Texas Stream Team
Caring for Our Waters

8^{th} – 12^{th} – Dissolved Oxygen (Activity 1)

Objectives for All Dissolved Oxygen Activities
- Link the concept of oxygen in the student's life to the levels of oxygen in the aquatic ecosystem.
- Discuss why dissolved oxygen is such an important measure of water quality.
- Students should be able to perform the Winkler titration to test for Dissolved Oxygen.
- Explain the reasons for and circumstances associated with changes in dissolved oxygen level.

Materials Activity 1
- Large Milk Shake Straws
- Regular Straws
- Stirring Straws

Background
Dissolved oxygen is the form of oxygen in water that is freely available to aquatic plants and animals. Dissolved oxygen is vital to fish and other aquatic life and for the prevention of odors. Oxygen is transferred from the atmosphere into surface waters, as well as being produced by aquatic plants, algae and phytoplankton as a by-product of photosynthesis. Once dissolved in water, oxygen diffuses throughout a water body very slowly since distribution depends on the movement of aerated water by turbulence and currents, water flow and thermal upwelling.

Traditionally, the level of dissolved oxygen has been accepted as the single most important indicator of a water body's ability to support desirable aquatic life. The amount of oxygen required varies according to species and the stage of life of a species. Usually, dissolved oxygen levels of 5.0 to 6.0 part per million (ppm) are required for growth and activity. Dissolved oxygen levels below 3.0 ppm are stressful to most aquatic organisms. When levels fall below 2.0 ppm for an extended period of time, most fish will not survive. Oxygen is a particularly sensitive constituent because its availability during different times of day and times of year is influenced by temperature, other chemicals present in the water, and biological processes. Temperature plays a major role in influencing the amount of dissolved oxygen in water. Water at a temperature of 31°C (typical for Texas' summer days) will only hold about half as much dissolved oxygen as the same water on a cold winter day at 1°C. Cold water has the ability to contain more oxygen than warm water.

Oxygen is transferred from the atmosphere into the surface waters at the point of contact where the surface of the water interfaces with air. Points of contact are increased by the action of the wind through a process called physical aeration. Oxygen is also added as a by-product of plant photosynthesis. As a result, floating and rooted aquatic plants increase DO levels through the process of photosynthesis. Since the existence of plants depends on the availability of light, the oxygen producing processes only occur near the surface or in shallow waters where light can penetrate. Consumption of oxygen can be most damaging at night and on very cloudy days when

when sunlight necessary for photosynthesis is lacking and when the production of oxygen by photosynthesis does not occur. Nearly all aquatic organisms use oxygen dissolved in water when using the energy to grow and move. This process is called respiration. Respiration is a 24-hour a day process for plants and animals. Aquatic organisms can reduce oxygen concentrations, sometimes to harmfully low levels, through the process of respiration.

Through respiration, an overabundance of aquatic plants and animals can at times consume most of the oxygen in the water. This overabundance of aquatic plant or algal growth may occur when there are elevated concentrations of nitrogen and phosphorus in the water. This process is called over-fertilization. Phosphorous and nitrogen are two nutrients essential for plant growth. Over-fertilization is caused by runoff from farmlands, fertilized yards, and golf courses or by the release of treated sewage effluent into a waterway.

Measuring Dissolved Oxygen (DO)

The DO test tells how much oxygen is dissolved in the water. But it does not tell how much dissolved oxygen the water is capable of holding at the temperature which it is tested. When water holds all the DO it can at a given temperature, it is said to be 100 percent saturated with oxygen. The following table shows the relationship between various temperatures and the solubility of oxygen in milligrams/liter (mg/L) or parts per million (ppm).

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The concentration of oxygen dissolved in water decreases as salt concentrations increase. For example, water from Corpus Christi Bay that is 100% saturated with oxygen at a temperature of 25ºC, and that has a salinity of 15 parts per thousand (conductivity of 24,900), has a dissolved oxygen concentration equal to 7.6 mg/l. From the table above, you will see that freshwater that is 100% saturated with oxygen at a temperature of 25ºC, has a dissolved oxygen concentration equal to 8.4 mg/l.

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Titrating the sample involves the addition of several chemicals to the fixed sample resulting in a DO value. Using this method, several samples can be collected, fixed in the field, and then carried back to a testing station or laboratory for titration. Fixed samples should be stored in the dark and away from hot or very cold temperatures. Titration should be completed no longer than 4 hours following fixation.

Activity 1: Available Oxygen Introduction Activity

1. Gather materials. Cut each straw into 3 sections, and cut enough straws so each student has 3 sizes of straws.

2. Tell students you are going to limit a very necessary ingredient for life, oxygen. They will be allowed to breathe whatever oxygen they can pull through the straw.
    a. Hand out the large straws. Ask students to breathe only through the straw in their mouth.
    b. Have them stand and jog in place.
    c. Hand out the medium sized straws and repeat the steps. Hand out the small straws and ask the students to only breathe through the small straws. Caution them to sit down if they get dizzy.

3. Discuss with students the difference between terrestrial life’s access to oxygen and aquatic life’s access to oxygen. Just like the straws restricted oxygen amounts, aquatic life has restricted pathway for oxygen to dissolve into water.

8^{th} – 12^{th} – Dissolved Oxygen (Activity 2)

Materials Activity 2
- 2 Clear Glasses
- Heat Source
- Pan
- Saran Wrap
- Feeder Goldfish or Fish Sensitive to Low Amounts of Dissolved Oxygen

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Activity 2: Oxygen Biomonitoring

1. Gather materials.

2. Prepare a sample of water low in D.O., by boiling pond water for five minutes, carefully adding it to a glass and covering it with Saran Wrap as it cools. If the water splashes when being poured, it will introduce oxygen back into the sample. Cover with Saran Wrap by floating the wrap on the surface of the water as it cools. Fill another glass with untreated pond water.

3. Have students place a monitoring organism into each glass of pond water. Do Not Allow The Organism To Die. Ask the students to count gill movements for 2 minutes, before transferring fish back to an aquarium.

4. Graph the data.

5. Discuss with the students what gill movement might represent in fish (intake of gases for breathing.)

NOTE: You may also use guppies for this experiment. Guppies can be obtained at any pet store. Allow the animals to acclimate in an aquarium for the week preceding the experiment. How many fish will your aquarium hold? A good rule of thumb is 1” of fish per gallon of water. Use the same source of water for both boiled and non-boiled treatments. Cups used in the experiment should hold a minimum of 12 ounces of water, and fish should not be left in the cups for more than 3 minutes. Make sure both cups of water are room temperature, as is the aquarium water where you will be removing the fish. Low oxygen is stressful; do not repeat the experiment using the same fish on the same day.

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8^{th} – 12^{th} – Dissolved Oxygen (Activity 3 and 4)

Materials Activity 3/4
- Winkler Titration Kits
- Disposable Gloves
- Protective Eyewear
- Collected Pond or River Water

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Activity 3: Dissolved Oxygen Testing Part I

1. Gather materials. Have enough equipment for students to break up into groups to conduct their activity. Laminate D.O. Testing – Part I instruction sheet and place with each equipment set up.

2. Discuss with the students:
   - Ways that the natural environment may impact D.O. levels
   - Ways that nonpoint source pollution may impact D.O. levels

3. Perform the test procedure for dissolved oxygen for the students to observe.

4. Have students break into groups and conduct the test themselves. (They will SAVE the samples for D.O. Testing –Part II.)

5. Pass out D.O. Worksheet – Part I (1.1). Either in class time or as homework, have students complete D.O. Worksheet – Part I.

Activity 4: D.O. Testing Part II

1. Perform the test procedure for dissolved oxygen for the students to observe.

2. Have students break into groups and conduct the test themselves.

3. The students will calculate the D.O. for their sample using the following method:
    - Calculate the average of the two tests, and record the average (rounded off to the nearest 0.1 mg/l).
    - If the values for the titration differ by more than 0.5 mg/l oxygen, repeat the titration on one of the sample bottles.
    - If the range between the two sample bottles is still more than 0.5 mg/l oxygen, repeat the titration on the remaining sample bottle.

4. Pass out D.O. Worksheet – Part II (1.2). Either in class time or as homework, have students complete D.O. Worksheet – Part II.

8th – 12th – Dissolved Oxygen (Activity 5)

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Materials Activity 5
- Winkler Titration Kits
- Disposable Gloves
- Protective Eyewear
- Clear Jars with Screw-on Lids
- Pond Water
- Aquatic Plant-elodea
- Rock Salt
- Ice
- Heat Source

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Activity 5: Dissolved Oxygen Influences

1. Gather materials. Prepare the following containers of water 2 days prior to the lesson:
    a. Jar with closed lid and elodea sprig, set in windowsill.
    b. Jar with closed lid and elodea sprig, set in a dark cupboard.
    c. Jar with heated water, tightly closed.
    d. Jar with dissolved rock salt (not cloudy) - agitate equally with jar (e & f).
    e. Jar with collected water, untreated - agitate equally with jar (d & f).
    f. Jar of water, stored on ice, uncovered - agitate equally with jar (d & e).

2. Label the water treatment jars with letters only.

3. Have students run D.O. tests on the water samples in each jar.

4. List on the board the different water treatments. Have students indicate if treatments will increase or decrease D.O. in collected water. Ask them to match treatments with D.O. values derived from the testing procedures.

Assessment/Evaluation (Pre-/Post-Test)

1. To assess the students' knowledge and understanding of the importance of oxygen levels in an aquatic ecosystems - ask the students to explain the significance of terrestrial life's access to oxygen and aquatic life's access to oxygen and how they may be different.

2. The student's graph of gill movement may be used as an assessment to determine their knowledge of how significant D.O. is as a measure of water quality.

3. To assess the student's conceptual knowledge of D.O. Testing – D.O. Worksheet 1.1 and 1.2 may be used as an assessment.

4. Allow students to use the testing procedure on samples with a variety of D.O. levels.

5. Have students explain the reasons why D.O. levels change in an aquatic ecosystem.

TEKS

Science 8.1A, 8.2A, 8.2B, 8.2C, 8.2D, 8.2E, 8.3A, 8.4A, 8.4B, 8.11B, 8.11C
Aquatic Science 1A,2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 3A, 4A, 5B, 5D, 6A, 9C, 11B, 12A
Biology 1A, 2E, 2F, 2G, 2H, 3A, 11B, 12F
Chemistry 1A, 2E, 2F, 2G, 2H, 2I, 3A, 10A, 10B
Earth and Space Science 1A, 2E, 2H, 2I, 3A
Environmental Systems 1A, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 3A, 4E, 5B, 9B, 9C

Dissolved Oxygen Testing – Part I
Collecting & Fixing the Sample

Step 1 Rinse both sample bottles and caps ***twice*** with the water to be tested.

Step 2 Collect the sample by submerging the bottles upside down in the sampling bucket and invert.
- Flip the bottle right side up, and the sample bottles will fill with water.
- When the bottles fill, check that there are no air bubbles in the bottle or in the caps.
- If there are, tap the sides of the bottle or cap until the bubbles rise out.
- Then, cap the bottles while everything is still under water.

Step 3 Remove the capped bottles from the water and closely inspect for air bubbles. If there are any air bubbles, throw out the current sample and get a new sample using the above procedures.
- ✓ ***Put safety goggles and gloves on at this point***

Step 4 Place bottles on a flat surface and remove the caps.

Step 5 Add 8 drops of Manganese Sulfate solution
Then add 8 drops of Alkaline Potassium Iodide to each bottle.
When these chemicals are added, carefully count the drops and do not allow the chemical bottles to touch the sample bottles.

Step 6 Cap the bottles and invert 25 times to mix the chemicals and the sample. Look for a precipitate to form as the bottles are inverted.

Step 7 Sit the bottles back on the flat surface and wait for the precipitate to settle below the 'shoulder' of the bottle. The shoulder of the bottle is the part on the sample bottle where the bottle changes from thin to wide.
- ✓ ***When testing in the field, you may want to do other tests while the precipitate is settling.***

Step 8 Once the precipitate is settled (about 1 or 2 minutes later), invert the bottles 25 times again.

Step 9 Wait for the precipitate to settle down to the shoulder again.

Step 10 Uncap both bottles and add 8 drops of Sulfuric Acid to each.
- Recap the bottles and invert them until all precipitate dissolves (about 3 minutes).
- Depending on the amount of oxygen in the water, the samples at this point should be a clear yellow (little oxygen) to brown-orange (a lot of oxygen).

Step 11 The samples are now "fixed" because at this point the oxygen in the sample is stuck in the bottle and will not be affected by exposure to the air in the room. Therefore, after this point, samples can be saved in a dark, cool place for up to 4 hours.

Name: ____________________
Date: ____________________

Worksheet 1.1

Dissolved Oxygen Worksheet – Part I

1. Short Answer: Please answer in complete sentences.

What is D.O.?

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____

Explain the significance of "fixing a sample" out in the field:

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____
____

2. Place the following selected steps of collecting and fixing water samples in order.

______ Check for air bubbles

______ Look for precipitate

______ Invert until precipitate dissolves

______ Submerge bottle upside down in bucket

______ Save in a dark, cool place for up to 8 hours

______ Add 8 drops of sulfuric acid

______ Add 8 drops of Manganese Sulfate & 8 drops of Alkaline Potassium

3. At what level of D.O. concentration did the test organisms experience stress?

____
____
____

4. How can the level of D.O. be increased?

____
____
____

Dissolved Oxygen Testing – Part II

Step 1 Take the fixed sample and use some of the contents to rinse the glass titration vial ***twice***.

✓ ***Use only a small amount of fixed solution in rinsing the titration vial. You want to leave enough fixed sample to perform the titration a second time if necessary.***

Step 2 Pour exactly 20 ml of the fixed solution from one of the sample bottles into the vial.

- This amount will correspond to the white line on the vial.
- To use this container as the measuring tool, be sure that the meniscus (dip in the water) is even with the white line, not the highest point of the water in the container.
- Put the cap on the vial.

Step 3 Fill the titrator (the small syringe) with the Sodium Thiosufate solution so that the very tip of the plunger is at the 0.0 mark.

- Look for air bubbles, and remove them from the titrator by tapping the sides or by moving the plunger up and down.

✓ ***Because the titrator syringe never comes into contact with any other reagents, it should not be necessary to rinse the titrator EXCEPT when your Sodium Thiosulfate Solution has become out of date OR if you think your titrator may have become contaminated. In these cases you should rinse the titrator twice with a small amount of Sodium Thiosulfate Solution, disposing of the rinse directly into your waste container.***

Step 4 Put the now-filled titrator in the hole in the cap of the glass titration vial.

- Add one drop of Sodium Thiosulfate from the titrator to the vial.
- Swirl the glass vial thoroughly to mix.
- Add another drop of Sodium Thiosulfate, and again mix thoroughly.
- Continue to add the Sodium Thiosulfate until the yellow-brown solution in the vial turns to a pale straw color.

Step 5 Remove the cap from the vial with the titrator still attached to the hole.

Step 6 Add 8 drops of Starch Solution to the titration vial while holding the dropper vertically and while not touching the dropper to the vial's contents.

- Swirl (but do *not shake*) the titration vial very thoroughly to mix.
- This step should see the contents turn from the pale yellow to a dark blue.

✓ ***The final part of the titration is best conducted against a white background (such as a white sheet of paper) so that you can clearly see when all the blue color has disappeared.***

Step 7 Put the cap and titrator back on the vial.

- Add the remaining Sodium Thiosulfate until the solution turns from *blue* to *clear*.

- After each drop, swirl the vial thoroughly and gently.
- Be careful to swirl after each drop because the number of drops it takes to make the water change from blue to clear will be directly related to the level of DO in the reading.

✓ ***If you use 9 ml of Sodium Thiosulfate and the color is still blue STOP and refill your titrator with additional Sodium Thiosulfate. Remember to add the 9ml to the additional amount of Sodium Thiosulfate used to complete the DO test.***

Step 8 Using the scale on the side of the titrator, count the total number of units to the nearest 0.1 of Sodium Thiosulfate.

- Each line of the titrator represents 0.2 mg/1 (ppm) of oxygen.
- That number equals the number of parts per million (ppm) or milligrams per liter (mg/l) of oxygen dissolved in the water.
- This value will be the number to record on the data sheet under the "1st test" space on the Dissolved Oxygen row.
- Then pour the liquid in the titration vial into the waste container.

✓ ***Any remaining sodium thiosulfate in your titrator after your second titration should be disposed of by injecting it into the titration vial or your waste container. Do not put excess back into the sodium thiosulfate bottle. Store titrator with plunger pulled away from the tip, or it will stick eventually.***

Step 9 The second bottle of fixed water sample now must be tested in the same manner as the first was tested. Simply repeat the steps one through eight.

Step 10 The difference between the two values for DO should be no more than 0.5 mg/l.

- If there is a large difference, most likely the test procedures were not followed properly.
- It is recommended that the test be redone for whichever sample bottle was probably incorrect.
- If the values are within 0.5 mg/l, then calculate the average of the two titrations and record that value in the "average" space.
- Round off the answer to the nearest 0.1 mg/l.

✓ ***If the values are more than 0.5 mg/l oxygen apart, it is suggested that you perform the entire DO test a second time and record only the second test results on your Monitoring Form. If this is not possible, please indicate all four initial DO values on your Monitoring Form, but do not average the results.***

✓ ***A sealable waste container should be used for collection of all test samples. Safely dispose of all out of date or waste chemicals by flushing them down a sanitary sewage system drain with plenty of water. Do not dispose of chemicals into a septic waste system, water body, or onto the ground.***

Name: ______________________________
Date: ______________________________

Worksheet 1.2

Dissolved Oxygen Worksheet – Part II

1. What part of the titrating procedure was the most difficult to complete? Explain.

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2. Put the following selected steps of titration in order.

_____ Add 8 drops of starch solution
_____ Add sodium thiosulfate to the vial until the solution turns light yellow
_____ Rinse the glass titration vial
_____ Add sodium thiosulfate to the vial until the solution turns from blue to clear

3. What is the importance of dissolved oxygen in aquatic ecosystems?

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4. Briefly summarize how the dissolved oxygen in rivers and streams in your watershed may be affected by naturally occurring phenomena or human interactions with the environment.

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