

## APPENDIX 2

### GUIDE TO INDICATORS AND MONITORING METHODS

This appendix contains general information about watershed indicators and methods.

#### Descriptions of Common Water Sampling and Analysis Indicators and Methods Used in this Handbook

##### General Terms Used In Water Sampling

- ◆ **Grab Sampling:** Samples are collected in some type of container by dipping the container in the water and filling it to some pre-determined level.
- ◆ **Integrated Sampling:** Samples are collected from various depths or locations across a transect that are combined into one sample for analysis.
- ◆ **Multiple Depth Sampling:** Individual samples are collected at various depths and analyzed separately.
- ◆ **Direct Measurement:** The indicator is measured directly from the water without collecting a sample.

##### Sample Handling Requirements

From Standard Methods				
Indicator	Container Type	Minimum Size (mL)	Preservation	Max. Holding Time
Alkalinity	P, G	200	Ref.	24 h
Bacteria	P, G (S)	200	Ref.	6 h
BOD	P, G	1000	Ref.	6 h
Chlorophyll	P, G	500	Dark	30 d
Conductivity	P, G	500	Ref.	28 d
N-Ammonia	P, G	500	ASAP or acidify	7 d
N-Nitrate	P, G	100	ASAP or ref.	48 h
N-Kjeldahl	P, G	500	Ref., acidify	7d
Oxygen	G-BOD	300	Fix	8 h
PH	P, G	-	ASAP	2h
Phosphate	G(A)	100	Ref.	48 h
Solids	P, G	-	Ref.	7 d
Turbidity	P, G	-	Ref., Dark	24 h
Abbreviations P = Plastic, G = Glass G(A) = acid-rinsed glass (S) = sterile Ref. = refrigerate h = hours, d = days				

## General Terms Used In Water Analysis Methods

This section describes the basic laboratory methods used to analyze water samples. These methods are referred to in the next section on methods for each indicator.

- ◆ **Titration:** Determining the concentration of an indicator in a sample by adding to it a standard reagent of known concentration in carefully measured amounts until a color change or electrical measurement is achieved, and then calculating the unknown concentration. Common indicators measured this way are dissolved oxygen and alkalinity.
- ◆ **Colorimetric:** Determining the concentration of an indicator in a sample by adding to it a reagent that causes a color change in direct proportion to the concentration of the indicator being measured. The intensity of the color (as measured by the extent to which it absorbs or transmits light) is either read with a visual color comparator or measured using a meter and either read directly in appropriate reporting units or read in “% absorbance” or “% transmittance” units and converted to reporting units. Common indicators measured this way are nutrients.
- ◆ **Electrometric:** Determining the concentration of an indicator in a sample by using a meter with an attached electrode that measures the electric potential (millivolts) of the sample. This amount of electric potential is a function of the activity of ions or molecules in the sample and proportional to the concentration of the indicator being measured. The electrode is selected based on its response to specific ions (known as an “Ion Selective Electrode” (or ISE), general ionic activity (conductivity) or molecules (for example, a Membrane Electrode). The meters can either display results in millivolts (mV) or in appropriate reporting units. Common indicators measured this way are dissolved oxygen, pH, conductivity and nutrients.
- ◆ **Gravimetric:** Determining the concentration of an indicator in a sample by filtering a specified quantity of the sample and determining the weight of the material retained on the filter. Common indicators measured this way are total solids and total suspended solids.
- ◆ **Nephelometric:** Determining the clarity of a sample by measuring the intensity of light scattered by particles in the sample and comparing this with a known solution. The higher the intensity of the scattered light, the higher the turbidity reported in nephelometric turbidity units (NTU's).
- ◆ **Membrane Filtration and Incubation:** Determining the bacteria concentration of a water sample by filtering a specified quantity through a specified 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 specific nutrient medium and then incubated at a specified temperature for a specified length of time. Colonies of a specified color growing on the filter are then counted.

## Acidity

Acidity is the water's ability to resist a change in pH when a base is added. This is largely caused by carbon dioxide, salts of strong acids and weak bases and other factors. Above a pH of 8.3, there is no measurable acidity. Acidity is the reverse of the alkalinity buffering effect. It is measured as the concentration of CaCO<sub>3</sub>.

### **Analytical Methods**

Acidity is measured by titrating a sample to either 3.7 (methyl orange acidity using bromphenol blue indicator) or 8.3 phenolphthalein acidity using phenolphthalein indicator). Acidity (in mg/L as CaCO<sub>3</sub>) is calculated from the amount of titrant (sodium hydroxide) needed to bring the sample to either pH.

## Alkalinity, Total

This is a measure of the water's ability to neutralize acids -- the higher the alkalinity levels, the more acid-neutralizing capacity the water has. This is important for aquatic ecosystems because it protects against changes in pH, which can harm aquatic life.

### **Analytical Methods**

Basic methods use titration. The advanced method uses a meter to measure the pH at two endpoints.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<i>Sulfuric Acid Titration w/ Bromocresol Green/Methyl Red</i> <ol style="list-style-type: none"><li>1) The sample is collected and treated with bromocresol green-methyl red.</li><li>2) It is then titrated with sulfuric acid until the solution turns pink.</li><li>3) The amount of acid added to reach this point is converted to total alkalinity.</li></ol>	<i>Double End Point Sulfuric Acid Titration w/ pH Meter</i> <ol style="list-style-type: none"><li>1) The sample is collected and the pH measured</li><li>2) It is then titrated with sulfuric acid until the pH is 4.5</li><li><b>3)</b> The amount of acid added to reach this point is converted to total alkalinity.</li></ol>

## Biochemical Oxygen Demand (BOD)

BOD is a measurement of the amount of oxygen consumed by organic matter and associated microorganisms and through chemical oxidation in the water over a period of time, usually five days. Measuring the biochemical oxygen demand (BOD) of the water tells us whether oxygen demanding wastes might cause low dissolved oxygen (DO) levels at times.

### **Analytical Methods**

Basic and advanced methods use a version of Winkler Titration. As with dissolved oxygen (DO), the main difference is the type of titrator or use of a meter.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Modified BOD-5 Day Method (Hach via Mitchell &amp; Stapp)</i></p> <ol style="list-style-type: none"> <li>1) Two samples are collected in glass-stoppered BOD bottles (one clear and one black) as in the DO method.</li> <li>2) The DO is determined for the clear bottle, using Modified Winkler Titration with a syringe or eyedropper.</li> <li>3) The black bottle is placed in the dark and incubated for five days at 68°F.</li> <li>4) The DO for this sample is then determined the same way.</li> <li>5) BOD is determined by subtracting the DO level of the black bottle from the clear bottle.</li> </ol>	<p><i>Modified BOD-5 Day Method w/ modified Winkler Titration or Meter (Standard Methods #521 O-B or equivalent)</i></p> <ol style="list-style-type: none"> <li>1) Two samples are collected in glass-stoppered BOD bottles (one clear and one black) as in the DO method.</li> <li>2) The DO is determined for the clear bottle, using Modified Winkler Titration with a buret, syringe, digital titrator or meter</li> <li>3) The black bottle is placed in the dark and incubated for five days at 68°F.</li> <li>4) The DO for this sample is then determined the same way.</li> <li><b>5)</b> BOD is determined by subtracting the DO level of the black bottle from the clear bottle.</li> </ol>

## Chlorophyll a

Chlorophyll a is a green pigment found in all plants. It is used to quantify the abundance of algae in water. When chlorophyll a degrades, it converts to pheophytin. The ratio of chlorophyll a to pheophytin is used to determine the health of the algae sampled.

### **Analytical Methods**

Measuring chlorophyll a involves a sophisticated process for which there are no simple methods.

<b>Basic Methods</b>	<b>Advanced Methods</b>
None	<p data-bbox="846 495 1451 600"><i>Pigment extraction followed by spectrophotometry (Adapted by Paul Godfrey from Standard Methods #10200 H)</i></p> <ol data-bbox="846 617 1451 1291" style="list-style-type: none"><li data-bbox="846 617 1451 688"><b>1)</b> Collect an integrated water sample using a clean container (at least one quart).</li><li data-bbox="846 695 1451 800"><b>2)</b> Filter subsample (quantity depends on a Secchi reading) using a glass fiber filter and vacuum pump.</li><li data-bbox="846 806 1451 877"><b>3)</b> Analyze filters immediately, frozen or dried.</li><li data-bbox="846 884 1451 1031"><b>4)</b> Extract pigment by grinding the filter, steeping the ground mass in 90% acetone, and centrifuging in tubes to de-suspend fibers from the solution.</li><li data-bbox="846 1037 1451 1108"><b>5)</b> Read color with a spectrophotometer and calculate concentration.</li><li data-bbox="846 1115 1451 1186"><b>6)</b> Add hydrochloric acid to the sample to convert all chlorophyll a to pheophytin.</li><li data-bbox="846 1192 1451 1291"><b>7)</b> Read color again with a spectrophotometer and calculate the concentration of pheophytin.</li></ol>

## Chlorine, Total Residual

Chlorine is a gas in its natural state. It is toxic to microbes and animals and is widely used to disinfect drinking water and wastewater. It can also combine with a wide variety of organic and inorganic chemicals to produce toxic compounds. It does not appear to be toxic to humans, except when it combines with other compounds. Total Residual Chlorine consists of Free Chlorine (hypochlorite ion, hypochlorous acid – the disinfecting agent) and chloramines (formed when chlorine reacts with ammonia or nitrogen).

### **Analytical Methods**

Amperometric Titration: Chlorine is tricky to sample because it is volatile (tends to convert to gas quickly) and has tricky flow patterns. We recommend consulting with your regional DEP biologist. The analytical method involves titrating a prepared sample with Phenylarsine Oxide with an Amperometric Titrator to an endpoint.

## Conductivity

This is a measure of the water's ability to pass an electrical current. This ability depends on the presence of inorganic dissolved solids made up of ions (particles that carry a positive or negative electrical charge). Since it measures a wide range of materials, its primary importance is as an indicator of general pollution, rather than a specific pollutant.

### **Analytical Methods**

Basic and advanced methods use a conductivity meter. This meter contains a probe with two electrodes. The probe is lowered into the water, voltage applied, and the drop in voltage caused by the resistance of the water is measured and converted to conductivity. The basic method uses a relatively inexpensive "pen." The advanced method uses a high-quality meter to measure the conductivity.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<i>Electrometric Using A Conductivity Pen</i> 1) Collect water sample or measure directly with pen. 2) Measure collected sample with pen.	<i>Electrometric Using A Conductivity Meter (EPA Method 120.1)</i> 1) Collect water sample or measure directly with meter. <b>2)</b> Measure collected sample with meter.

## Dissolved Oxygen (DO)

DO is the presence of oxygen gas molecules in the water. Since it is critical to many biological and chemical processes in the water and essential for aquatic life, dissolved oxygen is an indicator of the capability of the aquatic ecosystem to support life.

### **Analytical Methods**

Basic and advanced methods use a version of Winkler Titration. The main difference is the size of the increment of titrant added to the sample – smaller increments increase sensitivity.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Modified Winkler Titration with a syringe or eyedropper (Hach via Mitchell &amp; Stapp)</i></p> <p>This is essentially the Modified Winkler Titration described above, with some changes. The titrant is phenylarsine oxide solution and the titrator is an eyedropper. The eyedropper gives less accuracy and sensitivity than other titrators because it dispenses larger drops -- each drop equals 0.5 mg/l.</p>	<p><i>Modified Winkler Titration with a buret, syringe or digital titrator: Standard Method #4500-OG (or equivalent)</i></p> <ol style="list-style-type: none"> <li><b>1)</b> Collect surface samples in 300 mL “BOD” bottles with glass stoppers so that no air bubbles are trapped. In lakes, an integrated sample is collected using a length of garden hose.</li> <li><b>2)</b> Samples must be analyzed immediately or fixed and analyzed within eight hours.</li> <li><b>3)</b> The level of oxygen in the sample is “fixed” by adding reagents which produce a chemical reaction producing iodine in direct proportion to the amount of oxygen in the water.</li> <li><b>4)</b> Sodium thiosulfate is then added incrementally using a digital titrator (Hach) or syringe (Lamotte). 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 the oxygen) in the sample.</li> </ol>
	<p><i>Meter (Membrane Electrode) Method: Standard Methods #4500-OG (or equivalent)</i></p> <ol style="list-style-type: none"> <li>1) A membrane-covered electrode probe is lowered into the water.</li> <li>2) The meter electronically measures the diffusion of oxygen from the water across a membrane-covered electrode, which is directly proportional to the DO concentration.</li> </ol>

*Notes On Methods*

- ◆ Water samples for dissolved oxygen should be collected in glass-stoppered BOD bottles or other container designed so that no air is trapped in the sample.
- ◆ If you have a limited budget for this indicator (<\$300), we recommend that you use the Hach or Lamotte Adaptation of Winkler Titration. The Hach digital titrator dispenses smaller increments of the sodium thiosulfate than the Lamotte syringe and therefore increases the sensitivity. But, it’s more expensive.
- ◆ If you have the budget (\$600-800) to purchase a meter and you need frequent (or continuous) measurements from a few sites, a meter will work best. However, if you have a large number of sites and can only use one meter, we recommend titration.

## **Fecal Coliform and *E. coli* Bacteria**

Fecal coliforms and *E. coli* are bacteria that are common in the intestines and feces of warm-blooded animals. They are used both as an indicator of the presence of sewage or animal manure in the water and as an indicator of the health risk of swimming and other water contact recreation. Fecal coliforms are the indicator used in Pennsylvania's water quality standards. *E. coli* (a species of fecal coliforms) are used in other states, per their water quality standards.

### ***Analytical Methods***

The basic methods will either detect the presence of bacteria (but won't give you a count) or they give you an estimate of density (but not a reliable count). Advanced methods are EPA-approved and give reliable counts. The most reliable count is produced by the mTEC method. Note that the mColiBlue method does not identify fecal coliforms. *E. coli* colonies are used as the equivalent, but these counts will underestimate fecal coliforms.



<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Estimates of Density</i></p> <p>These methods estimate the number of bacteria in a sample in various ways:</p> <ol style="list-style-type: none"> <li>1) Easygel: Using one subsample size, usually 1-5 mL, bacteria are grown on nutrient medium without filtration. Colonies of specified color are counted. Estimates total coliforms and <i>E. coli</i>. Fecal coliforms are estimated using <i>E. coli</i> counts.</li> <li>2) Colilert: A reagent is added to various subsample sizes, which turns yellow if coliforms are present and fluoresces if they are <i>E. coli</i>. A statistical table is used to estimate density based on which subsamples turn yellow and/or fluoresce.</li> </ol>	<p><i>Fecal coliforms and E. coli: Membrane Filtration Using mTEC: EPA Method #1103.1</i></p> <ol style="list-style-type: none"> <li>1) Collect water sample in sterile container.</li> <li>2) Filter several subsample sizes through 0.45 micron filters.</li> <li>3) Dry incubate on mTEC nutrient medium in petri plates at 35°C for two hours.</li> <li>4) Incubate at 44.5°C in a water bath for 22 hours.</li> <li>5) Count fecal coliforms after incubation.</li> <li>6) Incubate at room temperature for 20 minutes on a urea solution and count <i>E. coli</i>.</li> </ol>
<p><i>Presence-Absence</i></p> <p>These methods detect the presence of selected bacteria types by whether or not bacteria grows on the plate (as detected by a certain color) or whether a special broth turns color when exposed to sample water. These methods tell you bacteria is present, but do not indicate the number of bacteria</p>	<p><i>Fecal Coliforms: Membrane Filtration Using mFC: Standard Methods #9222 D</i></p> <ol style="list-style-type: none"> <li>1) Collect water sample in sterile container.</li> <li>2) Filter several subsample sizes through 0.45 micron filters.</li> <li>3) Incubate at 44.5°C in a water bath for 24 hours on mFC nutrient medium in petri plates.</li> <li>4) Count fecal coliforms after incubation.</li> </ol>
	<p><i>Fecal Coliforms: Membrane Filtration Using mColiBlue24 (Hach)</i></p> <ol style="list-style-type: none"> <li>1) Collect water sample in sterile container.</li> <li>2) Filter several subsample sizes through 0.45 micron filters.</li> <li>3) Incubate at 44.5°C in a water bath for 24 hours on mColiBlue nutrient medium in petri plates.</li> <li>4) Count <i>E. coli</i> colonies after incubation (use to estimate fecal coliforms).</li> </ol>

## Hardness

Hardness is a measure of the calcium and magnesium positively charged ions in the water. These ions reduce the surface tension of the water, and soap does not produce lather in hard water. When hardness is primarily calcium, it is closely related to alkalinity. Hardness frequently reduces the impacts of metals on aquatic life: the higher the hardness, the lower the toxicity. Hardness is reported as mg/l of CaCO<sub>3</sub> at a given pH.

### **Analytical Methods**

There are no basic methods. The advanced method involves either a titration or calculating the hardness from previously-determined calcium and magnesium concentrations.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<b>None</b>	<i>EDTA Titration Method (Standard Method 2340C, EPA Method 130.2)</i> <ol style="list-style-type: none"><li>1) Collect water sample.</li><li>2) Add dye (EBT indicator) which turns sample purple.</li><li>3) Titrate with EDTA reagent until solution turns blue.</li><li>4) Calculate hardness from amount of titrant used as CaCO<sub>3</sub>.</li></ol>

### **Nitrogen**

Nitrogen is a gas in the atmosphere. It combines with oxygen or hydrogen to produce various compounds -- ammonia, nitrites and nitrates. It is an essential nutrient for plant growth and metabolic reactions in plants and animals. Together with phosphorus, it is the primary source of food energy in the aquatic ecosystem. Too much of certain forms of nitrogen can cause too much biological activity and cause undesirable effects. It is also toxic to babies in high concentrations. Nitrogen occurs in various forms, both organic and inorganic in the water, some of which are more available for plant growth than others. In some waters, nitrogen is the nutrient in short supply, so that relatively small amounts can cause impacts. Three forms of nitrogen are recommended as indicators in this handbook: ammonia, nitrates and total. Their descriptions and methods follow:

#### **Nitrogen - Ammonia Nitrogen**

Ammonia (NH<sub>3</sub>) is produced when organic nitrogen and/or urea break down. It is a byproduct of sewage decomposition. It is naturally present in surface waters, and can be toxic to aquatic life at relatively low concentrations (<1.0 mg/l).

#### **Analytical Methods**

Measuring ammonia involves a sophisticated process for which there are no simple methods. The Hach adaptation is the easiest, though it uses the distillation step only if known interferences are present.

<b>Basic Methods</b>	<b>Advanced Methods</b>
None	<i>Distillation followed by Nesslerization (Standard Methods #4500-NH<sub>3</sub> C or equivalent)</i> <b>1)</b> Add borate solution to sample for buffering. <b>2)</b> Distill <sup>1</sup> sample using a distillation apparatus. This removes certain interferences. <b>3)</b> Nesslerization. This involves pretreatment to remove turbidity-producing compounds and adding a Nessler reagent. <b>4)</b> This produces a yellow to brown color that is measured with a spectrophotometer. <b>5)</b> The reading is compared with a set of standard concentrations and reported as mg/l NH <sub>3</sub> -N.

### **Nitrogen - Nitrate Nitrogen**

Nitrate (NO<sub>3</sub>) is produced naturally by nitrogen-fixing plants and lightning acting on atmospheric nitrogen or ammonia. Nitrate is a form of nitrogen readily used by plants. In excess, it can cause excessive biological activity in surface waters and can be toxic to infants.

#### **Analytical Methods**

Basic and advanced methods use a variation on the same procedure, except that in the basic method the color is read using a visual color comparator. In the advanced method, the color is read using an electronic meter.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<i>Cadmium Reduction followed by Color Comparator (Hach via Mitchell &amp; Stapp)</i> <b>1)</b> A cadmium reduction reagent is added to a water sample. This causes a chemical reaction and turns the sample pink. <b>2)</b> The sample color is matched to colors labeled in pH units on a color comparator. <b>3)</b> The analyst determines the closest color match and records the nitrate concentration.	<i>Cadmium Reduction followed by spectrophotometry (Standard Methods #4500-NO<sub>3</sub>- E or equivalent)</i> <b>1)</b> A cadmium reduction reagent is added to a water sample. This causes a chemical reaction and turns the sample pink. <b>2)</b> This color is measured with a spectrophotometer. <b>3)</b> The reading is compared with a set of standard concentrations and reported as mg/l NO <sub>3</sub> -N.

#### *Notes on Methods*

- ◆ The basic method should be considered an approximation only. This method is not acceptable for federal and state agency assessment, but is fine for education and awareness and some community assessments.

### **Nitrogen - Total Kjeldahl Nitrogen (TKN)**

This refers to the total of organically bound nitrogen and ammonia. By analyzing samples for both ammonia and total Kjeldahl nitrogen, organic nitrogen can be calculated. This enables you to

<sup>1</sup> Distillation involves boiling the sample and collecting the steam.

estimate how much nitrogen in the system is in organic form, intermediate form (ammonia) and inorganic form (nitrate). It may tell you how much comes from sewage, versus fertilizer, for example.

**Analytical Methods**

Measuring TKN involves a sophisticated process for which there are no simple methods. The Hach adaptation is the easiest, though it uses the distillation step only if known interferences are present.

Basic Methods	Advanced Methods
None	<p><i>Digestion followed by Nesslerization followed by spectrophotometry (Standard Methods #4500-Norg B or equivalent)</i></p> <ol style="list-style-type: none"> <li><b>1)</b> Digest<sup>2</sup> water sample to convert organic and ammonia compounds to ammonia nitrogen.</li> <li><b>2)</b> Ammonia is then measured using the Nesslerization Method (see ammonia methods).</li> <li><b>3)</b> The reading is compared with a set of standard concentrations and reported as mg/l TKN.</li> </ol>

**pH**

pH is a measure of the acidity of the water. Since pH affects many biological and chemical reactions in the water and most organisms have a preferred range, it is a good indicator of capability of the aquatic ecosystem to support life.

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<sup>2</sup> The process of disintegration by means of chemical action, heat, and/or moisture.

## Analytical Methods

Basic methods use colorimetry. The advanced method uses a meter.

Basic Methods	Advanced Methods
<p><i>Colorimetric Method (Hach via Mitchell &amp; Stapp)</i></p> <ol style="list-style-type: none"> <li>1) This method involves the addition of pH indicator solution to a water sample which changes color according to the pH.</li> <li>2) The sample color is matched to colors labeled in pH units in a color comparator.</li> <li>3) The analyst determines the closest color match and records the pH. This should be considered an approximation only.</li> </ol>	<p><i>Electrometric Method (EPA Method 050.1 or Equivalent)</i></p> <ol style="list-style-type: none"> <li>1) Collect sample or measure directly with a meter.</li> <li>2) Measure on a collected sample using a laboratory-quality meter with an electrode suitable for ionic-strength of waters.</li> <li>3) There are less expensive pH pens or “pocket pals” on the market. These should be checked against a reliable, laboratory-quality meter to establish accuracy and precision.</li> </ol>
<p><i>pH Paper</i></p> <p>This is similar to the colorimetric method, except that a specially coated paper is dipped in the sample and turns color according to the pH. This should be considered an approximation only.</p>	

### Notes on Methods

- ◆ For waters that are low in ionic strength, accurate pH measurements require a probe that will respond in these types of waters.
- ◆ pH samples should be collected so that no air is trapped in the sample.
- ◆ The colorimetric method is subject to variation in the light source and the judgments of the analyst. It is inherently imprecise.

## Phosphorus

Phosphorus is an essential nutrient for plant growth and metabolic reactions in plants and animals. Together with nitrogen, it is a key element in the aquatic ecosystem. Too much phosphorus can cause too much biological activity and cause undesirable effects. Phosphorus occurs in various forms in the water, some of which are more available for plant growth than others.

### Phosphorus - Total Orthophosphates

This is primarily the dissolved and most available form. It is a good indicator of enrichment from various sources, such as sewage, manure or fertilizer.

#### Analytical Methods

Basic and advanced methods are basically the same colorimetric method. The main difference is that the advanced method measures the color of the treated sample using an electronic instrument.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Ascorbic Acid Method</i></p> <ol style="list-style-type: none"> <li>1) Collect a sample in a phosphorus-free container.</li> <li>2) Analyze by adding ascorbic acid reagent which turns the sample blue (ascorbic acid method) in proportion to the amount of phosphorus in the sample.</li> <li>3) Compare this blue color to various shades using a visual color comparator to get a concentration.</li> </ol>	<p><i>Ascorbic Acid Method (EPA Method #365.2 or equivalent)</i></p> <ol style="list-style-type: none"> <li>1) Collect a sample in a phosphorus-free container.</li> <li>2) Analyze by adding ascorbic acid reagent which turns the sample blue (ascorbic acid method) in proportion to the amount of phosphorus in the sample.</li> <li>3) Measure the intensity of this blue color using a spectrophotometer or colorimeter and compare with results for a set of standard concentrations.</li> </ol>

*Notes on Methods*

- ◆ The basic method should be considered an approximation only. This method is not acceptable for federal and state agency assessment, but is fine for education and awareness and some community assessments.

**Phosphorus, Total**

Total phosphorus includes all the forms. It is a good indicator of enrichment from various sources, such as sewage, manure, or fertilizer.

**Analytical Methods**

Basic and advanced methods are basically the same colorimetric method. The main difference is that the advanced method measures the color of the treated sample using an electronic instrument.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Persulfate Digestion Followed by Ascorbic Acid Method</i></p> <ol style="list-style-type: none"> <li>1) Collect a sample in a phosphorus-free container.</li> <li>2) Boil, acidify and oxidize a sub-sample to convert all forms of phosphorus to orthophosphate (persulfate digestion).</li> <li>3) Analyze Orthophosphate by adding ascorbic acid reagent which turns the sample blue (ascorbic acid method) in proportion to the amount of phosphorus in the sample.</li> <li>4) Compare this blue color to various shades using a visual color comparator to get a concentration.</li> </ol>	<p><i>Persulfate Digestion Followed by Ascorbic Acid Method (EPA Method #365.2 or equivalent)</i></p> <ol style="list-style-type: none"> <li>1) Collect a sample in a phosphorus-free container.</li> <li>2) Boil, acidify and oxidize a sub-sample to convert all forms of phosphorus to orthophosphate (persulfate digestion).</li> <li>3) Analyze Orthophosphate by adding ascorbic acid reagent which turns the sample blue (ascorbic acid method) in proportion to the amount of phosphorus in the sample.</li> <li>4) Measure the intensity of this blue color using a spectrophotometer or colorimeter and compare with results for a set of standard concentrations.</li> </ol>

### Notes on Methods

- ◆ The basic method should be considered an approximation only. This method is not acceptable for federal and state agency assessment, but is fine for education and awareness and some community assessments.

## Phosphorus, Total Dissolved

Total dissolved phosphorus includes all the forms after a sample is filtered. It is a good indicator of the available forms from various sources, such as sewage, manure or fertilizer.

### **Analytical Methods**

Basic and advanced methods are basically the same as the method for total phosphorus, with the addition of filtering the sample before digestion and analysis.

## Solids

Solids include materials that are dissolved, suspended or settled in the water column. *Total solids* include all of these.

## Solids - Total Suspended

Total suspended solids consist of solids that are filtered out of a water sample. Suspended solids affect water clarity and can reduce photosynthesis and cause higher temperatures.

### **Analytical Methods**

Measuring total suspended solids involves a sophisticated process for which there are no simple methods.

Basic Methods	Advanced Methods
None	<p><i>Gravimetric Method: Total Suspended Solids Dried at 103-105° C (Standard Methods #2540D)</i></p> <ol style="list-style-type: none"><li>1) Weigh a glass fiber filter.</li><li>2) Filter a sample through the filter, and transfer it to a Gooch crucible.</li><li>3) Dry filters and crucibles in an oven at 103-105°C for an hour.</li><li>4) Weigh filters and crucibles again.</li><li>5) Calculate total dissolved solids by subtracting the weight of the filter and crucible from the weight after filtering and drying. Results are reported as mg/l.</li></ol>

## Solids - Total Dissolved

Dissolved *solids* include various ions of calcium, chlorides, nitrate, phosphate, iron, sulfur and others that will pass through a two-micron pore. These affect the water balance in the cells of aquatic organisms, making it difficult for them to maintain position in the water column.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<i>None</i>	<p><i>Gravimetric Method: Total Dissolved Solids Dried at 180°C (Standard Methods #2540C)</i></p> <ol style="list-style-type: none"> <li><b>1)</b> Filter a sample through a glass fiber filter.</li> <li><b>2)</b> Weigh a ceramic dish.</li> <li><b>3)</b> Pour the filtered sample into the dish.</li> <li><b>4)</b> Evaporate the water in an oven at 180°C, and weigh the dish plus residue.</li> <li><b>5)</b> Calculate total dissolved solids by subtracting the weight of the dish from the weight of the dish with residue. Results are reported as mg/l.</li> </ol>

### **Secchi Depth Transparency (for lakes only)**

Transparency describes scattering and absorption of light by small particles and molecules in the water. 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. Reduced transparency has the same effects as elevated turbidity.

<b>Basic Methods</b>	<b>Advanced Methods</b>
<p><i>Secchi Disk</i></p> <ol style="list-style-type: none"> <li><b>1)</b> Lower Secchi disk into the water until it disappears from sight.</li> <li><b>2)</b> Bring disk up until it appears again.</li> <li><b>3)</b> The average of these two depths is the Secchi depth transparency.</li> </ol>	<i>Same as basic</i>

### **Temperature**

Since temperature affects many biological and chemical reactions in the water and most organisms have a preferred range, it is a good indicator of capability of the aquatic ecosystem to support life. It is measured in degrees Fahrenheit (°F) or degrees Celsius (°C).

#### **Analytical Methods**

<b>Basic Methods</b>	<b>Advanced Methods</b>
<i>Direct measurement with thermometer</i>	<i>Direct measurement with thermometer, thermocouple, thermistor or a multi-use meter</i>

### **Turbidity (for streams only)**

Turbidity describes how the particles suspended in the water affect its clarity by scattering light. It is an indicator of the presence of suspended sediment from erosion, which can decrease biological activity, raise water temperatures and clog fish gills and gravel spawning areas. Turbidity results are usually reported as nephelometric turbidity units (NTUs).



## Analytical Methods

The basic methods involve measuring transparency, which includes both light scattering and absorption. The advanced method measures just light scattering. Thus, the results of basic and advanced methods are not comparable with each other. The “advanced” method is actually fairly simple, though it involves an expensive meter.

Basic Methods	Advanced Methods
<p><i>Turbidity Tubes (Lamotte)</i></p> <ol style="list-style-type: none"> <li>1) Two graduated cylinders with black dots on the bottom are filled to a specific volume -- one with sample water the other with turbidity-free water.</li> <li>2) A reagent is added to the turbidity-free water cylinder, until the visibility of the dot on the bottom is equivalent to that of the cylinder with the sample.</li> <li>3) The results are reported in unspecified units.</li> <li>4) This method actually measures absorbance plus scattering, so the results are not actually NTUs.</li> </ol>	<p><i>Nephelometric Method (Standard Methods #2130 or equivalent)</i></p> <ol style="list-style-type: none"> <li>1) Turbidity is measured by collecting and analyzing a water sample using a nephelometer.</li> <li>2) A nephelometer consists of a light source that projects a beam of light through the water sample and a photo-electric cell that measures the intensity of light scattered by particles at a 90° angle from its original path.</li> <li>3) The results are reported as nephelometric turbidity units (NTUs).</li> </ol>
<p><i>Turbidity Tube (Tennessee Valley Authority)</i></p> <ol style="list-style-type: none"> <li>1) These tubes are marked in increments of NTUs on the side and a wave pattern on the bottom.</li> <li>2) The sample is poured into the tube until the wave pattern disappears.</li> <li>3) The NTU increment level of the sample is reported.</li> <li>4) This method actually measures absorbance plus scattering, so the results are not actually NTUs. In fact, they should be reported in centimeters or inches.</li> </ol>	

### Notes on Methods

- ◆ Measure turbidity in rivers.
- ◆ Make sure that the meter you purchase is a nephelometer that measures light scattered at a 90° angle.
- ◆ Turbidity tubes are not acceptable substitutes for a nephelometer, since they actually measure transparency (light scattering and absorption), rather than just light scattering. Because of this, they are unreliable in colored waters, which absorb light, though may not be turbid at all. They are really more analogous to Secchi disks, in that your eye responds to absorption. If you use these tubes, report your results as a depth (in centimeters or inches) rather than NTUs.

## **Water Column Metals and Other Elements Recommended for Sampling Only (Advanced Assessments)**

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Many naturally-occurring metals are toxic to aquatic life, when present in high enough concentrations. The most common process is when they bind to gill surfaces on fish and insects. The metals of concern are mostly those that are “available” as dissolved ions in the water column.

The following metals and other elements are recommended by several of the advanced assessments. However, the analysis methods require techniques and equipment beyond the means of community-based groups and schools (except universities). Therefore, we recommend sampling only. Even for sampling, we recommend consulting with your regional DEP biologist as to sampling procedure

### ***Sampling (Standard Methods 3010B)***

Because some metals are toxic in very small amounts (micrograms per liter), the methods must be able to detect very low concentrations. Contamination of sampling containers is a real concern. Samples for metals are usually collected in special acid-rinsed containers made of polypropylene, linear polyethylene or borosilicate glass. Samples to be analyzed for dissolved metals are immediately filtered through a 0.45 micron filter. Otherwise they are preserved by acidifying with concentrated nitric acid to a pH of <2.

### ***Analytical Method (EPA Method 200.7)***

Various forms of metals can be analyzed, depending on how the sample is treated:

- Dissolved Metals: The filtered sample is analyzed.
- Suspended Metals: Metals are trapped on the filter.
- Total Metals: Metals are detected in an unfiltered sample after digestion or the sum of dissolved and suspended metals are added together.
- Acid-extractable Metals: Metals in solution after treatment of the sample with hot mineral acid.

Metals are usually analyzed as a suite using *Inductively Coupled Plasma - Atomic Emission Spectrometry*. An inductively coupled plasma source (a machine) vaporizes the sample and heats it to about 6000-8000°C. Molecules separate and atoms become active and ionized (reactive). In this state, each element produces a unique spectral (colored) pattern which is read by a spectrometer.

Needless to say, this is an extremely technical and expensive method beyond the reach of volunteer monitoring programs.

### **Aluminum (sampling only)**

Aluminum is a naturally occurring element in rocks, soils and the waters in contact with them. It occurs as a soluble salt, a colloid or an insoluble compound. Aluminum toxicity for aquatic life depends on pH.

### **Arsenic (sampling only)**

Arsenic is a naturally occurring element in rocks, soils and the waters in contact with them. Recognized as a toxic element for centuries, arsenic today also is a human health concern because it can contribute to skin, bladder and other cancers (National Research Council, 1999).

Arsenic is widely distributed throughout the earth's crust and is used commercially, primarily in alloying agents. It is introduced into water through the dissolution of minerals and ores, from industrial effluents and from atmospheric deposition; concentrations in groundwater in some areas are elevated as a result of erosion from local rocks

Arsenic is highly toxic, though its toxicity is dependent on its form and environmental conditions.

Used in groundwater assessment only.

### **Barium (sampling only)**

Barium is a lustrous, machinable metal that exists in nature only in ores containing mixtures of elements. It is used in making a wide variety of electronic components, in metal alloys, bleaches, dyes, fireworks, ceramics and glass. In particular, it is used in well drilling operations where it is directly released into the ground.

EPA has found barium to potentially cause gastrointestinal disturbances and muscular weakness when people are exposed to it at unsafe levels for relatively short periods of time

In water, the more toxic soluble barium salts are likely to be converted to insoluble salts that precipitate. Barium does not bind to most soils and may migrate to groundwater. It has a low tendency to accumulate in aquatic life and does not seem to be an aquatic life health concern.

Used in groundwater assessment only.

### **Cadmium (sampling only)**

Cadmium is a metal found in natural deposits as ores containing other elements. The greatest use of cadmium is primarily for metal plating and coating operations, including transportation equipment, machinery and baking enamels, photography and television phosphors. It is also used in nickel-cadmium and solar batteries and in pigments.

EPA has found cadmium to potentially cause vomiting, diarrhea, muscle cramps, nausea, salivation, sensory disturbances, liver injury, convulsions, shock and renal failure when people are exposed to it at unsafe levels for relatively short periods of time. Cadmium has the potential to cause kidney, liver, bone and blood damage from a lifetime exposure at unsafe levels.

Cadmium occurs naturally in zinc, lead, copper and other ores that can serve as sources to ground and surface waters, especially when in contact with soft, acidic waters. Major industrial releases of cadmium are due to waste streams and leaching of landfills, and from a variety of operations that involve cadmium or zinc. In particular, cadmium can be released to drinking water from the corrosion of some galvanized plumbing and water main pipe materials.

Some cadmium compounds are able to leach through soils to groundwater. When cadmium compounds do bind to the sediments of rivers, they can be more easily bioaccumulated or re-dissolved when sediments are disturbed, such as during flooding. Its tendency to accumulate in aquatic life is great in some species, low in others. Toxicity increases as hardness decreases.

### **Calcium (sampling only)**

Calcium is a naturally-occurring element that enters surface water from surrounding rocks. It is a vital micro-nutrient for both plants and animals. In various compounds, calcium is an important part of the water's buffering system (see alkalinity, acidity, hardness). Used in groundwater assessment only.

### **Chromium (sampling only)**

Chromium is a metal found in natural deposits as ores containing other elements. Though chromium occurs in nature mostly as chrome iron ore and is widely found in soils and plants, it is rare in natural waters. The greatest use of chromium is in metal alloys such as stainless steel; protective coatings on metal; magnetic tapes; and pigments for paints, cement, paper, rubber, composition floor covering and other materials. Its soluble forms are used in wood preservatives.

Short-term: EPA has found chromium to potentially cause skin irritation or ulceration when people are exposed to it at unsafe levels for relatively short periods of time. Chromium has the potential to cause damage to liver, kidney, circulatory and nerve tissues; and cause skin irritation from a lifetime exposure at unsafe levels.

When released to land, chromium compounds bind to soil and are not likely to migrate to groundwater. They are very persistent in water as sediments. There is a high potential for accumulation of chromium in aquatic life.

### **Copper (sampling only)**

Copper is a metal found in natural deposits as ores containing other elements. It is widely used in household plumbing materials.

Copper is an essential nutrient, required by the body in very small amounts. However, EPA has found copper to potentially cause stomach and intestinal distress, liver and kidney damage, and anemia when people are exposed to it at high levels for relatively short periods of time.

Copper may occur in drinking water either by contamination of the source water used by the water system, or by corrosion of copper plumbing. Corrosion of plumbing is by far the greatest cause for concern. Copper is rarely found in source water, but copper mining and smelting operations and municipal incineration may be sources of contamination.

All water is corrosive toward copper to some degree, even water termed non-corrosive or water treated to make it less corrosive. Corrosivity toward copper is greatest in very acidic water. Many of the other factors that affect the corrosivity of water toward lead can also be expected to affect the corrosion of copper.

Copper is toxic to aquatic life at high levels, though it does not appear to accumulate in the edible portions of freshwater fish. Toxicity increases as hardness decreases.

### **Iron, Total (sampling only)**

Iron is a metal common in rocks and soils and in varying quantities in surface water. It is an essential trace element required by both plants and animals. Iron is not considered a problem for aquatic life, except for physical effects from iron precipitate.

### **Lead (sampling only)**

Lead is a metal found in natural deposits as ores containing other elements. It is sometimes used in household plumbing materials or in water service lines used to bring water from the main to the home.

Lead can cause a variety of adverse health effects when people are exposed to it at unsafe levels for relatively short periods of time. These effects may include interference with red blood cell chemistry, delays in normal physical and mental development in babies and young children, slight deficits in the attention span, hearing and learning abilities of children, and slight increases in the blood pressure of some adults. Lead has the potential to cause stroke, cancer and kidney disease from a lifetime exposure at unsafe levels.

Lead may occur in drinking water either by contamination of the source water used by the water system, or by corrosion of lead plumbing or fixtures. Lead is rarely found in source water, but lead mining and smelting operations may be sources of contamination. Corrosion of plumbing is by far the greatest cause for concern.

When released to land, lead binds to soils and does not migrate to groundwater. In water, it binds to sediments. It does not accumulate in fish, but does in some shellfish, such as mussels. Toxicity increases as hardness decreases.

Used in groundwater assessment only.

### **Manganese (sampling only)**

Manganese does not occur naturally as a metal. It is frequently found in various salts and minerals, frequently with iron. It is a vital micro-nutrient for both plants and animals. It occurs in surface waters in soluble or suspended form, but rarely in concentrations considered toxic to aquatic life.

### **Potassium (sampling only)**

Potassium is an abundant element found in many minerals. It is an essential plant and animal nutrient and is also common in fertilizers. Used in groundwater assessment only.

### **Silica (sampling only)**

Silicon is an abundant element and most waters contain it in the form of silica ( $\text{SiO}_2$ ) and silicates. The chief concern is deposits on industrial equipment. Used in groundwater assessment only.

### **Sulfate (sampling only)**

Sulfate occurs in natural waters in a wide variety of concentrations. It can occur in high concentrations in mine drainage from the oxidation of pyrite and the use of sulfuric acid.

### **Zinc (sampling only)**

Zinc is common in natural waters, but is increased by the deterioration of galvanized pipes. It is essential to human metabolism, but can be toxic to aquatic life.

# Descriptions of Common Physical and Stream Channel Indicators and Methods Used in this Handbook

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## Lake Level

Lake level is the elevation of the water surface elevation relative to a fixed elevation. This is typically done by fixing a staff gauge (a stick marked in inch or centimeter increments) to an object anchored to the lake bottom, such as a dock or pier support. Levels are read directly off the gauge. Frequently, lake level gauges are located at lake outlet dams.

## Rainfall

Rainfall amounts can be measured using a rain gauge, or received from the National Oceanic and Atmospheric Administration (NOAA). Rain gauges are essentially collection devices marked in inches. The amount collected in the gauge is read and recorded at the time interval of interest (daily, hourly, etc.). NOAA data is collected at various locations throughout the country. If one of these stations is in your watershed, this data may serve your needs. This information is available from the National Climatic Data Center: <http://cdo.ncdc.noaa.gov/plclimprod/plsql/poemain.poe>. This will take you to a page where you can begin your search for data. There is a charge for this data. The National Climatic Data Center (NCDC) is part of the Department of Commerce, National Oceanic and Atmospheric Administration (NOAA). However, since rainfall patterns can vary over a region, you may need to set up your own gauges that more accurately reflect conditions in your areas of interest.

## River Channel Characteristics (wadeable waters only)

River channel characteristics are the various physical features of the river channel that reflect geological and hydrological changes over time. The river channel is a dynamic land form that is constantly moving as water erodes the land surface. It also responds to human-caused changes in watershed land use and alterations of the river channel. These characteristics form the physical foundation of the river system and provide habitat for aquatic life. Monitoring these characteristics must be a long term, on-going effort. Characteristics recommended in this guide are Bottom Composition, Embeddedness, Channel Cross Section and Longitudinal Profile. These characteristics should be surveyed at both pool (low energy) and riffles (high energy) habitats. These measurements can be done only in wadeable waters.

*Bottom Composition (Wolman Pebble Count, US Forest Service Stream Channel Reference Sites Guide)*

Bottom composition is the percent of the bottom in various size classes: sand, gravel, cobble and boulder. It is measured using the pebble count procedure. This involves measuring the intermediate axis (neither the shortest nor the longest of the sides) of randomly selected particles on the stream bottom along transects where cross sections are measured. Each particle is placed in a size class, from sand (<2mm) to very large boulders (2048-4096 mm). This data can be plotted in various ways to represent bottom composition. The USFS recommends plotting cumulative percent (cumulatively adding the percent of the total count in each size class percent from smallest to largest) versus particle size. The changes in particle size over time will reflect the effects of erosion and deposition.

*Embeddedness (EPA Environmental Monitoring and Assessment Program)*

Embeddedness is the extent to which larger particles (especially cobbles) are surrounded by sand and silt. It is measured by estimating the percentage of the particle surface (the same particle used in the pebble count) that is surrounded by sediment. The area that was buried is typically lighter in

color than that which was exposed. Changes in embeddedness can indicate scouring and deposition.

*Channel Cross Section (US Forest Service Stream Channel Reference Sites Guide)*

A channel cross section is the shape of a “slice” of the channel -- its width and depth. It is also the location where flow and bottom composition are measured. A channel cross section is measured at locations that represent typical channel form, clear channel features, clear indicators of bankfull (top of the bank flow) and active floodplain, clear terraces and a straight reach. It is measured by locating and determining the elevations of endpoints on either side of the channel, measuring the depths (using a surveyor's level and rod) from a line stretched across the endpoints to the channel bottom and water surface. The measurements are plotted as distance versus elevation to depict the cross-section. Changes in channel cross-section will reflect scouring, deposition and channel movement.

*Longitudinal Profile (US Forest Service Stream Channel Reference Sites Guide)*

A longitudinal profile measures and plots the slope of a 300-500 foot reach of the river. It is measured by first locating and marking important channel and related features (such as terraces, riffles, pools, vegetation changes, etc.). Elevations at the marked features are measured using a surveyor's level and rod. Elevations of the channel bottom, water surface, terraces and floodplains can all be gathered. The data are plotted as elevation versus distance. Changes in channel cross-section will reflect scouring and deposition.

## **Stream Flow**

This is the volume of water passing a point expressed in cubic feet or meters per second. Flow affects the river physical characteristics, such as erosion and sedimentation, bottom composition, amount of the bottom covered with water, etc. Historical and current flow data can be found at the USGS' Pennsylvania Web Site: <http://pa.water.usgs.gov/>. If no data are available for your waters, you may have to collect your own.

*Embodiment Float Method (EPA Volunteer Stream Monitoring Method Manual/Pa. Senior Environment Corps)*

Flow is measured by first calculating cross-sectional areas (width times average depth) of two transects in a 20-foot section of stream. Then current velocity is measured by how long it takes a float (typically an orange) to travel the length of the 20-foot segment. Flow is calculated by multiplying the average cross sectional area times a constant (for rocky or muddy stream bottoms) times the length (20 ft.) and dividing by how long it takes a float (typically an orange) to travel the length of the 20-foot segment. Flow is reported in cubic feet per second.

## **Visual Field Surveys**

Visual surveys involve observations, inventories and estimates of river, riparian, lakeshore and watershed characteristics, uses, values and threats:

- ◆ A pollution source inventory.
- ◆ Water color, odor and appearance.
- ◆ Corridor land uses.
- ◆ Evidence of pollution.
- ◆ Habitat types.
- ◆ Pipe Survey.
- ◆ Channel and shoreline vegetation.
- ◆ Bottom composition.
- ◆ Condition of shorelines.
- ◆ Water uses.
- ◆ In-stream or in-lake plant growth.

The area surveyed should include the watershed zones of interest -- the water column, river banks, riparian areas or upland areas. Typically, the presence or absence of these characteristics is noted, the quantity or extent visually estimated, and location mapped.

### ***Methods Options***

There are a variety of visual survey methods available. Sources of these methods include:

- ◆ DEP
- ◆ Pennsylvania Senior Environment Corps
- ◆ ALLARM
- ◆ Alliance for Chesapeake Bay
- ◆ Pennsylvania Bureau of State Parks
- ◆ River Network
- ◆ EPA
- ◆ Adopt-A-Stream Foundation

These agencies and organizations have methods that have been field tested and found to produce useful information and can be taught to and carried out by volunteers and schools. Select a method that will provide information useful to your data users and meets your data quality goals.



# Descriptions of Common Aquatic Life and Habitat Indicators and Methods Used in this Handbook

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## Benthic Macroinvertebrates

These are organisms without backbones that live on the river bottom. They include aquatic insects (such as mayflies), mollusks, crustaceans and worms. They are good indicators of ecological conditions and human impacts, since they are integral to the river's food web. The community present reflects both water and habitat quality.

### *Terms Used In Benthic Macroinvertebrate Sampling*

- ◆ Qualitative Net Collection: A sample is collected directly off the bottom using a net. The level of effort is not standardized.
- ◆ Semi-Quantitative Net Collection: A sample is collected directly off the bottom using a net. The level of effort is standardized by collecting from a specified area in front of the net. Since the area is not precisely delineated, the method is not strictly quantitative.
- ◆ Quantitative Surber or Hess Sampler: A sample is collected directly off the bottom using a sampler which delineates the area from which samples will be collected. The level of effort is standardized by collecting from this delineated area
- ◆ Rock Basket or Multi-Plate Samplers: A sample is collected by placing rock-filled baskets or stacked tiles on the bottom or in the water column and allowing them to be colonized. The time they are left out is standardized at six weeks and the colonization area in each basket is roughly the same. This is the most quantitative collection method.

Note that there are many variations on the sampling methods. The ones listed below should be considered basic templates that can be modified to fit different conditions.

Methods for each are described below.

<b>Basic Methods</b>	<b>Advanced Method</b>
<p data-bbox="198 210 857 310"><i>Streamside Benthic Macroinvertebrate Assessment (Pa. Senior Environment Corps, River Network or equivalent)</i></p> <ol data-bbox="198 325 836 703" style="list-style-type: none"> <li><b>1)</b> This assessment is carried out entirely in the field.</li> <li><b>2)</b> It involves the qualitative collection of one composite sample from three spots in riffle habitats with a seine or net with a 0.6 mm mesh.</li> <li><b>3)</b> Organisms are identified to major group and the relative abundance estimated in the field.</li> <li><b>4)</b> Three primary habitat characteristics are estimated or measured.</li> </ol> <p data-bbox="198 714 828 924">This survey produces a quick estimate of conditions, based on the presence and relative abundance of key indicator organisms. This method is not acceptable for federal and state agency assessment, but is fine for education and awareness and some community assessments.</p>	<p data-bbox="881 210 1409 310"><i>Intensive Benthic Macroinvertebrate Assessment: Net Collection (River Network, Stroud Water Research Center)</i></p> <ol data-bbox="881 325 1453 976" style="list-style-type: none"> <li><b>1)</b> This method is carried out in the field and lab.</li> <li><b>2)</b> Semi-quantitative samples are collected with a metal frame net with an opening of 18" wide by 8" high with 0.6 mm nylon mesh<sup>3</sup> OR</li> <li><b>3)</b> Quantitative collection with a Surber or Hess sampler.</li> <li><b>4)</b> Collection with the specified device of three composite samples from two fast and two slow spots in riffle habitats.</li> <li><b>5)</b> This sample is preserved in alcohol for later lab identification.</li> <li><b>6)</b> Twenty-two habitat characteristics are estimated or measured in the field.</li> <li><b>7)</b> Critters are identified to family and counted in the lab.</li> </ol> <p data-bbox="881 987 1453 1302">This survey produces a fairly sensitive assessment of conditions based on a number of numerical analyses of community composition, functional feeding groups, pollution tolerance of families, and allows numerical site to site comparisons. It can detect shifts in families within major groups that might result from pollution or habitat alteration.</p>

<sup>3</sup> This mesh size is the standard recommended by the U.S. EPA. This size catches the smaller critters (like midges) but does not quickly plug up with sediment.

<b>Basic Methods</b>	<b>Advanced Method</b>
<p data-bbox="201 216 821 279"><i>Basic Benthic Macroinvertebrate Assessment (River Network)</i></p> <ol data-bbox="201 296 846 747" style="list-style-type: none"> <li>1) This assessment is carried out in the field and lab.</li> <li>2) It involves the semi-quantitative collection with a specified seine or net (0.6 mm mesh) of one composite sample from one fast and one slow spot in riffle habitats.</li> <li>3) This sample is preserved in alcohol for later lab identification.</li> <li>4) Twenty-two habitat characteristics are estimated or measured in the field.</li> <li>5) Organisms are identified to major group and counted in the lab.</li> </ol> <p data-bbox="201 758 857 1003">This survey produces a somewhat sensitive assessment of conditions based on a number of numerical analyses of community composition, gross pollution tolerance of major groups and allows site to site comparisons (if the communities are different enough to produce dramatically different results).</p>	<p data-bbox="885 216 1393 317"><i>Intensive Benthic Macroinvertebrate Assessment: Rock Basket Collection (River Network)</i></p> <ol data-bbox="885 331 1463 926" style="list-style-type: none"> <li>1) This assessment is carried out in the field and lab.</li> <li>2) Quantitative samples are collected with rock baskets consisting of a coated wire mesh basket filled with similar sized rocks (4 to 12 cm in diameter). Organisms colonize the rock basket over a period of five weeks. Two or three samples are collected from riffle and run habitats.</li> <li>3) Samples are preserved in alcohol for later lab identification.</li> <li>4) Twenty-two habitat characteristics are estimated or measured in the field.</li> <li>5) Organisms are identified to family and counted in the lab.</li> </ol> <p data-bbox="885 936 1458 1255">This survey produces a fairly sensitive and more quantitative assessment of conditions based on a number of numerical analyses of community composition, functional feeding groups, pollution tolerance of families, and allows more precise numerical site to site comparisons. It can detect shifts in families within major groups that might result from pollution or habitat alteration.</p>

## **Benthic Macroinvertebrate Habitat**

Benthic macroinvertebrates exist in a wide range of locations in the river:

- ◆ Shallow, fast moving rocky bottom areas known as *riffles*;
- ◆ Deeper, slower moving sandy and gravelly bottom areas known as *runs*; and
- ◆ Slow moving muddy-bottom areas known as *pools*.

However, the number and diversity of organisms present is greatest in riffles. Habitat quality must be assessed in order to separate the influence of water column chemistry and biology from habitat on the community. While all of these are affected by human activities, natural variations in habitat might produce changes that might be mistaken for human-caused changes. So, a habitat assessment is a critical part of a benthic macroinvertebrate assessment.

*Habitat Assessment (River Network Adaptation of EPA Rapid Bioassessment Protocol II)*

A habitat assessment is the estimate and measurement of 10 selected physical characteristics of the river in order to determine the overall quality of the habitat for benthic macroinvertebrates. Examples of these characteristics include the velocity of the current; the composition of the river bottom; depth; and the nature and extent of riffles. Together with water quality, these characteristics determine the kinds and numbers of macroinvertebrates that can live there. Both habitat, quality and water quality are affected by human activities in the river or on lands in the watershed. This includes physical characteristics of the river that provide habitat for the invertebrates such as bottom composition, sedimentation, current velocity, shading, extent of riffle habitat, and others. Results for each site from the “Benthic Macroinvertebrate Habitat Assessment Field Sheet” are scored, totaled and compared with the total score from the reference site (least impaired upstream conditions).

## **Aquatic Vegetation (lakes)**

Aquatic vegetation is an important part of a lake ecosystem, especially in near-shore areas. They provide habitat for aquatic animals and are an important source of oxygen. Some are nuisance plants that cause dramatic habitat alterations and interfere with recreational uses. The plant types, density, diversity and growth patterns are important characteristics to assess.

*Aquatic Vegetation Mapping/ Identification*

Aquatic vegetation mapping and identification involves visual observation and mapping and collection of specimens for identification. For mapping, monitors take a tour of the lake shoreline and observe areas of the lake where aquatic vegetation is at or near the surface. The location and extent of vegetation beds is drawn onto a map. For identification, vegetation samples are collected along a transect using a weighted rake. The samples are sorted, a qualitative estimate is made of the percentage and density of each type of plant, and specimens of each type are bagged for shipment to a botanist for identification.