DISSOLVED OXYGEN (DO or Dissolved O_2)

Among the most important variables affecting stream health is the concentration of dissolved oxygen present in the water. It is essential to numerous life forms and is readily affected by many human activities. In this activity, students will measure the amount of dissolved oxygen in a water sample from the Huron River watershed.

Although the pre and post discussions are necessary to provide important information about each topic, it is the activity that is most vital to this unit. Be sure to allow plenty of time to complete the activity.

Pre-Activity Discussion (Answers can be found in the Background Information section below)
Before beginning the experiment ask the students: 1) What is dissolved oxygen and why is it important? How does it differ from the combined oxygen in H_2O? 2) What are some natural sources of dissolved oxygen in water? 3) What natural phenomena affect the level of dissolved oxygen in stream water? 4) What human activities affect the level of dissolved oxygen in stream water?

The Activity

Equipment

- Hach field test kit for dissolved oxygen: Azide-modified Winkler method
- Scissors or small cutting pliers to open powder pillows in Hach kit
- Small table
- Long-handled dipper (if you need to sample water from a steep bank)
- Display board
- Data sheets and clipboard
- Wastewater jug for disposal of spent solutions
- Safety glasses and gloves (rubber, vinyl)

The Process

You will be using a Hach portable field test kit for this measurement. The procedure is below and is easy to follow if you do each step carefully. But the chemistry, explained in the following paragraph, is complicated.

In brief, you will be removing interfering NO_2 (nitrite ion) from the water sample with alkaline sodium azide. Manganous sulfate ($MnSO_4$) and potassium iodide (KI) are then added to the sample. A white precipitate of manganous hydroxide ($MnOH_2$) is formed which rapidly reacts with any dissolved oxygen present to form brown manganese dioxide (MnO_2). The solution is then made acid by adding sulfamic acid (H_3NSO_3). In this acidic solution the manganese dioxide reacts with iodide ion (from the potassium iodide added earlier) to form an amount of iodine (I_2) chemically equivalent to the amount of dissolved oxygen originally present in the sample. Lastly, the released I_2 is titrated using a carefully measured amount of sodium thiosulfate ($Na_2S_2O_3$) solution. This converts the yellow brown I_2 to colorless I^- leaving a colorless solution. The amount of sodium thiosulfate needed to turn the water colorless is proportional to the concentration of dissolved oxygen in the original sample.

Toxicity and Disposal Information for Activity Leaders and Students

These chemicals are toxic (some quite toxic) and very irritating to the eyes. Wear safety goggles, avoid spills, DO NOT TASTE ANY OF THE CHEMICALS, and rinse your hands well with water after completing the activity. If you get any of these chemicals in an eye, wash the eye with clean water (such as drinking water) for 5 minutes and then seek medical attention at once. Dispose of liquid wastes from the activity in the jug provided for later disposal in a sink or toilet.

Set-up

Select a site with space for a small table and easy access to the stream. You will need to be upstream from activities that may cause a lot of turbidity (macroinvertebrate collection, stream speed). The water should be deep enough to allow you to hold the sample bottle 6-12 inches under the surface of the water while filling it.

Student Roles

In order to keep the students involved, give as many as possible a task. One student can handle the data sheet. Another can fetch water samples and dispose of treated water in a container. One student can read the steps of the procedure to the group. A simplified version of the steps is included on a separate page for this purpose. It follows the background information. Be sure to print it out. Lastly, one or two students can wear latex gloves and add the chemicals to the water sample. While the students are following the steps below, you can give an explanation as to what is happening.

Procedure (Technical information is in parentheses)

1. Fill the glass-stoppered bottle with sample water, allowing the water to overflow for a minute and making sure there are no bubbles in the bottle. If possible, fill this bottle by holding it 6-12 inches below the surface of the water in the stream.

2. Tip the bottle slightly and stopper it WITHOUT trapping any air bubbles. The concentration of oxygen in air is roughly 30 times its concentration in water so a small air bubble can throw off your results making them high.

3. Remove stopper and add the contents of a DO 1 reagent envelope. (This envelope contains sodium azide that will prevent nitrite interference, and sodium hydroxide to make the solution alkaline.) Also add a DO reagent 2 envelope. (This envelope contains manganous sulfate and potassium iodide.) Stopper the bottle carefully to avoid trapping any bubbles.

4. Invert the bottle several times to dissolve the powders. A precipitate (floc) will form. It will be brown if oxygen is present. (The floc is a mixture of white manganous hydroxide and dark brown manganese dioxide.) Let the floc settle to about half the bottle volume.

5. Invert the bottle once more to mix and again let the floc settle to about half the bottle volume. This gives time for the reaction between manganous hydroxide and dissolved oxygen to go to completion.

6. Remove the stopper and add the contents of one DO 3 reagent powder pillow. (This pillow contains sulfamic acid. It is much safer than sulfuric acid and just as effective). Invert the bottle several times to mix. Allow the precipitate to settle at least 5 min. The sample will turn clear yellow-brown if oxygen was present.

7. Add one full measuring tube- the small round tube- of the sample to the square bottle.

8. Add sodium thiosulfate solution one drop at a time to the square bottle, swirling it after the addition of each drop and counting the number of drops added. Stop when the color of the solution changes from yellow to colorless. This is easiest to see if done on a white background. A piece of white paper is fine. (The thiosulfate reacts with the colored iodine to produce colorless iodide ion.)

9. The number of drops of thiosulfate solution added to the sample is equal to the DO concentration in the water in mg/L. (milligrams per liter)

Examining the Results
Compare the results of the testing to the chart below.

Table 1: Minimum DO requirements for some aquatic organisms

Organism	Minimum D.O. requirement (mg/L)
Trout	6.5
Smallmouth bass	6.5
Caddisfly larvae	4.0
Mayfly larvae	4.0
Catfish	2.5
Carp	2.0
Mosquito larvae	1.0

Note: DO requirements for fish depend on a number of factors, so the numbers in Table 1 are not precise and other sources may give slightly different figures. Nevertheless Table 1 provides useful approximate information about the DO needs of aquatic life.

Post-activity discussion questions:

1) What do our results tell us about the stream water?
2) Are there any conditions nearby that might affect the amount of dissolved oxygen concentration in the portion of the stream being sampled?
3) The chart below shows how the ability of water to hold dissolved oxygen decreases as water temperature rises. What are some activities that can cause water temperatures to rise? What can we do to prevent the warming of stream waters?
4) What other-actions can be taken to protect against low levels of dissolved oxygen?

Table 2: Oxygen solubility in water at 1 atmosphere (760 mm Hg) pressure of air

Temperature ^{o}C	Temperature ^{o}F	Oxygen solubility: mg/L
0	32	14.6
10	50	11.28
20	68	9.09
30	86	7.56
40	104	6.41

Background Information

Dissolved oxygen is essential for fish and benthic macroinvertebrates to live. When there is not enough oxygen, the food web for the whole ecosystem is affected. It must be remembered that the oxygen that hooks up with hydrogen to make water is not available for respiration. Although the concentration of oxygen in the air is quite high, O_2 is not very soluble in water. At a pressure of one atmosphere (atm) of air, the oxygen concentration in water at room temperature is about 8.2 mg/L or 8.2 parts per million (ppm) at saturation level.

Levels of DO are affected by discharges from industrial facilities and water treatment plants. They are also affected by water released from dams. DO concentrations in Michigan waters are monitored by the Michigan Department of Natural Resources. Violations are investigated and corrective action is taken. According to the laws of the State of Michigan, the minimum permissible DO in most Michigan streams is 5.0 mg/L. This is enough to support most aquatic life (but not trout or small-mouth bass) however the margin is not large. Another reason that the DNR monitors DO levels is to determine where to stock fish and the kind of fish to stock.

Dissolved oxygen can enter stream or lake water in many ways. Below are some common sources.

1) Diffusion from the atmosphere
2) Aeration as water moves over rocks and debris, riffles, rapids, waterfalls, etc.
3) Aeration from wind and waves
4) Photosynthesis of aquatic plants

There are a number of factors that affect the DO concentration including:

1. Efficiency of re-aeration from the atmosphere: Oxygen is easily transported from air to water in shallow, turbulent streams. It is poorly transported in deep, slow-moving or stagnant streams.

2. Organic materials in water material such as food processing wastes, human and animal feces and urine, paper mill wastes, dead and decomposing algae and leaves, etc. can affect the levels of DO in water. These materials when present in water are referred to as Biochemical (or Biological) Oxygen Demand (BOD) and can be used as food by bacteria naturally present in surface waters. As the bacteria feed upon these materials, they use oxygen. They also multiply. If there is sufficient BOD present, its metabolism by the stream bacteria will use up all of the dissolved oxygen in the water. At this point fish and most benthic macroinvertebrates die of suffocation.

3. Temperature: The solubility of oxygen in water decreases with increasing temperature. In other words, colder water is capable of holding more dissolved oxygen than warmer water. For example, at 14^{o}C the solubility of oxygen in pure water (no dissolved salts) is 10.30 mg/L, while at 30^{o}C it is only 7.56 mg/L.

There are a number of human-caused conditions that can cause stream temperature to rise. Some strategies for remediation for each of these are listed.

1) Runoff: Impervious surfaces, cultivated fields and lawns cause water to run into lakes and

streams quickly. When this happens, the water is much warmer than if it soaks into the ground and slowly moves as groundwater to enter streams and lakes. Buffer zones, retentions ponds, rain barrels and water gardens can be helpful. Greater use of permeable asphalt that allows water to penetrate through to the soil would also reduce runoff.

2) Discharged water: Water that is discharged from industrial operations or water treatment plants is generally warmer than the bodies of water it is discharged into. Here, too, retention ponds for cooling could be helpful.

3) Health of Riparian Zones: Water that is shaded is cooler than water that is exposed to sunlight. When trees and shrubs that shade streams and rivers are removed, the increase in sunlight warms the water. Restoring streamside vegetation with a mix of trees, shrubs, grass and other plants will create more shade and also reduce erosion of stream banks.

4) Erosion: When soil enters the water through erosion, the increased concentration of sediment (dirt in the water) increases turbidity. Water heats up more quickly when it has high turbidity. Good vegetation buffer zones to prevent soil from entering streams will improve stream health in many ways.

For this and other units, advanced level information is available if desired. Contact the HRWC and request an electronic version of the unabridged manual.

Dissolved Oxygen- Procedure

1. Fill the glass-stoppered bottle with sample water. Be sure there are no air bubbles.

2. Add the contents of DO 1 reagent envelope. Cover and turn back and forth several times to mix. Then add DO 2 reagent and mix again. (Be careful to avoid trapping air.)

3. A precipitate will form called floc. Let it settle to the bottom until it fills ½ of the volume of the bottle.

4. Tip the bottle back and forth again and then let the floc resettle.

5. Remove stopper and add DO 3 reagent. Mix and then let settle for at least 5 minutes. Watch the reaction.

6. Add one full measuring tube (the small round tube) of the sample to the square bottle.

7. Add sodium thiosulfate one drop at a time to the solution in the square bottle until the solution is colorless. (Swirl after adding each drop.) BE SURE to count the drops.

8. The number of drops = mg/l of DO

Dissolved Oxygen- Lesson Narrative

Transfer of O2 from the atmosphere to water is a slow, inefficient process. It is faster when the water is shallow and turbulent (riffles, rapids, windy conditions). Michigan's standard for DO is 5 mg/L, below which fish and other aquatic life may suffer or die.

DISSOLVED OXYGEN MEASUREMENT IN WATER
Activity Leader Information and Commentary.

1. My name is ________, and I'm a volunteer with the Dissolved Oxygen station. Names?

2. Most organisms need oxygen to survive, except anaerobic bacteria. What percentage of air is oxygen? *(~21% is oxygen).* Is this a large amount? *(yes, a large fraction of air is oxygen with mostly nitrogen)*

3. How do organisms living underwater get oxygen? *(gills, skin; some beetles store bubbles)*

4. What is the molecular formula of oxygen and is it colorful or detectable by *our senses? (O_2 , is not detectable; except its absence will cause our death.)*
Possible Anecdote: traveling showmen did parlor experiments in 1760s with birds dropping unconscious in a partial vacuum ("air pump"): painting 1768 An Experiment on a Bird in the Air Pump by Joseph Wright where they knew something in air was essential for life, but had not yet identified oxygen as a component. Oxygen identified by J. Priestly and others, published after 1776) with air having an inert component of nitrogen (1772). For example, nitrogen is left after all the oxygen is used to support burning paper in a closed tube.

5. What is your mental picture of oxygen in air versus water? *(random collisions of molecules in both cases, but not much oxygen is in the water ,H_2O, due to poor solubility)*

6. How does oxygen get into the water? *(aeration from wind, waves, rapids, waterfalls; direct diffusion into surface (slow); aquatic plants release oxygen)*

7. Oxygen has some equilibrium concentration in water which depends on temperature. Do you think hot or cold water holds more oxygen? Hint: Do you think boiling water drives out the dissolved air? *(cold water has more)*

8. Optional: Oxygen solubility at 1 atmosphere pressure (less in high mountains) is :
50 ^{o}F :11.28 mg/L; 68 ^{o}F :9.09 mg/L; 86 ^{o}F :7.56 mg/L; 104 ^{o}F: 6.41 mg/L

This is an exponential type of drop with increasing T. T of water is very important control of oxygen.
How do these numbers compare with oxygen in air? *(recall 22.4 L/mol, <MW> air is 29 g/mol or 1290 mg/L at 21% oxygen or about 1290 x 0.21 = 270 mg/L or about 30 times more in air than in water).*

Carbonated drinks lose some of their fizz, carbon dioxide gas, in warmer temperatures. They are bottled at cold temperatures for that reason.

How can fish and critters (benthic macroinvertebrates) even survive ? *(Lungs vs gills?)*
How can we measure such a small amount of oxygen in ABSOLUTE units?

9. Anyone been in a hospital or emergency room where they put an oxygen sensor on your finger?
What are they measuring and how? *(measuring ratio of oxygenated hemoglobin (redder color) vs total hemoglobin where non-oxygenated hemoglobin is a bluer color. This is a RELATIVE measurement not ABSOLUTE). (This is a COLOR measurement, a common method of detecting molecules by absorption spectroscopy, which in this case compares intensity changes of two colors through your skin).*

Student Copy

MEASUREMENT of absolute oxygen concentration in water.

OUR PROBLEM: Convert a colorless molecule to something we can measure with an ABSOLUTE method. We will use color with a classic chemical titration method, just like your chemistry class.

Safety: Wear safety glasses and use gloves when handling the reagents.
Dispose of all solutions in designated waste bottle.
Wash hands after procedure and before handling food.

PROCEDURE: Use the Hach portable field kit using a modified Winkler chemistry.

A. Obtain a Water Sample.

1. Dip up a water sample from a flowing part of the stream. Watch out for poison ivy and slippery mud and rocks!
Students note the presence of riffles and rocks and places that might have more or less local oxygen. (Look for the "bubble line" in the stream below rapids that has the most oxygen, most critters, and most fish eating the critters.) Students can note their observations of the stream on the data sheet. These can include relative water speed, presence of rocks or other objects, riffles, etc.

B. Remove the most common interfering ions in the water.

1. Use the bottle with a glass stopper. Rinse it and the stopper 2 times with sample water to remove impurities. Slowly pour sample into bottle to not create air bubbles and slowly stopper to not trap air bubbles.

2. Rubber gloves and safety glasses. Add DO1 reagent that will remove any nitrite ions, NO_2, from the water. This uses alkaline sodium azide. Cut top of packet and pinch to make easy pouring (minimize getting on sides of bottle) stopper and invert several time while holding stopper down with a gloved finger. Do not get extra air bubbles when stoppering.

C. Use manganese (Mn) metal to react with oxygen and convert it to a MnO_2 precipitate.

1. Remove stopper and add DO2 reagent. Then stopper carefully and invert several times and watch the precipitate form a fine suspension of insoluble MnO_2 [brown] and Mn $(OH)_2$ [white] crystals. Make another mix by inverting, and then let it settle for a few minutes to be sure all oxygen has been converted.

2. The chemistry here is redox or reduction-oxidation chemistry where electrons are moved during the reactions. The first step is making Mn $(OH)_2$ which then reacts with the oxygen. The brown color is not well defined due to the presence of crystals, and is not easy to measure quantitatively. We need to make some other chemical that is a colorful solution and keeps the quantitative relationship.

D. Use sodium iodide and acid to make a solution of yellow brown iodine, I_2 , from the MnO_2

1. Wearing gloves, add DO3 reagent which has sulfamic acid. This allows DO2 included sodium iodide (NaI) to now react and make the iodine molecule I_2. This is a molecule that is often used in tablets to purify water, as it can oxidize bacteria. It is a reddish orange color and stays dissolved in the water. Each MnO_2 [brown] molecule reacts to form one I_2 molecule so we have a quantitative conversion of O_2 **.**

2. KEEP INVERTING the bottle as the excess of reagents should dissolve all the crystals to make a clear solution.

E. Use a standardized solution of sodium thiosulfate to react with the iodine; a quantitative titration.

1. We need a known volume of the orange solution to make a titration. Fill the open top measuring tube to the top and transfer to the square bottle.

2. (Now the standardized sodium thiosulfate solution can be used to get a quantitative measurement. This reagent reacts with the iodine molecule to make iodide ions which are colorless. The concentrations are adjusted so that 1 drop equals 1 mg/L of oxygen in the original solution.)

   Add one drop of the reagent to the bottle. Put bottle on white paper and swirl bottle. Continue to add drops and swirl, keeping count of the number of drops. Repeat until the solution becomes colorless. The number of drops required is comparable to the dissolved oxygen concentration in mg/L (milligrams per liter)

3. Fill out data on the data sheet.

Student Copy

Procedure for Determining Amount of Dissolved Oxygen in a Water Sample

Safety: Wear safety glasses and use gloves when handling the reagents.
Dispose of all solutions in designated waste bottle.
Wash hands after the procedure and before handling food.

1. Fill bottle with the sample of water. Be sure no air is trapped inside, even when you put in the stopper.
2. Remove stopper and add DO 1 reagent. (DO= dissolved oxygen) Invert the bottle several times.
3. Add DO 2 reagent. Replace stopper without trapping air and, with a thumb on top of the stopper to hold it securely in place, turn the bottle upside down and back several times so that the chemicals mix and dissolve.
4. Set the bottle down and watch while a precipitate (floc) settles. Let it settle until the bottle is about ½ filled with the precipitate.
5. Holding the stopper securely, turn the bottle upside down and back a few times and then let the precipitate settle once more until the bottle is half filled.
6. Remove the stopper and add DO 3 reagent. Allow the precipitate to settle. The water will turn yellow-brown if O_2 is present. Keep inverting the bottle as the excess of reagents should dissolve all the crystals to make a clear solution.
7. Fill the measuring tube to the top with the liquid and then pour it into the square bottle.
8. Add sodium thiosulfate one drop at a time. Swirl the bottle after each drop. Count the drops of sodium thiosulfate as they are added. Stop adding the sodium thiosulfate when the solution is clear.
9. Each drop of sodium thiosulfate =1mg of O_2 per liter of water.

Dissolved Oxygen (DO) Data Sheet

Student observations:

Minimum DO requirements for some aquatic organisms

Trout	6.5 mg/L
Smallmouth bass	6.5 mg/L
Caddisfly larvae	4.0 mg/L
Mayfly larvae	4.0 mg/L
Catfish	2.5 mg/L
Carp	2.0 mg/L
Mosquito larvae	1.0 mg/L

1. Drops of thiosulfate solution added to decolorize one measuring tube of sample: _____DO concentration = ______ mg/L

2. How does oxygen from the atmosphere get into the water?

3. Name 3 factors that affect or can change Dissolved Oxygen concentration in a stream.

4. Does this water meet the Michigan state requirement of 5.0 mg/L as the minimum acceptable DO concentration?

5. Based on your DO measurements, could trout and smallmouth bass live in this stream?

6. Would you expect similar DO concentrations in this stream during the summer? Why or why not?

Does that change your answer to #5? If yes, explain why.

7. How could the DO concentration over time affect the <u>types</u> and <u>numbers</u> of critters (BMIs)?

8. What can people do to improve the DO concentration in our watershed

KEY Dissolved Oxygen Student Page

1. **Answers will vary**
2. How does oxygen from the air get into the water?
   Oxygen can dissolve into the water at the edge (surface) between the water and the air. This happens more when the water gets turbulent, such as in a waterfall, or riffle.

3. Name 3 factors that can change Dissolved Oxygen concentration
   a. Efficiency of re-aeration from the atmosphere. Efficiency of oxygen transport from air to water is high in shallow, turbulent streams; it is poor in deep, slow-moving or stagnant streams.

   b. Temperature. The solubility of oxygen in water decreases with increasing temperature. For example, at 14^{o}C the solubility of oxygen in pure water (no dissolved salts) is 10.30 mg/L, while at 30^{o}C it is only 7.56 mg/L.

   c. Presence of Biochemical (Biological) Oxygen Demand, BOD. BOD consists of organic material (food processing wastes, human and animal feces and urine, paper mill wastes, dead and decomposing algae and leaves, etc.) that can be used as food by bacteria naturally present in surface waters. As the bacteria feed upon the BOD, they use oxygen. They also multiply. If there is sufficient BOD present, its metabolism by the stream bacteria will use up all of the dissolved oxygen in the water. At this point fish and most benthic macro-invertebrates die of suffocation.

4. Does this water meet the Michigan state requirement of 5.0 mg/L as the minimum acceptable DO concentration?
   Answers will vary

5. Based on your DO measurements, could trout and smallmouth bass live in this stream?
   Answers will vary

6. Would you expect similar DO concentrations in this stream during the summer? Why or why not?
   Likely answer is no. Higher water temperatures mean that less oxygen is dissolved in the water, lowering the DO.
   Does that change your answer to #5? If yes, explain why.
   Lower O_2 concentrations make it less likely these can survive

7. How could the diversity (number of kinds) of aquatic critters (BMI's) relate to the DO concentration over time?
   Answers may vary but student predictions could hypothesize that higher DO concentrations over time would lead to a greater diversity in BMI's. However, the key idea is that the minimum DO requirements ALWAYS must be met. This is a case in which the average is not enough.

8. What can people do to improve the DO concentration in our watershed?

a. **Help maintain natural streamsides by limiting erosion,** leaving rocks in river bottoms. Remove un-needed dams since sediments can increase stream temperatures.
b. **Help maintain natural streamsides by limiting erosion**
c. **Rain gardens reduce runoff and so reduce both erosion and organic (dog poop) pollution into streams**