Title the graph and supply the following information:

   a. The independent variable is *temperature*.

   b. The dependent variable is *dissolved oxygen*.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

Sample Graph



2. Based on your class data, what is the relationship of temperature to DO?
   As temperature increases, DO decreases. DO is inversely related to temperature.

3. Read the percent saturation from the middle scale of the nomograph. Record this number in Table 1.
   Answers should reflect the data collected. See Sample Table 1 for representative data.

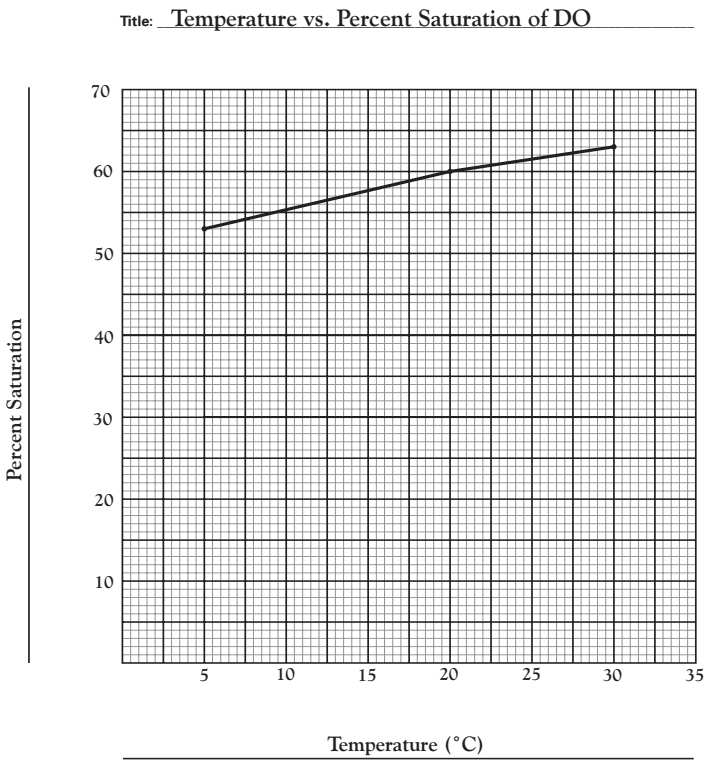
4. Plot the Class Averages for Percent Saturation from Table 1. Title the graph and supply the following information:

   a. The independent variable is *temperature* (or *mg O_2/L*).

   b. The dependent variable is *percent saturation*.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

Sample Graph



5. Based on your data and graphs, what is the relationship of temperature to percent saturation?
   As temperature increases, percent saturation increases.

6. What inference can you draw from your answer to #5 that would help explain the relationship of temperature to DO given in your answer to #2?
   The solubility of oxygen in water decreases with temperature. As a result, the total amount of DO that can be held by the water, the point at which the water is saturated with DO, must also decrease. Thus, even if the amount of DO remains constant, as temperature increases, the percent saturation of DO will increase.

Activity B: Primary Productivity

Procedure

Sample Table 2: Group Productivity Data R = 2.50 mg/L

Bottle	DO	Net Productivity	Gross Productivity
Baseline (Initial)	7.40		
Dark	4.90		
Light (0 screens)	10.90	3.50	6.00
1 screen	10.60	3.20	5.70
3 screens	8.95	1.55	4.05
5 screens	7.80	0.40	2.40
8 screens	5.10	–2.30	0.20

Actual results should reflect the data collected.

Analysis of Results

1. Calculate the loss of oxygen due to respiration and record here: _____ mg/L

   $R = I - D$

   where
   R = loss due to respiration
   I = DO baseline
   D = DO dark bottle

   Answers should reflect the data collected.

2. Calculate net productivity of the other samples and record the data in Table 2.

   $P_n = L - I$

   where
   P_n = net productivity
   I = DO baseline
   L = DO sample

   Answers should reflect the data collected. See Sample Table 2.

3. Calculate gross productivity of each sample and record in Table 2.

   $P_g = P_n + R$

   where
   P_g = gross productivity
   P_n = net productivity
   R = loss due to respiration

   Answers should reflect the data collected. See Sample Table 2.

4. Determine class averages for net and gross productivities. Record the data in Table 3.

   Answers should reflect the data collected. No "class averages" sample data is provided here (i.e., there is no Sample Table 3). Note that the Sample Graph in 5, below, is a graph of the group data from Sample Table 2. This is representative data, for reference only.

5. Graph the data for Average Gross and Average Net productivities from Table 3. Title the graph and supply the following information:

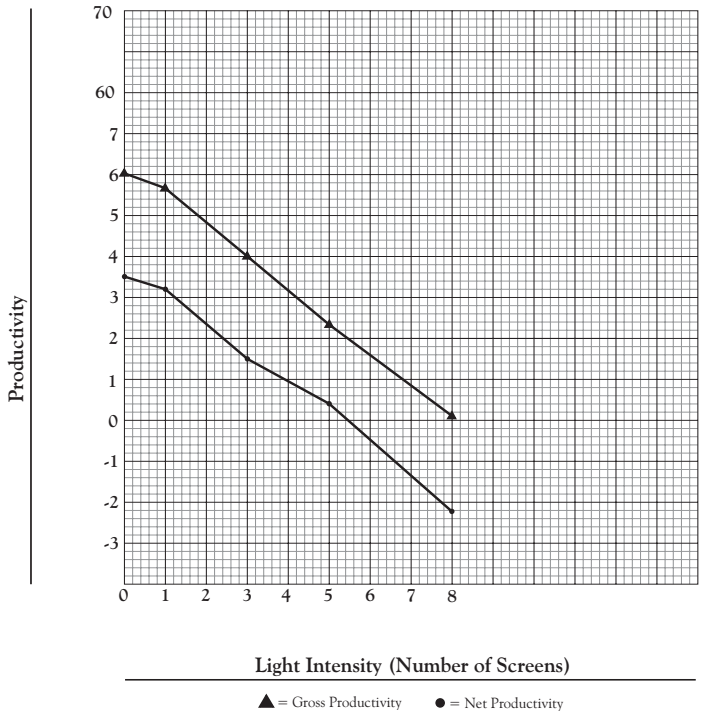
   a. The independent variable is *number of screens* (or *light intensity* or *depth*).

   b. The dependent variables are *net productivity and gross productivity.*

Plot the independent variable on the x-axis, and the dependent variables on the y-axis.

Sample Graph

Title: Productivity vs. Light Intensity



6. From your graph:

   a. At approximately what light intensity does the rate of respiration equal the rate of photosynthesis?

   Answers will vary according to data collected. Using the sample data given above, this occurs at a light level just below 2%. Notice that productivity may not drop in the 1- and 3-screen bottles. This is because phototrophs are unable to utilize all the light energy available to them. Thus, productivity may not fall until much of the light is blocked. If your students are fortunate enough to obtain this result, it presents a wonderful opportunity to discuss limiting factors.

b. At approximately what depth in the simulated pond does this occur?

Answers will vary according to data collected. Using the sample data given above, this occurs at a depth just below 4 meters.

7. Two researchers, one at Toolik Field Station in northern Alaska and the other at La Selva Biological Station in Costa Rica, are studying populations of aquatic arthropods in freshwater pools during July. The researcher in Alaska determines an average of 280 arthropods per m^3 of water in the pools she is studying. The researcher in Costa Rica determines an average of 125 arthropods per m^3 at his study site. In terms of the current lab, how would you account for this difference?
Instruction Note: Toolik and La Selva are real science stations. You may wish to allow students to research them and incorporate their findings into their answer.

The primary answer is that productivity is greater at the Alaska site than at the site in Costa Rica, and this supports a greater number of consumers. Secondary explanations: The difference may be due to the extended daylight and lack of forest cover in northern Alaska. In July, Costa Rica would have a shorter day. The Costa Rica site would also have a dense forest cover, which would decrease light intensity in pools. A student might also argue that the high rainfall at the Costa Rica site would result in lower nutrient levels in the pools, decreasing productivity. A weaker but still acceptable argument is that the cooler water at the Alaska site contains more DO for animals. Extra credit answer: The difference could result from a difference in the average size of aquatic arthropods at the two sites. In this case, the data must be expressed as grams of arthropods per m^3 of water before an answer can be given.

8. You are the Water Quality Director for Derry County. In this capacity, you are asked to review reports of two fish kills in the county. Both involve artificial ponds with surface areas of approximately 0.8 hectares and maximum depths of 7 meters. Tests performed immediately after the fish kills detected no pesticides or other poisons. The dead fish showed no signs of fungal attacks or other disease. Case A involves a pond stocked with bass and used for recreational fishing. Meteorological records show that the kill occurred after 4 weeks of hot weather in which daytime temperatures reached 35–40°C. Case B involves a pond stocked with bluegill and used to irrigate pastureland. This kill occurred in the spring, before the heat wave and 9 days after a heavy rain. The file for Case B contains a photo showing dead fish floating in the pond. You also notice what appear to be mats of decaying algae floating on the surface of the water. A call to the farmer reveals that he applied ammonium nitrate to the pastureland the week before the rain. State your judgment as to the probable causes of these fish kills, and describe the chain of events that led to each.

In both cases, the fish probably suffocated and died from a lack of dissolved oxygen.

Case A: The solubility of oxygen in water decreases with temperature. As the heat wave continued, water temperature in the small pond also rose, but at a slower rate. After several weeks, the water reached a point at which it could not

hold enough oxygen for the size of the fish population. As oxygen levels continued to drop, fish suffocated and died. Some may point out that the shallow depth of the pond could have been a factor. A deeper pond might maintain a layer of colder, oxygen-rich water in its depths, creating a refuge for the fish.

Case B: The heavy rain produced runoff from the pastureland into the pond. The runoff carried with it ammonium nitrate in solution, which stimulated an algae bloom. At night, the increased population of algae used oxygen, dropping DO levels. Also, as the nitrogen was used up, the increased population of algae could not be maintained, and the algae began to die. The dead algae were broken down by decomposers, which used still more of the DO and further deprived the fish, resulting in the fish kill.

Optional Activities

1. In Activity A, students may be unfamiliar with the use of a nomograph (syn. nomogram). Basically, a nomograph is a chart that allows the conversion of one quantity into another. A simple example can be demonstrated with many rulers in which one edge of the ruler is scaled in cm and the other in inches. In practice, a nomograph is used when one value is determined by two or more values, and the calculation of the desired value is complex. Students can research and report on the many uses of nomographs. A mathematically talented student might even report on the complexities of nomograph design or design an original nomograph.
2. Students can design and conduct an experiment to test the possible effect of a solute or pH on DO.
3. For Activity B, some prefer to take a water sample from a local lake or pond rather than use the *Chlorella* culture. If this option is available to you, we highly recommend it. However, keep in mind that the light and dark bottle method only works well for eutrophic and mesotrophic waters that have the nutrient levels required to support large populations of phytoplankton. Oligotrophic waters will contain too few photosynthetic organisms to show a difference between the light and dark bottles. Irrigation ponds surrounded by fields or pasture and subject to fertilizer runoff would be a good choice. Even in an oligotrophic pond or lake, you may be able to collect from a "green water" area near the margin. You might also add a water-soluble fertilizer to the collected sample and incubate it under light until it becomes green.

   Some teachers maintain an artificial pond in their classroom. This consists of an aquarium set up near a window or under a light bank. Filtration is not necessary. Simply add to the aquarium any aquatic organisms (algal and protozoan cultures, elodea, planaria, daphnia, guppies, etc.) left over from classroom activities. In time, the water should turn green. If not, add a fertilizer and/or increase the light. This pond will provide a continuing source of aquatic organisms for classroom use. When using water from sources that contain non-photosynthetic organisms, remember that the loss due to respiration will include respiration by autotrophs and heterotrophs, so technically, net primary productivity cannot be measured. In practice, this is usually ignored and the calculated figure for gross productivity is accurate in any case.

4. Currently, primary productivity is more often measured as moles of carbon consumed by using radioactive carbon (^{14}C). Students can research how this is done and why it is preferred to the DO method. Students can also convert their data from mass of O_2 produced to mass of C consumed from the general formula for photosynthesis:

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

   From this formula, for each mole of O_2 released, one mole of C is consumed. The conversion factor is then 12 mg C/32 mg O_2 = 0.375.

5. Aquatic primary productivity is often expressed as mg/m^3 of water. The conversion is mg/L × 1000 L/m^3. Notice that the values for respiration and productivity determined in Activity B are actually rates, so you may prefer that your students express the quantities as mg/L/day or mg/L/hr. Other manipulations of the data are possible. An Internet search can provide additional ideas for conversions.

6. Students can research and report on methods for estimating terrestrial primary productivity. Of what importance is primary productivity to agriculture?

CAROLINA

Student Guide

Name/Group # _____

Date _____

AP® Biology Laboratory 12
Dissolved Oxygen and Aquatic Primary Productivity

Objectives

- Measure dissolved oxygen in a water sample using the Winkler Method
- Measure primary productivity
- Investigate some factors that can affect the primary productivity of a system

Background to Activity A

Because cellular respiration requires oxygen (see AP® Biology Lab 5, Cellular Respiration), the dissolved oxygen (DO) concentration in a body of water is critical to the water's ability to support most forms of aquatic life. Thus, DO is often used as an indicator of water quality. You have probably read or seen news reports of fish kills that have been linked to sewage spillage. Aquatic microorganisms metabolize the sewage, using up DO. As DO levels drop, fish cannot acquire the oxygen they need, and they die.

DO concentration is expressed in parts per million (ppm) or mg/L. One ppm DO is equivalent to 1 mg of oxygen per L of water, so the two are interchangeable. Desirable fish species such as trout and perch require a minimum of 8 mg/L dissolved oxygen to survive. Less-desirable fish such as carp can survive at dissolved oxygen levels as low as 2 mg/L. Below 2 mg/L, only invertebrates such as sludge worms and mosquito larvae can survive.

How does oxygen enter water? One way is by diffusion. Oxygen is more concentrated in air than in water; thus, oxygen diffuses from the atmosphere through the water's surface. (See AP® Lab 1, Diffusion and Osmosis.) If both the air and water are static (nonmoving), the concentration of DO falls rapidly with distance from the surface. However, if the water is in motion due to winds, currents, tides, etc., DO can be mixed throughout the water column, increasing the total amount of oxygen dissolved in the water. Other physical factors that might influence DO concentration in a water sample include temperature, pH, and the presence and concentration of solutes. In this activity you will investigate the effect, if any, of temperature on DO.

Activity A: Temperature and Dissolved Oxygen

Materials

3 BOD bottles, gloves, manganous sulfate, starch indicator, sulfamic acid and measuring spoon, alkaline potassium iodide azide, sodium thiosulfate, 2 titration syringes, 2 20-mL sampling vials, waterproof marker, thermometer, 60-mL syringe with tubing attached (optional; see Procedure, 2b).

Caution: *Use extreme care when handling chemicals. Sulfamic acid and alkaline potassium iodide azide can irritate or burn the eyes, skin, and mouth. Avoid all skin contact with these and other chemicals. Do not put any chemical in or near your mouth. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.*

Introduction

In Activity A, you will investigate the effect of temperature on the concentration of DO and on the ability of water to hold dissolved oxygen. You will begin with a single water sample, divide it into three portions, and let each portion equilibrate at a different temperature. Then you will use the Winkler Method to measure DO for each sample. The Winkler Method is a series of reactions that incorporates and removes from solution the oxygen dissolved in the water and releases free iodine. The amount of free iodine released is proportional to the amount of free dissolved oxygen in the original sample. The amount of free iodine in the sample is then determined by adding a starch indicator solution to the sample, which turns blue in the presence of free iodine, then titrating with sodium thiosulfate to a colorless endpoint. The amount of sodium thiosulfate needed to titrate the iodine is directly proportional to the concentration of dissolved oxygen in the original sample.

Procedure

1. Label 3 BOD bottles, one *4*°C, one *25*°C, and one *30*°C.
2. Fill one BOD bottle with water of the matching temperature. It is important that you do not trap air in the bottle and avoid introducing any turbulence. Improper filling will mix air into the sample and increase the dissolved oxygen level. Consider the following methods:
   - **(a)** Fill the bottle by submerging it in the sample. Allow the bottle to fill, then cap it while it is still submerged.
   - **(b)** Use a 60-mL syringe with a piece of tubing attached. (This method works well if the sample container is deep or has a narrow mouth.) Place the end of the tubing at the bottom of an upright BOD bottle and introduce the sample gently. To ensure that there is no air trapped in the bottle to give elevated oxygen readings, fill the bottle until it overflows significantly. Cap the bottle tightly after filling.
3. Determine the DO of the sample by following the Winkler Method Protocol below. Record the DO for that temperature sample in Table 1.
4. Repeat this procedure for each of the other water temperatures.

Winkler Method Protocol

Step 1: Oxygen Fixation

a. Uncap the BOD bottle.

b. Add 8 drops of manganous sulfate solution to the bottle.

c. Add 8 drops of alkaline potassium iodide azide to the bottle.

d. Cap the bottle and mix. A precipitate will form. Allow the precipitate to settle to the shoulder of the bottle before proceeding.

e. Use a 1-g spoon to add 1 gram (1 spoonful) of sulfamic acid powder to the bottle.

f. Cap the bottle and mix until reagent and precipitate dissolve. The sample is now fixed.

Note: *This is an optional stopping point. Samples can be stored at room temperature until you are ready to continue.*

Step 2: Titration

a. Uncap a BOD bottle and use it to fill the titration sampling vial to the *20 mL* line. Be accurate; variations in filling from group to group and from bottle to bottle will result in inconsistent data.

b. Fill the titration syringe to the top of the scale (1.0 mL) with sodium thiosulfate. Read the volume across the concave edge of the plunger.

c. Add one drop of sodium thiosulfate at a time to the sample, swirling between each additional drop until the sample becomes a faint yellow color.

d. Remove the titration syringe and the cap together, without disturbing the syringe. Add 8 drops of starch indicator solution.

e. Replace the lid of the titration tube and swirl the sample. The solution should turn blue.
Note: If the solution does not turn blue, either there is not a measurable amount of oxygen present, or too much sodium thiosulfate was added in Step 2c. Pour out the sample, refill the titration tube from the BOD bottle, and start the titration again at Step 2b.

f. Continue the titration with the sodium thiosulfate already in the syringe. Add one drop at a time, swirling the sample after the addition of each drop, until the blue color disappears. If the blue color does not disappear after the addition of the whole syringe of sodium thiosulfate, refill the syringe and continue. When the titration is complete, add the amount from the first syringe to the amount added from the second syringe to get the total amount of sodium thiosulfate used.

g. Read the syringe at the bottom of the plunger. Each 0.1 mL of sodium thiosulfate used in the titration equals 1 ppm DO, or 1 mg DO per L of water. Record your data in Table 1.

Table 1: Temperature and Dissolved Oxygen

Temperature	Group DO	Class Average DO	Group % Saturation	Class Average % Saturation

Analysis of Results, Activity A: Temperature and Dissolved Oxygen

1. Plot the Class Averages for DO from Table 1. Title the graph and supply the following information:

   a. The independent variable is _____.

   b. The dependent variable is _____.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

2. Based on your class data, what is the relationship of temperature to DO?

   _____

   _____

   _____

3. Rarely is water saturated with oxygen. Usually, the amount of DO in a water sample is only part of what the water could hold. You can estimate the percent saturation of the water samples with oxygen using the Class Averages for DO from Table 1 and the nomograph shown in Figure 1. Use a pencil to mark the temperature of the water on the top scale of the nomograph and the amount of dissolved oxygen on the bottom scale, then use a straightedge to draw a line connecting the two marks. Read the percent saturation from the middle scale of the nomograph. Record this number in Table 1.

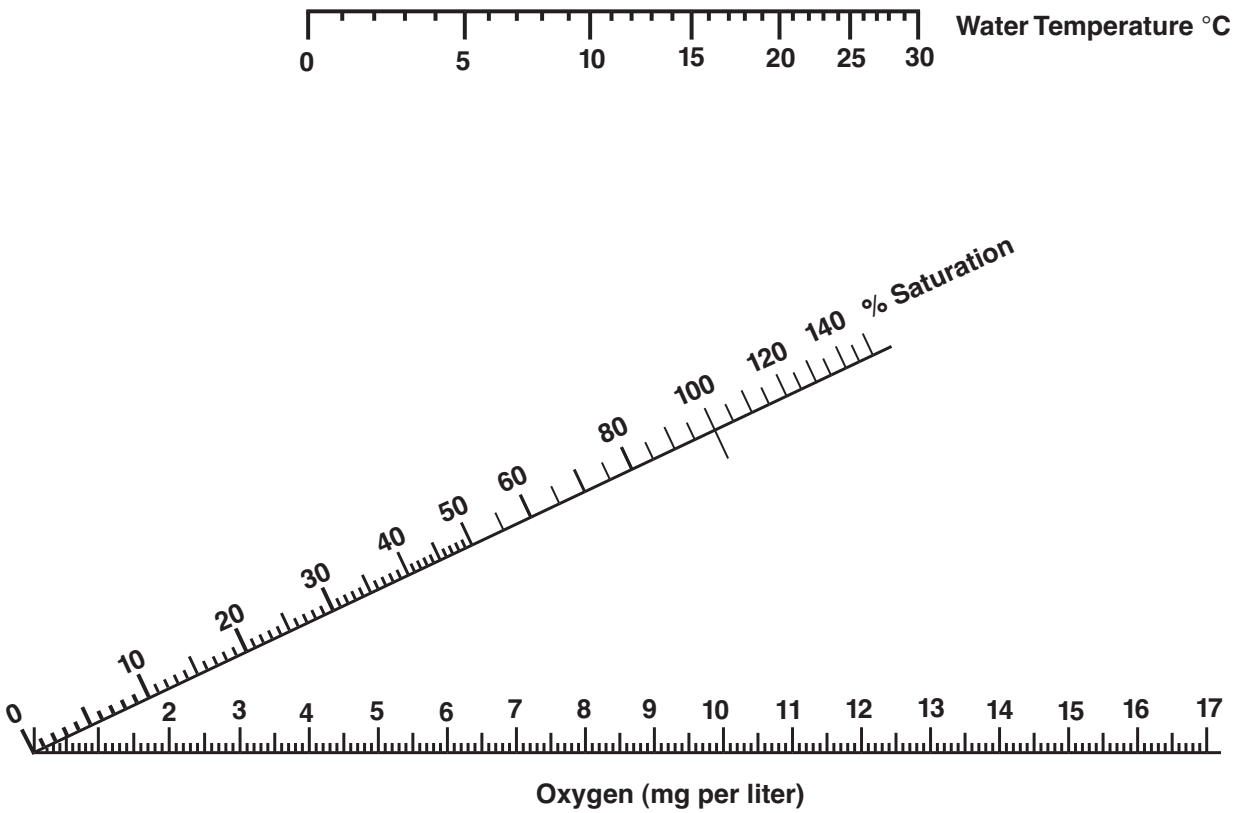


Figure 1: Nomograph of Oxygen Saturation

4. Plot the Class Averages for Percent Saturation from Table 1. Title the graph and supply the following information:

   a. The independent variable is _____.

   b. The dependent variable is _____.

   Plot the independent variable on the x-axis, and the dependent variable on the y-axis.

5. Based on your data and graphs, what is the relationship of temperature to percent saturation?

   _____

   _____

   _____

6. What inference can you draw from your answer to #5 that would help explain the relationship of temperature to DO given in your answer to *#2?*

   _____

   _____

   _____

   _____

   _____

   _____

Activity B: Primary Productivity

Background

Primary productivity is one of the key concepts of ecology. It refers to the rate at which autotrophs (producers) store organic materials. If the primary productivity of an ecosystem is high, the ecosystem can support a large biomass of autotrophs, which in turn will support a substantial (but smaller) biomass of heterotrophs (consumers and decomposers). If primary productivity falls, biomass at all levels must decrease as well. Thus, measuring the primary productivity of an ecosystem, especially if the measurement is repeated over time, can reveal a great deal about what is happening and what will happen in that ecosystem.

In most ecosystems, primary productivity is driven by the rate of photosynthesis in green plants and/or photosynthetic protists (collectively, phototrophs). Recall from AP® Biology Lab 4, Plant Pigments and Photosynthesis, the basic equation for photosynthesis:

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2$$

From this equation, we see that primary productivity can be determined by measuring the rate of carbon dioxide consumption, the rate of formation of organic compounds, or the rate of oxygen production. In an aquatic ecosystem, the oxygen produced by photosynthesis commonly goes into solution, increasing the DO of the water. In this activity, you will use DO levels as a measure of primary productivity.

Determining productivity is complicated by the fact that phototrophs engage in both photosynthesis and cellular respiration. Phototrophs produce oxygen and glucose through photosynthesis, which requires light and carbon dioxide. Phototrophs also use some of the glucose they manufacture as an energy source through respiration, which requires oxygen. Phototrophs respire constantly, but photosynthesize only when light is available. It is therefore necessary to distinguish gross (total) primary productivity from net primary productivity.

You will use a light and dark bottle method to determine the primary productivity of a model pond ecosystem. In this method, two identical samples are incubated, one in light, the other in darkness. At the end of incubation, you will measure the DO of each sample. You will assume that the rate of respiration is the same in both samples. Thus, the sample in the light measures net primary productivity, and the sample in the dark measures the loss due to respiration. Adding the two will give the gross productivity. Most ponds vary in depth. You will simulate samples taken from differing depths of the model pond ecosystem by wrapping sample bottles with screens to block some of the light during incubation.

Materials

7 BOD bottles, *Chlorella* culture, gloves, manganous sulfate, starch indicator, sulfamic acid and measuring spoon, alkaline potassium iodide azide, sodium thiosulfate, 2 titration syringes, 2 20-mL sampling vials, 17 fiberglass screens, square of aluminum foil, waterproof marker, rubber bands, 60-mL syringe with tubing attached (optional; see Activity A Procedure).

Caution: *Use extreme care when handling chemicals. Sulfamic acid and alkaline potassium iodide azide can irritate or burn the eyes, skin, and mouth. Avoid all skin contact with these and other chemicals. Do not put any chemical in or near your mouth. Your teacher will instruct you about the proper safety procedures for handling hazardous materials.*

Introduction

In this activity, you will measure oxygen production by the photosynthetic protist *Chlorella* under various light intensities, using the light and dark bottle method.

The first step involves measuring the DO of an initial sample of the simulated pond water. This will give you a baseline DO to which you can compare all other measurements of DO for the simulated pond. You will then prepare a light bottle sample and a dark bottle sample. These will be identical except that you will wrap the dark bottle in aluminum foil to block all light. You will simulate samples taken from different depths of a pond by preparing four additional samples, wrapping each bottle with one or more fiberglass screens.

Procedure

Day One

1. **Determining the Initial (Baseline) DO.** Fill a BOD bottle with water from the model pond (*Chlorella* culture). Use the same procedure for filling that you used in Activity A, and cap the bottle. Determine the DO by following the Winkler Method Protocol. Record your data as the Baseline in Table 2. **Note:** If your sample contains a heavy algae load, the algae will form a black precipitate that will not go into solution. This will not affect your results.
2. **100% Light and Dark Bottle Preparation.** Fill two BOD bottles with water from the model pond and cap them. Wrap one bottle, which will be the dark bottle, in aluminum foil to exclude all light. The other bottle will be the 100% light bottle. Label the bottles with your group's name and the appropriate treatment. Lay the bottles on their sides under a fluorescent or grow light, seam side down, and leave them overnight.
3. **Preparation of Simulated Depth Samples.** Prepare four additional BOD bottles with model pond water. Cover each one with one or more screens according to the table below. Secure the screens with rubber bands. Label the bottles with your group's name and the appropriate treatment. Lay the bottles on their sides under a fluorescent or grow light, seam side down, and leave them overnight. Notice that the light bottle prepared in Step 2 above serves as 100% light.

Number of Screens	Percent Light	Simulated Depth
0 (prepared in Step 2)	100%	0.0 m
1	65%	1.0 m
3	25%	2.0 m
5	10%	3.0 m
8	2%	4.0 m

Day Two

Determine the DO for each of your sample bottles by following the Winkler Method Protocol. Record your data in Table 2. **Note:** If your sample contains a heavy algae load, the algae will form a black precipitate that will not go into solution. This will not affect your results.

Table 2: Group Productivity Data

Bottle	DO	Net Productivity	Gross Productivity
Baseline (Initial)			
Dark			
Light (0 screens)			
1 screen			
3 screens			
5 screens			
8 screens			

Analysis of Results, Activity B: Primary Productivity

1. Calculate the loss of oxygen due to respiration and record here: _____ mg/L

   $R = I - D$

   where
   R = loss due to respiration
   I = DO baseline
   D = DO dark bottle

2. Calculate net productivity of the other samples and record the data in Table 2.

   $P_n = L - I$

   where
   P_n = net productivity
   I = DO baseline
   L = DO sample

3. Calculate gross productivity of each sample and record in Table 2.

   $P_g = P_n + R$

   where
   P_g = gross productivity
   P_n = net productivity
   R = loss due to respiration

4. Determine class averages for net and gross productivities. Record the data in Table 3.

Table 3: Class Averages for Gross and Net Productivities

Bottle	Average Net Productivity	Average Gross Productivity
Light (0 screens)		
1 screen		
3 screens		
5 screens		
8 screens		

5. Graph the data for Average Gross and Average Net productivities from Table 3. Title the graph and supply the following information:

   a. The independent variable is _____.

   b. The dependent variables are _____.

   Plot the independent variable on the x-axis, and the dependent variables on the y-axis.

6. From your graph:

   a. At approximately what light intensity does the rate of respiration equal the rate of photosynthesis?

   _____

   _____

   _____

   b. At approximately what depth in the simulated pond does this occur?

   _____

   _____

   _____

7. Two researchers, one at Toolik Field Station in northern Alaska and the other at La Selva Biological Station in Costa Rica, are studying populations of aquatic arthropods in freshwater pools during July. The researcher in Alaska determines an average of 280 arthropods per m^3 of water in the pools she is studying. The researcher in Costa Rica determines an average of 125 arthropods per m^3 at his study site. In terms of the current lab, how would you account for this difference?

   _____

   _____

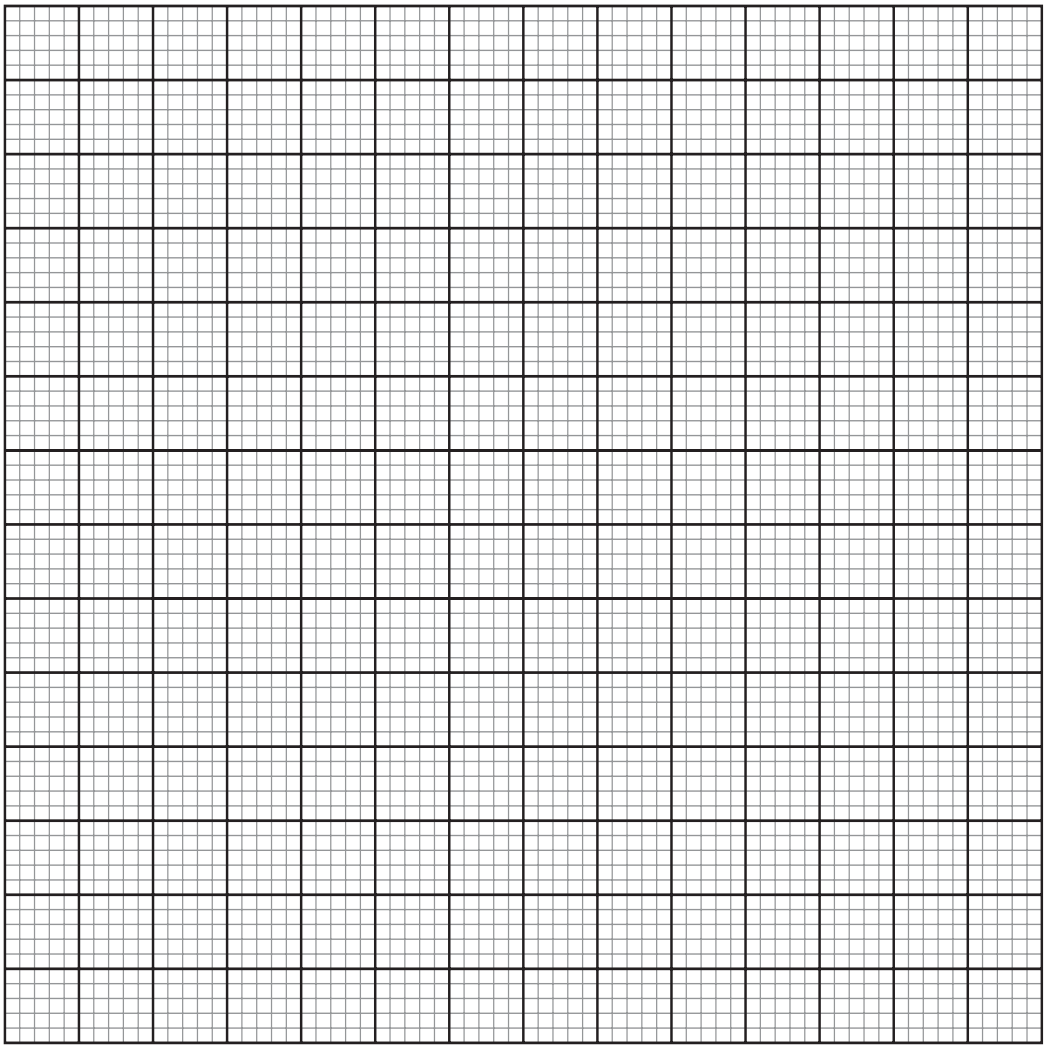
   _____

   _____

   _____

8. You are the Water Quality Director for Derry County. In this capacity, you are asked to review reports of two fish kills in the county. Both involve artificial ponds with surface areas of approximately 0.8 hectares and maximum depths of 7 meters. Tests performed immediately after the fish kills detected no pesticides or other poisons. The dead fish showed no signs of fungal attacks or other disease. Case A involves a pond stocked with bass and used for recreational fishing. Meteorological records show that the kill occurred after 4 weeks of hot weather in which daytime temperatures reached 35–40°C. Case B involves a pond stocked with bluegill and used to irrigate pastureland. This kill occurred in the spring, before the heat wave and 9 days after a heavy rain. The file for Case B contains a photo showing dead fish floating in the pond. You also notice what appear to be mats of decaying algae floating on the surface of the water. A call to the farmer reveals that he applied ammonium nitrate to the pastureland the week before the rain. State your judgment as to the probable causes of these fish kills, and describe the chain of events that led to each.

Title: _____



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