



Merrimack River Initiative

Watershed Connections

Training Manual for Core VEMN Monitoring Parameters and Methods

Final Report

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THE MERRIMACK RIVER INITIATIVE

The Merrimack River Initiative began in 1988 as an agreement between the U.S. Environmental Protection Agency, the State of New Hampshire, the Commonwealth of Massachusetts, and the New England Interstate Water Pollution Control Commission to collaborate on water quality issues. This initiated a dialogue to examine issues and problems in the watershed, thereby resulting in a proposal for funding and further work toward expanding the watershed approach. The watershed approach is different from other water resource planning efforts in several ways. First, the approach is "resource based" using the watershed as the management unit rather than looking at a specific portion of a river, as is usually the case. This allows planners to examine the cumulative impact of all activity in the watershed. Second, it strives to be a holistic approach. It considers issues of surface and groundwater quality and quantity along with human use and natural functions in the watershed. Lastly, it builds partnerships. The **Initiative** brings together public and private groups, state and federal agencies, industry and environmental groups all with a common goal.

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Central NH Regional Planning Commission, 329 Daniel Webster Hwy., Boscawen, NH 03303
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Training Manual for Core VEMN

Monitoring Parameters and Methods

First Edition, Spring 1996

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This manual is based on two existing manuals for volunteer water quality monitors:

1. “River Water Sampling and Analysis Manual” prepared by River Watch Network in June of 1995
2. “Manual for Volunteer Water Quality Monitors” prepared for the Massachusetts Water Watch Partnership in the Spring of 1994

Additional work for this manual on the Health Risk Assessment section was done by a collaboration of River Watch Network with Cynthia Lopez, a Harvard School of Public Health Ph.D. candidate.

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INTRODUCTION

The **Merrimack River Initiative** was started in 1988 to promote holistic management of natural resources throughout the Merrimack watershed. Participants recognize the dual benefits of developing a watershed-wide base of information and of building a strong watershed constituency by supporting citizen groups who want to conduct their own surveys. To meet these objectives, the **Volunteer Environmental Monitoring Network** (VEMN) was organized.

The VEMN provides services to volunteer environmental monitoring groups in the Merrimack River Watershed. These services include guidance in the form of documents and manuals, training, consulting, and connecting volunteers groups to resources in their communities. The purpose of the VEMN is to coordinate all the volunteer monitoring efforts and to develop a complete picture of water quality throughout the watershed.

The VEMN is developing an array of organizational and training services for new and existing groups. One major aim of the VEMN is to more closely integrate volunteer monitoring programs into environmental management decisions that are made in the Merrimack Watershed every day at every level of human activity.

The guidelines laid out in this and in other VEMN handbooks, will help citizen groups build effective local programs that also contribute to a watershed-wide view of the health of the Merrimack watershed.

This “Training Manual for Core VEMN Monitoring Parameters and Methods” was designed to work hand-in-hand with two other VEMN handbooks:

1. “Guidelines For Subwatershed Groups On Preparing Scientific Study Designs”. This is a “how to” document.
2. “Designing a Volunteer Water Monitoring Study in the Merrimack River Watershed”. This is information that will be included in a study design but it is not “how to” do a study design.

The first handbook listed above is a step by step manual on how to prepare a monitoring study. The studies that groups design using this manual will enable them to answer the questions that best address the issues that they determine to be important. It also includes worksheets that will help groups complete each step of the process.

The second document provides guidance on how to select indicators and methods, sampling location, sampling frequency and quality assurance measures for your study design. It will be useful to volunteer monitoring groups interested in designing studies which will provide data to users from state and federal agencies to local town citizens and businesses. This document includes:

- A Complete Guide To Identifying Issues and Questions That Monitoring Can Address
- A Guide To Matching Data Users and Uses With Data Quality Goals and Objectives
- A Guide To Selecting Indicators, Methods, Sites, Frequency of Monitoring and Quality Assurance/Quality Control

While these manuals guide monitoring groups in developing study designs, sometimes the hardest part of writing a study design is determining which issues to address by monitoring. To make this task easier, the VEMN recommends **three basic assessments** that address issues and questions we feel will be of interest throughout the watershed:

1. Preliminary Watershed Assessment - a visual survey and evaluation of some basic watershed characteristics to help focus your other monitoring activities.
2. Water Quality Standards Assessment - water sampling and analysis of river or lake water quality indicators that the states of Massachusetts and New Hampshire use to determine how closely our waters comply with state standards for their designated uses.
3. Health Risk Assessment - a combination of water sampling and data gathering on actual disease occurrence to see if there's a relationship between water quality, contact with the water and illness.

These core assessments will help the VEMN and participating monitoring groups:

- gather basic information on watershed characteristics,
- assist communities and water users to assess health risks associated with water contact, and
- contribute to federal and state information on the quality of our waters.

This manual includes background, procedures, and tips needed to carry out these assessments. You will find that this manual follows the order of the assessments as listed above.

ORIGINS OF THIS MANUAL

This manual is based on two existing manuals for volunteer water quality monitors:

1. "River Water Sampling and Analysis Manual" prepared by River Watch Network in June of 1995
2. "Manual for Volunteer Water Quality Monitors" prepared for the Massachusetts Water Watch Partnership in the Spring of 1994

Background information on dissolved oxygen, temperature and Secchi depth transparency for lakes in the Water Quality Standards Assessment section was borrowed from the University of Rhode Island's (URI's) 1994 Watershed Watch Report. Watershed Watch is a citizen volunteer lake monitoring program housed in the URI Department of Natural Resources Science. Watershed Watch receives staff support and laboratory facilities from the URI Cooperative Extension.

Additional work for this manual on the Health Risk Assessment section was done by a collaboration of River Watch Network with Cynthia Lopez, a Harvard School of Public Health Ph.D. candidate.

PRELIMINARY WATERSHED ASSESSMENT

This section provides some background information on watersheds, describes comprehensive watershed assessment, and how to carry out a preliminary watershed assessment.

Introduction

Watershed assessment is the collection of new and existing information on watershed conditions and processes at a watershed level. This information is used in order to make informed management decisions at all levels, from the individual landowner to state and federal permits. We define two levels of watershed assessment: preliminary watershed assessment and comprehensive watershed assessment.

Preliminary watershed assessment is really just the first step in a comprehensive watershed assessment program. A preliminary watershed assessment consists of two parts:

- 1) a compilation of *existing* information from reports and interviews, and
- 2) easily-gathered visual observations on various watershed characteristics, conditions, and activities.

Comprehensive watershed assessment includes gathering new information through field monitoring of the physical, chemical and biological characteristics of the water column, river channel or lake basin, shoreline, corridor and upland areas. Obviously, comprehensive watershed assessment is a big job. Should you decide to undertake it, you'll need to make some choices about what and how to monitor.

Why Do A Preliminary Watershed Assessment?

The two main purposes for a preliminary watershed assessment are 1) to identify watershed improvement and protection actions and 2) to help you decide whether or not to carry out a comprehensive watershed assessment and to help you decide those characteristics and areas that are most important. We suggest starting any monitoring program with the preliminary watershed assessment.

The preliminary watershed assessment helps answer two of the VEMN's basic questions:

- 1) *Where are problem areas that should be a high priority for remediation?*
- 2) *Where are the special natural and cultural resources?*

The answers can be used for several things:

- Identify areas where data that are needed to make management decisions are lacking,

- Identify problems and conflicts which need to be resolved by some management decision,
- Identify special areas in need of protection
- Plan and implement specific projects to address problems identified in the assessment

The preliminary watershed assessment also prepares your group for monitoring by identifying issues, characteristics, conditions, processes, human activities, and problem areas that you might wish to monitor. You can then design a program to monitor the selected characteristics that are most important to your watershed, thereby maximizing your group's financial and time resources.

About Watersheds

A *watershed* is an area of land that drains into a water body. Its boundaries are the highest points of land around the water body. Water running over the land's surface or through the ground eventually gathers into a river, stream, lake, or pond. Streams and rivers carry the surface and ground water from higher elevations to lower elevations, eventually draining into the ocean. Lakes and ponds are formed when surface runoff fills a natural or human caused depressions or backs up behind a natural or human-caused dam.

Surface water bodies may be *permanent* -- they have water in them year round -- or they may be *intermittent* -- they don't have water in them during the drier months of the year.

Watersheds range in size from a few acres to millions of square miles. Small watersheds occur within large watersheds. A large watershed like the Mississippi (about 1.2 million square miles) can contain hundreds of small watersheds, each drained by a tributary.

The River Network

The joining of river channels in a watershed forms the river network. As more and more tributaries join the main stem, the channels get progressively larger as the volume of water increases. The amount of water in a river channel depends on the amount of precipitation and the size of the drainage area. The resulting pattern looks somewhat like the veins in a leaf or the trunk and branches of a tree.

The characteristics of this drainage network change upstream to downstream:

The *Upper Reach or headwaters* are the small streams that are the beginning of the river, at higher elevations at the rim of the watershed. In mountain areas, they are usually forested and most or all of the stream is shaded, cold, and fast-moving. Headwaters streams are high energy water bodies that readily erode soft banks and channels. The food source for aquatic life comes primarily from

streamside vegetation and insects. Habitat is primarily rocky streambed that provides spawning areas for some fish and has limited living spaces for aquatic insects. Large woody debris may form temporary dams with pools behind them.

In the *mid-reach*, the stream becomes wider and is no longer totally shaded by vegetation. Floodplains develop in the flatter reaches. These floodplains are areas where active erosion and deposition occurs and the stream channel may move and meander throughout the area. In agricultural or developed areas, there may be little or no bank vegetation. The river current slows in places, forming pools, and the water may be warmed considerably by the sun. The food source comes from upstream, instream growth of plants, and streamside vegetation. The bottom may vary from rock and cobbles, to sand and gravel and woody debris. This diverse habitat provides an abundance of different types of living places for aquatic insects and rearing places for fish.

Still farther downstream, the *lower reach* is wide with extensive deposition in floodplains and in deltas near the confluence with larger water bodies. The water is deeper and frequently less clear. The food source is primarily from upstream and some small plants and animals in the water column. The bottom is often mud or silt. This type of bottom provides limited living space for aquatic insects and limited shelter for fish, though other types of habitat may exist at the margins along the river banks. This reach is primarily a migration corridor for fish.

Comprehensive Watershed Assessment

This is a long-term and very intensive effort to gather new and existing information on watershed conditions and processes. Existing information includes reports, studies, and other documentation. New information includes field monitoring of watershed characteristics or indicators.

In organizing a comprehensive watershed assessment, it's helpful to think of watershed characteristics/indicators and zones. Watershed characteristics or indicators are specific things that are measured in the field that reveal conditions and processes at work. Watershed zones are the different areas, from the water to the highest point of land. Starting in the water, these zones are the water column, the channel or basin, the shoreline, the corridor, and upland areas. Following are some of the characteristics that are commonly assessed in each of these zones:

The Water Column

The physical, chemical, and biological properties of the water itself are commonly measured as part of a field monitoring program. Following are examples of commonly measured characteristics:

Examples of Physical Characteristics:

- width
- depth
- current velocity
- color
- temperature
- sediment load
- clarity

Examples of Chemical Characteristics:

- dissolved oxygen
- acidity
- nutrients
- alkalinity

Examples of Biological Characteristics:

- bacteria
- algae
- plants
- plankton
- invertebrates
- chlorophyll a

Other information which should be gathered includes:

- human water uses
- attitudes towards the water
- pollution sources

The Channel or Basin

The channel or basin extends from the top of one bank to the other. It is the channel that carries the surface runoff. It includes the river or lake bed (the bottom of the channel) and the banks. Important channel characteristics include:

- bottom/bed composition
- width
- erosion rate
- bank composition
- bank alteration
- rooted aquatic plant coverage
- gradient
- depth (independent of the water)
- sinuosity (the path it takes)
- bank stability
- bank vegetative cover
- habitat types and quality

The Shoreline

The shoreline generally extends from the top of the channel to the next height of land. It comprises the land area immediately adjacent to the river or lake that drains directly into it. Depending upon the slope, this area may carry floodwater regularly and would be called a floodplain. The first height of land is frequently a formation that the river created long ago. It may be the top of an old river bank when the river was at a higher elevation -- known as a river terrace. It may be a glacially-deposited ridge. This area has a large influence on the river.

Important characteristics include:

- vegetative cover and type
- slope
- groundwater flow
- habitat types and quality
- shading of the water
- the types of soils
- land use

The Corridor

The corridor is generally defined as the area from the first river or lake terrace to the first ridge. This is a vague definition, especially in low gradient coastal watersheds. Think of it as the immediate valley that contains the lake or through which the river flows. Surface runoff flows directly into the water body, perhaps through short intermittent channels.

Important characteristics include:

- amount and type of vegetation
- the types of soils
- land use
- slope
- groundwater flow
- habitat types and quality

Upland Areas

Upland areas include the rest of the watershed. Runoff from these areas may not have the same direct impact on the water body that runoff from shorelines or channels will.

Important characteristics include:

- amount and type of vegetation
- the types of soils
- land use
- slope
- groundwater flow
- habitat types and quality

A watershed is a lot more than just the water flowing or standing in a basin. It is a complex web of physical, chemical and biological characteristics.

Preliminary Watershed Assessment: The Process

As you can see from the partial lists of watershed characteristics described above, a full-blown watershed assessment is a huge undertaking. We suggest the preliminary watershed assessment as a starting point. There are numerous types of field assessments that we would call preliminary out there. Most assess many of the characteristics listed in the previous section using simple visual methods, rather than measuring the characteristics. Most involve at least the water column, the channel or basin, the shorelines, and the corridor. There is no single “best” preliminary assessment methodology that we recommend. Therefore, we suggest the following process:

STEP 1: IDENTIFY AND DELINEATE YOUR WATERSHED OF INTEREST

The first step is to determine the geographic scale of your preliminary assessment. What is the area you will assess? Is it the immediate watershed around a lake? Or does it include all the feeder streams as well? For larger watersheds, you might want to start with a tributary watershed within the larger watershed.

Get a map of watersheds within the Merrimack River watershed. These are available from the NH DES, the MA DEP, New England Interstate Water Pollution Control Commission, and the Merrimack River Watershed Council. We suggest that you pick a watershed that you'll be able to cover adequately with your assessment, given your groups resources, time availability, and energy. Delineate this on a topographic map, and use this map as your reference map.

STEP 2: RESEARCH EXISTING INFORMATION ON YOUR WATERSHED:

Find out what's already known about your watershed of interest. Here are a few ideas for information you'll find helpful:

- a. Water Classifications and Uses:** All water bodies in NH and MA are classified according to their designated uses. These uses include:

- | | |
|----------------------|-----------------------|
| • Swimming | • Fishing |
| • Irrigation | • Public Water Supply |
| • Waste Assimilation | • Canoeing/Kayaking |

These designated uses are goals -- the water is managed to support these uses. Class A waters are managed as public water supplies. Class B waters are managed for fishing and swimming. Another designation is cold water versus warm water fisheries. This relates to the type of fish species that the water should be able to support. Warm water fisheries are managed for species such as pike, perch and bass. Cold water fisheries are managed for species such as trout and salmon. Identify and map the classifications and fisheries designations for each water body in your watershed.

- b. State Water Quality Assessments:** Also known as 305(b) reports, these are prepared every two years as reports to Congress on the extent to which the waters of the state support their designated uses. They usually identify water bodies that fully support, partially support, or don't support their designated uses. They usually identify the cause of partial or non-support. They may also contain monitoring results. Identify and map the water bodies in your watershed that only partially support or don't support their designated uses.
- c. State Watershed Assessments:** Both Massachusetts and New Hampshire periodically prepare assessments on a watershed basis. These assessments usually involve water column sampling, with some limited biological

monitoring. These usually identify problem areas that need action or further study.

The Massachusetts DEP has produced a watershed information template that lists sources of information on a variety of watershed features, from topography to land use, population patterns, water quality data, etc. It's a more comprehensive list than your group may ever need, but it does serve as an excellent reference that provides the "universe" of possible information.

STEP 3: IDENTIFY THE CONDITIONS AND PROCESSES THAT YOU WISH TO ASSESS AND WHERE YOU WISH TO ASSESS THEM

Once you've researched what's known about your watershed, you may have identified specific problems or issues for specific water bodies or portions of your watershed. The preliminary watershed assessment is a tool. It's up to you to figure out how and where to use it.

You should decide which conditions and processes that you want to assess. For example, has anyone ever done a pollution source inventory for a specific lake or stream segment? Are erosion problems documented? Has anyone ever looked at the location of different river habitat types? This will help you decide on an appropriate preliminary assessment methodology.

You also need to decide where you wish to carry out the preliminary assessment. You may want to carry out a preliminary watershed assessment in problem areas identified by the state in order to "ground truth" or verify them in the field. Or you may wish to focus on other parts of the watershed that have not been studied recently, if ever. You should also decide in which watershed zone(s) you wish to focus: water column, channel or basin, shoreline, corridor, or upland areas. Some preliminary assessments focus on all of these.

STEP 4: SELECT AND CARRY OUT A PRELIMINARY WATERSHED ASSESSMENT METHODOLOGY

There are numerous methodologies available. These include the following:

- *Windshield Watershed Survey for Volunteers*: Massachusetts Watershed Partnership
- *Shoreline Survey for Volunteers*: Massachusetts Riverways Programs
- *Watershed Non-point Source Evaluation and Site Assessment*: University of New Hampshire Cooperative Extension Service.
- *River Walk*: River Watch Network and Vermont Department of Environmental Conservation
- *Streamwalk*: EPA Region 10

None of these methodologies includes all of the watershed characteristics listed in the section describing the comprehensive watershed assessment. At a minimum, though, look for methodologies that:

- 1) Address the conditions and processes you wish to assess. At a minimum, they should include the following:
 - *A pollution source inventory*
 - *Water color, odor, and appearance*
 - *Corridor land uses*
 - *Evidence of pollution*
 - *Habitat types*
 - *Channel and shoreline vegetation*
 - *Bottom composition*
 - *Condition of shorelines*
 - *Water uses*
 - *In-stream or in-lake plant growth*
- 2) Include the watershed zones of interest.
- 3) Have been field tested and found to produce useful information.
- 4) Can be taught to and carried out by volunteers and schools.

When Planning A Watershed Assessment

A preliminary watershed assessment can be a relatively quick and simple process, or it may involve significant expenditures of time, expertise, and money. The route you choose depends on your information needs and your resources.

Groups considering doing a preliminary watershed assessment should contact their local environmental agency representative, regional planning commission, area colleges, town boards and others to determine what information already exists or to explore a collaborative venture. For example, Massachusetts DEP watershed teams typically perform watershed assessments at the beginning of their 5-year watershed schedule in any given watershed. Regional planning agencies commonly perform assessments under the federally-funded Water Quality Management Planning Program (section 604(b) of the Clean Water Act).

Some communities or non-profit organizations contract with college interns or consultants to collect or compile information on such wide-ranging subjects as watershed uses and values, economic impacts of watershed use, topographical features, demographic trends, or local regulations and by-laws that might affect watershed health.

Where To Get Help

- * *Massachusetts Department of Environmental Protection, Office of Watershed Management.* P. O. Box 116, N. Grafton MA. 508-792-7470.
- * *Massachusetts Water Watch Partnership.* Blaisdell House, University of Massachusetts, Amherst MA 01003. 413-545-2842. 413-545-2304 Fax.
- * *Massachusetts Riverways Program.* MA DFWELE, 100 Cambridge Street, Boston MA 02202. 617-727-1614.
- * *River Watch Network.* 153 State Street, Montpelier VT 05602. 802-223-3840. 802-223-6227 Fax.
- * *Volunteer Environmental Monitoring Network.* C/O Merrimack River Watershed Council, 56 Island Street, Lawrence MA 01840. 508-681-5777. 508-681-9637 Fax.
- * *UNH Cooperative Extension.* To inquire about their *Watershed Nonpoint Source Evaluation Method* manual, contact Jeff Schloss, UNH CES, 109 Pettee Hall, UNH, 55 College Road, Durham, NH 03824-3599. 603-862-3848. 603-862-1585 Fax.

US EPA. Region 10, 1200 Sixth Avenue, Seattle, WA 98101. To obtain a copy of *A Watershed Assessment Primer*, ask for document EPA 910/B-94/005

Also:

- * *The Volunteer Monitor* 1318 Masonic Avenue, San Francisco, CA 94117-4012. 415-255-8049. 255-0199 Fax. A twice-yearly newsletter on volunteer monitoring, sponsored by the EPA.
- * *EPA's Office of Water.* 401 M St. SW, Washington DC 20460. 202/260-7018. 260-7024 Fax. Publishes several guidance documents, including *Volunteer Water Monitoring: A Guide for State Managers*; *Volunteer Lake Monitoring: A Methods Manual*; and *Volunteer Estuary Monitoring: A Methods Manual*. They also support an electronic bulletin board with a special interest group on volunteer monitoring.

References:

Euphrat, F.D. and B.P Warkentin, 1994, "A Watershed Assessment Primer," U.S. EPA #910/B-94-005, EPA Region 10, Watershed Section, Seattle WA.

Hynes, H.B.N. 1970, "The Ecology of Running Waters," University of Toronto Press.

Leopold, Luna, 1994, "A View of the River" Harvard University Press, Cambridge MA.

Vannote, Robin L. , 1980. The River Continuum Concept, Canada Journal of Fisheries and Aquatic Science, Volume 37.

Water Quality Standards Assessment

This chapter describes the background and procedures for doing a water quality standards assessment.

Introduction

A water quality standards assessment involves water sampling and analysis for river or lake water quality indicators that the states of Massachusetts and New Hampshire use to determine whether these waters support their designated uses. This information is used by the states and the US EPA in their biennial reports to Congress on the condition of our waters relative to the Clean Water Act. Water quality standards and their uses are described in the “Why Do A Water Quality Standards Assessment?” section below.

The VEMN Water Quality Standards Assessment includes river and lake water sampling for the following indicators:

- 1) Bacteria
- 2) Dissolved Oxygen
- 3) pH
- 4) Temperature
- 5) Turbidity (for rivers)
- 6) Secchi Depth Transparency (for lakes)

These indicators were selected because they are included in each state’s water quality standards and because they are relatively easy to monitor. The background and procedures for each of these indicators are described in separate sections of this chapter.

Why Do A Water Quality Standards Assessment?

The main purpose for doing a water quality standards assessment is to provide additional data that Massachusetts and New Hampshire can use to assess whether the surface waters in the watershed meet their water quality standards. This assessment is reported in “305(b) reports.” This is important because these reports help the states and EPA determine how to allocate their pollution control resources to achieve and protect the human and aquatic life uses of our waters. To help you understand how your results can help, we’ll briefly describe what water quality standards and 305(b) reports are.

General Background On Water Quality Standards and 305(b) Reports

The Federal Clean Water Act, passed in 1972 (and amended roughly every five years thereafter), required the states to pass standards which describe the

beneficial uses for surface water and the water quality conditions required to support them. Section 305(b) requires that the EPA and the states report every two years on the extent to which the waters meet these standards.

About Water Quality Standards

The water quality standards that apply to the Merrimack River Watershed are contained in the “Surface Water Quality Regulations” issued by the NH Department of Environmental Services (NH DES) and the “Surface Water Quality Standards” issued by the MA Department of Environmental Protection (MA DEP).

The water quality standards contain *designated uses*, *classifications*, and *criteria*:

Designated uses: The uses of the water -- such as swimming, public water supply, fishing, aquatic life habitat, irrigation, and industrial processing and cooling -- that are to be achieved and protected.

Classifications: All the waters in the watershed are segmented and each segment is assigned to a classification: A or B in NH and A, B, or C in MA. Designated uses are assigned to each classification. It’s important to note that the uses assigned to each classification are not necessarily uses that are *actually achieved*. Rather, they are uses *to be achieved* and protected.

Criteria: For each classification, water quality criteria describe the conditions which need to be achieved in order to support the designated uses. These conditions are described for various water quality indicators such as bacteria, temperature, dissolved oxygen, pH, etc. There are two types of criteria: numerical and narrative

- * Numerical Criteria specify a level or a range of levels for each indicator needed to support the designated uses for each class. For example, in New Hampshire, Class B waters can contain no more than 406 *Escherichia coli* bacteria per 100 mL to support swimming.
- * Narrative Criteria are general statements about the conditions for each indicator needed to support the designated uses for each class. For example, for color and turbidity in Massachusetts for Class B waters: “These waters shall be free from color and turbidity in concentrations or combinations that are aesthetically objectionable or would impair any use assigned to this Class. ”

See *The State Water Quality Standards* section below for a description of the designated uses, classifications, and criteria for Massachusetts and New Hampshire.

To determine whether the waters support their designed uses, waters are either *monitored* or *evaluated* for each of the indicators listed in the water quality criteria.

Unfortunately, these terms are defined differently in each state. However, the following will serve as basic definitions:

Monitored Waters: The water quality indicators are measured and the results are compared with the numeric criteria.

Evaluated Waters: If monitoring data are not available (or are out of date), the states may determine the level of use support with land use data, pollution source inventories, citizen complaints, fish and game surveys, and predictive models.

Volunteer monitoring programs can be a valuable source of data to determine the extent to which our rivers and lakes actually support their designated uses.

About 305(b) Reports

The US EPA is required by section 305(b) of the Clean Water Act to report to Congress on the status of the Nation's surface water every two years.¹ EPA compiles reports from all the states into a national assessment of whether our surface waters are meeting the requirements of the Clean Water Act. These state and EPA reports are known as "305(b) Reports."

305(b) reports use the most recent and best available water quality data and compare the results to the criteria in the water quality standards. They then assess whether each use in each class is supported by current water quality conditions. The reports then consolidate these individual assessments into an overall assessment for each waterbody. Finally, each waterbody is placed in one of the following use support categories:

Fully Supporting: All designated uses are fully supported. In other words, there are no known violations of state water quality standards.

Threatened: One or more designated uses are threatened and the remaining uses are fully supported.

Partially Supporting: One or more designated uses are partially supported and the remaining uses are fully supported. In other words, the actual water quality does not meet all of the criteria some of the time (e.g. when combined sewer overflows occur).

Not Supporting: One or more designated uses are not supported. In other words, there are known violations of the state's water quality standards.

Not Attainable: The State performs a study and documents that support of one or more designated uses is not achievable due to natural conditions or human activity that cannot be reversed without imposing widespread economic and social impacts.

¹This will probably be changed to every 5 years when the Clean Water Act is re-authorized.

305(b) reports are used to determine pollution control and management priorities at the state and national level.

Volunteer monitoring programs can be a valuable source of data for these reports, particularly as state and federal resources devoted to monitoring dwindle. According to Greg Comstock (the NH 305(b) Officer), the most important things for volunteer groups to remember when carrying out water quality standards assessments (if they wish their data to be incorporated into 305(b) reports) are:

1. develop and implement effective quality control measures,
2. be in touch with state agencies and deliver data to them in a timely manner and in a format that they can use.

If adequate quality control measures are not used for sampling and lab analysis, state agencies may still be able to use volunteer data for 305(b) reports. However, your river or lake may be considered as *evaluated* rather than *monitored*.

The Massachusetts and New Hampshire Water Quality Standards

In most cases, fresh water volunteer monitoring groups monitor Class B waters (recreational waters that people contact directly as in for swimming). Most of the waters in MA and NH fall into that category. Class A waters are usually designated drinking water supplies and are therefore monitored by the drinking water suppliers. For Massachusetts Class C waters are designated so because they are too polluted to risk direct human contact and it would be too costly to clean the water to a point where it could be reclassified as B. Most volunteer groups do not need to bother monitoring Class C waters because they are not usually heavily used for recreation and are not considered as valuable a resource as Class A or B waters.² Therefore, the standards shown in the tables below are for Class B waters only.

If your group wishes to know the classification for a water body that you will be monitoring, or the standards for a Class A or C water, in NH call the DES at (603)271-2457 and in MA call the DEP at (508)792-7470.

New Hampshire Classes, Uses and Criteria

Designated Uses for Class B waters: Acceptable for fishing, swimming and other recreational purposes and, after adequate treatment, for use as a drinking water supply.

²New Hampshire does not have a C classification for surface waters and Massachusetts does not currently designate any surface waters in the C classification.

Table 1. NH Water Quality Standards

Indicators	Criteria
Coliform Bacteria	<ul style="list-style-type: none"> no more than 126 <i>E. coli</i>/100 mL based on a geometric mean of 3 samples obtained over a 60 day period no more than 406 <i>E. coli</i>/100 mL in any one sample <p>In designated beach areas:</p> <ul style="list-style-type: none"> no more than 47 <i>E. coli</i>/100 mL based on a geometric mean of 3 samples obtained over a 60 day period no more than 88 <i>E. coli</i>/100 mL in any one sample
Dissolved Oxygen	<ul style="list-style-type: none"> not less than 75% saturation
pH	<ul style="list-style-type: none"> 6.5 to 8.0 or as naturally occurs
Temperature	<ul style="list-style-type: none"> no increase that would appreciably interfere with the designated uses
Turbidity	<ul style="list-style-type: none"> not to exceed naturally occurring conditions by 10 Nephelometric Turbidity Units (NTUs)

Massachusetts Classes, Uses and Criteria

Designated Uses for Class B waters: These waters are designated as a habitat for fish, other aquatic life, and wildlife, and for primary and secondary contact recreation. Where designated they shall be suitable as a source of public water supply with appropriate treatment. They shall be suitable for irrigation and other agricultural uses and for compatible industrial cooling and process uses. These waters shall have consistently good aesthetic value.

Table 2. MA Water Quality Standards

Indicators	Criteria
Coliform Bacteria	<ul style="list-style-type: none"> no more than 200 fecal coliform bacteria/100 mL based on a geometric in any representative set of samples no more than 10% of the plates shall exceed 400 fecal coliform bacteria /100 mL in any one sample

Dissolved Oxygen	<ul style="list-style-type: none"> • not less than 6.0 mg/L in cold water fisheries • not less than 5.0 mg/L in warm water fisheries unless background conditions are lower • not below 75% of saturation in cold water fisheries • not below 60% of saturation in warm water fisheries • site-specific criteria may apply where background levels are lower than specified levels, to the hypolimnion (bottom layer) of stratified lakes (see temperature section) or where the Director determines that designated uses are not impaired
pH	<ul style="list-style-type: none"> • 6.5 to 8.3 • not more than 0.5 units outside of the background range • there shall be no change from background conditions that would impair any use assigned this Class
Temperature	<ul style="list-style-type: none"> • not to exceed 68°F (20°C) in cold water fisheries • not to exceed 83°F (28.3°C) in warm water fisheries • the rise in temperature due to a discharge shall not exceed 3°F (1.7°C) in rivers and streams designated as cold water fisheries • the rise in temperature due to a discharge shall not exceed 5°F (2.8°C) in rivers and streams designated as warm water fisheries • the rise in temperature due to a discharge shall not exceed 3°F (1.7°C) in the epilimnion (top layer) of lakes and ponds (see temperature section)
Temperature (cont'd)	<ul style="list-style-type: none"> • natural seasonal and daily variations shall be maintained. There shall be no changes from background conditions that would impair any use assigned to this Class, including site-specific limits necessary to protect normal species diversity, successful migration, reproductive functions or growth of aquatic organisms

Turbidity	<ul style="list-style-type: none"> • free from turbidity in concentrations that are aesthetically objectionable or would impair any use assigned to this Class
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E. coli/Fecal Coliform – background

Fecal Indicator Bacteria and Why They Are Important

Fecal indicator bacteria are different types of bacteria that are common in the intestines and feces of both warm and cold-blooded animals. They are used to indicate the presence of a variety of other, more difficult to detect, disease-causing microscopic organisms.

Micro-organisms are present everywhere in our environment. They are also present in our bodies, as well as those of all other living things. The vast majority of these micro-organisms are beneficial to their hosts. However, some are harmful and cause diseases. Disease-causing micro-organisms are said to be *pathogenic*. Examples of micro-organisms that can be pathogenic include bacteria, viruses, and protozoa (see the box below).

Disease-Causing Micro-organisms:

Bacteria are one-celled micro-organisms with no membrane around their nucleus and with a circular strand of DNA that contains the hereditary information necessary for cell life. Bacteria vary in their shape, oxygen and nutritional requirements, and movement. They may be free-living or they may live on or in dead or live organisms. Examples of bacteria which cause disease are *Salmonella typhosa* which causes typhoid fever, *Escherichia coli* (a specific type) which causes acute diarrhea, and species of *Streptococcus* which cause scarlet fever and sore throat.

Protozoa are any of a large group of one-celled, usually microscopic animals. Examples of protozoa which cause disease include *Giardia lamblia* which causes giardiasis, and *Entamoeba histolytica* which causes amoebic dysentery.

Viruses are simple parasites of plants, animals, and bacteria that often cause disease and that consist essentially of a core of RNA or DNA surrounded by a protein coat. Unable to replicate without a host cell, viruses are typically not considered living organisms. Examples of viruses that cause disease include hepatitis A, which causes hepatitis, polio, which causes poliomyelitis, and Norwalk group which causes gastroenteritis.

Monitoring water for the presence of pathogenic micro-organisms is difficult because there are so many different types. So, instead of testing for each type, we test for the presence of *fecal indicator bacteria*. The indicators are associated with human waste which is the most likely carrier of pathogenic organisms. The most

common types of fecal indicator bacteria used are the *fecal coliform* group and the *fecal streptococcus* group. Both commonly inhabit the intestines of humans and other animals. Most fecal coliform bacteria are not pathogenic. However, they live with other bacteria, viruses and protozoa that are. Many types of fecal streptococci, on the other hand, are pathogenic. They destroy red blood cells and cause various diseases.

Fecal indicator bacteria, along with their pathogenic associates, are excreted in digestive waste material, called *feces*. If fecal material gets into surface water and you come in contact with that water, you run the risk of getting sick. Pathogens can enter your body if you accidentally swallow the water or through cuts. Testing water for fecal indicator bacteria enables you to assess the risk of getting sick.

Fecal Indicator Bacteria Types and What They Tell You:

The most commonly tested fecal indicator bacteria types are total coliforms, fecal coliforms, *Escherichia coli* (*E. coli*), and enterococci. Below is a description of these types:

Total Coliforms: This is a group of bacteria that includes the fecal coliforms and other non-fecal bacteria. The total coliform group is widespread in the environment. They are typically rod-shaped. All types may occur in human feces, but some may also be found in animal manure, soil, submerged wood and other places outside the human body. Thus, the usefulness of total coliforms as an indicator of sewage is limited. They are not usually used as an indicator of the health risk of recreational water contact, but are the standard test for drinking water contamination since their presence indicates contamination of a water supply by an outside source.

Fecal Coliforms: This is a more fecal-specific subset of total coliform bacteria. However, there is one genus, *Klebsiella*, in this group with species that are not necessarily fecal in origin. *Klebsiella* are commonly associated with textile, pulp and paper mill wastes, in the absence of fecal contamination. The fecal coliform group was the primary bacterial indicator for recreational waters until relatively recently when EPA began recommending *E. coli* and enterococci (see below) as better indicators of health risk from water contact. Fecal coliforms are still being used in many states as the fecal indicator bacteria.

***Escherichia coli* (*E. coli*):** This is the only species of fecal coliform bacteria that is consistently and exclusively associated with the intestines of warm-blooded animals, including humans. Other members of the fecal and total coliform groups may be found in the intestines of warm-blooded animals, however, they may be found elsewhere as mentioned in the total coliform section above. Most *E. coli* strains are not pathogenic, though one strain causes acute diarrhea. *E. coli* occurs in high densities in human feces and has

been used as an indicator of fecal contamination for many years. It does not grow in the natural environment under ordinary circumstances. Studies from the US EPA in 1986 showed a close correlation between high *E. coli* counts and the incidence of gastroenteritis (digestive tract illness) at swimming areas. EPA recommends *E. coli* as the best indicator of health risk from water contact in freshwater used for recreation and some states have changed their water quality standards and monitoring accordingly. For this reason, the VEMN recommends that *E. coli* be measured as well as fecal coliform by volunteer groups in Massachusetts and New Hampshire, even though Massachusetts does not have an *E. coli* standard.

Enterococci: Is a subgroup of the fecal streptococcus group (a different group than fecal coliforms). These are round shaped bacteria that occur in pairs or chains. Many species destroy red blood cells and cause various diseases in human beings, including scarlet fever and septic sore throat. Unlike the fecal coliform bacteria, they have the ability to survive in salt water and in this respect more closely mimic many pathogens than the other indicators. They are generally more human-specific than the larger fecal streptococcus group which is associated with all warm-blooded animals. EPA recommends this bacteria as the best indicator of health risk in salt water used for recreation and as a useful indicator in fresh water as well.

Sources of Bacteria In Surface Water

Sources of fecal contamination to surface waters include wastewater treatment plants, on-site septic systems, domestic and wild animal manure, and urban runoff. Fecal indicator bacteria and associated pathogens enter the river and lake system in one of two ways: 1) they are discharged from pipes or excreted directly, 2) they are carried by surface runoff following rainfall events.

Direct discharge from pipes is a relatively rare occurrence these days. Most human sewage is removed by centralized wastewater treatment plants or on-site septic systems. However, on occasion, wastewater treatment plants fail for a variety of reasons and discharge inadequately treated sewage to our waters. A relatively common failure results from “combined sewer overflows.” This occurs in communities where the sewer pipes also carry stormwater runoff from streets, yards, and parking lots. During and after precipitation, these pipes bring both stormwater and untreated sewage to the treatment plant. If the runoff is particularly heavy, it may exceed the treatment plant’s capacity to handle the flows. In this case, the sewage and rainwater may be diverted directly to the receiving waters without treatment. A number of cities and towns have separated the stormwater and sewage water drain lines to avoid this problem. A number of cities have constructed stormwater treatment systems to address the stormwater problem as well.

Surface runoff following rainfall events is the other pathway for pathogens. This runoff can carry animal manure from farms, feedlots, streets, and yards. It can also carry sewage from failing on-site septic systems. This is known as “non-point” pollution, since it does not come out of a pipe. In this case, the “discharge point” may be a temporary waterway or small tributary.

Rainfall and the flow level of the river have a direct relationship to the presence of fecal indicator bacteria. In general, if there are consistently high bacteria counts during low flow periods, this might indicate fecal pollution from a steady source such as a failing sewage system. High bacteria counts during high flow periods might indicate non-point source pollution (runoff from agriculture and urban areas).

How Fecal Indicator Bacteria Levels Affect Rivers and Lakes

If fecal indicator bacteria are doing their job, then higher levels of fecal indicators in our waters should correlate with a higher level of fecal material in the waters. In addition to causing health risks, fecal material can have other impacts on rivers and lakes: cloudy water, unpleasant odors, and an increased oxygen demand as saprophytic bacteria decompose the fecal matter (see the section on dissolved oxygen). Sewage sources that carry fecal material also contain nutrients like phosphorus and nitrogen that can act as fertilizers to aquatic plants like algae and lily pads in the same way that manure fertilizes agricultural lands. This fertilizer can promote plant growth to such a degree that water bodies can become choked with weeds or unpleasantly covered with algae. When this plant material dies, it provides more food for the saprophytic bacteria and again increases the oxygen demand. This problem is more pronounced in lakes where in late summer, all the oxygen in the bottom waters can be used up in this decomposition killing many of the fish that can only live in these cooler bottom waters (see the sections on temperature and dissolved oxygen).

How We Measure Coliform Bacteria

For all fecal indicator bacteria a common method of lab analysis is membrane filtration. This method allows bacterial colonies to be counted (enumerated). A sample of water is collected and several volumes are filtered. The filter catches the bacteria. This filter is then placed on a growth medium which encourages the growth of the indicator bacteria and inhibits the growth of other bacteria. The filters and growth medium are then incubated at a specified and stable temperature for a specified period of time (usually 24 hours). Theoretically, each individual bacterial cell on the filter will multiply into colonies which become visible to the eye. We then count the number of colonies and report the number per 100 milliliters (mL) of water.

Another common enumeration method is the *multiple-tube fermentation method*. This involves adding specified quantities of the sample to tubes containing a nutrient

broth, incubating the tubes at a specified temperature for a specified time period, and then looking for the development of gas and/or turbidity that the bacteria produce as they consume the nutrients in the broth. The presence or absence of gas in each tube is used to calculate an index known as the Most Probable Number (MPN) of colonies per 100 mL. The most probable number is an estimate, not an actual count.

Monitoring Considerations

Bacteria are an excellent indicator to study, if you are concerned about the health risk of water contact recreation, possible sewage pollution, and runoff from urban streets or farm lands.

To monitor fecal indicator bacteria, there are several decisions you will need to make: 1) which fecal indicator bacteria to monitor, 2) which analytical method to use, 3) which sampling method to use. These are briefly discussed below.

Which Fecal Indicator Bacteria To Monitor

Which bacteria you test for depends on what you want to know. Bacteria are commonly used to either determine the health risk of water contact, (through compliance with state water quality standards designed to protect human health) or the presence of fecal matter from humans or animals to help determine the impact of point or non-point pollution sources on a river.

Studies conducted by the Environmental Protection Agency (EPA) to determine the correlation between different bacterial indicators and the occurrence of digestive system illness at swimming beaches suggest that the best indicators of health risk from recreational water contact in fresh water are *E. coli* and enterococci. For salt water, enterococci are best. Interestingly, fecal coliforms as a group were determined to be a poor indicator of the risk of digestive system illness. However, many states continue to use fecal coliforms as their primary health risk indicator.

New Hampshire uses *E. coli* as its indicator, Massachusetts uses fecal coliforms. If your water is in Massachusetts and you want to know whether the water meets state water quality standards, use fecal coliforms. However, if you want to know whether water contact is safe, the results of the EPA studies suggest that you should consider switching to *E. coli* or enterococci in your monitoring. For freshwater, the VEMN recommends *E. coli*. Volunteers in Massachusetts can easily measure both fecal coliform (for comparison to state standards) and *E. coli* with the same water sample by using the mTEC analytical method explained below.

Which Analytical Method To Use

The VEMN recommends the mTEC method (see box) to enumerate fecal coliforms and/or *E. coli*. At one step in the procedure, you count fecal coliforms.

At a subsequent step, you count *E. coli*. Studies have shown that mTEC is the best overall method for the enumeration of either fecal coliforms or *E. coli*.

Overview of *E. coli*/Fecal Coliform Membrane Filtration Procedure using mTEC Medium :

Several volumes of a sample are pulled through a membrane filter with a maximum pore size of 0.45 microns, which retains the bacteria. After filtration, the filter containing the bacteria is placed on a nutrient medium, "mTEC," which promotes the growth of coliform colonies. The filter is then dry incubated at 35°C for 2 hours to resuscitate injured or stressed bacteria, and then incubated in a water bath at 44.5°C for 22 to 24 hours. Following incubation fecal coliforms can be counted as the colonies that are yellow, yellow-brown, and yellow-green. To determine which are *E. coli* colonies, the filter is transferred to a pad saturated with a urea and phenol red solution. After 15 minutes, some of the colonies may turn reddish or reddish purple and the yellow or yellow-brown colonies remaining are counted as *E. coli*.

Which Sampling Method To Use

Water samples for bacterial analysis are collected in pre-sterilized containers. These can be factory-sterilized disposable bags, plastic or glass bottles that you sterilize yourself, or plastic samplers that filter a small sample directly from the water.

Consider the volume of sample you'll need to collect. Most analytical methods require that numerous portions of the sample be filtered to obtain a desired number of colonies on the filter (20-80 colonies) for reliable counting. So, your lab might filter a 100 mL portion, a 10 mL portion, and a 1 mL portion. One of these will likely have the right number of colonies for reliable counting. They may need to analyze the same sample several times or send a portion to another lab as a quality check. In general, the larger the sample you collect, the better. 500 mL is a good minimum. The small plastic samplers filter a very small portion of the water -- usually 1 mL. This is fine if the water has high bacteria counts. However, as counts on the filter drop below 20 colonies, they become more statistically unreliable. So, these samplers may not produce accurate counts if your water has less than 2000 colonies per 100 mL (which is a fairly high count for class B surface water).

If you are sampling below a wastewater treatment plant that uses chlorine as a disinfectant, you should consider adding sodium thiosulfate to your sample container. This compound binds with the chlorine, which might otherwise kill the bacteria in your sample.

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Escherichia coli/Fecal Coliform Membrane Filter Procedure Using mTEC Medium

Summary of Method: A water sample is first collected in the field in a sterile container. Refrigerated (in a cooler) samples are transported to a lab for analysis within 6 hours. A 100 mL (or smaller quantity) subsample is pulled through a gridded membrane filter, which retains the bacteria and other particles larger than 0.45 microns. After filtration, the membrane containing the bacterial cells is placed on a nutrient medium, "mTEC," which promotes the growth of fecal coliform and *E. Coli* colonies. The filter is then incubated at 35°C for 2 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5°C for 22 to 24 hours. Following incubation, yellow fecal coliform colonies can be counted. The filter is transferred to a filter pad saturated with urea substrate. After 15 minutes, yellow or yellow-brown *E. coli* colonies are counted with the aid of a fluorescent lamp and a magnifying lens.

Type of Container: Sterilized Polypropylene Bottle or Whirl-Pak Bags

Sample Volume: Approximately 500 mL

Maximum Holding Time: 6 hours (refrigerated)

Equipment: see list at end of procedure

..... Procedure

Preparation

1. **Sample Containers:** Factory-sealed, pre-sterilized, disposable Whirl-Pak bags need no preparation. *If you're sampling below a wastewater treatment plant, use Whirl-Pak bags with a sodium thiosulfate pellet (they may be purchased with the pellet in them).* This neutralizes any residual chlorine that might be present in your sample, which could kill bacteria before it's analyzed. Re-used sample containers and caps (and all glassware used in this procedure) must be rinsed and sterilized at a temperature of 121°C and a pressure of 15 lb. per square inch for 15 minutes using an autoclave.

2. **Labware: Wash labware (if not done after previous sample run):** Wash the following items with non-phosphate detergent, rinse well with tap water and final rinse with distilled water:
 - _ Flasks
 - _ Beakers
 - _ 500 mL autoclavable wash bottles
 - _ filter funnels and membrane filter holder base
 - _ #8 blue rubber stopper
 - _ tweezers
 - _ graduated cylinders .

3. **Incubators:**
 - a. **Place the thermometers in the incubators.**
 - b. **Pour distilled water into the water bath to the level recommended by the manufacturer,** usually about 2" from the top. Remember that the petri plates and weights will raise the level when they are placed in the water.
 - c. **Turn on and Check Incubators:** At least two hours prior to beginning the sample analysis, turn the water bath on and set to 44.5°C ($\pm 0.2^\circ\text{C}$). Turn the dry air incubator on and set to 35° C ($\pm 0.5^\circ\text{C}$). (Note: the first time you use an incubator, follow the manufacturer's instructions for how to set temperature and upper temperature limit controls.) After the water bath reaches 44.5°C, record this temperature on the "Water Bath Temperature Check Record" form. **Check and record two more times, at half hour intervals to assure that the temperature fluctuation does not exceed $\pm 0.2^\circ\text{C}$ - easier to maintain if the water bath has a cover.** If the temperature does fluctuate, adjust controls accordingly, until you get two consecutive readings of 44.5°C. Check and record the dry air incubator the same way on the "Dry Air Incubator Temperature Check Record" form. Adjust the controls accordingly.

4. **Clean and disinfect work area.**

5. Prepare Buffered Rinse Water

Ingredients (for approximately 30 filtrations):

–	Sodium Dihydrogen Phosphate	0.58 g
–	Sodium Monohydrogen Phosphate	2.50 g
–	Sodium Chloride	8.50 g
–	Distilled water	1.0 Liter

Preparation:

- Dissolve and mix the ingredients in distilled water in a 1000 mL plastic Erlenmeyer flask w/screw cap or an aluminum foil cover.
- Check the pH – should be 7.4 ± 0.2 .
- Cover the flask, if you are using a screw top do not tighten!
- Autoclave at 121°C (15 lb. pressure) for 15 minutes per procedure below.
- Check the pH again by pouring a small quantity of the cooled buffer solution into a beaker – final pH should be 7.4 ± 0.2 .
- Dispense into several 500 mL sterilized autoclavable wash bottles.

6. Prepare Nutrient Medium – mTEC Agar (Difco 0334-15-0)

Ingredients: (for approximately 200 plates)

–	Dehydrated mTEC medium	45.26 g
–	Reagent Water: distilled water	1.0 Liter

Preparation:

- Add dehydrated mTEC medium to 1 L of reagent water in a 3 L Pyrex Erlenmeyer flask and heat to boiling, until ingredients dissolve (the size of the flask will prevent boil over).
- Place foil or screw cap on flask and screw a few turns – do not tighten!
- Autoclave (see step 8) at 121°C (15 lb. pressure) for 15 minutes and cool in a $44\text{-}46^{\circ}\text{C}$ water bath.
- Check the pH – it should be 7.3 ± 0.2 .
- Pour approximately 4-6 mL of the medium into each petri plate and allow to solidify.

You can prepare a proportionately smaller or larger amount. Store the plates inverted (mTEC medium side closest to the top) in a refrigerator for up to one month.

7. **Prepare Urea Substrate Medium**

Ingredients: (for approximately 25-30 plates)

–	Urea	2.0 g
–	Phenol red	0.01 g
–	Hydrochloric acid (0.1N) ³	few drops
–	Sodium hydroxide (0.1N) ⁴	few drops
–	Distilled water	100 mL

Preparation:

- a. Add dry ingredients to 100 mL distilled water in a 250 mL Erlenmeyer flask and use a magnetic stirrer to dissolve. Note, it takes a while for the phenol red to dissolve.
- b. Adjust to a pH of 5.0 with a few drops of 1N hydrochloric acid (HCl) or 1N sodium hydroxide (NaOH). The substrate solution should be a straw yellow color at pH 5.0 ± 0.2 .

Store at 6°C - 8°C for up to 1 week.

- 8. Autoclave labware and rinse water at 121°C (250°F) for 15 minutes.** Wrap all items (except liquid reagent containers) in aluminum foil. Place autoclave tape on all items. Follow the autoclave manufacturer's instructions. For pressure cooker style autoclaves, see Appendix 2 for instructions.⁵

Note on Autoclaving Liquids: Remember that liquids must be cooled before you release the pressure in the autoclave -- otherwise they'll boil out of their containers. For this reason, you may want to autoclave them separately, especially if you don't need them right away.

After autoclaving is complete, check the autoclave tape on all items. It should have brown diagonal stripes indicating successful sterilization. If not, autoclave these items again, using new tape. *HINT: Leave this tape on all unused items so you know they've been sterilized.*

³ Purchase or make. To make 500 mL, place 8.5 mL of 6N HCl acid in a 500 mL volumetric flask and bring up to volume with distilled water. Store in a 500 mL Erlenmeyer flask with a screw cap.

⁴ Purchase or make. To make 500 mL, place 2.0 grams of dried NaOH in a 500 mL volumetric flask, add 100 mL of distilled water, swirl to mix thoroughly, and add enough distilled water to bring up to volume. Store in a 500 mL Erlenmeyer flask with a screw cap.

⁵Note that Standard Methods recommends the use of autoclaves with an accurate thermometer mounted so as to register the minimum internal temperature within the chamber. Pressure cooker autoclaves are not recommended, because of the difficulty of adjusting and maintaining sterilization temperatures and the potential hazard.

Sampling Procedures

In General: For rivers and streams, sample away from the bank in the main current, for lakes and ponds, take a sample at the deepest point or if you are sampling a bathing area, wade about waist deep into the bathing area and take a sample at an arms length depth. In any case, avoid sampling stagnant water! The outside curve of a river is often a good place to sample since the main current tends to hug this bank. In shallow areas, wade into the water carefully to collect the sample. If wading is not possible, tape your sample bottle to an extension pole or use a boat. Reach out from shore or boat as far as safely possible.

A boat will be required for deep sites. Try to maneuver the boat into the center of the lake or main river current to collect the water sample.

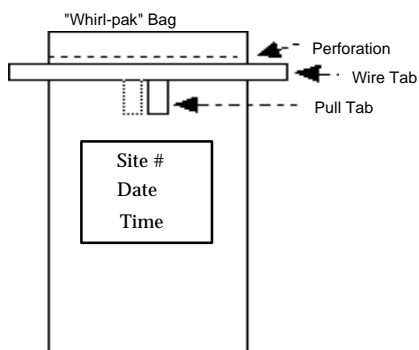
Wash your hands before starting your sample run – if you sample in an area that may have high fecal levels rinse hands before handling the next sample bottle. Be careful not to touch your hands to yourself before you have cleaned them in order to avoid coming into contact with possible pathogens. If you are sampling from waters known to be contaminated with sewage wear latex gloves to protect yourself.

For Screw-cap Sample Bottles:

1. **Remove the cap** from the bottle **just before** sampling. **Avoid touching the inside of the bottle** or the cap. If you accidentally touch the inside of the bottle, use another one!
2. **Wading:** try to disturb as little bottom sediment as possible. In any case, be careful not to collect water that has sediment from bottom disturbance. Stand facing upstream. Collect the water sample on your upstream side.
Boat: Carefully reach over the side and collect the water sample on the upstream side of the boat.
3. **Hold the bottle near its base and plunge it (opening downward) below the water surface.** If you are using an extension pole, remove the cap, turn the bottle upside down and plunge it into the water, facing upstream. Collect a water sample 8” to 12” beneath the surface or mid-way between the surface and bottom if shallow.
4. **Turn the bottle underwater into the current (for rivers) and away from you.** In slow-moving river reaches, push the bottle underneath the surface and away from you in an upstream direction.
5. **Leave a one inch air space.** Do not fill bottle completely (so that the sample can be shaken just prior to analysis). Recap the bottle carefully – remember, don’t touch the inside!
6. **Fill in the bottle # and/or site # on the appropriate field sheet. This is vital!** It’s the only way the lab coordinator will know which bottle goes with which site.
7. **Place the sample in the iced cooler.** Bring all samples to the lab for analysis. Samples should arrive at the lab within six hours of sampling. Late or warm samples may yield inaccurate results.

For Whirl-Pak Bags

1. **Label bag with site #, date and time, using an indelible marker.**
2. **Tear off the top of the bag** along the perforation above the wire tab just prior to sampling. Avoid touching the inside of the bag! If you accidentally touch the inside of the bag, use another one!
3. **Wading:** Try to disturb as little bottom sediment as possible. Be careful not to collect water that contains bottom sediment. Stand facing upstream. Collect the water sample on your upstream side.
Boat: Carefully reach over the side and collect the water sample on the upstream side of the boat.
4. **Hold two white pull tabs in each hand and lower the bag into water on your upstream side** with the opening facing upstream. Open the bag mid-way between the surface and the bottom by pulling the white pull tabs. The bag should begin to fill with water. You may need to "scoop" water into the bag by pushing it upstream and away from you. **Fill the bag no more than 3/4 full!**
5. **Bring the bag out of water.** Pour off excess water. Pull on the wire tabs to close the bag. Continue holding the wire tabs and flip the bag over at least 4-5 times quickly to seal the bag. **Don't squeeze the air out of the top.** Fold the ends of the wire tabs together at the top of the bag, being careful not to puncture the bag. Twist them together forming a loop. Label the bag with the site number, date, and time and put the sample on ice in a cooler.
6. **Fill in the bag or site # on the field sheet. This is vital!** It's the only way to know which bottle goes with which site.
7. **Place the sample in the cooler.** Bring all samples to the lab for analysis.



***Remember to label
the bag with Site #,
date, and time!***

8. Return Field Sheets and Samples To Lab Or Drop-off Point

Samples for bacteria must be analyzed within 6 hours of collection. Keep the samples on ice and bring them to the lab or drop-off point as soon as possible.

Filtration

1. **Place a sterile filter base on the receiver flask.**
2. **Dip tweezers in 70% ethanol and flame in burner.** Allow tweezers to cool before touching membrane filter. **Keep ethanol away from burner.**
3. **Remove a membrane filter from its sterile wrapping with the tweezers.** If you accidentally touch the membrane filter discard it and start over with a new one.
4. **Place filter grid-side up on the filter base and attach the funnel to the base.**
The membrane filter is now held between the funnel and the base. For screw-type funnels, be careful not to tighten the funnel too much – it will wrinkle or tear the filter. If this happens, or if the filter melts from heat of tweezers or if a piece breaks or it cracks, replace it with another filter.
5. **Transfer a portion of the sample into the funnel:**
For 10 mL or less subsamples of water samples: First, squirt 20-30 mL of sterile buffered rinse water into the funnel.⁶ *Shake sample bottle or bag vigorously for 10 seconds.* Open the sample bottle or bag and use the 10 mL pipette to transfer 1 mL or 10 mL of sample into filter funnel.
For the subsamples greater than 10 mL: *Shake sample bottle or bag vigorously for 10 seconds.* Open the bottle or bag and use a 100 mL graduated cylinder to transfer 50 mL or 100 mL of sample into filter funnel. Sterilize the graduated cylinder for 3 minutes after each use in ultraviolet light or boiling water.
6. **Use a hand or electric pump** to create a vacuum and pull the water through the filter into the receiver flask.
7. **Rinse the sides of the funnel at least twice with 20-30 mL of sterile buffered rinse water** and allow the vacuum to pull the water through the filter into the receiver flask. Turn the pump off and release the pressure.
8. **Detach the filter funnel from the base.**
9. **Carefully remove the lid of the petri plate** containing your mTEC medium and place the lid so the opening is face down on the counter.
10. **Sterilize the tweezers by dipping them in alcohol and flaming.**
11. **Remove filter from the holder base.** Be careful to hold just the edge of the filter with tweezers. Grab filter with the tweezers no more than 1/8 inch from filter edge – do not touch the surface area of the filter that may have bacteria trapped on it.
12. **Carefully remove the membrane filter from the filter base and roll it onto the mTEC agar** in the petri plate. Make sure the filter is perfectly flat and that there are no air pockets between membrane filter and medium. Reseat the membrane if air pockets occur. Use the forceps to gently press the filter down on the agar around the edges.

⁶This encourages the random distribution of bacteria on the filter.

13. **Without touching the inside of the lid, replace it on the bottom of the plate.** Turn the plate over so that the filter is facing down to prevent condensation which may have formed on the lid from dripping on the filter.
14. **Mark the bottom of the plate (the wider half with the flange) as follows:**
 - _ If this is a water sample, mark the plate with the site number, the amount of sample filtered (e.g. 1 mL or 50 mL) and the date.
15. **Sterilize the graduated cylinder (if using), filter base and funnel after each filtration:** Place the filter funnel and base in a container of sterile, boiling water for several minutes or into an ultraviolet (UV) light sterilization chamber. Either wait a few minutes until the funnel and base are sterile or use another sterile set. Use two or three filter funnels and bases, and rotate them through the boiling water or UV sterilization chamber so you always have a sterile unit waiting. Be sure to allow the boiled funnel and base to cool down enough before using so you don't injure the bacteria.
16. **Seal the plates in Whirl-Pak bags for incubation.** For every 6 plates, place them in an 18 oz. Whirl-Pak bag and fold the top over at least three times to seal. It is very important that each fold be wrinkle free. Then fold over each end of the twist tie toward the middle of the folds to clamp the folds together. There should be a slight amount of air trapped in the bag. Give the bag a gentle squeeze between the palms of your hands to test the seal. If you notice air leakage re-seal the bag. It is this air pressure that keeps water from seeping in the bag. For optional added protection, place each sealed Whirl-Pak, into a larger Zip-lock sandwich bag and seal. Store the plates in a refrigerator until you're ready to incubate them.
17. **Repeat the above procedure from step 1 for the each control plate and the subsamples of each water sample.**

Incubation

1. **Dry air incubation:** Place the marked petri dishes inverted (filter side down) in a dry air incubator for two hours incubation at 35°C. Remove the plates from the dry incubator after two hours.
2. **Water bath incubation:** Place double-bagged plates inverted (filter side down) in a 44.5°C water bath for 22 hours. The plates must be weighted down to be immersed in the water. One way to do this is to place them in or under some sort of weighted rack.
3. **Remove the plates from the water bath after 22 hours.**

Counting and Recording Colonies

Guidelines for counting: Plates with more than 80 bacteria colonies are unreliable to count because colonies may actually grow on top of each other and inhibit growth. Therefore, the counts may be unreliable.

- * If you count 80 colonies on a filter and you're nowhere near the end, stop counting and record the results as "TNTC" (too numerous to count) in the "filter count" column of the lab sheet. You can, if you wish, note the approximate % of the filter area from which you counted. Record this in the "comments" column on the lab sheet. This will enable a rough estimate of the number of colonies.
- * If you count 80 colonies on a filter and you're near the end, continue counting and record the count in the "filter count" column of the lab sheet.
- * If it's obvious that there are more than 80 colonies on the filter, don't try to count. Just record "TNTC."

Fecal Coliforms

1. **Count and record Fecal Coliform colonies:** After 15-20 minutes, count the number of yellow, yellow-brown or yellow-green colonies on the membrane filters. Record the count in the "FC filter count" column of the lab sheet.

E. coli

1. **Place the filters on urea substrate:** Remove the lids of the petri plates and place absorbent pads in them. Saturate with approximately 2-3 mL of urea substrate until the pad is just saturated (no puddles). With sterile forceps, transfer the filters to the urea-saturated absorbent pads and let them sit for 15-20 minutes.
2. **Count and record *E. coli* colonies:** After 15-20 minutes, count the number of yellow, yellow-brown or yellow-green colonies on the membrane filters. Record the count in the "filter count" column of the lab sheet. Pink, red or purple colonies are not *E. coli* and should not be counted.

Calculating Results

1. **To calculate colonies per 100 mL:** For each sample look at the membrane filter counts for the various subsample sizes you filtered (e.g. 50 and 1 mL). Select the subsample with the filter count within, or closest to, the ideal range (20-80). Calculate the colonies per 100 mL according to the general formula:

$E. coli/100 \text{ mL} = \frac{\text{\# of } E. coli \text{ colonies counted}}{\text{Volume in mL of sample filtered}} \times 100 \text{ mL}$
<p><u>Example:</u></p> $E. coli/100 \text{ mL} = \frac{65 \text{ colonies counted}}{10 \text{ mL of sample filtered}} \times 100 \text{ mL} = 650$
<p>Record the result on the lab sheet as "650."</p>

2. **Guidelines for calculating and reporting results:** In all cases, if you have an actual filter count for the larger subsample size, and the filter count for the smaller subsample is less than 20, use the actual filter count from the larger subsample.

If both counts are “TNTC” assume the filter count is 200

$$E. coli/100 \text{ mL} = \frac{200}{\text{Smallest volume in mL of sample filtered}} \times 100 \text{ mL}$$

Example:

$$E. coli/100 \text{ mL} = \frac{200}{1 \text{ mL of sample filtered}} \times 100 \text{ mL} = 20,000$$

Record the result on the lab sheet preceded by an “E” for estimated: “E20,000.”

If both counts are <20: e.g. 2 (for 1 mL) and 15 (for 50 mL), use the filter count closest to 20:

$$E. coli/100 \text{ mL} = \frac{\text{Count closest to 20}}{\text{Volume in mL of sample filtered}} \times 100 \text{ mL}$$

Example:

$$E. coli/100 \text{ mL} = \frac{15 \text{ colonies counted}}{50 \text{ mL of sample filtered}} \times 100 \text{ mL} = 30$$

Record the result on the lab sheet as “30.”

If one count is “TNTC” and the other is <20: e.g. 15 (for 1 mL) and TNTC (for 50 mL), use the filter count closest to 20:

$$E. coli/100 \text{ mL} = \frac{\text{Count closest to 20}}{\text{Volume in mL of sample filtered}} \times 100 \text{ mL}$$

Example:

$$E. coli/100 \text{ mL} = \frac{15 \text{ colonies counted}}{1 \text{ mL of sample filtered}} \times 100 \text{ mL} = 1500$$

Record the result on the lab sheet as “1500.”

Clean Up

1. Place petri plates back in Whirl-Paks and sterilize in autoclave. Then discard as you would any other trash.
2. Wash and sterilize lab ware and re-used sample bottles for next sampling run.

Quality Assurance / Quality Control For Lab Analysis

Whenever You Make a New Batch of mTEC Medium

It is important that quality checks are done each time a new batch of media is made. This assures that every bacteria present in a sample will be counted when it is analyzed and that your media is not contaminated. Remember, your results are only as good as your quality control. The types of control testing you will need to perform on each new batch of media are called **positive, negative** and **overall media quality** control.

Positive Control Testing:

A positive control checks that your media and incubators are working properly. This test should be performed every time a new batch of media is made and every day that samples are analyzed. In a nutshell, this test involves filtering a sample known to contain fecal coliform or *E. coli* bacteria and incubating the plate as usual. If you find that bacteria grew on the plate as expected, the test results are satisfactory. Your **overall media quality** control testing when you make a new batch of media will satisfy the **positive** control requirements so a separate positive control test will not need to be performed when testing a new batch of media (see overall media quality control testing section below).

Negative Control Testing:

A negative control, also known as a blank or a buffer blank, checks the sterility of your media and your buffer solution. This test should be performed every time a new batch of media is made and every day that samples are analyzed. In a nutshell, this test involves filtering approximately 100 mL of sterile buffered rinse water using a sterile filter apparatus through one of your sterile membrane filters and incubating it on a plate as usual. Nothing should grow on this plate after incubation. If something does, it indicates that either the media or buffer are not sterile. If you suspect the buffered rinse water may not be sterile, you may repeat this test with a new batch of sterile buffered rinse water. If you still find growth on the filter after incubation, it indicates that your media or petri plates are contaminated and you must throw away the batch of media and start again.

Overall Media Quality Control Testing:

This quality check on your media can be done in one of two ways:

1. **Run an analysis on a known sample.** Known samples may be obtained from the EPA at no charge. There are explicit instructions that come with the sample explaining how to analyze it. This type of known sample requires that serial dilutions of a freeze dried sample of bacteria are made before filtrations are performed. Serial dilutions involve having at least five sterile bottles each containing exactly 99 mL of sterile buffered rinse water available. If you have these containers available, the instructions that come with the sample are fairly straightforward and must be followed exactly. After filtering appropriate quantities of sample (as per the instructions), incubate the plates in exactly the same way as you would any other sample. If your media is of good quality and

functioning properly, you should end up with a number of bacteria per 100 mL within a range described in the instructions.

If you would like to order a free sample from the EPA QA/QC lab, you can call 513-569-7325. Rumor has it, however, that with EPA budget cuts, it may take up to 6 months to receive a sample if they even send you one at all. If you don't have 6 months to wait for a sample, you might opt to spend a little money purchasing a few pre-made mTEC plates from Difco that have already been certified. Then you can compare your media to those plates using the method outlined below.

2. **Compare an older batch of media that met quality control standards to a new batch.** It is important to think ahead if you use this method of quality control because you will need to save some plates from an old batch of media to use for comparison. **Remember, bacterial media can be stored for only one month in the refrigerator.** If your media is approaching one month in age, make a new batch of media right away so that you can use the old batch to compare quality. In this method, quality is checked by using five plates from an old batch of media and five plates from your new batch.
 - 1) Obtain some sample for which you know the approximate concentration of bacteria. Usually, your local sewage treatment plant can help with this because they will probably know the approximate concentration of *E. coli* or fecal coliform bacteria in their influent, primary effluent and secondary effluent. The secondary effluent may be the easiest to use because the concentration of bacteria is lower and therefore fewer dilutions will have to be made in order to filter an amount from which you will get plates within the counting range of 20 and 80 colonies.
 - 2) Calculate the volume of sample you will need to filter in order to get plates within the counting range. The treatment plant may give you a range rather than an exact number unless they have a sample that they have already tested on hand which they can give you. Therefore you may have to use a few different volumes of sample in order to get countable plates.

The formula for determining volumes to filter for a good count and an example are shown in the box below.

$$\text{Volume of sample to be filtered} = \frac{\text{\# of bacteria you want per plate}}{\text{known concentration of bacteria}} \times 100 \text{ mL}$$

Example: Known Sample Concentration = 250,000 *E. coli*/100 mL

$$\text{Volume of sample to be filtered} = \frac{50 \text{ colonies wanted}}{250,000 \text{ } E. coli} \times 100 \text{ mL} =$$

0.02 mL = 2 mL of a 100 X dilution to be filtered

As shown in the example above, it is likely that dilutions of the original sample must be made in order to get numbers of colonies on your plates within the counting range.

Making Dilutions:

Making dilutions requires that you have some sterile bottles and some sterile buffered rinse water on hand. Add 99 mL sterile buffered rinse water to as many sterile bottles as you need (using a sterile graduated cylinder or sterile pipettes). Adding 1 mL of sample to one of these bottles will give you a 100 X dilution (a dilution of 0.01 or 10⁻²). Adding 11 mL of sample to one of these bottles will give you a 10 X dilution (a dilution of 0.1 or 10⁻¹).

For the example above, you would need to make a 100 X dilution and filter 2 mL for every plate in order to get a count of 50 colonies per plate.

If larger dilutions are required, you can make dilutions of dilutions in subsequent bottles as necessary. If you need a 1,000 X dilution (a dilution of 0.001 or 10⁻³), you would add 1 mL of sample to the first bottle (giving you a 100 X dilution) and then 11 mL of that dilution into the next bottle. Be sure to mark the dilutions on each bottle so you don't get confused. Also, be sure to cap and shake the bottles well before making dilutions and before filtering samples.

- 3) Filter the calculated amount of sample for each of 3 plates of the new and old media according to the filtration procedure. You do not need to sterilize your filter funnel between filtrations because you are using the same sample and cross contamination will not be a problem. If you have calculated a range of sample you might need rather than just one specific number to get a good plate count, use three plates of both the new and old media at each sample volume you calculated. This way, you can be sure that you will get three plates of both new and old media within the counting range (this assumes that your new media is of equal quality to the old stuff). Be sure as always, to label the bottoms of each plate so you know exactly which dilutions you used and which media is new and which is old.
- 4) Incubate your plates as usual.
- 5) Examine your plates and count the three of both new and old media that are closest the 20 to 80 colony counting range.
- 6) Calculate the mean and standard deviation for the counts on the old media. Then do the same for the new media. If the mean values for the new and old media are within one standard deviation of each other, your new batch of media is just as high quality as the old batch and you may rest easy that you will get accurate plate counts using it.

Whenever You Analyze Samples

Each day on which you perform sample analysis, you must also perform quality control checks to make sure that your equipment and lab technicians are working properly. The types of quality control testing you will need to perform are **positive** and **negative controls**, **replicate** and **laboratory split** analyses.

Positive Control Testing:

A positive control checks that your media and incubators are working properly. This test involves filtering a sample known to contain fecal coliform or *E. coli* bacteria and incubating the plate as usual. If you find that bacteria grew on the plate as expected, the test results are satisfactory.

For the positive control plate: Obtain a sample of influent, primary or secondary effluent (if you use secondary effluent, make sure it is pre-disinfection secondary effluent) from your local wastewater treatment facility. Measure about 10 mL of buffered rinse water into a filter funnel on which you have mounted a membrane filter. It's not necessary to measure an exact amount, since we're not going to count bacteria colonies we're just checking for their presence. Then measure approximately 1 mL of your sewage sample into the filter funnel. Filter and incubate the membrane filter as usual. There's no need to count the colonies on the positive plates – record as TNTC (too numerous to count).

Negative Control Testing:

There are two types of negative controls you should perform on each sample analysis day. The first was described in the “Whenever You Make a New Batch of mTEC Media” above. This type of negative control checks the sterility of your filter funnels and other labware, your media and your sterile buffered rinse water. The second is more of a check on the lab technician than the equipment or materials but it is a negative control nevertheless because the result should be a complete absence of bacterial growth on the plate. It is a test performed on a used, unsterilized filter funnel to check that the technician effectively rinsed all bacteria completely from the walls of the filter funnel.

For negative control plate 1: Squirt about 100 mL of buffered rinse water into the filter funnel. It's not necessary to measure an exact amount, since we're not going to count bacteria colonies we're just checking for their absence. Filter and incubate as usual. Be sure to mark the bottom of this plate with the date and a “-1.”

For negative control plate 2: Squirt about 30 mL of buffered rinse water into a filter funnel that was just used to analyze a sample. Do not sterilize the filter apparatus before running this negative control. Filter and incubate as usual. Be sure to mark the bottom of this plate with the date and a “-2.”

Replicate Analysis:

A replicate is an internal check for a laboratory. It involves analyzing a sample twice in exactly the same way. Replicate analysis must be performed on at least 5% of all samples analyzed in a given day. The results of the replicate should be within the same log of the original sample. Mark your replicate plate as you would your original sample plate but add an “R” to it.

Laboratory Split Analysis:

A laboratory split is an external check for a laboratory. It involves taking a portion of your actual water samples and having them analyzed by an outside laboratory (a quality control lab) to check the accuracy of the results of your lab. Laboratory split analysis should be performed on at least 5% of all samples analyzed in a given day. The results of the split should be within the same log of the original sample. Compare the results from your lab to those of the quality control lab.

How To Calibrate and Standardize Thermometers and Incubators

1. Check temperatures in incubators daily to insure operation within stated limits. Keep a record of these daily temperature checks in a log book.
2. Check thermometers at least annually against an National Bureau of Standards (NBS) certified thermometer or one traceable to NBS. Check mercury columns for breaks.

Equipment Needed *E. coli* / Fecal Coliform Sampling and Analysis

Sampling

- _____ **Sterile sample containers** (1 per site plus extra)
- _____ **Sterile sample containers w/ sodium thiosulfate** (to sample below wastewater treatment plants, 1 per site plus extra)
- _____ **Indelible marker** (to label whirl-Pak bags)
- _____ **Small cooler with ice** (to keep samples cool)
- _____ **Written procedures and field sheets**
- _____ **Waders or footwear** that can get wet
- _____ **Rubber gloves** (if sampling polluted waters)
- _____ **Towel and a dry, warm change of clothes**
- _____ **Clip board and pencil**
- _____ **First Aid kit**

Preparation of Reagents

- _____ **Balance** (accurate to 0.01 g. to weigh reagents)
- _____ **Weigh Dishes** (up to 2.4g, to weigh dry reagents)
- _____ **Hot Plate** (w/ magnetic stirrer, to dissolve dry reagents)

- **Buffered Rinse Water** (for ~ 30 filtrations):

- _____ **Balance** (accurate to 0.01 g. to weigh reagents)
- _____ **Sodium Dihydrogen Phosphate** (0.58 g)
- _____ **Sodium Monohydrogen Phosphate** (2.50 g)
- _____ **Sodium Chloride** (8.50 g)
- _____ **Distilled water** (1.0 Liter)
- _____ **pH meter** (to check final pH)
- _____ **1 L volumetric flask** (to measure distilled water)
- _____ **1 L Erlenmeyer flask** w/screw cap (autoclavable)
- _____ **500 mL squirt bottles** (autoclavable, 2 per batch)

_ **mTEC Agar** (for ~ 30 plates):

- _____ **Dehydrated mTEC medium** (45.26 g)
- _____ **Distilled Water** (1.0 Liter)
- _____ **1 L volumetric flask** (to measure distilled water)
- _____ **3 L Pyrex Erlenmeyer flask** (to boil agar and autoclave)
- _____ **1 L Erlenmeyer flask w/screw cap** (autoclavable)
- _____ **pH meter** (to check final pH)
- _____ **Heat Resistant Gloves**

_ **Urea Substrate** (for ~ 30 plates):

- _____ **Urea** (2.0 g)
- _____ **Phenol red** (0.01 g)
- _____ **Distilled water** (100 mL)
- _____ **100 mL volumetric flask** (to measure distilled water)
- _____ **Hydrochloric acid** (1N, to adjust pH)
- _____ **Sodium hydroxide pellets** (to mix 1N solution, to adjust pH)
- _____ **250 mL Erlenmeyer flask w/ screw cap** (to mix and store substrate)

Sterilization

- _____ **Autoclave** (sterilizer)
- _____ **Aluminum Foil** (to wrap items or cover flasks and bottles for sterilization)
- _____ **Autoclave tape** (to indicate proper temperature and pressure were achieved)
- _____ **Distilled water** (generates steam)
- _____ **Petroleum jelly** (for pressure cooker style, lubricates seal)
- _____ **Heat Resistant Gloves**

Filtration Equipment & Supplies

- _____ **Vacuum air pump** (hand or electric, one per station)
- _____ **1L receiver flask w/ side arm** (autoclavable, to hold filtrate, 1 per station)
- _____ **Flat tipped tweezers** (1 per station)
- _____ **Alcohol** (to flame tweezers)
- _____ **50 mL Pyrex beaker** (to hold alcohol and tweezers, 1 per site)
- _____ **Sterile filter funnel** (2 per station)
- _____ **Filter holder base** (2 per station)
- _____ **#8 blue rubber stopper** (2 per station, to seal base to receiver flask)
- _____ **10 mL disposable pipettes** (to transfer 10 mL and/or 1 mL subsamples to filter funnel, 1 per subsample)
- _____ **Pipette pump** (for 10 mL pipettes)
- _____ **100 mL graduated cylinders** (to transfer the subsamples to the funnel, 2 per station)
- _____ **Membrane filters** (0.45 micron, 47 mm diameter, gridded, 1 per subsample)
- _____ **Petri plates with mTEC agar** (47 mm, 1 per subsample)
- _____ **UV sterilizer** (254 nm, 2250 μ watts per sq. cm) **or a pot of boiling water** (to sterilize funnels and bases between filtrations, 1 per station)
- _____ **500 mL squirt bottle with buffered rinse water** (autoclavable, to hold and dispense rinse water, 1 per station)
- _____ **Lighter or flame** (to flame tweezers, 1 per station)
- _____ **Water-proof fine-tipped marker or grease pencil** (to mark petri plates)
- _____ **Lab Sheet** (to record filtrations)

Incubation Equipment & Supplies

- _____ **Water bath incubator** (approx. 4 gallon cap., $\pm 0.2^\circ\text{C}$ rating)
- _____ **Dry air incubator** ($\pm 0.5^\circ\text{C}$ rating)
- _____ **Thermometers** $0-50^\circ\text{C}$ in $.5^\circ$ incr. (1 for each incubator)
- _____ **18 oz. Whirl Pak bags** (to hold plates in water bath)
- _____ **Rack and weights** (to keep plates submerged in water bath)

Counting and Recording Equipment & Supplies

- _____ **Absorbent pads**, sterile (to hold urea and filter)
- _____ **Urea substrate** (to confirm *E. coli*)
- _____ **Lighted magnifier** (to aid counting)
- _____ **Lab Sheet** (to record results)

Dissolved Oxygen – background

What Is Dissolved Oxygen And Why Is It Important?

Dissolved oxygen (DO) is the concentration of oxygen gas molecules (O_2) in the water column. It is an important indicator since oxygen is necessary for most living things and for many of the biological and chemical processes that happen in the water. Animals take in oxygen during respiration. Terrestrial animals breathe the oxygen gas in the air. Most aquatic animals breathe the oxygen gas dissolved in the water. Different species of aquatic organisms have varying requirements for the amount of dissolved oxygen. These requirements can change with the stage of their life cycle. Waters of consistently high dissolved oxygen are usually considered healthy ecosystems capable of supporting many different kind of aquatic organisms.

Water is composed of H_2O . It is important to note, that the oxygen that animals and plants use, is not from the molecular structure of water. They cannot break down these water molecules to remove the oxygen. It is the gas, O_2 dissolved in the water that the plants and animals use.

Dissolved Oxygen in the Aquatic Ecosystem

Rivers:

The river system both produces and consumes oxygen. Oxygen enters the river system by diffusion from the surrounding air and by the photosynthesis of aquatic plants. Churning, running water dissolves more oxygen than still water (it mixes more with the air). Natural processes that consume oxygen are: respiration by aquatic animals, decomposition of organic matter by microorganisms, and various chemical oxidation reactions. The combination of all the processes that consume oxygen is called “biochemical oxygen demand” or BOD for short. If more oxygen is consumed than produced or dissolved from the atmosphere, then dissolved oxygen levels decline.

River water can become saturated with oxygen when it can't hold any more at a given temperature. In this case, it is 100% saturated and is at equilibrium with the atmosphere. Supersaturation occurs when there are more oxygen molecules dissolved in the water than it is capable of holding at the current temperature. For example, supersaturated solutions of oxygen frequently occur in cold and turbulent streams. The turbulence forcibly mixes oxygen into the water from the air. The cold water is capable of dissolving a certain amount of oxygen. If the water warms up, the oxygen will diffuse out of the water into the atmosphere because the water is no longer capable of holding that amount of oxygen at the higher temperature. Therefore, when testing for dissolved oxygen, it is possible to have more than 100% saturation.

Dissolved oxygen levels vary with water temperature and altitude. Warm water holds less oxygen than cold water. That's because the action of water molecules is faster in warmer water and that forces the oxygen molecules out of the water. Water at a higher altitude holds less oxygen due to lower atmospheric pressure to hold it in solution.

Dissolved oxygen levels fluctuate daily and seasonally. The lowest levels of dissolved oxygen occur in the early morning just before dawn. Throughout the night, the processes that consume oxygen (respiration, oxidation, and decomposition) have continued, while photosynthesis has stopped. As the sun rises, and plants begin to photosynthesize, the oxygen level will increase to a high point in the afternoon.

In larger deeper rivers, as with lakes (see following lakes section), some vertical variation in dissolved oxygen levels may occur. Changes in dissolved oxygen are more likely to occur along the course of the river. That's because the processes that add and consume oxygen change upstream to downstream. For example, in high altitude, forested, mountain streams, high aeration from falling water and cooler water temperatures will tend to cause high and relatively stable dissolved oxygen levels. In lower elevations, less shading, warmer air temperatures, less aeration, and more biological activity may produce wide fluctuations. The DO levels in and below riffle areas or water falls are typically higher than in pools and slower moving stretches.

River discharge (the volume of water flowing past a point in the river over a given time) has an effect on DO levels. During dry periods, discharge may be severely reduced, causing warmer water and decreased DO levels.

Lakes:

The principles of how oxygen is used by aquatic life is the same for lakes and ponds as it is for rivers. However, in contrast to rivers, where dissolved oxygen levels are fairly constant throughout the water column because the water is usually well-mixed, the DO levels in lakes are more likely to vary vertically in the water column. Also, the ways in which oxygen enters and mixes with the water in lakes differs from rivers. Groundwater entering a lake or pond, which may be naturally low in oxygen, can decrease the overall oxygen levels.

In shallow ponds there is usually complete mixing of the pond waters by wind and wave action, during most of the year. However, during hot, windless summer days, oxygen depletion can occur near the bottom of shallow ponds. As with rivers, in these ponds the lowest levels of dissolved oxygen are found just before dawn, since oxygen consumption has been occurring throughout the night, without the replenishment of oxygen as a result of photosynthesis (see rivers section). During long winters, when the pond is covered by ice, oxygen depletion may also occur.

The situation is very different in deep ponds and lakes, defined for our purposes as having a maximum depth of greater than 5 meters. In deep lakes, rapid changes in water density due to thermal stratification (see temperature background - lakes section) limit the movement of oxygen from the oxygenated surface into the deeper waters. When the water cools down in the fall and eventually reaches the same temperature at all depths, the water column mixes and the bottom waters become resupplied with

oxygen. This is also discussed in the section on temperature (see Temperature Changes In Aquatic Ecosystems - Lakes).

If the deep waters remain oxygenated, there is a good chance for cool water fish, such as trout, to survive and reproduce. If the deep waters lose their oxygen, fish must move to warmer, shallower water in order to survive, or may die if their systems cannot adapt to such a change. In water without oxygen, nutrients are released from the bottom sediments and suspended in the water column. When the surface water temperature cools in the fall and the stratification is broken down, an algal bloom can result from the nutrients now resuspended throughout the water column.

Depletion of dissolved oxygen in the bottom waters deep lakes and ponds is an indication of the productivity of that water body. Oxygen is used up in the breakdown of organic matter and the more productive lakes (the ones that produce more organic matter) will have greater oxygen depletion than a less productive lake. Organic productivity in fresh waters is often related to the concentration of the element phosphorus or one of its related compounds such as phosphate. This is because all plant life requires phosphorus for growth and it is usually the element that is least available in fresh waters and therefore considered a “limiting” nutrient. When phosphorus is supplied to a fresh water (such as from runoff from lawn and garden fertilizers or sewage or septage) plant growth is allowed to flourish and a water body becomes very productive. Productivity in a lake can be beneficial because more plant life makes more animal life possible, however, it also means that more organic matter will be found in the water column which depletes the oxygen and reduces the ability of most animal life to survive.

Because of the problems noted above, volunteers who monitor lakes may want to add phosphorus to their list of monitoring parameters. The VEMN did not include phosphorus as a core parameter, because it is not a parameter for which there are any state water quality standards, and therefore does not fit into that assessment.

How Do Humans Affect the Amount of Dissolved Oxygen in Water?

The human causes of changes in DO fall into five main categories:

- 1) **addition of oxygen consuming wastes:** These include organic material such as sewage and animal manure, organic fibers from textile and paper processing, and food wastes from food processing. Organic materials are decomposed by microorganisms which use oxygen in the process (aerobic bacteria). The amount of oxygen needed by these organisms to break down the waste is known as the biological oxygen demand or BOD. Wastewater from sewage treatment plants and failing septic systems, stormwater runoff from farmland or urban streets, feedlots, and discharges from food processing plants, dairies, and meat packing plants are all sources of organic waste.
- 2) **addition of nutrients:** Nutrients can cause dramatic increases in biological activity that both add and remove oxygen from the water. These stimulate the extensive growth of algae and other aquatic plants (eutrophication). When these die, bacteria

consume oxygen to decompose them. A significant part of urban and agricultural runoff is fertilizers.

- 3) **addition of chemicals:** Some chemicals and many salts react with oxygen, effectively making it unavailable for aquatic life.
- 4) **changing the flow of water:** This affects the physical aeration process. Examples include the flooding of riffles and waterfalls behind a dam, reducing the flow from withdrawals for various uses (e.g. water supply and irrigation), and flow management for hydropower generation, and the warming of water in the pools behind the dam by sunlight. A sample site behind a dam will probably have much lower oxygen levels than one immediately downstream of the spillway. The site below the spillway will probably have higher levels than in a quiet section of the river upstream of the water impounded by the dam.
- 5) **activities which raise water temperatures.** These include removing the vegetation from stream-banks, (this increases the level of sunlight on a river, increasing the temperature of the water), thermal pollution from the release of heated water used as a coolant in industrial processes and power production heats the water, and low flow rates caused by dam controlled water and water removal for irrigation or snow-making flow management may cause warmer water in the summer months.

What Effect Does Dissolved Oxygen Have On The Aquatic Ecosystem?

Dissolved Oxygen is critical for many of the biological and chemical processes in the river. It's necessary for respiration, for decomposition, for converting nutrients to useful forms, for converting some chemicals to less harmful forms, and other essential functions.

All aquatic animals have mechanisms for taking in dissolved oxygen. Many have gills such as fish and some insect larva and salamanders. Others can absorb oxygen directly through their skin. Some, such as the predaceous diving beetle actually rise to the surface, capture an air bubble and carry it with them. The amount of dissolved oxygen determines the suitability of an area for specific organisms. Some animals can survive with less oxygen, such as carp and catfish. Others require more, such as pike and trout. Most fish need more dissolved oxygen in the early stage of their life cycle, just after egg hatch through their juvenile stage. Waters too low in dissolved oxygen or subject to changes will be unsuitable for oxygen-sensitive species.

Too much dissolved oxygen can be a problem which can occur mostly in rivers. Highly turbulent waters in hydro dam turbines and at spillways can create supersaturated conditions that are dangerous to fish. This is called gas bubble disease. Fish can get this by swimming through turbine blades or over the spillway to get upstream. As fish breathe in this supersaturated environment, the oxygen level in their blood rises. When the fish leave these areas, the oxygen gas bubbles dissolve out of their blood and the fish may die.

Supersaturated conditions will not last as the water flows downstream. As the water slows or warms up, the excess dissolved oxygen will diffuse into the air.

How is Dissolved Oxygen (DO) Measured?

Dissolved oxygen can be measured using either a meter or titration. The meter electronically measures the diffusion of oxygen across a membrane. Titration involves “fixing” the amount of oxygen in the sample through a chemical reaction which produces iodine in direct proportion to the amount of O₂ in the water and turns the sample a brown-yellow color. Sodium thiosulfate is then added incrementally. The amount of sodium thiosulfate it takes to turn the solution clear is proportional to the amount of iodine (which has taken the place of DO) in the sample.

DO is measured in milligrams per liter (mg/L) or “percent saturation”. Milligrams per liter is the amount of oxygen dissolved in a liter of water. Percent saturation is the amount of oxygen in a liter of water relative to the total amount of oxygen that the water can hold at a given temperature.

Monitoring Considerations

It is important to note the time of day when you test for dissolved oxygen. Because the concentration of oxygen in the water changes during the course of the day, it is important to try to collect samples at the same time each day. The most critical time for many aquatic animals is early morning on hot summer days, when river flows are low, water temperatures are high, and dissolved oxygen levels are likely to be at their lowest. If monitoring the lowest levels of oxygen are important to you, then testing in the early morning is important. Make sure to record the time on your field sheet.

Sampling Considerations

The DO levels in and below riffle areas or waterfalls are typically higher than in pools and slower moving stretches. A sample site behind a dam will probably have much lower oxygen levels than one immediately downstream of the spillway. The site below the spillway will probably have higher levels than in a quiet section of the river upstream of the water impounded by the dam. If you wanted to measure the effect of the dam, dissolved oxygen data from all three sites would be important to monitor. Since DO is critical to fish, sample sites might be located in the pools which fish tend to favor, or in the spawning areas they use.

An hourly time profile of dissolved oxygen levels at a sample site is a valuable set of data because it shows the change in DO levels from its low point just before sunrise to its high point sometime in the mid-day. However, this may not be practical for a volunteer monitoring program. So, it is important to note the time of your DO sampling to help judge when in the daily cycle the data occurred.

DO samples are collected by filling a special bottle with a ground glass stopper, called a BOD bottle. The bottle can be filled directly in the stream or lake if it is wadeable or boatable, or by using a sampler which is dropped from a bridge or boat into water deep enough to submerge the sampler. Samplers can be made or purchased.

Dissolved Oxygen Procedure

This section contains procedures for two methods to monitor dissolved oxygen:

- 1) Modified Winkler Titration
- 2) Meter and Probe

1) Summary of Method: Modified Winkler Titration

In this method, dissolved oxygen is analyzed using the Hach Azide Modification of Winkler Method with a Digital Titrator. The sample is collected in a 300-mL BOD bottle and “fixed” in the field using Manganous Sulfate and Alkaline Iodide-Azide. This produces manganese hydroxide (a brown flocculent). Sulfamic Acid is then added. The acid converts the floc to its equivalent of iodine in proportion to the oxygen concentration. At this point, the sample can be titrated in the field or transported to a lab and held in the dark up to 8 hours. Sodium thiosulfate is then added to this solution until the solution becomes colorless. The amount of dissolved oxygen in the sample is calculated from the quantity of sodium thiosulfate used.

Type of Container: 300 mL BOD bottle

Sample Volume: 300 mL

Maximum Holding Time: Fix in field, analyze in field or lab within 8 hours.

Equipment: see list at end of this procedure

2) Summary of Method: Meter and Probe

A dissolved oxygen meter is an electronic device that converts signals from a probe that is placed in the water into milligrams per liter of dissolved oxygen. The probe is filled with a salt solution and has a selectively permeable membrane that allows DO to pass from the water into the salt solution. The DO that has passed into the salt solution changes the electric potential of the solution. This change is sent as an electric signal to the meter which converts it to milligrams per liter. Most DO meters also measure temperature.

Type of Container: The probe is placed directly into the river water.

Sample Volume: not applicable

Maximum Holding Time: not applicable

Equipment: DO meter

. **Winkler Titration Procedure**

Collecting a Water Sample - RIVERS

Use a 300 mL BOD sample bottle. The water sample must be collected in such a way that you can cap the bottle while it is still submerged. That means that you must be able to reach into the water with both arms and the water must be deeper than the sample bottle.

- 1) Carefully wade into the stream. Stand so that you are facing one of the banks. Take and record the temperature of the water.
- 2) Remove the cap of the BOD bottle. Slowly lower the BOD bottle into the water, pointing it downstream, until the lower lip of the opening is just submerged. Allow the water to fill the bottle very gradually, avoiding any turbulence (this will add oxygen to the sample). When the water level in the bottle has stabilized (it won't be full because the bottle is tilted), slowly turn the bottle upright and fill completely. Keep the bottle under water for a minute to ensure that no air bubbles are trapped in the sample.
- 3) Cap the bottle while it is still submerged. Lift it out of the water and look around the "collar" of the bottle just below the bottom of the stopper. If you see an air bubble, pour out the sample and try again.

Analyzing a Water Sample - RIVERS

- 4) "Fix" the sample immediately by:
 - a) Removing the stopper and adding the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Powder Pillow.
 - b) Immediately insert the stopper so air is not trapped in the bottle and invert several times to mix. **This solution is caustic – rinse your hands if you get any solution on them.** An orange-brown flocculent precipitate will form if oxygen is present.
 - c) Wait a few minutes until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled. This insures complete reaction of the sample and reagents.
 - d) Remove the stopper from the sample and add the contents of one Sulfamic Acid Powder Pillow. Immediately insert the stopper so air is not trapped in the bottle and invert several times to mix. The floc will dissolve and leave a yellow color if oxygen is present. The oxygen in the sample is now "fixed" and the sample can be stored in the dark for up to eight hours.

If you want to finish the test in the lab:

1. Cap the bottle firmly and seal it by pouring a small amount of water into the flared lip area. Place the bottle in a cooler in an upright position.
 2. Record the site # on the bottle and the field sheet.
 3. Transport the bottle in an upright position with the stopper firmly in place in a dark place, such as a cooler. Maximum holding time is 8 hours.
-
- 5) Examine the water samples. If any have brown particles in the bottom, shake the bottle, use a stir rod, or use a magnetic stirrer to dissolve them.
 - 6) Insert the 0.2N Sodium Thiosulfate cartridge into the digital titrator and insert a clean delivery tube into the cartridge.
 - 7) Over a sink, hold the titrator with the cartridge tip pointing up. Turn the delivery knob to eject air and a few drops of titrant. Be sure no air is trapped in the cartridge. Reset the counter to 0 and wipe the tip.
 - 8) Measure 100 mL of the sample volume from the “fixed” sample in the 300 mL BOD bottle and pour into a 250-mL Erlenmeyer flask.
 - 9) Place the delivery tube tip into the solution and swirl the flask while turning the delivery knob. Keep turning the knob until the solution turns a pale yellow color.
 - 10) Add two **full eyedroppers** of Starch Indicator Solution and swirl to mix the solution. This will turn the solution very dark blue.
 - 11) Continue titrating until the liquid turns clear. Record the number of digits required.
 - 12) Calculate mg/L of DO = Digits Required X .02 and record on lab sheet.
(0.02 is the correct digit multiplier for the 0.2N concentration of the Sodium Thiosulfate cartridge)

Collecting a Water Sample - *LAKES*

The following is a method for using a Modified Wisconsin Sampler to collect a DO sample. Other samplers (including home-made ones) and methods are valid and produce scientifically “good” data, however, we could not list every possible method here. Modify this method according to your equipment instructions. Between the river and lake methods listed, you should be able to find adequate guidance for collecting and analyzing DO samples.

DO samples, like other samples collected from lakes, should be taken at the deepest point in the lake.

- 1) Determine the depth at which you will collect your samples. A convenient way to do this is to lower the Secchi Disk until the line goes slack. Retrieve the line until all slack is removed. Record this bottom depth on your data sheet. Subtract 0.4 meters (two marks on the sampler's lowering line) from this depth. This will be the depth to collect the dissolved oxygen sample.
- 2) Lower Wisconsin sampling bottle to desired depth. Check that no bubbles are coming from the sampler. Give a short, sharp yank on the lowering line to pull intake and outlet plugs. Wait until all bubbles disappear at the surface. Retrieve the sample bottle.
- 3) Carefully unscrew and remove sampler top to avoid spilling any water. Extract D. O. bottle from sampler. Place the thermometer in the Wisconsin sampling bottle (not in the D. O. bottle).

Analyzing a Water Sample - *LAKES*

- 4) “Fix” the sample immediately by:
 - a) Adding the contents of one Manganous Sulfate Powder Pillow and one Alkaline Iodide-Azide Powder Pillow to the sample. Do not be concerned if a small amount of water overflows the bottle, but do be careful not to introduce any bubbles.
 - b) Immediately insert the stopper so air is not trapped in the bottle and invert several times to mix. **This solution is caustic – rinse your hands if you get any on them.** An orange-brown flocculent precipitate will form if oxygen is present. (You will notice that more of this will form with higher D.O. samples than low.)
 - c) Wait a few minutes until the floc in the solution has settled. Again invert the bottle several times and wait until the floc has settled. This insures complete reaction of the sample and reagents.
 - d) Carefully remove cap of D.O. bottle by twisting slightly and lifting. Add the contents of one Sulfamic Acid Powder Pillow. Immediately insert the stopper so air is not trapped in the bottle and invert several times to mix. The floc will dissolve and leave a yellow color if oxygen is present. The

oxygen in the sample is now “fixed” and the sample can be stored in the dark for up to eight hours.

- 5) As the sample temperature will rise in your boat, it is advisable to use a depth electronic thermometer. If you don't have one, however, read the manual thermometer in the WI sampler and record in °C on the field data sheet. Repeat this procedure at this site and depth, filling and fixing a second (replicate) D.O. bottle.
- 6) Uncap sample bottle and pour sample into 50 mL volumetric flask to the line or, if volumetric flask has been specially cut off at 50 mL line, to overflowing. Careful measurement is crucial to accurate measurement of dissolved oxygen. Simply reading the meniscus incorrectly can introduce an error of 2-5%. No special care needs to be taken to avoid introducing bubbles into the sample.
- 7) Pour sample from volumetric flask into beaker or standard flask, insert magnetic stirring rod and place on bright white paper on the magnetic stirrer. Set stirrer to mix sample without splashing.
- 8) Set digital titrator at zero.
- 9) Begin titrating the standard solution. (You can go pretty quick at first). Continue until the solution turns from a strong yellow to a pale yellow.
- 10) Add a 5 drops of the starch indicator solution (enough to turn the standard solution from pale yellow to dark blue).
- 11) Continue titrating, adding about 5 to 10 digits every few seconds. As the color turns to light blue, slow down, adding about 2 digits every few seconds until the blue just disappears.
- 12) Write the number of digits down, but then continue titrating by adding one more digit as you look carefully for a blue swirl. Keep doing this every few seconds, one digit at a time until your last digit causes no visible change. Record the previous digit as the correct amount. For instance, if you saw a change at 194 digits, but none at 195, write down 194 as the units of titrant used.
- 13) Calculate the dissolved oxygen of the sample by this formula:
Dissolved oxygen (milligrams per liter) = digits of titrant x .04
Example: $194 \times .04 = 7.76$ mg/L DO, or 7.76 parts per million
- 14) Rinse sample bottle, volumetric flask and beaker once with distilled water.

..... Meter Procedure

Preparing the Meter

- 1) Check cable connections between the probe and the meter
- 2) Make sure that the probe is filled with electrolyte (salt) solution, that the membrane has no wrinkles, and that no air bubbles are trapped on the surface of the membrane.
- 3) Calibrate the meter according to the manufacturer's directions.
- 4) Leave the meter on until samples are analyzed.

Analyzing a Water Sample

- 1) Place the probe in the water below the surface.
- 2) Set the meter to measure temperature, and allow the temperature reading to stabilize. Record on the field sheet.
- 3) Switch the meter to read dissolved oxygen.
- 4) Allow the reading to stabilize and record on the field sheet.

..... Clean Up

1. Wash all glassware with a brush and non-phosphate detergent.
2. Rinse with hot tap water
3. Rinse three times with distilled, de-ionized water.

..... Troubleshooting Hints for the Lab.

- 1) Some brown particles may remain when sample is ready for titration. This can cause variable results because chemicals in the sample are now unevenly concentrated.

TO AVOID THIS: Carefully observe the BOD bottle after adding all three reagents. If particles are visible, or if there is a deposit on the bottom of the bottle, try shaking the bottle to dissolve any remaining solid matter. If this doesn't work, use a plastic, Teflon, stainless steel or glass stirring rod or spatula to stir up the bottom sediments. This should allow the acid in the solution to fully dissolve the particles. You are then ready to titrate.

NOTE: Make sure you rinse the stirring rod well after trying this, to avoid corrosion of your utensil and dry the rod so any clinging drops of rinse do not dilute the sample.

- 2) If your results seem wildly inaccurate, check to see you are using the correct Sodium Thiosulfate cartridge. Some folks have been known to use a sulfuric acid cartridge by mistake (that cartridge is used for pH and alkalinity analysis).
- 3) If you have titrated a quality control sample from the standard solution received from MassWWP and your value seems very high (remember, DO almost never goes above 14 mg/L in natural settings), it may be because you added powder pillow #1 to the standard before titrating. This QC test only uses pillows #2 and #3.
- 4) Sometimes an old cartridge can give an inaccurate reading, particularly if it has been left uncapped and allowed to evaporate somewhat. If you suspect the cartridge, try using a new one. Starch indicator bottle and thiosulfate cartridges should be refrigerated when not in use. This slows the deterioration that occurs rapidly at room temperature.

..... Calculating Percent Saturation

Some water quality standards are expressed in terms of percent saturation. To calculate the percent saturation of the sample:

1. Find the temperature of your water sample as measured in the field.
2. Use the table below to find the maximum concentration of dissolved oxygen in water at that temperature.
3. Divide your actual dissolved oxygen measurement by the maximum dissolved concentration at the same temperature.

$$\frac{\text{mg/L DO measured in your sample}}{\text{mg/L maximum DO at the same temperature}}$$

4. Record the percent saturation in the appropriate column on the lab sheet.

Example:

You measured a dissolved oxygen concentration of 5 mg/l at 20 ° C.

Divide 5 mg/l by 9.07, the maximum concentration at 20 degrees C.

The percent saturation would be 55%.

Note about percent saturation: It is possible to get more than a hundred percent saturation. The sample can be supersaturated if your sample is in a fast moving area over rocks or it is a sunny day causing a lot of photosynthesis.

Table 3. Maximum Dissolved Oxygen Concentrations

<i>Temperature</i>	<i>Dissolved Oxygen</i>	<i>Temperature</i>	<i>Dissolved Oxygen</i>
°C	mg/L	°C	mg/L
0	14.60	23	8.56
1	14.19	24	8.40
2	13.81	25	8.24
3	13.44	26	8.09
4	13.09	27	7.95
5	12.75	28	7.81
6	12.43	29	7.67
7	12.12	30	7.54
8	11.83	31	7.41
9	11.55	32	7.28
10	11.27	33	7.16
11	11.01	34	7.05
12	10.76	35	6.93
13	10.52	36	6.82
14	10.29	37	6.71
15	10.07	38	6.61
16	9.85	39	6.51
17	9.65	40	6.41
18	9.45	41	6.31
19	9.26	42	6.22
20	9.07	43	6.13
21	8.90	44	6.04
22	8.72	45	5.95

. . . . Equipment Needed for Winkler Titration

___ DO Test Kit

Hach DO Test Kit (for 300 mL samples - *RIVERS*) (for 60 mL samples - *LAKES*)

___ 300 mL BOD Bottles (1 per site) - *RIVERS*

___ Modified Wisconsin Sampler with 60 mL Dissolved Oxygen bottle inside - *LAKES*

___ Extra 60 mL Dissolved Oxygen bottle for replicate samples - *LAKES*

___ Calibrated Line for Modified Wisconsin Sampler - *LAKES*

___ Manganous Sulfate and Alkaline Iodide Azide Powder Pillows

___ Nail Clipper (to open powder pillows)

___ Field Thermometer

___ Cooler with ice packs

___ Hach Digital Titrator

___ Magnetic Stirrer (optional)

___ Sulfamic Acid powder pillows

___ Hach Sodium Thiosulfate Titration Cartridge 0.2 N: for DO in the 0-10 mg/l range

___ Starch Indicator Solution

___ Graduated Cylinder, 250 mL

___ Erlenmeyer Flask, 250 mL

or comparable test kit from another manufacturer

___ Lab Sheet

pH – background

What is pH and why is it important?

pH is a measure of how acidic a solution is in terms of its hydrogen ion concentration. Ions are charged atoms that come from various compounds, including water, and react with other charged atoms or molecules. Some water molecules (which are composed of one oxygen atom and two hydrogen atoms) dissociate (break apart) and produce hydrogen ions (H^+) and hydroxide ions (OH^-). When water dissociates, it produces equal number of hydrogen and hydroxide ions. Other compounds that enter the water might also break apart, adding ions to the solution. The ions of water and those of the compound will combine (react) with each other. Sometimes there are more hydrogen ions left after this reaction than hydroxide ions. In this case, the solution is acidic. At other times there are more hydroxide ions than hydrogen ions. In this case, the solution is basic or alkaline.

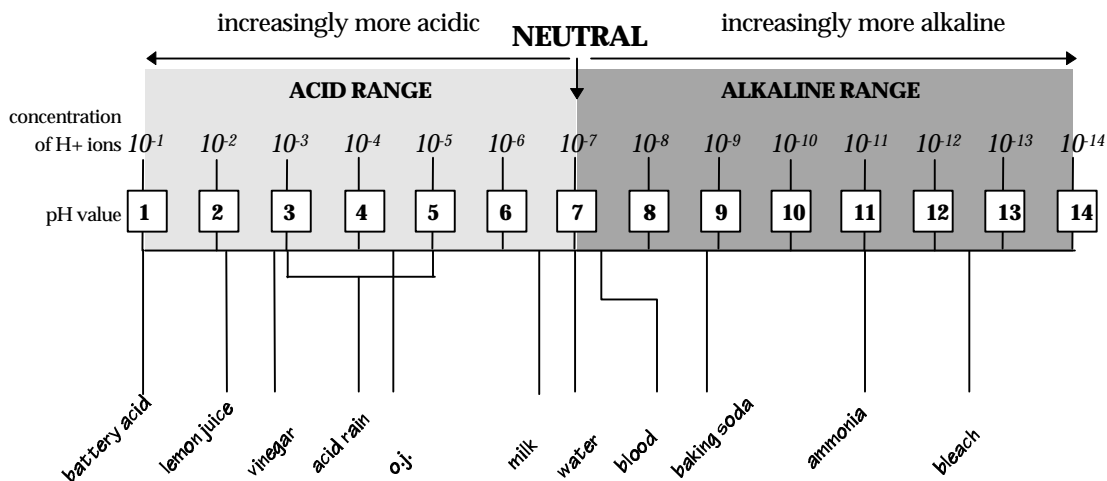
The term pH (which comes from the Latin Expression “potentia hydrogenii” -- hydrogen potential) is another way to express the concentration of hydrogen ions.

The pH is critical to the aquatic ecosystem because it affects many chemical and biological processes in water.

How do we measure pH?

pH is measured on a scale of 0-14. A pH of 7 has equal amounts of both hydrogen ions (H^+) and hydroxide ions (OH^-) and is considered neutral. Numbers less than 7 are acidic, and have more H^+ ions. Numbers greater than 7 are alkaline or basic and have more OH^- ions. The lower the number, the stronger the acid. The higher the number, the stronger the base. An pH of 1 is a very strong acid, a pH of 14 is a very strong base.

The pH Scale:



pH is actually a mathematical expression of the concentration of hydrogen ions in the water. It is the negative logarithm of this concentration. pH is displayed on a logarithmic scale. A pH of 1 has 10^{-1} (0.1) concentration of hydrogen ions (one H⁺ ion for every 10 water molecules), a pH of 2 has 10^{-2} (0.01) concentration of hydrogen ions (one H⁺ ion for every 100 water molecules) and so on. We use the pH scale because these numbers are easier to work with than the actual concentrations.

There is 10 fold increase in acidity for every change of one unit of pH. If one solution has a pH that is two units lower than a second, the first one is actually 100 times more acidic than the second. A three unit change creates a thousand times difference in acidity, and so on. This means that a river with a pH of 5 is 100 times more acid than a river with a pH of 7.

What causes a pH change?

Changes in pH can be caused by atmospheric deposition (acid rain, snow, and dry particles), the surrounding soil and rock, and wastewater discharges from business and industry. A major flush of acid can occur in streams and ponds in the spring if the snow melt is acidic. Acid deposition is caused by the burning of fossil fuels by cars, factories, and smelters.

How does pH affect aquatic ecosystems?

pH affects many chemical and biological processes in water, such as availability and toxicity of nutrients, metals, and other important compounds.

Different organisms have different ranges of pH in which they flourish. The largest variety of aquatic animals prefer a range of 6.5-8.0. pH outside of this range reduces the diversity in the stream. Lower pH's particularly affect the immature stages of aquatic insects and fish. A low pH creates conditions where heavy metals leach (dissolve) into the water from the soil and sediment. These can cause deformities in immature fish and can accumulate on fish gills, effecting how well they take in oxygen.

Important in determining the effects of acid in water is its "acid neutralizing capacity" or alkalinity. Water with a high acid neutralizing capacity has the ability to accept large inputs of acids and maintain a relatively constant pH level. For that reason, alkalinity is an important indicator to measure along with pH, although because it is not a parameter for which there are state standards, alkalinity is not one of the recommended core VEMN monitoring parameters.

Monitoring Considerations

There are a variety of methods to measure pH. Choose the method that is most appropriate for your purposes. Below is a description of the options you have for measuring pH.

pH Meters and probes:

A pH meter measures the electric potential (energy expressed as millivolts) across an electrode when immersed in a water sample. This electric potential is a function of the hydrogen ion activity in the sample. Therefore, pH meters can display results in either millivolts (mV) or pH units.

A pH meter consists of a *potentiometer* which measures electric current, a *glass electrode* which senses the electric potential where it meets the water sample, a *reference electrode* which provides a constant electric potential, and a *temperature compensating device* which adjusts the readings according to the temperature of the sample (since pH varies with temperature). The reference and glass electrodes are frequently combined into a single probe called a *combination electrode*.

There is a wide variety of meters, but the most important part is the electrode. Buy a good, reliable electrode and follow the manufacturer's instructions for proper maintenance. Infrequently used or improperly maintained electrodes are subject to corrosion, which renders them highly inaccurate.

A good quality pH meter measures to 0.01 pH units.

“Pocket pals” are cheaper than a meter/electrode, but do not last as long, and only measure to 0.1 pH units.

pH Paper:

Paper strips are coated with a solution called an indicator, which changes color as the pH changes. This color change is then compared to a scale. The pH paper is the cheapest option, but it measures to only 1 pH unit at best.

Sampling Lakes V. Rivers

Rivers mix continually unlike lakes which layer in the warm weather (see Temperature Background). Therefore the pH sampling procedure for lakes and rivers is quite different. In lakes it is important to take ***an integrated sample*** - a well mixed vertical “slice” of water. The procedure for obtaining an integrated sample will immediately follow this section. After sample collection, laboratory analysis of both river and lake samples are identical.

pH Procedure For *Lake* Sample Collection - The Integrated Sample

Summary of Method: Conceptually, you will be taking a core or slice of water from the surface to a particular depth. You will mix this vertical slice of water and conduct one analysis.

Type of Container: polyethylene or glass

Sample Volume: 500 mL

Maximum Holding Time: analyze immediately

Equipment:

- Integrated sampler (garden hose weighted at one end)
- Calibrated line
- Two 500 mL sample bottles
- Mixing bucket (calibrated to at least 4 quarts)
- Cooler and Koolits or ice

..... **Lake** Sample Collection Procedure

- 1) Rinse the mixing bucket and the 500 mL bottles three times with surface water at the sampling site.
- 2) Lower the integrated sampler straight down to a depth three times the Secchi disk depth or top to bottom if three times the Secchi disk depth is more than the depth of the lake. Tossing the hose over the side of the boat will not work: pretend that the tube is rigid and must be lowered vertically.
- 3) Crimp the top of the sampler by hand (the crimp may be held by needle-nose vise grips or a valve may be inserted in the top end).
- 4) With the tube firmly crimped, pull the submerged end of the integrated sampler back up to the boat. The submerged end should emerge from the water with the opening facing up and with little loss of water.
- 5) Place the previously submerged end into the mixing bucket and raise the crimped end of the integrated sampler above the height of the mixing bucket. Then release the crimp. Working your way toward the previously submerged end, raise the sampler to drain the water into the bucket.

Collect enough integrated samples at each sampling site to fill the mixing bucket to the four quart mark or above.

- 6) Swirl the mixing bucket to mix the samples.
- 7) Pour a mixed sample from the bucket into one 500 mL sample bottle and store in cooler.
- 8) Repeat steps 1) - 7) for a replicate sample using the other 500 mL sample bottle.

pH Procedure For Laboratory Analysis

Summary of Method:

Type of Container: polyethylene or glass

Sample Volume: 100 mL

Maximum Holding Time: analyze immediately

Equipment:

- 250 mL beaker
- Wash Bottle with de-ionized water: to rinse pH meter electrode
- pH meter w/ combination temperature and reference electrode
- pH standard buffer solutions: pH 4.00 or 4.01 and pH 7.00 (we'll refer to these buffers as 4 and 7 for the rest of this section)

Choosing Equipment: There are a multitude of ways to measure pH. Before you decide which equipment to use, you need to determine the level of pH accuracy you need. If you are using it solely for background information, you can use the paper strips. The degree of sensitivity for strips is whole units, 1-14. It is the least expensive method. Pocket Pals measure to the tenth decimal point and tend to last 1-2 years. pH meters measure to the hundredth decimal point. The probe that comes with the meter needs replacing approximately 1-2 years.

If you are measuring for alkalinity as well, it is important to measure pH to the tenth decimal point, so you'll need to use either a pocket pal or the meter. Often universities and schools have old meters. If you are using an old meter, make sure that it is accurately calibrated before using it (see calibrating instructions in the meter manual).

..... Lab Procedure

Using the Meter

Calibrate the pH Meter:

Install the electrode(s) as described in the pH meter manual. Prior to calibration, the electrode should soak in the pH 4 buffer for at least one day. Then, the electrode must be rinsed with deionized water just before calibrating the meter. The pH meter should be calibrated prior to sample analysis and after every 10 samples per the instructions in the meter manual or if the meter has been unused for a significant period of time (such as after winter storage). Usually, instructions are as follows:

- 1) Use two pH standard buffer solutions: 4 and 7.
- 2) Insert the electrode in pH 7 buffer and adjust the calibration dial until exactly pH 7.00 shows on the meter.
- 3) Remove the electrode and rinse with deionized water.
- 4) Place the electrode in pH 4 buffer and adjust the slope until the meter shows pH the appropriate value (4.00 or 4.01 depending on your buffer). Rinse with deionized water.
- 5) Test the pH 7 buffer again.
- 6) If necessary, repeat the calibration.

Notes regarding buffers:

- * The buffer solutions should be at room temperature (68°F) when you calibrate the meter.
- * Do not use buffers after their expiration date.
- * Do not use buffers that have mold growing in them.
- * Always cap the buffers during storage to prevent contamination.
- * Do not pour used buffer back into the bottle.
- * Buffer pH values change with temperature, so it is best to use a meter with a built-in temperature sensor that automatically standardizes the pH when calibrating the meter. If your meter does not have a built-in temperature sensor, record the temperature of your buffer solution and refer to the chart below (interpolating the expected value if your buffer temperature is between the temperatures listed) before standardizing the meter.

<u>Temperature °C</u>	<u>Temperature °F</u>	<u>pH 4.0</u>	<u>pH 7.0</u>
0	32	4.003	7.119
5	41	3.998	7.086
10	50	3.996	7.058
15	59	3.996	7.035
20	68	3.999	7.015
25	77	4.004	7.000
30	86	4.011	6.988

To measure pH in the lab

- 1) If samples have been refrigerated prior to analysis, allow them to come to room temperature before analyzing them (about one hour). Keep the bottle capped while it is warming up, to prevent the sample from coming into contact with the air.
- 2) Rinse the 250 mL beaker with de-ionized water prior to each measurement. This is particularly important if you are analyzing multiple samples.
- 3) Measure 100 mL of the water sample in the 250 mL beaker and place on a magnetic stirrer, or stir manually when measuring the sample.
- 4) Rinse the electrode well with de-ionized water.
- 5) Follow the manufacturer's instructions for using the meter. Place the pH meter or electrode into the sample. Read the temperature and pH and record in the appropriate columns on the data sheet. Rinse the electrode well with de-ionized water.
- 6) Measure the pH of the 4.01 and 7.0 buffers periodically to assure that the meter is not drifting off calibration. If it has, you'll need to recalibrate.

To measure pH in the field

- 1) Place pH meter probe in the water and read pH or ;
- 2) Collect a sample and measure pH immediately using instructions above.

A Note About Quality Control:

Calibrating your meter with a buffer solution prior to measuring pH and alkalinity provides an important quality control check. However, do not assume that a meter that measures a commercial buffer solution accurately will also provide accurate measurement of your field sample. Buffer solutions generally have a high ionic strength (ions other than H⁺ ions). The high concentration of ions tends to “improve the connection” for the pH meter, and thus the meter responds more quickly instead of drifting slowly, so it is easier to measure pH. Your meter may be able to measure the standard pH 4 and 7 buffered solutions easily, but still have trouble with river and lake water samples, which are likely to have much lower concentrations of ions. For this reason, the quality control samples which the Massachusetts Water Watch Partnership (MassWWP) sends to participating Massachusetts monitoring groups contain low ionic strength. Contact MassWWP for information about joining its QC program.

CARE OF YOUR PH METER

Note: This section should be used with a pH meter and its manual in front of you.

General electrode care and handling procedures are very important in your lab, because pH measurements will only be as good as the condition of your electrode(s). For greater accuracy in your measurements and longer electrode life, there are a few areas of electrode care with which you should be familiar.

Storage

Glass combination or separate pH and reference electrodes should be kept wet. The reference electrode requires a free-flowing junction, so be sure to maintain the reference filling solution at a level significantly above the storage or sample solution level at all times. This will provide a positive head pressure, which forces the filling solution out through the junction rather than the storage solution into the probe.

For long-term dry storage, the sleeve or plug should cover the filling hole to reduce any flow of filling solution. During calibration or short-term storage in pH 4 buffer, this sleeve or plug must be slid away or removed to allow flow of the reference solution into the sample.

To obtain a faster electrode response, the glass electrode should be stored in a slightly acidic solution. In the protective cap for the glass electrode, put a drop or two of pH 4 buffer and put the cap on the electrode, carefully. Distilled water extracts ions from the bulb causing a slower response; pH 7 buffer over a long time period ages the electrode slightly.

If using a separate reference electrode, the best solution would be to place the reference electrode in its own filling solution but this can be messy. Providing

KCl to both sides of the junction keeps it flowing freer. To reduce the salt crust of saturated solution, an approximately 0.1 M KCl solution may be used, but for storage only. Experience indicates that simply covering the filling hole with the protective sleeve and storing dry suffices in most instances as long as the soaking procedure is followed.

For combination electrodes, store the electrode in a combined solution of approximately 0.1 M KCl in pH 4 buffer.

If the reference electrode is to be stored for more than four months, it should be emptied of liquid and stored dry in a sleeve. During the sampling season, keep it immersed in pH 4 buffer.

One day or more prior to analysis, soak both electrodes in fresh pH 4 buffer. During analysis, place the electrodes in the same buffer and store them in the buffer when not in use.

Reference Electrode Filling Solution

Read the instructions that came with your electrodes carefully. When filling electrodes or replacing the solution, use whatever solution is called for in the instructions. When in doubt, call MassWWP and we will advise you. Be sure to ascertain which filling solution is correct for your electrode(s) and double check that your filling solution matches these requirements.

NOTE: Due to their unique micropore junction, it is recommended that permanently filled or Gel electrodes be stored hanging dry.

Preliminary Electrode Response Testing

If your electrode exhibits slow response, poor span between two buffer values or undue sensitivity to movement of the electrode, rejuvenation may be necessary to improve performance.

Response varies with the electrode and the solution it is in. Generally working electrodes reach 0.05 pH units of the final reading in buffer within 10 seconds. A stable reading (less than 0.01 pH units per minute change) should be reached in fresh water samples within a minute or two. If you have to wait too long (5 minutes or more) then the pH itself may change due to the contact of the water sample with air.

Electrodes may also require adjusting the slope to values significantly different from 100% for two point calibration. Perform the following test if in doubt:

Set your meter to 100% slope and room temperature, then standardize as usual with pH 7 buffer. Without moving the slope dial, read a pH 4 buffer. It should read between 3.85 and 4.15; set the slope to read pH 4, the slope should be 95% to 105%.

If your electrode exhibits either of the above problems or is sensitive to movement, rejuvenation is in order.

Glass Electrode Rejuvenation

To treat the bulb of the pH electrode:

Contact us on how to obtain 2 bottles of acid and base (0.1N). BE CAREFUL WHEN HANDLING THESE SOLUTIONS - USE RUBBER GLOVES AND WEAR PROTECTIVE EYEWEAR. IF YOU GET ANY ON YOU RINSE IT OFF WITH LOTS OF WATER.

To treat, simply dip the bulb into the acid and immediately into the base. Repeat this several times. Then rinse the electrode under tap water and let sit in pH 4 buffer for 1/2 hour. Rinse the electrodes and restandardize as you normally do with pH 7 and pH 4 buffers. You may need to do this several times a year.

Another treatment is to gently wash the pH bulb with a tissue soaked in methanol. Rinse with water and soak in pH 4 buffer.

To treat the reference electrode:

Replace the 4M KCl solution in the reference electrode and get rid of crystals that may have formed. If there are lots of crystals, then shake out the solution and put deionized pure water into the filling hole and soak the electrode tip in hot tap water for 15 minutes or so until the crystals have dissolved. Then shake all the liquid out of the filling hole in the reference electrode and refill with fresh 4 M KCl. Let the electrode sit at room temperature for 1/2 hour before use. Frequently add more 4M KCl solution to the reference electrode since it will continually leak out and evaporate. The solution in the electrode should be within 1/2 inch of the filling hole. The hole should be open when reading pH but close it when you are through for the day or else the solution will evaporate and new crystals will form. If you still have problems with slow response, try rubbing the tip on your blue jeans or on very fine (600 grit) sandpaper.

Final Test For Linearity

Standardize the meter as described below. Rinse the electrodes and your sample cup with pure deionized water. Then titrate 100.0 mL of deionized water with your 0.16N acid as follows: Make sure your digital titrator is working and reset to zero. Add 10 digits of acid, record digits and pH, increase acid to 20 digits, record pH; repeat until you have added 100 digits of acid and stop. Send the results to us and we will send you a report. If you want to see the results yourself, try plotting the hydrogen ion concentration ($H = 10^{-(pH)}$) vs. digits and see if the line is straight.

Movement Sensitivity

If your meter gives wild readings and is sensitive to your touch, it may not be properly grounded. Try using a three prong power plug or attach a wire from the meter to a cold water pipe. Sometimes a problem of fluctuating readings or consistently wrong readings can be solved by disconnecting and reconnecting the electrode connectors several times. Apparently an oxide layer can sometimes cause these symptoms.

Temperature – background

A. What Is Temperature and Why Is It Important?

Water temperature is a simple and important indicator to measure, because it affects the rates of many of the river's biological and chemical processes. The well-being of aquatic life in the river, from microbes to fish depends on temperature. Two kinds of exposures to temperature are important: *maximum* and *optimum*. The maximum is the uppermost temperature that fish can tolerate before they die or their growth and reproduction is adversely affected. The optimum temperature is that temperature at which growth and reproduction occurs most efficiently.

Temperature Changes In Aquatic Ecosystems

Rivers:

Water temperature is affected by a number of factors: air temperature, the temperature of groundwater inflows to the river, stormwater runoff temperature, the cloudiness of the water, amount of shading, and exposure to sunlight.

Temperature may vary across the width and depth of a river. The magnitude of this variation increases with the size and depth of the river. Small upland streams have a more consistent temperature than larger rivers due to the churning and relatively uniform mixing of the water in the upland stream. Upland streams often also are cooler due to shading from adjacent forests. In larger, deeper rivers, the water across the river and from top to bottom of the water column may not mix as uniformly. The surface tends to be warmer than the bottom; shaded areas near the bank and shaded reaches may be cooler than exposed reaches on sunny days.

Temperature fluctuates considerably from one season to another, with the warmest temperatures occurring in the summer months. Daily changes may be significant (as much as 6°C) in smaller streams, with the warmest temperatures being in the afternoon and the coolest at night. However, this varies depending on the character of the stream. Deeper streams, spring-fed streams, and shaded streams do not heat up as quickly. Larger streams do not fluctuate as rapidly because the more water there is, the longer it takes to heat up or cool down.

Lakes:

Note: The following information was borrowed from the 1994 University of Rhode Island Watershed Watch Report.

During the summer, lakes do not have uniform temperature from top to bottom, nor does temperature change uniformly with depth. Rather, they develop "stratification" which simply means two layers of water: an upper warmer and less dense layer that is fairly uniform in temperature (the epilimnion) and a lower colder and more dense layer that is also fairly uniform in temperature (the hypolimnion). Between the two is a region of sharp temperature change called the thermocline. In many respects, the two

layers are isolated from each other. The sharp temperature and density barrier prevents mixing of the two layers. Consequently, nutrients may accumulate as organisms die, settle to the bottom and decompose. Decomposition in the bottom waters will also reduce dissolved oxygen critical for fish life, even though the surface waters are saturated with oxygen. There are many other differences that make the measurement of the temperature with depth (temperature profile) critical to understanding a lake.

The thermocline depth changes throughout the summer and will differ between lakes. The size of the lake, its orientation to the prevailing wind and the weather (temperature and wind) determine the depth of the thermocline. In spring, after ice out, the thermocline begins to form near the surface as the lake warms. Increased warming will increase the thermocline depth. As the summer progresses, the thermocline deepens. By late summer, the temperature difference between top and bottom layers is greatest. But even when the summer ends and air and surface water temperatures cool, the thermocline continues to deepen, although the temperature differential between top and bottom layers begins to get smaller. Eventually, the temperature difference gets so small that the wind can force the thermocline to the bottom of the lake and completely mix the lake's water (see Figure 1).

Human Effects On Temperature

Human alterations that create an increase of temperature to streams and rivers include; removal of shading river bank vegetation, impoundments, discharge of warmed water used to cool industrial processes (thermal pollution), and urban storm water. The removal of trees and other bank vegetation reduces shade, allowing more direct sunlight to penetrate and heat the water. Impoundments, such as dams, cause the river to slow down and absorb more of the heat from the sun. If the pool behind the dam is larger than the original river channel, more of the water surface is exposed to sunlight. Storm water from developed areas is warmer because it absorbs heat from the paved surfaces (streets, parking lots) it runs off of before entering the stream. Nuclear power plants and other manufacturing industries may use water to cool the equipment, and discharge the heated water into the river. Erosion can also cause an increase in water temperature. The more erosion there is, more particles are in suspension in the water. These particles absorb heat from the sun and warm the water.

How Temperature Changes Affect Aquatic Life

Thermal pollution is the human input of warmed water into an aquatic ecosystem. This input, as well as an increase in temperature due to the activities listed above can have a great affect on the river ecosystem. Temperature affects the oxygen content of the water (warmer water holds less oxygen than cooler water, decreasing the amount of oxygen for the animals to breath); the rate of photosynthesis by aquatic plants; the metabolic rates of aquatic organisms; and the sensitivity of organisms to toxic wastes, parasites, and diseases. Every aquatic organism has a temperature range that is optimal for its health.

Growth of fish is affected by temperature because it directly affects their metabolic rate (the rate at which they process food). Fish and most aquatic organisms are cold-blooded. Therefore, their metabolic rate changes with the temperature. Some fish process food most efficiently in colder-water, while others prefer warmer waters. Each species of fish has a maximum average weekly average temperature for growth. Temperatures above this average will slow their growth.

Spawning (mating and laying eggs) success also depends on temperature. Each species has its own preferred temperature range for spawning. The survival of newly hatched embryos are dependent on certain temperature ranges for their optimal health. If temperatures are above this maximum for a prolonged time they will die. Fish migration is often triggered by temperature changes.

Fish in northern climates can usually withstand a wide range of temperatures. However, they must have time to adjust and cannot withstand sudden changes.

Since temperature is related to dissolved oxygen, the impacts of changes in dissolved oxygen fluctuations described in the dissolved oxygen section apply to temperature as well. Since colder water usually has more dissolved oxygen than warm water (all other things being equal), those fish that need a lot of oxygen such as trout, will live in colder water.

The invertebrates that live on the bottom of the river (benthic macroinvertebrates) are also sensitive to temperature and different species will move in the stream to find their optimal temperature.

Table 4 displays the maximum and optimum temperatures for different life stages of fish: growth, spawning, and survival of embryos. For overall survival, the table shows the “short term maximum” (perhaps a matter of hours) above which fish will die.

How We Measure Temperature

Temperature is measured directly in the river with a thermometer or a multi-use meter. It is measured in degrees Fahrenheit (°F) or degrees Celsius (°C). Alcohol- filled thermometers are preferred over mercury-filled because they are less hazardous if broken. Armored thermometers for field use can withstand more abuse than unprotected glass thermometers and are worth the additional expense. Meters for other tests, such as pH or dissolved oxygen, may also measure temperature and can be used instead of a thermometer.

Monitoring Considerations

Temperature should be measured as a part of any monitoring effort. If you wish to calculate dissolved oxygen as percent saturation, you will need to measure temperature in addition to dissolved oxygen. Several measurements from the top to the bottom of the water column should be made to obtain a temperature profile.

Table 4. Maximum Weekly Average Temperatures for Growth and Short-Term Maximum Temperatures for Selected Fish (Centigrade and Fahrenheit)

Species	Maximum Weekly				Maximum for			
	Average for Growth		Short-term Maximum		Embryo Survival			
	20° C (68° F)		23° C (73° F)		5° C (41° F)		7° C (45° F)	
Atlantic salmon	20° C (68° F)		23° C (73° F)		5° C (41° F)		7° C (45° F)	
Bigmouth buffalo					17	(63)	27	(81) a
Black crappie	27	(81)						
Bluegill	32	(90)	35	(95)	25	(77)	34	(93)
Brook trout	19	(66)	24	(75)	9	(48)	13	(55)
Carp					21	(70)	33	(91)
Channel catfish	32	(90)	35	(95)	27	(81)	29	(84)
Coho salmon	18	(64)	24	(75)	10	(50)	13	(55)
Emerald shiner	30	(86)			24	(75)	28	(82) a
Freshwater drum					21	(70)	27	(81)
Lake herring (Cisco)	17	(63)	25	(77)	3	(37)	8	(46)
Largemouth bass	32	(90)	34	(93)	21	(70)	27	(81)
Northern pike	28	(82)	30	(86)	11	(52)	19	(66)
Rainbow trout	19	(66)	24	(75)	9	(48)	13	(55)
Sauger	25	(77)			10	(50)	21	(70)
Smallmouth bass	29	(84)			17	(63)		
Smallmouth buffalo					17	(63)	21	(70)
Sockeye salmon	18	(64)	22	(72)	10	(50)	13	(55)
Striped bass					18	(64)	24	(75)
Threadfin shad					18	(64)	34	(93)
White bass					17	(63)	26	(79)
White crappie	28	(82)			18	(64)	23	(73)
White sucker	28	(82)			10	(50)	20	(68)
Yellow perch	29	(84)			12	(54)	20	(68)

a - upper temperature for spawning.

Source: EPA Water Quality Criteria, 1984

References:

- Green, Linda Taylor and Elizabeth Herron. 1994. URI Watershed Watch: 1994, Kingston, Rhode Island.
- Hynes, H.B.N., 1970. The Ecology of Running Waters, University of Toronto Press, Toronto Canada.

Temperature Procedure

Summary of Method: Temperature sensor pH meter or thermometers

Maximum Holding Time: measure immediately directly in the stream, lake, sampler (if using one) or a separate sample container.

Type of Container: polyethylene or glass

Sample Volume: 25 mL

. Lab Procedure - **Rivers**

If possible, take the temperature directly in the water. If not, then collect a sample and take the temperature immediately. **Do not use this as the sample for the lab analysis of other indicators.**

1. If possible, take the temperature in the main current of a river, not along the sides.
2. Place the thermometer a few inches in the water.
3. Wait about 30 seconds, until the thermometer stabilizes.
4. Read the thermometer immediately after removing it from the water.
5. Take the temperature 2-3 more times and average.
6. Record the average on the lab sheet.

. Lab Procedure - **Lakes**

The upper layer of lake water is where most of the algal growth occurs because it receives sufficient light for photosynthesis and the bottom layer is where most of the effects of decomposition, such as oxygen depletion, are observed. The volume of these respective layers will determine much of the chemistry and biology of the lake (translation: can the lake support trout? Is the lake becoming eutrophied?). Consequently, we want to be able to determine the depth of this layer.

One could take a sample every meter or so from top to bottom, but this would take quite awhile. To save time, we suggest a different approach. We will use the fact that the upper and lower layers are each relatively uniform in temperature.

1. If possible, take the temperature at the deepest point in the lake.

2. Take a surface measurement by holding the thermometer for a few minutes at elbow's length below the surface. When you collect your bottom dissolved oxygen sample, take another temperature reading as instructed in that section. Record both of these on your data sheet.
3. Take another temperature reading of the integrated sample while it is in the mixing bucket (DO NOT PUT YOUR HANDS IN THE WATER, ONLY THE TIP OF THE THERMOMETER!).

If the temperature of the surface and the integrated sample differs by less than 1°F, take a sample with your Wisconsin sampler at a depth 2 meters deeper than 3 times the Secchi disk depth (2 meters deeper than the bottom depth of the integrated sample). Apply the same test of a 1°F difference. If there is not a one degree difference, sample another 2 meters deeper until a degree difference is observed. When you reach that point, take two more samples, one 1 meter higher and another 1 meter lower. Record all temperature readings.

4. Read the thermometer immediately after removing it from the water.
5. Take the temperature 2-3 more times and average.
6. Record the average on the lab sheet.

If the temperature difference between the surface and integrated sampler is greater than 1°F, reverse the process by taking samples 2 meters higher than the bottom of the integrated sampler. When you find the area where the temperature change seems to be occurring, take a sample one meter above and one meter below as described above.

If there is less than a 2°F between the top and bottom, the lake is not stratified. Record the top and bottom measurements; they are all that is necessary.

When you take your first sample of the year, you will have no idea where the thermocline lies, if there is one; but, after that, you can be reasonable assured that it will be in the same place or lower. The smaller and more wind-sheltered your lake, the more certain you can be. So, for the next sampling period, start your search where you last found the thermocline and work down from there. Lakes do have an interesting habit of sloshing about (both temperature layers behaving a little differently) when exposed to wind, so be a little flexible about your search. In late fall, the temperature difference between top and bottom layers will get smaller and smaller, and consequently harder to find. Luckily, the thermocline is less necessary to find at this time, too. Use your own judgment on how hard to search for the thermocline in late fall.

Hopefully, this process will help you find the thermocline with a minimum number of samples. If you discover a better way, we are all ears!

Alternatively, if you have an electronic temperature probe, measure the temperature at one meter intervals from top to bottom.

.....Calibrating Your Thermometers.....

The following protocol is used by the Chesapeake Bay Citizen Monitoring Program to calibrate new field thermometers before they are distributed to volunteers.

The Chesapeake Bay Citizen Monitoring Program uses a non-certified precision thermometer, available for about \$30.00, for the calibration procedure. If a greater degree of accuracy is required, you may wish to check your precision thermometer against a certified thermometer. Certified thermometers are very expensive, but you may be able to find an agency or university lab that will let you bring your thermometer in and check it against their certified thermometer.

Maintenance checks on the calibrated thermometers are performed during one of the two annual quality control sessions that are held for volunteers. At the session, all the volunteers' thermometers are put into a water bath at the same time and checked to ensure that they all read within one degree Celsius of the precision thermometer.

.. Equipment Needed for Thermometer Calibration ..

- Depth electronic thermometer or precision-grade thermometer that reads in an increment of 0.1°C
- Calibrated line - *LAKES*
- Insulated Cooler
- Wide-mouthed jar, such as a one-quart mayonnaise jar
- String or twine
- Ice

..... Calibration Procedure

The day before you will be calibrating the thermometers, fill the insulated cooler with tap water. Suspend the precision thermometer in the water by tying it with a string to a cabinet door or some other stable object above the cooler. Allow the water to equilibrate overnight.

The following day, use string to loosely tie together the field thermometers that you want to calibrate. Calibrate no more than ten thermometers at a time.

1. Room-temperature bath calibration:
 - suspend the thermometers in the water in the cooler
 - let stand for fifteen minutes
 - read and record the values for all the thermometers, including the precision thermometer

2. Ice-bath calibration:
 - prepare an ice bath in the wide-mouth jar (make sure the ice to water ratio is such that the jar is packed with ice at the bottom)
 - suspend precision and field thermometers in the bath
 - let stand for fifteen minutes
 - read and record the values for all the thermometers, including the precision thermometer
 - let stand for another fifteen minutes, adding more ice if the ice is floating above the bottom of the jar
 - take second reading

Analysis of Results:

The thermometers should read within 1 degree of the precision thermometer. Any thermometer outside of this range should not be used.

Water Clarity – Turbidity and Secchi Depth background

What Is Water Clarity and Why Is It Important?

Water clarity is a general term that integrates various phenomena that determine how clear the water appears. The two main phenomena are the light scattering and light absorption characteristics of the water. Light gets scattered and absorbed by particles in the water. It gets absorbed by certain molecules that give it color.

There are two common measures of water clarity: turbidity and transparency.

Turbidity describes the light scattering component of the clarity of the water. This clarity is expressed in terms of the intensity of light scattered by particles in the water and measured by a device known as a “nephelometer” (also commonly known as a turbidimeter). In this device, a beam of light is shown through a portion of your water sample. The meter measures the intensity of that portion of the light that is reflected at a 90° angle from its original path. The meter is adjusted so that it compares the intensity of light scattered in your sample with that scattered in a solution of known turbidity. The results are reported as “nephelometric turbidity units” or “NTUs” for short.

Transparency is a more integrated measure that describes scattering and absorption at the particulate and molecular level. This is most commonly expressed as the depth at which a black and white patterned device known as a secchi disk disappears from sight. The more transparent the water, the lower the depth at which the disk disappears.

To the naked eye, turbidity appears as cloudy or muddy water. It’s different than color. Water can have high color and low turbidity. For example, cherry Kool Aid is very red, but you can see through it. Turbidity levels in rivers vary from less than 1.0 NTUs in mountain streams to more than 50.0 NTUs in larger rivers after rainfall events.

What Causes Reduced Clarity?

Reduced clarity may be caused by soil particles (clay and silt), algae, plankton, microorganisms, and other substances that color the water. These materials can enter the water column directly from a pipe or through erosion of the river channel and surrounding land. They can also result from biological activity.

Sources of reduced clarity in aquatic systems include natural erosion of river channels and erosion accelerated by human activities. Some rivers (and the lakes they feed) are naturally turbid because they flow through highly erodible material and carry a large load of suspended sediment. Examples include many rivers in the arid Southwest that flow through soft sand and sandstone. Rainfall and surface runoff events in these river systems carry a heavy sediment load to these rivers. Others, like many in the Northeast, flow through hard bedrock channels that erode very slowly. Some natural biological activity causes turbidity. For example, some bottom feeding organisms stir up the sediments as they feed. Natural algae and plankton growth causes turbidity.

Human activities that can cause turbidity include flow management, direct discharge of particles to the water, any activity in the river channel that disturbs the river bed or banks, or any activity on the surrounding land that disturbs the soil so that it gets carried by surface runoff into the river. Common land uses that can cause erosion, sedimentation, and turbidity include logging, cropping, grazing, road construction, road drainage, sand application to roads in the winter, excavation for buildings, extraction of gravel from the river channel, and mineral extraction in riparian areas.

Other human activities cause turbidity as a secondary effect. For example, dissolved nutrients in the water column from waste discharges can cause algae and plankton blooms in the water column.

Turbidity is closely related to rainfall and water flow, since the energy of falling and flowing water the main way that sediment gets dislodged and carried into rivers. So turbidity often increases sharply during and after a rainfall, especially in developed watersheds.

The Effects of Reduced Clarity On Rivers and Lakes

Reduced clarity can affect the aquatic ecosystem in a number of ways. It can cause higher water temperatures because suspended particles absorb more heat. This, in turn, can reduce the concentration of dissolved oxygen since warm water holds less dissolved oxygen than cold water. It can reduce the amount of light penetrating the water column which reduces photosynthesis and the production of dissolved oxygen. It can also reduce the growth of algae and other types of food for aquatic organisms on the bottom. Suspended materials can clog fish gills reducing resistance to disease, lowering growth rates, and affecting egg and larval development. Suspended particles also may provide a place for harmful microorganisms to breed. These particles may also carry attached pollutants such as nutrients and toxic materials. As suspended particles settle in slower areas, they can smother fish eggs. Particles can settle into the spaces between the rocks on the bottom and decrease the amount and type of habitat available for

macroinvertebrates. For drinking water, suspended particles can support the growth of harmful microorganisms.

Monitoring Considerations

Turbidity measures the effects on water clarity of the particles in the water column. However, it does not actually measure the concentration of these particles per unit of volume. An example of this type of measurement would be milligrams per liter of total suspended solids, where the suspended particles in a given volume of sample would actually be weighted. So turbidity can not be used to quantify the amount of sediment in the water. But it is a useful indicator of suspended sediment and can be used to assess the impacts of erosion and sedimentation over time and space.

Unlike turbidity, transparency is influenced by both color and scattering. Highly colored waters are less transparent than non-colored waters because the color absorbs light. While very red cherry Kool Aid is not any less turbid than clear water, it is less transparent. So, the secchi disk is a more integrated measure of clarity than the nephelometer.

A secchi disk is impractical for monitoring flowing water since it needs to sink vertically. The VEMN recommends the secchi disk for lakes and the nephelometer for rivers.

A turbidity meter consists of a light source that illuminates a water sample and a photoelectric cell that measures the intensity of light scattered at a 90 degree angle by the particles in the sample. Meters can measure turbidity over a wide range from 0 to 1000 NTUs. A clear mountain stream may have a turbidity of around 1 NTU, whereas a large river like the Mississippi may have a dry weather turbidity of around 10 NTUs. These values can jump into hundreds of NTUs during runoff events. Therefore, the turbidity meter to be used should be reliable over the range in which you will be working.

Turbidity Procedure

Summary of Method: Direct nephelometric measurement with Hach Turbidimeter. This measures the light scattering properties of the sample.

Type of Container: polyethylene or glass

Sample Volume: 30 mL.

Maximum Holding Time: 48 hours refrigerated

Equipment and Supplies

- Secchi Disk
- Hach 2100P portable turbidimeter and manual
- 4000 NTU Formazin standard (for making calibration standards)
- Gelex Secondary Standards: 0-10, 0-100, 100-1000 NTU
- Silicone oil
- Lint-free cloth
- 3 sample cells
- Distilled (turbidity-free) water

. Lab Procedure w/Turbidimeter

Preparation:

The First Time You Use the Meter:

The Hach turbidity meter is calibrated at the factory. New meters do not need to be calibrated. However, you should do the following:

- **Determine the True Value of the Gelex Standards**

Gelex Standards are vials with a gel inside. Each vial contains gel with a different turbidity and the range within which the turbidity of the gel falls. The turbidity of these must be measured using a meter calibrated to formazin standards. These are then used to check if the meter has “drifted” from its calibration.

- 1) Measure the turbidity of each of the three Gelex standards following procedure on pages 46-47 of the Hach manual (provided with the meter).
- 2) Record these values *in pencil* in the white diamonds on each standard.

- **Choose and Orient the Sample Cell**

- 1) Select one of the three sample cells provided with the meter. **This will be the cell used for all the samples.**

- 2) Each side of this sample cell may have different optical properties which will yield different turbidity readings. To correct for this, test each cell is by rotating it 45-degrees and recording the turbidity reading after each rotation. Place a mark on the side that yields the lowest turbidity reading. **Each time you use this cell to measure a sample, place it in the meter with this mark aligned with the raised mark on the meter.** Follow the directions on pages 13-14 of the manual to find the proper orientation of all three sample cells.

The First Use of the Season: Calibrate Meter to Formazin Standards

If you're using the meter for the first time in a season, you must **calibrate the Meter To Formazin Standards**.

Turbidimeters must be calibrated to a standard solution with known turbidity. The solution used is known as "formazin." Formazin has optical properties that give consistent turbidity readings. Therefore, formazin solution is known as a "**primary standard**." Hach supplies a 4000 NTU formazin stock solution with the meter. This stock solution is used to make up standard solutions that are used to calibrate the meter. Use the procedure described on pp. 31-37 of the Hach manual:

- 1) Make up formazin dilutions (800, 100, and 20 NTU) using the 4000 NTU stock solution supplied with the meter and distilled low turbidity water. Prepare low turbidity water by filtering through a 0.45 micron membrane filter. See table 1 on page 31 of the Hach manual for proportions.
- 2) Calibrate the meter following the steps on pages 32-37 of the Hach manual.
- 3) Determine the true value of the Gelex standards following the procedure described in the above section and record them on the white diamonds.

Measure Turbidity

- 1) Check the calibration of the meter: measure the NTUs of the Gelex standards. If they are off by more than 5% from the values you marked after the last calibration, calibrate the meter.
- 2) Allow samples to come to room temperature.
- 3) For the first sample, prepare the sample cell by applying a thin coating of silicone oil with a soft, lint-free cloth. (This masks any scratches, which the meter would read as turbidity).
- 4) Gently turn over the sample bottle several times to mix the sample. Do not shake the sample – air bubbles are read by the meter as turbidity.
- 5) Gently and slowly fill a sample cell to the fill line (15 mL) with a portion of the sample. If you must handle the sample cell, handle it only at the top to avoid smudging the lower part of the cell (which the meter would read as turbidity). Cap the cell.

- 6) Check the sample in the cell for air bubbles and condensation. If there are air bubbles, uncap it and let it sit for a few moments before measuring. If the air bubbles persist, try refilling the sample cell more slowly. If they still persist, use the degassing kit to remove them. If condensation forms on the outside of the cell, wipe it off with a Kimwipe or lint-free cloth. If it immediately reforms, allow the sample to come to room temperature.
- 7) Set the meter to “auto range” (see page 28 of the Hach manual).
- 8) Insert the sample cell into the turbidimeter and line up the orientation mark on the cell with the raised tab on the meter. Record the reading in the “replicate 1” column of the lab sheet. Remove the sample cell from the meter and pour it out.
- 9) Repeat steps 4-8 using the **same sample**: Record the reading in the “replicate 2” column of the lab sheet. Remove the sample cell from the meter. Calculate the average of the two readings and record in the average column.
- 10) Rinse the sample cell 3 times with de-ionized water and shake out remaining water drops inside the cell.
- 11) Use the same sample cell with the same orientation and read all the samples following steps 4-8.
- 12) If the turbidity of the sample is above 40 NTU, it must be diluted and read again. Follow the instructions in the manual to do this.

Secchi Depth Transparency - Procedure

Summary of Method: Visual measurement of disappearance and reappearance of Secchi disk below the water surface while using a view scope.

Equipment and Supplies

- Secchi Disk
- Calibrated Line
- View Scope
- Two Spring Clip Clothespins
- Field Data Sheet

. Procedure

Your measurements should be taken between 10 a.m. and 2 p.m. If possible, a team of two should perform the Secchi disk measurement. One should lower the Secchi disk and the other should submerge the end of the viewscope and watch the lowering.

- 1) Lower the Secchi disk into the water. When the disk just disappears (according to the person looking through the viewscope), a clothespin should be clipped on the calibrated line at the water's surface.
- 2) Lower the disk another one or two feet and then slowly bring it up toward the surface until it can just be seen again. Another clothespin should be clipped on the line at the water's surface.
- 3) The Secchi disk depth is the average of these two measurements to the nearest tenth of a meter.
- 4) Repeat steps 1) - 3) with the team members reversing roles.
- 5) Record the Secchi disk reading of each viewscope operator. Average the two measurements and record on the field data sheet.

WATER CONTACT HEALTH RISK ASSESSMENT

The main purpose of this assessment is to evaluate the health risk of coming in contact with the river or lake water -- by swimming or wading in it, eating the fish from it, or ingesting it. Put more simply: if you come in contact with the water in any of these ways, what is the risk that you'll get sick? Or, if you got sick, what is the likelihood that it is associated with contact with contaminated water?

The design of a water contact health risk assessment is tailored to the location, issues, problems, and situation in specific areas. So, unlike other sections in this manual, this section is not a step by step procedure that you can carry out on your own. Rather, this section describes the principles and process of assessment design. This process will result in specific data gathering tools and procedures that you can use. ***This process should be undertaken with the assistance of a resource person experienced in the design of community health surveys.*** This will help you avoid common procedural errors that might needlessly frighten community members and/or bias⁷ your sampling.

AN OVERVIEW OF THE HEALTH RISK ASSESSMENT PROCESS

Assessing the health risk of water contact involves the study of a representative sampling of a population by assessing their exposure to disease-causing agents in the water and the actual presence of disease. Exposure to disease agents can be assessed from behavior reports (such as reports of water contact), water sampling and analysis for contaminants or indicators of concern, occupational or medical records, or even biological samples (which can be analyzed for indicators of prior exposure to suspected disease agent(s)). The presence of disease can be determined in many ways, including the collection of biological samples such as blood and/or tissue, physician exams, or symptom reports. Once exposure and disease status are known, unexposed and exposed groups are compared to determine if they have a different statistical "risk" of getting disease. The results measure association (i.e. the disease is associated with or occurs in exposed populations at a higher rate than in unexposed populations), not causation (i.e. exposure causes the disease). However, the stronger the association, if there is a dose-response relationship (the exposed person responds to the contaminant by getting sick), and if results are replicable, the greater the evidence for a causal relationship. The type of assessment described above is termed "epidemiological." Epidemiology is the study of the causes, distribution, and control of disease in populations.

⁷ A statistical sampling error caused by systematically favoring some outcomes over others.

HEALTH RISK ASSESSMENT STEPS

Health assessments involve at least two main elements:

- 1) **Collection of water samples and their analysis for the contaminant(s) of interest (for example, *E. coli* bacteria).** This data is used to assess human exposure to the contaminant(s).
- 2) **Information on the behavior and health status of the target group (e.g. a community, a sub-group) of interest or a reasonable substitute.** The types of information collected will typically include indicators of health status, perhaps behaviors associated with exposure to the contaminant(s), and any exposures to other types of contaminants.

STEP A: ACKNOWLEDGE COMMUNITY HEALTH CONCERNS

Meet with local public and environmental health professionals, medical personnel, community leaders, activists, and individuals concerned about the human health consequences of river contamination. These key players should have valuable information and theories about existing concerns and the history of disease in the community. Collect both anecdotal and factual information on diseases in the community.

STEP B: IDENTIFY ILLNESSES IN THE COMMUNITY

Identify unusual prevalences or rates of disease, temporal or spatial clusters of disease, or the presence of rare diseases in the community. Of these diseases, identify those that may be linked with known or potential water contaminants. Examples include gastro-intestinal or diarrheal diseases of the digestive system or unusual skin rashes.

STEP C: COLLECT AND ANALYZE WATER SAMPLES

Either before, or in conjunction with steps A and B, establish a water monitoring plan and infrastructure (i.e. volunteers and a coordinator, equipment and lab, steering committee, etc.). Collect and analyze river and/or well water samples for the indicator bacterium *E. coli* and other contaminant(s) of concern such as metals and/or toxics. Note that the USEPA recommends *E. coli* bacteria, rather than fecal coliforms, as the preferred health risk indicator in fresh water. For *E. coli* bacteria, use the procedures described in this manual to collect and analyze samples at your project lab. For metals or toxics, use the procedures recommended by your consultants. You will likely be able to collect the samples yourself. However, they will probably need to be analyzed at an EPA or state-certified lab.

Collect and analyze water samples preferably before, during, and after Step D below. The objective is to determine the presence and levels of contaminants that

may produce a human health impact when humans come into contact with the contaminated water.

STEP D: DESIGN A DATA GATHERING STRATEGY

Determine the appropriate strategy to gather information on the health status and water contact from a sample of community residents. In the case of rare, yet easily identifiable and diagnosed diseases, a “case-control” strategy (sampling the people who have the disease of interest and asking them about their exposures) may be the best. Where many people are exposed to a contaminant that may cause a variety of diseases, a “cross-sectional” strategy (sampling a representative cross section of the population at one point in time to determine their disease and exposure) may be more appropriate. If it is suspected that the rate of contamination or illness may change over time, a “prospective cohort” strategy (sampling a cross section of the population at multiple points over a specified period of time to determine their disease and exposure) has the potential to identify trends over time. Each strategy will require a different sample size of community residents, affecting the time and cost of data gathering.

STEP E: GATHER DATA

Information can be gathered in a number of ways and these methods may be combined. Some options include: mail or face-to-face survey; medical examination; or review of hospital and/or medical records. Each option also requires the informed consent of the participants. Each option also requires the training of survey administrators and/or record reviews to ensure reliability. Care must be taken to avoid bias, particularly selection, recall, and observer biases:

Recall Bias: the interviewees selectively remember their exposure to support their notion that they’ve got the disease,

Observer Bias: the interviewer knows the disease or exposure status of the people being interviewed and asks leading questions to elicit the desired response,

Selection Bias: people who are more likely to have the disease or exposure are purposely included in the sample.

As an example, we’ve included in this manual a questionnaire⁸ that was used to gather community health data in settlements along the U. S. Mexican border that lack basic water and sewer services. The questions were developed specifically

⁸ Adapted from a survey developed by Cynthia Lopez.

for conditions and issues in these settlements. It will need to be modified for use in communities in the Merrimack River Watershed with the advice of a community health survey advisor. ***A survey like this should only be administered by trained interviewers!***

The objective is to gather enough data, systematically and without bias, to determine if those people coming into contact with water contaminants are more likely to contract disease, OR if those people who experienced disease were more likely to have come into contact with contaminated water in their past.

STEP F: DATA ENTRY

To facilitate data analysis, data gathered from interview or record reviews should be coded and entered into an easy to use and inexpensive database software package. The Center for Disease Control (CDC) provides the software “Epi Info” at minimal cost (\$25). To ensure data reliability, double entry (two people enter the data separately) or random review of entered records is recommended.

STEP G: DATA ANALYSIS

Once checked for errors, the data are ready for statistical analysis. In almost all cases, statistics that describe the data set in various ways will be computed. The selection of appropriate statistics describing associations and risk will depend upon the nature of the data. Most statistical software packages allow the computation of various statistical analyses.

STEP H: REVIEW RESULTS

Results of the statistical analyses may indicate an “association” between exposure to the water you’re testing and disease. If so, it is important to notify local health authorities. It is also crucial to communicate results with residents in a manner that will provide them the greatest information without unnecessary fear.

It is possible that no association may be found between exposure and disease. This does not mean that no association exists in truth, but that the study may not have had enough power (ability to detect a difference between two populations) to find the relationship. In this case, a follow-up study may be best.

It is also possible that another exposure, such as air pollution, may be associated with the disease of interest. Or that the original exposure of interest, water contamination, may be associated with another disease that, at the beginning, was not suspected in the community. This information is valuable. It allows the community to focus on the more important health risks to the community, to “prioritize” risk.

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Community Health Survey Example

Adapted from A Survey Developed by Cynthia Lopez
for River Watch Network

Note: This survey was developed for settlements along the U.S. Mexican border known as "colonias." These settlements lack basic water and sewer services. The questions were developed specifically for conditions and issues in these settlements. It will need to be modified for use in communities in the Merrimack River Watershed. The survey was in Spanish and English. Spanish was removed for this example.

A survey like this should only be administered by trained interviewers!

Date ____/____/____

Interviewer Code/Key: 1 2 3 4

Survey Number: ____

1. **Colonia Name:** _____

2. **What relationship do you have in the family?**

Housewife____ Head of Household ____ Grandmother____

Mother-in-law____ Other Person, Who? _____

(Instruction: If there is more than one person answering, indicate all. If there are only children present in the house, terminate the survey.)

3. **What is your first name?** _____

4. **How long have you been living in this colonia (more or less)?**

Months?____ Years?____

(Instruction: If less than 3 months, terminate the survey. If respondents lived elsewhere during the past three months, their exposures would differ, and their health effects would more likely be associated with these different exposures.)

5. **What was the last year of school you completed?**

None____ Elementary 1 2 3 4 5 6 Jr./High School 1 2 3 4 5 6

Technical____ Professional____ University____

Other, Explain _____

6. **How many people in total, including children and adults live in this place/house?** ____adults ____children ____total

Note: The total and number listed in the tables for questions 17 and 18 should agree.

7. How many families live in this place/house? 1 2 3 4 5+

Note: Defining separate families is difficult. Often, there is one large extended family. When grandparents and unmarried siblings reside in one home, I am counting them as one family. However, if married siblings with children, cousins with children, nephews/nieces with children, live in the same home, they are counted as a separate family. The residents themselves may or may not consider themselves as separate family units in these cases. Therefore, this question often prompts discussion between the respondent and the interviewer.

8. How many rooms do you have in your house, including the kitchen?

1 2 3 4 5+

9. Of these rooms, how many do you use for sleeping? 1 2 3 4 5+

10. Do you have a working refrigerator? yes___ no___ sometimes___

11. What type of stove do you use?

Nothing___ Oil___ Gas___ Wood___

Other, Explain_____

12. Do you have a heater that works? yes___ no___ don't know___

(Instruction: If the answer is "no," omit question 13)

13. What type of heater do you have?_____

14. Do you have electricity? yes___ no___ sometimes___ don't know

15. How do you dispose of your trash?

Burn it___ Bury it___ Truck Picks It Up___

Throw it out___ Other, Explain_____

(Instruction: You can indicate more than one answer)

16. What are the principal materials of the walls of your house?

Adobe___ Cement Block___ Wood___

Carton___ Palettes___ Trash/Disposal Material___

Other, Explain_____

17. What is the principal material of the floor of your house?

Cement___ Wood___ Earth___

Other, Explain_____

18. Please tell me the first names and ages, of each person that lives here, whether they work outside the house and the type of work they do, starting with the youngest and finishing with you.

#	First Name	Age	Work Outside House?	Type of Work?
1)				
2)				
3)				
4)				
5)				
6)				
7)				
8)				
9)				

For Type of Work:

M = Maquila, A = Agriculture, C = Construction, D = Domestic, O = Other (Explain)

(Note: a maquila is a type of manufacturing plant)

Indicate for each person that works in a maquila or in agriculture:

If you work in maquila, doing what specifically?

If you work in agriculture, with what crop?

If your work in a maquila or in agriculture, do you work with chemicals (such as pesticides)?

Have you or anyone in your family had any of the following illnesses during the last two weeks?

- D = diarrhea (diluted, runny excrement three or more times in 24 hours), S = rash,**
- J = red, irritated eyes,**
- R = respiratory infection,**
- O = other infection (explain)**

#	First Name	Were You Ill In the Last 2 Weeks?	Type of Illness
1)			
2)			
3)			
4)			
5)			
6)			
7)			
8)			
9)			

Indicate for each person whether the interviewer observed these illnesses.

In your opinion, what caused the illness(es) in your family? (indicate above for each illness)

19. Have you or someone in your house visited a doctor, nurse, midwife, healer, or pharmacist in the last three months?

yes____ no____ don't know____

(If person answers "no," omit questions 20 and 21)

20. Why did you and/or someone in your house visit a health care provider? (indicate for each visit and for each person)

#1_____ #2_____

#3_____ #4_____

21. What illness was diagnosed? (Indicate for each visit)

#1_____ #2_____

#3_____ #4_____

22. In your opinion, what are the illnesses most frequent/common in your community?_____

23. How many people smoke tobacco in the house? 0 1 2 3 4+

24. Where do you get your drinking water?

Tap water, piped into the house____

From a public tap____ Where is it?_____

Well, private, on my property____ Or outside of my property____

If a well, Depth?__meters If outside, Where?_____

Pipe from River, Stream____

Purchased Bottled Water?__always?__sometimes?

Other, Explain_____

(Instruction: You may indicate more than one response)

25. Do you think that the water you drink from the community is safe/sanitary?

yes__ no__ don't know__ Why?_____

26. Where do you keep the drinking water in the house?

Nowhere, it's directly from the pipe or well_____

5 gallon plastic tank without a spout_____

Small Jar/Bottle_____

55 Gallon Drum_____

Cement Basin/Trough_____

Tank_____ Metal?_____

Industrial?_____Agricultural?_____

Other, Explain_____

27. Do you purify your drinking water? yes___ no___

sometimes___ don't know___

*(Instruction: If the answer is "no," omit questions 28 and 29)***28. How do you purify your drinking water?**

Add Chlorine_____ Boil It_____for how much time? ___ minutes

Bottled Purifying Solution_____

Other, Explain_____

29. How many hours or days has it been since you purified the drinking water last? ___hours ___days**30. Where do you get your water for cooking?**

Tap water, piped into the house_____

From a public tap_____ Where is it?_____

Well, private, on my property_____ Or outside of my property_____

If a well, Depth?___meters If outside, Where?_____

Pipe from River, Stream_____

Purchased Bottled Water?___always?___sometimes?

Other, Explain_____

*(Instruction: You may indicate more than one response)***31. Do you purify your cooking water?** yes___ no___

sometimes___ don't know___

*(Instruction: If the answer is "no," omit question 32)***32. How do you purify the water for cooking?**

Add Chlorine_____ Boil It_____for how much time? ___ minutes

Other, Explain_____

33. Where do you get your water for washing and bathing?

Tap water, piped into the house____

From a public tap____ Where is it?_____

Well, private, on my property____ Or outside of my property____

If a well, Depth?__meters If outside, Where?_____

Pipe from River, Stream____

Purchased Bottled Water?__always?__sometimes?

Other, Explain_____

*(Instruction: You may indicate more than one response)***34. Do you or anyone in your family fish in the river or streams?**

yes__ no__ sometimes__ don't know__

35. Do you or anyone in your family bathe/swim in the river or streams?

yes__ no__ sometimes__ don't know__

36. What type of sanitary service do you have. In other words, what do you use to defecate?

Latrine____ Sanitary System with Water____

A Simple Hole____ Free Air____ Nothing____

Portable Toilet____ Other, explain_____

*(Instruction: You may indicate more than one response)***37. Do you have a septic system or are you connected to the municipal drainage system? yes__ no__ don't know__**

Interviewer's Observation

38. What are the most common/frequent problems that you see in the water, or in obtaining it?_____

39. In your opinion, what are the most important public health/sanitation and environmental problems that affect this community?

40. Have any relatives from your house died over the past year? (include infant mortality and stillbirths) yes___ no___ don't know___

If the answer is "yes," How many?___ What ages?___

What was the cause of death? _____

Observations/Notes

Notes⁹ On the Community Health Survey Example

Interviewer Code/Key: Each interviewer is assigned a "key" (number). They typically circle their interviewer key before conducting the survey. With this information, it can be determined if responses differ significantly by interviewer.

Survey Number: Each survey has a unique coding number that I assign prior to administration.

1. **Colonia Name:** This is to verify the colonia boundaries. On some occasions, residents view themselves not as a member of the larger colonia, but more as a member of a smaller neighborhood. They may name the neighborhood rather than the colonia per se.
2. **What relationship do you have in the family?** It is important to indicate who responds to the survey (or if a group responds). With the information as to who responds, responses by family member can be analyzed to determine if there exists any significant differences. If there are only children present in the home, the survey is terminated.
3. **What is your first name?** This information is primarily gathered to facilitate a return to the household/respondent in the event follow-up study is needed.
4. **How long have you been living in this colonia (more or less)?** This question is important because health information is asked retrospectively, over the past two weeks to three months. The investigator must be sure that respondents' exposures are from the colonia of interest, and that health effects can be reasonably assumed to be associated with exposures in the colonia. If respondents lived elsewhere during the past three months, their exposures would differ, and their health effects would more likely be associated with these different exposures.
5. **What was the last year of school you completed?** The response to this question serves as a proxy measure for knowledge, literacy, and socio-economic status (SES).
6. **How many people in total, including children and adults live in this place/house?** The response to this question is used to determine density in the home, and as a check to question numbers 17 and 18 where family member work and health status is requested. The total and number listed in the tables for questions 17 and 18 should agree.

⁹These notes are from Cynthia Lopez, author of the survey example, personal communication, 11/95.

7. **How many families live in this place/house?** Sometimes more than one family lived in the same "compound" (several homes/rooms on one lot) or house. Also, census takers may underestimate border peoples because they assume that there is only one family per home; census surveys are designed for only one family to fill out.

However, defining separate families is difficult. Often, there is one large extended family. When grandparents and unmarried siblings reside in one home, I am counting them as one family. However, if married siblings with children, cousins with children, nephews/nieces with children, live in the same home, they are counted as a separate family. The residents themselves may or may not consider themselves as separate family units in these cases. Therefore, this question often prompts discussion between the respondent and the interviewer.

8. **How many rooms do you have in your house, including the kitchen?** The response to this question can serve as a proxy measure of SES. It can also be used with the response to question 7 to obtain a measure of density in the home. Over-crowding in the home may be a confounding variable influencing health status.
9. **Of these rooms, how many do you use for sleeping?** This response also serves as a proxy SES measure and a density measure.
10. **Do you have a working refrigerator?** Many residents have refrigerators but do not have electricity for it, or it may be broken. This is also a proxy measure of food hygiene, as those people without working refrigerators are more likely to ingest contaminated foods.
11. **What type of stove do you use?** This is another proxy of SES. It's also important to determine if wood burning occurs in the home as it is associated with respiratory disease.
12. **Do you have a heater that works?**
13. **What type of heater do you have?**

Responses to questions 12 and 13 are other proxy measures of SES and indicators of wood burning in the home. Again, wood burning in the home is associated with respiratory disease.

14. **Do you have electricity?** A measure of SES and an indicator of the provision of municipal services. Many colonias do not have access to electricity as well as other public services.

15. How do you dispose of your trash? The response to the question is a measure of household hygiene. In particular, if the trash is buried in the home, it may contribute to well contamination. It's also possible that burning trash may be associated with respiratory illnesses.

16. What are the principal materials of the walls of your house?

17. What is the principal material of the floor of your house?

Responses to questions 16 and 17 are also proxy measures of SES and hygiene.

18. Please tell me the first names and ages, of each person that lives here, whether they work outside the house and the type of work they do, starting with the youngest and finishing with you. This question is designed to obtain information about family size and occupation of family members. Of particular interest is determining how many family members work in agriculture and in the maquiladoras. Of these, how many may have exposures to pesticides or industrial chemicals? These exposures could be considered "confounders." It is expected that those who are exposed to contaminated water supplies and work with toxics would be more likely to exhibit adverse health effects.

However, there is a healthy worker effect here, as those who are able to work in the maquilas or in agriculture are typically younger and healthier than other family members. Hence, they should be compared to younger family members living in colonias who work but in other occupations.

Have you or anyone in your family had any of the following illnesses during the last two weeks? These are illnesses which may or may not have prompted a visit to a health care provider. The acute illnesses are diarrhea, rash, respiratory illnesses (like the flu or bronchitis) or any other illnesses.

19. Have you or someone in your house visited a doctor, nurse, midwife, healer, or pharmacist in the last three months?

20. Why did you and/or someone in your house visit a health care provider?

21. What illness was diagnosed?

Questions 19, 20 and 21 are designed to obtain more information about health experiences in order to learn about more serious illness experiences of the family. Because many colonia residents may have limited access to health care, particularly to a physician's care, the definition of health care is broad.

22. **In your opinion, what are the illnesses most frequent/common in your community?** This is an opinion question, a “fishing expedition.”
23. **How many people smoke tobacco in the house?** Tobacco use is a potential confounder.
24. **Where do you get your drinking water?** This question and various responses assess potential exposure to a contaminated water source.
25. **Do you think that the water you drink from the community is safe/sanitary?** This is another opinion question.
26. **Where do you keep the drinking water in the house?**
27. **Do you purify your drinking water?**
28. **How do you purify your drinking water?**
29. **How many hours or days has it been since you purified the drinking water last?**
30. **Where do you get your water for cooking?**
31. **Do you purify your cooking water?**
32. **How do you purify the water for cooking?**
33. **Where do you get your water for washing and bathing?**
34. **Do you or anyone in your family fish in the river or streams?**
35. **Do you or anyone in your family bathe/swim in the river or streams?**
36. **What type of sanitary service do you have. In other words, what do you use to defecate?**
37. **Do you have a septic system or are you connected to the municipal drainage system?**
38. **What are the most common/frequent problems that you see in the water, or in obtaining it?**

Questions 24 through 38 assess the exposure of residents to possibly contaminated water.

39. **In your opinion, what are the most important public health/sanitation and environmental problems that affect this community?** This is another opinion question.

- 40. Have any relatives from your house died over the past year?** Another health outcome question, mortality over the past year. It's preferable to ask this question before the exposure question to blind interviewers to exposure status. However, this might be a sensitive question to ask and should be asked last.

