VI THE TESTS

TEST 1: Temperature

Brief Summary

Water and air temperatures are measured with a shielded Celsius thermometer.

Note: *To obtain the most accurate results, this test should be completed in the field.*

Background

Most aquatic organisms have evolved to take advantage of one of water's most unique features: its heat capacity, or its unusual ability to absorb thermal energy (heat) with only minimal changes in temperature. Most fish, amphibians and sea mammals require fairly constant water temperature, and can only survive within certain temperature ranges. They can handle seasonal fluctuations because water temperatures change much more slowly than air temperatures. If water temperatures change more than 1° to 2°C (2° to 4°F) within 24 hours, aquatic animals can suffer thermal shock, which can injure or kill them, and it can also make fish more susceptible to disease and parasites. In addition, the stages of these creatures' life cycles are highly sensitive to seasonal changes in water temperature. Changing temperatures signal many animals to mate, lay eggs or migrate.

Unfortunately, many of the side effects of our industrial society affect water temperature, usually raising it. These increases in water temperature often have a negative effect on water quality.

TEST 1: Temperature

Natural Factors That Influence Water Temperature

- The size (volume) of the body of water: large bodies change temperature more slowly, so a small stream or pond will vary in temperature more than a large lake or ocean.
- The depth of the water: deep waters are cooler because they warm up more slowly; the deeper the water, the less sunlight warms it and the cooler it stays.
- The color and turbidity of the water: dark waters convert more sunlight to heat.
- The temperature of tributary water: rivers or lakes receiving water from snow-fed mountain streams will stay cooler than those fed by streams meandering through flatlands.
- The amount of overhanging vegetation: during the summer, shaded water will stay cooler than water exposed to sunlight.
- The direction of a stream: streams that run south are exposed to more sun than those running east/west.
- The latitude, season, and time of day.

Human Factors That Influence Water Temperature

- Industrial facilities and power plants discharge water used for cooling.
- Storm runoff contains water warmed by urban surfaces, such as streets, sidewalks, and parking lots.
- Cutting trees along banks exposes water to more sunlight.
- Soil erosion increases the amount of suspended solids, making water turbid, and turbid water absorbs more heat from the sun.

Effects of Raising Water Temperature

- Warmer water holds less oxygen, yet produces conditions that require more oxygen.
- The rate of photosynthesis by algae and larger aquatic plants increases. The result of this increase is a higher level of BOD because of the increased amount of decaying organic material.
- The metabolic rates of aquatic organisms increase. As their metabolism increases, they become more active and consume more oxygen, reducing the DO level.
- Organisms become more sensitive to toxic chemicals, parasites and diseases.
- Bacteria (including pathogenic bacteria) and parasites can sometimes grow.

Testing for Temperature

Overview: In this test, you will first measure the air temperature. Then you will either measure the temperature of the water at the same location and depth from which the water samples are taken for the other tests, or you will examine variations in water temperature in a body of water.

You may hold the thermometer directly in the body of water itself or in a container of sample water. If you test water from a container, you should measure its temperature before the water has time to warm up or cool down.

Length of Test: 5 -10 minutes (including waiting time)

Difficulty of Test: Simple

Protective Clothing: Rubber gloves

Suggestion: Always predict the water temperature before taking it. You may be surprised fairly often.

PROCEDURE

1. Measure the air temperature by holding the thermometer in the shade for about two minutes. Do not let the thermometer rest on surfaces that might transmit their own heat.

2. Record the air temperature on the *Data Recording Form.*
3. Measure the water temperature by submerging the thermometer into the body of water (or a container of sample water) and holding it there for two minutes. The thermometer should be at least four inches beneath the surface and should not touch the bottom. If possible, try to hold it at the same depth from which you drew the samples for the other tests.

   Note: *If you are working from a bridge, you should measure the temperature of sample water from a container. Regardless of how fast you pull up the thermometer, air temperature and evaporative cooling will distort your measurement.*
4. Record the water temperature on the *Data Recording Form.*
5. Wash off the thermometer with tap water and wipe it dry before returning it to the kit.

Interpreting Results

What factors at the site might contribute to the water's temperature:

- size, depth, and flow of the water?
- turbidity and color?
- shade and vegetation?
- direction of stream?
- season, time of day and recent weather?

TEST 1: Temperature

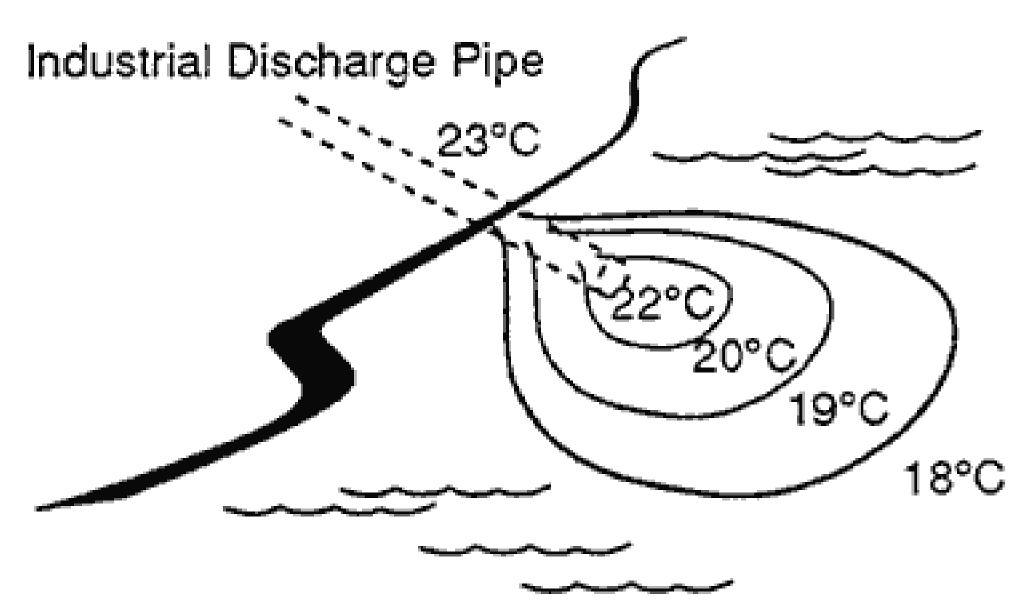
Did you find any indications of thermal pollution (activities that raise the water temperature above its natural level) such as industrial discharges, storm drain discharges, or discharges from electrical generating plants? These influences can raise water temperature in the ocean as well as in freshwater bodies.

Water temperature affects several other water quality factors:

- *Dissolved oxygen* levels are higher in colder water.
- Higher temperatures speed up chemical reactions in plants and animals. Fish increase their metabolic rate in warm water and need more oxygen.
- Some fish can only live in certain temperature ranges, for example:
  - Native brook trout live where water remains below 13°C (55°F) in the summer;
  - Rainbow trout and salmon need water temperatures of 13° - 20°C (55° - 68°F) in the summer;
  - Carp and catfish prefer water above 20°C (68°F) in the summer;
- Plant life changes with water temperature:
  - Green algae bloom above 25°C (77°F) and blue-green algae bloom at 30°C (86°F). (Algae blooms occur when water has too many nutrients, and they lead to low oxygen levels.)
  - Many fresh water aquatic plants thrive at 20°C (68°F), while fewer plants live below 13°C (55°F).

Extension Activities

1. If you locate a site where two streams converge or if you find an outflow from an industrial site or a utility plant, take the water temperature from as many locations as you can and plot or draw the thermal plume. (See illustration.) Similarly, if you locate an area where runoff from paved areas enters a stream, take the water temperature upstream and downstream of this runoff, and measure the temperature of the runoff itself.



2. Bring a jar of water from your sampling site back to the classroom. Heat and cool different samples of the water to different temperatures. Perform the pH test, DO test, and salinity test to see if temperature affects the results.

TEST 2: pH

Brief Summary

A relative measure of water's alkalinity/acidity. This test uses a liquid reagent and color comparator.

Note: *To obtain the most accurate results, this test should be completed in the field.*

Background

Chemically, pH indicates the number of hydrogen ions. At a pH value of 7.0, water contains an equal number of hydrogen ions (H^+) and hydroxyl ions (OH^-). If there are more hydrogen ions than hydroxyl ions, the substance is acidic and has a pH level lower than 7.0. If there are more hydroxyl ions, the substance is alkaline, and it has a pH value higher than 7.0.

The pH scale is logarithmic, so each one-digit change in the scale indicates a ten-fold change in acidity or alkalinity. In other words, a substance with a pH of 3 is 10X more acidic than a substance with a pH of 4; 100X more acidic than a substance with a pH of 5, and 10,000 times more acidic than a substance that is neutral. Substances at the far ends of the pH scale, therefore, are extremely acidic or alkaline.

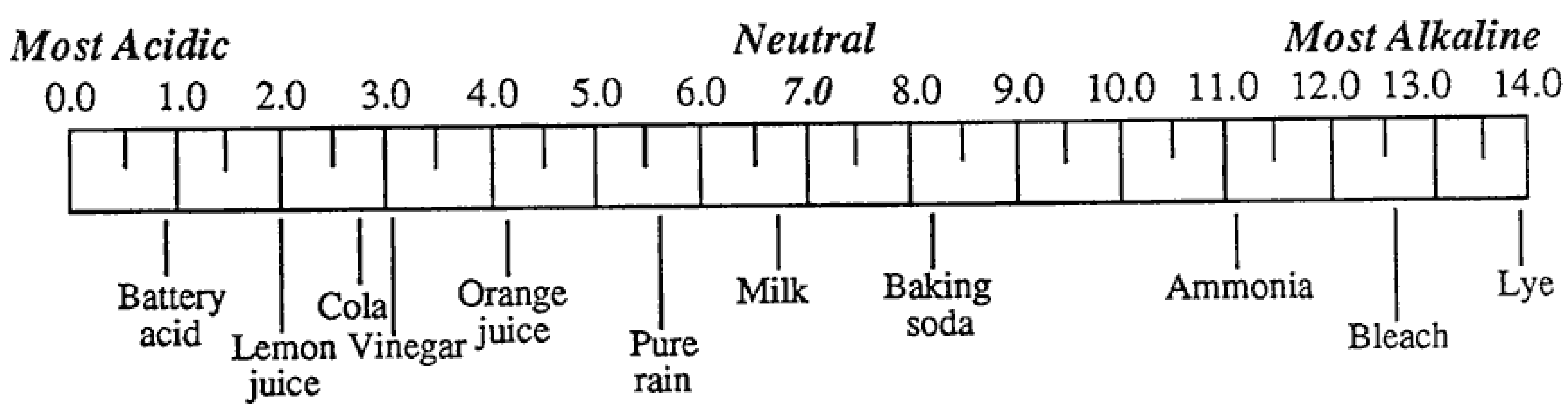


Table 1: *pH values of common substances*

Natural Factors That Influence pH

In nature, decomposition of organic materials releases carbon dioxide, and as that carbon dioxide mixes with water, it forms carbonic acid. Alkaline minerals, such as limestone, however, can buffer that acidity. At the time of their formation, most lakes and ponds had high pH, but over time carbonic acid has lowered their pH. Fresh water is much more susceptible to changes in pH than sea water because the minerals in sea water act as almost infinite buffering agents. Sea water, in fact, tends to maintain a steady pH of between 7.7 (in deeper waters) and 8.1 (at the surface).

Human Factors That Influence pH

The industrial revolution brought a variety of new combustion technologies which released varying amounts of sulfur dioxide (SO_2), oxides of nitrogen (NO_x), and carbon dioxide (CO_2) into the atmosphere. These compounds go through a series of chemical reactions in the atmosphere and eventually return to earth as acid precipitation. Where limestone exists in the soil, the acid is readily neutralized; where it does not exist, such as in large portions of the northeastern U.S., many lakes and streams have acidified. Interestingly, many of the acidic lakes are beautifully clear, but that clarity is caused by an alarming lack of life.

Testing for pH

Overview: This test consists of a test tube of sample water to which you will add 10 drops of a wide-range indicator. You will then compare the color of the solution with colors in an octet comparator. The test contains two comparators: one ranges from 3.0 to 6.5; the other, from 7.0 - 10.5.

Length of Test: <5 minutes

Difficulty of Test: Simple

Protective clothing: Eye protection, rubber gloves

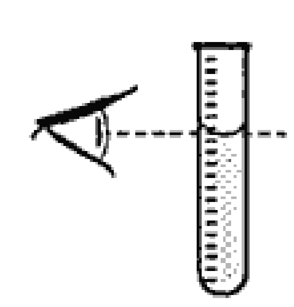
Suggestion: Reading the colors on the octet comparator is rather subjective. Given the ease and speed of this test, therefore, we recommend that each person in the group read the comparator, and that several different groups carry out the test. If the results vary, take a class average and discuss the inherent difficulties of testing variability.

Expected Results:

- Sea water maintains a pH of around 8.0. In shallow coastal waters, that number may go as low as about 7.0.
- Fresh water in the region generally ranges from about 5.5 to 6.5. Snow and rainwater may be lower.

PROCEDURE

1. Fill one test tube with sample water to the level of the line marked on it (5 mL).

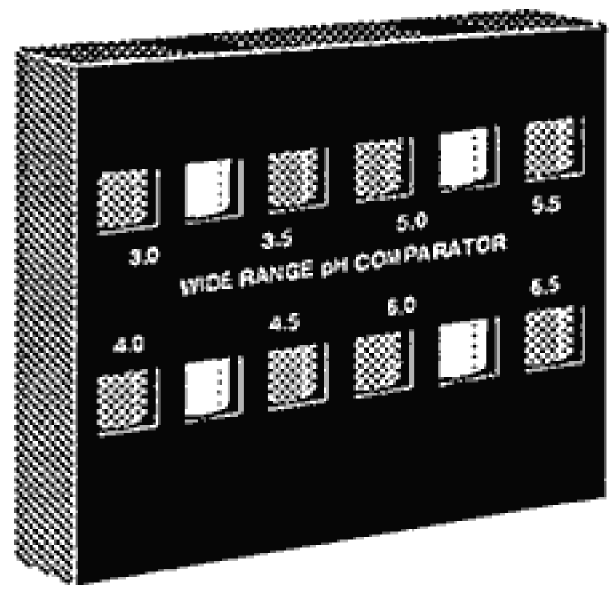


2. Carefully add 10 drops of the wide-range indicator to the test tube.
3. Cap the test tube with the small blue plastic cap and shake the solution gently until the color is uniform. (DO NOT cover the tube with your fingers. It is bad lab practice and it might change the pH of the solution.)
4. Select the appropriate octet comparator and insert the test tube into the appropriate slot.

USING THE OCTET COMPARATOR

- The test contains two comparators: one ranges from 3.0 to 6.5; the other, from 7.0 - 10.5.
- After adding indicator solution, the water sample will change colors. The color indicates the level of acidity/alkalinity.

Comparator #1	
Pinks	= 3.0 - 3.5
Oranges	= 4.0 - 5.0
Yellows	= 5.5 - 6.5



Comparator #2	
Greens	= 7.0 - 8.5
Blue-Greens	= 9.0 - 9.5
Blues	= 10.0 - 10.5

5. Hold the comparator so you are looking into a light source (preferably natural light) before comparing the color of the sample to the colors in the comparator.
6. Record the pH value on the *Data Recording Form.*
7. Have a lab partner (or a second person) take an independent reading so you can double-check each other.
8. The waste from this test is harmless to the environment and the water system, so you may pour it out in the field or wash it down the drain.
9. Clean and dry the equipment and carefully replace everything into the kit.

Interpreting Results

Did you predict the results you found? What conditions at the site or in the watershed area might have led to this pH level? What type of fish and plant life can and cannot survive at this level? Refer to Table 2 on page 38 for additional details.

Extension Activities

1. Test liquids throughout the school using the pH paper in the kit.

   Procedure for using pH paper

   a. Wet the pH paper in the sample.
   b. Compare the pH paper with the color chart in the box of pH paper.

   Test whatever sample liquids you want. Before testing a liquid, predict what you think the pH might be. Here are a few suggestions with some comments about their chemistry:

   Colas: All carbonated beverages contain carbonic acid, so they are acidic.
   Aspirin (dissolved in water): Aspirin is acetylsalicylic acid.

TEST 2: pH

TABLE 2: The Effects of pH on Freshwater Aquatic Life*

More harmful | Less harmful | Beneficial | Less harmful | More harmful

Few, if any, fish can survive for more than a few hours. Some plants and invertebrates can live at this level.	Lethal to salmonids (salmon and trout).	Few fish, frogs or insects can survive.	Most fish eggs will not hatch.	Bottom-dwelling bacteria–those that decompose organic material–begin to die. Leaf litter and detritus accumulate, locking up nutrients. Plankton disappears, and there are few snails and clams. Mats of fungi replace bacteria. Metals such as aluminum and lead are released in forms toxic to aquatic life.	Most fish and plant life survive.	Optimal for most organisms.	Not directly harmful to most fish, but some chemicals, such as ammonia, become more toxic at higher pH levels.	Harmful to salmonids and perch.	Lethal to salmonids; harmful to carp and perch.	Rapidly lethal to all species of fish.

3.0 3.5 4.0 4.5 5.0 5.5 6.5 8.2 9.0 10.5 11.0 11.5

pH level

ACIDIC | **NEUTRAL** | **ALKALINE**

*Because sea water is pH-steady, this chart does not include marine life.

Adapted from *The Monitor's Handbook* from the LaMotte Company.

Bicarbonate of soda (dissolved in water): People often take bicarbonate of soda to neutralize the unpleasant effects of too much stomach acid. What do you think its pH value is?

Alka-Seltzer or Buffered Aspirin (dissolved in water): Both of the these substances contain acetylsalicylic acid and bicarbonate of soda, an acid and an alkali.

2. Think of ways to alter the pH of a sample. For example, you could make solutions of dilute lemon juice and bicarbonate of soda, and gradually add drops of those solutions to samples to see if they can change the pH.

3. Measure the pH of water in nature, such as rainwater, snow, and parking lot runoff. Let rainwater seep through a sample of soil in your area and compare the pH level both before and after.

4. Chart the pH level of precipitation during a single storm or over several storm events. Note whether the pH level changes during the storm and the direction from which the storm came. Does rain from a "nor'easter" (which comes from the northeast where there are few sources of industrial pollution) have a different pH level than rain from the south or west (where industrial regions emit sulfur dioxide and oxides of nitrogen)?

TEST 3: Dissolved Oxygen (DO)

Brief Summary

A measure of the amount of oxygen dissolved in water. The test uses a two step process: in the first step, the sample is "fixed"; in the second, it is titrated to determine the level of DO in parts per million (ppm). The samples must be neutralized with an alkaline solution before disposal.

Note: *To obtain the most accurate results, the sample should be fixed in the field. The second half of the test may be completed in the classroom for as long as eight hours after the sample is fixed.*

Background

Water is capable of holding a reasonably large amount of dissolved oxygen, and that oxygen is essential for life. Oxygen enters the water in two primary ways: movement, such as waves, mixes atmospheric oxygen with water, and plants release oxygen to the water through photosynthesis.

As long as water is capable of holding oxygen, oxygen dissolves into it very quickly. Upon nearing the saturation point, though, the speed slows dramatically.

Several factors affect the amount of oxygen in water:

Salinity: Less salinity → higher DO

Agitation and Turbulence: More contact with atmospheric oxygen → higher DO

Temperature: Lower temperature → higher DO

(This temperature variant is compounded by the fact that organisms increase their activity in warmer water, increasing the demand for oxygen. Thus, water is more apt to become oxygen starved in summertime when water's capacity to hold oxygen is at its lowest and organisms' demand for oxygen is at its highest.)

Minerals: Higher mineral content → lower DO.

Plant Life: More photosynthesis → higher DO.

(During the daylight hours when photosynthesis occurs, DO levels can be quite a bit higher than at other times. DO is generally at its lowest just before dawn, and it peaks in the late afternoon.)

Organic Wastes: More waste → lower DO.

(The aerobic bacteria that decompose organic wastes (such as leaves and feces) consume oxygen.)

These factors often affect each other. During extended warm sunny periods, for example, algae can grow profusely. If the sunny period is then followed by several days of thick clouds, the algae does not receive adequate sunlight, and much of it dies. The bacteria that decompose algae consume oxygen, causing the levels of available oxygen to decrease. If the levels of DO become too low, fish die. This scenario is particularly common in shallow water during summertime.

Acceptable Levels of Dissolved Oxygen

Fish need a minimum level of DO to survive. In general, most aquatic animals can withstand DO levels as low as 2.0 ppm only for very short periods. Few fish can survive for extended periods at DO levels below 3.0 ppm, and at DO levels below 5.0 ppm, fish grow and develop more slowly. The DO level in most areas of Boston Harbor is adequate to support a healthy fish population.

DO level	
2.0	Fish can live for short periods.
<3.0	Few fish survive for extended periods.
<5.0	Fish grow and develop slowly.
6.0	Healthful for most fish.

Whether or not a given body of water has a minimum level of DO depends on the temperature, salinity and level of pollution of the water. A higher percentage of oxygen can dissolve in water with low temperature and low salinity.

Simply measuring the level of DO in a body of water indicates whether or not it can support a healthy fish population at a given moment. The "quality" of that body of water may more accurately be determined by comparing the level of DO to the saturation limits of the water. The following chart shows 100% saturation at various temperatures and various levels of salinity.

Temperature	Salinity (ppt)				
°C	0	9	18	27	35
0°	14.6 ppm	13.7 ppm	12.9 ppm	12.1 ppm	11.4 ppm
5°	12.8 ppm	12.0 ppm	11.3 ppm	10.7 ppm	10.1 ppm
10°	11.3 ppm	10.7 ppm	10.1 ppm	9.5 ppm	9.0 ppm
15°	10.1 ppm	9.5 ppm	9.0 ppm	8.5 ppm	8.1 ppm
20°	9.1 ppm	8.6 ppm	8.2 ppm	7.7 ppm	7.3 ppm
25°	8.2 ppm	7.8 ppm	7.4 ppm	7.1 ppm	6.7 ppm
30°	7.5 ppm	7.2 ppm	6.8 ppm	6.5 ppm	6.2 ppm
35°	6.9 ppm	6.6 ppm	6.3 ppm	6.0 ppm	5.7 ppm

Table 3: *100% Saturation Levels of Dissolved Oxygen*

Example: If the salinity of a brackish water source is 18 ppt and the temperature is 5°, you would expect a DO level approaching 11.3 ppm. If the measured DO level was 4 ppm, it would have a saturation level of 35% ($4 \div 11.3$). Fish may be able to survive, but something is probably amiss with the health of the water.

Low oxygen levels are often caused by decaying organic material, such as untreated or poorly treated sewage. The bacteria that cause decay consume a great deal of oxygen as they break down the organic material. (See the BOD test.) Dense populations of very active fish can also cause a condition of low oxygen, as can sunless periods in bodies of water with dense growths of algae because the algae will consume oxygen when there is no photosynthesis taking place.

Table 3 shows how much oxygen can dissolve in water under certain temperature and salinity conditions. The numbers shown represent 100% saturation, and DO levels in nature are generally somewhat below those levels. The DO level, however, can also be substantially higher than the level shown if there is unusual opportunity for oxygen to mix with the water. Three common conditions lead to supersaturation. When cold, well-aerated water is rapidly warmed, the water could change temperature faster than the oxygen can leave it. In extremely choppy conditions ("white water"), turbulence mixes large quantities of oxygen with water. Finally, bright sunlight coupled with dense plant growth causes rapid photosynthesis which increases plant respiration.

Testing for Dissolved Oxygen

Overview: The dissolved oxygen test uses a titration known as the "Winkler Method." It is reliable and accurate, but it requires care and accuracy. In the "fixing" stage of the test, the oxygen present in the sample converts some of the test chemicals to free iodine. That iodine exists in direct proportion to the amount of oxygen in the water. Because of the iodine, the fixed sample appears as a yellow to yellow-brown liquid.

Once the sample has been fixed, it is titrated with a specific concentration of sodium thiosulfate. The sodium thiosulfate causes the yellow color to disappear, but the color changes can be minute and difficult to discern. Therefore, a starch indicator is added to the fixed solution prior to the titration. As long as any free iodine exists, the indicator remains deep blue-purple. As soon as the titration is complete, the blue color disappears and the solution becomes clear.

The titrator is calibrated from 0 to 10 in units of .2 mL (so the titrator holds 2 mL of titrant). Each unit represents 1 ppm of dissolved oxygen. If the scale on the titrator reads 5.5 when the starch indicator turns clear, the water sample has a dissolved oxygen level of 5.5 ppm. If the DO level of the sample is greater than 10 ppm, you will have to refill the titrator during the test.

Length of Test: Fixing: 10 minutes (to be completed as quickly as possible in the field)
Titrating: 10 minutes (in the field or the classroom)

Time Restrictions: The fixing phase of this test must be carried out in the field. Once the solution has been fixed, the sample may be returned to the classroom for the titration. A fixed sample should be tested within eight hours.

Difficulty of Test: Difficult (Requires care, patience and precision, and involves the use of acids.)

Protective Clothing: Eye protection, rubber gloves, apron

Safety Procedures:

- Wash immediately if any reagent spills or drips onto the skin.
- If an accident occurs, follow the directions on the Material Safety Data Sheet (MSDS) for that particular chemical.

Suggestions:

- Sample must not be "aerated" during collection. In other words, do not allow air to bubble into the sample container. If possible, gently tap the sample bottle while it is under water to let air bubbles escape.
- The sample should be "fixed" in the field as quickly as possible. The oxygen level in the bottle can change very quickly.
- For accuracy, test at least two samples at each site. (The kit contains extra DO sample bottles for additional tests and for BOD testing.)
- During fixing, it is OK for a small quantity of sample to overflow. The overflow will not affect the results, and it is important that no oxygen be introduced during the testing process.
- Follow the directions carefully. Not allowing the floc to settle and not mixing for the specified time could result in inaccurately low readings.

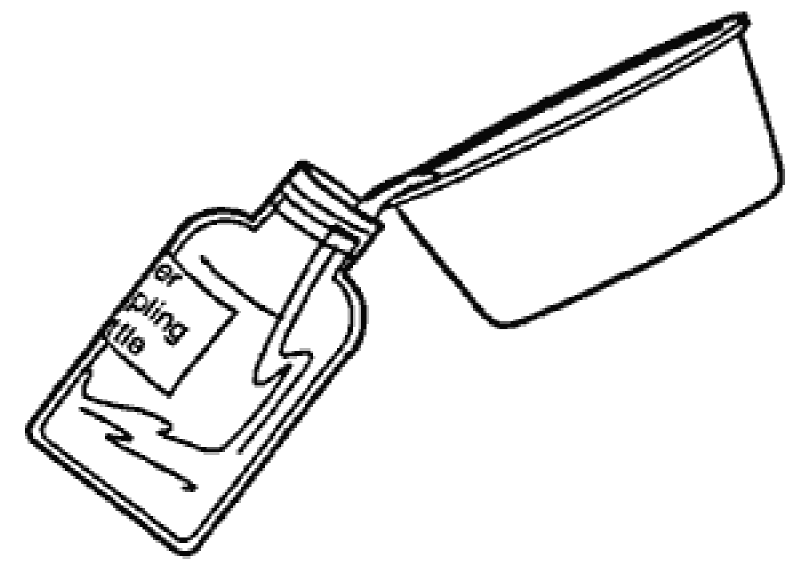
Expected Results:

- See Table 3 on page 40. The results for healthy water should be reasonably close to the limits indicated on the chart.

PROCEDURE

Fixing the Sample

1. Fill a DO water sampling bottle to overflowing with sample water. Pour the sample water down the side of the sampling bottle so it does not slosh or mix air bubbles in with the sample, because bubbles will change the test results.



2. Cap the bottle briefly. (The cap contains a small insert that will force a tiny amount of sample out of the bottle, thus reducing overflow as chemicals are added.)

   NOTE: *During fixing, it is OK for a small quantity of sample to overflow. This overflow will not affect the results. It is far better to have some overflow than an air bubble, because an air bubble could cause oxygen to be introduced to the sample during the testing process, thus altering the results.*

3. Remove the cap and carefully add 8 drops of the Manganous Sulfate solution and 8 drops of the Alkaline Potassium Iodide Azide. A "floc," or white precipitate, will form.

4. Cap the bottle and mix it gently for about 30 seconds. Do not shake it vigorously because shaking could introduce oxygen. Let the bottle sit for a few minutes while the floc settles to beneath the shoulder of the bottle.

   NOTE: *If you rush this part of the process by not mixing the solution or not allowing the floc to settle, the results will be falsely low.*

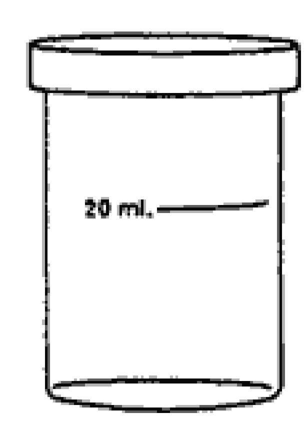
5. Carefully add 8 drops of the sulfuric acid solution and replace the caps on both containers.

6. Gently mix the solution by continuously inverting the bottle until the precipitate is gone. The color of the sample should be yellow to yellow-brown. The sample may have to be mixed for ten minutes or more. A clear solution indicates either the total absence of oxygen or improper fixing, and the precipitate will not disappear completely if the sample has high turbidity. The fixed sample may be kept for up to 8 hours before completing the test.

Titrating the Fixed Sample

1. Fill the titration tube to the 20 mL line with the fixed water sample.

2. Add 8 drops of the Starch Indicator Solution.

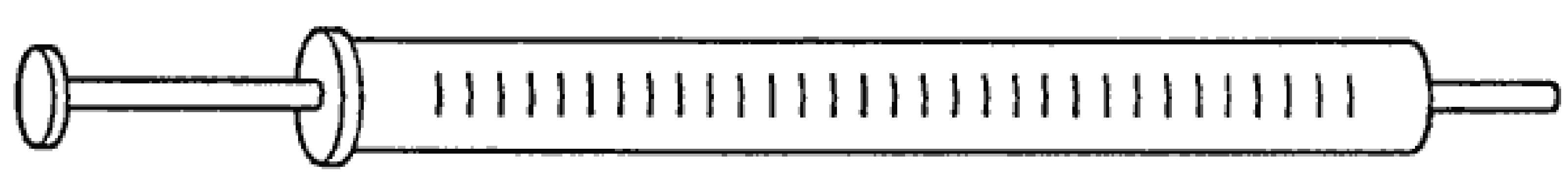


Titration tube

3. Cap the titration tube and gently swirl the solution to mix it. The solution will be deep blue-purple.

   NOTE: *The yellow color of the fixed solution is caused by iodine. The amount of iodine in the fixed solution is directly proportional to the amount of oxygen in the sample. The blue-purple color is the result of the starch indicator being in contact with the free iodine. When there is no more iodine in the water, the color will disappear.*

4. Fill the titrator to the "0" mark with Sodium Thiosulfate. Fill the titrator by putting its tip through the small hole in the top of the Sodium Thiosulfate bottle. Then turn the bottle upside down, hold the titrator at eye level (while wearing eye protection), and slowly pull out the plunger until the liquid fills the titrator to the "0" line. If any large air bubbles remain in the titrator, hold the titrator upside down and gently tap the side; the bubbles will rise to the surface. Once it is full, turn the titrator right-side-up again. THE TITRATOR IS GLASS, SO TREAT IT GENTLY.



Titrator

5. Insert the tip of the titrator into the hole in the top of the titration tube.

6. Add titrant to the blue solution <u>one unit at a time</u>. Swirl the tube gently after each addition. When the solution starts to get lighter, carefully add only <u>one drop at a time</u>.

7. When the solution becomes clear, stop adding titrant, and read the number on the side of the titrator. That number represents the dissolved oxygen (in parts per million) in the water sample.

   NOTE: *The titrator only holds enough titrant to measure 10 ppm of DO. If your solution has greater than 10 ppm of DO, the solution will not have turned clear when the titrator is empty. Refill the titrator and continue with the titration, then add 10 to your results. For example, if, after refilling, your final reading is 3, you have a DO level of 13 ppm.*

   DO NOT RETURN THE UNUSED SODIUM THIOSULFATE REAGENT TO THE STORAGE BOTTLE. Empty the titrator into the sample solution and dispose of it according to the instructions below.

8. Record the DO of the sample on the *Data Recording Form.*

9. Refer to Table 3: Saturation levels of Dissolved Oxygen on page 40. Find the temperature and level of salinity closest to the sample. (Fresh water will have a salinity level of 0 ppt.)

10. Determine the oxygen saturation of the sample by dividing the DO level of the sample by the saturation level on the chart.

measured DO level ÷ saturation level = % of saturation

11. Record the percent of saturation on the *Data Recording Form*..

12. Neutralize the fixed sample and the titrated sample by adding 5 - 10 drops of the neutralizing solution (bicarbonate of soda) included with the kit. Once the solutions are at pH 5.5 or higher, they may be flushed down the drain with plenty of water. The MWRA does not permit water to enter the sewer system that is less than pH 5.5 or greater than pH 10.

    Disposal is a very important part of the testing procedure. *Be sure you check the pH of the neutralized solution before disposing of it.*

13. Clean and dry all the equipment and carefully replace everything into the kit.

Interpreting Results

Does the water have enough oxygen to support a healthy fish population?

DO Level	
2.0	Fish can live for only short periods
< 3.0	Few fish can survive for extended periods
<5.0	Fish grow and develop slowly
6.0	Healthful for most fish

Water that contains 80% to 125% of the possible oxygen content is considered ideal for healthy fish life. (See Table 3 on page 40.)

Extension Activities

1. Examine the effects of temperature and salinity on water's ability to hold oxygen.

   Procedure

   a. Make four different water samples:
      cold salt water (roughly 0° - 10°)
      cold fresh water
      warm salt water (roughly 25° - 35°)
      warm fresh water

   (Ocean water contains 35,000 ppm of salt, so you can create an ocean water substitute in your classroom by adding one level teaspoon of salt to 1 3/4 cups of water. You will need 20 mL of water for each test.)

b. Just before fixing each solution, aerate it by shaking it vigorously for one full minute.

c. Plot and compare the results of each of the four tests.

2. If you have access to deep water, test for DO from a surface sample and a deep water sample (ten meters or more). DO should be higher at the surface as a result of atmospheric mixing and sunlight, which results in photosynthesis. The temperature in deeper water tends to be several degrees cooler, so the saturation point will be somewhat higher, but that should not have a large effect on the results.

3. If you have an aquarium with an aerator in your classroom or school, measure the DO of the water with the aerator running, then test it again after the aerator has been turned off for a few hours.

TEST 4: Biochemical Oxygen Demand (BOD)

Brief Summary

A measure of the oxygen-consuming life in a water sample. The testing procedure for BOD is the same as for DO, except that the DO sample is fixed in the field then titrated immediately, while the BOD sample is left unfixed and stored for five days in the dark at room temperature. After five days, the sample is fixed and titrated. The results of the test are subtracted from the oxygen level found in the DO test, and the result is Biochemical Oxygen Demand.

Note: *Do not fix the sample until it has incubated for five days, then fix it just as you would for the DO test.*

Background

The bacteria that decompose organic materials (such as aquatic plants and sewage) consume oxygen. The amount of oxygen consumed over a fixed period of time in a controlled environment indicates the amount of organic matter – or biodegradable waste – in the water. That measure is called Biochemical Oxygen Demand, or BOD. High BOD indicates pollution; low BOD suggests good water quality. If BOD is high, it is because bacteria living in the water used up the oxygen as part of the process of decomposing organic material. Thus, the BOD serves as an important clue to water quality.

Interpreting BOD results:

1 - 2 ppm	Very clean water, little organic decay
3 - 5 ppm	Moderately clean water, some organic decay (probably from plant life)
6 - 9 ppm	Much organic decay (possibly from algae blooms)
10+ ppm	Very unhealthy levels of organic decay (often from untreated sewage)

Testing for BOD

Overview: The basic testing procedure for BOD is identical to that of Dissolved Oxygen. The water sample that you will test, though, will have been incubated in the dark for five days at 20°C before being tested for DO. The difference in the results of the initial DO test and the five-day DO test represent BOD. When measuring BOD, do not fix the sample until you are ready to carry out the test.

$$\begin{array}{rl} \text{DO} & \text{(at time of initial sampling)} \\ \underline{-\ \text{DO}} & \text{(after five-day incubation period)} \\ \text{BOD} & \end{array}$$

TEST 4: Biochemical Oxygen Demand

Length of Test:	Test: Fixing- 10 minutes, Titrating - 10 minutes (same as DO) Incubation period: 5 days
Difficulty of Test:	Difficult
Protective Clothing:	Eye protection, rubber gloves, apron
Safety Procedures:	• Wash immediately if any reagent spills or drips onto the skin. • If an accident occurs, follow the directions on the Material Safety Data Sheet (MSDS) for that particular chemical.
Suggestion:	All of the suggestions for the DO test apply.

PROCEDURE

1. Fill a DO water sampling bottle to overflowing with sample water.
2. Do not fix the sample immediately. Instead, store the full bottle in darkness at 20°C for five days. Wrapping the bottle in aluminum foil works fine.
3. After five days, test the sample for dissolved oxygen. (Follow the procedure for the dissolved oxygen test.)
4. Subtract the five-day results from the initial DO results to find the BOD, and record the BOD on the *Data Recording Form.*
5. Follow the instructions from the DO test for disposing of the fixed and tested samples. Be sure the samples are neutralized to a pH of between 5.5 and 10.0.

Interpreting Results

The BOD results indicate the amount of organic material in the water sample. The more organic material, the more bacteria will consume oxygen to decompose that organic material. An unusually high BOD level might indicate the presence of pollution or sewage.

BOD Level	
1-2 ppm	Very clean water, little organic decay
3-5 ppm	Moderately clean water, some organic decay (probably from plant life)
6-9 ppm	Much organic decay (possibly from algae blooms)
10+ ppm	Very unhealthy levels of organic decay (often from untreated sewage)

Extension Activity

1. Rather than conducting a single BOD test after five days of incubation, incubate five bottles of sample water, and measure the level of dissolved oxygen each day for five days. Plot the results on a graph.

TEST 5: Nitrates

Brief Summary

A measure of a common nutrient. In the test, several reagents are added to the water sample. After twelve minutes of waiting time, the tester uses a color comparator to determine the level of nitrates in parts per million (ppm).

Note: *The tested samples contain a cadmium residue. They must be stored in a special container and returned to the MWRA for proper disposal.*

Background

Nitrogen is abundant not only in aquatic ecosystems, but also throughout nature; in fact, 79% of the air we breathe is nitrogen. Nitrogen is considered a "nutrient," because it is essential for plant growth. The other nutrient most often found in aquatic systems is phosphorus, but problems caused by phosphorous are less common in New England waters, so this test focuses on nitrogen as a representative nutrient.

Nutrients are part of an important cycle of life. As part of the process of biosynthesis in aquatic environments, these nutrients are taken up by plankton and seaweeds which enter the food chain. When plants and animals die, their tissues are decomposed by bacteria, and the nutrients re-enter the water. Plants do not use nitrogen (N_2) in a pure state; rather they use it in the form of nitrate (NO_3) or ammonia (NH_3). Chemically, bacteria initially break tissue down into ammonia (NH_3). That ammonia is then oxidized by other bacteria into nitrites (NO_2) and nitrates (NO_3), thus completing the cycle.

In addition to this chemical cycle, nitrogen enters the water in other ways. Animal excrement is rich in ammonia. While the amount of nitrogen added to water by fish excrement is small, the amount added by larger animals living near the water, such as seagulls, ducks and geese can be quite large. Fertilizers, sewage, and poorly maintained or overused septic tanks, are the main human-made sources of nitrates.

Algae Blooms and Oxygen Levels

The most common signs of artificially high levels of nutrients are algae blooms and excessive aquatic plant growth. Algae blooms and increased plant growth would seem to benefit fish, because plants produce oxygen as a by-product of photosynthesis. In reality, though, algae blooms can be dangerous, and they can lead to unhealthy water quality for fish by depleting the water's oxygen. Here is how that works:

High levels of nutrients result in increased plant growth, which increases photosynthesis. During photosynthesis (which occurs only in the presence of sunlight), plants produce oxygen and carbohydrates. Plants respire, "burning" these carbohydrates for energy, consuming oxygen and producing carbon dioxide. Once the sun sets, photosynthesis and oxygen production end, but the plants still respire, consuming oxygen and producing carbon dioxide. Algae blooms can actually consume more oxygen at night than they produce during the day! Thus, the water in algae-rich ponds, stagnant streams, bays and estuaries can have dangerously low oxygen levels.

In addition, more plants living also means more plants dying. The bacteria that decompose the dying plants become more abundant, and these bacteria also use up much of the available oxygen. As a result, fish and other aquatic animals may not have enough oxygen to thrive. To make matters worse, these algae blooms often occur during the summer, when the warmer water holds less oxygen and the more active fish require more oxygen.

Eutrophication

Eutrophication means "enrichment with nutrients," and it can lead to the death of a lake or pond. In nature, the process can take thousands of years; human activities that introduce excessive nutrients greatly speed up the natural process.

At the beginning of the process, nutrients slowly build up in the water. More and more plant life grows in the lake, initially providing a good environment for fish. Oxygen-consuming, or "aerobic," bacteria break down the organic wastes from these plants and animals. Eventually the decaying organic material and respiring plants cause the oxygen levels to drop, and fish begin to die. Without oxygen, aerobic bacteria can no longer break down the waste, so the nutrients stay trapped in the waste. The lake can no longer support fish or animal life. Bacteria that do not require oxygen, "anaerobic" bacteria, begin to break down the organic waste very slowly. After hundreds of years, the lake fills with decaying organic materials and becomes a swamp.

Human-caused eutrophication can affect sea water as well as freshwater. As sewage and agricultural runoff enter the ocean, the concentrations of nutrients increase. This overfertilization can cause an increase in plankton and encourage the growth of aquatic plants, seaweed, and phenomena such as "red tide." The effects are most obvious in harbors, bays and estuaries because they tend to be relatively shallow and sheltered.

Testing for Nutrients

Water-borne plants use nutrients very quickly. As a result, if you test in an area with algae blooms, your tests may not reveal high nitrogen levels because the algae would have already used it up. The nutrients may have entered the water upstream where the algae would not grow because the water was moving too swiftly. Testing upstream from the bloom might reveal the source of the nutrients.

If several cloudy days follow a period of sunny days during which algae or plankton have grown wildly, the plants' respiration can cause a dramatic drop in the dissolved oxygen level. In addition, the reduced sunlight will cause some algae/plankton to die. Aerobic bacteria will then feed on the decaying plants, decreasing the level of dissolved oxygen even more.

Health Effects of One Specific Form of Nitrogen: Nitrites

Nitrites (NO_2) are a short-lived but dangerous product of the nitrogen cycle. High nitrite levels in the blood reduce its ability to carry oxygen. Fish can develop "brown blood disease," caused by this lack of blood oxygen. In humans, nitrites react with the hemoglobin in the blood, creating a condition called methemoglobinemia in which blood cells cannot carry oxygen. This condition is called "blue baby" disease because babies can suffocate from lack of oxygen. Children have even been poisoned by drinking formula made with water containing high nitrite levels. Many of these poisoning cases involve private wells where excess nitrogen from fertilizers or poorly functioning septic tanks has seeped into the ground water.

Testing for Nitrates

Overview: In this test, a water sample is mixed with a mild acid solution and special "nitrate reducing agent." The level of nitrates is then read on a color comparator.

Length of Test: 20 minutes (including 12 minutes of waiting time)

Difficulty of Test: Moderate

Protective Clothing: Eye protection, rubber gloves, apron

Safety Procedures:

- Wash immediately with soap and water if any reagent spills or drips onto the skin.
- Dispose of the waste sample by sending it back to the MWRA in the appropriate bottle.
- If an accident occurs, follow the directions on the Material Safety Data Sheet (MSDS) for that particular chemical.

Suggestion: When testing algae-laden waters, test upstream from the bloom as well as near it. This is important because water-borne plants use nutrients very quickly, and algae do not grow well in moving water. As a result, nutrient levels may not be elevated in areas with rich algae blooms. The nutrients may have entered the water upstream, and the algae did not grow until the water settled down.

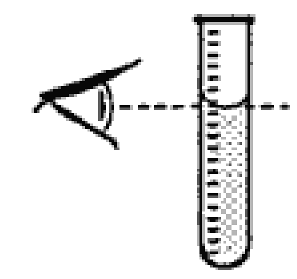
Expected Results:

- Unpolluted water should have a nitrate level of 1 ppm or less.

TEST 5: Nitrates

PROCEDURE

1. Fill the water bottle with sample water. (This bottle enables you to fill the test tube with sample water more accurately and more safely.)
2. Fill one of the test tubes to the 2.5 mL line with water from the sample water bottle. Hold the tube at eye level and read the level from the lowest point of the meniscus.



3. Carefully continue filling the test tube up to the 5 mL line with the mixed acid reagent.
4. Put the cap on the test tube, mix gently, and wait two minutes.
5. Using the small white measuring spoon, carefully add one level scoop (.1 g) of Nitrate Reducing Agent.
6. Put the caps back on the test tube and the bottle of Nitrate Reducing Agent. Invert the test tube gently 50 - 60 times in one minute, then wait ten minutes.
7. Put the tube into the color comparator, and match the color of the sample as closely as possible to one of the standards.
8. Record your results on the *Data Recording Form*.
9. The Nitrate Reducing Agent used in this test contains Cadmium, so the liquid waste from the test must be disposed of properly. Pour the liquid waste into the waste collection bottle contained in the kit and return it to the MWRA. **DO NOT ADD WASTE FROM ANY OTHER TEST TO THE BOTTLE, AND DO NOT FLUSH THE SAMPLE FROM THE NITRATE TEST DOWN THE DRAIN OR POUR IT ON THE GROUND.**

   Upon receiving the contaminated sample, the MWRA will evaporate the liquid, collect the solid residue which will contain cadmium, and dispose of it as a hazardous waste according to state and federal regulations.
10. Clean and dry all the equipment and carefully replace everything into the kit.

Interpreting Results

The color comparator in this test measures "NO_3-N," which is "nitrogen that is in the form of nitrate." Fresh water with a nitrate level below 1 ppm is considered optimal for aquatic life, while in salt water environments, that level is lower, at about .6 ppm. Nitrate levels of about 10 ppm are unsafe for drinking.

Determining the precise level of nitrates at which problems begin to occur in nature is extremely difficult because of other factors involved. For example, if a body of water has a very low pH, it may have virtually no plant life. Plants grow best in warm water, so even low levels of nitrates in a warm, fertile environment can cause problems with excessive plant growth. Nutrients tend to have little effect in fast-moving water, so high nutrient levels in a moving brook may have no effect, while low levels in a stagnant pond may have a huge effect.

These figures from the National Oceanographic and Atmospheric Administration (NOAA) offer guidelines for understanding the effect of nitrates on water quality:

	Sea Water	Fresh Water
High quality	<.6 ppm	<1.0 ppm
Fair quality	.6 - 1.0 ppm	1.0 - 1.8 ppm
Fair to poor	1.0 - 1.8 ppm	1.8 - 2.8 ppm
Poor quality	>1.8 ppm	>2.8 ppm

Extension Activities

1. Conduct empirical studies of nitrate levels on a body of water. For example, compare nitrate levels during the winter (when they should be low) and during the summer (when more fertilizer is being used).

   If you find a situation of excessive algae growth, test for both nitrates and dissolved oxygen. For nitrates, try to track the relationship between plant growth and nitrate levels. Test for DO during the day and at night. During the day, when photosynthesis is taking place, the DO level should be high, and at night, when plants are consuming oxygen, it should be lower.

2. If you find water with large quantities of algae or water with high levels of nitrates, continue testing water from farther upstream to try to identify the source of the nutrients. Look for areas where human or animal waste or agricultural runoff, such as from golf courses or farms, might enter the waterway.

TEST 6: Total Dissolved Solids (TDS) and Salinity

Brief Summary

The test uses a digital meter that measures "micromhos," a measure of electrical conductivity. When measuring TDS (fresh water only), the meter reading must be multiplied by .5, which is a standard conversion factor. When measuring salinity, the sample must first be diluted, then multiplied by the level of dilution and the standard conversion factor.

Background

As a universal solvent, water can dissolve a wide range of substances, such as calcium, sodium, phosphorous, iron, sulfate, carbonate, nitrates, chlorides, and other ions. Solids from urban runoff often include soluble contaminants, such as road salts and fertilizers.

Although scientists do not completely understand why the ocean is salty, it is thought that some salts enter the ocean from river runoff. Another possible source is the ocean floor itself. The ocean's salinity has been consistent for as long as scientists can tell.

Some solids in water are essential to maintain health – phytoplankton, in fact, depend entirely on dissolved nitrates and phosphates – but high concentrations of solids can lower water quality. High concentrations of solids can increase turbidity, which decreases the rate of photosynthesis. These solids can also bind with toxic compounds and heavy metals, and lead to an increase in water temperature as a result of greater absorption of infrared radiation from sunlight.

Fresh water, brackish water, and sea water differ primarily in their level of total dissolved solids. Fresh water contains up to 1 part per thousand (ppt) of dissolved solids. Brackish water contains 1 - 35 ppt, and sea water contains 35 ppt (35,000 ppm). Water in protected harbors and bays that are fed by rivers often have salinity levels lower than 35 ppt. In Boston Harbor, for example, the salinity level is often around 28 ppt.

Rainwater generally has a TDS level of around 10 ppm. MWRA tap water is generally around 40 ppm. Levels of TDS greater than 500 ppm can be harmful to water systems.

Testing for Total Dissolved Solids and Salinity

Overview: The traditional test for TDS requires evaporating a precise quantity of liquid, then weighing the residue. This process, however, is slow and it requires a very precise scale.

In lieu of the traditional test, therefore, this kit contains a digital tester. Two electrodes on the tester measure the water's conductivity in microsiemens (µs). A microsiemen is a micromho per centimeter, and a micromho is the inverse of a microohm (one one-millionth of an ohm). (Notice that the "mho" in micromho is "ohm" – the measure of resistance – spelled backwards.)

Dissolved solids in water raise its conductivity. Multiplying the water's conductivity by a specific factor, therefore, gives the total dissolved solids in parts per million. The factor varies according to the solids dissolved in the water, and it ranges between .5 and .9. This test uses a factor of .5. Your results will be reasonably accurate using that factor.

Length of Test: 5 minutes

Difficulty of Test: Moderate (Requires some math. Salinity test requires a dilution.)

Additional Material: A 250 ml beaker or a cup.

Protective Clothing: Rubber gloves

Suggestions:

- When testing fresh water for TDS, the procedure is as simple as putting the meter into the sample, taking a reading, and multiplying by .5. The result is TDS, measured in ppm.
- If the meter reads "1.0", the conductivity is higher than the range of the tester, and you will have to dilute the sample and follow the directions for testing brackish or sea water.
- Tap water and bottled water contain some dissolved solids (usually <100 ppm). When making a dilution you must use demineralized water that you can make by using the demineralizer bottle included in the kit.
- The test for brackish water or sea water is a little more complicated than that for fresh water. The meter registers conductivity only up to a level of 1900 µs (which equals about 950 ppm of dissolved solids). To test water with conductivity greater than 1900 µs, therefore, you must dilute the sample. We recommend a dilution of 1:50. After measuring the conductivity of the diluted sample, multiply the meter reading by the dilution to find the conductivity of the sample. (For example, if a 1:50 dilution reads 1,100µs on the meter, the conductivity is 1100 X 50 = 55,000µs.)

ABOUT DILUTIONS
A 1:50 dilution means that there is one part of a substance per 50 parts of the total. In other words, there are 49 parts of other substances to the one part of the measured substance. To make a 1:50 dilution for this test, you should add 1 mL of sample water to 49 mL of demineralized water (NOT 1 mL of sample water to 50 mL of demineralized water).

PROCEDURE

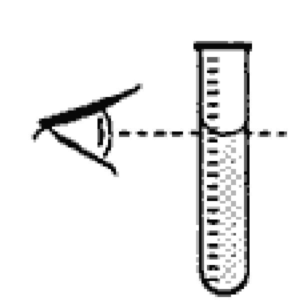
FRESH WATER

1. Pour some water sample into the 250 beaker or a cup to a level of 1 or 2 cm.
2. Push the white button on the digital tester to turn it on. When it is ON, it will read 000µs.
3. Put the tip of the digital TDS tester into the sample water. DO NOT SUBMERGE THE TESTER ABOVE THE BROWN LINE.
4. Multiply the meter reading by .5. The result is the total dissolved solids (TDS) in parts per million (ppm).
5. Record your results on the *Data Recording Form.*
6. Wash the meter's electrodes with tap water, and empty and rinse the beaker.
7. Push the white button to turn the meter OFF. When it is OFF, the digital face will be blank.

BRACKISH WATER AND SEA WATER

1. Using demineralized water and the graduated cylinder, make a 1:50 dilution of your sample water by filling the graduated cylinder to the 49 mL mark with demineralized water then adding 1 mL of sample water. When pouring water from the demineralized water bottle, DO NOT REMOVE THE CAP; pull out the cap and squeeze the bottle.

   To fill the cylinder to the proper level, hold it at eye level and read the water level from the lowest point of the meniscus.

2. Pour the diluted sample from the graduated cylinder into the 250 ml beaker or cup.

MAKING DEMINERALIZED WATER

The test kit contains a demineralized water bottle, which is a plastic squeeze bottle with a pull-out cap and a green resin inside. The resin is a demineralizing agent, and the cap contains a special filter so the resin will not escape the bottle. <u>When making a dilution for this test, only use demineralized water.</u>

If the demineralizer bottle is not full, follow this procedure. If it is full, go to #1 of the procedure.

a. Fill the demineralized water bottle with fresh (tap) water.
b. Put the cap on the bottle, be sure the spout is closed, and shake it for 30 seconds.
c. Leave the bottle full when storing it in the kit. (NOTE: Do not let the "ion exchange resin" dry out.)
d. If you notice the "ion exchange resin" changing colors from dark green to amber yellow, notify the MWRA that it is time to replace the resin.

3. Push the white button on the digital tester to turn it on. When it is ON, it will read 000µs.
4. Put the tip of the digital TDS tester into the diluted sample water. DO NOT SUBMERGE THE TESTER ABOVE THE BROWN LINE.
5. Multiply the meter reading by the amount of dilution (50 for a 1:50 dilution) to find the total conductivity of the sample in microsiemens (µs).
6. Multiply the conductivity (the answer from #5) by .5 (which is a standard factor for converting conductivity to salinity). The answer will be salinity in parts per million (ppm).
7. Convert the salinity in parts per million (ppm) to salinity in parts per thousand (ppt) by dividing by 1000.
8. Record your results on the *Data Recording Form.*
9. Wash the meter's electrodes carefully with tap water; empty and rinse the beaker.
10. Push the white button to turn the meter OFF. When it is OFF, the digital face will be blank.

Interpreting Results

For fresh water, the total dissolved solids count will tell you how many salts and minerals are dissolved in the water. (The TDS count is different from the turbidity level in that turbidity refers to undissolved solids.)

For sea or brackish water, the TDS level determines the salinity (saltiness) of the water. In estuaries, bays, tidal rivers, and salt water marshes, the salinity level will change with the ebb and flow of the tide and the amount of fresh water that runs into these waters.

0 ppm		Demineralized water or "pure" water
10 ppm		Rain water
40 ppm		Tap water
500 ppm		Harmful to plumbing in water systems
<1000 ppm	(<1 ppt)	Fresh water
1000-35,000 ppm	(1 - 35 ppt)	Brackish water
35,000 ppm	(35 ppt)	Sea water

Sample Calculation (Brackish water)

Sample meter reading for a 1:50 dilution = 675μs

1. Multiply by the level of dilution
   675 X 50 = 33,750 μs
2. Multiply by the standard factor of .5
   33,750 X .5 = 16,875
3. Put the results into the proper units
   Salinity = 16,875 ppm OR 16.9 ppt
   (Both of these are correct. TDS is generally measured in ppm; salinity is generally measured in ppt.)

Extension Activity

1. Follow an estuarial river or stream as far as you can. At the inland end of the estuary the water will contain <500 ppm of total dissolved solids. At the ocean end, it will contain around 35 ppt of salinity (or around 28 ppt in the harbor). Plot the concentrations of salinity throughout the mixing zone.

TEST 7: TURBIDITY

Brief Summary

The measure of water's cloudiness. This test measures turbidity by comparing a turbid sample with a clear sample, then adding drops of a special clouding solution to the clear sample until it appears as cloudy as the turbid sample. The results are measured in Jackson Turbidity Units (JTUs).

Background

Turbidity is a measurement of how cloudy water appears. Technically, it is a measure of how much light passes through water, and it is caused by suspended solid particles that scatter light. These particles may be microscopic plankton, stirred up sediment or organic materials, eroded soil, clay, silt, sand, industrial waste, or sewage. Bottom sediment may be stirred up by such actions as waves or currents, bottom-feeding fish, people swimming or wading, or storm runoff.

Clear water may appear cleaner than turbid water, but it is not necessarily healthier. Water may be clear because it has too little dissolved oxygen, too much acidity or too many contaminants to support aquatic life. Water that is turbid from plankton has both the food and oxygen to support fish and plant life. However, high turbidity may be a symptom of other water quality problems.

Effects of Turbidity

- Turbidity diffuses sunlight and slows photosynthesis. Plants begin to die, reducing the amount of dissolved oxygen and increasing the acidity (decaying organic material produces carbonic acid, which lowers the pH level). Both of these effects harm aquatic animals.
- Turbidity raises water temperature because the suspended particles absorb the sun's heat. Warmer water holds less oxygen, thus increasing the effects of reduced photosynthesis. In addition, some aquatic animals may not adjust well to the warmer water, particularly during the egg and larval stages.
- Highly turbid water can clog the gills of fish, stunt their growth, and decrease their resistance to diseases.
- The organic materials that may cause turbidity can also serve as breeding grounds for pathogenic bacteria. When drinking water reservoirs are turbid, the water treatment plant usually filters the water before disinfecting it.

- Industrial processes and food processing require clear water. Turbid water can clog machines and interfere with making food and beverages.

Causes of Excessive Turbidity

- Algae blooms caused by excess nitrogen (from agricultural runoff, septic tanks, or sewage outflows).
- Weather and seasons can contribute to turbidity. Water that may be clear in the spring may grow turbid by August because of plant growth enhanced by warmer days and longer sunlight hours. Similarly, heavy rains or spring snow melts can stir up soil and sediments, increasing turbidity.
- Contamination from sewage, industrial waste, or urban runoff.

Standard Measures of Turbidity

There are several ways to test turbidity and several ways to standardize the results. The most scientific measurement uses a *nephelometer* to electronically measure the amount of light scattered by the suspended particles in the sample, reporting the results in Nephelometric Turbidity Units (NTUs). (*Nephele* is the Greek word for "cloud"; *metric* means "measure." *Nephelometric*, therefore, means "measuring cloudiness.")

Using a Secchi Disk (a 20 cm diameter disk with black and white quadrants), the observer notes how deep the disk can be lowered in the water until it disappears from sight and reports the results in meters.

A more versatile test for the classroom and shallow water uses two tubes with targets at the bottom. The observer compares the fuzziness of the target through clear water and sample water and reports the results in Jackson Turbidity Units (JTUs), named after the now-archaic method of holding a long glass "Jackson" tube over a lit candle. This test measures turbidity in JTUs.

A Note On Color

Turbidity can cause color in water, but color itself is not turbidity. Color may be caused by dissolved substances or reflections from rocks and vegetation.

Testing for Turbidity

Overview: In this test, you will pour measured amounts of your sample water and clear water into identical cylinders, each with a black dot at the bottom. You will add small increments of a turbidity reagent to the clear water until the dot appears as cloudy as the dot in the sample water. You will then record the amount of reagent you added, and convert that amount to Jackson Turbidity Units (JTUs). The test relies on observation and comparison, and can be subjective. Several groups should perform this test to confirm the results. If the results vary widely, take a class average.

Length of Test: 5 minutes

Difficulty of Test: Simple

Protective Clothing: Rubber gloves

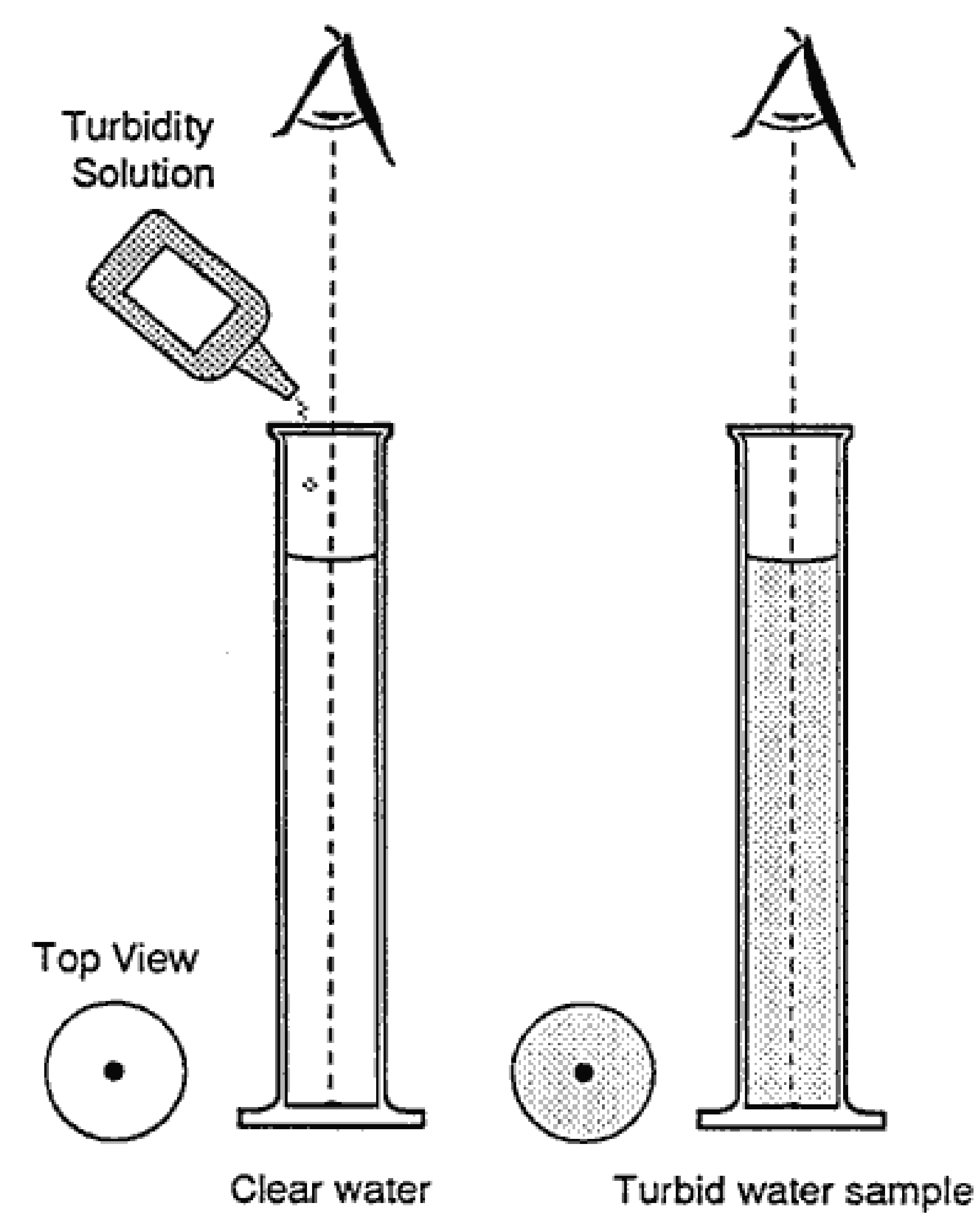
PROCEDURE

1. Fill one turbidity tube to the 50 mL line with clear water, either from the tap or bottled water.

2. Fill the other turbidity tube to the 50 mL line with the sample water.

3. Hold the tubes side-by-side in the light, and look through them vertically (from the top down). Compare their cloudiness by observing the fuzziness of the black dot at the bottom of the tube.

4. Shake the Standard Turbidity Reagent vigorously and add 0.5 mL (which is marked by a line on the pipette) to the tube of *clear water*. The reagent will make the clear water turn slightly cloudy or turbid. Stir the water in each tube with the stirring rods.



5. Compare the turbidity again by looking down into the water in each tube at the black dot. If the sample water is still more turbid than the clear water, continue adding the Turbidity Reagent by 0.5 mL increments until both tubes appear equally cloudy.

6. Compute the Jackson Turbidity Units of the sample. Each 0.5 mL of Turbidity Reagent that you added to the clear water equals 5 Jackson Turbidity Units.

.5 mL = 5 JTU

NOTE: *If your sample is so turbid that you cannot see the dot when the tube is filled to the 50 mL line, only add 25 mL of sample water to the tube. With half as much sample liquid, each 0.5 mL of Turbidity Reagent equals 10 JTUs.*

7. Record your results on the *Data Recording Form.*
8. Dispose of the waste liquid by pouring it down the drain.
9. Wash, dry and put away the test equipment.

Interpreting Results

- Do you consider the amount of turbidity you found to be a sign of a healthy or unhealthy water system?
- What do you think accounts for the level of turbidity you found?
- If you find high turbidity, look around the site to try to find the source of turbidity
- Would you expect to find more or less turbidity during another season?

Water bodies with only sparse plant and animal life	0 JTU
Drinking water	<0.5 JTU
Typical Groundwater	<1.0 JTU

Extension Activities

1. Look at your water sample under a microscope. Can you detect plankton? Plant debris? Sand? Sediment? From what you observe, discuss the sources and causes of the turbidity. Are they natural or human-made? Beneficial or harmful? Could people do anything to decrease the turbidity? Should we?
2. Experiment with other methods of testing turbidity. If you have time and opportunity during the site visit, try to measure the turbidity using a Secchi Disk and compare your results.

TEST 8: Total Coliform Bacteria

Brief Summary

A simple presumptive test that indicates the presence of total coliform bacteria in a water sample. After 48 hours of incubation at room temperature, a lactose broth solution changes colors from purple to yellow if coliform is present.

Background

Coliform is a group of generally nonpathogenic bacteria that live throughout the environment. One type of coliform, fecal coliform, lives in the intestinal tract of warm blooded animals and helps digest foods. It is not harmful in and of itself, but it serves as an *indicator* of the presence of pathogenic bacteria. Fecal coliform bacteria are abundant in human feces, so they are easy to find whenever sewage is present. If you find coliform bacteria in water, you can be sure that other bacteria, viruses or parasites are also present. Some of these might cause diseases.

Testing for particular pathogens in water is usually difficult and time consuming. Coliform bacteria, however, are fairly easy to test for, and they usually occur with other pathogens. That is why people who monitor water quality use this group as an indicator. In water with high total coliform counts, about 10% of the coliform will be fecal coliform.

This kit uses a simple presumptive test for the presence of total coliform bacteria. The test uses a lactose broth that changes color from purple to yellow in the presence of coliform bacteria. Since it is a presumptive test, it is impossible to calculate the quantity of coliform in the water, but this test does not require special equipment or incubation.

> WATER-BORNE DISEASES ARE STILL WORLDWIDE KILLERS
>
> According to the World Health Organization, throughout the world, 4.3 million people, mostly children, die every year from diarrhea caused by water-borne bacteria! That is more than the population of Norway. Other water-borne diseases, such as typhoid, cause seven million deaths and seven billion illnesses each year. Proper water treatment and disinfection of sewage prevent large-scale epidemics.

TEST 8: Total Coliform Bacteria

Sources of Coliform

- Combined sewage systems carry both sanitary wastes - from toilets, washers and sinks - and storm runoff. During rains, sewer pipes are too small to handle the combined flow, so part of it is diverted into a river, bay or harbor. This storm overflow contains untreated or inadequately treated sewage.
- Agricultural and rural runoff carries wastes from birds and animals.
- Improperly working septic tanks and cesspools can allow untreated wastewater to seep into the groundwater.

Testing for Total Coliform Bacteria

Overview: The conventional test for total coliform bacteria requires special equipment, precise lab procedures, incubation, and culture counting. This kit contains only a simple presumptive test for total coliform bacteria. It does not measure the type or quantity of coliform bacteria, only their presence. The coliform testing tubes contain a purple-colored lactose broth that is sensitive to coliform bacteria. If coliform bacteria are present, the purple liquid will turn yellow within 48 hours when incubated at room temperature.

Length of Test: 2 minutes in the field; 48 hours incubation time.

Difficulty of Test: Simple

Protective Clothing: Rubber gloves

Expected Results: Most natural water sources will contain some coliform bacteria, so expect a positive result. Tap water and bottled water should not contain any coliform bacteria. If a tap water sample tests positive, let the water run for a few minutes and test it again. If it continues to test positive, you may want to call the health department. If bottled water from a supermarket tests positive, retest it. If it continues to test positive, report it to a local health official. If bottled water from your water cooler tests positive, retest it. Your water cooler may need disinfecting, and your water distributor can provide instructions. After cleaning the cooler, test the water again using another bottle of water.

PROCEDURE

1. The coliform tubes are capped and about one-half full of purple liquid. Before removing the cap, write the date, time, and source of the water sample on the tube itself. (Either use tape or a permanent marking pen.)

2. Remove the cap from one of the coliform testing tubes. Fill the tube with your water sample to about 1 cm from the top. (The tube has a small shoulder at that level.)

3. Replace the cap.

4. Leave the tube in a safe, warm place for 48 hours.

   NOTE: *Cool temperatures will not change the results of the test; temperatures of less than 20°C (68°F), however, may slow down the color change if coliform is present.*

5. After 48 hours, examine the tube and record the results on the *Data Recording Form.*

   Purple = No coliform bacteria present
   Yellow = Coliform bacteria in the water sample

6. Add several drops of chlorine bleach to the tube to kill the bacteria. Flush the contents of the tube down the drain and discard the tube. DO NOT dispose of the sample without first disinfecting it.

Interpreting Results

Positive results indicate the presence of total coliform bacteria from warm-blooded animals and various soil organisms. People should not drink that water! Total coliform counts are usually about 10 times higher than fecal coliform. Thus, if you find total coliform bacteria, your sample probably contains fecal coliform and other disease-carrying pathogens. You cannot tell whether fecal coliform bacteria came from human sewage or animal waste without performing other laboratory tests.

Extension Activities

1. Rinse your hands in clean water (water not contaminated with coliform), then test that wash water. Were you carrying coliform on your hands? The results might be a strong argument in favor of regular hand washing.

2. Disinfect a sample of coliform-contaminated water and test it again. You may disinfect it by boiling the water sample or adding a drop of chlorine bleach. Try several different disinfection techniques and see if they all work equally well.