Chapter 4 -
Chemical Monitoring

Chemical Parameters

Many types of chemical tests can be performed to assess varying aspects of stream water quality. However, volunteer monitoring programs are faced with both financial and technical limitations. Given these constraints, Hoosier Riverwatch trains volunteers to conduct eight of the chemical parameters considered by the National Sanitation Foundation to be most useful in determining stream water quality (as well as a few additional tests):

Dissolved Oxygen
pH
Biochemical Oxygen Demand
Orthophosphate
***E. coli* and Coliform Bacteria**
Water Temperature Change
Nitrate and Nitrite
Transparency/Turbidity

Riverwatch Chemical Testing Instructions

Hoosier Riverwatch does not require volunteers to use a standard set of equipment or methods for chemical testing. However, the majority of volunteer groups actively participating in the program have received equipment through the Riverwatch Equipment Application program. The chemical testing instructions provided are for the most common methods used by volunteer stream monitoring groups in Indiana. They are also the methods presented during Hoosier Riverwatch training sessions.

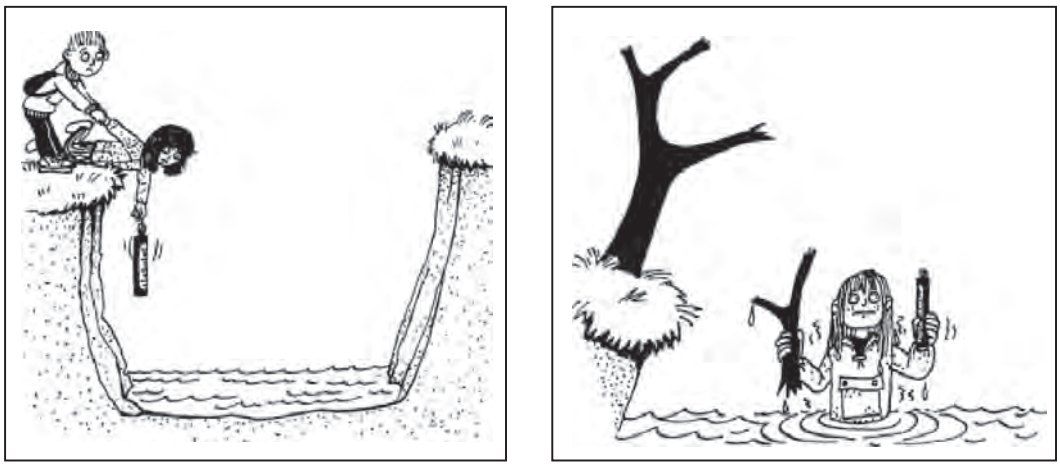
Tips on Collecting Water Samples

How you physically obtain the water sample depends on the size, depth, and banks of your stream. Hoosier Riverwatch volunteers should sample wadeable streams. If you are wading, make sure that you collect water from a point upstream of where you are standing, being careful not to stir up any sediment. The sample must be collected in a clean container to avoid contamination. Collecting water directly from the stream with the container used for the chemical test is preferred. Lower your container down 3 to 5 inches below the surface of the water (or until your wrist is completely submerged) so that your sample is representative of the whole stream. Rinse your collection container three times with sample water before collecting your final sample.

Deep water or steep banks are dangerous (Figure 15). Depending on conditions at your site, you may need to use alternative sampling techniques. If you have a bridge, you may be able to lower a sampling container or bucket down to the stream. When sampling with a bucket and line, it is helpful to have a small (~6 oz.) weight fastened to the rim of the bucket to tip it over. Or you may be able to use an extension rod or a cup on a stick (see Appendix A) from the edge of the stream. Regardless of the method, sample water should be collected from the *main stream flow*.

Figure 15

What not to do



Images from GLOBE 1997

Chemical Monitoring Critical Thinking Questions

(For Use During Hoosier Riverwatch Basic Training Workshops)

What is / are:

- Dissolved oxygen?
- Biochemical Oxygen Demand 5 day?
- pH?
- Nutrients (N and P)?
- Turbidity
- *E. coli*

What are sources of:

- Nutrients?
- Turbid water?
- *E. coli*?

What problems can result from:

- High BOD_5?
- Excess nutrients?
- Excessive turbidity?

What other parameters are associated with or affected by:

- Dissolved oxygen?
- pH?
- High nutrients?
- High *E. coli?*

Notes:

Hints For Performing Chemical Tests

- Practice, practice, practice! The more familiar you are with the tests, the easier they will be to perform, and the more accurate your results will be.
- Do not store chemical testing kits in your car, in direct sunlight, or in any extreme temperatures. The chemical reagents will degrade.
- Perform each test multiple times or have another volunteer read the results to assure precision.
- Wear protective gloves and safety goggles. Do not wear sunglasses when reading the test results.
- Rinse testing tubes or bottles with *sample* water before collecting the sample.
- Obtain your water sample from the stream's main stream flow (usually in the middle). Take the sample 3-5 inches under the surface.
- Rinse testing tubes and bottles with *distilled* water after completing each test.
- Wash your hands when you are finished.

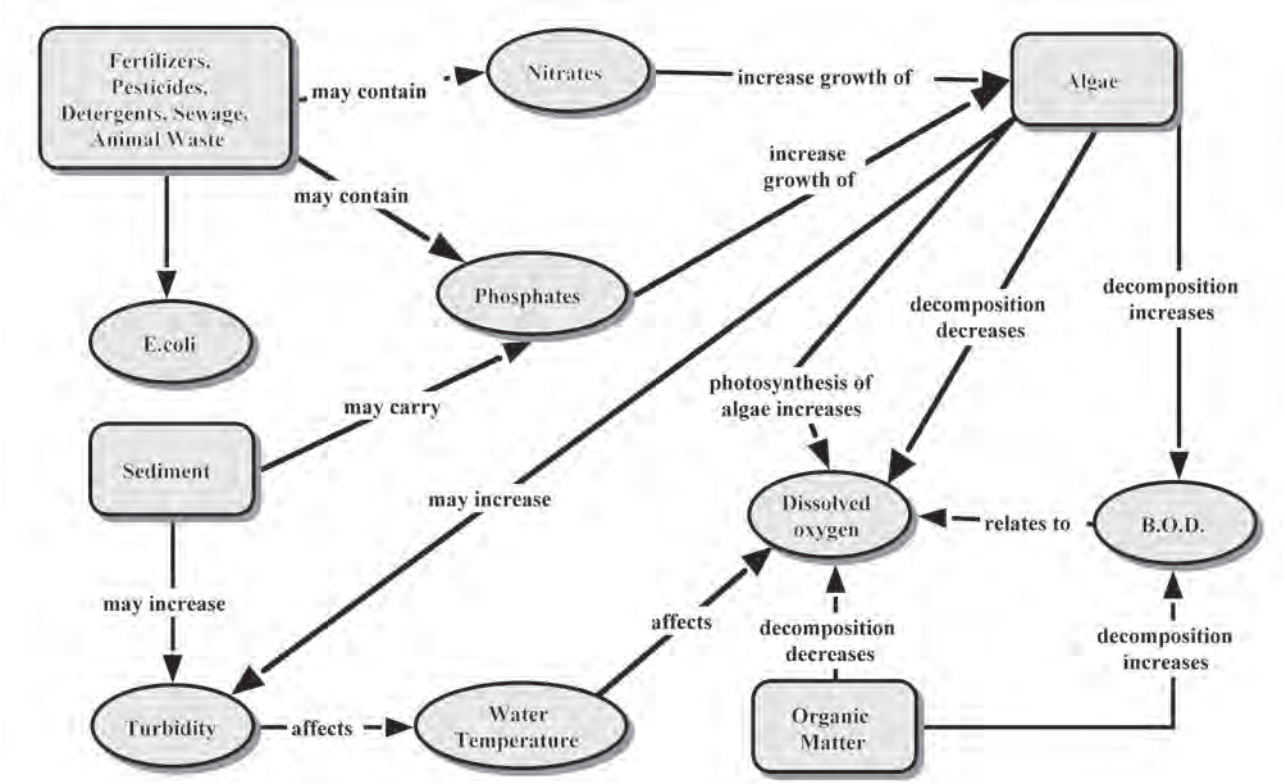
How to Discard Chemical Waste

Label a plastic container with a secure lid (such as a margarine or milk container) with "Chemical Waste". Place liquids and solids in the plastic container along with kitty litter. The chemical waste is in a solid form and can be discarded with your regular trash.

Water Monitoring Parameters are Interrelated

Aquatic chemistry is complex and is influenced by many interrelated factors. The simplified concept map below (Figure 16) may help in understanding these relationships in an aquatic environment. The rectangles represent watershed inputs into a river or stream, while the circles represent chemical parameters we measure to determine water quality.

Figure 16



Units of Measurement and Indices

(Information modified from Rivers Curriculum Guide: Biology)

Parameter	Unit of measurement
Water Temperature Change (1 mile)	°C
Dissolved Oxygen	mg/L and % Saturation
Biochemical Oxygen Demand (BOD)	mg/L
pH	Standard Units
Orthophosphate	mg/L
Nitrate/Nitrite	mg/L
Transparency/Turbidity	cm or NTU
E. coli and general coliforms	cfu/100 mL

Units of Concentration (ppm vs mg/L)

What does part per million (ppm) mean? How much are we talking about? The following examples are listed on "Water on the Web" **https://www.wateronthewеb.org/resources/conversiontables.html** to provide further understanding of these units of concentration. One part-per-million is equal to:

- one inch in 16 miles
- one minute in two years
- one ounce in 32 tons
- one cent in $10,000
- one car in bumper-to-bumper traffic from Cleveland to San Francisco

So, how can it be that one part per million (ppm) of something in water (e.g. dissolved oxygen) is the same as one milligram per liter (mg/L)? It is because a liter of water weighs 1000 grams and a milligram is 1 one-thousandth of a gram. This is true for freshwater since the density of freshwater is 1 g/mL (1 g/mL = 10^{-3} g/103 mL = 10^{-6}, or 1 ppm), but it does not hold for saltwater because density increases with salinity. The units mg/L and ppm are equal in freshwater. They are used interchangeably throughout this chapter.

Index

An index is a rating system that assigns a value to an object or process, or to specific qualities it may possess. Grades are indices of academic achievement; other index examples include movie guides, TV ratings, wind chill factors, and pollen counts. An index easily allows you to observe and quantify fluctuations in river or stream water quality. Using an index ratio over a period of time can indicate whether the water is becoming more polluted or cleaner. The indices used in studying a river or stream offer a mathematical picture that reduces many values having different units to one or two overall numbers.

Chemical Monitoring Water Quality Index: To compare apples and oranges, you must find a unit that is common for both (e.g., apples and oranges are both fruits). The same is true for comparison of water quality parameters. Water quality experts have developed a unit common to all eight water quality tests performed by Hoosier Riverwatchers – it is called a Q-value. Determining overall water quality or comparing the results of different types of tests requires converting results from each of the eight tests to the common Q-value. Each test for water quality has its own Q-value chart and table that facilitates this conversion. Each Q-value chart appears after the instructions for each test and is also listed in Appendix C.

Chemical Testing Instructions

Background information and instructions were copied or modified with permission from CHEMetrics, Inc., Water Works, Inc., Earth Force-GREEN, and the Student Watershed Research Project/Saturday Academy of Oregon.

Typical Ranges

After each set of test instructions, you will find values representing the likely ranges into which your chemical test results may fall. These ranges were taken from the 2012 Monitoring Water in Indiana: Choices for Nonpoint Source and Other Watershed Projects, also known as the Environmental Indicators Manual. This manual can be found at https://engineering.purdue.edu/watersheds/monitoring/MonitoringWaterinIndiana.2012.1.pdf. Data from existing monitoring sites in Indiana have been compiled to provide target ranges (also in Appendix F, page 143). These are here to give you a better idea what might be found in Indiana rivers and streams. The data used to compile ranges comes from IDEM's Fixed Station Data (rather than volunteer data) compiled by IDEM staff or Purdue University. In addition, the Indiana water quality standards for rivers are included for each applicable parameter.

Times and Locations for Completing Tests

The table below provides estimated times needed to perform each test and whether or not they should be completed on-site. If samples are taken off-site, they must be kept on ice or refrigerated until testing is completed (except BOD and turbidity). All tests should be completed the same day, except BOD and *E. coli*, and as soon as feasible to obtain the best possible results.

Test	Time to Complete	Location
Water Temperature Change (1 mile)	5 minutes	On-site
Dissolved Oxygen	5 minutes	On-site
Biochemical Oxygen Demand-5 day (BOD_5)	5 days to incubate, then 5 minutes to test	On-site/Off-site
pH	2 minutes	On-site
Orthophosphate	5 minutes	On-site
Nitrate/Nitrite	2 minutes	On-site
Transparency/Turbidity	5 minutes	On-site
***E. coli* and general coliforms**	24 hours to incubate, 15+ minutes to count	On-site/Off-site

Other Results

Other water chemistry test results sometimes obtained by volunteers include ammonia, total solids, chlorine, chloride, conductivity, alkalinity, hardness, heavy metals, or pesticides. Any water quality results you obtain (either by your own testing or from a laboratory) may be recorded on the data sheet and in the database. Some chemical tests require extremely sensitive and expensive equipment, and are not usually performed by volunteer monitors. A few examples of these tests include: mercury, PCBs, some pesticides, DNA source-tracking of bacteria, and pharmaceuticals. Contact your Watershed Specialist (page 155) to find out what type of data has been collected in your area.

Chemical Monitoring Data Sheet

Why use the chemical monitoring data sheet?

The chemical monitoring data sheet can be taken into the field to record the results of multiple samples. Use of the data sheet is optional. Hoosier Riverwatch recommends that volunteers take multiple samples to assure higher quality stream monitoring results. Up to three replicates can be recorded on this data sheet. Obvious outliers (results that are drastically different from other values) should not be recorded or used in calculations. The average of the test results is calculated then used in the average column on the Chemical Monitoring Data Sheet.

How the water quality index (WQI) works

The Chemical Monitoring Data Sheet utilizes a Water Quality Index (WQI). The Water Quality Index provides a simple analysis of the results of eight of the chemical test parameters. **If you complete at least six of the eight test parameters** you can derive a single score that will let you know if the stream results are: Excellent, Good, Medium, Bad, or Very Bad for that particular monitoring session. You can also use this value to track changes in your site over time, or compare the quality with other stream sites.

Each of the test parameters is weighted according to its level of importance to the overall water quality (in this particular index). Dissolved oxygen has the highest weighting factor (0.18); therefore, the oxygen results are the most important value in determining the water quality rating using the index. The weighting scheme allows analysts to condense complex test results into a common water quality measurement that can be readily communicated to the public and to other volunteers. The Water Quality Index score is like a final grade - weighting the results of multiple tests and exams.

How to use the Q-value charts

In order to obtain a WQI Rating, you must first determine the Q-value for each test. Each parameter (except Orthophosphate and Nitrite) has its own Q-chart immediately following the instructions. To find the Q-value locate your test result on the bottom of the appropriate chart (x-axis). Draw a vertical line up from your test result until it intersects the curved line (Q-line). From this point of intersection draw a line across to the left hand side (y-axis). Read the number on the left side of the chart closest to this intersection; this is the Q-value for that particular test result. Record the Q-value in the second column of the Chemical Monitoring Data Sheet.

You can also check the Q-value table (as an alternative to reading the graph) if your result is close to a given value. In addition, the Riverwatch database will calculate your Q-value when you submit chemical data online.

What does a Q-value mean?

You can think of a Q-value as a "Quality-value." It helps interpret your results in terms of the overall health or water quality of your stream. Think of it like a grade. The higher the Q-value, the better the test results (100 is the maximum value; 0 is the minimum).

Water Quality Index Instructions

As you complete each chemical test (or average your results for up to three test events for a parameter from the Chemical Monitoring Data Sheet), record the values on the chemical monitoring data sheet. Use the Q-charts or Q-tables in this chapter to derive the Q-values for the average of each parameter. Record those in the Q-value column. After the Q-values have been determined and recorded in the appropriate column, multiply the Q-value for each test by the Weighting Factor provided and record the value in the Calculation column. Once the calculations are completed for each parameter, you can then sum the Weighting Factor column and the Calculation column. Divide the total of the Calculation column by the total of the Weighting Factor column to obtain the Water Quality Index (WQI). See example on page 40.

If you complete all eight parameters, the total of the Weighting Factor column is 1.00 (or 100%). If you are missing one or two test parameters (but no more than two) you can calculate an adjusted Water Quality Index (WQI) Rating. Follow the same procedures. Divide the total of the Calculation column by the total of the Weighting Factor column for the tests you completed to obtain the adjusted WQI. In the example on page 41, if the Total Phosphate and *E. coli* tests were not completed, the total of the Weighting Factor column would be 0.72, and the total of the Calculation column would be 55.9. This results in a WQI score of 77.6, compared to 72.93 on page 40.

Hoosier Riverwatch
Chemical Monitoring Data Sheet

Date _____ / _____ / _____ Volunteer ID _____ Site ID _____

Stream Name _____ Latitude _____ Longitude _____

Time _____ : _____ AM / PM Time Sampling _____ hrs Air Temp.: _____ °C

Current Weather: ☐ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

Worst Weather (past 48 hours): ☐ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

	Units	Sample # 1	2	3	Avg.	**Q-Value** x Weighting = **Calculation** Factor *(Q-value x Wt. Factor)*
Temperature						
Water Temp at Site	°C					
Water Temp 1 Mile Upstream						
Water Temp Change: *Site Temp - Upstream Temp*						0.11
Dissolved Oxygen						
Dissolved Oxygen	mg/L					
DO% Saturation: *Determine from chart or table/equation*	%					0.18
BOD						
Avg. Dissolved Oxygen: *(Calculated Above)*	mg/L					
Dissolved Oxygen after 5 days						
BOD *Avg DO (original)-DO after 5 days*						0.12
pH						
pH						0.12
Nutrients						
Orthophosphate	mg/L					
Total Phosphate *(boil in acid)*	mg/L					0.11
Nitrate (NO3) *multiply by 4.4*	mg/L					0.10
Nitrite (NO2) *multiply by 3.3*	mg/L					
Turbidity						
Transparency *(from tube)*	cm					
Turbidity *(convert from chart/table)*	NTU					0.09
Bacteria						
E.Coli Bacteria	cfu/100 mL					0.17
Fecal Coliforms						

Use Average DO value for BOD calculation.

Remember to convert your reading from the tube to NTUs.

Add the calculation column.

Add Weighting Factors for test completed.

TOTALS

Divide Total of Calculation Column by Total Weighting Factor Column

WQI

WQI Ratings

Excellent	90 - 100%
Good	70 - 87%
Medium	50 - 69%
Bad	25-49%
Very Bad	0-24%

Hoosier Riverwatch
Chemical Monitoring Data Sheet

Date 10 / 04 / 2009 Volunteer ID 1000 Site ID 1000

Stream Name Example Stream Indiana Latitude 39.52533 Longitude -85.76369

Time 12 : 15 AM / PM Time Sampling 2.5 hrs Air Temp.: 29.5 °C

Current Weather: ☐ Clear/Sunny ☒ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

Worst Weather (past 48 hours): ☒ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

	Units	Sample # 1	2	3	Avg.	Q-Value x Weighting = Calculation Factor (Q-value x Wt. Factor)		
Temperature								
Water Temp at Site	°C	22.0	22.0	22.0	22.0			
Water Temp 1 Mile Upstream	°C	22.0	21.0	21.0	21.3			
Water Temp Change: *Site Temp - Upstream Temp*	°C	0.0	1.0	1.0	0.7	90	0.11	9.9
Dissolved Oxygen								
Dissolved Oxygen	mg/L	8.0	7.0		7.5			
DO% Saturation: *Determine from chart or table/equation*	%				86.2	92	0.18	16.6
BOD								
Avg. Dissolved Oxygen: *(Calculated Above)*	mg/L	7.5	7.5	7.5	7.5			
Dissolved Oxygen after 5 days	mg/L	6.0	5.0	5.5	5.5			
BOD *Avg DO (original)-DO after 5 days*	mg/L				2.0	80	0.12	9.6
pH								
pH		8.0			8.0	82	0.12	9.8
Nutrients								
Orthophosphate	mg/L	0			0			
Total Phosphate *(boil in acid)*	mg/L	0.06			0.06	98	0.11	10.8
Nitrate (NO3) *multiply by 4.4*	mg/L	10			10	51	0.10	5.1
Nitrite (NO2) *multiply by 3.3*	mg/L	0			0			
Turbidity								
Transparency *(from tube)*	cm	25	26	27.5				
Turbidity *(convert from chart/table)*	NTU	30	29	25	28	54	0.09	4.9
Bacteria								
E.Coli Bacteria	cfu/100 mL	215	185		200	37	0.17	6.3
Fecal Coliforms	cfu/100 mL	440	320		382			

Use Average DO value for BOD calculation.

Remember to convert your reading from the tube to NTUs.

Add the calculation column.

Add Weighting Factors for test completed.

TOTALS 1 72.93

Divide Total of Calculation Column by Total Weighting Factor Column

WQI 72.93 - Good

WQI Ratings

Excellent	90 - 100%
Good	70 - 87%
Medium	50 - 69%
Bad	25-49%
Very Bad	0-24%

Hoosier Riverwatch
Chemical Monitoring Data Sheet

Date 10 / 04 / 2009 Volunteer ID 1000 Site ID 1000

Stream Name Example Stream Indiana Latitude 39.52533 Longitude -85.76369

Time 12 : 15 AM / (PM) Time Sampling 2.5 hrs Air Temp.: 29.5 °C

Current Weather: ☐ Clear/Sunny ☒ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

Worst Weather (past 48 hours): ☒ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (heavy)

	Units	Sample # 1	2	3	Avg.	Q-Value x Weighting = Calculation Factor (Q-value x Wt. Factor)		
Temperature								
Water Temp at Site	°C	22.0	22.0	22.0	22.0			
Water Temp 1 Mile Upstream	°C	22.0	21.0	21.0	21.3			
Water Temp Change: *Site Temp - Upstream Temp*	°C	0.0	1.0	1.0	0.7	90	0.11	9.9
Dissolved Oxygen								
Dissolved Oxygen	mg/L	8.0	7.0		7.5			
DO% Saturation: *Determine from chart or table/equation*	%				86.2	92	0.18	16.6
BOD								
Avg. Dissolved Oxygen: *(Calculated Above)*	mg/L	7.5	7.5	7.5	7.5			
Dissolved Oxygen after 5 days	mg/L	6.0	5.0	5.5	5.5			
BOD *Avg DO (original)-DO after 5 days*	mg/L				2.0	80	0.12	9.6
pH								
pH		8.0			8.0	82	0.12	9.8
Nutrients								
Orthophosphate	mg/L	0			0			
Total Phosphate *(boil in acid)*	mg/L						0.11	
Nitrate (NO3) *multiply by 4.4*	mg/L	10			10	51	0.10	5.1
Nitrite (NO2) *multiply by 3.3*	mg/L	0			0			
Turbidity								
Transparency *(from tube)*	cm	25	26	27.5				
Turbidity *(convert from chart/table)*	NTU	30	29	25	28	54	0.09	4.9
Bacteria								
E.Coli Bacteria	cfu/100 mL						0.17	
Fecal Coliforms	cfu/100 mL							

Use Average DO value for BOD calculation.

Remember to convert your reading from the tube to NTUs.

Add the calculation column.

Add Weighting Factors for test completed.

TOTALS 0.72 55.9

Divide Total of Calculation Column by Total Weighting Factor Column

WQI 77.6 - Good

WQI Ratings

Excellent	90 - 100%
Good	70 - 87%
Medium	50 - 69%
Bad	25-49%
Very Bad	0-24%

Water Temperature

Water temperature is very important to overall water and stream quality. Temperature affects:

1. **Dissolved Oxygen Levels** – Colder water can hold more dissolved oxygen than warmer water, thus colder water generally has higher macroinvertebrate diversity. Warmer water has less dissolved oxygen. Lower oxygen levels weaken fish and aquatic insects, making them more susceptible to illness and disease (Figure 17).

2. **Rate of Photosynthesis** – Photosynthesis by algae and aquatic plants increases with increased temperature, this leads to an extremely high amount of oxygen produced when sunlight is present and a sag during the dark hours. Increased plant/algal growth leads to increased death and decomposition, resulting in increased oxygen consumption (BOD_5) by bacteria.

3. **Metabolic Rates of Aquatic Organisms** – Many animals require specific temperatures to survive. Water temperature controls their metabolic rates, and most organisms operate efficiently within a limited temperature range. Aquatic organisms die when temperatures are too high or too low. Water temperature varies naturally with changes of the seasons, the amount of rainfall and flow rates. Thermal pollution (artificial temperature increases such as, through the addition of cooling waters or cutting down shade trees) can threaten the balance of aquatic ecosystems. To determine if your river or stream is thermally polluted you must take a temperature reading at two different locations. Increased water temperature may be caused by many sources, some of which are listed below. If water temperature decreases within a mile of the sampling site, there may be a source of cold water, such as a spring, entering the stream.

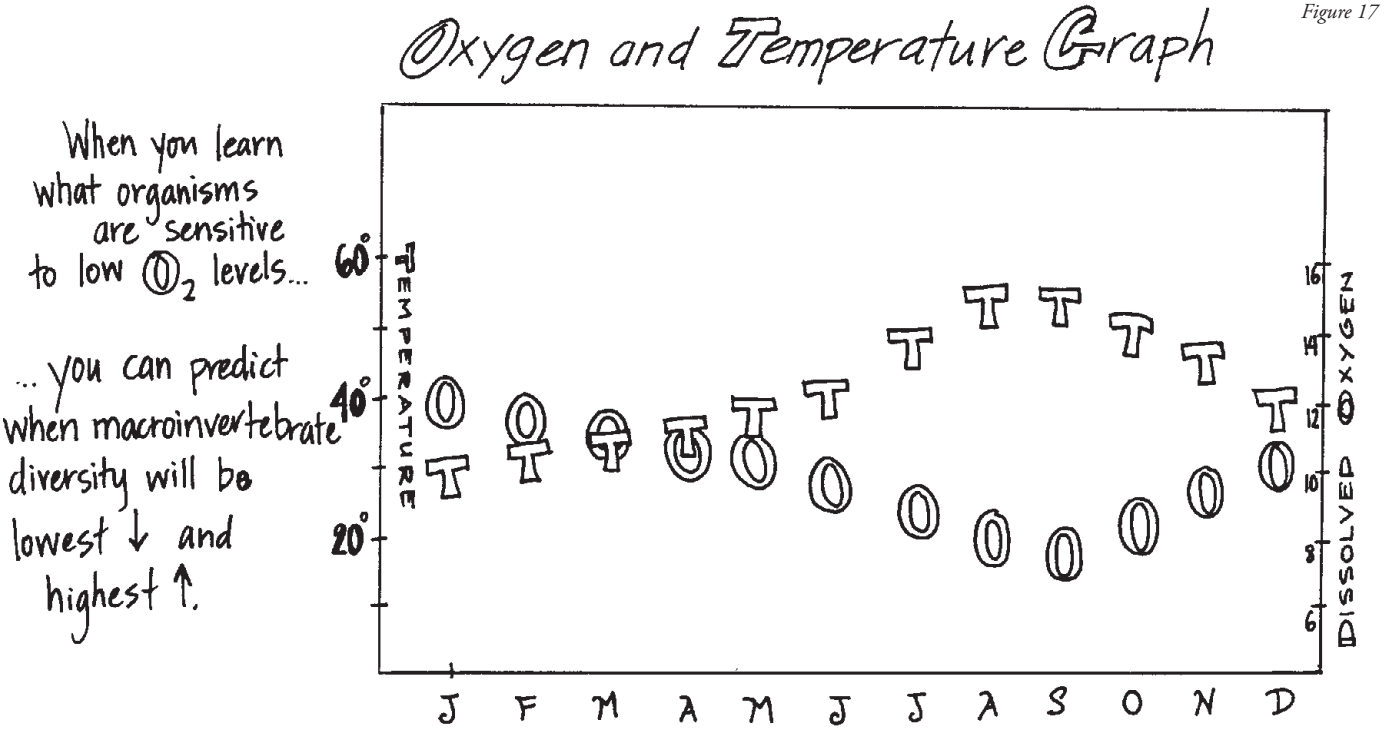
Problem:

Aquatic organisms have narrow optimal temperature ranges. In addition warmer water holds less dissolved oxygen.

Causes:

- Loss of shading by trees in the riparian zone and the watershed.
- Runoff from roads and parking lots.
- Discharges from municipal wastewater and industrial sources.

Figure 17



The *air temperature* needs to be taken while the thermometer is completely dry, **so do that first.** Hang the thermometer somewhere where it's not leaning against a solid object and where it is protected from direct wind and sunlight. *The thermometer will take 5-10 minutes to equilibrate.* **Record the result.**

Temperature Change Instructions

1. Place the thermometer below the water's surface (e.g., the same depth at which other tests are performed). If possible, obtain the temperature reading in the main streamflow.
2. Swirling gently, hold the thermometer in the water for approximately 2 minutes or until the reading stabilizes.
3. Record your reading in Celsius. (Note: If you are using a thermometer that reads only in Fahrenheit, look at Figure 18 or use the following equation to convert to Celsius):

$$C = (F - 32.0)°/1.8$$

4. Choose a portion of the stream with roughly the same degree of shade and velocity as in Step 1, and conduct the same test approximately 1 mile upstream as soon as possible using the same thermometer.
5. Calculate the difference between the downstream and upstream results. Record the temperature change in Celsius and note if the change is positive or negative.

Example:

Downstream Temp (Your Site)
- Upstream Temp (~1 mile away)
= Temperature Change (+/-)

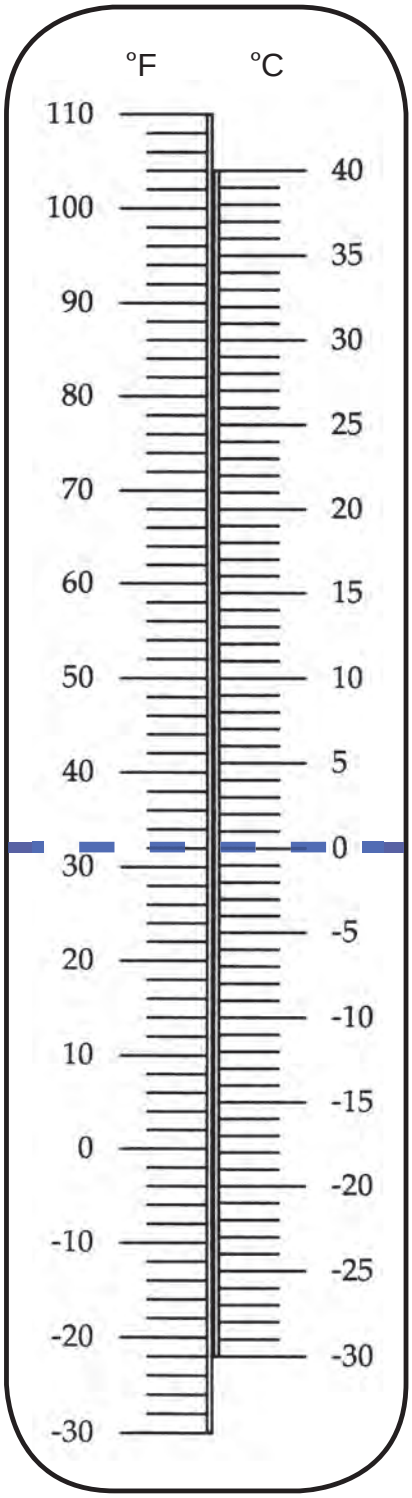
Because water temperature is influenced by time of day, season, and thermal inputs, typical values do not exist.

The maximum temperature rise at any time or place above natural temperatures shall not exceed **State Water Quality Standard:**

<5° F (approximatively 2.8° C)

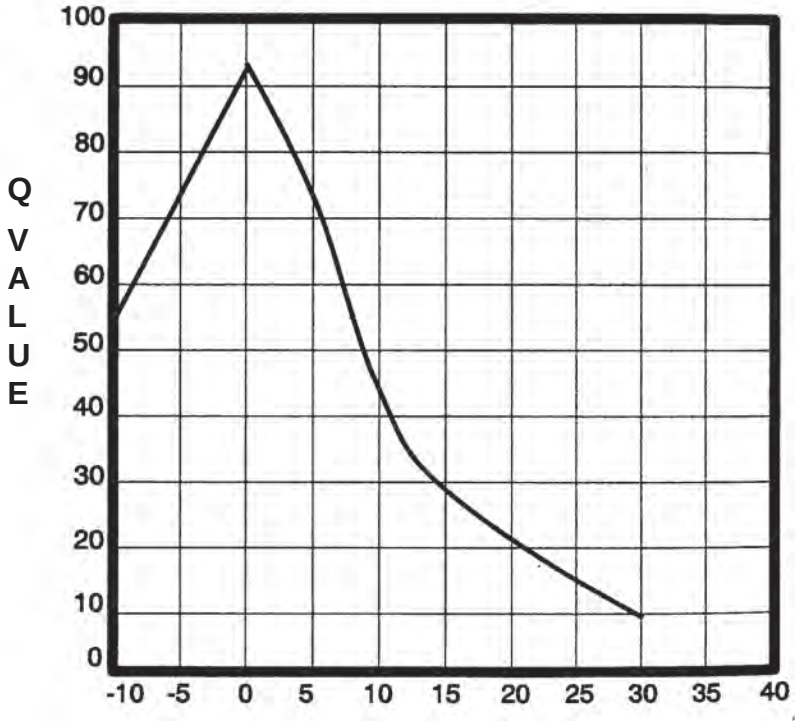
<2° F (approximatively 1.1° C) for trout streams

Figure 18



Temperature conversion image and air temp instructions provided by Friends of Casco Bay, ME.

Temperature Change Q-Values



Change in Temp. (ºC)	Q-Value
-10	56
-7.5	63
-5	73
-2.5	85
-1	90
0	93 (max)
1	89
2.5	85
5	72
7.5	57
10	44
12.5	36
15	28
17.5	23
20	21
22.5	18
25	15
27.5	12
30	10

Dissolved Oxygen

Oxygen is as important to life in water as it is to life on land. Most aquatic plants and animals require oxygen for survival. Although oxygen atoms are present in the water molecule (H_2O), most aquatic life require oxygen in the free elemental state (O_2) as a dissolved gas. The amount of oxygen in water is called the dissolved oxygen (DO) concentration. Oxygen dissolves into the water from the atmosphere until the water is saturated. Aquatic plants, algae, and plankton also produce oxygen as a by-product of photosynthesis; which is why oxygen levels rise during the day and fall at night during respiration. DO is an important measure of stream health. Presence of oxygen in water is a positive sign, while absence of oxygen from water often indicates water pollution. Aquatic organisms require different levels of DO. Dissolved oxygen levels below 3 ppm are stressful to most aquatic life. DO levels below 2 or 1 ppm will not support fish. Levels of 5 to 6 ppm are usually required for healthy growth and activity of aquatic life. Some of the factors affecting DO are:

- Temperature (water can't hold as much dissolved oxygen at higher temperatures)
- Altitude/atmospheric pressure
- Turbulence
- Plant growth/photosynthesis
- Amount of decaying organic material

Problem:
Lack of sufficient dissolved oxygen required by most aquatic organisms to breathe. Lack of oxygen increases the toxicity of other chemicals (e.g., hydrogen sulfide and ammonia).

Causes:

- Rapid decomposition of organic materials, including dead algae, shoreline vegetation, manure or wastewater decreases oxygen.
- High ammonia concentrations in the stream use up oxygen in the process of oxidizing ammonia (NH_4+) to nitrate (NO_3-) through nitrification.
- Less oxygen can dissolve in water at higher temperatures
- Lack of turbulence or mixing to expose water to atmospheric oxygen results in low dissolved oxygen concentrations.

Percent (%) Saturation

Two pieces of information are needed to interpret dissolved oxygen levels: the DO concentration (in ppm or mg/L) and the water temperature. From these two values, the percent saturation can be determined. Percent saturation expresses the current amount (in milligrams) of oxygen gas dissolved in one liter of water at a given temperature compared with the maximum milligrams of oxygen gas that can remain dissolved in one liter of water at the same temperature and pressure. The table on page 48 shows the mg/L of DO that represents 100% saturation at each given temperature. Cold water can hold more dissolved oxygen than warm water.

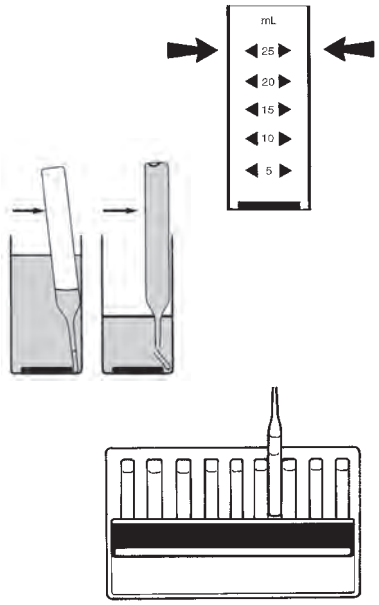
For example, water at 27°C is 100% saturated with 8 ppm dissolved oxygen. However, water at 8 °C can hold up to 11.8 ppm DO before it is 100% saturated. Thus, daily and seasonal temperature changes, as well as thermal pollution, greatly impact oxygen levels and aquatic life in streams and rivers.

Supersaturation

High levels of bacteria or large amounts of rotting organic material can consume oxygen very rapidly and cause the percent saturation to decrease. Conversely, water may become supersaturated for short periods of time, holding more than 100% of the oxygen it would hold under normal conditions. Supersaturation is often caused by high levels of photosynthesis in streams overloaded with aquatic plants and algae. Supersaturation may also occur at the base of dams due to increased pressure. Supersaturation can be harmful to aquatic organisms, causing gas bubble disease, a condition similar to "the bends", which scuba divers may get if they surface too fast. Supersaturation during daytime hours, along with evidence of high photosynthetic activity may indicate a possible oxygen sag during the evening hours, which would limit aquatic life use.

Dissolved Oxygen Instructions

These instructions are for use with the CHEMetrics Dissolved Oxygen Test Kit K-7512.



1. Triple rinse the sample cup with water to be tested. Fill the sample cup to the 25mL mark.
2. Place the CHEMet ampoule in the sample cup. Snap the tip by squeezing the ampoule against the wall of the cup. The ampoule will fill itself, leaving a small bubble to facilitate mixing.
3. Mix the contents of the ampoule by inverting it five times, allowing the bubble to travel from end to end each time. Do not place your finger over the broken tip. Wipe all liquid from the exterior of the ampoule. **Wait 2 minutes** for color development.
4. Hold the comparator in a nearly horizontal position while standing beneath a source of bright light. You may remove the color comparator from the lid. Place the ampoule between the color standards until the best color match is found. If the ampoule is between two color standards, you can estimate half-way between these two values.
5. Use the equation on page 48 or the graph in Figure 19 to calculate percent saturation. On page 19, run a straight edge from the appropriate water temperature to DO (mg/L) to determine % saturation along the angled (middle) scale. If you took the temperature in Fahrenheit, use this conversion equation, C = (F – 32.0)/1.8, or use the diagram on page 48 to obtain Celsius degrees.
6. Record the dissolved oxygen concentration to the nearest mg/L as well as the percent saturation. Rinse the glass tip out of sample cup into a waste container along with the spent ampoule.

Examples:

DO = 8 mg/L Temp = 16 ºC
Look on chart (page 47) = 81% Saturation

Or use table and equation on page 48:

$$\frac{8.0 \text{ mg/L} \times 100\%}{9.9 \text{ mg/L}} = 81\% \text{ Saturation}$$

Important Note:

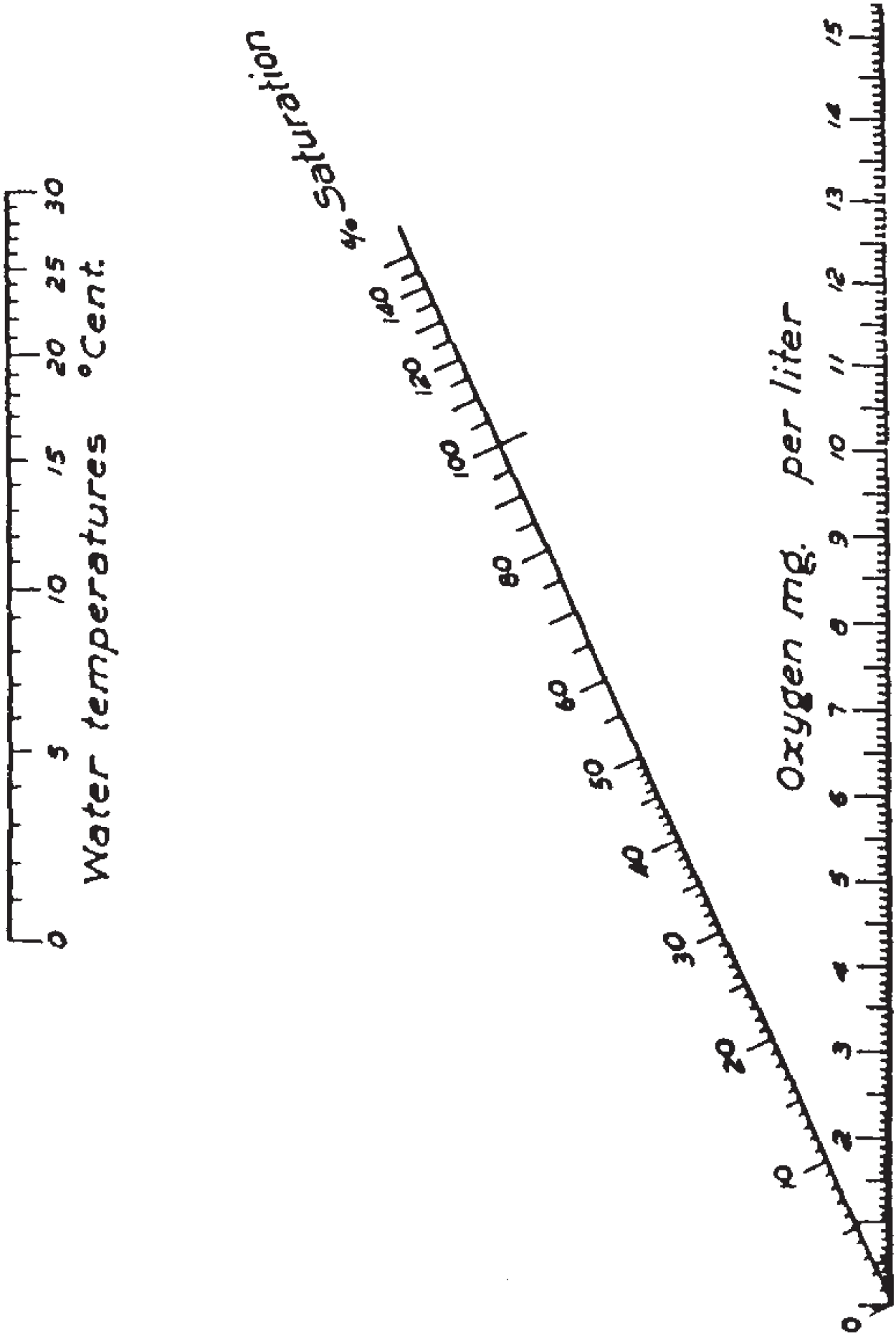
The CHEMet ampoules and color standards contain a reagent which deteriorates upon prolonged exposure to light. They will remain stable only if stored in the dark. The reagent should be a light straw color with no hint of blue or green when the ampoule is removed from the box. The normal shelf life of the color standards is two years.

Typical range for DO =
1.2 to 22.3 mg/L
Indiana Average = 9.6 mg/L

State Water Quality Standard: 4.0 mg/L - 12.0 mg/L
Min: 6.0 mg/L in coldwater fishery streams
Min: 7.0 mg/L in spawning area of coldwater fishery streams

Dissolved Oxygen % Saturation Chart

Figure 19



Approximate amount of Dissolved Oxygen (mg/L) needed for your water sample to be 100% Saturated at the given temperature.*

Temp °C	Dissolved Oxygen (mg/L)	Temp °C	Dissolved Oxygen (mg/L)
0	14.6	15	10.1
1	14.2	16	9.9
2	13.8	17	9.7
3	13.5	18	9.6
4	13.1	19	9.3
5	12.8	20	9.1
6	12.5	21	8.9
7	12.1	22	8.7
8	11.8	23	8.6
9	11.6	24	8.4
10	11.3	25	8.3
11	11.0	26	8.1
12	10.8	27	8.0
13	10.5	28	7.8
14	10.3	29	7.7

Calculating Percent Saturation:

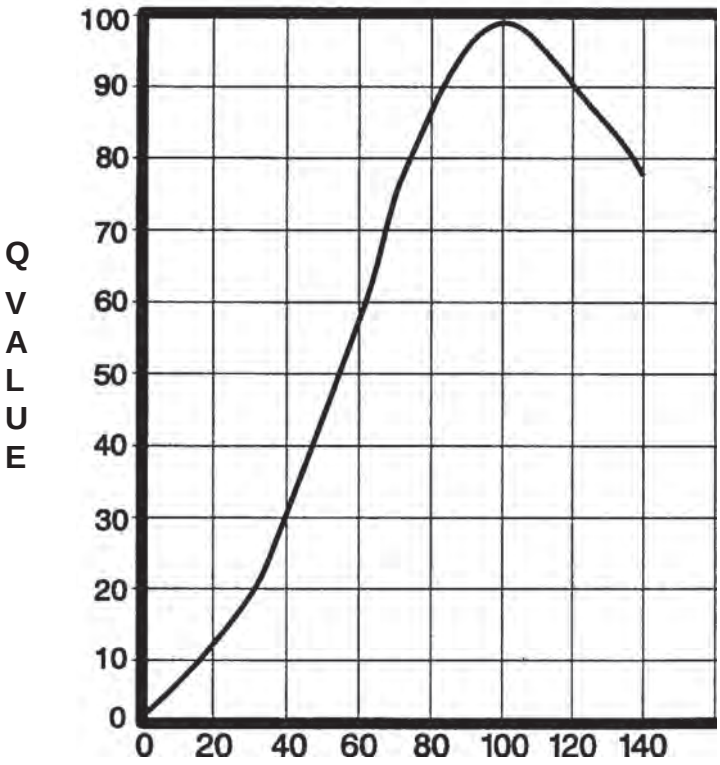
$$\frac{\textbf{DO mg/L (your sample)}}{\textbf{Max DO mg/L (from chart at left determined by water temperature)}} \times 100\%$$

Example at 16 °C:

$$\frac{8.0 \text{ mg/L}}{9.9 \text{ mg/L}} \times 100\% = 81\%$$

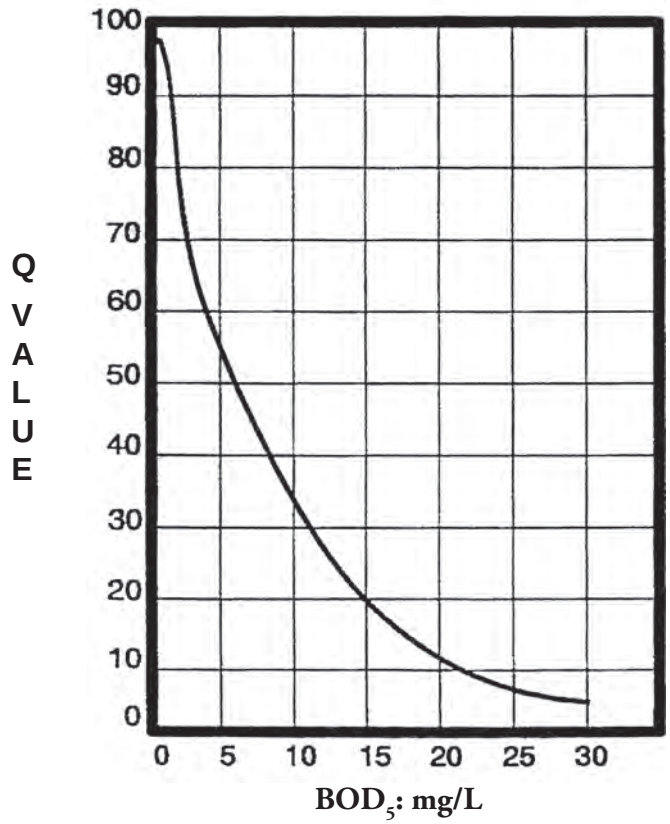
*for fresh water at sea level

Dissolved Oxygen Q-Values



DO (% Saturation)	Q-Value
0	0
10	8
20	13
30	20
40	30
50	43
60	56
70	77
80	88
85	92
90	95
95	97.5
100	99
105	98
110	95
120	90
130	85
140	78
>140	50

BOD_5 Q-Values



BOD_5 (mg/L DO)	Q-Value
0	96
1	92
2	80
2.5	73
3	66
4	58
5	55
7.5	44
8	40
10	33
12.5	26
15	20
17.5	16
20	14
22.5	10
25	8
27.5	6
30	5
>30	2

Biochemical Oxygen Demand (5-day)

Biochemical oxygen demand 5-day (BOD_5) is a measure of the amount of oxygen used by aerobic (oxygen-consuming) bacteria as they break down organic wastes over five days. Polluted streams, or streams with a lot of plant growth (and decay), generally have high BOD_5 levels. High levels indicate that large amounts of organic matter are present in the stream. Streams that are relatively clean and free from excessive plant growth typically have low BOD_5 levels. In slow moving and polluted waters, much of the available dissolved oxygen (DO) is consumed by bacteria, which rob other aquatic organisms of the oxygen needed to live. Streams with higher DO levels, such as fast-moving, turbulent, cold-water streams, can process a greater quantity of organic material. Therefore, interpretation of BOD_5 levels depends upon the conditions of the stream sampled, as some streams can "handle" more waste than others. However, in general, a healthy stream has high DO levels and low BOD_5 levels. Be careful not to confuse the two.

The following is a rough guide to what various BOD_5 levels indicate:

1-2 mg/L BOD_5	Clean water with little organic waste
3-5 mg/L BOD_5	Fairly clean with some organic waste
6-9 mg/L BOD_5	Lots of organic material and bacteria
10+ mg/L BOD_5	Very poor water quality. Very large amounts of organic material in water.

Instructions

In addition to a darkened (light-free) bottle, use the CHEMetrics Dissolved Oxygen Test Kit K-7512.

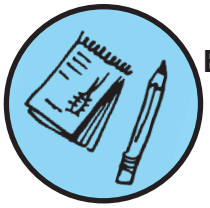
1. Rinse, then lower a stoppered dark (light-free) bottle below the water's surface. Allow water to flow into the bottle for approximately 2 minutes. Ensuring that no air bubbles exist, replace the stopper or lid while the bottle is underwater. Remove bottle from the water.
2. Place the BOD sample in a light-free location (e.g., desk drawer or cabinet) at room temperature and allow it to sit undisturbed at approximately 20 ºC (68 ºF) for 5 days.
3. After 5 days, remove the BOD bottle and perform Steps 1 through 4 of the DO test (page 46) using the BOD sample water.
4. Determine the BOD_5 level by **subtracting** the mg/L of the BOD sample from that of the original DO sample taken 5 days earlier. This difference is what gets recorded on the data sheet.

Problem:
High levels of organic matter - including leaves, dead fish, garbage, some industrial waste, fertilizer, pet waste, and sewage from poor functioning septic systems or combined sewer overflows - and some ions (ammonia in particular) can lead to rapid exhaustion of dissolved oxygen.

Causes:

- Municipal wastewater and septic tank effluent that has not been completely treated will use up oxygen.
- Eutrophication and hot weather can cause algae blooms. When bacteria decompose dead algae, oxygen is consumed which increases BOD.

Typical range for BOD_5 =
0.4 to 33 mg/L
Indiana Average = 2 mg/L



Example:

11 mg/L (DO Day 1)
- 6 mg/L (DO 5 days later)
= 5 mg/L (BOD_5)

pH

The pH test is one of the most common analyses in water testing. Water (H_2O) contains both hydrogen ions (H+) and hydroxide ions (OH-). The relative concentrations of these ions determine whether a solution is acidic or basic.

The activity of the hydrogen ions is expressed in pH units (pH = power of Hydrogen). The concentration of H+ ions is used to estimate pH. The pH scale ranges from 0 (most acidic) to 14 (most basic), with 7 being neutral. If the solution has more H+ ions than OH ions, it is acidic and has a pH less than 7. If the solution contains more OH- ions than H+ ions, it is basic with a pH higher than 7. It is important to remember that pH is measured on a logarithmic scale; it is reported as the negative log of the hydrogen ion concentration (-log [H+]). A change of 1 pH unit means a ten-fold change in the ion concentration. For this reason, pH units are not normally averaged; however, to simplify calculations, Riverwatch allows volunteers to average pH.

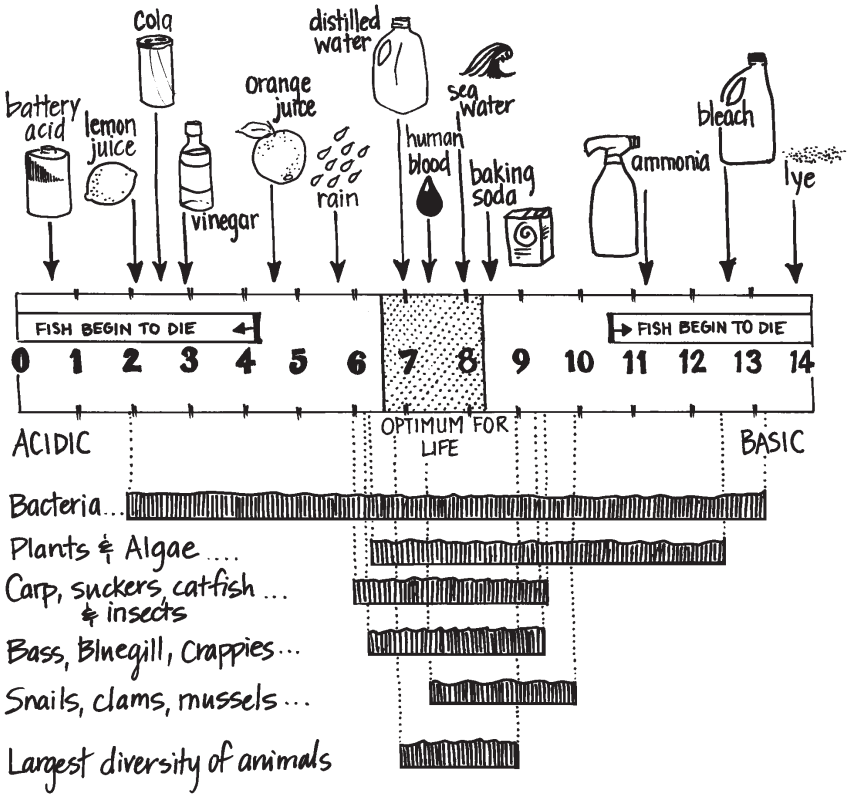
The pH level is an important measure of water quality because aquatic organisms are sensitive to pH, especially during reproduction. Adult organisms may survive, but young will not be produced. A pH range of 6.5 to 8.2 is optimal for most organisms (Figure 20).

Many natural processes affect pH. Waterbodies with higher temperatures have slightly lower pH values. Also, algae blooms remove carbon dioxide (CO_2) from the water during photo-synthesis, which may raise pH to 9 or more.

Runoff from abandoned mine lands can produce acid mine drainage which lowers pH. Lower pH values increase the solubility of some heavy metals, such as copper and aluminum, allowing them to dissolve in water and become toxic to aquatic organisms.

Most natural waters have pH values of 5.0 - 8.5. Freshly fallen rainwater has a pH of 5.5 - 6.0 due to the presence of CO_2 in the atmosphere. But air pollution from automobiles and coal-burning power plants creates acid rain which is even more acidic. Alkaline soils and minerals (limestone) buffer the effects of acid rain and may raise pH to 8.0 - 8.5.

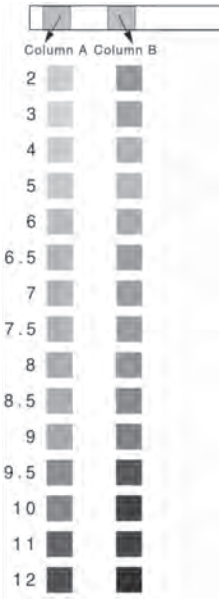
Figure 20



pH Instructions

For use with WaterWorks™ pH Test Strips (#481104).

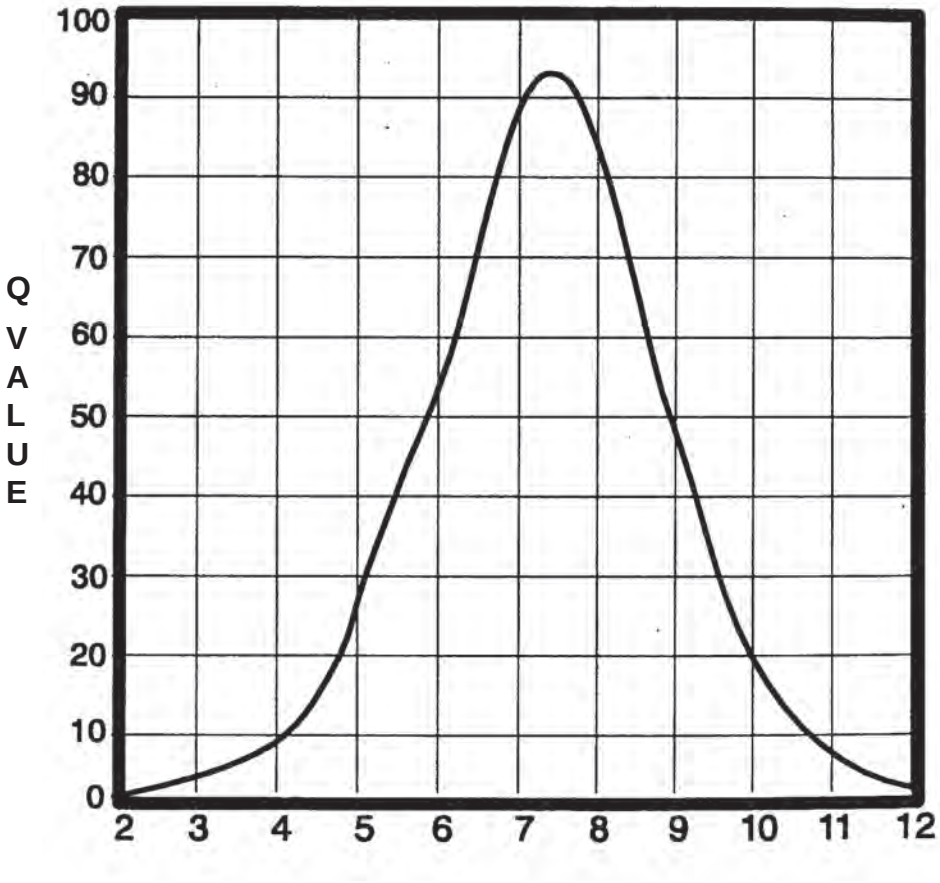
1. Triple rinse sample collection container with water to be tested, then collect a sample.
2. Dip one test strip into sample for 10 seconds with a constant, gentle back-and-forth motion.
3. Remove the strip and shake once, briskly, to remove excess sample.
4. Wait 20 seconds and match with the closest colors on both charts for Columns A and B at the same time.
5. For best performance, complete the reading within 10 seconds.
6. Record the pH level.



Typical range for pH = **7.2 to 8.8**

Indiana Average = 8.0

State Standard = between 6 - 9
Due to the state's limestone geology, Indiana surface waters will typically have a pH that is relatively basic (> 7).



pH Q-Values

pH (SU)	Q-Value
<2	0
2	2
3	4
4	8
5	24
6	55
7	90
7.2	92
7.5	93 (max)
7.7	90
8	82
8.5	67
9	47
10	19
11	7
12	2
>12	0

Orthophosphate

Phosphorus (P) is essential to plant and animal life, and its presence in the environment is natural. Problems with phosphorus as a water pollutant result not from its presence, but from excessive amounts. Aquatic ecosystems develop with very low levels of phosphorus. The addition of seemingly small amounts of phosphorus can lead to problematic algal blooms in freshwater. Research has indicated nitrogen leads to algal bloom in saltwater systems.

Phosphorus enters surface waters in organic matter (dead plants and animals, animal waste) attached or adsorbed to soil particles, or in a number of man made products (detergents, fertilizers, industrial wastes). Phosphorus is an important nutrient in commercial fertilizer because it increases terrestrial plant growth (vegetation). When transported into aquatic systems, phosphorus increases aquatic plant growth (e.g. algae, weeds), as well (Figure 21).

Phosphorus occurs in nature in the form of phosphates (PO_4). Phosphate levels higher than 0.03ppm contribute to increased plant and algae growth. Orthophosphates are one form of phosphates. Orthophosphates are dissolved in the water and are readily available for plant uptake. Thus, the orthophosphate concentration is useful as an indicator of current potential for algae blooms and eutrophication.

Figure 21

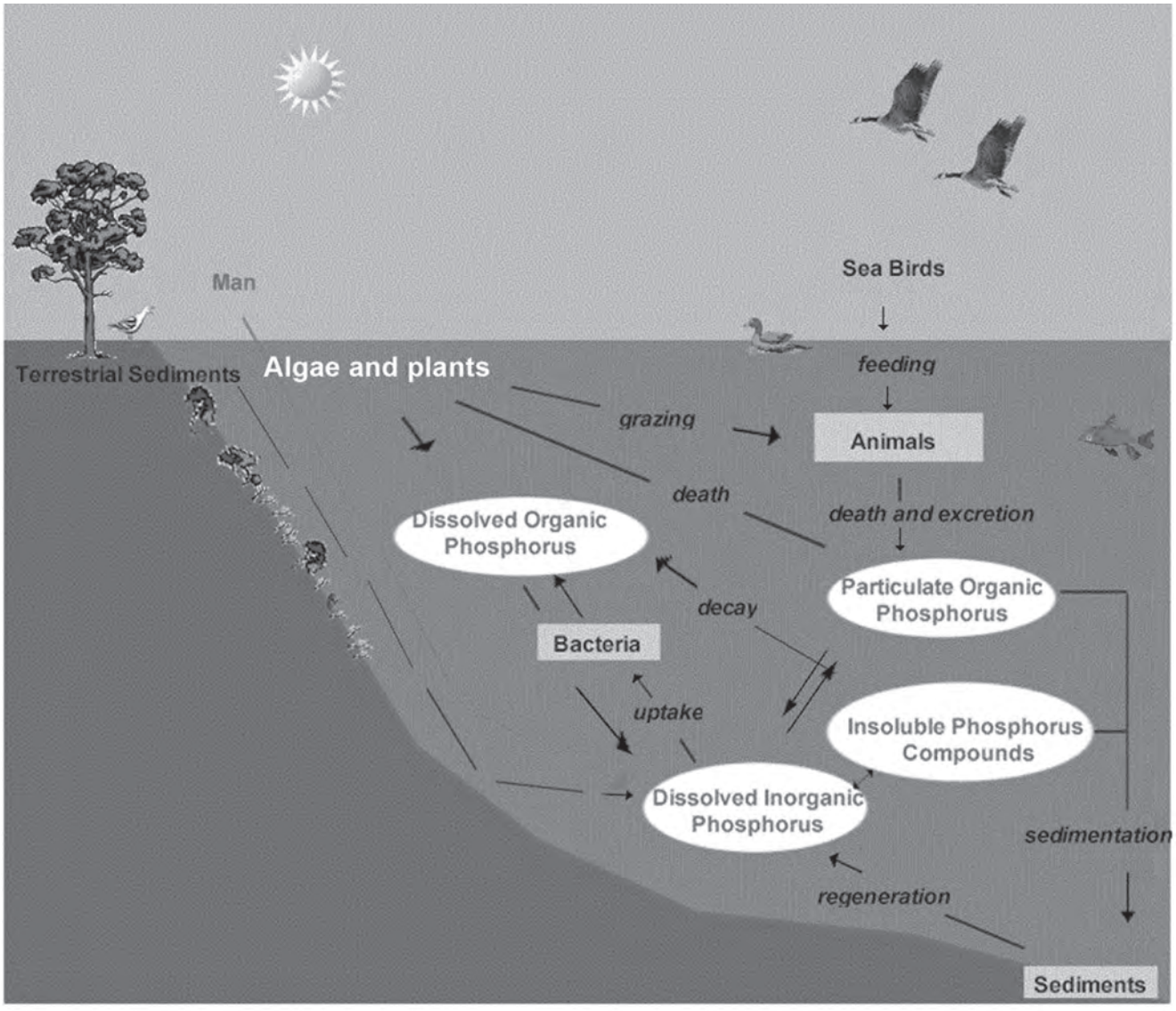
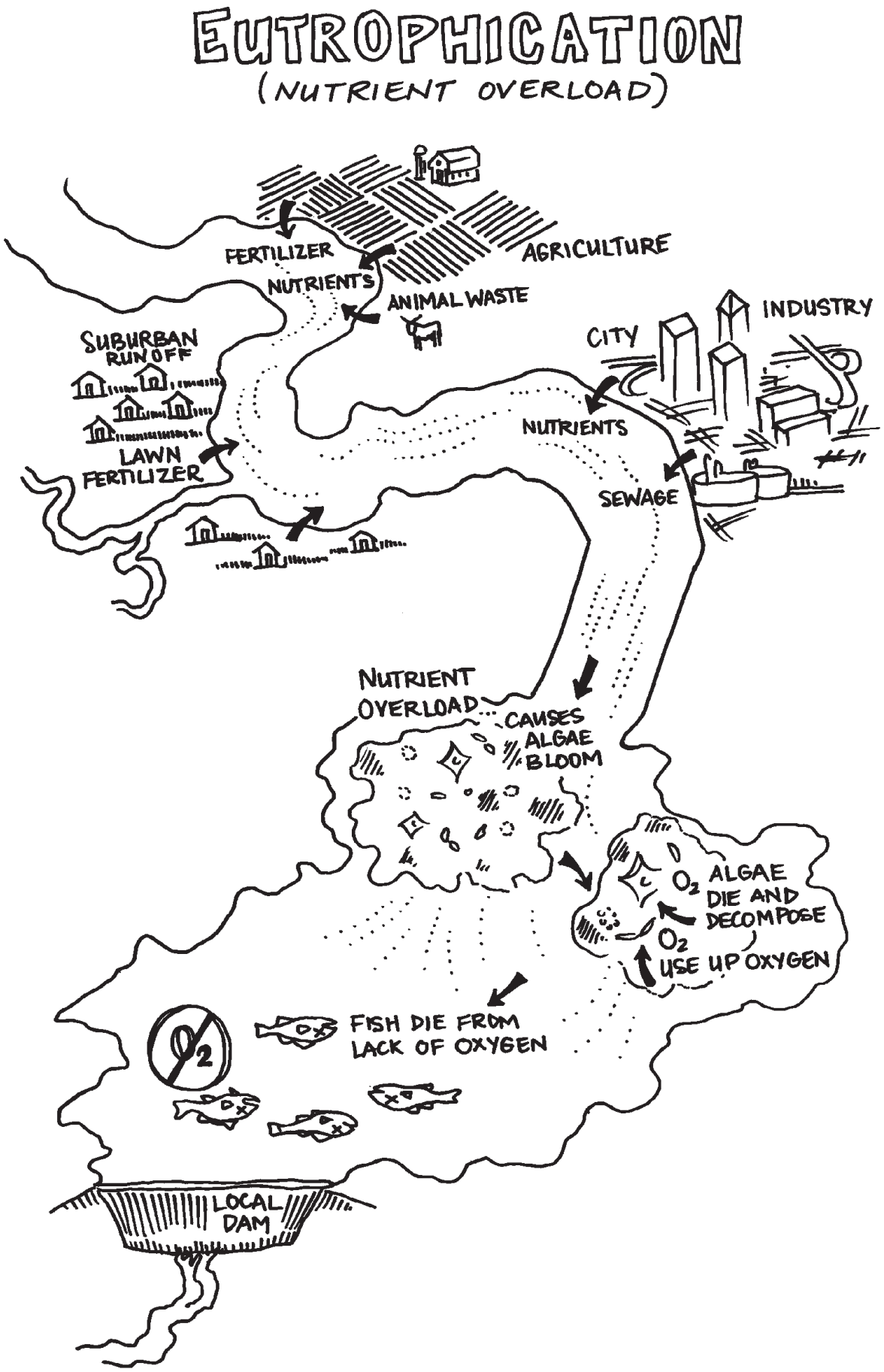


Image from Virginia Estabrook, Michigan Water Research Center

Figure 22


EUTROPHICATION
(NUTRIENT OVERLOAD)
FERTILIZER
NUTRIENTS
AGRICULTURE
ANIMAL WASTE
SUBURBAN RUNOFF
LAWN FERTILIZER
CITY
INDUSTRY
NUTRIENTS
SEWAGE
NUTRIENT OVERLOAD
CAUSES ALGAE BLOOM
O_2
ALGAE DIE AND DECOMPOSE
O_2
USE UP OXYGEN
O_2
FISH DIE FROM LACK OF OXYGEN
LOCAL DAM

When phosphorus levels are too high, excess plant and algal growth creates water quality problems. Plants begin to die and decompose, depleting the dissolved oxygen supply in the water - a condition called **hypoxia**, which can lead to fish kills in some cases. Phosphorus is also released from the sediments and decomposing plants back into the water, continuing the cycle. The reaction of the aquatic system to an overloading of nutrients is known as **eutrophication** (Figure 22). Hypoxia and eutrophication, to some extent, occur within many of our lakes and stream every year, and, on a larger scale, such as in the western basin of Lake Erie.

Unlike nitrogen and other nutrients, phosphorus does not have a gaseous phase. Once it is in an aquatic system, it remains there and cycles through different forms unless physically removed (e.g. by dredging). Over time some of the other forms of phosphates attached to particles in the water column and in the sediments (including organic forms) can be changed into orthophosphates, becoming available for plant growth. For this reason, it is useful to test for total phosphate levels.

The chemistry methods currently utilized by Hoosier Riverwatch do not include a means for obtaining total phosphate results.

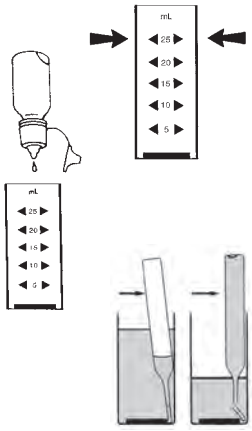
Problem:
Most fresh water has naturally low phosphate levels, and this limits algal growth. If excessive phosphates enter surface water, it can support rapid algal growth. When the algae die, their decomposition by bacteria uses up oxygen and may produce odors and algal toxins.

Causes:

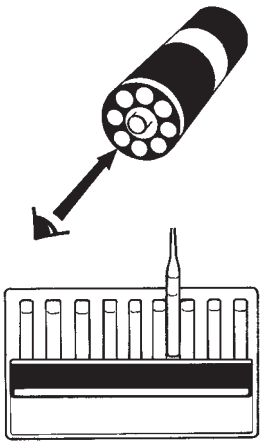
- Phosphorus occurs naturally in soil. Sediments from soil erosion and runoff are often a significant source of phosphorus. These may enter the stream via bank erosion or runoff from forestry, agriculture, and urban lands. Phosphorus can desorb from soil particles and enter solution.
- Phosphorus can come from manure sources, such as treatment lagoons, over-fertilized agricultural fields, or waterfowl.
- Urban sources of phosphorus may include: storm drains, parking lot and road runoff, construction sites, inadequately treated municipal wastewater and septic tank effluent, and lawn fertilizer.

Orthophosphate Instructions

These instructions are for use with the CHEMetrics Phosphate Test Kit K-8510.



1. Triple rinse the sample cup and cap with water to be tested. Fill the sample cup to the 25 mL mark with the sample.
2. Add **2 drops** of A-8500 Activator Solution. Place cap on sample cup and shake it briefly to mix the contents well.
3. Place the CHEMet ampoule into the sample cup. Snap the tip by squeezing the ampoule against the side of the cup. The ampoule will fill leaving a small bubble to facilitate mixing.
4. Mix the contents of the ampoule by inverting it ten times, allowing the bubble to travel from end to end each time. Do not place your finger over the broken tip. Wipe all liquid from the exterior of the ampoule. Wait **2 minutes** for full color development.
5. Use the appropriate comparator to determine the level of orthophosphate in the sample. If the color of the CHEMet ampoule is between two color standards, you can estimate half-way between the concentrations.



   a. Low-range (0-1 ppm) Place the ampoule, flat end downward into the center tube of the low range comparator (broken tip pointing away from you.) Direct the top of the comparator up toward a source of bright light while viewing from the bottom. Rotate the comparator until the color standard below the ampoule shows the closest match.

   b. High Range (0-10 ppm) Hold the high range comparator in a nearly horizontal position while standing directly beneath a bright source of light. You may remove the comparator from the lid. Place the ampoule between the color standards until the best color match is found. If the ampoule is between two color standards, you can estimate half-way between the concentrations.

6. Place ampoule and sample in waste container. Record the results in mg/L on the Chemical Monitoring Data Sheet. There is no Q-value for Orthophosphate, and this result may not be entered on the Water Quality Index Data Sheet.

Note: Results of the Orthophosphate test may be entered on the Chemical Monitoring Data Sheet & submitted to the online database. There are no state water quality standards for Orthophosphate.

However, we do know the Total Phosphate typical range (0-0.85 mg/L) and Indiana average (0.05 mg/L) values.

We generally expect orthophosphate values to be less than total phosphate, since orthophosphate is but one component of total phosphate.

Important Note:

The CHEMet ampoules and color standards contain a reagent which deteriorates upon prolonged exposure to light. They will remain stable only if stored in the dark. The reagent should be completely clear when the ampoule is removed from the box.

Nitrate & Nitrite

Nitrogen makes up about 80% of the air we breathe, and it is found in all living things. Nitrogen occurs in water as nitrate (NO_3), nitrite (NO_2), and ammonia (NH_3). It enters the water from human and animal waste, decomposing organic matter, and runoff of fertilizer from lawns and crops. Nitrates are an essential nutrient for plant growth. Similar to phosphates, these are a main ingredient in fertilizers and can lead to increased aquatic plant growth and eutrophication. Nitrogen is a leading cause of hypoxia in salt waters. Hoosier Riverwatch reports nitrates in units of mg/L (ppm) of the nitrate molecule itself, which is 4.4 times greater than nitrat-as N reported as mg/L of nitrogen.

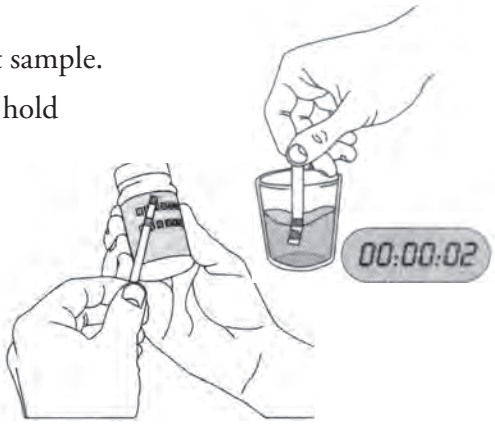
Problem:
Nitrogen works with phosphorus to increase algae growth and cause eutrophication.

Causes:

- Nitrogen can come from manure, such as treatment lagoons and over fertilized fields.
- Nitrogen is the most abundant nutrient in commercial fertilizers. Runoff from agriculture, golf courses, and lawns is high in nitrogen, especially if it rains soon after fertilization.
- Sewage is another source of nitrates in Indiana's surface water.

Instructions

For WaterWorks™ Nitrate/Nitrite Test Strips (#480009)



1. Triple rinse sample collection container with water to be tested. Collect sample.
2. Dip one test strip for 2 seconds without motion. Remove the strip and hold horizontally. Do NOT shake off excess sample water.
3. Wait 1 minute for colors to develop.
4. Match Nitrite as N (pad nearest handle) to the closest color. Then match Nitrate as N (end pad) to the closest color. Record these results separately. Complete color matching within 1 minute.
5. Apply conversions to results before recording:

   Conversion Ratio:
   A) To convert nitrite nitrogen as N to just nitrite (NO_2), multiply the test result by 3.3.

   Example: 1.5 mg/L (test strip) x 3.3 = 4.95 mg/L nitrite (NO_2)

   B) To convert nitrate nitrogen as N to just nitrate (NO_3), multiply the test strip result by 4.4.

   Example: 5 mg/L (test strip) x 4.4 = 22 mg/L nitrate (NO_3)
6. Record the converted values on Chemical Monitoring Data Sheet.

Important Note:
Store test strips in dry, cool place (< 30 ° C) and away from direct sunlight. Use by date printed on package.

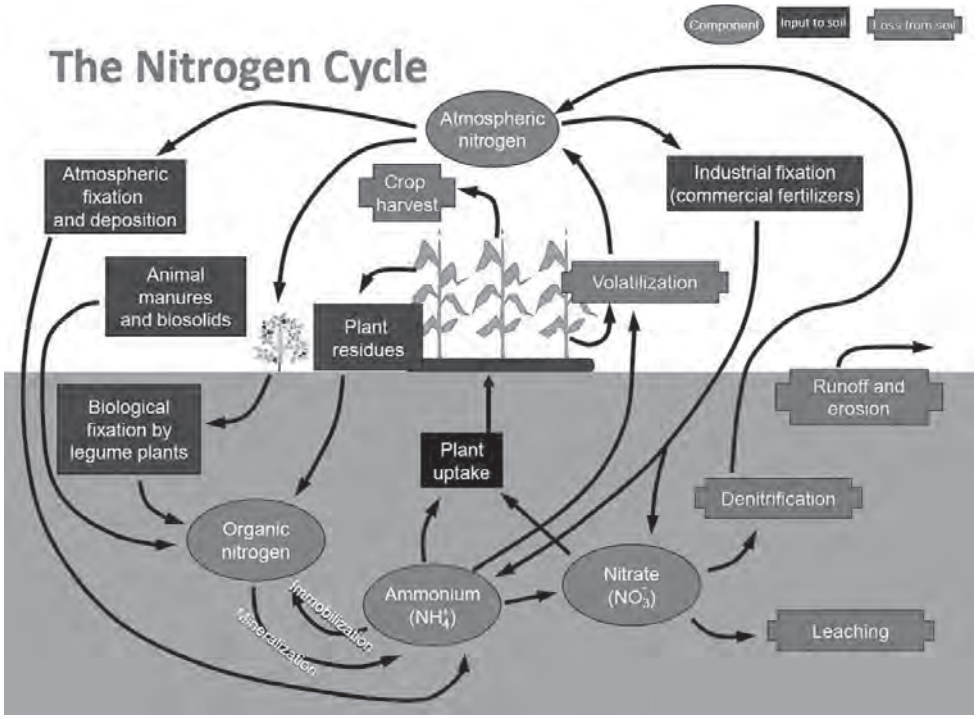
Typical range for nitrate (NO_3) = 0 to 36.08 mg/L

Indiana Average = 12.32 mg/L

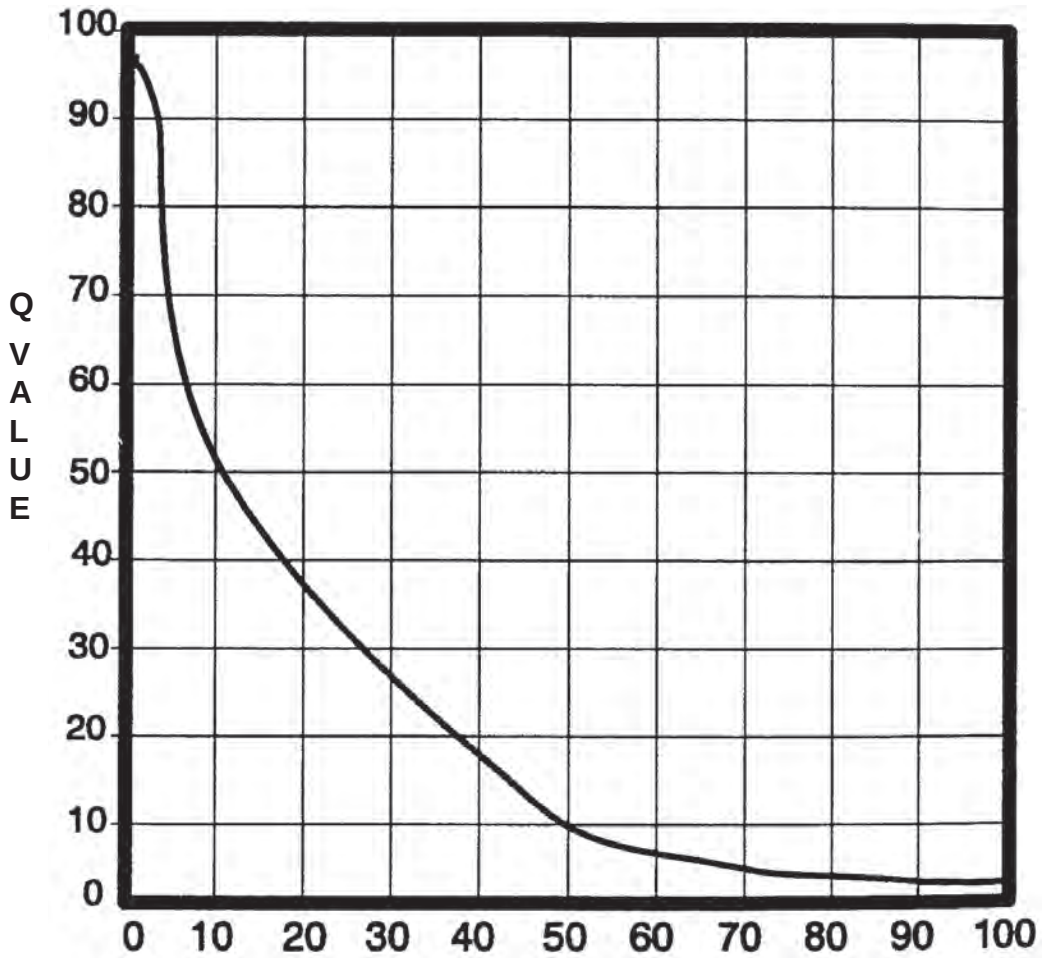
EPA recommends 1.5 mg/L as the dividing line between mesotrophic and eutrophic streams.

Nitrate/NO_3 (the converted value after the result has been multiplied by 4.4) is used in the Q-Value chart and the Water Quality Index Data Sheet.

Figure 23



Nitrate (NO_3): mg/L Q-Values



Nitrate (mg/L NO_3)	Q-Value
0	98
0.25	97
0.5	96
0.75	95
1	94
1.5	92
2	90
3	85
4	70
5	65
10	51
15	43
20	37
30	24
40	17
50	7
60	5
70	4
80	3
90	2
100	1
>100	1

There is no Q-value chart for Nitrite (NO_2)

Turbidity and Transparency

Turbidity is the relative clarity of the water and is measured by shining a light through the water column. Turbid water is more cloudy, and is caused by suspended matter including clay, silt, organic and inorganic matter, and algae. These materials scatter and absorb light, rather than allowing it to shine through the water column in a straight line. Turbidity should not be confused with color, since darkly colored water (like tea) can still be clear and not turbid.

Turbid water may be the result of soil erosion, urban and agricultural runoff, algal blooms, and bottom sediment disturbances caused by boat traffic or abundant bottom feeding fish. If a stream is very turbid, light will not reach through the water column and many reactions, especially photosynthesis, will be limited. When water is turbid, the floating particles absorb heat from the sun, raising water temperature and thus lowering dissolved oxygen levels. The particles can also kill fish and aquatic invertebrates by clogging their gills and smothering their habitat (Figure 24).

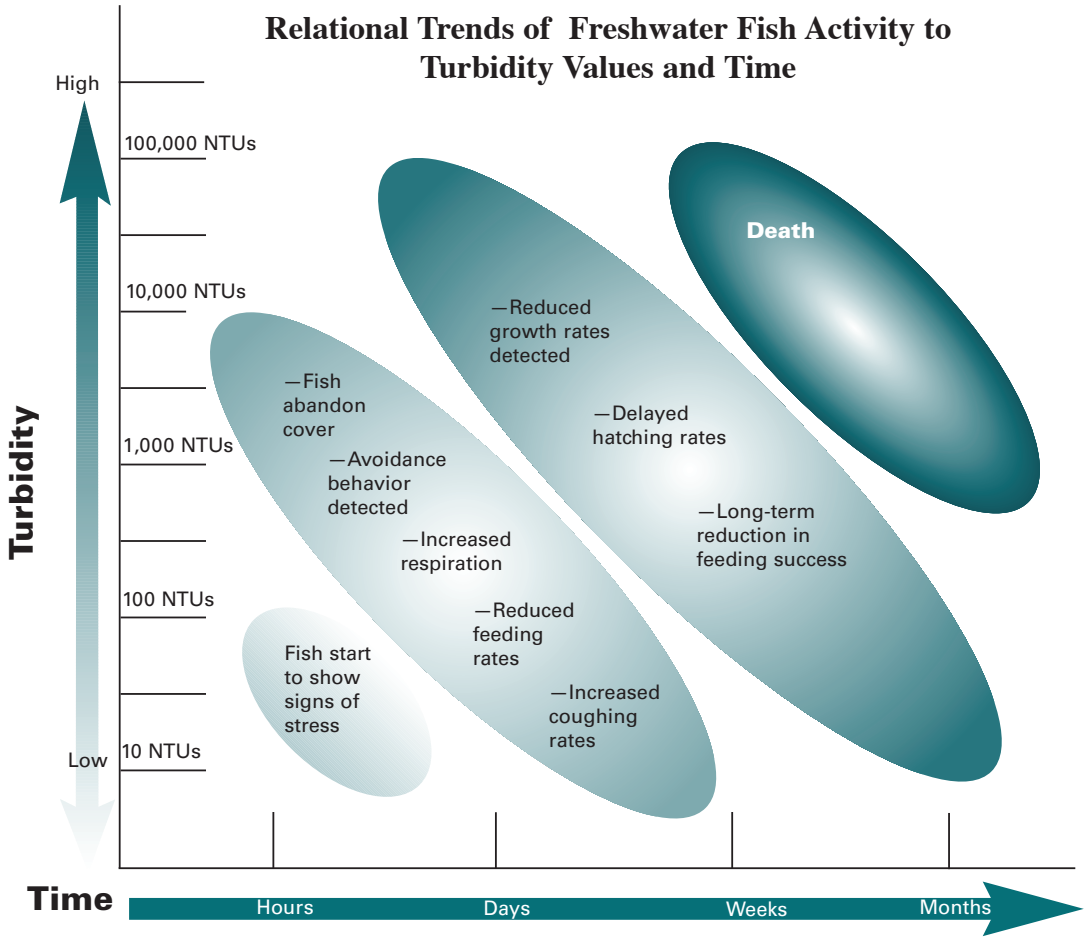
Transparency measures the scattering of light and is observed by the depth at which we can see an object in the water column. We measure the transparency of our water sample, and use a pre-determined relationship to convert our transparency results (cm) to units of turbidity (NTUs).

Problem:
The water looks "dirty." Photosynthesis is limited because organisms in the water column receive no light. Temperature is increased due to light absorption.

Causes:

- Soil erosion and runoff from agricultural fields, lawns, parking lots, construction sites, or the stream bank itself.
- Algae and organic matter also contribute to turbidity.

Figure 24



Transparency Instructions

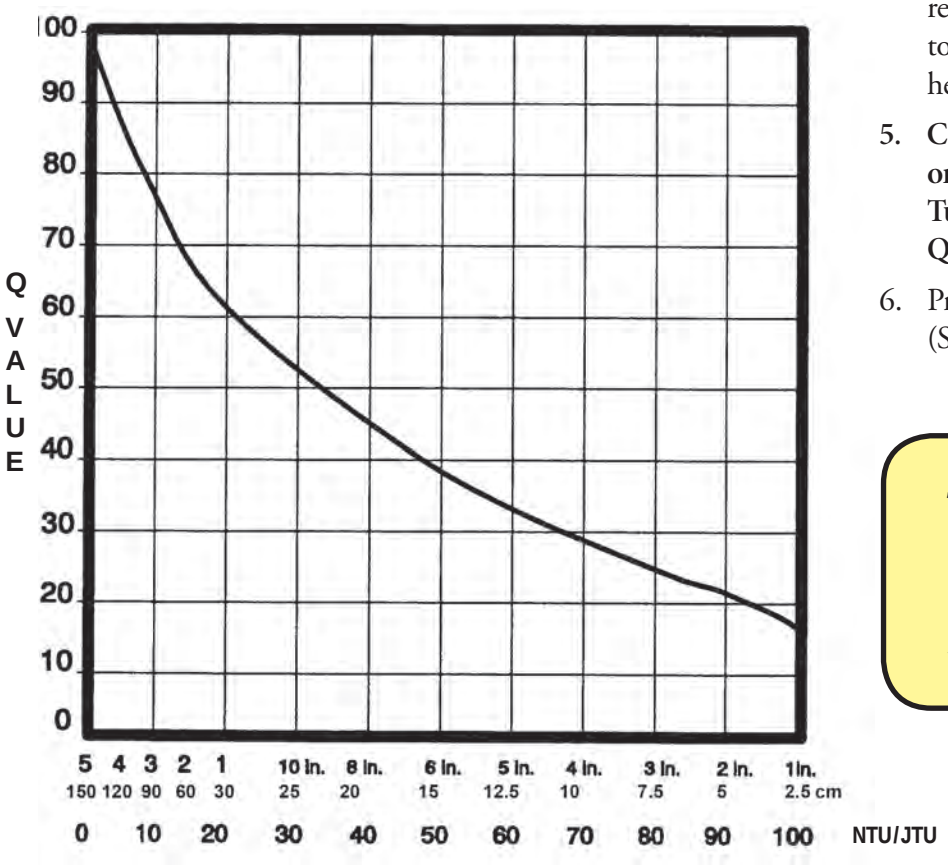
Turbidity can be assessed with a very accurate but expensive electronic turbidimeter. Transparency can be assessed with many types of equipment, including a homemade Secchi disk or transparency tube. As a side note, Secchi disks are usually used for lentic waters, like lakes. A transparency tube is used for lotic waters, like rivers and streams. See Appendix A for information about purchasing or making your own transparency tube.

For use with a Transparency Tube:

1. Rinse sample container with sample water. Collect sample water in a bucket or other container from which you can pour the water into a calibrated transparency tube. (*Note:* Avoid stirring bottom sediments when sampling at midstream.)
2. Avoid direct sunlight by turning your back to the sun. Swirl the water in your bucket to mix and slowly pour sample water into the tube.
3. While looking vertically down into the tube, release water until the point at which you can barely see the "X" on the bottom of the tube, and record the result in centimeters or inches. (*Note*: Do not wear sunglasses while taking this measurement.)

Transparency (cm) Reading from Tube	Turbidity (NTU) *(Approximate)*	Q Value
150	0	97
120	5	85
90	10	76
67.5	13	70
60	15	68
30	20	62
27.5	25	57
25	30	53
22.5	35	48
20	40	45
15	50	39
12.5	60	34
10	70	28
7.5	80	25
5	90	22
2.5	100	17
<2.5	>100	5

Turbidity Q-values



Turbidity: inches or cm or NTU/JTU

4. Repeat the above steps to verify the result. (Note: Allowing one or two people to repeat the test or view the tube may help obtain a more accurate result.)
5. **Convert the tube reading from inches or centimeters to Nephelometeric Turbidity Units (NTUs) using the Q-Value chart on this page.**
6. Properly clean your transparency tube. (See Appendix A)

Typical range for Turbidity:
0 to 2150 NTU

Indiana Average = 15 NTU

EPA recommends 10.4 NTU

E. coli Bacteria

Fecal coliform bacteria are found in the feces of warm-blooded animals, including humans, livestock, and waterfowl. These bacteria are naturally present in the digestive tracts of animals, but are minimal in unpolluted waters. Fecal coliform bacteria typically enter water via wildlife, livestock access to streams, combined sewer overflows (CSOs), poor septic systems, and stormwater runoff from agricultural and urban lands. The bacteria can enter the body through the mouth, nose, eyes, ears, or cuts in the skin.

E. coli is a species of fecal coliform bacteria that is used as an overall indicator of fecal contamination, per Indiana's state water quality standards. Thirty-eight percent (16,027 miles) of Indiana streams do not support primary contact recreation due to high *E. coli* bacteria levels *(Source: IDEM Integrated Water Quality Monitoring and Assessment Report, 2012)*.

Bacteria & Human Health

Occasionally strains of *E. coli* can lead to illness in humans. While not all strains of *E. coli* are pathogenic themselves, they occur with other intestinal tract pathogens that may be dangerous to human health. We test for the presence of *E. coli* as an indicator of overall fecal contamination, which may include many other dangerous bacteria, viruses, protozoa, and microbes which are not so easy to test for.

The U.S. EPA has determined that *E. coli* bacteria counts above 235 colonies per 100 mL indicate that more than 8 people out of 1,000 who come into contact with the water may become sick. But it is important to remember that as *E. coli* counts go up, it is the chance that someone will get sick that goes up. Still, there are many other things that determine if a person will become sick, such as:

- How long someone has been in contact with the water
- If water comes into contact with a person's eyes or mouth
- If the person has skin abrasions or wounds
- The age and health of the person, as that can determine a person's susceptibility to illness.

(Source: USGS Chattahoochee BacteriALERT website at - https://www.usgs.gov/science-explorer-results?es=bacteria)

Hoosier Riverwatch participated in a six-state research project from 2004-2006 in conjunction with Purdue Extension to determine the most accurate and usable method for detecting *E. coli* and coliform bacteria by volunteers. Details of the study may be found by attending an advanced Riverwatch workshop on this topic or search for Citizens Monitoring Bacteria: A Training Manual for Monitoring E. coli by Kris Stepenuck as part of the CMB regional partnership.

Problem:
High levels of *E. coli* indicate fecal contamination and the potential presence of pathogens that could cause human illness.

Causes:

- Human waste from poorly functioning septic systems, wastewater treatment systems, or combined sewer overflows.
- Pet waste, wildlife (including waterfowl).
- Livestock or manure runoff from fields.

E. coli Testing Instructions – Coliscan Easygel

The following instructions are adapted from those provided by Micrology Laboratories, Inc. for use with the Coliscan Easygel method. For details on use and interpretation of results, please refer to the manufacturer's instructions. **Be sure to request a copy of the color ID photo examples when ordering.**
Contact them (toll-free) at 1-888-EASYGEL or www.micrologylabs.com.

Coliscan media incorporates a patented combination of color-producing chemicals and nutrients that make *E. coli* colonies appear blue, coliform bacteria that are not *E. coli* as a pink magenta and non-coliforms as white or teal green colonies.

Checklist:

- ☐ Pre-treated petri dish from Micrology Labs
- ☐ Sterile pipettes, Whirl-pac bag or other sterile collection container
- ☐ Bottle(s) of Coliscan Easygel (thawed)
- ☐ Permanent marker (e.g. Sharpie)
- ☐ Tape, rubber gloves, ice and cooler (if needed)
- ☐ Bleach and water-tight bag for disposal
- ☐ Incubator

Do not rinse these materials before or after use! They are specially pre-treated or sterilized for use. Be sure to follow the instructions provided!

1. **Preparation** - Thaw Coliscan®Easygel® at room temperature by removing from freezer before sampling. Label the bottom of Petri dishes using a permanent marker. This label should include site ID, date and time of sample collection, volume of water collected, and sample number. Before plating, you may also secure the top and bottom of the petri dish with one piece of tape to make a "hinge."

The amount of sample used will vary according to the suspected conditions of the water you are testing. For Easygel methods, .25 mL is the minimum and 5.0 mL is the maximum amount of sample you can use. If you suspect a high bacteria count after a recent rainfall event, transfer only 0.5-1.0 mL of sample. Typically, 3-5 mL is appropriate. Your goal is to have < 200 colonies in the petri dish.

2. **Collection** - Wearing gloves and using only sterile collection equipment, obtain a sample slightly below the water's surface in one of two ways:
    a) Take a measured sample directly from the source using a sterile pipette and immediately place it into the bottle of Coliscan Easygel, or
    b) Collect your sample in a sterile container (e.g. Whirl-pak Bag) and transport the water to an appropriate test site.

3. **Plating** - Transfer a measured volume of sample water into the bottle of Coliscan Easygel. Gently swirl and invert the bottle to distribute the Easygel and then pour the mixture into the *bottom half* of a Micrology Labs *pre-treated* petri dish. (If you hold the petri dish up to a light, you can see the gelling agent.) Being careful not to splash over the side or onto the lid, gently swirl the dish until the mixture is evenly distributed across the bottom.

Plating offsite is recommended. *Water samples and Easygel bottles containing samples kept longer than 10 minutes prior to plating should be kept on ice in a cooler or in a refrigerator until plating. Samples must be plated within 24 hours.*

While its contents are still in liquid form, place the dish right-side-up directly onto a level location out of direct sunlight. Solidification will occur in approximately 45 minutes.

4. **Incubation** - Turn the petri dish upside down (to reduce condensation) and incubate at 35° C (95° F) for 24-hours.
5. **Counting/Analysis** - After the appropriate incubation period, inspect the dish. Count all of the purple/blue-violet colonies in the dish and record the results in terms of *E. coli* per 100 mL of water. You may also count all of the pink and magenta colonies and record these as coliforms. Do not count pin-point colonies < 1mm in size, and disregard any light blue, teal, or white colonies, as these indicate other types of bacteria.

To report the total number of *E. coli* and coliform bacteria colony forming units (CFU) per 100mL, first divide 100 by the number of mL you used in your sample, then multiply that figure by the # of colonies you counted in your petri dish.

Example:
You used 3 mL of stream water and you counted 4 purple colonies in your dish.

First divide 100 by 3 = 33.3.
Then multiply 33.3 x 4 = 133.2 colonies / 100mL.

6. **Disposal** - To prepare your sample bottle and petri dish for disposal in normal trash, place 5 mL (about 1teaspoon) of bleach onto the surface of the plate. Allow to sit for at least 5 minutes. Place in a watertight plastic bag and discard in trash.

Expiration -
Coliscan Easygel bottles (not petri dishes) need to be stored in a freezer. Coliscan Easygel medium is good for 1 year, and can be refrozen if thawed.

E. coli Testing Instruction – 3M Petrifilm

Storage

Store unopened Petrifilm plate pouches at temperatures <8°C (46°F) – **REFRIGERATE!**

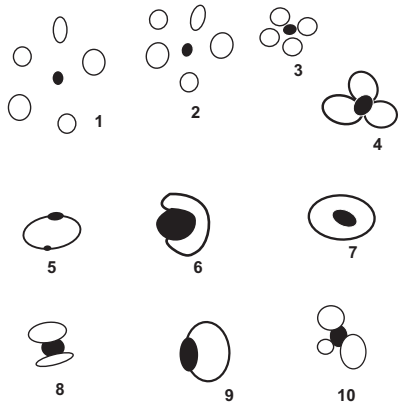
Store plates from opened packages in sets of no more than 8 in a small "snack-size" Ziploc, Glad, or similar type storage bag. Place a weight on top of the package to keep it from curling. Plates may be stored for up to a year.

Checklist:

- ☐ 3M Petrifilm
- ☐ Sterile pipettes, Whirl-pac bag or other sterile collection container
- ☐ Permanent marker (e.g. Sharpie)
- ☐ Rubber gloves
- ☐ Bleach and water-tight bag for disposal
- ☐ Incubator

1. **Preparation** - Allow pouches to come to room temperature before opening – at least 10-15 minutes. Do not use plates that show orange or brown discoloration. Expiration date and lot number are noted on each package. (Example, expiration date: 2015-10, would expire in the 10th month (October) of 2015. The lot number is also printed on individual plates.)
2. **Collection** -Wearing gloves and using only sterile collection equipment, obtain a sample slightly below the water's surface.
3. **Plating** - Inoculate and spread one Petrifilm plate before inoculating the next plate.
   - Place a Petrifilm plate on a level surface.
   - Lift the top film and dispense **1 ml only** of sample or diluted sample on the center bottom film.
   - Slowly roll the top film down onto the sample to prevent trapping air bubbles.
   - Leave plate undisturbed for at least one minute to permit the gel to solidify.
4. **Incubation** - Incubate plates in a horizontal position, with the clear side up in stacks of up to 20 plates. Incubator should be humidified with distilled water. Incubate 24 hours at 35 ºC (95° F) for 24-hours.
5. **Counting/Analysis** - After the appropriate incubation period, inspect the film. Count blue colonies with gas bubbles after 24 hours at 35 ºC (95° F). Do not count artifact bubbles. Approximately 95% of *E. coli* produce gas. In general, *E. coli* colonies are blue to blue-purple and closely associated (approximately one colony diameter) with entrapped gas. General coliform colonies are bright red and closely associated (approximately one colony diameter) with entrapped gas (Figure 25). **Only count blue colonies that have a gas bubble.**
6. **Disposal** - Place in a sealed Ziploc or similar type bag with bleach. The excess bleach will spill out and disinfect the Petrifilm plates, too. Discard with regular trash.

Figure 25



All 10 examples depict various bubble patterns associated with gas producing colonies. Each numbered picture would be counted as one colony. *(From 3MPetrifilm interpretation guide)*

Typical range for *E. coli* =
2 to 1,204 K colonies/100mL

Indiana Average =
210 colonies/100mL

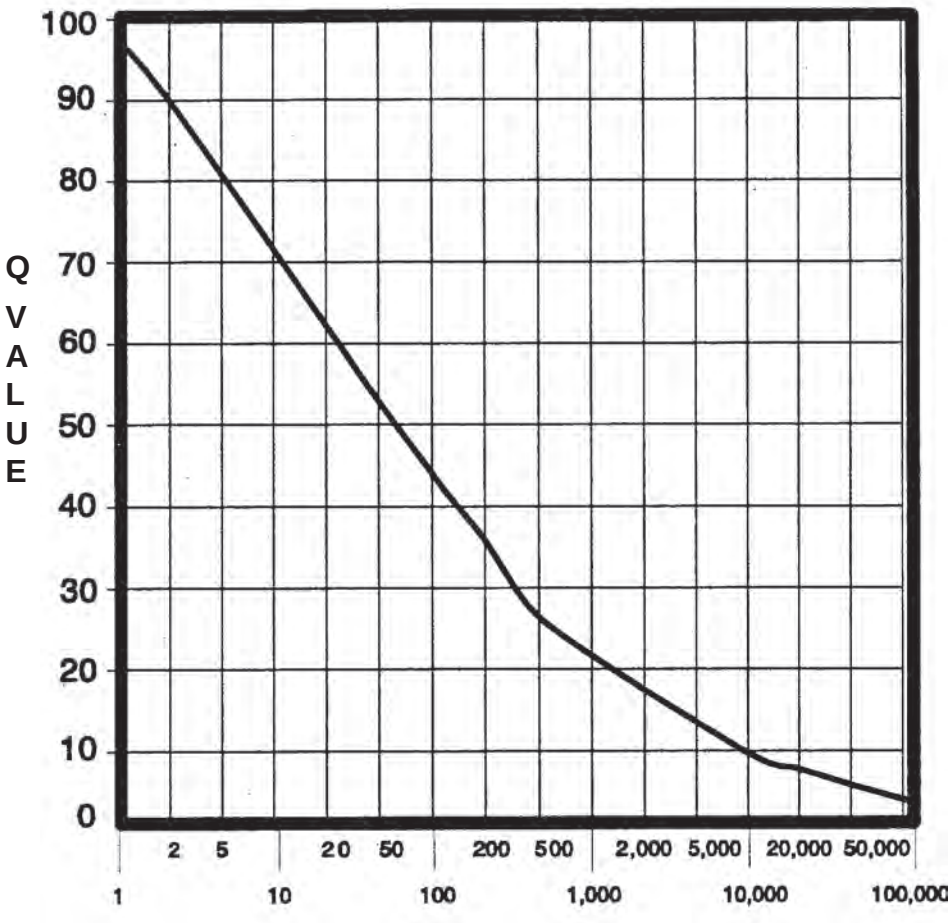
State Water Quality Standard for total body contact recreation:

<235 CFU/100 mL (a single sample)

OR

<125 CFU/100 mL (Geometric mean of 5 samples equally spaced over 30 days)

E. coli Q-Values



E. coli: colonies/100mL

E. coli (colonies/100mL)	Q-Value
0-1	98
2	89
5	80
10	71
20	63
50	53
100	45
200	37
500	27
1,000	22
2,000	18
5,000	13
10,000	10
20,000	8
50,000	5
100,000	3
>100,000	2