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

12. 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 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:

mg O_2/L x 0.698 = ml O_2/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.

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.

B. Measurement of Primary Productivity

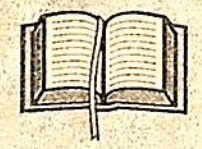
The productivity per square meter of a water column in an aquatic environment can be measured using the light–dark bottle oxygen method. In this experiment, the reduction of natural light levels at various depths will be simulated with screen filters. Temperature is constant so only a single variable,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

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



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

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 O_2/L) – Dark Bottle (ml O_2/L)
Net Productivity = Light Bottle (ml O_2/L) – Initial Bottle (ml O_2/L)
Respiration Rate = Initial Bottle (ml O_2/L) – Dark Bottle (ml O_2/L)

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:

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

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

$$2Mn(OH)_2 + O_2 + 2H_2O \longrightarrow 2Mn(OH)_4$$

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

$$2Mn(OH)_4 + 4H_2SO_4 \longrightarrow 2Mn(SO_4)_2 + 8H_2O$$

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

$$2Mn(OH)_4 + 4KI \longrightarrow 2MnSO_4 + 2K_2SO_4 + 2I_2$$

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:

$$4Na_2S_2O_3 + 2I_2 \longrightarrow 2Na_2S_4O_6 + 4NaI$$

Collect the class data and determine both the mean gross productivity and mean net productivity. Enter the values in Table 4 in the Analysis section.

Convert your mean gross productivity data (ml O_2) for your samples to carbon productivity (mg C/m^3) using the following formulas:

ml O_2/L = 0.698 x mg O_2/L
mg C/L = 0.536 x ml O_2/L

To convert liters to meters cubed, divide liters by 0.001.