Fundamentals of Limnology
Oxygen, Temperature and Lake Stratification

Prereqs: Students should have reviewed the importance of Oxygen and Carbon Dioxide in Aquatic Systems

Students should have reviewed the video tape on the calibration and use of a YSI oxygen meter.

Students should have a basic knowledge of pH and how to use a pH meter.

Safety: This module includes field work in boats on Raystown Lake. On average, there is a death due to drowning on Raystown Lake every two years due to careless boating activities. You will very strongly decrease the risk of accident when you obey the following rules:

1. All participants in this field exercise will <u>wear</u> Coast Guard certified PFDs. (No exceptions for teachers or staff).

2. There is no "horseplay" allowed on boats. This includes throwing objects, splashing others, rocking boats, erratic operation of boats or unnecessary navigational detours.

3. Obey all boating regulations, especially, no wake zone markers

4. No swimming from boats

5. Keep all hands and sampling equipment inside of boats while the boats are moving.

6. Whenever possible, hold sampling equipment inside of the boats rather than over the water. We have no desire to donate sampling gear to the bottom of the lake.

7. The program director has <u>final say</u> as to what is and is not appropriate safety behavior. Failure to comply with the safety guidelines and the program director's requests will result in expulsion from the program and loss of Field Station privileges.

I. <u>Introduction to Aquatic Environments</u>

Water covers 75% of the Earth's surface. We divide that water into three types based on the **salinity**, the concentration of dissolved salts in the water. Salt-water has the highest concentration of dissolved salts (greater than 17.0 parts per thousand). Brackish water has a moderate amount of dissolved salts in the water (salinity values range from 0.5 to 17 parts per thousand). Brackish water and saltwater make up 97.2% of the earth’s water resources and are typically associated with **Marine** environments, those environments associated with oceans. The study of Marine environments is referred to as Marine Science.

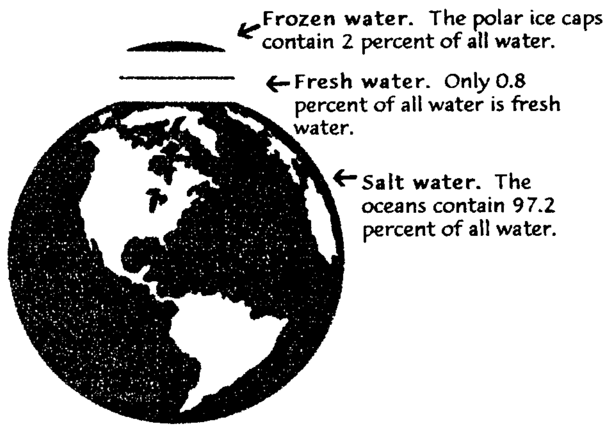


Fig. 1. Illustration of the earth's water.

Freshwater has the lowest concentration of dissolved salts in the water (less than 0.5 parts per thousand). Freshwater is subdivided into frozen and liquid freshwater; frozen water makes up 2% of the earth's water and liquid freshwater makes up only 0.8% of the earth's water resources. Freshwater environments are typically associated with inland resources and the study of inland waters is referred to as **Limnology** from the Greek word *limne*. meaning pool, marsh or lake. Remember, not all inland waters are freshwater, such as the Great Salt Lake in Utah (Fig. 1).

(Insert Graphic)

Freshwater environments come in two forms **Lentic** (running water) and **lotic** (standing water). Lentic habitats include streams and rivers. They are usually shallow enough to have plenty of light, and have sufficient nutrients from runoff of the river banks to maintain healthy populations of plants and animals. Lotic environments include lakes and ponds. A pond is shallow enough to receive light to all depths. Lakes however, are deep enough that light does not reach the bottom. As light does not penetrate to the deepest waters in a lake, plant growth is limited to an area near the surface referred to as the **Euphotic zone**. The euphotic zone is the region of a lake in which sunlight is of sufficient intensity to allow for photosynthesis. The euphotic zone is divided into two regions, the **Littoral Zone** and the **Limnetic Zone.** The Littoral Zone is the shallow area around the edge of a lake where light penetrates to the bottom. In many lakes, this area is characterized by rooted aquatic plants. The limnetic zone is the area of surface water that receives sufficient light for plant growth but is directly above deep, unlit waters rather than a shallow lighted bottom (Fig. 2).

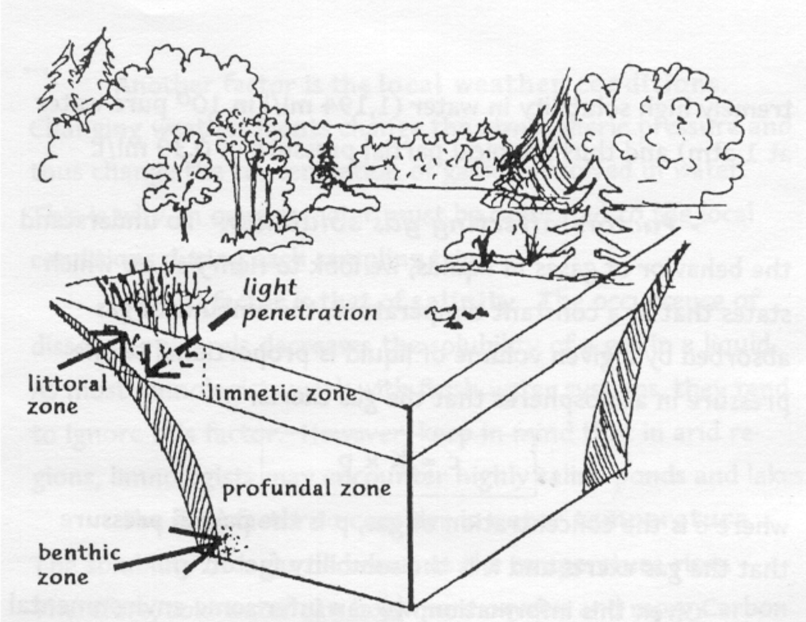


Fig. 2. Different zones found in lakes.

The waters directly below the Limnetic Zone are the cool dark waters referred to as the **Profundal Zone**. The bottom of the lake, whether below the Littoral Zone or the Profundal Zone, is referred to as the **Benthic Zone**.

II. Basics of Lake Chemistry

In this module, we are primarily interested in two important dissolved gasses in water which are critical to living organisms. These gases are Oxygen (O_2) and Carbon Dioxide (CO_2). Oxygen is abundant in the atmosphere (21%) and has a moderate solubility (38.46 ml/l in 10^o pure water at 1 atm) and a typical partial pressure of 8.06 ml/l. Carbon Dioxide is not nearly as abundant in the atmosphere (0.033%) but has extremely high solubility in water (1,194 ml/l in 10^o pure water at 1 atm) and thus a typical partial pressure of 0.39 ml/l).

Factors affecting gas solubility.

To understand the behavior of gases in liquids, we look to 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:

$$\mathbf{c = K \times p}$$

where c is the concentration of gas, p is the partial pressure that the gas exerts and K is the solubility factor.

Given this information, we can infer some environmental factors which will affect the concentration of O_2 and CO_2 in a lake. The first of these is **altitude**. Increases in altitude decrease the pressure of the atmosphere and thus decrease the concentration of gasses dissolved in water. This explains why an oxygen meter must be calibrated for the specific altitude at which the sampling is being conducted. Printed on the back of many oxygen meters is a chart which provides correction factors in oxygen solubility for given altitudes.

Species of Fish	Lowest DO amount (mg/L) at which fish survived for 24 hrs (summer)	Lowest DO amount (mg/L) at which fish survived for 48 hrs (winter)
Northern Pike	6.0	3.1
Black Bass	5.5	4.7
Common Sunfish	4.2	1.4
Yellow Perch	4.2	4.7
Black Bullhead	3.3	1.1

Fig 4. Dissolved Oxygen (DO) chart of fish survival rates taken by permission from “Water Quality Criteria,” the Resources Agency of California, State Water Quality Board, 1963, Pub. No. 3-A

Another factor is the **local weather conditions**. Changing weather fronts change the atmospheric pressure and thus change the concentration of gasses dissolved in water. This is why an oxygen meter must be calibrated to the local conditions during each sampling trip.

A third factor is that of **salinity**. The occurrence of dissolved minerals decreases the solubility of a gas in a liquid. As most limnologists work with fresh water systems, they tend to ignore this factor. However, keep in mind that in arid regions, limnologists may encounter highly saline ponds and lakes.

The final factor to consider is **water temperature**. The solubility of a gas decreases as the temperature rises. Therefore, cold water can hold more oxygen and more Carbon Dioxide than warm water. This does not mean that cold water will always have a greater concentration oxygen than warm water. There has to be a source of available oxygen to dissolve in the water.

III. Sources and Sinks of Oxygen and Carbon Dioxide in Lakes

In order to organize our discussion of O_2 and CO_2 in aquatic environments, it is useful to think in terms of sources and sinks of these gases. **Sources** are things or processes which put a given gas into the environment and **sinks** are things or processes which remove a given gas from the environment.

Oxygen Sources in the Euphotic Zone

In the euphotic zone, there are two main sources of O_2, **atmospheric mixing** and **photosynthesis**. When the atmosphere comes in contact with water, some oxygen goes into solution.

Wind and wave action will facilitate this process and cooler water will take up more oxygen than warm water. For oxygen to readily diffuse throughout a lake, some agitation of the water is required. For example, in an **anaerobic** body of water (no oxygen present), if oxygen was distributed from the surface by only simple molecular diffusion, it would require years for oxygen to become detectable 5 m below the surface.

The second and more important source of oxygen in the euphotic zone is photosynthesis. **$6CO_2$ + $6H_2O$ yields $C_6H_{12}O_6 + 6O_2$.**

The reaction is powered by light and the oxygen comes from the water rather than the CO_2. In most lakes, **phytoplankton**, tiny floating plants, provide most of the oxygen production as their populations are very dense in the limnetic zone. In the littoral zone, rooted aquatic plants and benthic algae contribute significantly to the oxygen production. Incidentally, many persons believe that the bulk of the oxygen produced on the planet comes from the Rain Forests. In fact, most of the oxygen (88%) is produced by phytoplankton in the euphotic zones of the oceans (Small 1972). Rain Forests cover less than 2% of the planet whereas oceans cover over 68% of the planet.

Oxygen Sinks in the Euphotic Zone

In the euphotic zone there are only two major oxygen sinks. These sinks are **respiration** and oxygen loss to the atmosphere. Some O_2 may be lost to chemical reactions in the water but the amount is usually negligible.

Just as oxygen may be gained in water from atmospheric mixing, it can also be lost. As water becomes saturated with oxygen, through photosynthesis for example, oxygen will be given off to the atmosphere. Wind and wave action facilitate this process. Typically, people think of cold rushing mountain streams as the most oxygen rich aquatic environments. However, as soon as a stream reaches 100% oxygen saturation, it easily gives off its excess oxygen to the atmosphere in riffles and falls. Small, algae rich stagnant pools, (often associated with nutrient pollution), produce lots of oxygen which diffuses out slowly. Such pools can reach saturation levels of up to 120%.

In many lakes, the primary oxygen sink is **respiration**, the metabolic process that breaks down sugars and consumes O_2 to produce energy and CO_2. Students tend to think of fish when they consider respiration occurring in lakes. However, all living organisms respire including plants, animals and *bacteria*? Respiration demands on oxygen tend to be greater at night, when no photosynthesis is occurring, and in the winter when ice and snow block sunlight from the water. Respiration demands on oxygen also tend to be greater when there is a lot of substrate and nutrients for bacterial growth. In this way, nutrient pollution of an aquatic system can result in fish kills as bacterial growth depletes the oxygen supply.

Oxygen Sources in the Profundal Zone

As the profundal zone is below the level of light penetration sufficient for photosynthesis, photosynthesis does not serve as an oxygen source in that zone. Furthermore, the profundal zone is insulated from atmospheric mixing by the littoral zone. Molecular diffusion of oxygen is very slow so the only significant source for the profundal zone is from possible water currents from the littoral zone which might carry oxygen down.

Oxygen Sinks in the Profundal Zone

The primary sink of oxygen in the profundal zone is respiration of fish and bacteria. Many of these organisms feed upon the rain of detritus from the littoral zone. Respiration demand is particularly great in the benthic zone where bacteria are abundant in the sediment. Oxygen content of the water at the bottom of a lake is often significantly lower than the water a few cm above. Lakes with gentle bottom contours and high sediment loading tend to have greater oxygen demand through respiration than lakes with steep contours and lower sediment loadings.

Measuring Oxygen *(Side Box)*

There are two methods at our disposal with which to measure oxygen. The first method is termed a Winkler Titration, developed by L.W. Winkler of Budapest in 1888. This clever and accurate method depends on the oxidation of manganous hydroxide by the oxygen dissolved in the water, resulting in the formation of a tetravalent compound. When the tetravalent compound is acidified, free iodine is liberated. The amount of iodine liberated is equalivent to the amount of dissolved oxygen present, and can be determined by titration with sodium thiosulfate (Wetzel and Likens 1991).

An easier, although less accurate method, is with a Clark polarographic oxygen sensor. The sensor consists of a platinum anode and a gold plated cathode in an electrolyte-filled housing separated from the water by an oxygen permeable membrane. Oxygen passes through the membrane and electrolyte solution and is reduced on the electrode resulting in an electrical current. The amount of oxygen reduced is proportional to the current which is measures with a meter (Wetzel and Likens 1991).

Carbon Dioxide

Carbon takes a variety forms in aquatic systems and the relationship among the various carbon compounds is quite complex. For the purposes of this investigation, we present a very simplified version of carbon sources and sinks.

Carbon Dioxide Sources in the Littoral Zone

There are several sources of Carbon Dioxide in the Littoral Zone. Although CO_2 has a low partial pressure in the atmosphere, its extremely high solubility in water significantly contributes to its abundance in natural waters. Another atmospheric source of carbon dioxide is through rain water (0.55 to 0.60 mg/l) and through runoff trickling through soil (which carries even greater concentrations of CO_2).

Another important source of carbon dioxide in the littoral zone is the decomposition activities of **aerobic** (oxygen consuming) bacteria and from respiration by plants and animals.

The introduction nutrients into an aquatic system may accelerate plant growth and / or increase decomposition activities. Such changes result in increased CO_2 production in the littoral zone.

Carbon Dioxide Sinks in the Littoral Zone

Carbon dioxide is lost to the Ecosystem in a variety of ways. The primary CO_2 sinks are through photosynthesis incorporating the carbon as organic matter and through chemical reactions. Although there are many chemical reactions involving CO_2 in natural waters including bicarbonate and monocarbonate, an important reaction for the purposes of this study is the formation of carbonic acid.

$$\mathbf{CO_2 + H_2O \text{ yields } H_2CO_3}$$

Although only a small proportion of CO_2 dissociates in this manner (less than 1%), the carbonic acid can again dissociate into bicarbonate and hydrogen ions resulting in a lowering of the pH. This process is similar to "carbonation" of soft drinks. (Coca Cola has a pH of ___) The lowering of pH is a typical occurrence when CO_2 is dissolved in natural waters.

Carbon Dioxide Sources in the Profundal Zone

Sources of carbon dioxide in the profundal zone are similar to those of the littoral zone. Respiration of animals and the decomposition activities of aerobic bacteria are major contributors. Although there is not direct input from rainwater or runoff, subterranean water sources such as springs are usually rich in Carbon Dioxide.

Carbon Dioxide Sinks in the Profundal Zone

There are not as many sinks for carbon dioxide in the profundal zone. Diffusion to the atmosphere and photosynthesis are not options due to the depth and inadequate light. The primary sinks for carbon dioxide in the profundal zone are thus through the formation of chemical compounds.

Measuring Carbon Dioxide *(Side Box)*

During this module we will not measure the concentration of carbon dioxide *per se.* Rather, we will obtain a rough index of CO_2 concentration by measuring the product of its dissociation into carbonic acid and then into hydrogen ions. A convenient and simple method to measure the concentration of hydrogen ions is with a pH meter. A pH probe consists of a glass electrode filled with HCl and a reference electrode filled with an electrolyte solution (usually KCl). The glass electrode discriminates H^+ ions and an electrical potential develops between the water sample and the HCl in the glass probe. The stream of KCl from the reference electrode completes the circuit and the electrical potential is registered by the meter.

The glass probe is very sensitive and **extreme care must be used to prevent abrasion or desiccation of the probe**.

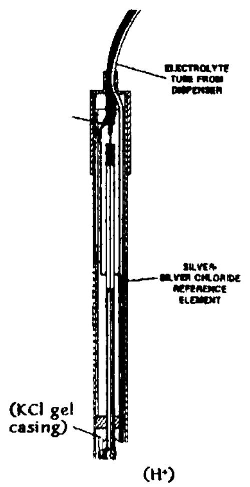


Fig. 4. Illustration of a pH probe (courtesy of the Hach Company).

Questions: Would CO_2 concentrations be affected by the time of day?

Does plant respiration contribute to CO_2 production in the profundal zone?

IV. Research Question

For the purposes of this module, our research questions are:

1. Is there a difference in oxygen concentration between the euphotic and profundal zone of Raystown Lake?

2. What is the relationship between dissolved oxygen distribution and water temperature in Raystown Lake?

3. Is there a difference pH between the euphotic and profundal zone of Raystown Lake?

V. Hypothesis Formation

With the above information, you the student should be able to make predictions about the relative concentrations of Oxygen and Carbon Dioxide in the Littoral Zone and in the Profundal Zone of a given lake. Will carbon dioxide (and thus hydrogen ions) be in greater concentration in the littoral zone than in the profundal zone? Work in teams of two or three students to make your predictions about carbon dioxide and oxygen. Record your predictions in your student notebook.

VI. Field Testing your Hypotheses

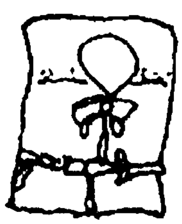
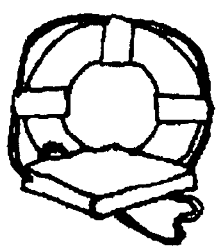
We will now go out to the lake to gather data on Oxygen and pH in an attempt to support or refute your hypotheses. We will also gather information on water temperature and water clarity which may affect oxygen concentrations.

Approach

We will travel by boat to a sampling point on the lake. The destination will be determined by weather conditions, boat traffic and time available. Most sampling points are approximately 20 m in depth. At the sampling point, we will examine the following parameters:

1. **Oxygen Profile** (Samples at 1 m increments from the surface to the bottom)
2. **Temperature Profile** (Samples at 1 m increments from surface to bottom)
3. **% Oxygen Saturation** (Samples at 1 m increments from surface to bottom)
4. **pH** (6 replicate samples from the surface, 10 m depth and the bottom)
5. **Secchi Disk Depth** (7 replicate samples)

Please Review the Boating Safety Notes Listed at the Beginning of this Module


Oxygen, Temperature and % Saturation (add diagram of oxygen meter)

We will measure Oxygen, Temperature and % Saturation with a YSI Oxygen Meter. The Oxygen probe is attached to a 30 m cable and marked at 1 m increments. First, calibrate the meter, according to the instructions printed on the back of the meter. Lower the probe into the water so that the probe is just submerged below the surface. This corresponds to the "surface" designation on the Oxygen / Temperature data sheet on page ___ of your student field book. Using the selector knob on the Oxygen Meter, record Oxygen, Temperature and % Saturation. Then lower the probe to the 1 m mark and repeat the process. In this manner, collect data from 1 m increments for the surface to the bottom. It is important to determine the depth at which you are sampling in order keep the probe off of the bottom sediments of the lake. Due to microbial action, the layer of water within 3 cm of the bottom tends to have significantly lower oxygen levels that the rest of the profundal zone. You have no way of knowing if the probe in lying on the bottom unless you know the exact depth. (on occasion, students have recorded 20 m oxygen profiles in 4 m of water). Take five replicate oxygen readings from the surface and the lowest depth. Only one reading from the remaining 1 m increments is necessary.

pH

Calibrate the pH meter according to the instructions accompanying the instrument. Recording the pH is easy. Merely place the electrode in the sample and record the reading. (page __ of your student field book). The trick is in getting the water samples.

To collect water samples from the desired depths, we will use two types of deep water samplers: *Alpha Bottle* and *Kemmerer Bottle*. (Fig __). First, arm the bottles according to the directions provided by your instructor. Lower the bottle to the desired depth and then send down the "messenger" to trigger the bottle to collect the sample. Bring the bottle back to the surface to extract your water sample.

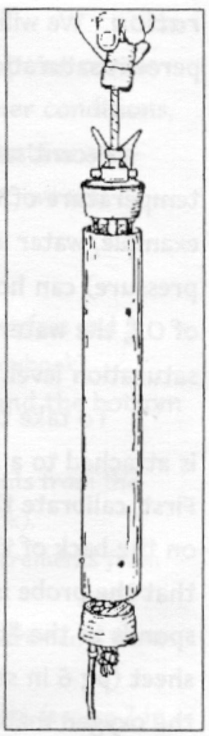


Fig. 5. Illustration of a Kemmerer Bottle (courtesy of the Hach Company).

(Put tips in box)

Tips - always have an additional person hold onto the cable reel to feed you line while using the bottles. Thus, if you lose your grip, you won't lose an <u>expensive</u> instrument!

- a replicate sample consists of replicate bottles, not replicate samples from one bottle.

- keep the bottles off of the bottom. The bottles will not trigger if they are laying on their side and sediments in the bottles will contaminate the samples.

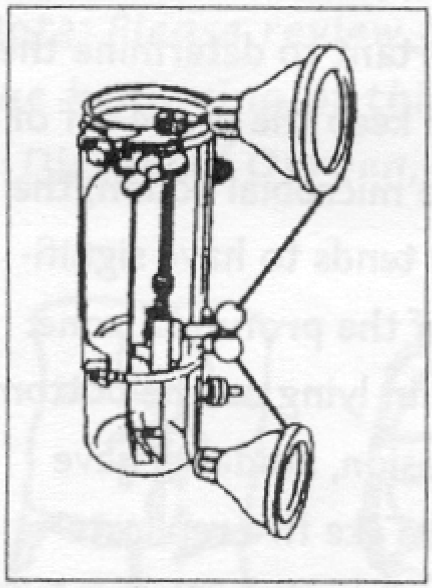


Fig. 6. Illustration of an Alpha bottle (courtesy of Wildco Company).

Take seven replicate pH readings form each of the three depths, (surface, 10 m, bottom)

Sechi Disk (Fig ___)

The Secchi disk allows for a simple measure of water clarity (Turbidity). Lower the disk into the water until it disappears. Then draw the disk up through the water until you can first distinguish the difference between the light and dark quarters of the disk. The depth at which you can discern the difference between the quarters is the Sceehi value. The cable is marked in 10ths of meters and the depth should be reported in meters. There is a great deal of observer variability associated with technique and standardization of method is important. Lower the disk from the shaded side of the boat when possible. As with the deep sampling bottles, always have an additional person hold onto the cable reel to feed you line while using the Secchi disk. Take seven replicate Secchi Depths and record them on page __ of you Field Notebook.

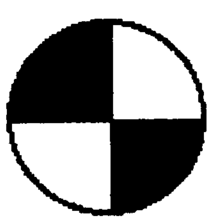


Fig. 7. Illustration of a Secchi disk.

VII. Data Analysis

After we have collect all of the required data, we will return to the Field Station. Make certain that all meters are shut off and packed properly.

Procedure

1. Upon returning to the Field Station, graph the Oxygen and Temperature Profiles together on one graph. We will provide you with graph paper and an example format.

2. Conduct a two sample t-test between the bottom and surface for Oxygen, Temperature, and pH. We will provide instruction on how to perform these tests.

3. We will then, as a group, discuss our findings and how to interpret them.

References

Small

Wetzel, R. G. and G. E. Likens. 1991. Limnological Analysis