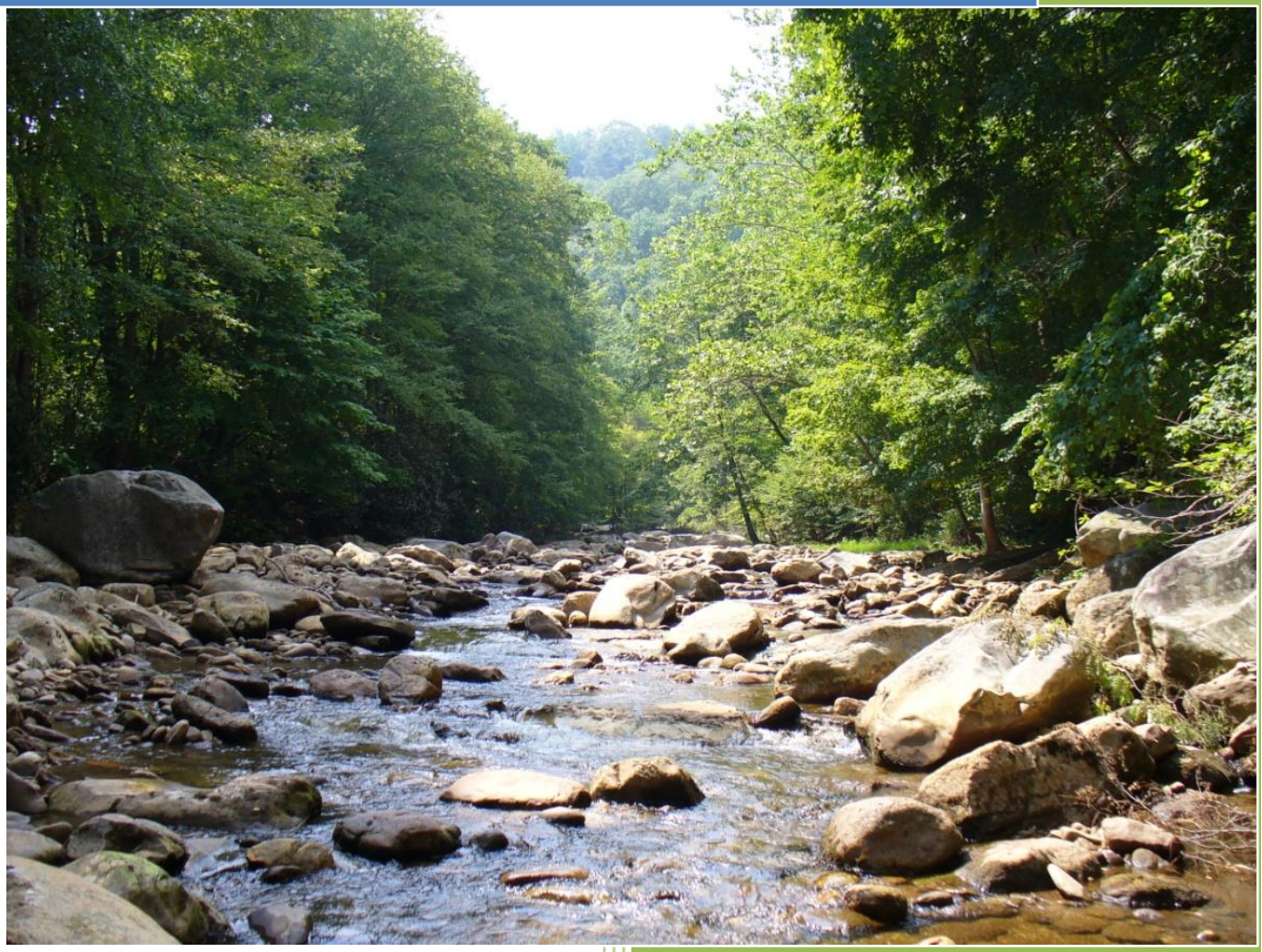
West Virginia Department of Environmental Protection

Watershed Branch 2010 Standard Operating Procedures



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Acknowledgements

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Since our sampling activities are always evolving and growing, this document is a "living document" that grows accordingly each year. It is intended as a reference document not only for agency staff, but also for collaborating federal, state, or local agencies and other government organizations or entities, the private sector (*e.g*., industry consultants), and public organizations (*i.e*., watershed groups) and individuals.

While reviewing or consulting this document, should you encounter a typographical or grammatical error or wish for some guidance or clarification in methodology please feel free to contact us at:

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Disclaimer

While this document does cover the use and care of some of the instrument models that WVDEP uses, any instructions in this document are meant to be used only as a quick reference for those already familiar with the operation and specialized maintenance of the instrument. Any information in this document is not meant to supersede the instrument manufacturer's operations manual but only to supplement it.

List of Abbreviations and Acronyms

AMD-Acid Mine Drainage or sometimes Alkaline Mine Drainage
AML-Abandoned Mine Lands
AWQN-Ambient Water Quality Network
COC-Chain-of-Custody
CWA-Clean Water Act
DO-Dissolved Oxygen
DS-Downstream
USEPA-United States Environmental Protection Agency
GIS-Geographic Information Systems
GPS-Geographic Positioning System
HUC-Hydrologic Unit Code
IBI-Index of Biotic (or Biologic) Integrity
LTMS-Long-Term Monitoring Station
NHD-National Hydrographic Dataset
NPS-Non-Point Source
PS-Point Source
QA/QC-Quality Assurance/Quality Control
RBP-Rapid Bioassessment Protocols
RTE-Rare, Threatened, or Endangered
SOP-Standard Operating Procedure
TMDL-Total Maximum Daily Loads
US-Upstream
USGS-United Stated Geological Survey
WAB-Watershed Assessment Branch
WQ- A general shorthand for water quality
WV-West Virginia
WVDEP-West Virginia Department of Environmental Protection
WVDNR- West Virginia Division of Natural Resources
WVEQB-West Virginia Environmental Quality Board
WVSCI-West Virginia Stream Condition Index

Chapter I. INTRODUCTION TO WATERSHED ASSESSMENT BRANCH SAMPLING ACTIVITIES

Function of the Watershed Assessment Branch

The purpose of the Watershed Assessment Branch (WAB) is to collect waterbody (*i.e.*, streams, rivers, and lakes) data in order to determine their quality in West Virginia according to the Federal Clean Water Act (CWA). This is accomplished by visiting hundreds of streams and lakes throughout the state collecting water and biological samples (*e.g.*, fish, benthic macroinvertebrates, and periphyton) and assessing the quality of the instream and streamside habitat. The data collected is used to determine which streams and lakes are in violation of water quality standards or impaired biologically.

All waterbodies (*i.e.*, streams, rivers, lakes, reservoirs, ponds, navigable waters, wetlands, etc.) in the state are grouped into 32 watersheds based on the USGS 8-digit HUCs (Hydrologic Unit Codes). These watersheds are sampled on a five-year rotation (aka the rotating Watershed Basin Schedule) so that any given year approximately one-fifth of the watersheds are being intensively sampled and assessed. The data produced by the sampling efforts of WAB provides information regarding the severity of pollution, the potential for cleanup, and supports the implementation of management and control measures.

Sampling Programs of the Watershed Assessment Branch

WAB consists of many different sampling programs that are each unique in their sampling methods, protocols, and intensities of habitat assessment. The sampling programs include:

Wadeable Streams Monitoring occurs on streams that are considered to be wadeable (*i.e.*, easily traversed without having to use a boat). This applies to almost all 1^{st}-4^{th} order streams, but may include some smaller 5^{th} and 6^{th} order streams. The components of sampling include water quality and biological assemblage samples (mainly benthic macroinvertebrates and periphyton, but sometimes fish) as well as an intensive habitat assessment. Two differing strategies of wadeable stream monitoring are as follows:

> *Random (Probabilistic) Sampling* is a sampling subset within the Watershed Assessment Branch designed to allow unbiased, statistical interpretations of water quality using water chemistry, biological, and habitat data. The state is further subdivided into Level III Ecoregions statewide and examined on a 100k scale. The sample stations include 1-4th order streams (based on the NHD Plus

stream coverage-100k scale) and are weighted based on the relative abundance of those orders in WV. Sampling does not coincide with the rotating Watershed Basin Schedule and occurs primarily in the Spring/Early Summer (April-Early June). Fish surveys to monitor populations & communities will be conducted on stations that are target and have watershed drainages greater than 2000 acres (+/- 10%). The fish surveys will occur later in the summer during a fish index period.

Targeted Sampling is designed to investigate:

1. Streams that have no previous data collected,
2. Streams that have only outdated data collected,
3. Streams with data previously collected that rendered inconclusive results (*e.g.*, streams with IBI scores that are uncertain or "gray" or streams with prior collections),
4. Streams that have known impairments (*i.e.*, legacy 303(d), AMD or Acid Mine Drainage, Biological impairments),
5. Streams of particular public interest (*i.e.*, high-quality streams, trout streams, streams undergoing restoration projects).
   This targeted sampling is driven by the rotating Watershed Basin Schedule and sampling is a one-time event that occurs mainly in the Summer/early fall (June-October). Fish surveys occur on a limited number of select larger streams.

TMDL stands for Total Maximum Daily Load. A targeted sampling strategy is used to gather information about the full extent of pollution impairments (*i.e.*, which streams are problem areas or not and what are the sources of pollution). The resultant data is used to develop and calibrate TMDL models for streams listed on the CWA Section 303(d) list. Candidate streams for TMDL development coincide with the rotating Watershed Basin Schedule and sampling occurs monthly for one year. The components of sampling include water quality samples and a limited habitat assessment. At streams with biological impairments sampling includes a one-time biological sample and intensive habitat assessment.

Ambient Water Quality Network (AWQN) is a bimonthly statewide trend monitoring program at 26 targeted stations on major rivers and streams (both wadeable and non-wadeable) for water quality constituents. The ambient network is perhaps the oldest program within the Watershed Assessment Branch with data existing as far back as the 1960s. The bimonthly components of sampling include water quality samples and limited habitat observations. These sampling activities are covered mainly in ***Chapter X. AMBIENT WATER QUALITY NETWORK PROTOCOLS starting on page 224***.

Long Term Monitoring Stations, or **LTMS**, are sampled to develop long-term biological trend data at targeted wadeable streams scattered throughout the state.

Stations are selected to represent a wide array of unique and varying impairments (*e.g*., Acid Mine Drainage, Acid Rain, Sediment, Nutrient Enrichment, etc.) as well as represent best attainable or reference conditions. Ambient Network stations (or nearby proxy stations) that are wadeable in the summer months are also included in this monitoring effort. Sampling occurs once per year for approximately five years to establish a baseline and then once every two to three years to monitor for changes. Sampling includes biological, intensive habitat, and water quality components. Some selected stations may also be surveyed for fish.

Lake Monitoring uses the rotating Watershed Basin Schedule much like TMDL sampling and the targeted Wadeable Stream Monitoring. Sampling occurs on targeted lakes (within the watershed group for that year) four times during the summer months (June - September or May - August). The number of stations per lake varies and is generally proportional to the size of the lake. The components of sampling include a vertical water chemistry profile (including the physiochemical properties, nutrients, and turbidity measurements), chlorophyll-*a* fecal coliform sampling, Secchi depth, and some limited habitat and disturbance observations. These sampling activities are covered mainly in ***Chapter XI. LAKE SAMPLING PROTOCOL starting on page 260***.

Special Surveys or **Projects** are temporary targeted sampling designs conducted on request from internal West Virginia Department of Environmental Protection (WVDEP) programs, external agencies, private industries, or public groups/individuals that are concerned about the water quality of particular streams or segments of streams and require additional data to supplement their own data. These surveys or projects are often done in association with land transactions, spills, pending legal actions/litigation, permit applications/renewals, water quality improvement projects (*e.g*., mitigation projects, infrastructure improvements), emerging pollution issues, or as a part of larger studies. Special Surveys are more limited in scope in that they concentrate on a very specific area and the stations are only visited once or twice. Special Projects are more long term and widespread. They may involve monthly sampling at a large number of sites over the course of a year or two. The components of sampling vary greatly depending on the survey or project needs and may include any combination of the following: simple habitat observations, water quality samples, deployable sondes, biological samples, limited habitat assessments, or intensive habitat assessments.

Deployable Sondes are often used to provide continuous water quality data (time-series) in support of other sampling programs (*e.g.*, TMDL, Special Projects). Deployment and retrieval of the sonde may be accompanied by a water quality sample and habitat observations at targeted locations.

Monitoring Programs in development:

Wetlands Monitoring
Non-Wadeable Streams and Large Rivers

Scope of SOP for Watershed Assessment Branch Sampling Programs

The following Standard Operating Procedure (SOP) chapters and sections are designed primarily for use with the **Wadeable Streams Monitoring (Random and Targeted), TMDL, AWQN**, **LTMS** and **Lake Monitoring** programs which cover the bulk of sampling activities by WAB**.** Since these sampling programs may share aspects/components with the other sampling programs (*e.g.*, Coordinate data collection, Sonde Calibration and Use, Flow Measurement, Photography) their individual sections in this document may refer to other sections for further reference.

In some cases, a **Special Survey** or **Project** may be unique enough that it may require the development of its own SOP document. However, the majority of the special surveys or projects can adequately rely on this document to cover its sampling components.

This document represents the first time the AWQN, Lake Monitoring, Golden Algae Sampling, and Fish Collection Protocols have been integrated into this document (*i.e*., prior to this document, these protocols resided within their own distinct documents or as draft protocols). The SOP documents for the **Deployable Sondes Protocols** are still in development and not included in this document version.

General Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols presented in this SOP document and calibrated to sampling standards. These sessions occur at a field location to provide real examples and situations. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors.

In the field, individuals who are more experienced in using these sampling protocols will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to work solo or lead a sampling team.

Several staff meetings also occur throughout the year to update field personnel (those collecting the data) and office personnel (those using, analyzing, and distributing the data) with any running changes to protocol and address reoccurring problems and issues in front of the two groups. These staff meetings also serve as communication

forums between field and office personnel to help each group better understand where and how the data is collected, how the data is used in fulfilling WVDEP's Clean Water Act requirements, and the specific needs of each group.

This SOP document is annually reviewed for completeness and accuracy coinciding with the mandatory training sessions and printed hard copies are provided to all program personnel for review and use in the field. In addition, any changes that occur between annual reviews of the SOP document are updated in the SOP document's electronic format and marked with a revision number. The revised SOPs are announced via email and made available internally via the WVDEP computer network at:

Q:\WATER RESOURCES\WAB\SOP'S\SOP2010

The field personnel are to print copies of the revised SOP pages and insert them into their existing hard copy for use until a new annual hard copy is provided.

Chapter II. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS)

Overview

The most important aspect of sampling that the Watershed Assessment Branch (WAB) does is the careful documentation of the location and conditions during a sampling event. This may be as simple as documenting the general conditions of the water (*i.e.*, was it turbid, did it smell, did it rain recently). Or it may be as complex as physically measuring various aspects of the stream habitat.

The following is an instruction of how use the Wadeable Benthic Stream Assessment Form to evaluate various stream assessment parameters. This chapter is intended to provide information on interpreting each parameter as well as identifying the value(s) of resultant data. Some of the parameters from other assessment procedures (*e.g.*, Benthic Sampling, Sonde Readings, GPS, etc.) are recorded on the form as well. You should consult the appropriate chapters and sections of this SOP to gain further knowledge about those parameters.

Also, since the Wadeable Benthic Stream Assessment Form is the most complex and complete that WAB uses (others like the TMDL forms are more limited in that they may only contain certain elements of what is seen on the Wadeable Benthic form), this chapter should adequately cover how to fill out the other forms as well.

Section A. Setting up the Site

Part 1. Initial Site Survey

A field crew typically consists of two individuals charged with collecting habitat and biological/physicochemical data (*i.e.*, water quality). In the case of some sampling that involves only physiochemical and some limited habitat data (*e.g.*, TMDL sampling) the field crew may consist of just one individual operating on a solo basis. This usually only occurs after the sampling station has been thoroughly established after some sort of initial visit.

Throughout the following discussions, the term "Geomorph" will be used to describe the crewmember in charge of collecting habitat information. "Biomorph" is the term used to describe the crewmember in charge of collecting biological and physicochemical data. In the case of a solo sampler, these roles are both played out by the same individual.

USGS topographic maps with a 1:24,000 scale will be used to navigate to sampling sites (GIS or Geographic Information System maps on Laptop, County Maps, or Gazetteer Maps are supplemental). The map coordinator should have marked all sites or stations (pink for random sites, yellow for target sites) before sampling begins. After the location of the stream site has been confirmed, the Geomorph is responsible for establishing a 100-meter assessment area and will actively traverse the stream from one end to the other taking note of pertinent habitat information and measuring the 100 m reach. **Note that the Geomorph will avoid walking in the stream until physicochemical samples have been collected and avoid stepping in riffles that may be used in macroinvertebrate and periphyton sampling. THERE SHOULD BE NO DEVIATION FROM THE ABOVE PROTOCOL. THE GEOMORPH MUST COVER THE ENTIRE 100 m STREAM REACH TO ACCURATELY COMPLETE THE HABITAT FORM. THIS CANNOT BE DONE STANDING AT ONE END OF THE REACH OR FROM THE VEHICLE!** The Geomorph will perform other duties concurrent with the establishment of the 100 m assessment reach (***outlined in Chapter II. Section C. Part 1. Description of Wadeable Benthic Stream Assessment Form starting on page 29***). Procedures specific to each sample type are discussed below.

Part 2. Accessing the Site

Due to the remoteness of some sites (usually reference and random), traversing to the sample site may require long strenuous hikes over difficult terrain; NOT DANGEROUS TERRAIN! If a long hike is necessary to get to a site, carefully consider the terrain and your personal ability and health to access the site. If you feel it is too difficult (*e.g.,* too far to hike or too deep to wade) or dangerous (*e.g.,* steep banks) to get to the site or assess it, do not attempt it. Discuss it with other sampling teams who may be willing to try to get the site later**. DO NOT NAVIGATE TO ANY ASSESSMENT SITE THAT PRESENTS A DANGEROUS SITUATION TO YOU OR ANOTHER TEAM MEMBER!**

A. Random Sites (EPA Probabilistic Sites)

An attempt should be made to access random sites no matter how far the hike unless it appears dangerous or too difficult to do so. The map coordinator should be notified and consulted about all sites which were not accessed due to dangerous or difficult conditions as a visit to that site may be attempted by another sampling team that may be better able to reach the site.

Beginning in 2007 the Random Sampling Program switched from a statewide watershed specific sampling effort to a statewide ecoregional effort based on Omernik's ecoregions. The state has been divided into 3 major ecoregions going West to East:

1. 70-Western Allegheny Plateau
2. 69-Central Appalachians
3. 67-Ridge and Valley

Twenty-six (Thirteen new sites and Thirteen revisits from 5 years prior) in each of the 3 ecoregions must be fully sampled for water quality, benthos, periphyton, and habitat each year. Additionally, we will be conducting fish surveys at sites that have drainage

areas of 2000 acres (+/- 10%) or greater. Target sites are defined as riffle/run habitat, wadeable, and can be sampled using kick protocols that result in comparable data.

The site lists for each ecoregion will consist of about 5-8 samples. ***See Table 1 below for an example of a site list***. Since you know you will be visiting all of the sites on the list, they may be sampled in any order. This will allow you to work more efficiently, as some sites may not be adjacent on the list but not necessarily in numerical order. For example (***referring to Table 1 below***): If you were working the stream list from the mouth up, you might sample Job Run and Badger Fork first, since they are close to each other, but not in random order.

Coordinates for the site are included in the stream list. In addition, GIS data of the sites will be available for use on the field laptops. These coordinates should approximately match what is plotted out on topographical maps. Unfortunately, these coordinates are based on stream GIS data that is not updated as quickly as a stream can cut or move through the landscape (naturally or human assisted). So you must do your best (*i.e.*, use best professional judgment) to translate the coordinates to a real stream site on the ground. ***See Locating the X-Site below for more information****.*

Alternate Sites

During the process of visiting the sites on the list, there will be a few that cannot be sampled for various reasons (*e.g.*, dry, too deep, landowner access denial or extreme physical barriers, etc.). To replace these sites, new alternate sites will be added to the work load. These sites are from the same randomly selected pool of sites as the primary sites and will be chosen to replace sites bumped off the primary list by ecoregion (*i.e.*, a site not done in ecoregion 70 will be replaced by a site in ecoregion 70). In addition, new sites will replace new sites and revisit sites will replace revisit sites. Some alternate sites may be handwritten on to site lists that have not yet been taken to the field. Others will be assembled into alternate site lists after the primary lists are completed (a deviation from prior random sampling efforts) to prevent inefficiencies that may arise from multiple teams working in one ecoregion and not being able to communicate what sites have been sampled. At some point, there will be a final alternate sampling list for each ecoregion that will be used to obtain the final sites needed to meet the per ecoregion goal of twenty-six sites. It is important to note that these lists will need to be completed in the order of the random numbers to maintain the unbiased probabilistic design.

Table 1. An example of a typical Random Site List

Western Alleghney Plataeu-Lower Middle

R#	ANCODE	STREAM NAME	Latitude			Longitude			TOPONAME	Date	Initials
R#5008	WVKC-39-{2.4}	Sang Run	38	41	0.42	81	9	25.99	Tariff		
R#5010	**WVK-34-{32.0}**	**SPRING CR**	**38**	**51**	**22.11**	**81**	**20**	**15.18**	**Spencer**		
X site is just DS of Elk Run, may need to slide reach to exclude this stream											
R#2085-R	WVK-46-B-{1.2}	Hog Jowls Run	39	5	2.40	81	8	11.44	MacFarlan		
R#5088	WVKC-10-P-1-A-{2.1}	Job Run	38	56	34.54	80	57	45.72	Tanner		
R#5104	**WVKC-31-G-{1.9}**	**McGregor Run**	**39**	**18**	**44.41**	**81**	**1**	**52.57**	**Ellenboro**		
Field Blank at this site											
R#5137	WVKC-10-T-15-A-{1.8}	Badger Fork	39	11	0.59	81	32	43.58	South Parkersburg		
Perform Duplicate Sampling at this Site											
2007 Random Parameters: Acidity (Hot) Alk, Sulfate, Fecal Coliform, TSS, Tot. Phosphate, TKN, NO2-NO3-N, Ba, Mg, Al (T&D), Cu (D-low det) Fe (T&D), Mn, Zn (D-low det), Ca, Total Se, Chlorides											

Bold/Green text indicates potential fish sites.

Locating the X-Site

Random sampling stations are marked with an **X (highlighted in pink)** on USGS 1:24,000 scale topographic maps. **Note that these maps are recycled and older sites (both targeted and random) may appear on the topographic maps. Therefore, you should take great care in matching up the stream name, AN-Code, and random number written next to the site with what is on the stream list.** This spot is referred to as the **X-site** and is the downstream end of a 100 m reach that is to be assessed. Some situations require sliding the reach and thus the X is not at the downstream end (***see Sliding the Reach below for details***). **Note: Always collect physicochemical samples and GPS coordinates at the X-site for random stations. If possible, get coordinates from the center of the stream channel and let the GPS run for several minutes (5-10) before recording the latitude and longitude.** Sampling teams should use all available means to ensure that they are at the correct location; including Laptop GIS programs, topographic, county, and/or gazetteer maps, or (as in the case of revisit sites) previous visit photocopies which include directions to the site, hand-drawn maps, and photos. GPS units should also be used to confirm the X-site latitude and longitude that is provided on the list for each random station. Using your GPS, if you can get one half of the coordinates to match almost exactly and the other half within a reasonable distance (no more than a couple of seconds), and then you have adequately located the random site. If the GPS coordinates and the given X-site coordinates differ by more than a couple of seconds, re-check your position. **You should make an attempt to get an exact match if possible.**

> ⇒ **NOTE: For revisit sites use the coordinates provided on the site list only as the coordinates on the previous visit photocopy may be in a different datum. Nevertheless, the hand-drawn map from the previous visit photocopy will be very useful to locating the exact same X-site that was established during the previous visit. You should make an attempt to get an exact match to the previous visit's X-site.**

There will be stations where the GPS unit will not track satellites and thus confirmation of the X-site coordinates may be impossible. Team members should collaborate in these instances and utilize their best professional judgment (BPJ) to decide where the X-site is located. In such a case, finely tuned map reading skills are important.

After the X-site has been confirmed (or located via best professional judgment), the Geomorph will establish a 100-meter assessment area based on the X-site. If there are no riffle/run habitats within 100 m reach, the site is considered non-target for random sites and should not be sampled. **For random sites, our target stream has riffle/run habitat, is wadeable, and can be sampled using kick protocols that result in comparable data**. If you are denied access to a site either by landowners (*i.e.,* direct verbal communication or by best professional judgment that you should not ignore posted signs or fences) or by physical barriers (not gates or fences, but natural

obstacles that involve dangerous conditions like steep gorges, forest fires, or floods), classify the site as "target" or "not-target" based on best professional judgment and clues that may be gathered about the stream. A good example is an agriculture stream where you are denied permission to the site but can see it well enough to properly classify it. If you cannot see the site, use GIS coverage data, information from locals, what you know about other streams nearby, and what you can gather about the stream from other accessible points up or downstream. It is better that you make an educated guess in the field rather than someone making a wild guess in the office. **If you get coordinates at your location and it is not at the X-site, put the coordinate information in the drawing and site verification notes. DO NOT PUT COORDINATES FROM A LOCKED GATE OR A LANDOWNERS HOUSE IN THE COORDINATES SECTION FOR THE X-SITE!**

Sliding the Reach

There are some conditions that may require "sliding" the 100 m stream reach around features we do not wish to sample across. Do not proceed upstream into a lower order stream or downstream into a larger order stream when laying out the stream reach. The map coordinator will note on the stream list any random 100 m reach that might require sliding due to the confluence of streams. If such confluence is encountered, note the distance and mark the confluence as the reach end. Make up for the loss of the reach length by sliding the other end of the reach an equivalent distance away from the X-site, ***as shown in Figure 1 below.*** **Note: the confluence must be within the initial 100 m reach for this sliding to apply**. Do not slide the reach to avoid human disturbances like bridges, culverts, rip/rap, or channelized areas. If you have to slide the reach, make sure it is documented on the stream assessment form and include why it was moved and where. Include this information in the sketch of the assessment area.

Additionally, if the reach contains a lake, reservoir, or pond, mark the water body as the reach end and make up for the loss of the reach length by moving the other end of the reach an equivalent distance from the X-site (***See Figure 1 below***). However, if the X-site is completely within a lake, pond, or valley fill, no sampling can occur and only the front page of the habitat form needs to be filled out describing the situation thoroughly. **Be sure to take photographs of the situation including the reach slid downstream of the X-site and the area above the X-site.**

In some cases a randomly site's X-site is located below a source or tributary with a significant water quality impact to the stream and there is inadequate room to collect benthos in the area below the sources. In such situations, it would be best to treat the source or tributary with significant water chemistry issues using the same rules as sliding the reach downstream around the X-site to avoid crossing stream orders **(*see below in Figure 1*)** so that the X-site and benthic collection area are in similar water quality.

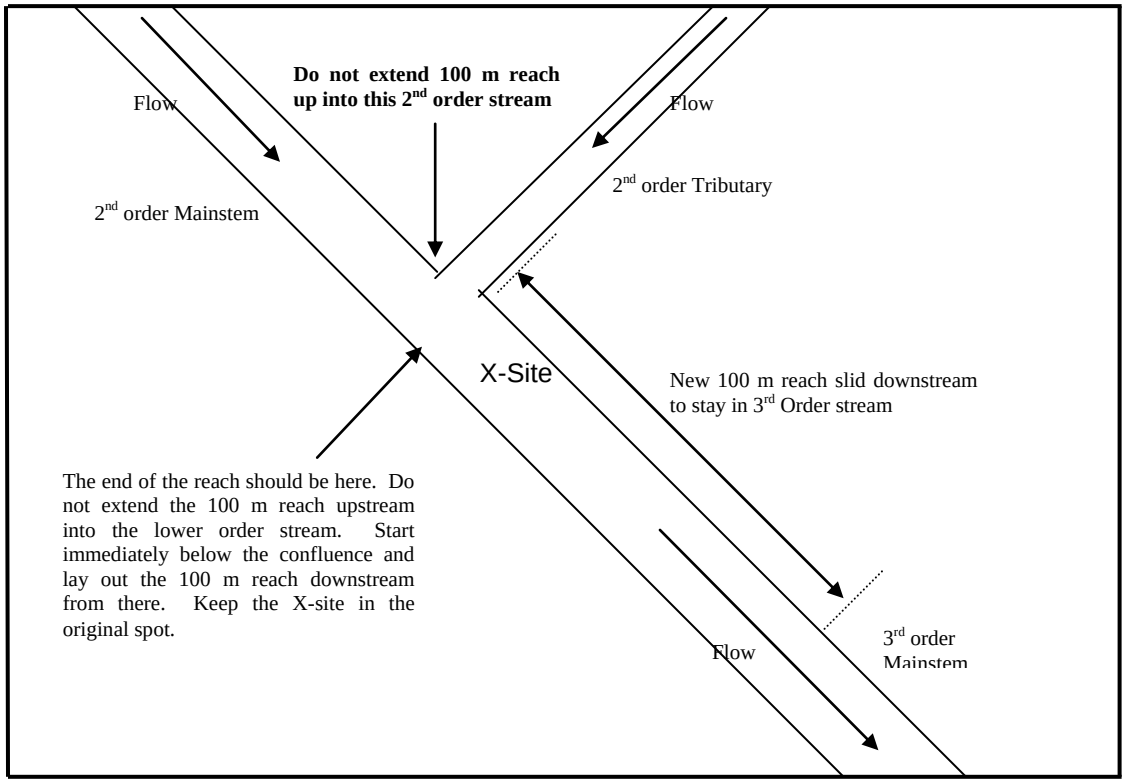

Figure 1. An example of sliding the reach to avoid larger/small confluences, lakes, ponds, etc. (used FOR RANDOM SITES ONLY).

It is important to describe in detail on the assessment form any deviations from the standard layout.

In order to determine the stream reach, the Geomorph will actively traverse the stream (NOTE: the Geomorph will avoid walking in the stream until physicochemical samples have been collected and avoid stepping in riffles that may be used in macroinvertebrate and periphyton sampling) from one end to the other taking note of pertinent habitat information and measuring the 100 m area. The Geomorph will perform other duties concurrent with the establishment of the 100 m assessment area (outlined below in detail). Random sites have specific requirements for physicochemical sampling. ***The list of parameters that must be collected at all random sites can be found under Chapter III. Section B. Part 3. Common Water Quality Parameter Suites starting on page 131 and on the CHEAT SHEET.***

B. Target Sites

Target sites should be assessed if at all possible, even if they are more than one mile from the vehicle, unless it appears dangerous or too difficult to do so. Some sites that are suspected of this may have notes relating to the acceptable distance and conditions under which the site may be moved. The map coordinator should be notified and

consulted about all sites which were not accessed due to dangerous or difficult conditions as an alternate site may be inserted to replace that site.

Target sampling stations are marked with an **X (highlighted in yellow)** and with the sample year on USGS 1:24,000 scale topographic maps. **Note that these maps are recycled and older sites (both targeted and random) may appear on the topographic maps. Therefore, you should take great care in matching up the stream name, AN-Code, and sample year written next to the site with what is on the stream list.** If possible, the assessment reach should be established above bridges. Additionally, bridges should not be included in the assessment reach, if possible. Target sites include high quality, severely impaired, moderately impaired, non-impaired, unassessed, and 303(d) listed streams. These sites differ from random sites as indicated by the following:

1) There are no predetermined coordinates for the X-site unless otherwise noted on the stream list. The latitude and longitude will be determined after the sample site has been chosen.
2) There is more latitude in making decisions on where to conduct the stream assessment (*i.e.*, you can more easily and readily make micro adjustments to the stream reach location).
3) Latitude and longitude (coordinates) and physicochemical samples are always collected at the downstream terminus of the 100 m assessment reach at all times (sliding the reach is not applicable).
4) In general, streams are sampled at the first readily accessible riffle/run upstream from the mouth and/or above tributaries or potential sources of interest.
5) Assessments are conducted upstream of and should not include road bridges/culverts if possible.

It is important to keep in mind that riffle/run sites are preferable to MACS sites when it comes time to report data as they are more abundant and only riffle/run data can be used to calculate a comparable WVSCI score. For example, if a riffle/run site can be found a ¼ mile further upstream without going above a <u>significant tributary or changing land use (agriculture, etc)</u>, then go and sample the riffle/run site. In general, do not collect a MACS sample unless the stream list indicates that the site is of special concern and should be sample regardless of the habitat type present. Describe in detail the type of MACS habitat present in case a future visit is scheduled.

<u>Note</u>: If a site is moved from the location marked on the map then the form should be filled out appropriately noting why the original intended site was not suitable (*see Section C. PAGE 1-Site Verification starting on page 30 for more info*). In addition, you should also indicate on the topographic maps provided in the

stream list packet where the site was moved to with an arrow drawn from the original site to the new site.

Some conditions may require establishing the stream reach around features we do not wish to sample across. Do not establish a 100 m reach that includes a nasty discharge (*e.g.,* AMD tributary, point source outfall, etc.). If a water quality impaired tributary is encountered within the chosen stream reach, move above the confluence a short distance, establish a new 100 m reach, and perform all WAB protocols. Additionally, fill out a form and collect appropriate physicochemical samples downstream of the confluence and from the mouth of the polluted tributary or outfall/source. If the nasty tributary is not on your stream list or the stream list for other sampling crews, conduct a full WAB assessment on the nasty tributary. **Provide detailed notes and document the specifics of the assessments and samples collected for all of the above**.

There is no definitive list of physicochemical parameters for target sites other than field readings (water quality sonde parameters) and fecal coliform bacteria. Sampling for specific parameters either indicated on the stream list or is determined on-site and is based on the surrounding land usage (*i.e.*, total phosphorus in agricultural areas when a problem is suspected, or metals in areas of mining). GIS software and data on laptops detailing the land use of each stream will be provided to the team with the topographic maps and stream list. These maps should be consulted to provide insight as to what parameters should be measured at the site. Another important way to get information about the land use is to ask and start a dialogue with local landowners and listen carefully to what they say about the stream and its upstream uses. These talks will often provide vital clues as to what may be occurring in the stream. You may also observe what is in the upstream watershed if you pass through it on the way to the site or the next site.

In some instances, a stream may appear to have an excellent water quality and habitat upstream of the targeted site. If this is the case, make all attempts to sample the segment as a potential reference site or make notes about the stream segment and report it to other sampling teams and personnel to determine if it is a possible reference site candidate later **(*see Reference Sites and Potential Reference Sites below*)**.

C. Duplicate Sites

In order to fulfill quality assurance and quality control or QA/QC requirements (***see Chapter XII. Section A. Field Blanks and Duplicates starting on page 285***), a select number of duplicate sites will be assessed in each watershed. The stream list will indicate where to conduct a duplicate sample. However, it should be noted that the stream listed is only a randomly picked site at which to complete a duplicate and serves as a reminder to conduct a duplicate sample. In fact, a duplicate can be performed at any site that meets certain needs. The assessment area should contain a large enough

riffle/run area to obtain two complete benthic macroinvertebrate samples without any overlap (4 kicks versus 4 kicks). Make sure the instream substrate & velocity of the duplicate benthic sampling sites match as closely as possible (*i.e.*, do not have one person kick all gravel/sand riffles, and the other kick all boulder/cobble riffles). If the stream does not have an adequate amount of riffle/run habitat to collect two full samples, it will be necessary to substitute a replacement at the next stream that does have adequate habitat. If the first site you visit on a list provides enough good habitat to do a duplicate, then sample it as a duplicate. Do not wait until the end of a week or list to sample for a duplicate stream.

During a duplicate, both team members will complete the habitat forms, collect benthic macroinvertebrates, and obtain appropriate physicochemical samples as if they are the only person there. **DO NOT PUT YOUR BENTHIC SAMPLING DATA ON THE OTHER PERSONS FORM!** Water quality sonde and flow readings should be recorded on the DUP 1 assessment form only. GPS coordinates can be shared between the two forms. **Make sure the name of the collector (not both team members) is written on the sample containers as well as a "-Dup 1" or –"Dup 2" at the end of the AN-Code**. If the names of both team members are written on the containers there will be no way of determining the actual collector and thus no way of comparing the results for quality. If for some reason the designated duplicate is not sampleable, the team should replace the duplicate site with another stream in the same week.

D. Reference Sites and Potential Reference Sites

Potential reference sites and established reference sites should be assessed no matter how far the hike unless it appears dangerous or too difficult to do so.

Reference conditions are thought to represent the characteristics of stream reaches that are least disturbed by human activities and are used to define attainable chemical, biological and habitat conditions for a region. The development of reference conditions is a key component of environmental impact evaluations. In most West Virginia streams, historic data were not collected prior to human disturbances and activities. A logical method of determining the health of streams is to compare them to established reference conditions. **Therefore, it is extremely important for sampling teams to conduct assessments on several (as many as possible) undisturbed streams that meet reference conditions**.

The map coordinator will provide each team with a list of potential reference sites and already established reference sites. A considerable amount of time is invested each year in the process of selecting candidate reference sites, conducting field assessments on them, analyzing resultant data, and elevating them to full reference site status. This includes time spent to maintain the reference site database and improve methodologies used to identify them. Candidate reference sites were established by examining GIS

land use data and marking the stream segments that appear to have the least amount of disturbance. Preference is often given to sites with minimal disturbance such as agriculture and urban land cover. Because the GIS data may not be current or complete, many of these candidate sites will not meet reference criteria **(*see Reference Site Criteria below*)** and, thus, should not be assessed unless otherwise directed on the stream list.

Reference Site Criteria

The following selection criteria are used to determine reference site status after assessments have been conducted and all the chemical, habitat, biological, and reconnaissance information is entered into a database. Each site is evaluated to see if it meets these reference site criteria. If all of the criteria are met, the site is given reference site status.

Note: It will be impossible to utilize all of these criteria while in the field. However, it will be useful to consider these criteria while making decisions on whether to conduct an assessment on a candidate reference site.

***** ***Indicates criterion that can be determined in the field.***

1. *Point source discharges* - Because reference sites presumably represent least disturbed conditions, point source discharges (NPDES) located upstream of an assessment site generally disqualify it from becoming a reference site. GIS data provides easy access to the locations of many permitted point sources. However, extra effort is taken in the field to ensure that point sources do not exist above the site.*
2. *Anthropogenic disturbances* within the stream assessment area are evaluated visually. Best professional judgment is employed to make reference site inclusions based on the number and type of disturbance(s). For example, a surface mine site would generally be considered a greater disturbance than an ATV trail and small road combined and could exclude the site from reference condition consideration. However, impacts from the ATV trail and/or road may be considered so minor that they do not exclude the site from reference consideration. The information gathered in the field on anthropogenic disturbance helps validate the GIS data used to select the candidate sites (***see Section C. Part 1. PAGE 2-Site Activities and Disturbances (Including Roads) starting on page 36***).
3. * *NPS* - Obvious sources of NPS are documented within the assessment area. If sources of NPS are documented for areas above the assessment site, they are also considered. Livestock feedlots, parking lots, and road runoff are common sources of NPS. Best professional judgment is employed to make reference site inclusions based on the type and intensity of the NPS. For example, a livestock feedlot with direct drainage to the stream would likely exclude the site from reference consideration. In contrast, a small road drain may not be significant enough to exclude a site from consideration.

4. * *Primary WQ criteria*:
   a. *D.O.* ≥ 5.0 mg/l - The criterion for dissolved oxygen was taken from "WV Water Quality Standards" as developed by the State Water Resources Board (SWRB).
   b. *pH* between 6.0 and 9.0 Standard Units (S.U.) - The criterion for pH was taken from "WV Water Quality Standards" as developed by the State Water Resources Board (SWRB).
5. *Secondary WQ criteria*: (used as flag values)
   a. * *Conductivity* < 500 µmhos/cm – Criterion for conductivity was established from analysis of WVDEP data and from best professional judgment of several experienced field employees. A value greater than 500 may indicate the presence of dissolved ions (such as sulfate, chlorides, and metals) exceeding the background levels for the area. It is important to note that a full water quality analysis that includes all possible chemical constituents is not within the resource pool of the program. Consequently, the conductivity reading of a site can be used as a means of flagging the site for further investigation before it can be considered a reference site. Note: Region specific criteria for conductivity are currently being examined to address natural differences in ambient conductivity. This may result in having lower or higher conductivity thresholds based on ecoregion, watershed (8-digit HUC), etc. Currently, best professional judgment is used when conductivity for a site is conspicuously higher than expected for the area.
   b. *Fecal coliform bacteria* < 800 colonies/100 ml - The fecal coliform value of 800 colonies/100ml is double the maximum set by the WV Environmental Quality Board (WV EQB) which states that fecal coliform shall not exceed 400/100ml in more than 10 percent of all samples taken during the month. This value was raised to 800/100ml for reference criteria due to the lengthy holding times of fecal samples (24 hours in many cases). Additionally, experienced field personnel have encountered fecal coliform bacteria counts exceeding the standard in streams where no human impacts were apparent or known. Thus, a value of 800/100ml would decrease the possibility of excluding some undisturbed (anthropogenically) streams from reference consideration. Similar to the criterion for conductivity, fecal coliform bacteria can be used as a means of flagging the site for further investigation before it can be considered a reference site.
6. *No known violations of state water quality standards* – If there is a violation of a water quality criterion standard as established by the (WV EQB), the site is eliminated from reference site consideration. **Note: This does not include fecal coliform bacteria as described above.** Because of their toxicity, metals are the primary consideration when evaluating data for violations.
7. * *RBP habitat metric scores*: The habitat criteria below are adapted from the USEPA-RBP habitat assessment procedures (***see Section C. Part 1. PAGES 5, 6,***

5a, and 6a-EPA's Rapid Habitat Assessment Form starting on page 52). These criteria were selected because they are considered most indicative of anthropogenic disturbance.

≥ 11 (lowest score possible for sub-optimal rating) for following:

a. epifaunal substrate
b. channel alteration
c. sediment deposition

≥ 6 (lowest score possible for marginal rating) for following:

a. bank vegetative protection (right bank & left bank scored separately)
b. riparian vegetative zone width (right bank & left bank scored separately)

≥ 130 (mid-suboptimal score) for total habitat score

A value >10 indicates that stream habitat is at least sub-optimal for that particular parameter. The WAB sampling strategy dictates that many assessments are conducted at or near the mouths of streams. This strategy tends to bias the habitat scores (many sites are roadside accessible or below bridges) and in many cases results in relatively low scores for those parameters that are most indicative of human disturbance. It is for this reason that the minimum values are set to 11 (7 through 10) and 6 (parameter 11). Otherwise, few streams (if any) would meet the selection criteria.

All samples that meet these criteria can be elevated to what is called a **Level I** reference status as it passed all of the needed criteria. However, it must be understood that absolute pristine habitat conditions do not exist in most areas. Therefore, decisions must be made on what is an acceptable level of disturbance to represent reference condition. Additionally, acceptable conditions may differ among watershed regions because of factors such as local geology, vegetation, and predominant land use. In heavily disturbed watershed regions, undisturbed conditions may not exist. A large proportion of reference samples currently in the database are on first and second order streams because the potential for anthropogenic disturbance generally increases as stream size increases. Consequently, reference conditions may need to be determined based on the best available conditions. Because of this, a second tier of reference samples called **Level II**, it has been established**.** Level II reference samples meet most of the criteria above, but may barely fail to meet some of the criteria. A third tier of reference samples, called **Level III,** represent the best available conditions in a geographical area or stream size class and generally fail to meet as many of the criteria of Level II reference status. Generally, Level III reference samples are on larger order streams where it is more difficult to meet all of the reference criteria.

Also note that reference status is declared on a sample basis and not a site basis. The reasoning for this is: 1) the station may become altered to the point that it would no

longer meet any of the above reference categories; 2) the station may meet reference criteria in one season, but fail to meet it on other seasons. When multiple samples are available, every effort is made to consider the other samples in making a determination on the one. For example, the chronologically first sample may seem to meet all of the reference criteria, but future sampling efforts may reveal something that was missed during the first evaluation. In this case, the reference status may be downgraded or stripped entirely. In a situation where the site has been altered between the earlier and subsequent samples, the earlier samples may maintain reference status while the subsequent samples do not gain reference status.

Determining Candidate Reference Sites While In the Field

Aside from the numeric criteria that can be evaluated while in the field (*i.e.* Water Chemistry and RBP Habitat Scores), determining if a site is a candidate reference site can seem like a daunting task. As one samples more and more in the different regions of the state and becomes familiar with what is the best possible condition for an area, this task becomes easier. It also helps to pay careful attention when sampling a site that is already established as reference quality and try to imprint a visual of the characteristics of that site into one's mind.

Determine human disturbances by reconnaissance and using GIS land use maps. Choose stream segments with no major (or as little as possible) human disturbance, (*i.e.*, eliminate sites with strip mines, refuse piles, towns, major roads, active open fields or agriculture), impoundments, power-lines, non-point sources, etc**. Consult current and historic GIS land use, aerial photos, and topographic maps for determination of upstream disturbances.** Some of these disturbances are indicated on topographic maps. If possible choose candidate sites located within a State Park or other static land use type. In most cases, it will be necessary to choose candidate sites with limited accessibility (obviously due to the nature of the condition we are searching for) that requires some long hikes. If passable jeep trails or hiking trails are indicated on the map, try and choose sites within their paths and make the hiking distance as short as possible.

Anthropogenic disturbances within the stream assessment area should be evaluated visually. Best professional judgment is employed to make reference site inclusions based on the number and type of disturbance. For example, a surface mine site would generally be considered a greater disturbance than an ATV trail and small road combined and would exclude the site from reference condition consideration. However, impacts from the ATV trail and/or road may be considered minor so that they do not exclude the site from reference consideration. In particular, don't immediately eliminate a site as potential reference if it has a small road following along much of its length unless there is obvious erosion or areas of high sediment deposition. Many of our established reference sites do have roads running parallel to them or crossing them at some point(s). Also, consider where you are in the state when deciding on potential reference sites. The northwestern portion of West Virginia (Western Allegheny Plateau

– Ecoregion 70) should not be held to the same standard as the eastern mountainous section (Ridge and Valley – Ecoregion 67). In other words, the least disturbed conditions in Ecoregion 70 are not equal to those of Ecoregion 67. For example, some streams in the Upper Ohio South watershed in Ecoregion 70 have hilltop farms that may offer little if any impact to the stream located a down in the valley below. Some of these are established reference sites and represent the best possible conditions for the Ecoregion. In Ecoregion 67, there are many streams without any recent land disturbance (entirely forested). Many of these are established reference sites. A concerted effort should also be made to recognize some candidates on streams with larger watershed areas since the potential for anthropogenic disturbance generally increases as stream size increases.

All potential reference sites and already established reference sites should be reconned by vehicle to provide additional information about the watershed not available thru GIS data.

Sampling teams should note that they are by no means limited to the list of potential reference sites provided by the map coordinator. If a potential reference site is encountered while in the field, every effort should be made to conduct a full WAB assessment on that stream segment. If a potential reference site is also designated as a target site, then you should search for a place to sample that will satisfy the potential reference conditions. In other words, if a small disturbance is encountered at or near the mouth of a stream that is not designated potential ref on the stream list, move the site above the disturbance to conduct the assessment.

Always collect "RANDOM SITE" physicochemical parameters at all potential and established reference sites.

Because of the nature of reference sites (undisturbed), traversing to the sample site may require long strenuous hikes over difficult terrain; NOT DANGEROUS TERRAIN! This should not be a reason for eliminating the site for assessment. If you personally feel it is too difficult (or too far to hike) to get to the site, do not attempt it. Discuss it with other sampling teams who may be willing to give it a try**. DO NOT NAVIGATE TO ANY ASSESSMENT SITE THAT PRESENTS A DANGEROUS SITUATION TO YOU OR ANOTHER TEAM MEMBER!**

Section B. Site Documentation

Part 1. Coordinates and Global Positioning Systems (GPS)

GPS Overview

GPS units use satellite communications to accurately determine the latitude and longitude of a specific location. Since the GPS units use triangulation to determine

location, the more satellites it is in contact with, the more accurate the data. To function efficiently the GPS must be used in an unobstructed area and must have good signals with at least four satellites for a reading. In addition, taking a longer time for a reading will generally result in a better reading as sometimes the first four satellites selected are not necessarily the best ones. But one must be careful as sometimes there is often only a brief window where there are enough satellites above at certain sites. It is suggested that you attempt to obtain GPS coordinates first upon arrival at the site and try repeatedly during the duration of the sampling.

The Watershed Assessment Branch uses a variety of GPS unit models under the Garmin brand because of their ease of use, low cost, and rugged design. However, unlike some other, more expensive GPS units, Garmin GPS units do not store the readings to be differentially corrected at a later date. Recent advances in GPS technology have compensated for this somewhat (*e.g.*, the removal of Selective Availability, WAAS enabled receivers, etc.). To further compensate for this, Watershed Assessment Branch takes great care to QA/QC its coordinate data (***See GPS Quality Assurance/Quality Control below***).

It is standard procedure to take GPS readings at all sites visited. The GPS reading location should be noted on Page 1 of the Habitat Form (***see Section C. Part 1. PAGE 1-Site Verification starting on page 30***). Specifically, the coordinates should be taken at the location where the water quality parameters and constituents are collected. Should you take coordinates at a location other than the water quality sampling area (*e.g.*, because of poor GPS reception), be sure to thoroughly note this discrepancy on the paperwork and reach map.

Because of the frequency of visitation of some sites, it may not be necessary to take GPS readings during each visit. ***Table 2 below*** outlines some typical frequency of GPS readings for various sample types.

In addition, there may be some survey sampling designs that require multiple GPS coordinates for one sampling event because they involve the use of variable reach lengths (*e.g.*, Fish Surveys, Non-Wadeable Stream Surveys, etc.). In such cases it will be necessary to take GPS coordinates at the following locations: the water quality collection location or X-site, the downstream terminus of the reach, and the upstream terminus of the reach. Should the X-site coincide with either the downstream or upstream terminus of the reach, then make a note as such and just collect GPS coordinates for the downstream and upstream terminus of the reach.

Table 2. Typical Frequency of GPS Readings for various Watershed Assessment Branch Activities

Sample Type	Frequency of GPS Readings
Wadeable Benthic (Random, Targeted, and associated TMDL visit) and Fish Surveys	Every Visit
Long Term Monitoring Sites	Every Visit
Special Surveys	Every Visit
Lakes & Large Rivers (or other boatable activities)	Every Visit
TMDL	1st, 2nd, and Final Visits
Special Projects	1st, 2nd, and Final Visits
Ambient Network	Old Sites-Only when the site is moved (*e.g.*, moved us 30 m because of a new bridge) New Sites-1st and 2nd visit

Quick Operation of the Garmin III+ or V GPS Unit

These instructions are meant to be only meant to offer quick guidance in the operation of GPS units. These instructions do not supplant the original manufacturers' operations manual. Consult the owner's manuals for specifics or information on configurations other than these and for details on maintenance and trouble-shooting. These procedures assume the user has a basic knowledge of the instrument.

These directions are not intended for first-time users. Individuals with no prior experience should operate the unit with the assistance of an experienced user.

<u>Procedures for obtaining coordinates with a GARMIN GPS III+ or V</u>

A) Unfold the antenna.
B) Press the red light bulb button to turn unit on.
C) At the warning screen, press enter to proceed to the satellite screen.
D) Wait an adequate amount of time while the unit locks onto the satellites. The bars at the bottom of the screen will rise with increasing signal strength and will turn black when the signal is locked for that satellite.
E) When the unit has locked into enough satellites to get any reading it will display a map.
F) Push the "quit" button twice to get back to the satellite screen. If the reading is adequate, record the EPE (Ellipsoid Precision Error) or accuracy. This is a number in feet that ranges generally from 15-100 with a lower number being more accurate. Imagine a circle represents your location that is as wide in feet

as the number. The larger the number, the larger the circle and the less sure you are of your exact position. An EPE of 20-30 feet is really good and an EPE of 100 feet is really bad. The unit will also display accuracy by stating if it was in 2-D or 3-D. A 2-D reading is a one with only three satellites available. Therefore, elevation information is not available and your position may be pretty inaccurate on a two dimensional plane. 3-D means that four or more satellites were available and the elevation and your position in three dimensional space are relatively accurate. Be sure to indicate on the habitat form if the reading is in 2-D in addition to the EPE number.

G) If the EPE is not very good or in 2-D wait some more to see if it improves. If it does not, then proceed with what is available or utilize alternative means to determine coordinates (*e.g.*, GIS, Previous Visit, etc.).
H) Push the "quit" button until the latitude and longitude are displayed in the lower third of the screen.
I) Record the latitude and longitude as "field readings" on the habitat sheet

Procedures for checking/changing the datum with a GARMIN GPS III+ or V

Sometimes it may be necessary to check the datum being used by the unit (*e.g.*, when a unit has been without batteries for an extended amount of time or with the purchase of a new unit). Each datum is different and will dictate how the coordinates be displayed or recorded. Since most of our GIS needs in the office are fulfilled through WCMS, we need to make sure that any data taken or recorded in the same datum used by WCMS. The older 2.8 version of WCMS uses NAD 1927 CONUS for a datum. The newer WCMS version (9.x) uses NAD 1983 CONUS. Watershed Assessment transitioned to NAD 1983 as the standard in July 2006.

A) Unfold the antenna.
B) Press the red light bulb button to turn unit on. Wait for the "Acquiring Sats" screen to appear.
C) Press Menu twice to get the Main Menu.
D) Scroll down to Setup and press ENTER.
E) Scroll right along the tabs to Position or Location.
    1. Make sure that the Position or Location Format is "hddd^0 mm' ss.s".
    2. If "NAD83 CONUS" or "NAD83" is not displayed under Map Datum, then scroll down and select whatever is listed under Map Datum. This will cause a list to pop up on the left. Scroll down and select "NAD83 CONUS" or "NAD83"; press Enter. The proper datum should now be selected. Press QUIT twice to get back to the "Acquiring Sats" screen and turn off the unit.
    3. If "NAD83 CONUS" or "NAD83" is not displayed under Map Datum, then scroll down and select whatever is listed under Map Datum. This will cause a list to pop up on the left. Scroll down and select "NAD83 CONUS" or "NAD83"; press Enter. The proper datum should now be selected.
F) Press QUIT twice to get back to the "Acquiring Sats" screen and turn off the unit.

GPS Quality Assurance/Quality Control

Before use, each GPS unit should be examined for proper datum and battery levels and adjustments should be made as required.

The accuracy reading of the GPS coordinates is observed and recorded in the field to help in obtaining the best possible reading as well as indicate if there may have been an issue with the unit's ability to report the correct location.

The location of GPS coordinates are checked and validated by the sampling team immediately after sampling or later during data entry and proofing. The coordinates are plotted on GIS topographic map and aerial photo basemaps and then compared to the field documentation notes (*e.g.*, hand drawn site map, directions to the site, site descriptions, accuracy reading, etc.). Those coordinates that do not fall within a reasonable distance of the expected location are more extensively cross checked and researched. Any position that does not meet these expectations is recalculated by using the field documentation notes about the site to approximate the site location and using the Watershed Characterization and Modeling System ArcGIS extension to generate coordinates for that location.

Stations or sites that are visited more than once (*e.g.*, TMDL sampling, special projects, etc.) will have multiple GIS coordinates obtained to help reassure that the coordinates do indeed match the sampling location.

In addition, spatial GIS queries are used to filter out potential "bad" coordinates. These bad coordinates are double checked and either corrected by using field documentation notes about the site (*i.e.*, site map, directions to site, and location description) to or documented as to why they appear "bad".

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the use and collection of GPS coordinates is included. In the field, individuals who are more experienced in using GPS units will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to collect coordinates solo. This document is also provided to all program personnel for review and use in the field.

Part 2. Photographic Documentation

Photography Overview

The Watershed Assessment Branch needs quality photographs from every site to use as illustrations for our reports, presentations, and for general use. They are vital for illustration and clarification of the ideas presented as well as visual relief from all the words in the text. To achieve this we need the field personnel to take a variety of pictures while they are in the field. Along with the pictures we need a way to keep track of these photos on our field forms as well as in our database.

This "photography log" is essential for four reasons:

1. We need to know who took the picture
2. We need to know where the picture was taken
3. We need to know what the picture is of
4. We need to know what to call the photo

For information about how to take a photograph with a particular camera, use various features, and download the photos to a computer, consult the operation manual with the camera.

Procedures for In the Field

Don't hesitate to take more than one picture of the same scene or activity. Even pictures taken at non-target or dry sites are considered useful and valuable.

Also feel free to experiment by varying the picture by using the settings feature on the camera (*e.g.*, flash level, aperture speed, exposure, wide angle/telephoto, etc.). Always use the highest image size setting on the camera. This will take up more space, but it will provide us with the most useable pictures.

Obviously all pictures will not be used in the report for the watershed where they were taken. Or any other report for that matter. But they may be used later in a presentation, brochure, or report we haven't thought of yet. In addition, these photos may be valuable for the 303(d) narrative criteria listings, 303(b) assessments, or TMDL process (*e.g.*, clarify and extent of hydroxides in stream). We cannot have too many pictures to choose from.

We need pictures of such items as:

- Stream alteration or management practices
- Stream disturbances
- Waterfowl or other wildlife in or near streams
- Silt laden streams flowing into clear streams

- Scenic Views
- Field crews at work
- Distinctive views of streams, buildings along streams, industry along streams, dams, boats or barges or other water related pictures.
- Pollution sources and features (*e.g.*, point and non-point sources, metal hydroxides, poorly constructed roads, feedlots, etc.)

All pertinent information about a photo should be recorded on the field sheet under the photography log section (***see Chapter II. Section C. Part 1. PAGE 10-Photography Log on page 76***). This information includes:

Camera Type: The type of camera used (*e.g.*, Canon, Olympus, or Sony).

Camera Number: The assigned number of the camera used. This is usually marked on the camera with a black sharpie. **Do not confuse this with the jeep number often marked on the camera in white ink.** If for some reason the camera's instrument identification number is not apparent, then write down the manufacturer's Serial Number on the instrument so that the proper identification number can be tracked down later and remarked onto the camera. **This is required for all photos taken!**

Disk-Photo #: Each camera assigns these unique file names to photos in series from 0-99999 in a format associated with some letters (*e.g.*, a photo will have a file name of DSV-00456). Write down the number portion of the file name on the form. **Do not confuse this number with the photo count numbers on the cameras that indicate how many photos have been taken or can be taken, which reset once photos are removed or deleted from the camera.** In addition, it is important to note that how the photos are removed from the camera may change this file name.

Stream Name and/or AN-Code: The name of the stream featured in the photo. ***This is only required if the photo was not taken at a sample site. If a lake or other waterbody is sampled, use this space to put in this space.*** If known, write down the AN-Code of the waterbody featured in the photo.

Photo Description: A description of the photo as it relates to the stream (*e.g.*, looking upstream from X-site) and the features that may be found in the photo (*e.g.*, AMD, eroded bank, channelization, an optimal score for bank vegetative protection, a poor score for sediment deposition, etc.). **This is required for all photos taken!**

Date: The date the photo was taken. **This is only required if the photo was not taken on the same date as the sample or if it is not at a sample site.**

Photographer: The person who took the photo. **This is required for all photos taken!**

Procedures for In the Office

Tagging the Photos with a Photo ID

In order to keep track of so many photos, at the end of each sampling week each team will need to tag each photo with a unique photo ID number that is maintained in the database. The following are the steps required for to not only tag each photo with this photo ID, but also ensure that each photo ID will have a description in the database as well.

Photos that are taken at sampling sites

Most of the photographs that we take are of this type and require the least amount of time to prepare for the database.

A. Open the WABbase.
B. From the main switchboard, select the Form called “Photo Data Entry”.
C. Press the button called “Get New Photo ID”.
D. Enter “Yes” into the box called “Number Used”. Press the “Get Number” button. Once this button is pressed, a number will appear in the box called Assigned Photo ID.
E. Rename the photo using this number as the name (*e.g.*, 136.jpg, 456.jpg, etc.)
F. On your field sheet, write this number under Photo ID on the line where your photo information is recorded.
G. Go to step C above and repeat for more photos or close the database if done.
H. Copy/Cut/or Move all of the photos from your computer onto the network server at the following directory:

   Q:\WATER RESOURCES\WAB\Photos\Coded Photos

   In this directory, there are folders for each group of 1000 photos based on Photo ID. Put the photos in the appropriate folder. If a message appears asking if you want to replace a file, press no. If this happens, then someone has already named a photo by that name and the two photo names (yours and the one already on the server) need to be investigated and resolved.

All of the information on your field sheet will be entered in during the data entry process and can be linked to your photo by the photo ID. The data entry person will write the appropriate sample ID next to each photo taken at that site.

Photos that are not taken at sampling sites

Only a handful of photos that we take are of this type. Since they will not be tied into a Sample ID all data entry for these photos is the responsibility of those who took the pictures.

A. Open the WABbase.
B. From the main switchboard, select the Form called “Photo Data Entry”.

C. Press the button called “Get New Photo ID”.
D. Enter “Yes” into the box called “Number Used”. Press the “Get Number” button. Once this button is pressed, a number will appear in the box called Assigned Photo ID.
E. Rename the photo using this number as the name (*e.g.*, 136.jpg, 456.jpg, etc.)
F. On your field sheet, write this number under Photo ID on the line where your photo information is recorded.
G. Press the button called “Non-Sample Related Photo Info”
H. Begin entering the data in the red box at the top of the form (*i.e.*, Photo Description, Photographer, Camera Type, and Camera Number).
I. Enter the applicable site information in the orange box at the bottom of the form (*i.e.*, Stream Name, AN-Code, Mile Point, Descriptor, Date, Watershed, Latitude and Longitude).
J. If you have more photos, press the “Go to New Photo” button and repeat steps D thru J.
K. Copy/Cut/or Move all of the photos from your computer onto the network server at the following directory:

    Q:\WATER RESOURCES\WAB\Photos\Coded Photos

    In this directory, there are folders for each group of 1000 photos based on Photo ID. Put the photos in the appropriate folder. If a message appears asking if you want to replace a file, press no. If this happens, then someone has already named a photo by that name and the two photo names (yours and the one already on the server) need to be investigated and resolved.

Again, because these photos are not taken at a site, they will not be entered during the data entry process and assigned a Sample ID. They only way the information about these sites will be entered is if the crew who took them enters the data. And a photo without this information is not very useful.

Photography Quality Assurance/Quality Control

Before use, each camera should be examined for proper date, resolution settings, and battery levels and adjustments should be made as required.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the use and collection of photos is included. In the field, individuals who are more experienced in using taking photos will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Section C. Guidelines for Completing the Stream Assessment Forms

What is presented here explains what is found on the Wadeable Benthic Stream Assessment Form and its appendices. Other forms (*i.e.*, TMDL-Initial Visit, TMDL-Secondary Visit, TMDL-Final Visit, TMDL-Source, and General WQ) will contain some, but not all of the following. Nevertheless, the instructions on how to fill out the sections are the same as present here unless otherwise stated.

Part 1. Description of Wadeable Benthic Stream Assessment Form

The quality and quantity of habitat is a major determinant of aquatic community potential. Consequently, a thorough habitat characterization is essential for proper interpretation of biological (benthic macroinvertebrate, periphyton, & fish) assessment results.

> Important Note
>
> If a stream is considered "not target" (*e.g*., dry or too deep), Page 1 of this form must be completed. Also take photographs of the stream that display the reason why it was not considered target.
>
> If water quality only is collected, you must complete pages 1-4, 8 and 10, as best as you can and take plenty of photographs as well.

Front Side of All Pages

PHYSICAL & SEDIMENT CHARACTERIZATION>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>		Reviewers Initials	
AN-Code		Date	

Figure 2. Example of identification fields on front top of each field form.

REVIEWER'S INITIALS: All habitat forms must be reviewed and initialed for completeness by a second crew member. This review must be performed on-site. The Biomorph should point out any omissions to his/her partner and initial the page when all the data are complete. In the case of duplicate sites, this is an opportunity for each field worker to discuss discrepancies between the forms. However, all results should be considered final and should not be changed to match the other person's results.

AN-CODE: It is extremely important that the **correct** AN-Code be recorded on each sheet as this is one way to link all the sheets for a sample together if accidentally separated. ***See PAGE 1-Site Verification below for more details about AN-Codes***.

Date: Use mm/dd/yyyy format: *e.g.,* 04/29/1999. It is extremely important that the date be recorded on each sheet as this is another way to link all the sheets for a sample together if accidentally separated.

PAGE 1

ALWAYS FILL OUT THE FIRST PAGE OF THE HABITAT ASSESSMENT FORM, GET COORDINATES OF THE SITE, AND TAKE PHOTOGRAPHS, REGARDLESS OF WHETHER ANY TYPE OF SAMPLING WAS CONDUCTED (EVEN IF STREAM IS DRY, IMPOUNDED, OR INACCESSIBLE)! THIS IS IMPORTANT INFORMATION AND ASSISTS IN DATABASE MANAGEMENT. ***See Figure 3 below for an example of this page.***

Site Verification

Stream Name and Location Description: Make sure the stream name on the map corresponds with the assigned AN-Code from your printed stream list. If they do not match, make a note of it on the habitat sheet and printed list. Include a detailed description of the location such as: Greenbrier River US (abbreviation for Upstream) of Big Run at Hilldale Bridge, New River DS (abbreviation for Downstream) Lick Run at Glen Lynn, Red Creek Between Oak Run and Pine Run at Laneville, Piney Creek Upstream Beckley PSD 50m, Pinnacle Creek DS right UNT 0.5 miles south of Pineville, Bear Run near mouth south of Sissonville Upstream first bridge, Camp Creek at mouth in Camp Creek St. Forest at Campsite #2, etc. Be sure to include the receiving stream in the name of any source discharges (*e.g.*, Beckley PSD outfall discharging into Piney Creek US of Smock Run).

AN-Code: It is extremely important that the **correct** AN-Code (Alpha-Numeric Code) be recorded for each stream site. Mistakes in translation from the printed stream list to the habitat sheet must be avoided. Mistakes in this step create mass confusion and plenty of extra work during data entry. All streams will have an AN-Code with the mileage designated between brackets (*e.g.,* - {3.6}). If you are going to sample at a location other than those listed, create a unique AN-Code such as KG-3-{#1}. The mileage can be assigned to this AN-Code later using GIS by the field personnel or the map coordinator.

Date: Use mm/dd/yyyy format: *e.g.,* 04/29/2006

Time: Use military time (*e.g.,* 1315). This time stamp should reflect the time of WQ sample collection. In cases where multiple samples are being collected during one sample event, then this time represents the general start of sampling activities.

Geomorph: Initials of the team member completing the habitat form.

Biomorph: Initials of the team member collecting benthic macroinvertebrate, periphyton and water samples.

SITE VERIFICATION >>>	Reviewers Initials								
Stream Name (with location)									
AN-Code		Date		Time		Geo		Bio	
Basin		County		Quad					
GPS Type		EPE		Random #		XY's Proofed		By	
EPA or Corrected Lat at X	N	EPA or Corrected Lon at X	W						
Field Lat X-site	N	Field Lon X-site	W						

X-site field verified?	☐ Yes ☐ No	If no, Why?	☐ No Access-Physical Barrier (☐ *Permanent* / ☐ *Temporary*) ☐ No Access-Landowner Denial (☐ *Verbal Denial* / ☐ *Posted* / ☐ *Fenced* / ☐ *Private*) ☐ Other:
Is site target and kick sampleable? ANSWER EVEN IF YOU DID NOT SEE THE SITE!	☐ Yes ☐ No	If no, Why?	☐ Low Flow (☐ *Permanent-Not Drought* / ☐ *Temporary-Drought*) ☐ Ephemeral ☐ Too Deep (☐ *Permanent-Not Wadeable* / ☐ *Temporary*) ☐ No Riffle/Run ☐ Wetland ☐ Filled (☐ *Mining* / ☐ *Farm* / ☐ *Urban/Residential* / ☐ *Road* / ☐ *Industry*) ☐ Impounded (☐ *Lake* / ☐ *Mining* / ☐ *Farm* / ☐ *Beavers* / ☐ *Navigation* / ☐ *Industry*) ☐ No Stream Present (Map Error) ☐ Other:
Detailed notes on verification, access, & sampleability of site.			

Sampled?	☐ Yes ☐ No	Sample Type	☐ YSI ☐ Lab ☐ Fecal ☐ Habitat ☐ Bugs ☐ Periphyton ☐ Fish ☐ Flow ☐ BE/CP		
Dup Type	☐ None ☐ Lab ☐ Fecal ☐ Hab ☐ Bugs ☐ Periphyton	Dup #		Was site moved (non-random)?	☐ Yes ☐ No
Explanation?					
Directions To Site					

Sketch of Assessment Reach & Comments: Indicate North with (↑), indicate flow direction (↑), indicate upper end of reach with (us) and lower end with (ds), indicate bug sample (b) & periphyton (p), indicate water sample (wq), indicate lat and long site (X).

Notes		Single WQ Sample ID	

Page 1 WVDEP WAB Wadeable Benthic Stream Assessment Form (4/23/2010)

Figure 3. Example of the Site Verification section (Page 1) of the field form

Basin: *e.g.,* Upper Kanawha, West Fork, Lower New

County: *e.g.,* Hardy, WV

Quad: Enter the topographic quadrangle name, *e.g.,* Cass, Mt. Nebo, and Panther

GPS Type: If a Gamin unit is being used, record the word **Garmin**. If GIS software is used to determine the coordinates, indicate **GIS** on the form. If coordinates from a previous visit are being used, indicate **Previous Visit** on the form. If coordinates from a subsequent visit are being used, indicate **Subsequent Visit** on the form.

EPE: Record the Ellipsoid Precision Error (EPE) from the Garmin GPS after the coordinates have been recorded.

Random #: EPA Probabilistic (Random) sites are designated by a special number. This number (which will be on the stream list or topographic map) is entered here.

XY's Proofed: The type of basemaps used as a reference when the coordinates were cross-checked in GIS to ensure their location is accurate to what was indicated in the directions, hand-drawn map, and location descriptions. Common answers would be the use of the **24k-DRG** (24k topographic GIS coverage), or **03-DOQs** and **96-DOQs** (2003 and 1996 vintage aerial photos). ***See Section B. Part 1. GPS Quality Assurance/Quality Control starting on page 24 for more information about proofing coordinates***. This step is usually done in the office by an experienced GIS person.

By: The person that double-checks the coordinates for accuracy in the office

EPA or Corrected Latitude and Longitude at X-site: Either the coordinates provided on the stream list for EPA Probabilistic sites (random) are recorded here or corrected versions of the coordinates are recorded here in the office after they were proofed in the office (see XY's Proofed above).

Field Latitude and Longitude: Enter for all sites after obtaining readings in the field using Garmin or Trimble GPS units (***see Chapter II. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) starting on page 20***)

X-site Field Verified?: Answer appropriately; **YES** or **NO**. This must be answered at all sites.

If no, why?: Sometimes it is possible a stream site will not be physically visited. This may be due to one of two things: Landowner access denial or a physical barrier. Landowner denial could come in the form of a verbal denial, which is absolute, or in the

form of implied denial. Implied denial simply means that the crew has seen evidence that the property owner would not be agreeable to our presence in the stream and used best professional judgment to not sample the site. This evidence can come in the form of an abundance of posted signs (*e.g.,* at every fence post), by conversation context talking to a neighbor (*e.g.,* "He likes to shoot at trespassers."), heavily fenced and secured areas, or simply a private property (*e.g.,* the site is located in the back yard of a secluded cabin). Physical Barriers are those that may be temporary (*e.g.*, a water flooded road) or permanent (*e.g.*, high cliffs). Physical barriers are not gated roads or fences as these are better classified as types of landowner denial.

Is site target and kick sampleable?: Answer appropriately; **YES** or **NO**. **THIS MUST BE ANSWERED EVEN IF THE SITE WAS NOT SEEN OR PHYSICALLY VISITED BY THE FIELD CREW!!! AN EDUCATED GUESS OUT IN THE FIELD IS FAR BETTER THAN A WILD ONE MADE IN THE OFFICE!**

If not, why?: Sometimes a stream site will not be sampled for one reason or another. The following are possible reasons:

- **Low Flow-Permanent** (non-drought, *i.e*., subsidence) or **Low Flow-Temporary** (drought)
- **Ephemeral**
- **Too Deep-Permanent** (*e.g*., a larger stream or river that has a riffle/run habitat that is flowing but always will be over the net) or **Too Deep-Temporary** (*e.g*., a smaller stream that is over the net at that time possibly due to recent rainfall, but would potentially be at base flow at another time)
- **No Riffle/Run** habitat present (*i.e*., MACS type habitat)
- **Wetland** (stream is dominated by cattails and has no real channel)
- **Filled** by one of the following: Mining (valley fills, reclaimed concrete channels), Farm (stream plowed under for farm land), Urban/Residential (stream is culverted to make room for houses/yards/residential roads/airports), Road (stream is culverted for a major road like and interstate or 4 lane expressway), or Industry (landfills, fly ash dumps)
- **Impounded** by one of the following: Lake (recreational lakes or reservoirs), Mining (sediment or treatment ponds), Farm (farm ponds), Beavers (stream is impounded by beaver dams and activities), Navigation (stream is inundated by the backwaters of a river with locks and dams used for barge navigation), or Industry (landfill treatment ponds)
- **No Stream Present (Map Error)** (this is extremely rare and has only truly occurred one time)
- And **Other**. If other reasons arise, please comment in sketch area on page 1 when appropriate.

Detailed notes on verification, access, and sampleability of site: Notes concerning the above four items and the process that led to the answers above.

Sampled?: Answer appropriately; **YES** or **NO.** This must be answered. In some instances you may be sampling some aspect (*e.g.*, WQ only) even if the site is declared to be non-target.

Sample Type: Indicate which of the data types were collected (1) **YSI** (represents any type of water quality sonde), (2) **Lab Water**, (3) **Fecal**, (4) **Habitat** (*i.e.,* RBP Habitat), (5) **Bugs**, (6) **Periphyton**, (7) **Fish**, (8) **Flow**, (9) **BE/CP** (*i.e*., the Stream Bank Erodibility Factors/Estimated Channel Profile Form). **Do not include sonde readings as part of the lab water data. This refers to laboratory-analyzed samples only.**

⇒ **Note: Other forms may have specific lab water suites as options (*e.g.*, AMD, Acid Rain, Nutrients, Orthophosphate, etc.). Please fill out accordingly.**

Dup Type: If the site is assessed by each team member independently, the site is a duplicate site. **These sites should be treated as if each person was the only person assessing the site.** Indicate the type of duplicate it is 1) **None**, 2) **Lab Water**, 3) **Fecal**, 4) **Habitat**, 5) **Bugs**, 6) **Periphyton**. Water quality sonde readings should be recorded on the DUP 1 assessment form only. GPS coordinates can be shared. Make sure all sample containers are labeled with the person's name that made the collection, not both team members. This allows for tracking potential sampling errors resulting from poor technique or improper training.

Duplicate #: The number designation of the duplicate sample, that is, Dup **#1** or Dup **#2**.

Was site moved (non-random)?: Used mainly for Non-Random sites. However, it could be used to indicate if a random site's reach was slid around the x-site (***see Chapter II. Section A. Part 2. Sliding the Reach starting on page 11***). Answer **YES** or **NO.**

Explanation?: Explain why the site was moved and where the site was moved to. This may apply to random sites where sliding the reach is necessary. It can also apply to other sites that might be moved upstream or downstream from the point marked in order to obtain riffle/run habitat, etc. **If the site is moved, it is important to identify and mark the location of the new assessment site on a topographic map with date and initials of team and fill out a form for both sites.**

Directions to Stream Site: Give a detailed description on how the stream site was accessed. Include highway names & numbers, distances from prominent landmarks (manmade and/or natural), proximity to towns, etc. Indicate if contact with landowner/stakeholder/groundskeepers, etc., are necessary and note where, when, and why they should be contacted. Addresses of and other specifics about the

landowner/stakeholder/groundskeepers can be written down on page 8 under the section called Landowner/Stakeholder Information.

Bird's-eye-view Sketch of 100 meter Stream Assessment Area and General Comments: Provide a detailed sketch of the area and include stream flow direction, stream morphology (*i.e.*, riffles, runs, pools, bends, falls, large boulders, erosion scars), land use on left and right bank, upstream activities (if possible), proximity to permanent land marks, indicate direction by drawing a North arrow **(↑)**, and any observations which may provide pertinent information to the assessment and location of the stream area. Indicate where GPS coordinates are collected by marking the spot in the stream with an (**X**). **Coordinates should be obtained at the "EPA provided" latitude and longitude for random sites (usually downstream terminus). Coordinates should be obtained at the downstream terminus at all other sites if possible.** Indicate direction of flow with an arrow (↑). Mark the areas where benthic macroinvertebrates (**b**) and periphyton (**p**) are collected**,** and mark water sample collection areas with a (**wq**). Indicate the location of the preceding descriptive drawings within the 100 m assessment area and provide visual estimates of distance (try drawing it to scale). Indicate the upper end of the reach with an "**us"** and the downstream end with "**ds**" and attempt to correlate these with permanent landmarks. **Keep in mind that a different field crew may be revisiting the site in 5 years and will rely heavily on your description/drawing to get back to the same location. In other instances, it may be necessary to determine the location using GIS programs.** General comments can be very important when interpreting sample data. Therefore, any anomalies or outstanding attributes should be noted. If it is a random site and sliding the reach was necessary, indicate on the map the changes that were made and place an X in the drawing of the reach to indicate the X-site location.

- ⇒ **Note: Other forms (*e.g.*, TMDL, General WQ) are more concerned with the more general area of the stream site and not necessarily concentrating on the 100m assessment reach.**
- ⇒ **The information generated from drawing a stream map should help one keep track of various features and more accurately fill out other portions of the form (*e.g.*, the Total Habitat Type % Coverage for Reach, Riparian Intensities, RBP metrics etc).**

Notes: General notes about the sample or sample location (*e.g.*, the site is on a 303(d) listed stream, this site is taken at a previously sampled Gray WVSCI site, etc.). Additional personnel and their role or capacity in which they worked on the site can be documented here.

Single WQ Sample ID: If used, document the pre-assigned Water Quality Sample ID used with this sample. This ID is unique and comes pre-printed on labels. It is used whenever a lab water sample is collected. If multiple water quality samples are taken during the sampling event (*i.e*., a waterbody profile), then this information will be

documented on another page with the specific collection information (*i.e.*, depth, distance, transect, etc.).

PAGE 2

Site Activities and Disturbances (Including Roads)

The information obtained from these measurements will aid in providing insight as to what organisms may be present or are expected to be present, and the presence of stream impacts. This information is also invaluable when conducting 303(d)/305(b) assessments of streams, during stressor identification, and when analyzing the random data. ***See Figure 4 for an example of what this section looks like.***

Local Watershed Erosion: In the 100 m reach, note the **existing or potential** detachment of soil within the local watershed (that portion of the watershed that drains directly into the stream upstream and including the reach that you can visually see) and its movement into the stream. Indicate whether there is **None** or if erosion is **Slight**, **Moderate**, or **Heavy**. Look for roads, drains, tilled ground, hillside slips, staging areas, etc. **Do not confine your observations to the local stream banks in the reach.** If observations are made upstream of the 100 m reach, note them in the large "Comments Box" on the bottom left of the page.

Recent Stream Scouring: In the 100 m reach, note the **existing or potential** scouring of the substrate from recent high flow events and mark as **None**, **Slight**, **Moderate**, or **Heavy**. Look for scared or abraded substrate particles or the absence of periphyton in seemingly ok streams. Confer with the Biomorph after the first kick to determine if the benthos seems normal. Also consider other streams visited in the area. Information from locals can also be invaluable. If the stream does appear to be moderately or heavily scoured, confer with other crews or the office to determine if benthic sampling should continue or be postponed at the site.

Atmospheric Odors: Rate the any atmospheric odors based on the following scale: **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, or NR-Not Rated.**

Odor Description: Describe the nature of the odor. Examples include sulfates, creosote, manure, sewage, septic, dead animals, soap, etc.

Reviewers Initials		ACTIVITIES AND DISTURBANCES >>>			
Local Watershed Erosion		☐ None	☐ Slight	☐ Moderate	☐ Heavy
Recent Stream Scouring		☐ None	☐ Slight	☐ Moderate	☐ Heavy
Atmospheric Odors (Rate 0-4 or NR)		Odor Description			
Local NPS Pollution	☐ None ☐ Potential ☐ Obvious	If obvious, magnitude?	☐ Slight	☐ Moderate	☐ Heavy
Specify Obvious or Potential NPS (feedlot, etc.) other than Siltation/Sedimentation!					
Point Source Discharges	☐ Yes ☐ No	Pt. Source(s)			

Stream Reach Activities & Disturbances (Rate 1-Low, 2-Moderate, 3-High, 4-Extreme In Each Box That Applies)

Residential			Recreational			Agricultural			Industrial			Management	
	Residences			Parks/ Campground			Annual Row Crops			Industrial Plants			Liming
	Lawns			Parking Lots			Pasture			Surface Mine			Rip/Rap or Bank Stabilization
	Power Lines			Boat Access/ Dock			Hay Production			Deep Mine			Dredging
	Construction			Swimming			Orchards			Coal Prep			Channelized
	Pipes/Drains			Fishing			Poultry			Quarries			Fill
	Bridges/Culverts			Pipes/Drains			Livestock Access			Railroad			Dams/Impounded
Width	Surf Type	Road Intensity		Foot Trails			Irrigation			Logging		Multipurpose State or County Maintained Roads	
				ATV, Horse, Bike Trails			Pipes/Drains			Power lines		Width	
Codes for Width and Surface Type are Displayed on the Right Under Multipurpose State or County Maintained Roads.				Bridges/Culverts			Bridges/Culverts			Woodyards/ Sawmills			A=Single Lane
			Width	Surf Type	Road Intensity	Width	Surf Type	Road Intensity		Sanitary Landfill			B=Double Lane
										Waste H_2O Treatment			C=Multi-Lane
Elaborate on any of the Stream Reach Activities & Disturbances checked above. Which of the above is the greatest detriment to the stream?										Drinking H_2O Treatment		Surface Type	
										Pipes/Drains			A=Dirt
										Parking Lots			B=Rutted Dirt
										Bridges/Culverts			C=Applied Limestone
										Gas/Oil Well			D=Applied Non-Limestone
										Gas/Oil Lines			E=Asphalt
									Width	Surf Type	Road Intensity		F=Concrete
													Road Intensity

If known, what is the predominant land use(s) in this stream's drainage? Is it mostly forested, agriculture, mining, logging, houses, urban? If mining present, is it active or abandoned, deep or strip, valley fills, etc. What is the predominant NPS pollution? Are there point sources above the reach? Indicate if you used maps (GIS) or field verified comments. <u>DO NOT LEAVE THIS BOX BLANK!</u>	Road Notes:

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Figure 4. Example of the Site Activities & Disturbances section (Page 2) of the field form

Local NPS Pollution: Refers to problems and potential problems ***other than siltation/sedimentation*** in the 100 m reach (the siltation/sedimentation aspect of NPS pollution should be addressed above under Local Watershed Erosion). Non-point source pollution is typically defined as runoff from broad landscapes such as agricultural lands and urban areas (*e.g.,* shopping center parking lots). However, we are more concerned with the **regulatory definition of Nonpoint-source pollution** which includes any pollution that is not regulated thru a permitting process or permitted outfall (*i.e.*, pipes that aren't required to have a permit number posted near it). This would include the typical NPS types as well as others that may affect water quality are feedlots, artificial wetlands, septic systems, dams and impoundments, oily strips in center of roads, mine seepage and pre-law mine portals, gob-pile runoff, quarry runoff, landfill leachate, wood-yard runoff and leachate, acid deposition, etc. Indicate **None**, **Potential**, or **Obvious** sources.

If obvious, magnitude?: If the Nonpoint-Source Pollution is obvious, indicate how intense it is by checking **Slight**, **Moderate**, or **Heavy**.

Specify Obvious or Potential Sources of NPS (feedlot, etc.): Indicate the obvious or potential source of NPS that you observed in the 100 m reach. If it is located above the assessment reach, describe it in the large "Comments Box" on the bottom left of the page.

Point Source Discharges: Since Non-Point source pollution is covering the **regulatory definition of Nonpoint-source pollution**, Point Source (PS) pollution includes any pollution that is regulated thru a permitting process or permitted outfall (*i.e.*, the pipe should have a permit number posted near it). Indicate the presence any permitted discharges entering the streams within the 100 m reach? Indicate **Yes** or **No**.

Pt. Source(s)**:** If there is a point source or sources located in the assessment reach describe it here. If it is located above the assessment reach, describe it in the large "Comments Box" on the bottom left of the page.

⇒ **If you are unsure about if it is NPS and PS, describe it thoroughly it in the large "Comments Box" on the bottom left of the page.**

Stream Assessment Area Activities & Disturbances: Rate the intensity of any of the following disturbances that were observed in the 100 m stream assessment area in the corresponding box. The intensity scale is as follows: **1-Low**, **2-Moderate**, **3-High**, and **4-Extreme and is exclusive of any other stream reach activity (*i.e.*, a 4-extreme rating for Foot Trails does not equal a 4-extreme rating for a parking lot).** If the disturbance type was not observed, leave the box blank. Please be careful to consider if the activity listed is actually impacting the stream reach. For example, a road or house may be adjacent to a stream site, but actually drain into the stream upstream or

downstream of the site. Additionally, a house ½ mile up on a ridge line separated by forest from the stream will not have any impact on the stream even though you know it is up there. If one of the disturbances is observed above or immediately below the 100 m reach or needs further explanation, record it in the large "**Elaborate on any of the Stream Reach Activities & Disturbances checked above. Which of the above is the greatest detriment to the stream?"** box mid-page on the left side.

The Stream Assessment Area Activities & Disturbances section of the form is divided into the following major categories:

RESIDENTIAL: Note the presence of any of the listed residential disturbances adjacent to or near the stream.

RECREATIONAL: Record the presence of organized public or private parks, campgrounds, beaches, or other recreation areas around the stream assessment area. Look for evidence of informal areas of camping, swimming, or boating around the stream (*e.g.,* swimming hole).

AGRICULTURAL: Note the presence of cropland, pasture, orchards, poultry, and/or livestock. Small gardens should be included in this category as row crops and rated according to its size and activities (*i.e.*, pesticide applications).

INDUSTRIAL: Record any industrial activity (*e.g.,* chemical, pulp), commercial activity (stores, businesses) or logging/mining activities around the stream assessment area. This includes high-tension power lines. Businesses like Wal-Mart and strip malls should be considered as parking lots.

MANAGEMENT: Note any evidence of liming activity, water treatment, dredging or channelization, flow control structures, etc.

ROADS/TRANSPORTATION: The **RESIDENTIAL, RECREATIONAL, AGRICULTURAL**, and **INDUSTRIAL** categories each have a block for documenting the presence of roads. Roads under these categories have specialized uses. For example, residential driveways, access roads to fishing sites (recreational), farm roads (agricultural), or mine haul roads (industrial). State and county maintained highways are usually roads that serve numerous purposes. If you cannot determine what the specific use of a road is this category will mostly likely best apply. It may also be helpful later on to write down a description of the road (*e.g.,* haul-road, I-77, C.R. 52/3) under the box called Road Notes.

Using the key on the right side of the page under "Multipurpose State or County Maintained Roads", indicate the width and surface type of the road.

Width: Record the road size as **<u>A</u>=Single**, **<u>B</u>=Double**, or **<u>C</u>=Multi-Lane**. Use best professional judgment to judge the size of roads. If you think two cars can pass one another without steering onto the shoulder, designate the road as double lane. A single lane would require steering onto the shoulder to pass one another. Multi-lanes are large roads such as Interstate highways and some U.S. routes. Large industrial roads such as the ones built on strip mine operations may also be considered multi-lane.

Surface Type: Record the road type as **<u>A</u>=Dirt**, **<u>B</u>=Rutted Dirt**, **<u>C</u>=Applied Limestone**, **<u>D</u>=Applied Non-Limestone** (*e.g.,* some roads use red dog-a type of coal refuse as a surface), **<u>E</u>=Asphalt**, or **<u>F</u>=Concrete**.

<u>Elaborate on any of the Stream Reach Activities & Disturbances checked above. Which of the above is the greatest detriment to the stream?:</u> This area is provided for notes about any of the Stream Assessment Area Activities & Disturbances checked above.

<u>Comments Box</u>: "If known, what is the predominant land use(s) in this stream's drainage? Is it mostly forested, agriculture, mining, logging, houses, urban? If mining present, is it active or abandoned, deep or strip, valley fills, etc. What is the predominant NPS pollution? Are there point sources above the reach? Indicate if you used maps (GIS) or field verified comments. **<u>DO NOT LEAVE THIS BOX BLANK!"</u>**

⇒ **Note: This area is a good place to put comments about the land use observed from recon trips or gleaned from the GIS land use or topographic maps. If comments are based on the map, note them as such. Landowner comments about the upstream activities should also go here. The source of each bit of information should also be noted (*e.g.,* GIS, Topo, Recon, or Local or Landowner).**

PAGE 3

Physical Characterization

PHYSICAL & SEDIMENT CHARACTERIZATION►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►►							Reviewers Initials			
AN-Code					Date					
Stream Width (m)				Total Habitat Type % Coverage for Reach	Riffle	%	Run	%	Pool	%

Figure 5. Example of the Physical Characterization section (Top of Page 3) of the field form

<u>Average Stream Width (m)</u>: Measure the wetted width of the stream at three transects representative of the 100 m assessment area. In general, the three measurements should be made at the downstream terminus, middle, and upstream terminus of the 100

m assessment area. These measurements will be used to calculate (40 x average width) the reach length for sites with substrate characterization (pebble counts) scheduled. Streams greater than 30 m in width will require a visual estimate at three points following the above protocols (if stream conditions permit, try to get one actual reading). Record the measurements and calculate the average stream width (for pebble counts only). The **Geomorph** will take the measurements while establishing the 100 m assessment area (Note: do not walk in stream or take stream measurements until physicochemical data has been collected). A tape measure or measuring stick (thalweg pole) is provided for taking the measurements. The **Geomorph** must conduct this part of the assessment. The gathering of this information is important for several reasons. First, it provides data that is necessary to classify streams by size. Additionally, it requires the Geomorph to cover the entire 100 m reach that will allow for increased accuracy and consistency in the assessment of habitat.

Total Habitat Type % Coverage for Reach: Estimate the percent coverage of each habitat type (**Riffle**, **Run**, & **Pool**) for the 100m reach. **When considering the Pool coverage, remember to count biologically functional pools in smaller streams (*i.e.*, do not use the <0.5 m cutoff used in the deep flow regimes in the RBP). This parameter is best evaluated after completing the Dominant Substrate Type and Reach Characterization below.**

Sediment Characterization

Sediment Odors		Sediment "Oils"		Sediment Deposits			
	Normal		Absent		Silt		Sand
	Sewage		Slight		Fine Gravel		Marl (See Note at Left)
	Petroleum		Moderate		Relic Shells		Limestone Chunks or Fines
	Chemical		Profuse		Sludge		Paper Fiber
	Anaerobic (Septic)	Rate Sediment Deposits: 0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, NR-Not Rated			Coal Chunks and Fines		Red Dog
	Other:				Iron (Orange Hydroxide)	Probable Source: ☐ Mining ☐ Natural	
Note: Marl (crumbly, grayish, lightweight) **** Potomac Direct Drains Only**** Place Marl in Proper Size Class Under "Inorganic Substrate" on Page 3					Aluminum (White Hydroxide)	Probable Source: ☐ Mining ☐ Natural	
					Manganese (Black Hydroxide)	Probable Source: ☐ Mining ☐ Natural	
					Other:		
Sediment Notes & Comments (Describe other or intensity):							

Figure 6. Example of the Sediment Characterization section (Middle of Page 3) of the field form

Sediment Odors: Disturb the sediment and note any odors described (**Normal**, **Sewage**, **Chemical**, **Petroleum**, **Anaerobic (Septic)**, or **Other**) which are associated with sediment in the area of the sampling station. Examine depositional areas for this parameter and collaborate with the Biomorph in making the decision.

Sediment "Oils": Disturb the sediment and choose the term (**Absent**, **Slight**, **Moderate**, or **Profuse**) that best describes the relative amount of sediment oils observed in the stream sampling area. Examine depositional areas and collaborate with the Biomorph before making the decision. **It should be noted that Manganese will often form sheens on the surface of waters and in the sediment that can resemble oil, and thus why this category has oils in quotation marks.**

Sediment Deposits: Note the deposits described (Silt, Sand, Fine Gravel, Marl, Relic Shells, Limestone Chunks or Fines, Sludge, Paper Fiber, Coal Chunks or Fines, Red Dog, Iron, Aluminum, or Manganese Hydroxides) or any other deposits not listed which are present in the sampling area. Collaborate with the Biomorph before making the decision. Rate each sediment deposit as **0-None, 1- Low, 2- Moderate, 3-High, 4-Extreme, and NR-Not Rated (used if for some reason the substrate cannot be seen like when visiting a TMDL site during high turbidity events). Rate the intensities of the each type of metal hydroxide (Iron=Orange/Yellow/Red), Aluminum=White, Manganese=Black). Also indicate the probable source of any metal hydroxide as either Natural or Mining related. If the probable source it is not known, do not guess natural. If both seem likely, just select Mining as this is often the most detrimental to the stream.** Also note that the Limestone Chunks and Fines should include any non-native limestone (*e.g*., road gravel, rip-rap, etc.) that is found in the stream.

Sediment Notes & Comments: Provided as a space to describe unusual substrates or qualities of the substrate. Use this area to elaborate on metal hydroxide sources, limestone chunks and fines sources, trash like bricks, concrete, or asphalt chunks that are serving as benthic substrate.

Substrate Particle Layer Profile

Substrate Particles	Particle Codes	Size Class
Bedrock	BR	Smooth surface rock/hardpan (>4000 mm –bigger than a car)
Boulder	BL	Basketball to car (>250-4000 mm)
Cobble	CB	Tennis ball to basketball (>64-250 mm)
Coarse Gravel	CG	Marble to tennis ball (>16-64 mm)
Fine Gravel	FG	Ladybug to marble (>2-16 mm)
Sand	SA	Gritty – up to ladybug (>0.06-2 mm)
Silt & Fines	ST	Fine – not gritty (<0.06 mm)
Clay	CL	Slick/ hard clay or hard-pan clay
Metal Hydroxide	MH	Any Metal Hydroxide Deposits (Use only in the Substrate Layer Profile)

Substrate Particle Layer Profile			
Location[1]	Habitat Type[2]	Substrate Particle[3]	Sand & Silt Thickness (cm)[4]
Special Instructions: *1) Sample riffle habitat if available. 2) The location (left, middle, or right) is to be kept consistent for each consecutive visit if possible (e.g., High Flows).*			
R M L RDB LDB		Notes:	

Top

Bottom

Box above is 1cm wide

1. Location (left, middle, right) along a transect across the stream. 2. Habitat type (riffle, run, pool). 3. Substrate Particle (use Particle Codes) is determined by removing one particle at a time (documenting each as a separate layer) starting from the uppermost layer and working down to the bottom. Only one layer profile is required per visit. 4. The thickness in cm of the sand & silt layers present in the profile. *DO NOT LABEL TWO CONSECUTIVE LAYERS OF SAND OR SILT (e.g., 1-Sand, 2-Sand or 1-Silt, 2-Silt)!!!!!*

Figure 7. Example of the Substrate Particle Layer Profile section (Middle of Page 3) of the field form

Find a riffle habitat, if available, near the X-site as this is the preferred habitat for this measurement. Document the habitat type (Riffle, Run, and Pool) of the measurement. Choose a location along the cross-section (Right, Middle, or Left facing downstream) that is convenient and will be consistently available for measurement in future visits during all possible flow regimes. It is preferred that this is the Middle if possible. This exact location is to be kept consistent for each consecutive visit if at all possible. An example of an instance where the same location may not be available for a sample would be a high flow that prevents measurement in the same location as prior visits. If you do need to move to an alternate location, be sure that you are still within the normal stream channel (look for a lack of vegetation). If high flows keep you on the bank, do not take this measurement. Next, begin to remove and document the substrate (***using the Substrate Size Classification outlined in Figure 7 above or Table 3 below***) one layer at a time. If any sand or silt is documented, record the depth of that layer in cm. **Note: Do not document two layers of sand or silt in succession (e.g., Layer 1=SA-Sand, Layer 2=SA-Sand). Instead, document the thickness of these layers.** Repeat this until the top five layers are documented or until you reach the bottom of the biologically inhabitable zone (no more than 5-10 cm). Record any notes that may be necessary. **Note: The purpose of this evaluation is to document the colonization potential of the substrate relative to sedimentation. Therefore it is important to include Metal Hydroxides in the layer profile as they may have a smothering/cementing effect on the stream substrate in some situations. In addition, it is essential that the habitat, location, and silt/sand layer depths be recorded in order to calculate the final Substrate Layer Profile Score.**

Table 3. Substrate Size Classification for Substrate Layer Profile and Dominant Substrate Type and Reach Characterization

Class	Code	Size	Description
Bedrock	BR	>4000 mm	Bigger than car
Boulder	BL	>250-4000 mm	Basketball to car
Cobble	CB	>64-250 mm	Tennis ball to Basketball
Coarse Gravel	CG	>16-64 mm	Marble to Tennis ball
Fine Gravel	FG	>2-16 mm	Ladybug to marble
Sand	SA	>0.06-2 mm	Gritty between fingers
Silt & Fines	ST	<0.06 mm	Smooth, not gritty (silt & muck)
Clay	CL	>4000 mm	Slick/ hard clay or hard-pan clay bottom
Metal Hydroxides	MH		Any Metal Hydroxide Deposit (Use this class only in the Substrate Layer Profile)

Dominant Substrate Type and Reach Characterization

Dominant Substrate Type - Reach Characterization (See Above for Substrate Particle Size Codes)

Reach Location (m)	Habitat Type	Depth (m)	Dom Sub 1	% age 1	Dom Sub 2	% age 2	Reach Location (m)	Habitat Type	Depth (m)	Dom Sub 1	% age 1	Dom Sub 2	% age 2

1. Reach Location (from d.s. end), 2. Habitat type (riffle = rf, run = rn, pool = p), 3. Dom Substrate (class codes), 4. %age (estimate in the habitat)

Figure 8. Example of the Dominant Substrate Type and Reach Characterization section (Bottom of Page 3) of the field form

At various points along the stream reach where there is a sudden change in habitat type, substrate type, or depth, measure the stream depth (m) using the thalweg pole and document the position (relative to the downstream end of the reach) along the stream reach in meters, the habitat type (riffle, run, or pool), the 1st dominant substrate type (***using the Substrate Size Classification outlined in Table 3 above***) and its percent aerial coverage (what you see on top in your "cone of vision"), and then the 2nd dominant substrate type and percent aerial coverage. Take measurements throughout the reach (Geomorph responsibility). Some streams sites may not have all of the three habitat types (usually pools are missing). **When considering the Pool areas, remember to count biologically functional pools in smaller streams (*i.e.*, do not use the <0.5m deep flow regime cutoff used in the RBP). Note: Do not use Metal Hydroxides as a class when evaluating the dominant substrate type; look only at the functional size classes (*i.e.*, those that have size ranges).**

⇒ **Note: The information generated from this task should help one more accurately fill out other portions of the form (*e.g.*, the Total Habitat Type % Coverage for Reach, Sediment Deposit Intensities, and *the EPA's Rapid Habitat Assessment Form parameters Epifaunal Substrate, Sediment Deposition, and Riffle Frequency*).**

PAGE 4

Field Water Quality Measures

Reviewers Initials		*FIELD WATER & RIPARIAN VEGETATION ZONE MEASURES>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>*		
WQ Sample Location	☐Mid-Stream ☐Bank (☐Left ☐Right) ☐Thalweg (☐Left ☐Middle ☐Right) ☐Left Channel ☐Right Channel ☐Cross Section ☐Other:		WQ Type	☐Single ☐Profile ☐Other:
Sonde Method	☐Grab ☐ Sample Tube ☐Bucket	Lab Water Method	☐Grab ☐ Sample Tube ☐Bucket	

Flag	Physicochemical Parameters (for a Single Water Quality Sample)		Seasonal Water Level		Water Odors		Surface "Oils"		Turbidity	
		Temperature °C		Below Normal		Normal		None		Clear
		pH (std. Units)		Normal		Sewage (Not Septic)		Flecks		Slightly Turbid
		Dissolved Oxygen (mg/L)		Above Normal		Petroleum		Sheen		Moderately Turbid
		Conductivity (µmhos/cm)		Flooding		Chemical		Globs		Highly Turbid
Sonde I.D. #:______ If any problems occur with the Water Meter or any readings are suspect, record notes in the space to the right.			Notes:			Anaerobic (septic)		Slick	Water color:	
						Other:				
					Foam/Suds (Rate 0-4 or NR)					

ABOVE: Record readings in box for corresponding physicochemical parameter. Insert a √ in the box for other categories.

Precipitation Status and History					
Current		Past 24 Hours (If Known)		Major Rain Event in past week?	☐Yes ☐No

If it is raining or has rained recently, which of the following best describes the peak runoff (flush) condition of the stream at the site when water samples were collected?

< 1 Hour		1 to 4 Hours		4 to 12 Hours		12 to 24 Hours		>24 Hours		Unknown	

Is the stream level rising, falling, or at baseflow at the time of visit? ☐ Baseflow ☐ Rising ☐ Falling

Figure 9. Example of the Field Water Quality Measures section (Top of Page 4) of the field form

<u>WQ Sample Location:</u> Indicate the cross-sectional location of the water quality sampling: 1) **<u>Mid-Stream</u>**, 2) **<u>Bank</u>** (**Left** or **Right**), 3) **<u>Thalweg</u>** (**Left**, **Middle**, or **Right**), 4) **<u>Left Channel</u>**, 5) **<u>Right Channel</u>**, 6) **<u>Vertical</u>** (*i.e.*, vertical profiles done on lakes or rivers where samples are taken at multiple depths), 7) **<u>Cross Section-Lateral</u>** (*i.e.*, samples are taken across a stream channel at Left Bank, Mid-Stream, and Right Bank or specific measured distances), 8) **<u>Cross Section-Longitudinal</u>** (*i.e.*, samples are taken along the distance of a stream reach at specific measured distances), 9) **<u>Other</u>** (please describe).

<u>WQ Type:</u> Indicate type of water quality sampling: 1) **<u>Single</u>** (*i.e.*, a single sample is taken at one of the first six WQ Sample Locations mentioned above), 2) **<u>Profile</u>** (*i.e.*,

samples are taken at multiple locations, but kept separate as distinct samples), 3) **Composite** (*i.e.*, samples are taken at multiple locations, but combined into one sample), 4) **Other** (please describe).

Sonde Method: Indicate the type of collection method used with the water quality sonde: 1) **Grab** (*i.e.*, direct stream or water column measurement), 2) **Bucket with Crane**, 3) **Van-Dorn Bottle**, 4) **Sample Tube with Rope**, 5) **Bucket with Rope**, 6) **Deployable**, 7) **Other** (please describe).

Lab Water Method: Indicate the type of collection method used to obtain the lab water: 1) **Grab** (*i.e.*, direct stream or water column measurement), 2) **Bucket with Crane**, 3) **Van-Dorn Bottle**, 4) **Sample Tube with Rope**, 5) **Bucket with Rope**, 6) **Clean Hands** (*e.g.*, Mercury sampling), 7) **Other** (please describe).

Flag: Indicate if one of the recorded values was not accurate or suspected of being in error. This field may also be marked in by the data entry person (in pen) if they suspect inaccuracy of the instrument readings. ***Examples of Flag Codes used in the fields are in Table 4 below***.

Table 4. Examples of Flag values used on the field forms

I	Parameter not recorded or deleted due to instrument problems or maintenance issues
L	Parameter recorded but suspected to be incorrect value; There is a low probability that the value is incorrect
M	There is a moderate probability that the value is incorrect
H	There is a high probability that the value is incorrect

Physicochemical Parameters - Temperature, pH, D.O., Conductivity: Record the values for each of the physicochemical parameters indicated from the water probe. 1) **Temp**-^{O}C, 2) **pH**-Standard Units, 3) **D.O.**-mg/l, and 4) **Conductivity**-μmhos/cm.

Sonde I.D.: Record the sonde instrument identification number. This is usually marked on the sonde with a black sharpie. Do not confuse this with the jeep number often marked on the camera in white ink. **Do not record the number written on the display unit as this unit does not store calibration information.** If for some reason the sonde's instrument identification number is not apparent, then write down the WV Property Tag number (found on a blue tag) or the manufacturer's Serial Number on the instrument so that the proper identification number can be tracked down later and remarked onto the sonde.

Seasonal Water Level: Indicate the water level relative to the season as 1) **Below Normal**, 2) **Normal**, 3) **Above Normal**, or 4) **Flooding**. **Example**: in general, high water in autumn would be Above Normal.

Water Odors: Record the odors described (include any odors not listed) that are associated with water in the sampling area: 1) **Normal**, 2) **Sewage**, 3) **Petroleum**, 4) **Chemical**, 5) **Anaerobic (Septic)**, or 6) **Other**. Collaborate with the Biomorph in making the decision.

Foam/Suds: Rate the any Foam or Suds on the surface of the water based on the following scale: **0-None**, **1- Low**, **2- Moderate**, **3-High**, **4-Extreme**, and **NR-Not Rated.** The presence of foam in streams is usually a product of nature. The most common cause of "natural" foam streams is turbulence via riffles and runs. Foam may also occur when plants and small aquatic organisms decompose and release a variety of organic compounds. Organic compounds leached from the soil also cause foam. Natural foam has a somewhat earthy or fishy smell, and it breaks down rather quickly. Foam from silt or erosion will usually have a brown color. Foams formed in the presence of acid mine drainage will often take on the color of any metal hydroxides in the stream (most commonly orange from iron hydroxides). Suds, however, originate from soaps and detergents entering the stream via straight pipes and drainages. They can be easily distinguished from foam by their scent (*i.e*., they smell like soap) and the bubbles often have an iridescence.

Surface Oils: Note the term(s) that best describes the relative amount of water surface oils present: 1) **None**, 2) **Flecks**, 3) **Sheen**, 4) **Globs**, or 5) **Slick**. Collaborate with the Biomorph in making the decision. These are generally associated with urban, industrial, or oil/gas activities.

Turbidity: Indicate the term that best describes the amount of material suspended in the water column: 1) **Clear**, 2) **Slightly Turbid**, 3) **Moderately Turbid**, 4) **Highly Turbid** (or Turbid). It is usually best to look in the pools to evaluate this. Also, you can look at the water samples collected.

Water Color: Indicate whether the water color is normal (clear) or colored (*e.g.,* orange for iron impacted streams).

Precipitation Status: Describe **precipitation events only** for the area during the time of visit and within the last 24 hours if possible. Comment on any heavy rainfall events, snowmelt, or storms that might have an impact on the water quality during sampling. This information can also be gathered by questioning locals you encounter, especially if you are just arriving to the area at the beginning of the week.

Major Rain Event in past week?: If there were any major rain events in the past week answer **YES** or **NO.** A major rain event is defined as a precipitation event that would result in the rise of stream level and/or drastic change in the turbidity of the stream (clear to muddy). For example, in a small 1st order stream, a brief light shower will probably not result in a change of the water level or turbidity, but light showers that last all day might. However, in a large stream or river, the same all day light showers would probably not affect the water level or turbidity to any great extent.

Peak Runoff: If it is raining or has rained recently, which of the following best describes the peak runoff (flush) condition of the stream at the site when water samples were collected**:** 1) **<1 hour**, 2) **1-4 hours**, 3) **4 -12 hours**, 4) **12-24 hours**, 5) **>24 hours**, 6) **Unknown**. Unless you have monitored the rainfall prior to arriving, the most likely answer is Unknown during your first day in the area.

Is the stream level rising, falling, or at baseflow at the time of visit?: Indicate if the stream level is 1) at **Baseflow**, 2) **Rising**, or 3) **Falling**. This can be hard to judge if a major rain event has occurred in the past week or if you are just arriving to the area at the beginning of the week. Attempt to answer the best that you can.

No Flow?: If a flow was scheduled for the site and not performed, then indicate if one of the following applies: 1) **Dry**, 2) **Low Flow**, 3) **Too Deep/Too Fast**, 4) **Instrument Failure**, 5) **Frozen/Ice**, or 6) **Safety**. **Note that this box is not on the Wadeable Benthic form since. This is because a benthic sample would never be collected under most of these "No Flow" conditions. However, this box is found on the TMDL and General WQ forms.**

Stream Bank/Riparian Buffer Zone Vegetation/Cover Type

Riparian Vegetation Classification

This segment of the stream assessment form was originally developed to address certain objectives proposed in WAB's application for funding under the Wetland's Development Grant Program, 104(b)(3). The principal objective of the project is to assess the integrity of riparian vegetation zones in selected priority watersheds. The following parameters were indicated as possible measures for meeting the proposed objective:

1) Erodibility of riverbank soils
2) Density of bank vegetative cover
3) Riparian disruptive pressure
4) Riparian zone width
5) Percent trees, shrubs, herbs, (bank and riparian zone)

STREAM BANK VEGETATION performs a vital role in the control of erosion to streams. Trees and woody shrubs exhibit deeper and more permanent root systems than

grasses and herbaceous plants and are, thus, more effective in reducing erosion throughout the year.

THE RIPARIAN VEGETATIVE ZONE serves as a buffer zone to pollutants that may enter a stream through runoff, controls erosion, and provides stream habitat and nutrient input into the stream. Relatively undisturbed riparian zones with large dominant tree species reflect healthy stream systems and are generally considered indicative of the best possible conditions.

The following visual estimation procedures are a semi-quantitative evaluation of the type and amount of different types of stream bank and riparian vegetation. The assessment will be used to evaluate the health and level of disturbance of the stream corridor.

The following discussion applies only to the <u>Stream Bank / Riparian Buffer Zone Vegetation / Cover Type</u> section on PAGE 4 of the Stream Assessment Form. See Figure 10 below for an example of what this section looks like.

Stream Bank/Riparian Buffer Zone Vegetation/Cover Type					
→ → What is the dominant vegetation type in the reach? ☐ Deciduous ☐ Coniferous (i.e., Spruce, Pine, Hemlock, Rhododendron) ☐ Mixed Deciduous (>10-49% Coniferous) ☐ Mixed Coniferous (>10-49% Deciduous)				Score Codes: 0=Absent (0%) 1= Sparse (0-10%) 2=Moderate (10-40%) 3=Heavy (40-75%) 4=Very Heavy (>75%)	
Left & Right Bank While Facing Down-Stream	Determined Within The 1st 18 m (60 Ft) From Stream Edge	Canopy (>5 M High) (>15 Feet)	Understory (0.5 – 5 M High) (1.5-15 Feet)	Ground Cover (<0.5 M High) (≈1.5 Feet)	Bare / Barren Soil
		Big Trees such as Sycamore, Oaks, Maples, Box Elder, River Birch, Hemlock	*Small trees and shrubby Vegetation such as Willow, Alder, Knotweed (blue devil), Rhododendron, Wingstem*	*Ferns, Grasses, Mosses, Wildflowers*	*Exposed soil surface, Readily erodible – not rock faces or asphalt roads*
LEFT (18 m) (≈60 ft)					
RIGHT (18 m) (≈60 ft)					

Stream Surface Shading (%)	Indicate % based on cloudless day in summer at noon. Place a √ in box that applies.							
Fully Exposed (0-25%)		Partly Shaded (25-50%)		Partly Exposed (50-75%)		Fully Shaded (75-100%)	

General Comments (include land cover types outside of 18 m zone on left and right side that may impact water quality at the stream site). *Provide your impression of the buffering capacity of the riparian zone in the 100 m reach including width, allochthonous input, topography, and plant composition.*

Amphibian pool in riparian area?	☐ Vernal ☐ Mud Puddle ☐ Sed. Pond ☐ Farm Pond ☐ Ditch ☐ Lake ☐ Cattail Wetland ☐ Other Comments:

Figure 10. Example of the Stream Bank/Riparian Buffer Zone Vegetation/Cover Type section (Bottom of Page 4) of the field form

While standing in a position perpendicular to the stream, visually establish a distance of **18 meters** from the right and left stream edge. This 18 m zone (one on each side of stream) will run parallel with the stream throughout the entire 100 m assessment area. Aerial coverage (described below) of the vegetation types will be conducted within this 18 m zone. ***Remember, that the Riparian Buffer Zone evaluation is not based on stem density, but rather an aerial coverage estimate.***

What is the dominant vegetation type in the reach?: Determine the dominant vegetation type within the 100 m reach as 1) **Deciduous** (*i.e.,* Oak, Maple, Sycamore, Birch, Beech, etc. >90%), 2) **Coniferous** (*i.e.,* Spruce, Pine, Hemlock, Rhododendron, etc. >90%), 3) **Mixed Deciduous** (>10-49% Coniferous), or 4) **Mixed Coniferous** (>10-49% Deciduous) Determination is made by considering both banks together.

Right and left riparian areas are scored separately while looking downstream. Conceptually divide each side into three layers: the **CANOPY** layer (> 15 ft high or 5 m), the **UNDERSTORY** layer (1.5 to 15 ft high or 0.5 to 5 m), and the **GROUND COVER** layer (< 1.5 ft high or < 0.5 m). Note that any one individual plant can potentially occur in more than one layer (*e.g*., a tree with branches at the canopy and understory level or a shrub or herb at the ground cover and understory levels).

The **CANOPY** category includes big trees such as sycamore, silver maple, box elder, river birch, cottonwood, and hemlock. The **UNDERSTORY** layer includes small trees and shrubby vegetation such as willow, alder, rhododendron, knotweed, wingstem, and multiflora rose. **GROUND COVER** vegetation includes ferns, mosses, and grasses.

⇒ **Note: If you are evaluating the stream when the leaves are not on the trees (October-April/May), you need to visualize the CANOPY AND UNDERSTORY as if it was summer. This should not be too hard to do since the branches of the tree indicate where the leaves would be. However, the GROUND COVER cannot be visualized like this very well (especially in forested/wooded riparian areas) as many of the species composing the ground cover layer community are not up and fully visible from October to April/May. Therefore, you must evaluate the GROUND COVER as best as you can with what you can see on the day of sampling.**

Estimate the aerial cover provided by each of the three layers separately per side. **The aerial cover can be thought of as the amount of shadow provided by a particular layer**. The maximum cover in each layer is 100%, so the sum of the aerial cover for the combined three layers could add up to 300%. The four entry choices for aerial cover within each of the three vegetation layers are: **0 (Absent= Zero Cover)**, **1 (Sparse= <10%)**, **2 (Moderate= 10-40%)**, **3 (Heavy= 40-75%)**, or **4 (Very Heavy= >75%)**. These ranges are provided as a key on the Stream Assessment Form.

Also, indicate the percent of **BARREN OR BARE SOIL** within the same 100 m reach and 18 m zone. This refers to highly erodible surfaces and does not include rock cliff faces or asphalt/concrete roads.

Stream Surface Shading (%): Stream surface shading plays a significant role in maintaining water quality in streams. Exposed streams will often experience increased water temperatures that may be directly or indirectly limiting to some organisms and may be favorable to nuisance algae and result in decreased dissolved oxygen. Light intensity may be favorable to some organisms and limiting to others. In general, a partially shaded (50-75%) stream achieves the greatest diversity. A fully shaded stream may inhibit the growth and reproduction of herbaceous aquatic and riparian plants. This situation can potentially inhibit primary production, cover, and habitat. However, this situation does provide better temperature control and increased allochthonous (organic material from outside sources) food resources.

Estimate the percent of stream surface shading using the following categories: **Fully Exposed (0-25%)**, **Partially Shaded (25-50%)**, **Partially Exposed (50-75%)**, and **Fully Shaded (75-100%)**. Evaluate the shading based on a cloudless day in the summer at noon.

Riparian Vegetation Comments Box: *Describe your impressions of the condition of the riparian zone in the 100 m stream reach.* What is its' buffering ability? How intact is the riparian vegetation? Describe the vegetation species assemblage for both sides. Indicate the presence of human activities. Note the land cover type(s) immediately adjacent to the 18 m riparian vegetative zone on both left and right banks. Again, comments in this section are useful during 305(b) stream assessments.

Amphibian Pool Present in riparian area?: Indicate if any of the following amphibian habitat types were present in the riparian area of the stream assessment reach:

1) **Vernal Pools** - Vernal pools are an extremely scarce wetland habitat type occurring only where certain soil conditions are present. In late summer, fall and early winter, vernal pools appear as dry, dusty indentations mostly devoid of vegetation. Look for depressions filled with water along the stream bank and riparian zone.
2) **Mud Puddle** – small depressions in dirt roads are often great habitats for amphibian breeding.
3) **Sediment Ponds** - sediment ponds are built to trap runoff water. Sediment settles to the bottom of these ponds rather than accumulating in local creeks and streams. Typically found below valley fills and other mined areas.
4) **Farm Pond** – livestock watering hole or used for irrigation to crops.
5) **Ditch** – roadside ditches or channel-ways that trap water in low places.

6) **Lake** – larger than a pond.
7) **Cattail Wetland** – typical of waterbodies that are considered to be true wetlands (*i.e.*, Greenbottom Swamp or Canaan Valley).
8) **Other** – Include comments in the area provided to elaborate on any of these.

PAGES 5, 6, 5a, and 6a

EPA's Rapid Habitat Assessment Form

The habitat assessment approach used in this protocol is adapted from EPA's Rapid Bioassessment approach and refined from various applications across the country (***see Figure 11***). The approach focuses on integrating information from specific parameters on the structure of the physical habitat. Specific instruction and training are necessary for an adequate assessment of habitat quality. For each habitat parameter listed, carefully read the description under each ranking category and place the score in the left margin that best describes the condition of the 100 m stream assessment area.

EPA Rapid Habitat Assessment References

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. EPA 444-4-89-001. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

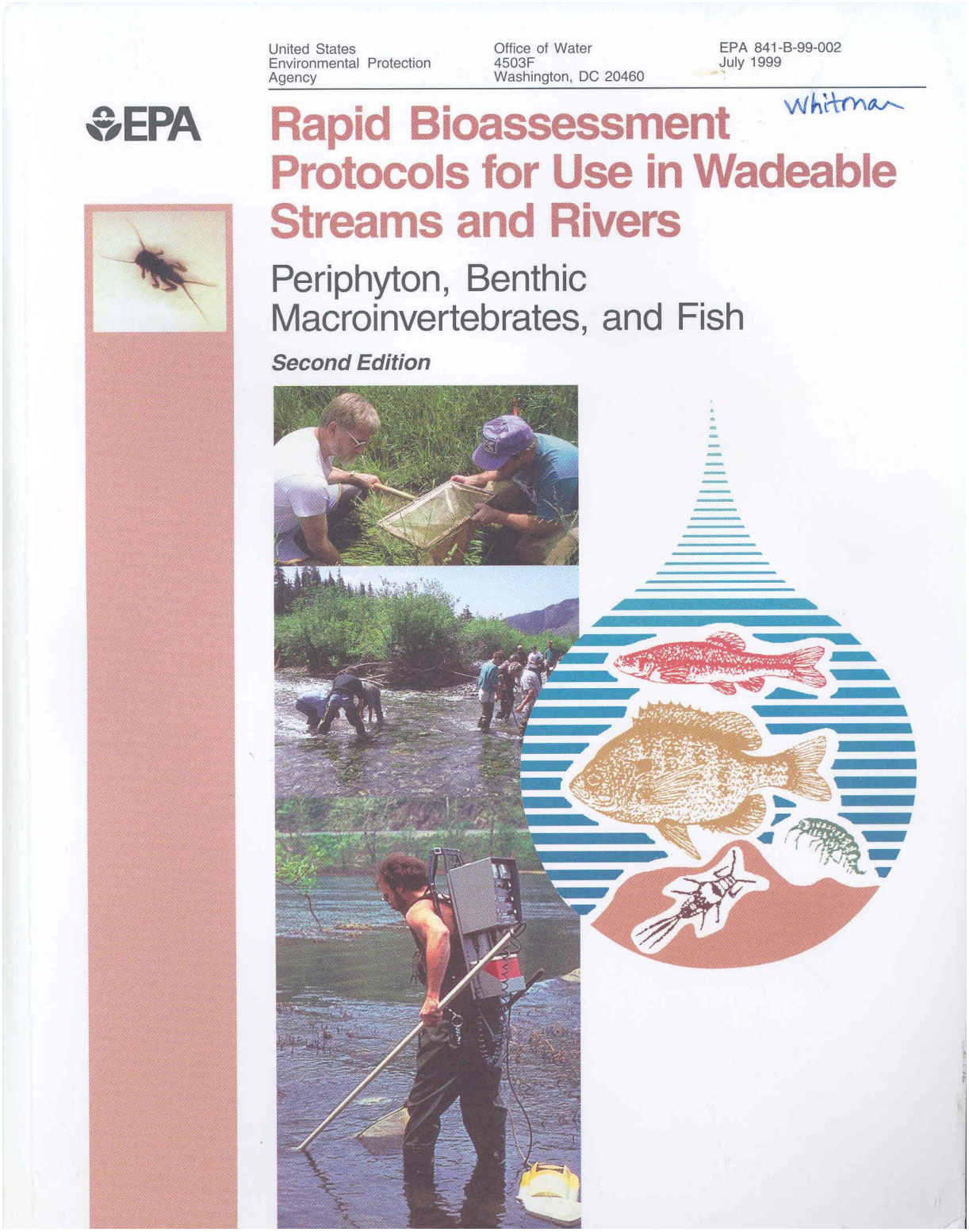

Figure 11. Cover of EPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Second Edition)

Different assessment forms are used for streams that are riffle/run prevalent versus those that are pool/glide prevalent. After making the initial survey of the stream assessment area, classify the stream as either riffle/run or glide/pool prevalent based on your visual assessment of the dominant habitat type (Note: glide/pool habitats will require "MACS" macroinvertebrate sampling methods for low gradient streams). The WAB sampling strategy dictates that a riffle/run habitat is sampled **if possible**. If a stream reach is mostly glide/pool but has a small area of riffle/run, sample the riffle /run if there is enough to obtain the 1 m^2 of substrate. Accordingly, fill out the **riffle/run** Rapid Habitat assessment form (***see Figure 12 and Figure 13 below***). A glide/pool habitat form should only be used when the MACS sampling method is used (***see Figure 14 and Figure 15 below***). **IMPORTANT: In general, MACS sites are not assessed unless indicated on the stream list or there is a special interest in obtaining data from the site. The MACS technique should only be used in streams that are truly "wetland like", such as sites impounded downstream and offer very little to no observable flow. A general rule of thumb is if you have a difficult time determining which direction the stream is flowing, then MACS methods are probably applicable. MACS methods can also be used on large streams that are too deep to wade. In these larger streams, samples are collected from the bank by jabbing the net into appropriate habitat types. Furthermore, if a stream is heavily embedded with sand but has a perceivable flow; it should <u>not</u> be sampled by MACS methods. Riffle/run protocols should be followed (*i.e.*, benthic samples should collected by kicking the sand). Also, MACS methods should only be used if there are enough good habitats to collect all 20 jabs/sweeps.**

⇒ <u>NOTE: In low water conditions, many of the RBP parameters will be rated lower than their potential. Do not try to envision a full stream channel (bank to bank) when rating the parameters. Rate the stream conditions as they exist on that day. For example, in low flow conditions the epifaunal substrate/available fish cover parameter would be rated lower than its potential simply because the habitat components are not covered with water during that visit.</u>

Riffle/Run Prevalence

RAPID HABITAT ASSESSMENT: RIFFLE/RUN>>				Reviewers Initials
AN-Code			Date	
HABITAT PARAMETER	**CATEGORY**			
	Optimal	**Sub-optimal**	**Marginal**	**Poor**
1. EPIFAUNAL SUBSTRATE/ AVAILBLE FISH COVER	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at a stage to allow full colonization potential (i.e., logs and snags that are not new fall and not transient.)	40 to 70% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall, but not yet prepared for colonization (may rate at high end of scale).	20 to 40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. EMBEDDED-NESS	Gravel, cobble and boulder particles are between 0 and 25% surrounded by fine sediment; layering of cobble provides a diversity of niche space.	Gravel, cobble, and boulder particles are between 25 and 50% surrounded by fine sediment.	Gravel, cobble and boulder particles are between 50 and 75% surrounded by fine sediment.	Gravel, cobble and boulder particles are over 75% surrounded by fine sediment.
		Rate in Riffles or Runs		
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. VELOCITY/ DEPTH REGIMES	Slow (<0.3 m/S or ≈1 FT/S), deep (>0.5 m or ≈1.5 FT); slow, shallow (<0.5 m); fast (>0.3 m/S), deep; fast, shallow habitats all present.	Only 3 of 4 habitat types present (if fast-shallow is missing, score lower than if other types are missing).	Only 2 of the 4 habitat types are present (if fast-shallow or slow-shallow are missing, score low).	Dominated by one velocity/depth regime (usually slow-deep).
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. CHANNEL ALTERATION	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e. dredging, (> than past 20 years) may be present, but no evidence of recent channelization.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40% to 80% of the stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach is channelized and disrupted. Instream habitat greatly altered or entirely removed.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. SEDIMENT DEPOSITION Upstream Watershed Total Score:	Little or no enlargement of island or point bars and less than 5% of the bottom is affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand, or fine sediment; 5 to 30% of the bottom is affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of bottom is affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition in pools prevalent.	Heavy deposits of fine material; increased bar development; more than 50% of bottom is changing frequently; pools almost absent due to substantial sediment deposition.
	Rate in Pools & Depositional Areas			
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Page 5 WVDEP WAB Wadeable Benthic Stream Assessment Form (4/23/2010)

Figure 12. Example of the Riffle/Run Rapid Habitat Assessment Part 1 Page 5) of the field form

1. EPIFAUNAL SUBSTRATE/AVAILABLE FISH COVER: Epifaunal substrates are essentially the amount of niche space or hard substrates (stones, snags) available for insects and snails. Numerous types of insect larvae attach themselves to rocks,

logs, branches, or other submerged substrates. The greater the variety and number of available niches or attachments, the greater the variety of macroinvertebrate life will exist in the streams. Rocky bottom areas are critical for maintaining a healthy variety of insects in most high-gradient streams.

Fish cover includes the relative quantity and variety of natural structures in the stream such as fallen trees, logs, and branches, large rocks, and undercut banks, that are available for refugia, feeding, or laying eggs. A large variety of submerged structures in the stream provide aquatic organisms with a large number of niches, thus increasing the diversity.

Note: The Benthic Macroinvertebrate Substrate parameter at the top of *PAGE 7-Non-RBP Parameters* should be considered when rating this parameter as it is essentially the same as rating just the Epifaunal Substrate half of the parameter.

2. EMBEDDEDNESS: refers to the extent to which rocks (gravel, cobble, and boulders) are covered or sunken into the silt, sand, or mud of the stream bottom. Generally, as rocks become embedded the surface area available to macroinvertebrates and fish (shelter, spawning, and egg incubation) is decreased. To estimate the percent of embeddedness, observe the amount of silt or finer sediments overlying and surrounding the rocks. If kicking does not dislodge the rocks or cobble, they may be greatly embedded. It is useful to observe the extent of dark area on the underside of a few rocks. **To avoid confusion with SEDIMENT DEPOSITION (habitat parameter number 5), observations of EMBEDDEDNESS should be taken in the upstream and central portions of riffles and cobble substrate areas. Collaborate with the biomorph on this parameter.**

3. VELOCITY/DEPTH REGIMES: examines the availability of each of the four primary current/depth combinations: (1) slow-deep, (2) slow-shallow, (3) fast-deep, and (4) fast-shallow. The best streams in high gradient regions will have all four habitat types present. The presence or availability of these four habitats relates to the stream's ability to provide and maintain a stable aquatic environment. The general guidelines are 0.5m depth to separate shallow from deep, and 0.3 meters/second to separate fast from slow.

4. CHANNEL ALTERATION: is a measure of large-scale changes in the shape of the stream channel. Many streams in urban and agricultural areas have been straightened, deepened, or diverted into concrete channels often for flood control purposes. Such streams have far fewer natural habitats for fish, macroinvertebrates, and plants than do naturally meandering streams. Channel alteration is present when a stream runs through a concrete channel; when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when

the stream is very straight is very straight for significant distances; when dams and bridges are present; and when other such changes have occurred. Scouring is often associated with channel alteration. In some instances, channel alteration may benefit the stream (*e.g.* K-dams). This parameter should be rated regardless of the intent of the channel alteration. *Note that in the example of K-dams, the channel alteration would be depressed by the presence of these structures, but the Epifaunal Substrate/Available Fish Cover and/or Velocity/Depth Regime score could possibly benefit from their presence.*

5. SEDIMENT DEPOSITION: measures the amount of sediment that has accumulated and the changes that have occurred to the stream bottom because of the deposition. Deposition occurs from large-scale movement of sediment caused by watershed erosion. Sediment deposition may cause the formation of islands, point bars (areas of increased deposition usually at the beginning of meanders that increase in size as the channel is diverted toward the outer bank) or shoals, or result in the filling of pools. Increased sedimentation also results in increased deposition. Usually this is evident in areas that are obstructed by natural or man-made debris and areas where the stream flow decreases, such as bends. High levels of sediment deposition create an unstable and continually changing environment that becomes unsuitable for many organisms.
   To avoid confusion with EMBEDDEDNESS (habitat parameter number 2), observations of sediment deposition should be taken in pools and slow water depositional areas.

   Upstream Watershed Sediment Deposition Total: In addition to rating the 100m reach, if any portion of the upstream watershed was observed, please rate the Sediment Deposition for the section observed. It is not required to drive up the watershed for observations, but rather as a way to take advantage of situations where the upstream area is observed on the way to or from a site.

6. RIFFLE FREQUENCY: is a way to measure the sequence of riffles occurring in a stream. Riffles are a source of high quality habitat and diverse fauna. Therefore, an increased frequency of occurrence greatly enhances the diversity of the community. The types and variety of riffles should also be considered once the riffle distance to stream width ratio is determined.

7. CHANNEL FLOW STATUS: is the degree to which the channel is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. When water does not cover much of the streambed, the amount of useable substrate for aquatic organisms is limited. Do not count extremely large substrate (giant boulders) particles that would rarely if ever be submerged or used by aquatic organisms.

Reviewers Initials		*RAPID HABITAT ASSESSMENT*: RIFFLE/RUN >>>

HABITAT PARAMETER	CATEGORY			
	Optimal	Sub-optimal	Marginal	Poor
6. RIFFLE FREQUENCY	Occurrence of riffles is relatively frequent; ratio of distance between riffles divided by the width of the stream is <7:1 (generally 5 to 7); variety of habitat is key in streams where riffles are continuous; placement of boulders or other large natural obstruction is important.	Occurrence of riffles is infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distances between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by width of the stream is greater than 25.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. CHANNEL FLOW STATUS	Water reaches the base of both banks and a minimal area of channel substrate is exposed.	Water fills more than 75% of the available channel; or less than 25% of the channel substrate is exposed.	Water fills 25 to 75% of the available channel; and/or riffle substrates are mostly exposed.	Very little water in channel, and mostly present as standing pools.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. BANK STABILITY Upstream Watershed Total Score:	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems (<5% of bank affected).	Moderately stable; infrequent, small areas of erosion mostly healed over; 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE:	**Determine left/right by facing downstream**			
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
9. BANK VEGETATIVE PROTECTION Upstream Watershed Total Score:	More than 90% of the stream bank surfaces and immediate riparian zones (crest-over) covered by vegetation including trees, understory shrubs, and non-woody plants (herbs, grasses, ferns, mosses); vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the stream bank surfaces are covered by vegetation, but one class of plants is not well represented; disruption evident, but not affecting plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the stream bank surfaces are covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the stream bank surfaces are covered by vegetation; disruption of stream bank vegetation very high; vegetation has been removed to 2 inches or less in average stubble height.
SCORE:		**Rate from bottom of bank to crest-over at top of bank-large roots count**		
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
10. WIDTH OF UNDISTURBED VEG. ZONE Upstream Watershed Total Score:	Width of undisturbed vegetative zone is >18 meters; human activities (parking lots, roadbeds, clear cuts, lawns, or crops) have not impacted this zone.	Zone width is between 12 and 18 meters; human activities have only minimally impacted this zone.	Zone width is between 6 and 12 meters; human activities have impacted the zone a great deal.	Width of zone is less than 6 meters; little or no un-disturbed vegetation due to man-induced activities.
SCORE:	**Undisturbed veg. is trees, shrubs, & non-woody macro-phytes**			
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
TOTAL:	Optimal=160-200	Sub-Optimal=110-159	Marginal=60-109	Poor=0-59

Estimated Mileage of Upstream Watershed Evaluated	

Page 6 WVDEP WAB Wadeable Benthic Stream Assessment Form (4/23/2010)

Figure 13. Example of the Riffle/Run Rapid Habitat Assessment Part 2 (Page 6) of the field form

8. BANK STABILITY: measures whether the stream banks are eroded (or have the potential for erosion). Steep banks are more likely to collapse and suffer from erosion than gently sloping banks and are therefore considered unstable. Signs of erosion include: crumbling, unvegetated banks, exposed tree roots, and exposed soil. However, exposed cliff faces or rocks provide a stable, non-erodible bank. In addition, the extent to which the bank has healed over with vegetation and roots (*i.e.*, the age of the erosional scars) must be considered. **This parameter is scored by considering right and left banks separately throughout the entire 100 m assessment area**. For example, after observing the right bank, it was determined that less than 5% of the total bank area in the 100 m assessment reach exhibited erosional scars. This would result in an optimal score in the range of 9-10.

   Upstream Watershed Bank Stability Total: In addition to rating the 100m reach, if any portion of the upstream watershed was observed, please rate the Bank Stability for the section observed. It is not required to drive up the watershed for observations, but rather as a way to take advantage of situations where the upstream area is observed on the way to or from a site.

9. BANK VEGETATIVE PROTECTION: measures the amount of the stream bank that is covered by natural vegetation for the area (large trees, small trees, herbaceous layer for most of WV streams). **For WAB assessments, the stream bank extends from the edge of the channel floor up to the crest-over at top of bank.** The top or "crest-over" of the bank can be determined by looking for an obvious slope break that differentiates the channel from a flat floodplain higher than the channel. The root systems of plants (trees, shrubs, grasses) growing on stream banks helps hold soil in place, thereby reducing the amount of erosion that is likely to occur. **Large roots should be considered when rating this parameter.** The Bank Vegetative Protection parameter supplies information on the ability of the bank to resist erosion, as well as additional information on the uptake of nutrients of by the plants, the control of in-stream scouring, and stream shading. Consideration must be given to the abundance and diversity of trees, shrubs, or grasses (grazed/mowed and un-grazed/un-mowed). The frequency or age of mowing and grazing can also be considered. Banks that have full, diverse, natural plant growth are better for fish and macroinvertebrates than are banks without vegetative protection or those shored up with concrete or riprap. However, the presence of exposed cliff faces or rocks should not detract from this score as they are natural structures that normally do not support vegetation. **This parameter is scored by considering right and left banks separately throughout the entire 100 m assessment area**.

   Upstream Watershed Bank Vegetative Protection Total: In addition to rating the 100m reach, if any portion of the upstream watershed was observed, please rate the Bank Vegetative Protection for the section observed. It is not required to drive up

the watershed for observations, but rather as a way to take advantage of situations where the upstream area is observed on the way to or from a site.

10. WIDTH OF UNDISTURBED VEGETATION ZONE: is a measure of disruptive changes to the natural vegetative zone (big trees, small trees, shrubs, & non-woody macrophytes or herbaceous layer for most of WV streams) because of grazing or human interference (*e.g.* mowing). In areas of high grazing pressure from livestock or where residential and urban development activities disrupt the riparian zone, the growth of a natural plant community is impeded. Residential developments, urban centers, golf courses, and pastureland are the common causes of anthropogenic effects on the riparian zone. **This parameter is scored by considering right and left banks separately throughout the entire 100 m assessment area.**

    Upstream Watershed Width of Undisturbed Vegetation Zone Total: In addition to rating the 100m reach, if any portion of the upstream watershed was observed, please rate the Width of Undisturbed Vegetation Zone for the section observed. It is not required to drive up the watershed for observations, but rather as a way to take advantage of situations where the upstream area is observed on the way to or from a site.

TOTAL: Total all of the scores for a final RBP score from 0-200. ***See Table 5 for the Total RBP Score Categories.***

Table 5. Total RBP Score Categories

RBP Total Score	Category
160-200	Optimal
110-159	Sub-Optimal
60-109	Marginal
0-59	Poor

Estimated Mileage of Upstream Watershed Evaluated: Indicate the approximate mileage of the upstream watershed that was observed for the Upstream Watershed scores.

Glide/Pool Prevalence Form (Low Gradient Macs-Type Sites Only)

RAPID HABITAT ASSESSMENT: GLIDE/POOL >>>	Reviewers Initials	
AN-Code	Date	

HABITAT PARAMETER	CATEGORY			
	Optimal	Sub-optimal	Marginal	Poor
1. EPIFAUNAL SUBSTRATE/ AVAILBLE FISH COVER	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other habitat and at a stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient.	30 to 50% mix of stable habitat; well suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of new fall; but not yet prepared for colonization (may rate at high end of scale.	10 to 30% mix of stable habitat; habitat availability is less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. POOL SUBSTRATE CHARACTER-IZATION	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation are common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation are present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. POOL VARIABILITY DEEP >1 m (3FT); SHALLOW <1m	Even mix of large[1] –shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. CHANNEL ALTERATION	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e. dredging, (> than past 20 years) may be present, but no evidence of recent channelization.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40% to 80% of the stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach is channelized and disrupted. Instream habitat greatly altered or entirely removed.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. SEDIMENT DEPOSITION Upstream Watershed Total Score:	Little or no enlargement of islands or point bars and less than 20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand, or fine sediment; 20 to 50% of the bottom is affected; slight deposition in pools.	Moderate deposition of new gravel, sand, or fine sediment on old and new bars; 50 to 80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
	Rate in Pools & Depositional Areas			
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

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[1] Large if length, width, or oblique dimension is greater than ½ of the stream width.

Figure 14. Example of the Glide/Pool Rapid Habitat Assessment Part 1 (Page 5a) of the field form

1. EPIFAUNAL SUBSTRATE/AVAILABLE FISH COVER: See No. 1 under PAGE 5a - RIFFLE/RUN PREVALENCE. In low gradient streams with muddy bottoms, the epifaunal substrate consists mostly of submerged logs or snags, and aquatic vegetation.

2. POOL SUBSTRATE CHARACTERIZATION: evaluates the type and condition of bottom substrates found in pools. Firmer sediment types (*e.g.,* gravel, sand) and rooted aquatic plants support a wider variety of organisms than a pool substrate dominated by mud or bedrock and no plants. In addition, a stream that has a uniform substrate in its pools will support far fewer types of organisms than a stream that has a variety of substrate types.

3. POOL VARIABILITY: rates the overall mixture of pool types found in streams, according to size and depth. The four basic types of pools are large shallow, large-deep, small-shallow, and small-deep. A stream with many pool types will support a wide variety of aquatic species. Rivers with low sinuosity (few bends) and monotonous pool characteristics do not have sufficient quantities and types of habitat to support a diverse aquatic community. As a general guideline, consider a pool deep if it is greater than 1 meter in depth and large if its length, width, or oblique dimension is greater than half the stream width.

4. CHANNEL ALTERATION: ***See No. 5 under Riffle/Run Prevalence***.

5. SEDIMENT DEPOSITION: ***See No. 6 under Riffle/Run Prevalence***.

6. CHANNEL SINUOSITY: evaluates the meandering or the relative frequency of bends in the stream. Streams that meander provide a variety of habitats for aquatic organisms, whereas straight stream segments are characterized by monotonous habitats that are prone to flooding. A high degree of sinuosity creates a variety of pools and reduces the energy from surges when the stream flow fluctuates. The absorption of this energy by bends protects the stream from excessive erosion and flooding.

7. CHANNEL FLOW STATUS: determines the percent of the channel that is filled with water. The flow status will change as the channel enlarges or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. The water will not cover as much of the streambed, thus decreasing the amount of living space for aquatic organisms. In muddy bottom streams, the decrease in water level will expose logs and snags, thus reducing the areas with good habitat.

8. BANK STABILITY: ***See No. 8 under Riffle/Run Prevalence***.

9. BANK VEGETATIVE PROTECTION: ***See No. 9 under Riffle/Run Prevalence***.

Reviewers Initials		*RAPID HABITAT ASSESSMENT:* GLIDE/POOL>>>

HABITAT PARAMETER	CATEGORY			
	Optimal	Sub-optimal	Marginal	Poor
6. CHANNEL SINUOSITY	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line.	The bends in the stream increase the stream length 2 to 3 times longer than if it was in a straight line.	The bends in the stream increase the stream length up to 2 times longer than if it was in a straight line.	Channel is straight; waterway has been channelized for a long distance.
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. CHANNEL FLOW STATUS SCORE:	Water reaches the base of both banks and a minimal area of channel substrate is exposed.	Water fills more than 75% of the available channel; or less than 25% of the channel substrate is exposed.	Water fills 25 to 75% of the available channel; and/or riffle substrates are mostly exposed.	Very little water in channel, and mostly present as standing pools.
	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. BANK STABILITY Upstream Watershed Total Score: SCORE:	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems (<5% of bank affected).	Moderately stable; infrequent, small areas of erosion mostly healed over; 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
	Determine left/right by facing downstream			
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
9. BANK VEGETATIVE PROTECTION Upstream Watershed Total Score: SCORE:	More than 90% of the stream bank surfaces and immediate riparian zones (crest-over) covered by vegetation including trees, understory shrubs, and non-woody plants (herbs, grasses, ferns, mosses); vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the stream bank surfaces are covered by vegetation, but one class of plants is not well represented; disruption evident, but not affecting plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the stream bank surfaces are covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the stream bank surfaces are covered by vegetation; disruption of stream bank vegetation very high; vegetation has been removed to 2 inches or less in average stubble height.
	Rate from bottom of bank to crest-over at top of bank-large roots count			
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
10. WIDTH OF UNDISTURBED VEG. ZONE Upstream Watershed Total Score: SCORE:	Width of undisturbed vegetative zone is >18 meters; human activities (parking lots, roadbeds, clear cuts, lawns, or crops) have not impacted this zone.	Zone width is between 12 and 18 meters; human activities have only minimally impacted this zone.	Zone width is between 6 and 12 meters; human activities have impacted the zone a great deal.	Width of zone is less than 6 meters; little or no un-disturbed vegetation due to man-induced activities.
	Undisturbed veg. is trees, shrubs, & non-woody macro-phytes			
LEFT:	10 9	8 7 6	5 4 3	2 1 0
RIGHT:	10 9	8 7 6	5 4 3	2 1 0
TOTAL:	Optimal=160-200	Sub-Optimal=110-159	Marginal=60-109	Poor=0-59

Estimated Mileage of Upstream Watershed Evaluated	

Page 6b WVDEP WAB Wadeable Benthic Stream Assessment Form (4/12/2010)

Figure 15. Example of the Glide/Pool Rapid Habitat Assessment Part 1 (Page 5a) of the field form

10. WIDTH OF UNDISTURBED VEGETATION ZONE: ***See No. 10 under Riffle/Run Prevalence***.

TOTAL: Total all of the scores for a final RBP score from 0-200. ***See Table 5 above for the Total RBP Score Categories.***

PAGE 7

Non-RBP Parameters

BENTHIC, AESTHETIC, & REMOTENESS RATINGS & EXTRA SPACE >>>>>>>>>>>>>>>>>>				Reviewers Initials	
AN-Code				Date	
PARAMETER	**Optimal**	**Sub-optimal**	**Marginal**	**Poor**	
BENTHIC MACRO-INVERTEBRATE SUBSTRATE	Preferred substrate abundant; stable, & at full colonization potential (riffles well developed & dominated by cobble; substrate not new or transient).	Substrate adequate for maintenance of populations; abundance of cobble with coarse gravel &/or boulders common; small areas of new &/or transient substrate particles (sand and fine gravel) may be present.	Preferred substrate uncommon; some cobble present but gravel or large boulders & bedrock prevalent; transient substrate areas may be frequent.	Preferred substrate virtually absent; gravel or large boulders & bed – rock dominant; transient areas may be dominant.	
	Rate for entire reach even if the reach is not representative of benthic sample area				
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0	
TRASH INDEX	Little or no evidence of human refuse present.	Human refuse present in small amounts.	Human refuse present in Moderate amounts.	Human refuse abundant and unsightly.	
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0	
REMOTENESS RATING	Stream assessment site more than ¼ mile from nearest Road; access difficult and little or no evidence of human disturbance.	Stream assessment site within ¼ mile of roadside; site with moderately wild character.	Stream within ¼ mile of roadside; development activities evident.	Segment immediately adjacent to roadside access; visual, olfactory, and/or auditory displeasure experienced.	
SCORE:	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0	

Figure 16. Example of the Non-RBP Parameter section (Top of Page 7) of the field form

BENTHIC MACROINVERTEBRATE SUBSTRATE: This parameter measures the quality of the benthic macroinvertebrate substrate throughout the 100m assessment reach. ***This measure is essentially the same as the Epifaunal Substrate half of the Epifaunal Substrate/ Available Fish Cover parameter at the top of PAGES 5, 6, 5a, and 6a.*** **Only the benthic macroinvertebrate substrate quality should be considered with this parameter.** The benthic macroinvertebrate substrate is essentially the amount of niche space or hard substrate (*i.e.*, stones, snags) available for insects, snails, worms, clams, and crustaceans to colonize. Numerous types of benthic organisms attach themselves to rocks, logs, branches, or other submerged substrates. The greater the diversity and abundance of available niches for attachment the greater the diversity and abundance of benthic macroinvertebrates in the stream. Rocky bottom areas are critical for maintaining a healthy variety of benthic macroinvertebrates in most high-gradient streams.

The relative amount of cobble drives this parameter as it is the most productive and optimal substrate size-class for benthic macroinvertebrates in riffle/run samples. As the prevalence of the benthic substrate drifts into size classes larger (boulder and bedrock) or smaller (gravels, sand, and silt) than cobble, the productivity decreases. Boulders and bedrock may be stable, but do have as much potential niche space as cobble. However, it is important to consider the size and texture of the boulders and bedrock as smaller boulders provide more niche space than larger boulders and rough/fissured boulders and/or bedrock provide more niche space than smooth boulders and/or bedrock. Gravels, especially coarse gravel, may provide niche space, but are more transient (*i.e*., unstable and susceptible to scouring) than cobble. Fine gravel, sand, and silt are especially bad as they provide minimal niche space and are extremely transient. Therefore, the relative amount and sizes of the transient particles is also important to consider when rating this parameter.

NOTE: Rate this parameter for the entire reach, even if the reach is not representative of benthic sample area. For example, you may have a stream reach that is 95% bedrock, but you were able to do all of the benthic samples in an isolated cobble-dominant riffle with the best benthic habitat you have ever seen. Since the reach is so dominated by bedrock, you would probably score the Benthic Macroinvertebrate Substrate Score in the Marginal to Poor categories (depending on the quality of bedrock as discussed above). ***The quality of the actual benthic macroinvertebrate substrate area that was sampled will be described in better detail in the middle of PAGE 9-Benthic Substrate Sample Composition***.

TRASH INDEX (AESTHETIC RATING): Record the aesthetic character of the stream assessment area **(NOT JUST IN THE STREAM)** based on the abundance of human refuse that is present in and around the stream bank. Consider any piece of trash that could potentially be washed into the stream by high flows or floods.

REMOTENESS RATING: Record the remoteness of the stream assessment area based on its wild character, proximity to roads, and development activities.

PRS and Stressor Info

Is Site a Potential Reference?: Answer **Yes** or **No**. Consider the Water Chemistry, Benthos, Habitat, Human Disturbance, Location (*i.e*., Ecoregion), Level I vs. Level II Reference Condition, etc. ***Refer to Determining Candidate Reference Sites While In the Field under Chapter II. Section A. Part 2. D. Determining Candidate Reference Sites While In the Field starting on page 19 for more information.***

Is Site A Potential Reference?	☐ Yes ☐ No (Consider Water Chemistry, Benthos, Habitat, Human Disturbance, Location (i.e., Ecoregion), Level I vs. Level II vs. Level III Reference Condition, etc.)
If not a Potential Reference, why?	
Stressor Info (Check all that apply and only those that are definite stressors).	☐ Sediment ☐ Fecal ☐ Nutrients ☐ Metals ☐ pH ☐ Sulfate ☐ Conductivity ☐ Other:
Please check Other if the site is located 1-2 miles downstream of any impoundment (e.g., lakes, ag. or mining ponds, flood control dams, beaver dams, low water ford/bridge dams) or a valley fill (mining or road) structures. Be sure to include type of structure (with type of impoundment release), distance upstream to the structure, number and size of tributaries in between that may alter the water chemistry (including dilution effects), and size of impoundment in m x m.	☐ Impoundment: ☐ Lake ☐ Ag Pond ☐ Mining Pond ☐ Flood Control ☐ Beaver ☐ Instream Pool ☐ Concrete Low Water Ford/Bridge Impoundment Release Type: ☐ Bottom ☐ Spillover ☐ Valley Fill: ☐ Mining ☐ Road (i.e., refuse from highway construction)
	Distance Upstream from Sample Site to Structure (Miles)
	Number of Tributaries Between Structure and Sample Site
	Size of Impoundment (m x m)

Figure 17. Example of the PRS and Stressor Info section (Middle of Page 7) of the field form

If not a Potential Reference, why?: Indicate whether this site appears to be relatively undisturbed and may be considered as a potential reference site (see reference site criteria). Also make notes as to why the stream does not satisfy reference site criteria in the space provided. **Note that a yes answer will not necessarily mean the site will achieve reference status as many other criteria that cannot be determined in the field are considered. Many sites that a person would typically say no to as a potential reference site still meet all of the reference criteria. Therefore it is important to consider only those criteria that can absolutely be determined in the field when answering this question.** ***Refer to Determining Candidate Reference Sites While In the Field under Chapter II. Section A. Part 2. D. Determining Candidate Reference Sites While In the Field starting on page 19 for more information.***

Stressor Info: Indicate all definite stressors that are believed to have an impact on the benthic macroinvertebrate community at the site. Options include: **Sediment**, **Fecal** and/or **Nutrients** (both considered Organic Enrichment), **Metals** (or acid metals which represent toxicity), **pH** (low pH playing a role in metal toxicity and high pH playing a role in ionic stress), **Sulfate** and/or **Conductivity** (both considered ionic stressors), and **Other** stressors. **Please check Other if the site is located 1-2 miles downstream of any impoundment (*e.g.*, lakes, agriculture or mining ponds, flood control dams, beaver dams, low water ford/bridge dams) or a valley fill (mining or road) structures. Be sure to include type of structure (with type of impoundment release), distance upstream to the structure, number and size of tributaries in between that may alter the water chemistry (including dilution effects), and size of impoundment in m x m.**

EXTRA SPACE FOR SPILL-OVER COMMENTS AND NOTES BELOW. ***See Figure 18 below***. When using this space, please indicate from which section of the form this is a continuation. For example, “More Sediment Notes” or “More Stream Reach Activities & Disturbances Notes” will allow the data entry person to associate this to the

appropriate subform in the database. Also be sure to indicate that there are additional notes here under the appropriate section (*e.g.*, "More Notes on Page 7")."

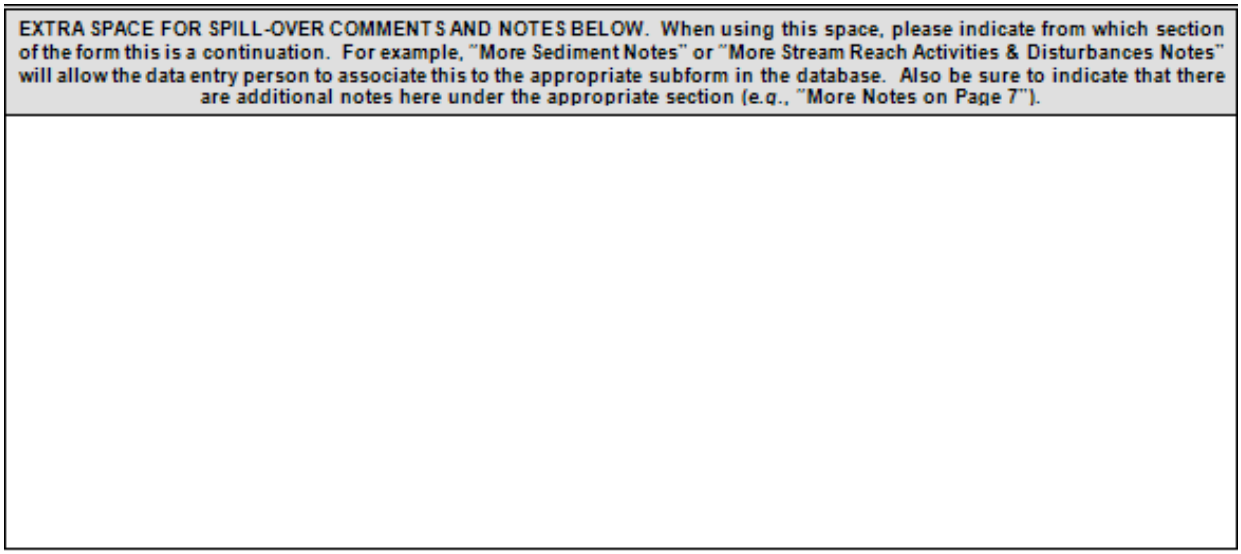


Figure 18. Example of the Extra Space for Comments and Notes section (Bottom of Page 7) of the field form

PAGE 8

Wildlife & Freshwater Mussel Observations

Note actual wildlife or plants observed or indications of their presence (*e.g.*, minnows are common, kingfisher observed, frog observed, etc.). **List any organisms/wildlife that were observed at the sample site that may be of interest. Any organisms observed and put into the Benthic Sample Jar should be noted on page 9 under Benthic Sample Notes. PLEASE NOTE ANY NON-TROUT FISH OR SALAMANDERS RELEASED FROM THE BENTHIC SAMPLE HERE! ALL TROUT SHOULD BE NOTED ONLY IN THE SECTION BELOW. REMEMBER TO DOCUMENT ANY SNAILS COLLECTED FOR DNR HERE!** ***See Figure 19 below for an example of this section of the form.***

Common Name: The common name of the organism observed.

Genus/Species: The genus or species of the organism observed.

Comments: Specific notes concerning the organism or evidence of organism observed.

Number Observed: The number of individuals of that organism observed.

Observed: The initials of the observer.

Reviewers Initials		WILDLIFE OBSERVATIONS >>>		
List organisms/wildlife that you observed at the sample site. Any organisms observed and put into the Benthic Sample Jar should be noted on page 9 under Benthic Sample Notes. PLEASE NOTE ANY NON-TROUT FISH OR SALAMANDERS RELEASED FROM THE BENTHIC SAMPLE HERE! ALL TROUT SHOULD BE NOTED ONLY IN THE SECTION BELOW! REMEMBER TO DOCUMENT ANY SNAILS COLLECTED FOR DNR HERE!				
Common Name	*Genus Species*	*Comments*	*Number Observed*	*Observer*
Did you see freshwater mussels?	☐ Yes ☐ No	☐ Alive ☐ Dead	Did you collect dead shells?	☐ Yes ☐ No

Figure 19. Example of the Wildlife & Freshwater Mussel Observations section (Top of Page 8) of the field form

<u>Did you see fresh water mussels?:</u> Answer **<u>Yes</u>** or **<u>No</u>**.

<u>Alive or Dead?:</u> Answer **<u>Alive</u>** or **<u>Dead</u>**.

<u>Did you collect dead shells?:</u> Answer **<u>Yes</u>** or **<u>No</u>**. Dead shells are submitted to Doug Wood for identification or further identification by WVDNR.

Trout Observations (For Sites that are <u>not actively being sampled</u> for Fish!)

This section is provided to help WVDEP obtain more information about the status and location of trout populations in the state. From this information, WVDEP can more effectively determine which streams would be good candidates for intensive surveying and monitoring of trout populations and ultimately if the stream qualifies for Trout Stream Status and Protection. This information will also be shared with the WVDNR

(West Virginia Division of Natural Resources) which is charged with the management of all fish in West Virginia. **Note that this section is only for documenting passive observations of trout (*i.e.*, casual sightings, trout that were inadvertently caught in the benthic net, etc.) and not for use with any sort of active fish sampling activities (*i.e.*, electrofishing, netting, etc.). All data from active fish sampling activities are documented via a different protocol and set of forms. *See Figure 20 below for an example of this section of the form.***

Trout Observations (For Sites that are not actively being sampled for Fish!)					
Did you see trout? ☐ Yes ☐ No	Comments				
Observation Method (i.e., Benthic Net, Free Swimming, Rod & Reel)	Species ID (i.e., Brook Trout, Brown Trout, or Rainbow Trout)	Count	Size (cm)	Notes (e.g., YOY, DELTs)	Photo #'s (Enter Photo Details on Page 10)

Figure 20. Example of the Trout Observations (Non-Fish Sites) section (Bottom of Page 8) of the field form

Did you see any trout?: Answer **Yes** or **No**. Do not answer if you were not positive you saw a trout.

Comments: Comments regarding what was or not observed. Would you expect to see trout at this stream reach based on the habitat and water quality information available?

Observation Method: How was the trout observed? Was it passively caught in the benthic net during a kick? Was it observed freely swimming in the stream? Was it caught by an angler in the stream reach?

Species ID: List the Common Name of the trout species (*i.e.*, Brook Trout, Brown Trout, Rainbow Trout, or Cutthroat Trout).

Count: The number of specimens of each trout species observed.

Size (CM): The size of the trout specimens in centimeters.

Notes: Notes about the trout specimens (*e.g.*, reproductive or life stage, DELTs (Deformities, Erosions, Lesions, or Tumors).

Photo #'s: Any photo numbers associated with the trout specimens. If the identification of the trout specimens is unsure, a photo may be a means of identifying the specimens at a later date. ***NOTE: All photo description information should still be entered on PAGE 10-Photography Log (see below). This field just links a photo to an individual or group of specimens.***

PAGE 9

Benthic Macroinvertebrate Collection Information

BENTHIC MACROINVERTEBRATE & PERIPHYTON COLLECTION INFO>>>>>>>>>>>>>>>>				Reviewers Initials	
AN-Code			Date		
Benthic sample collected?	☐ Yes ☐ No	If no, why?			
Benthic collection device	☐ Kicknet ☐ D-net ☐ Hand	Benthic habitat type & #	*Riffles*		*Runs*
Benthic sample comparability	Was benthic sample comparable with respect to riffle/run depth and velocity?			☐ Yes	☐ No
Is there evidence that the stream channel was scoured by recent flooding or high flows?				☐ Yes	☐ No
Is it possible that sample areas were dry or partially dry for an extended period before sample was taken?				☐ Yes	☐ No
Is there evidence that the stream is "wet-weather" and flowing only in response to recent rainfall?				☐ Yes	☐ No
Benthic kick area depths	m	m	m		m
Use the space below to describe the site and explain responses to the previous questions. What organisms were put in the jar? PLEASE NOTE ANY FISH, TROUT, OR SALAMANDERS RELEASED FROM THE BENTHIC SAMPLE ON PAGE 8!!!!					

Figure 21. Example of the Benthic Macroinvertebrate Collection Information section (Top of Page 9) of the field form

Benthic Sample Collected?: Answer **Yes** or **No**.

If no, why?: Provide reason why benthic sample was not collected.

Benthic collection device: Indicate which device was used to collect benthic macroinvertebrate samples (bugs). ***See Chapter V. Section A. Benthic Macroinvertebrate Sampling starting on page 154 for a more detailed description of each device and its applicability.*** Describe any deviations from the protocols below. 1) **Kicknet** (*i.e.*, Rectangular frame dip-net), 2) **D-net**, or 3) **Hand** (*i.e.*, Hand pick). **Note: Hand-pick methodology is not a comparable method and should only be used if indicated as an alternative on the stream list.**

Habitat Sampled and # of Each: ***See Chapter V. Section A. Benthic Macroinvertebrate Sampling starting on page 154 for a more detailed description***. 1) **Riffle**, 2) **Run**, 3) **Woody snags (MACS)**, 4) **Vegetated banks (MACS)**, 5) **Aquatic plants (MACS)**.

Benthic Sample Comparability: Was benthic sample comparable with respect to riffle/run depth and velocity?: Answer **Yes** or **No**. Sampling should generally occur only if the depth is at least 0.05 m deep and has enough velocity to push debris into the net.

Evidence of scouring?: Answer **Yes** or **No**. Consider asking locals, look at new or recently deposited materials on banks, consider recent precipitation and flood events for the area.

Evidence of dry conditions?: Answer **Yes** or **No**. Look for indications that the stream was dry or partially dry recently). Consider asking locals, past weather conditions, benthic macroinvertebrate density and diversity, and stream conditions while you are there.

Evidence of wet-weather stream?: Answer **Yes** or **No**. Consider asking locals, look for dirt channel, vegetation and roots in channel growing across the stream, jagged rocks in the stream, no easily definable U-shaped channel, over abundance of leaves in the stream for the season. Consider watershed area, consider benthic density, diversity, and community composition while collecting sample.

Kick Area Depths (m): Record the measured depth of water at each kick sample location (usually four locations).

A blank space is provided to describe the site and explain responses to the previous questions regarding the benthic sample comparability. Also, any organisms observed **in the sample** should be recorded here. ***Please note any fish, trout, or salamanders released from the benthic sample on PAGE 8-Wildlife & Freshwater Mussel Observations!!!!***

Benthic Substrate Sample Composition

Inorganic Substrate Components: ***Using Figure 22 below as a guide***, provide a visual estimate of the relative proportion of each of the seven particle types listed. **This assessment should be conducted only within the actual benthic collection area and should be done by the Biomorph**. Estimate the proportion of each substrate type within the $1m^2$ riffle/run area that was sampled using the following scale:

Low gradient (MACS) streams will require a visual estimate of the entire 100 m assessment area.

Inorganic Substrate (1 m² Of Kicked Substrate)	Class Codes	Size Class	% Composition
Bedrock	BR	Smooth surface rock/hardpan (>4000 mm – bigger than a car)	%
Boulder (BL)	BL	Basketball to car (>250-4000 mm)	%
Cobble (CB)	CB	Tennis ball to basketball (>64-250 mm)	%
Coarse Gravel (CG)	CG	Marble to tennis ball (>16-64 mm)	%
Fine Gravel (FG)	FG	Ladybug to marble (>2-16 mm)	%
Sand (SA)	SA	Gritty – up to ladybug (>0.06-2 mm)	%
Silt & Fines (ST)	ST	Fine – not gritty (<0.06 mm)	%
Clay (CL)	CL	Slick/ hard clay or hard-pan clay	%
Enter estimated % composition for each substrate type. ****MACS SITES: estimate over entire 100 meter stream reach.****			
Describe the benthic sampling substrate quality in terms of relative sizes (e.g., small-sized vs. large-sized cobble or boulders), shapes (globular vs. flat vs. angular), texture (e.g., rough vs. smooth bedrock), layering (i.e., was the cobble stacked) and embeddedness (embedded by pea gravel vs. sand/silt). Also mention any unusual substrate features (e.g., trash or unnatural substrate that was sampled as substrate) and provide general comments about the benthic sample substrate.			

Figure 22. Example of the Benthic Substrate Sample Composition section (Middle of Page 9) of the field form

Describe Quality of Benthic Substrate: Describe the benthic sampling substrate quality in terms of relative sizes (*e.g.*, small-sized vs. large-sized cobble or boulders), shapes (globular vs. flat vs. angular), texture (*e.g.*, rough vs. smooth bedrock), layering (*i.e.*, was the cobble stacked) and embeddedness (embedded by pea gravel vs. sand/silt). Also mention any unusual substrate features (*e.g.*, trash or unnatural substrate that was sampled as substrate) and provide general comments about the benthic sample substrate. Note outstanding features like “nice stacked flat medium-sized cobble”, “very sandy with lots of fine gravel”, “large–sized boulders with a some coarse gravel here and there”, “large amounts of partially broken down leaf packs among the cobble”, “embedded with pea gravel rather than sand”, “lots of rough, fissured bedrock”. Indicate if you think the benthic sample substrate is stable and capable of maintaining benthic populations.

Visual Estimation of Periphyton and Aquatic Plant Density

Indicate Abundance of each: Periphyton (Brown-slick; Diatoms), Filamentous Algae (green), Aquatic Vascular Plants, Aquatic Moss : Indicate the abundance of periphyton, algae, aquatic plants, and “aquatic” mosses in the stream assessment area as **0-None, 1- Low, 2- Moderate, 3-High, 4-Extreme, and NR-Not Rated.** ***See Figure 23 below for an example of this section of the form.***

Indicate abundance of each: 0=None, 1=Low, 2=Moderate, 3=High, 4=Extreme, NR=Not Rated	Periphyton (*Brown-slick; Diatoms*)		Filamentous Algae (Green)		Aquatic Vascular Plants		Aquatic Mosses	
Periphyton/Algae/Aquatic Plants & Mosses Notes:								

Figure 23. Example of the Visual Estimation of Periphyton & Aquatic Plant Density section (Middle of Page 9) of the field form

Periphyton is algae, diatoms, fungi, bacteria, protozoa, and associated organic matter associated with stream channel substrates. They are useful indicators of water quality because they respond rapidly and are sensitive to a number of human disturbances, including habitat destruction, contamination by nutrients, metals, herbicides, and acids. In this section of the WAB assessment, periphyton will include only the microalgae. These are the microscopic organisms that make the substrate slick and slimy. They usually leave a brownish-yellow stain on your hand when rubbed.

Although generally included in the broad class of periphyton (microalgae), filamentous algae (macroalgae) will be considered separately in this section. Filamentous algae include the long stringy types that are green in coloration and exhibit wavy undulations in stream currents. Note: during Periphyton collection, both the microalage and Filamentous Algae are collected (***see Chapter VII. PERIPHYTON COLLECTION PROTOCOLS starting on page 201***).

Aquatic plants are generally associated with larger streams such as the New River and Cacapon River. Riverweed is an example that would be included in the aquatic plant category.

Aquatic mosses are those mosses found growing naturally in the water. They should not be confused with terrestrial mosses that are growing near the stream or under the water level in a stream that is typically dry for extended periods (Note: that terrestrial mosses can be a good indicator of stream intermittency as well as an excellent benthic macroinvertebrate habitat). True aquatic mosses are much darker and look like they have a different texture compared to terrestrial mosses.

Periphyton Collection Information

Periphyton sample collected?	☐ Yes ☐ No	If no, why?						
Periphyton Habitat & #	*Riffles*		*Runs*		Shade and number of each	*Fully exposed (0-25%)*		*Partly shaded (25-50%)*	
						Fully shaded (75-100%)		*Partly exposed (50-75%)*	
Periphyton sample comparability	Was periphyton sample comparable? *(Consider questions above about benthic comparability)*						☐ Yes ☐ No	
Use the space below to describe the Periphyton sample. Explain any variances from the collection protocol that may affect comparability. Was the substrate stable and undisturbed? Could the substrate have been scoured? Dry?								

Figure 24. Example of the Periphyton Collection Information section (Bottom of Page 9) of the field form

Periphyton Sample Collected?: Answer **Yes** or **No**.

If no, why?: Provide reason why periphyton sample was not collected.

Periphyton Habitat and #: Record the number of rocks selected from riffles and from runs during periphyton collection.

Shade and number of each: Record the number of rocks selected from the various shade categories during periphyton collection: **Fully Exposed (0-25%)**, **Partly Shaded (25-50%)**, **Partly Exposed (50-75%)**, **Fully Shaded (75-100%)**. Example: 2 in Fully Exposed, 1 in Fully Shaded, and 2 in Partly Shaded. The shading ratings are estimates of the amount of shade (or conversely sunlight) at the stream site on the day of sampling throughout the duration of the day.

Periphyton Sample Comparability: Was periphyton sample comparable? (Consider questions above about benthic comparability): Answer **Yes** or **No**.

Periphyton Sample Notes: Use the space below to describe the Periphyton sample. Explain any variances from the collection protocol that may affect comparability. Was the substrate stable and undisturbed? Could the substrate have been scoured? Dry?

PAGE 10

Landowner/Stakeholder Information

If a landowner or stakeholder encountered during the sampling event you can keep track of contact information here by recording name address and/or phone numbers. ***See Figure 25 below for an example of this section of the form.***

> ⇒ **Note: If a landowner/stakeholder is interested in getting information about the stream, you must fill out a Landowner Data Request Card. This card has two portions, one on which you write down the mailing/email information and turn in with the paperwork to the map coordinator, and one on which you write down some of the instantaneous readings (*i.e.*, Sonde readings) and Total RBP score and give to the landowner/stakeholder before leaving the site. The cards were designed to speed up the process of returning information to the landowners**.

Name: Name or names of the landowner/stakeholder(s) or company that own, use, or manages the land.

Stream Data Requested?: Were the results from this sample requested by the landowner? Check **Yes** or **No**. Again, checking this box will not ensure prompt delivery of the stream data, so also use the **Landowner Data Request Card.**

Address: Mailing address of the landowner.

Reviewers Initials		*LANDOWNER/STAKEHOLDER INFORMATION & PHOTOS >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>*	
Name		Stream Data Requested	[ ] Yes [ ] No
		WS Report Requested	[ ] Yes [ ] No
Address		Phone:	( )
		ALT #:	( )
Other Pertinent Landowner Information And Comments (alternate landowner or addresses, etc.). Discuss the accessibility to the site including posted property, fenced, beside road, long walk over treacherous terrain, 4 x 4 needed, get key from landowner, etc.			
Check all that apply:	☐ Easy Access ☐ Difficult Access ☐ Private Property ☐ Posted ☐ Fenced ☐ Gated ☐ Get Key from Landowner ☐ Beside Road ☐ Short Hike ☐ Long Hike ☐ 4x4 Needed ☐ Other (explain)		

Figure 25. Example of the Landowner/Stakeholder Information section (Top of Page 10) of the field form

Watershed Report Requested?: Was a future watershed report from the watershed being sampled requested by the landowner? Check **Yes** or **No**.

Phone: The primary phone number of the landowner.

Alt #: A secondary phone number of the landowner (cell phone or work phone).

Site Accessibility: A set of check boxes is provided to give a quick indication of what may be involved in getting to the site. Check all that apply. These boxes include: **Easy Access**, **Difficult Access**, **Private Property**, **Posted**, **Fenced**, **Gated**, **Get Key from Landowner**, **Beside Road**, **Short Hike**, **Long Hike**, **4x4 Needed**, **Boat Ramp**, or **Other (explain)**.

Landowner and Accessibility Notes: A blank field is provided to discuss how to find the landowner and the accessibility of the site including elaborations on the Site Accessibility check boxes discussed above. This location is also be a good place to keep track of people you talked to while trying to track down the landowner. In the case of a mistake landowner identity, this chain of information will help alleviate any misunderstandings between the field crew and true landowner, who is usually very angry that you did not talk to them to get access to the stream. ***Any information about the watershed that may affect the stream water quality or sampling should be recorded on the bottom left of PAGE 2 under the Comment Box describing the source of the information as "Landowner".***

Photography Log

A more detailed description of the photography process can be found in ***Chapter II. Section B. Part 2. Photographic Documentation starting on page 25***. ***See Figure 26 below for an example of this section of the form.***

Photography Log >>>>>>>>>>>>>>>>>		Camera Type		Camera Number	
#	Photo ID (office)	Disk Photo # (field)	Stream Name and/or AN-Code	Photo Description (Use Key Words)	Date	Photographer
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

Figure 26. Example of the Photography Log section (Bottom of Page 10) of the field form

Camera Type: The type of camera used (*e.g.,* Canon, Olympus, or Sony).

Camera Number: The assigned number of the camera used. This is usually marked on the camera with a black sharpie. **Do not confuse this with the jeep number often marked on the camera in white ink.** If for some reason the camera's instrument identification number is not apparent, then write down the Manufacturer's Serial Number

on the instrument so that the proper identification number can be tracked down later and remarked onto the camera.

Photo ID # (Office): Obtained in the office after getting a unique identification number from the WABbase.

Disk-Photo # (Field): Each camera assigns these unique file names to photos in series from 0-99999 in a format associated with some letters (*e.g.*, a photo will have a file name of DSV-00456). Write down the number portion of the file name on the form. **Do not confuse this number with the photo count numbers on the cameras that indicate how many photos have been taken or can be taken, which reset once photos are removed or deleted from the camera.** In addition, it is important to note that how the photos are removed from the camera may change this file name. ***This is required for all photos taken!***

Stream Name and or AN-Code: The name of the stream featured in the photo. ***This is only required if the photo was not taken at a sample site. If a lake or other waterbody is sampled, use this space to put in this space.*** If known, write down the AN-Code of the waterbody featured in the photo.

Photo Description: A description of the photo as it relates to the stream (*e.g.,* looking upstream from X-site) and the keyword features that may be found in the photo (*e.g.,* AMD, eroded bank, channelization, an optimal score for bank vegetative protection, a poor score for sediment deposition, etc.). ***This is required for all photos taken!***

Date: The date the photo was taken. ***This is only required if the photo was not taken on the same date as the sample or if it is not at a sample site.***

Photographer: The person who took the photo. ***This is required for all photos taken!***

Part 2. APPENDIX FORMS

In addition to the main form, there are several appendix forms that cover observations and parameter sets that are not as commonly used. When needed, these additional appendix forms should be attached to the main form upon completion of sampling.

APPENDIX #1 - Stream Discharge (Flow)

⇒ **This appendix form is used whenever a flow measurement is required during sampling (Mainly TMDL sites and Special Surveys or Projects, but also at some Wadeable Monitoring Sites). Be sure to fill out the AN-Code, Date, and Reviewer Initials just like the front of all form pages so that it can later be attached to the appropriate form by the map coordinator.**

This area is provided to record measurement made with a flow meter and the resulting CFS (cubic feet per second). Record the Flow Meter I.D., measurer and the time of measurement. ***Instructions for determining stream discharge (flow) are presented in Chapter IV. STREAM FLOW MEASUREMENT. See Figure 27 below for an example of this section of the form.***

Measurer: Record the flow measurer.

Time: The time of the flow measurement.

Flow Meter I.D.: The assigned number of the flow meter used. **Do not confuse this with the jeep number often marked on the flow meter in white ink.** If for some reason the flow meters' instrument identification number is not apparent, then write down the WV Property Tag number (found on a blue tag) or Manufacturer's Serial Number on the instrument so that the proper identification number can be tracked down later and remarked onto the flow meter.

Distance: Record distance from one bank along the flow transect (measuring tape) where the measurement is occurring.

Depth: Record the depth at the point of the flow measurement.

Velocity: Record the velocity at the point of the flow measurement.

Measurement Notes: Any measurement specific notes (*e.g.*, a negative reading)

Final Discharge Reading (cfs): Record the total stream discharge by entering in the Distance, Depth, and Velocity data from each increment into the Flow Spreadsheet or record the reading from a gage.

FLOWMEASUREMENTS>>				Reviewers Initials	
AN-Code				Date	
Stream Discharge Measurement (Calculated In Cubic Feet Per Second – cfs)					
Measurer		Time		Flow Meter ID	
Location	Distance	Depth	Velocity	Measurement Notes	
Bank					
EEF					
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					
31					
32					
33					
34					
35					
EEF					
Bank					
Final Discharge Reading (cfs) =					
Do you think that this flow measurement is comparable?	☐Yes ☐No	If Not, Why?			

U.S.G.S. Gage Name:		U.S.G.S. Gage Number:		Time:		Gage Height or Control:	

Appendix WVDEP WAB Flow Form (4/23/2010)

Figure 27. Example of the Flow Measurements Appendix field form

Do you think that this flow measurement is comparable?: Answer **Yes** or **No**. Do you think that there were enough unusual circumstances that would make you want to consider the flow measurement not comparable (*e.g.*, too many shallow measurements below 0.1 ft depth, too many changes in the direction of flow vectors across the transect, etc.).

If not, why?: Why it is believed the flow measurement is considered not comparable.

USGS Gage Name: The name (usually the name of the closest town) of the USGS gage queried for flow data.

USGS Gage Number: The ID number of the USGS gage queried for flow data.

Time: The time the gage was read for the flow measurement.

Gage Height or Control: The Height of the water on the USGS Gage.

APPENDIX #2 - Stream Bank Erodibility and Channel Profile Measurements

⇒ **This appendix form is used whenever information about stream erodibility and channel profile is needed. It is mostly used in cases where changes can be tracked thru time (*e.g.*, at Long Term Monitoring Sites once per visit) or when additional information about sediment potential from erosion is required (*i.e.*, at TMDL sites once during all 12 visits). Be sure to fill out the AN-Code, Date, and Reviewer Initials just like the front of all form pages so that it can later be attached to the appropriate form by the map coordinator.**

Stream Bank Erodibility Factors

Bank erosion potential is determined by using the diagrams and descriptions provided (***see Figure 28 below***) to evaluate the conditions of the stream banks within your reach. Score (1-3 scale) the various factors that have a role in bank erosion **for each bank (left and right descending banks)**. Choose the illustration and descriptions that most closely matches what you see. Compare your selection with to the scale (Increasing numbers mean increasing erodibility; lower scores indicate better conditions) to determine the proper category. All measurements are broad generalizations about both banks in the 100m reach. These scores will be combined to calculate a Stream Bank Erodibility Index.

Do not attempt to rate these factors in atypical sections of the stream. You should record the most dominant bank condition by mentally averaging the bank condition for the reach.

Bankfull Height: Score the overall ratio of the Bankfull Depth vs. the Bank Height

1-High=Bankfull indicators very common throughout the reach; their elevations are mostly at or near the top of the bank; stream has access to its floodplain during high water and bankfull flow events as shown by leaf lines or debris in the floodplain.

2-Medium=Bankfull indicators somewhat common along portions of the reach; their elevations are usually below the top of the bank and more commonly at the middle or lower portions of the bank; channel may be somewhat incised.

3-Low=Bankfull indicators very infrequent throughout the reach; if observed, their elevations are in the middle and lower portions of the bank; channel is usually deeply incised.

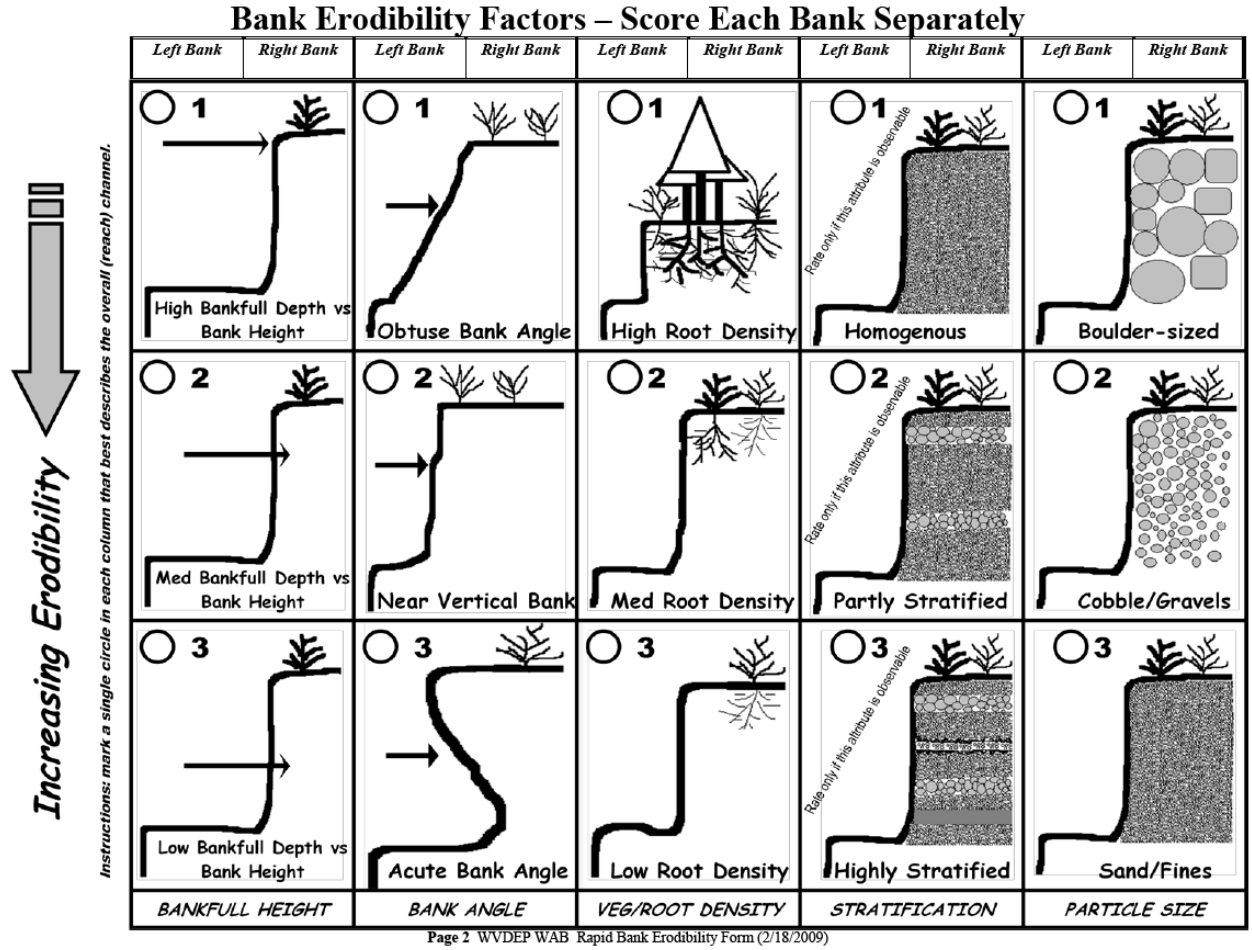


Figure 28. Example of the Stream Bank Erodibility Appendix field form

Bank Angle: Score the overall angle of the banks. Note that undercuts should be considered for their erosion potential. Many undercuts are shallow enough and associated with heavy root balls so that their erosion potential is minimal.

1-Obtuse=Banks have a slight to moderate angle throughout most of the reach; may have some areas of erosion (< 30%) but mostly the reach shows little sign of disturbance.

2-Near Vertical=Banks have a moderate to steep slope throughout much of the reach; some erosion is occurring (30-60%) within the reach. Note: some banks are often steep but very stable especially is covered by hard surfaces or vegetation.

3-Acute=Banks have a steep angle or are undercut to the extent that potential for sloughing is very high) throughout much of the reach (> 60%); there are obvious signs of erosions such as bare soils, exposed roots, etc. along with

many depositional features (point bars, islands, lateral bars, etc.) in the channel.

Veg/Root Density: Score the overall root density in and on the banks

1-High=More than 90% of the banks are covered by natural undisturbed vegetation (all layers are well represented); most roots systems probably extend to the lower portions of the bank.

2-Medium=60-90% of the banks are covered by natural vegetation (most layers represented but some may be absent); some disturbances such as mowed areas, pastures, trails, etc. are evident; most root systems probably extend to the lower or middle sections of the bank.

3-Low=<60% of the banks covered by natural vegetation (only one or two layers represented but most are missing); areas of disturbance very obvious throughout most of the reach or non-native species dominate.

Stratification: Score the overall stratification of the bank's materials (*i.e.*, layering). This factor is only rated if the bank is exposed and can be observed

1-Homogenous=Where visible, banks have an almost uniform composition with no apparent layering.

2-Partly Stratified=Where visible, banks have some level of distinct layering into differing size classes.

3-Highly Stratified=Where visible, banks have extremely obvious alternating layers of size class particles.

Particle Size: Score the overall particle size of the bank

1-Boulder=Banks consist primarily of large sized materials (large cobble and boulder); smaller materials may be present but these can be seen only at the tops of the banks or on floodplain or terrace surfaces.

2-Cobble/Gravel=Banks consist primarily of a mix of materials from large to smaller sizes (cobble to fine gravel); some sand may be intermixed but it usually makes up < 20%.

3-Sand/Fines=Banks are primarily made up of small materials (mostly fine gravel and sand); silts and clay may be present.

Estimated Channel Profile (Width to Depth Ratio)

Widths to depth ratios (W/D) are defined as the ratio of the bankfull surface width to the mean depth of the bankfull channel. W/D is a key measurement in understanding the energy dynamics within a stream channel. If a stream has a high W/D (*i.e.*, a really wide stream that is shallow), the distribution of energy within the channel is such that the stress is placed near the banks. As W/D increases, hydraulic stress against the bank increases and erosion will accelerate making the stream wider in respect to its depth. In turn, the erosion increases the sediment supply to the stream. Since the stream is overly wide and shallow, it does not have enough power to move the excess

sediment out and sediment deposition occurs in its center. This in turn reduces its depth, thus increasing the W/D and creating a feedback loop.

Using the diagrams provided on the form for guidance, measure the estimated Bankfull Width and Depth (*i.e*., Height) and the estimated Channel Width and Depth of the stream reach (**see Figure 29 below**).

Bankfull is defined as the water level that is achieved by moderate-sized flood events that occur every one or two years. A bankfull event will fill a portion of the stream channel to a certain width and depth (or height). Look for a variety of bank characteristics to determine the extent of the bankfull event. First, determine the location of the active floodplain. Next, look for an obvious slope break in the banks that differentiates the channel from a flat floodplain higher than the channel. A transition zone often exists between exposed substrate and vegetation, which marks the bankfull height. Look for a change from water-loving and scour-tolerant vegetation to more drought-tolerant vegetation. Also, it may be determined by moss or vegetation growing on rocks along the banks. A change from well-sorted stream sediments to unsorted soil materials is also a good indicator. In addition, indicators from the previous season's flooding are may be used if there have been no recent large floods or prolonged droughts: the presence of drift material (*e.g.*, leaves, trash) along the bank or on overhanging branches from the previous seasons flooding, the level where deciduous leaf-fall is absent on the ground because it was swept into the stream by flooding since the last leaf-fall, and unvegetated sand, gravel or mud deposits from previous seasonal flooding.

The channel depth (*i.e*., height) can be determined by the vertical distance from the bottom of the channel up to the level of the first major valley depositional surface that the stream channel would spill into during a greater than bankfull flooding event. This measure is an indicator of the degree of incision or downcutting of the stream below the general level of its valley. The channel width is how wide the stream channel is when it begins to spill out into the flood plain.

In cases where the channel is cutting a valley sideslope and has oversteepened and destabilized that slope, the bare "cutbank" against the steep hillside is not necessarily an indication of recent incision. In such a case, the opposite bank may be lower and a more obvious indicator of bankfull and channel heights and widths.

In streams in deep V-shaped valleys, the difference between the bankfull and channel depth may be indistinguishable due to a lack of stream incision. **Remember that the channel depth may be equal to the bankfull depth, (an indication that the stream channel is not incised or downcutting) but it should <u>never</u> be less than the bankfull height.**

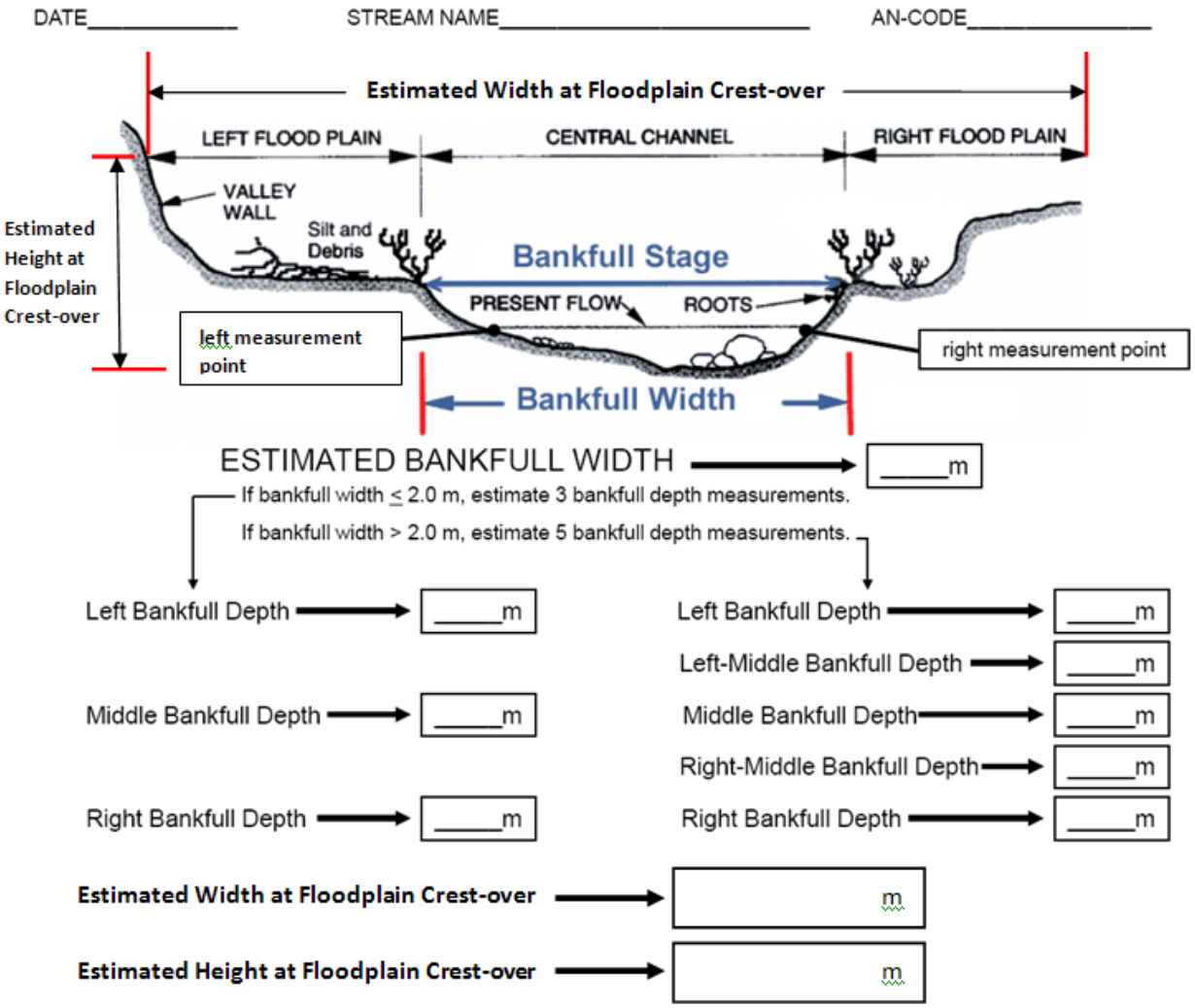


Figure 29. Example of the Channel Profile Measurements Appendix Field Form

All height and width measurements are in meters (tenths) and should be conducted in an area that is representative of the overall reach condition (*i.e.*, do not pick the one excessively wide or narrow section of the reach for these measurements). These estimates will assist in sediment load modeling.

Note: Do not confuse Bankfull Depth and Bankfull Height (a measure used in Relative Bed Stability classification). The Bankfull Depth=Bankfull Height + the Stream Depth at the observation location. In this instance, we are including the depth below the water surface in the bankfull estimates.

Estimated Bankfull Width: Measure the estimated bankfull width for the reach in meters.

Estimated Bankfull Depth:

If the Estimated Bankfull Width is <=2.0 meters, then estimate 3 bankfull depth measurements at the following locations:

1) Left Bankfull Depth: Measure the estimated Bankfull Depth in meters at the left (descending) edge of the wetted stream channel.
2) Middle Bankfull Depth: Measure the estimated Bankfull Depth in meters at the mid-point of the wetted stream channel.
3) Right Bankfull Depth: Measure the estimated Bankfull Depth in meters at the right (descending) edge of the wetted stream channel.

If the Estimated Bankfull Width is >2.0 meters, then estimate 5 bankfull depth measurements.

1) Left Bankfull Depth: Measure the estimated Bankfull Depth in meters at the left (descending) edge of the wetted stream channel.
2) Left-Middle Bankfull Depth: Measure the estimated Bankfull Depth in meters at the midpoint between the left (descending) edge of the wetted stream and the middle of the wetted stream channel.
3) Middle Bankfull Depth: Measure the estimated Bankfull Depth in meters at the mid-point of the wetted stream channel.
4) Right-Middle Bankfull Depth: Measure the estimated Bankfull Depth in meters at the midpoint between the right (descending) edge of the wetted stream and the middle of the wetted stream channel.
5) Right Bankfull Depth: Measure the estimated Bankfull Depth in meters at the right (descending) edge of the wetted stream channel.

Estimated Channel Height: Measure the estimated channel height for the reach in meters.

Estimated Channel Width: Measure the estimated channel width for the reach in meters.

APPENDIX #3 – TMDL/Wadeable Benthic Appendix Form

⇒ **This appendix form is used whenever a benthic survey is concurrently with a TMDL sampling event. There are just a few parameters that are rated at a TMDL site that are not covered on the Wadeable Benthic Form. Be sure to fill out the AN-Code, Date, and Reviewer Initials just like the front of all form pages so that it can later be attached to the appropriate form by the map coordinator.** ***See Figure 30 below for an example of this section of the form.***

Sketch of Assessment Reach and Comments: Indicate North with (↑), indicate flow direction, indicate water sample (wq), indicate lat and long site with (X). Draw the sketch with a coarse resolution to give an overall idea of the sample area beyond the typical 100m reach. **You only need to do this sketch if you are conducting a TMDL-Initial assessment concurrently with a Wadeable Benthic Assessment.** ***See Chapter II. Section C. Part 1. PAGE 1-Site Verification on page 30 to contrast the needs of this coarse resolution sketch versus the detailed sketch for the Wadeable Benthic Assessment form.***

Stream Debris

Dead Fish: Indicate the abundance of dead fish in and near the stream assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

Garbage: Indicate the abundance of garbage in and near the stream assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**. Be sure to consider all garbage than may be moved into the channel during high flows/flooding.

Gas Bubbles: Indicate the abundance of gas bubbles in the stream in the assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

Ice Cover: Indicate the abundance of ice cover on the stream in the assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

Oil-Grease: Indicate the abundance of oil or grease in the stream in the assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

Sewage: Indicate the abundance of sewage in the stream assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

Sludge: Indicate the abundance of sludge in the stream assessment area as **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated**.

TMDL-Wadeable Benthic Appendix Form

STREAM VERIFICATION >>>		Reviewers Initials	
AN-Code		Date	

Sketch of Assessment Reach and Comments: Indicate North with (↑), indicate flow direction, indicate water sample (wq), indicate lat and long site with (X). Draw the sketch with a coarse resolution to give an overall idea of the sample area beyond the typical 100m reach.

Stream Debris (0=None, 1=Low, 2=Moderate, 3=Heavy, 4=Extreme, NR-Not Rated)			
Dead Fish		Oil-Grease	
Garbage		Sewage	
Gas Bubbles		Sludge	
Ice Cover		Other/Notes	

WVDEP WAB TMDL-Wadeable Benthic Appendix Form (4/23/2010)

Figure 30. Example of the TMDL-Wadeable Benthic Appendix Field Form

<u>Other/Notes:</u> Indicate the abundance of any other notable Stream Debris (*e.g.*, recently fallen leaves) not mentioned as **<u>0</u>-None, <u>1</u>-Low, <u>2</u>-Moderate, <u>3</u>-High, <u>4</u>-Extreme, and <u>NR</u>-Not Rated**. Also make notes regarding any of the above.

APPENDIX #4 – Water Quality Profile

⇒ **This appendix form is used whenever a more than one water quality sample occurs during a single sampling event (*i.e.*, a water profile). Be sure to fill out the AN-Code, Date, and Reviewer Initials just like the front of all form pages so that it can later be attached to the appropriate form by the map coordinator. *See Figure 31 below for an example of this section of the form.***

WQ Profile Appendix Form

Reviewers Initials		ANCode		Date		SONDE PROFILE READINGS PART 1>>>										
Measurement	WQ Sample ID	Depth Description (e.g., Top, Middle Bottom, Thermocline, etc.)	Depth (in feet)	Distance Description (e.g., Left, Middle, Right)	Distance (in meters)	Reach Location (in meters)	Transect (e.g., A, B, C, etc.)	Time (Mandatory for each reading)	Temperature Flag	Temp (°C)	pH Flag	pH (S.U.)	Dissolved Oxygen Flag	Dis. Oxygen (mg/L)	Conductivity Flag	Specific Conduct (umhos/cm)	Measurement Notes
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	

Page 1 WVDEP WAB WQ Profile Appendix Form (5/13/2010)

Figure 31. Example of the WQ Profile Appendix Field Form

WQ Sample ID: This ID is unique and comes pre-printed on labels. It is used whenever a lab water sample is collected.

Depth Description: Record a general depth description (*e.g.*, Top, Middle, Bottom, Surface, Subsurface, etc.) of the water sample.

Depth: Record the depth of the water sample in feet.

Distance Description: Record a general distance description (*e.g.*, Left Bank, Middle, Right Bank, Left Channel, Right Channel, etc.) of the water sample.

Distance: Record the distance of the water sample from the left descending bank in meters.

Reach Location: Record the distance of the water sample relative to the X-site in meters.

Transect: Record the transect designation (*e.g*., A, B, C, D, etc.) of the water sample.

Time: Record the time the water sample was taken. This is mandatory for all water samples.

Temperature Flag: Record any temperature flags.

Temperature: Record the temperature measurement in ^{o}C.

pH Flag: Record any pH flags.

pH: Record the pH measurement in S.U.

Dissolved Oxygen Flag: Record any dissolved oxygen flags.

Dissolved Oxygen: Record the dissolved oxygen measurement in mg/L.

Specific Conductivity Flag: Record any specific conductivity flags.

Specific Conductivity: Record the dissolved specific conductivity in μmhos/cm.

Measurement Notes: Document any notes concerning the water quality measurements.

APPENDIX #5 – Substrate Characterization (Pebble Count) including Gradient

⇒ **This appendix form is used whenever a Substrate Characterization (or Pebble Count). This type of survey is very infrequent, but when it does occur, it will often accompany the Wadeable Benthic Form. Be sure to fill out the AN-Code, Date, and Reviewer Initials just like the front of all form pages so that it can later be attached to the appropriate form by the map coordinator.** ***See Figure 32 below for an example of this section of the form.***

This form is provided to record measurements made on the stream substrate and stream channel. Record the measurements in the spaces provided and make comments as necessary. ***See Chapter IX. RELATIVE BED STABILITY/SUBSTRATE CHARACTERIZATION PROTOCOLS (INCLUDING GRADIENT) for instructions on completing this section***.

Reach Length: Record the total reach length in meters (100m minimum to 500m maximum)

Measurer: Record the measurer's initials

Recorder: Record the recorder's initials

Gradient Method: Check the box corresponding to the gradient method used (**Water-Filled Tube** or **Hand-Level**)

Wetted Width: Record the wetted width in m for that transect

Left, Left Mid, Middle, Right Mid, and Right: Record the substrate classification scores for these locations on the transect using the scale in ***Figure 32 below.***

Thalweg: Record the thalweg depth in m for that transect

Bankfull Height: Record the bankfull height in m for that transect

Rise: Record the stream rise in m for the distance between transects

RELATIVE BED STABILITY (PEBBLE COUNT) >>>			Reviewers Initials	
AN-Code		Date		

Relative Bed Stability (Pebble Count)

Reach length	m	Measurer		Recorder		Gradient Method	☐ Water-filled tube	☐ Hand-level

Transect	Wetted Width (m)	Left	Left Mid	Middle	Right Mid	Right	Thalweg (m)	Bankfull Height (m)	Rise (m)
A									
B									
C									
D									
E									
F									
G									
H									
I									
J									
K									

Notes:

Substrate Classification

Class	Code	Size	Description
Bedrock	BR	>4000 mm	Bigger than car
Boulder	BL	>250-4000 mm	Basketball to car
Cobble	CB	>64-250 mm	Tennis ball to basketball
Coarse Gravel	CG	>16-64 mm	Marble to Tennis ball
Fine Gravel	FG	>2-16 mm	Ladybug to marble
Sand	SA	>0.06-2 mm	Gritty between fingers
Silt & Fines	ST	<0.06 mm	Smooth, not gritty (silt & muck)
Clay	CL	>4000 mm	Slick/ hard clay or hard-pan clay bottom
Leaves	LD	Regardless of size	Leaf packs
Wood	WD	Regardless of size	Root wads, snags, logs, sticks

Appendix WVDEP WAB Relative Bed Stability Form (4/12/2010)

Figure 32. Example of the Substrate Characterization (Pebble Count) including Gradient Appendix Field Form

Assessment Form Quality Assurance/Quality Control

During sampling, the team member who did not conduct the initial assessment performs an on-site review of every habitat assessment. The reviewer determines completeness and verifies that the information is correct through discussion with the other crew member. If the sampling team consists of one person, as is often the case during a TMDL assessment, the form is reviewed by the sampler for completeness before leaving the site. There is no need to submit a duplicate habitat form if working alone as you will be unable to duplicate habitat evaluations.

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Habitat data will be collected along with other activities at the designated duplicate WAB sites. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. Duplicate habitat sampling consists sampling the site by each individual as if no one else was there to help (*i.e.*, one person serves as both Biomorph and Geomorph). Sampling occurs in the usual fashion with the Geomorph doing the habitat assessment and the Biomorph collecting benthos. To duplicate, these individuals reverse roles while keeping their data and samples completely separate. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training. In addition the duplicate data is looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. WAB members will visit one or two stream sites and each person will complete a habitat assessment form at each site. The results of these evaluations will be compared and the group will discuss problems with variability. Retraining will be conducted, if major discrepancies are encountered. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in evaluating habitat data will be teamed up with the less

experienced to assure reinforcement of training and accurate results. This SOP document is also provided to all program personnel for review and use in the field.

Forms Used In the Watershed Assessment Branch

The forms used by the Watershed Assessment Branch (WAB) are available internally via the WVDEP computer network at:

Q:\ WATER RESOURCES\WAB\Forms\2010 Forms.zip

Chapter III. WATER COLLECTION PROTOCOLS

Section A. Water Quality Sondes: Calibration, Maintenance, & Use

Part 1. Sonde Calibration and Maintenance

The following procedures are a basic overview of YSI calibration for an YSI 600XL Sonde/650 MDS display combination and Hydrolab Quanta G. These instructions are meant as a quick reference guide to the steps involved in calibrating a sonde and do not supplant the manufacturers' operation manual. Consult the owner's manuals for specifics or information on configurations other than these and for details on maintenance and trouble-shooting. These procedures assume the user has a basic knowledge of the instrument.

These directions are not intended for first-time users. Individuals with no prior experience should calibrate with the assistance of an experienced user.

All calibration adjustments are documented in a permanent logbook. The date and time of calibration, name of the calibrator, the identification number of the unit, battery voltage and all adjustments/maintenance must be documented (***see example in Figure 33 below***).

Note: Rinsing the probe is a procedure that is frequently performed during calibration. To rinse the probe, install the calibration cup (which is the same as the storage cup on YSI and Quanta G sondes) and add about 1/2 cup of rinse solution, as specified in the directions (usually deionized water). Seal the open end of the calibration cup with the screw cap or rubber lid and shake the probe for 30 seconds. Discard rinse water and repeat according to directions.

All calibrations are performed with the probes in the pointing upward and at temperatures as close to room temperature as possible (25^{0}C). If calibration does not occur at room temperature (*i.e.*, a field calibration) then every attempt should be made to temperature adjust the calibration solutions according to the manufacturers' guidelines present below.

Sonde ID: _____ Date: _____ Time: _____ Calibrator's Initials: _____

Battery Check: _____ Field User's Initials (if different from Calibrator): _____

Temperature (°C): _____

Temperature should be recorded for all Calibrations (even single probe calibrations)!

DO Probe Calibration

Be sure to check the age of the DO probe. It should be no more than 2 years old for 600 XL YSIs (5-7 years for 556 YSIs).
Is the D.O. Membrane > 30 days old? If so, it needs to be changed regardless of the amount of use it has seen.
For those sondes that have it, is the D.O. charge <100? If not, then resurface the probe's electrodes with sandpaper.
Atmospheric Pressure (mm Hg): _____

Initial % Sat: _____ Final % Sat: _____

Initial DO (mg/L): _____ Final DO (mg/L): _____

Specific Conductivity Probe Calibration

1) *For best accuracy, Specific Conductivity should be calibrated according to the expectations of the streams/sites you will be sampling. It may be necessary to recalibrate between streams/sites due to extreme differences between streams/sites (e.g., 1000 vs. 10000 µmhos/cm).*
2) *Be sure to check the age of the Specific Conductivity probe. It should be no more than 3 years old.*

Conductivity Solution (µmhos/cm): _____

Initial Sp Cond (µmhos/cm): _____ Final Sp Cond (µmhos/cm): _____

Low-End Sp Cond Check (i.e., <5 µmhos/cm)
Solution: ☐ Deionized or ☐ Distilled Water Sp Cond (µmhos/cm): _____
Weekly Mid-Range Sp Cond Check (e.g., 500 or 1000 µmhos/cm)
Conductivity Solution (µmhos/cm): _____ Sp Cond (µmhos/cm): _____

pH Probe Calibration

1) *If the streams/sites you will be sampling are expected to fall within the same pH range (i.e., 4-7 vs. 7-10), pH should be calibrated using a two-point calibration for best possible accuracy. Should a stream site fall outside of the calibrated pH range, then recalibration is necessary. If expectations are unknown, use a three-point calibration.*
2) *Be sure to check the age of the pH probe. It should be no more than 12 to 18 months old.*

Initial pH (7): _____ Final pH (7): _____ mV (7): _____

The pH 7 mV should be in a range of +/- 30 mV. Between +/- 30-50 mV, the probe is still useable, but should be monitored closely for irregularities. After +/- 50mV, the probe should no longer be used.

Initial pH (10): _____ Final pH (10): _____ mV (10): _____

Initial pH (4): _____ Final pH (4): _____ mV (4): _____

Notes and Maintenance Performed: _____

_____

_____

_____

Figure 33. Example of Sonde Calibration Sheet

YSI 600XL Sonde/650 MDS Display Unit Calibration

These directions are very similar to the older Scout 2 Hydrolab and newer Quanta G directions. However, individuals with no prior experience should calibrate with the assistance of an experienced user.

1) YSI Display Unit

The YSI display unit uses a series of escapable menus in conjunction with several keys in the calibration process. Become familiar with the **Enter** key (which looks like a left arrow), escape, scroll, and alpha-numeric keys as these will be the most often used.

Maintenance of YSI Display Unit

The YSI display unit runs on a 4 alkaline C-cell battery system contained within the display unit. The battery power left is displayed on the screen.

Also of importance is the fact that the results of calibration for YSI units are stored in the sonde itself, not in the display unit. Switching the sonde and display units will not affect calibration. This may be especially helpful as one can calibrate several sondes with only one display unit as others may be recharging.

The display unit also features a Date/Time and an auto-shutoff function, which may be modified by selecting "System Setup" in the main menu and then selecting the appropriate function to modify.

2) Dissolved Oxygen

A) DO Probe Calibration

Note: With some of the newer sondes (2004-Present), you need to run the sonde just as if it was in the stream to get the initial or pre-calibration DO readings and then go thru the following steps to calibrate DO and get the post-calibration readings.

1. Remove the threaded lid to the calibration cup. Unlike the Hydrolab sondes, it is not necessary to dry the membrane on the D.O. probe by blotting it with a soft cloth or tissue, but rather only make sure that the membrane is **not inundated with water. In fact, YSI recommends against touching DO membranes when replacing or servicing them. There is a potential for oils or dirt to affect O_2 diffusion through the membrane.** Also, check the membrane for wrinkles, tears, bubbles, dirt, etc. and replace membrane, if necessary.

2. Reattach the calibration cup to the sonde and add no more than 1/8-inch of DI water. Try not pour water on the membrane, but if it does get wet, just make sure that the membrane is not totally inundated with water. Make certain that the DO and Temperature probes are not immersed in water.

3. Cover the calibration cup with the lid and engage only 1 or 2 threads. An alternative to cover the calibration cup with a moist paper towel, then place the lid on upside down on top of the cup with a small weight on top of the lid.

4. Turn on the unit and let sit for about 10 minutes so that the air inside the cup will saturate with water and come to thermal equilibrium.

   Note: If the sonde is from a newer manufacture year (2004-Present), you may need to do one-time adjustment of the sonde settings so that it will give you the initial or pre-calibration readings before continuing the calibration procedure. The manufacture year can be determined by reading the first two digits of the sonde's serial number (*e.g.*, 04=2004). If this is the case, this can be fixed by deactivating the Autosleep RS232 function in the following section of the menu: Sonde Menu > Advanced > Setup > Auto sleep RS232. Toggle the function off by pressing the enter button.

5. Turn on the unit and use the **Up** or **Down** keys to scroll to "Sonde Menu" and press **Enter**. Select "Calibrate" and press **Enter**.

6. Scroll to select "Dissolved Oxy" and press **Enter**.

7. Select "DO %", press **Enter**. One must keep in mind that this is actually calibrating based on O_2 air saturation, not water saturation.

8. Type in the Barometric Pressure displayed by the unit in the bottom right of the screen using the alpha-numeric pad; press **Enter**. Wait for both temperature and DO readings to stabilize; this may take up to 40 seconds (after waiting the initial 10 minutes for water vapor equilibration in the cup). The upper right of the screen should have the word "Calibrate". Record the initial or pre-calibration DO, temperature, and % air saturation in logbook. If the upper right of the screen has the word "Continue" instead of "Calibrate" then calibration has already occurred and the readings given are the final or post-calibration readings. This can be avoided for future calibrations by deactivating the Autosleep RS232 function in the following section of the menu: Sonde Menu > Advanced > Setup > Auto sleep RS232. Toggle the function off by pressing the enter button. This will permanently allow the initial or pre-calibration readings will be available prior to calibration.

9. Press **Enter** to finish calibration. Record the final or calibrated DO and % air saturation in log book.

10. The final % air saturation should be within the range of 98% air saturation at the lowest WV elevations to 83% at the highest WV elevations (+/– 2 %). The probe

should typically not read above 100% air saturation as this only occurs at sea level. A 100% reading may also be caused at low WV elevations by a high-pressure front or unusual weather in the area. Consult the attached sheet for air saturation values that should be found a different elevations or Appendix D (page 227) from the YSI operating manual (***see Table 6 below***). YSI probes may be calibrated at lower elevations and then brought to a higher elevations and still be accurate. However, calibration at an extreme elevation and transport to a lower elevation may require a recalibration at the lower elevation. If the barometer reading is extremely unusual for your local elevation, the internal barometer may require recalibration in the lab by a person familiar with that procedure.

11. The upper right of the screen will say "Continue". Press **Enter**. And it will take you back to the DO Calibration Menu.

Table 6. From Appendix D Table 2 of the YSI operating manual (page 227)

Pressure (mm Hg)	Altitude (ft)	Expected % Saturation (+/- 2 %)
760	0	100
752	278	99
745	558	98
737	841	97
730	1126	96
722	1413	95
714	1703	94
707	1995	93
699	2290	92
692	2587	91
684	2887	90
676	3190	89
669	3496	88
661	3804	87
654	4115	86
646	4430	85
638	4747	84
631	5067	83

Elevation at Harpers Ferry=249 ft and at Spruce Knob=4862 ft.

B) DO Probe Maintenance

The membrane on the DO probe should be examined for fouling and bubbles before calibration and during use. If the membrane is torn, dirty or wrinkled, or if there are bubbles under the membrane, the membrane must be replaced. YSI recommends the membrane be replaced at least every 30 days. **The membrane should be replaced 24 hours before use or calibration to allow time for the new membrane to relax. If, in an emergency, a DO probe must be used before the complete 24 hour relaxation period has lapsed, a minimum of 30 minutes must elapse prior to use. In addition, significant drift in its response should be**

expected due to shifting tension in the membrane. Therefore, the probe should be calibrated every hour it is used until the full 24 hour relaxation period has passed. For most TMDL and other short-duration sampling events, this means the user will most likely need to recalibrate the DO probe before every site.

To replace the membrane, remove the O-ring and old membrane and shake the remaining electrolyte (KCl solution) out of the probe. New KCl is available as an undissolved solid pre-aliquoted in a bottle and provided with each new DO probe or in an YSI maintenance kit. This bottle should be filled with deionized water to the **neck** to provide the proper working concentration. Add a few drops of fresh KCl solution to the probe. The tip of the probe should be filled to create a positive meniscus (looks like an "outie"), and should be free of bubbles. Hold new membrane between thumb and probe body. Use your free hand to stretch the membrane up, over, and down the opposite side of the probe. Secure the loose end with your forefinger. Roll the O-ring over the tip of the probe without touching the membrane with your finger. Cut off excess membrane. Document any membrane replacement in the logbook.

Caution: The KCl solution used under the DO membrane is especially corrosive to the electrical contacts on the probes and should not be allowed to contact these electrodes or come in contact anywhere near open probe ports when a probe is being removed or installed.

C) DO Probe Diagnostic

To check the quality of the calibration or diagnose a potential problem with the DO probe, an advanced function called DO charge may be used.

1. Press **Esc** to get the Main Menu.

2. Use the **Up** or **Down** keys to scroll and select “Report”.

3. Scroll down and select “Dochrg” and press **Enter**. When this is done, the symbol to the left of “Dochrg” should change from an empty to a black circle.

4. Press **Esc** twice to get the 650 Main Menu. Scroll up to “Sonde Run” and press Enter.

5. A new parameter should be visible on the screen called “DOc”. If the probe is in adequate condition and calibrated successfully, the number should range from 25 - 75 with a score of 50 being optimum.

6. If the probe reads in this range, then simply repeat this procedure to turn off the DO charge function (the black circle will change back into an empty circle).

If the DO charge is in the low end of the range or below this range, the KCl solution under the membrane may be contaminated with water. In this case the membrane and solution should be replaced.

If the DO charge is in the high end of the range several things may be wrong. First, the highly malleable Au electrode may be distorted or the silver-plating on the electrode may be "tarnished" and gray looking. In this case, the electrode may be reconditioned by buffing it using one of the **YSI provided buffing discs only**. THIS SHOULD ALSO BE DONE ONLY WITH STRICT ADHERENCE TO THE DIRECTIONS PROVIDED IN THE MANUAL FOR USING THIS BUFFING DISC ON THE PROBE SURFACE. IT MAY BE NECESSARY TO CONSULT WITH AN YSI REPRESENTATIVE BEFORE ATTEMPTING THIS ACTION. **YSI recommends running newly-buffed probes for 10-15 minutes continuously to realize good stability.**

A second possible cause of a high DO charge reading are cracks around the electrodes as a result of drying and rewetting of the surface. If this is the case, then the DO probe may need to be replaced.

YSI's 6562 Clark cell DO probes have an expected lifetime of 3-5 years from the date of manufacture. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe has passed its expected lifetime, it may simply be too old to give proper calibration and readings.

D) DO Probe Accuracy

The DO probe accuracy is +/- 0.2 mg/L (or ppm) O_2 or 2% of the reading (whichever is greater). The range for % saturation is or +/- 2 % or the reading or Air Saturation (whichever is greater).

3) Conductivity

A) Conductivity Probe Calibration

1. Remove the lid on the calibration cup and use the special brush designed to fit inside the conductivity probe's 2 end ports, vigorously scrub each port 5-10 times.

2. Rinse the probe 3 times with deionized water.

3. Rinse the probe 2 times with a small amount of **fresh** conductivity standard in the **1000-5000** microSiemens or μS (also known as micromhos or μmhos/cm) range. (The exact concentration of the standard will be written on the bottle.).

4. Fill cup with conductivity standard to within a centimeter of the top of the cup. Make sure that there are no bubbles in the measurement cell of the specific conductance sensor (*e.g.* gently inverting the sonde several times). Record the concentration of standard used to calibrate in the logbook.

5. Press **Escape**. Scroll to **conductivity**; press **Enter**.

6. Scroll to **SpCond**; press **Enter.**

7. Type in concentration of standard in milliSiemens (not microSiemens). 5000 microSiemens = 5.000 milliSiemens. Press **Enter**.

8. Allow the reading to stabilize, (a maximum of one minute, though the conductance probe response time is usually the fastest of all probes). Record the initial or pre-calibration readout in logbook. Press **Enter** to calibrate and record the final or calibrated readout in logbook. Press **Enter** again to continue back to the conductivity menu.

9. Now, after at least 3 rinses with deionized water, exit from the calibration menu, enter the discrete sampling mode (Sonde Run from the main menu), and take a specific conductance reading in a solution of known specific conductance less than 1000 microSiemens. This second point is a check only, not a calibration. For most checks, this will simply be distilled or deionized water (expected conductance should be < 4 microSiemens). However, in some cases a 2nd conductivity standard, of 500 microSiemens for example, will be available.

B) Conductivity Probe Maintenance

The openings that allow fluids to access the conductivity electrodes should be cleaned regularly (once a month at most) using the small acrylic brush included in the YSI calibration kit. Dip the brush in clean water and insert it into each hole 20-30 times. A mild detergent may be used with the brush, if deposits have formed on the electrodes.

C) Conductivity Probe Diagnostic

The conductivity probe on an YSI sonde can be checked using a function called Cal Constants.

1. Press **Esc** to get the Main Menu.

2. Scroll down and select "Advanced" and press **Enter**.

3. Scroll down and select "Cal Constants" and press **Enter**. The reading next to the "Cond:" should range from 4.5 – 5.5. IF THE READING IS NOT WITHIN THIS RANGE CONSULT THE YSI OPERATION MANUAL OR AN YSI REPRESENTATIVE.

4. To escape from this screen, press Esc repeatedly until the Main Menu appears.

YSI's Temperature/Conductivity probes have an expected lifetime of 2-3 years from the date of manufacture. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe has passed its expected lifetime, it may simply be too old to give proper calibration and readings. **Note that YSI 556 sondes have Conductivity Probes that are not interchangeable with the YSI 600 series.**

D) Conductivity Probe Accuracy

The Conductivity probe accuracy is +/- 0.5% +/- 1 μS/cm. For example, a solution that is 1000 microSiemens, the range would be 1000 x 0.005 +/- 1 microSiemen or 5 +/- 1 microSiemen.

4) pH

The pH probe on an YSI sonde can be calibrated using one three methods:

1) One-Point
2) Two-Point
3) Three-Point

If you know what side of neutral (*i.e*., pH=7) the majority of the streams you are going to be sampling will be on, it is recommended that you use a Two-Point calibration. A Three-Point Calibration may seem to be advantageous since it covers both sides of neutral (*i.e*., acidic and alkaline), but it has been observed that accuracy can suffer, especially near or beyond the two endpoints (*i.e*., 4 and 10 pH). Since the Three-Point calibration covers all three pH buffers, we will describe it below as an example.

A) pH Probe Calibration (Three-Point calibration)

1. Press **Escape** to get to Calibration mode.
2. Rinse probe three times with DI water.
3. Scroll down to **ISE1 pH**; press **Enter.**
4. Scroll down to **3 Point**; press **Enter.**
5. Rinse probe twice with DI water and once with 7.0 buffer solution.
6. Fill calibration cup with 7.0 buffer solution to within a centimeter of the top of the cup.
7. Because the exact pH of a buffer varies (depending on its constituents) with its temperature, a table provided by the manufacturer must be used to determine the exact current pH of the buffer solution. ***Refer to Figure 34 below or the chart hanging in the lab for the exact pH of a given buffer solution at the current temperature of the room.***

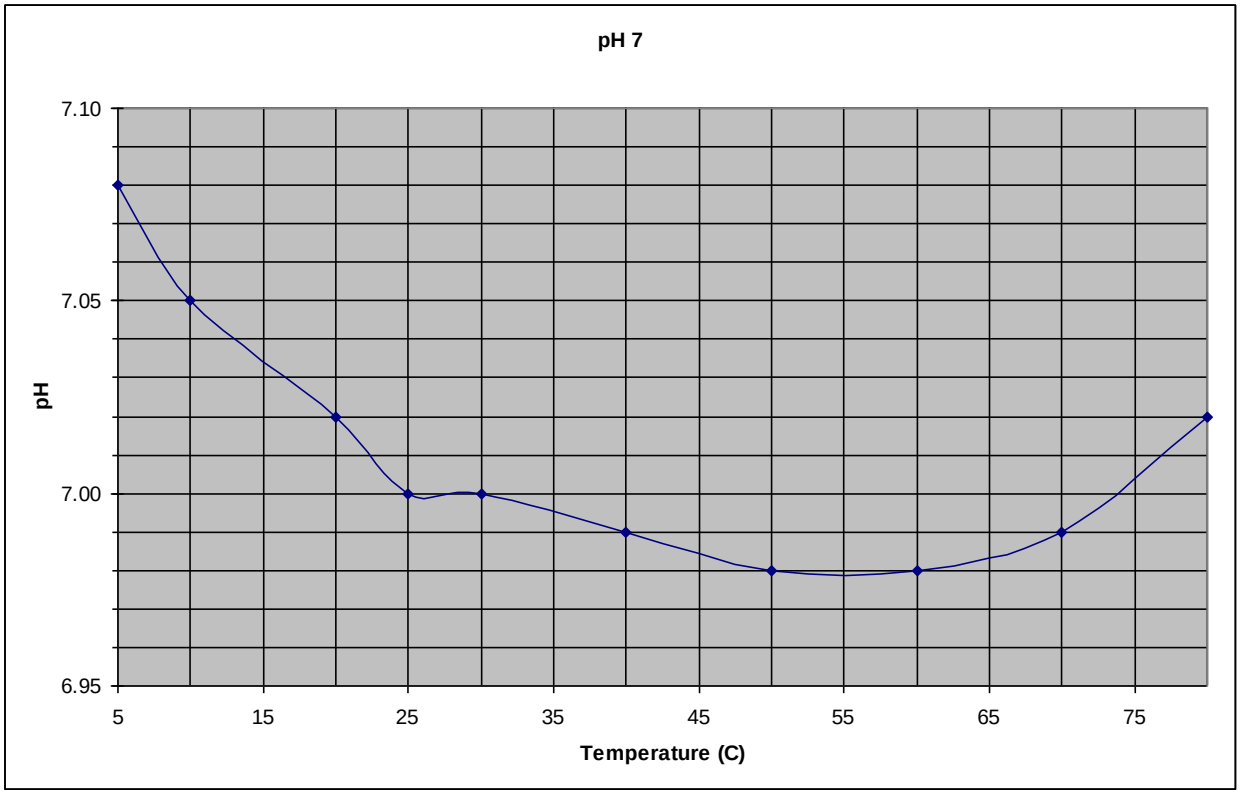


Figure 34. Temperature/pH curve for pH 7 Buffer Solution

8. Type in the exact pH for the 7.00 buffer solution for the current temperature; press **Enter**. Allow readout to stabilize (approximately one minute).

9. Record the initial or pre-calibration readout. Press **Enter** to calibrate.

10. Record the final or calibration readout and press **Enter** again.

11. Rinse probe 2 times with deionized water and once with pH 10.00 buffer solution.

12. Fill calibration cup with 10.00 buffer solution to within a centimeter of the top of the cup.

13. Determine the exact current pH of the 10.00 buffer solution from the table provided by the manufacturer or in ***Figure 35 below*** as in Step 7 above.

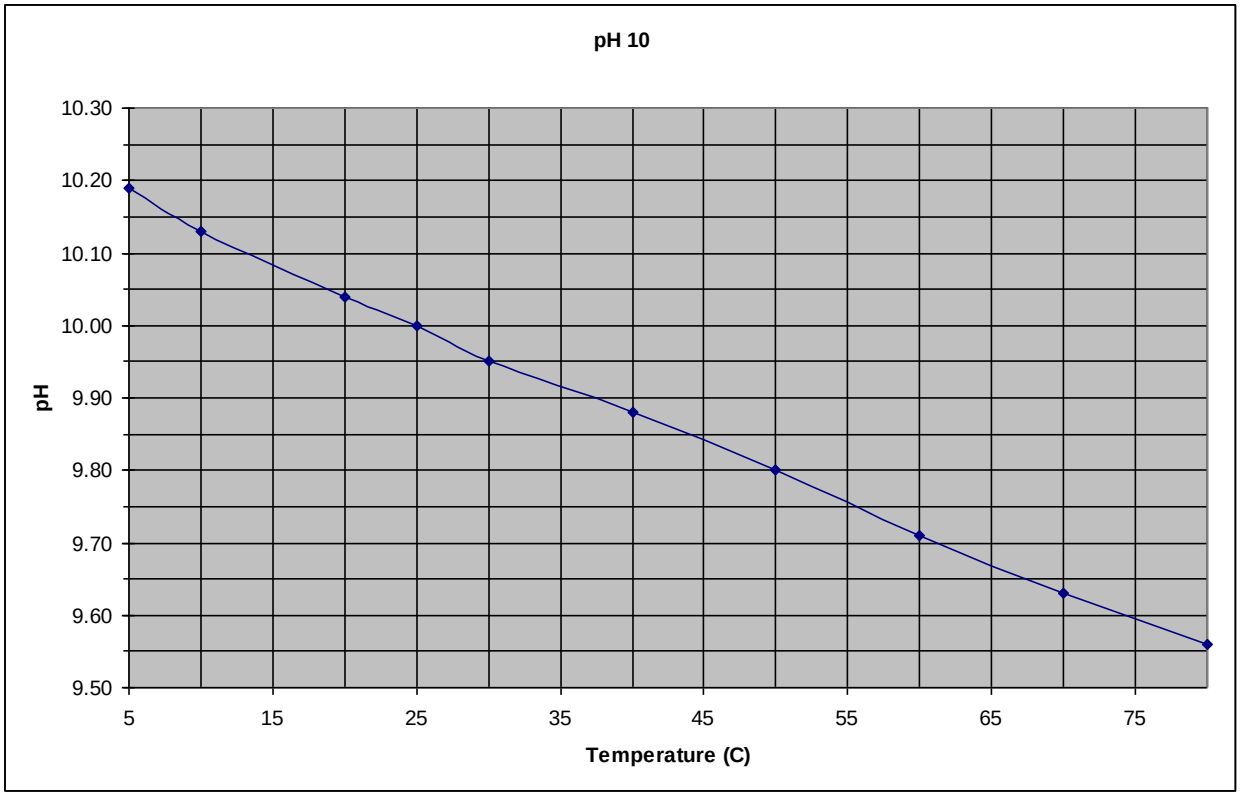


Figure 35. Temperature/pH curve for pH 10 Buffer Solution

14. Type in the exact pH for the 10.00 buffer solution given the current room temperature; press **Enter**. Allow readout to stabilize (approximately one minute).

15. Record the initial or pre-calibration readout; press **Enter** to calibrate.

16. Record the final or calibration readout and press **Enter** again.

17. Rinse probe 2 times with deionized water and once with pH 4.00 buffer solution.

18. Fill calibration cup with 4.00 buffer solution to within a centimeter of the top of the cup.

19. Determine the exact current pH of the 4.00 buffer solution from the table provided by the manufacturer or in ***Figure 36 below*** as in Step 7 above.

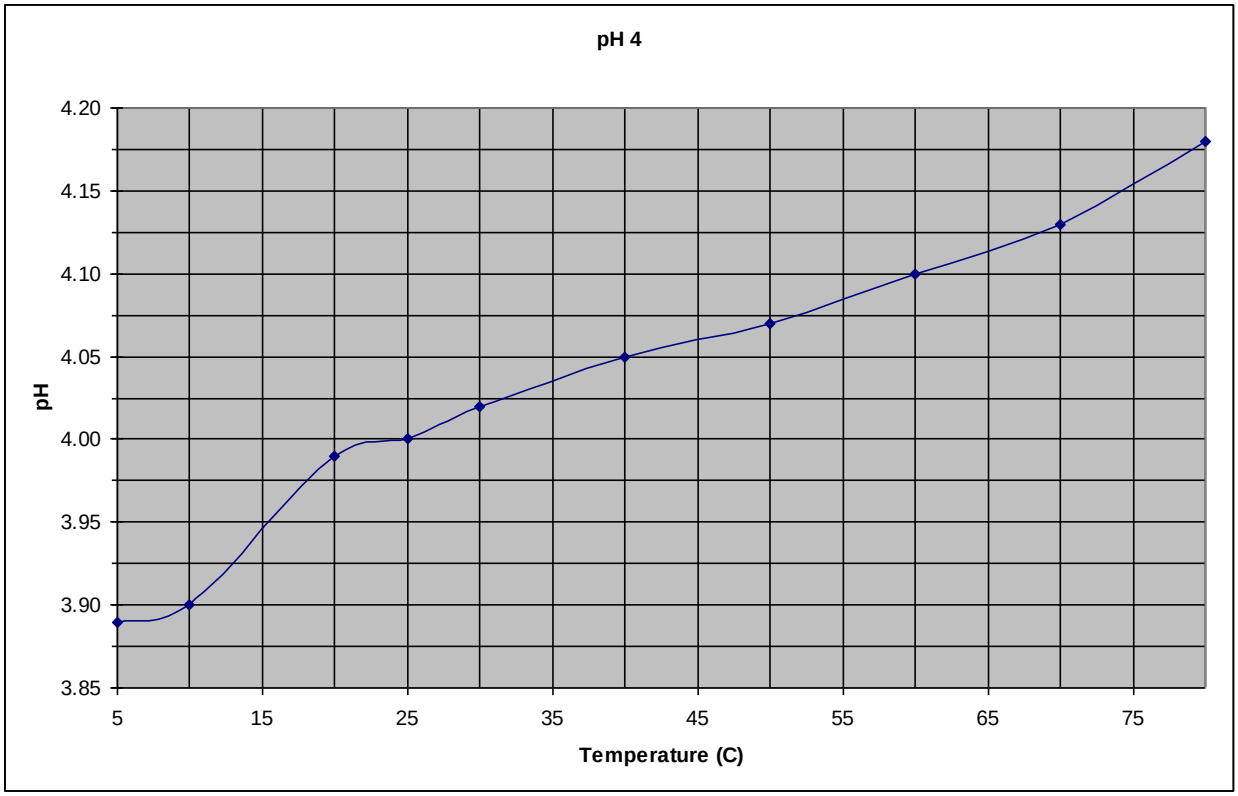


Figure 36. Temperature/pH curve for pH 4 Buffer Solution

20. Type in exact pH for the 4.00 buffer solution given the current room temperature; press **Enter**. Allow reading to stabilize (approximately one minute).

21. Record the initial or pre-calibration readout; press **Enter** to calibrate. Record the final or calibrated readout. Press enter to return to the pH calibration menu.

22. Pour out some of the 4.00 buffer solution from the calibration cup, leaving some behind to keep the air inside the cup moist. Preferably, the sonde should always be stored in some sort of high ionic solution (such as the pH buffer solutions) as this will prevent leaching of ions from the pH probe and prevent degradation to the probe's expected lifespan. Should you spill the buffer out of the calibration

cup while in the field, add an *extremely small* amount of stream water (just enough to keep the air inside the cup moist) to the storage cup. THE WATER SHOULD NOT COVER THE PH OR DO PROBE WHEN THE SONDE IS HORIZONTAL. This water should be replaced by buffer solution as soon as possible to prevent the aforementioned degradation of the pH probe.

B) Probe Maintenance and Troubleshooting

Sometimes slow response times or instability with the values (jumping as much as +/- 1.0 unit during calibration or field measurements) are observed with the pH readings. This may be caused by a number of factors and may or may not be indicative of a bad probe.

One consideration is the age of the probe. YSI's pH probes have an expected use lifetime of about 18 months. The date of manufacture is stamped on the side of the probe in the form of a 3-character code where the first two numeric digits indicate the year and the third character is a letter corresponding to the month (A = January, B = February and so on). If the probe is 18 months or older, then it is likely that it has passed its expected lifetime and it may simply be too old to give proper calibration and readings.

Another factor to consider is the temperature probe. The calculation of the pH by the sonde is a temperature dependent calculation. If the temperature probe is malfunctioning, then it may appear as if the pH probe isn't working right. Be sure to check the temperature to see if it returning a reasonable value. If it is not, then the temperature/conductivity probe may need to be replaced.

Water or sealant grease can also get in the connector when replacing a probe and can cause malfunctions and erratic readings. When replacing a pH probe, dry off the probe and sonde as much as you can before removing the probe to make sure water doesn't enter the fitting. Also, remove the pH probe with the sonde upside down so that water cannot run into the connections. Once removed, look inside the connector end of the probe and sonde to see if there is any water or grease in the fitting. If so, remove it with a can of compressed air and/or a paper towel. The important thing is to dry it out as much as possible. If there is excessive grease, then try to remove it with a towel. If the grease cannot be removed, an YSI maintenance expert may need to use a solvent for to break up the grease. Once dry, reconnect the probe using very little grease around the upper O-ring near the threads. A very thin coat making the O-ring look wet is sufficient for a proper seal.

Cleaning is required when response becomes slow or when deposits build up on the surfaces. To clean the glass bulb, remove the probe and use a soft cloth or tissue to wipe foreign material from the glass bulb and platinum button. Then use a moisten cotton swab to GENTLY remove any material blocking the reference electrode

junction. DO NOT WEDGE THE SWAB TIP BETWEEN THE GUARD AND THE GLASS SENSOR.

If response is still slow, soak the probe 10-15 minutes in clean water containing dishwashing liquid. Then wipe the probes gently with a cotton swab moistened with the cleaning liquid. Rinse in clean water, wipe once more with a clean swab and rinse again.

If response times continue to be slow, the probe may be cleaned in a 1:1 chlorine bleach solution for 1 hour. YSI recommends this procedure ever 6 – 12 months if the probe does not work well. This is usually as result of extreme conditions in which fowling of the probe is more probable.

Finally, if the probe still does not respond well, it may be soaked in one molar HCl for 30-60 minutes. THIS SHOULD BE USED AS A LAST RESORT METHOD ONLY. REFER TO THE YSI OPERATING AND MAINTENANCE MANUAL FOR DETAILS ON THESE PROCEDURES OR CONSULT AN YSI REPRESENTIVE.

C) pH Probe Diagnostic (Nernst Equation Calculation)

The pH probe on an YSI sonde operates using the Nernst Equation (***see Figure 37 below***). Simply put, a line running from 4 to 7 (or 7 to 10) pH on the x-axis should increase 180 mV (or 60 mV/pH unit) from 7 to 4 or (decrease 180 mV from 7 to 10) pH on the y-axis. See illustration below. If this slope flattens, the pH probe will lose resolution. This slope is a result of the probe condition as well as the quality of the calibration. A function called pH mV may be used to check this slope.

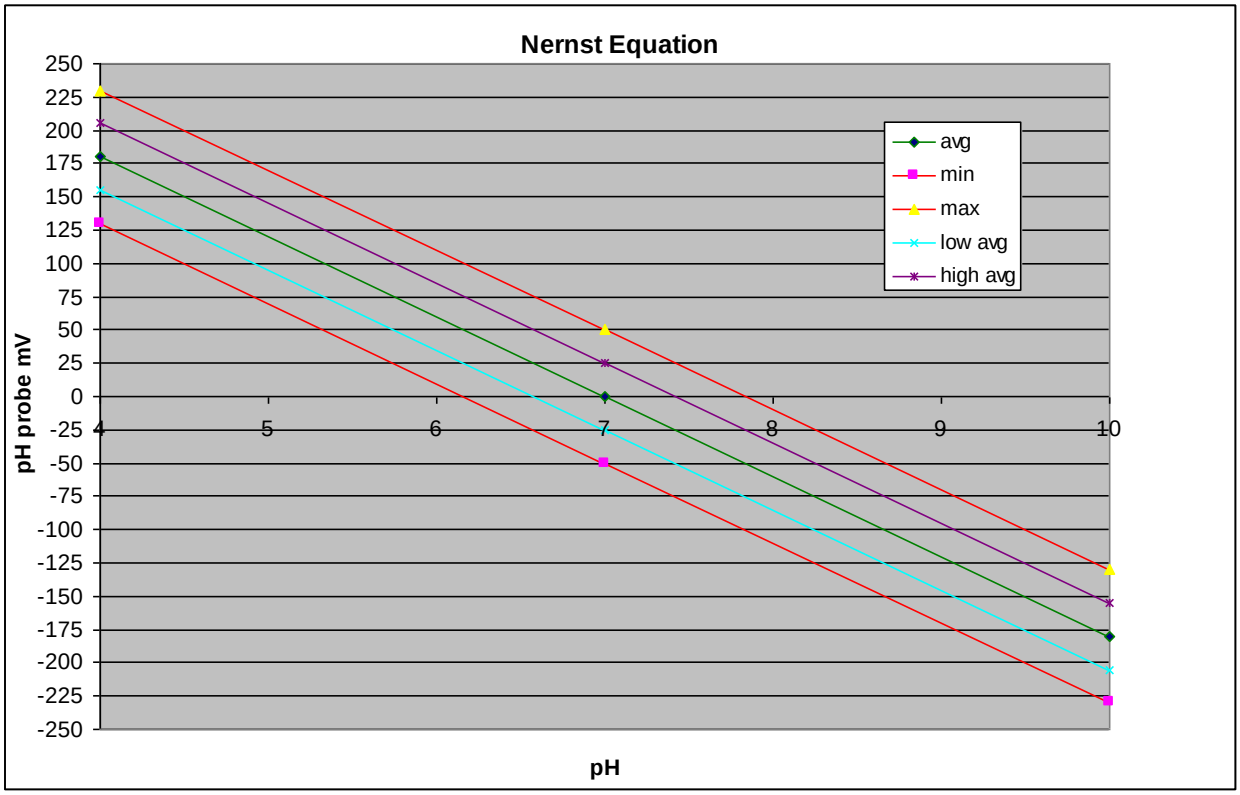


Figure 37. The Nernst Equation

Equation 1. The Nernst Equation

The Nernst Equation may be calculated by following these steps:

1. Press **Esc** to get the Main Menu.

2. Use the **Up** or **Down** keys to scroll to Report and press Enter.

3. Scroll down and select “pH mV” and press **Enter**. When this is done, the symbol to the left of “pH mV” should change from an empty to a black circle.

4. Press **Esc** twice to get the 650 Main Menu. Scroll up to "Sonde Run" and press Enter.

5. A new, second parameter called "pH mV" should be visible on the screen that reads well beyond 14 and may be positive or negative.

6. Use the rinse procedures from the pH calibration above to introduce the 7.00 buffer solution to the probe. Write down the second "pH" reading for the 7.00 buffer solution. It should be between –40 and 40, but may be slightly more (-50 to 50). The reading should stabilize within the aforementioned values in less than 30 seconds.

7. Repeat the rinse procedures for either 4.00 or 10.00 buffer solution. For either solution, the new reading should have a difference of 180 from the initial 7.00 buffer solution reading.

   For example, if an initial reading at 7.00 was -20.0, then the second reading at 4.00 should be around 160 (+/- 40)or, if using 10.00, the second reading should be around –200 (+/- 40).

8. If the probe reads in this range within 30 seconds, then the probe is ok and simply repeat this procedure to turn off the pH mV function (the black circle will change back into a **–**). If the probe reads outside this range or takes longer than 30 seconds, two things may be wrong. First, the calibration may be off and the calibration procedure should be repeated to check for this. Unfortunately, if recalibration does not correct the problem, this is an indication that the KCl solution inside the probe is contaminated with water and the whole probe will need to be replaced. ***See YSI Sonde Storage below for prevention of contamination of the KCl solution inside the pH probe.***

D) pH Probe Accuracy

The pH probe accuracy is +/- 0.2 pH units (*i.e.*, 6.8-7.2 is an acceptable reading for 7.0 buffer solution.

5) Temperature

Temperature Probe Accuracy

The Temperature probe accuracy is +/- 0.15^0 C.

YSI Sonde Storage

The pH probe on an YSI sonde operates using a polypropylene wick from the water (or sampling) side to a concentrated KCl side sealed inside the probe. To increase the life of a probe, proper storage of the probe must be implemented.

If the probe is being stored for a **short period of time**, place only a minute amount of water (1/8th of an inch is probably too much) in the cup for storage making sure that no water will inundate the pH probe. The other probes (*e.g.*, the DO probe) require only moist air to maintain proper function. A small damp sponge inside the cup would be adequate for such storage.

For **long-term storage** (*e.g.*, over winter), it is recommended that the cup be filled with a concentrated KCl solution. This will lengthen the life of the probe and help maintain the concentration of KCl inside of the pH probe. TO OBTAIN THE CONCENTRATION OF KCL SOLUTION, CONSULT THE YSI OPERATING MANUAL OR AN YSI REPRESENTATIVE.

Hydrolab Quanta G Calibration

These directions are very similar to the older Scout 2 Hydrolab directions. However, individuals with no prior experience should calibrate with the assistance of an experienced user.

1) Quanta G Display Unit

The Quanta G display unit uses a series of escapable menus in conjunction with several keys in the calibration process. Become familiar with the Enter key (which looks like a left arrow with a right angle), escape (Esc ∞), on/off (O | I), and arrow keys as these will be the most often used.

Maintenance of Quanta G Display

The Quanta G runs on 3 C batteries. Replace the C batteries as required. The Quanta G System provides at least 15 hours of continuous operation on one set of new batteries. A Battery Low icon will show the battery status.

2) Dissolved Oxygen

A) DO Probe Calibration

1. Remove calibration cup from probe and dry the membrane by blotting with a soft cloth or tissue. Check the membrane for wrinkles, tears, bubbles, dirt, etc. and replace membrane, if necessary.

2. Attach calibration cup to the Quanta and fill cup with room temperature tap water until the water surface is just level with O-ring on the D.O. probe. Do not pour water on the membrane. If the membrane gets wet, blot dry with a soft cloth or tissue.

3. Cover the calibration cup loosely using the black calibration cup cover placed upside down on the calibration cup.

4. Let the unit sit for about 10 minutes so that the air inside the cup will saturate with water.

5. Turn on the Quanta G using the **O | I** key and allow the D.O. reading to stabilize. If the circulator is on, press the **Esc** ∞ key to toggle the circulator off so that it doesn't splash the water in the cup onto the membrane. Record the initial or pre-calibration readings (mg/L) into the logbook. Also record the initial readout for temperature.

6. Press the **enter** key to toggle to the next screen and record the initial or pre-calibration % DO saturation in the logbook.

7. After power-up the Display's "Screen" icon in the lower center of the screen is blinking. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "DO" to blink and the press the **enter** key.

8. Determine the barometric pressure for entry as the calibration standard and record in the logbook. *Use the local barometric pressure. Many local weather bureaus correct the barometric pressure to sea level. Consult the operating manual for formulas to convert from sea level barometric pressure to local barometric pressure.*

9. Press the **arrow** keys to raise or lower the barometric pressure to match the calibration standard.

10. Press the **enter** key to finish calibration of Dissolved Oxygen. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen.

11. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration D.O. readings into the logbook. Press the **enter** key to toggle to the next screen and record the final or post-calibration % DO saturation in the logbook.

B) DO Probe Maintenance

If the D.O. will not calibrate, the membrane may be torn, wrinkled, dirty, damaged, or a bubble may be trapped in the probe. The membrane should be replaced whenever these conditions are observed. Frequent replacement of membranes can also lengthen the life of the probe.

To change the membrane, remove the calibration cup. Remove the O-ring that holds the membrane on the probe. Shake out the old electrolyte solution, rinse the probe with electrolyte solution, and refill with fresh electrolyte until a positive

meniscus rises above the probe surface. Make sure there are no bubbles in the probe. Install the new membrane (don't stretch the membrane while doing this), and replace the O-ring. If possible allow the probe to soak overnight in tap water to acclimate to its new shape.

C) DO Probe Accuracy

The DO probe accuracy is +/- 0.2 mg/L (or ppm) O_2 at ≤ 20 mg/L or +/- 0.6 mg/L (or ppm) O_2 at >20 mg/L.

3) Conductivity

A) Conductivity Probe Calibration

1. Remove the lid on the calibration cup and rinse the probe 3 times with deionized water.

2. Rinse the probe 2 times with a small amount of conductivity standard in the **1000-5000** microSiemens range. (The exact concentration of the standard will be written on the bottle.).

3. Fill cup with conductivity standard to within a centimeter of the top of the cup. Make sure that there are no bubbles in the measurement cell of the specific conductance sensor. Wait for the readings to stabilize. Record the concentration of calibration standard used and the initial or pre-calibration specific conductance readings in the logbook.

4. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "SpC" to blink and the press the **enter** key.

5. Press the **arrow** keys to raise or lower the specific conductance to match the calibration standard in mS/cm.

6. Press the **enter** key to finish calibration of specific conductance. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen

7. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration specific conductance readings into the logbook.

8. To check with a lower conductivity standard, repeat steps 1-3 with the lower standard.

B) Conductivity Probe Maintenance

Clean the oval measurement cell on the specific conductance sensor with a small, non-abrasive brush or cotton swab. Soap or rubbing alcohol may be used to remove grease, oil, or biological material. Rinse with water.

C) Conductivity Probe Accuracy

The Conductivity probe accuracy is +/- 1% +/- 1 μS/cm. For example, a solution that is 1000 MicroSiemens, the range would be 1000 x 0.01 +/- 1 MicroSiemen or 10 +/- 1 MicroSiemen.

4) pH

The pH probes on a Hydrolab sonde only offer one type of calibration: Two-Point.

A) pH Probe Calibration (a Two-Point calibration)

1. Rinse the probe 3 times with deionized water.

2. Rinse the probe 2 times with a small amount of the 7.0 pH standard.

3. Fill cup with 7.0 pH standard to within a centimeter of the top of the cup. Wait for the readings to stabilize. Record initial or pre-calibration specific conductance readings in the logbook.

4. Press either of the **arrow** keys to cause the "Calib" icon to blink instead of "Screen". Press the **enter** key to select calibration. Use the **arrow** keys to cause "pH" to blink and the press the **enter** key.

5. Press the **arrow** keys to raise or lower the pH to match the calibration standard for the given room temperature (***See Figure 34 above under YSI 600XL Sonde/650 MDS Display Unit Calibration pH***).

6. Press the **enter** key to finish calibration of pH. If the unit rejects the calibration, the display will show "FAIL" before returning to the "Calib" screen

7. Press the **Esc** ∞ key to return to the real-time data screen. Record the final or post-calibration pH readings into the logbook.

8. Repeat steps 1-7 for the second pH standard. This pH standard will depend on the types of streams that will be encountered. Use the 4.0 pH buffer if mainly acid streams will be encountered and use the 10.0 pH buffer if mainly alkaline streams will be encountered. The calibration standards exact pH at the given temperature can be found in ***Figure 35*** and ***Figure 36*** (***See above under YSI 600XL Sonde/650 MDS Display Unit Calibration pH***).

9. When finished with the second pH standard, add a *very* small amount of tap water (just enough to keep the air inside the cup moist) to the storage cup. THE STORAGE WATER SHOULD NOT COVER THE PH OR DO PROBE WHEN THE SONDE IS HORIZONTAL.

B) pH Probe Maintenance

Two electrodes are used to measure pH: a glass pH probe and a reference electrode enclosed in a reference sleeve. If the response time for pH seems slow, refer the owner's manual for cleaning instructions.

Glass pH probe: Little maintenance is required. Check the tip of the probe to make sure the glass is not broken or dirty. If the pH sensor is obviously coated with oil, sediment, or biological growth, clean the glass with a very clean, soft, non-scratching cloth wet with rubbing alcohol (a cotton ball will do). Rinse with tap water.

Reference electrode: Gently pull the reference sleeve away from the probe. The reference sleeve is the black tube with a porous Teflon Reference Junction attached. Discard the old electrolyte from the reference sleeve. Refill the sleeve to the top with reference electrolyte. With the probe pointed toward the floor, push the full reference sleeve back onto its mount until the sleeve has just covered the first o-ring located on the mount (just behind the silver electrode). Turn the probe so that the sensors point toward the ceiling and push the sleeve the rest of the way onto its mount. Rinse with tap water. The porous Teflon Reference Junction is the most important part of the pH performance. Make sure it is clean and passes electrolyte readily. If not, replace it. When seating the reference sleeve, trapped air and excess electrolyte is purged. This purging flushes and cleans the porous Teflon Reference Junction.

C) Conductivity Probe Accuracy

The pH probe accuracy is +/- 0.2 pH units (*i.e.*, 6.8-7.2 is an acceptable reading for 7.0 buffer solution.

5) Temperature

Temperature Probe Accuracy

The Temperature probe accuracy is +/- 0.2^0 C.

Quanta G Probe Storage

When not in use, the H20 should be stored with the storage cup containing about ½ inch of tap water. In an emergency, the cup can be filled with ½ inch of clean creek water. The creek water should be replaced with tap water when you return to the lab. The pH reference electrode should also be stored in saturated KCl solution under the plastic cap.

Part 2. Field Procedures

The readings from a water quality sonde are often referred to as instantaneous readings as they are taking immediately and directly from the water column.

While weekly calibration should be adequate to take care of the majority of the probes, the DO probe should be calibrated daily. In some cases, when travel to sites are greatly varying elevations, DO should be calibrated in each new elevation.

In the case of the Hydrolab Quanta, the pH should be recalibrated if the pH regime of the stream changes (*i.e.*, the Quanta was calibrated for 4.0 to 7.0 and the streams you are sampling are above 7.0 pH).

Setting up the Water Quality Sample Site

1. Attempt to locate a good sampling location with adequate depth and flow near mid-stream. If mid-stream is not available due to high flows or deep water, you may take deploy the sonde from the bank if you are sure that there is no plumes from pollution sources or tributaries that may be flowing along either bank. Additionally, if the cord of your sonde is long enough, you could attempt to deploy the sonde from a bridge. Another alternative is to deploy the sonde into a proxy like a bucket or sample tube that was lowered off of a bridge to collect water. In any case, be sure to document where and how you sampled on the habitat form. **IF YOU ARE COLLECTING WATER FOR ANALYSIS AT A LAB, DIRECTLY FROM THE STREAM, YOU MUST PLACE THE SONDE IN THE SAME FLOW VECTOR AS THE WATER SAMPLE COLLECTION.**

2. Remove the calibration cup from the end of the sonde, screw on the deployment guard, and deploy the sonde into the water column. **Be sure to not disturb the substrate above this point until all water data collection is completed.**

   Note: When deploying a sonde into the water, give it a little tap or shake once submerged. This will help dislodge any air bubbles inside the conductivity probe that will bias a reading. Make sure that all probes are submerged adequately.

3. Once fully submerged in the water turn the unit on. For YSI, turn on the unit with the power key and press **Enter** twice. For the Hydrolab Quanta, turn on the unit using the **O | I** key. Press the **Esc** ∞ key to toggle the circulator on and off if necessary.

4. Let the readings stabilize for a few minutes. This time could be used to fill out parts of the habitat form, collect water samples, or check on the GPS coordinates.

5. Record the readings onto the habitat form and turn the sonde off. Take off the deployment guard and replace the calibration cup. Always make sure sand and other particles are kept clear of the threads on the sampling weight, cap, storage cup, and sonde itself. These threads are plastic and will strip if sand is caught in the treads while screwing these parts on and off.

6. Store the sonde securely for future use. When storing the sonde between sites or sampling events, only a small amount of 4.0 pH buffer inside the cup is necessary to keep the air (and membranes) moist. If the pH buffer is spilled at the site, you can get away with a few drops of water inside the cup until you can replace it back at a vehicle or the lab. DO NOT STORE THE SONDE WITH A FULL CUP OF WATER, AS THIS WILL LESSEN THE LIFE OF THE pH PROBE.

Tips for usage of YSI probes in the field:

- If taking readings from an intermediate water container (*e.g.*, a bucket), make sure to keep the hole on the conductivity probe away from the edges of the container as this may cause stray signals from the probe and result in an inaccurate reading. Also attempt to keep the sample as sealed and isolated as possible to maintain the temperature and DO concentrations. It also may be necessary to swirl the sonde around to keep the water circulating.
- If a DO probe is suspected of being out of calibration, check the DO charge reading as well as the % air saturation. If the air saturation is not within an expected range for your current elevation, recalibration at that elevation may be necessary. It is also possible that the internal barometer needs recalibration (a manufacturer repair).

Sonde Quality Assurance/Quality Control

Before use, each sonde and probe should be examined for wear (*e.g.*, breakage, air bubbles, membrane tears or wrinkles) and adjustments should be made as required.

Calibration logbooks are maintained for each instrument and entered into a database. Any instrument failing to meet calibration requirements is repaired in house or shipped to the manufacturer. Meters are calibrated weekly, prior to sampling, and are recalibrated in the field, if conditions warrant. For example, if a Hydrolab has been calibrated for pH using the 7 and 10 buffers, recalibration is performed if a stream pH of 3 is encountered. Note that an YSI sonde has a 3-point pH calibration procedure available that is conducted in the lab since streams in the acidic and alkaline ranges are often both encountered during the course of a week of sampling. D. O. is calibrated daily. In addition, all sondes and probes are cross-checked against each other monthly for accuracy and stabilization speed.

Each meter has an identification number, which is recorded on the habitat assessment sheet each time the meter is used. Should any instrument fail to calibrate, readings

taken prior to the failed calibration will be examined for reliability and accuracy. Documentation of the instrument used at each site will help to keep data loss to a minimum. All repairs to sondes and probes are documented in a repair log including the serial numbers and manufacture dates of any replacement probes.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. A hands-on session concerning the calibration, maintenance, and collection of water quality sonde data is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in using water quality sondes will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to use the sondes solo. This document is also provided to all program personnel for review and use in the field.

Section B. Water Quality Sample Collection and Preservation

The water quality monitoring is the centerpiece of the Watershed Assessment Branch's (WAB) efforts to assess streams. It is extremely important that all of these methods are followed to maintain comparability between samplers and sampling events.

Materials and Reagents

1. Analysis Request Form with Chain-of-Custody (COC) - for sample identification and tracking of samples from the field to lab and results from the lab back to us.
2. Water Quality Sample Labels-featuring the unique WQ Sample ID for each distinct water sample.
3. Waterproof pen or sharpie - for labeling sample bottles.
4. Sterile Fecal bottles with Sodium thiosulfate tablet - for collecting bacteria samples.
5. Plastic Bottles (Cubitainers with Lids) - for collecting other water quality samples, except phenols.
6. Cooler - for sample preservation.
7. Wet Ice - for sample preservation.
8. Fixatives (nitric acid, sulfuric acid, and sodium hydroxide) - for sample preservation.
9. Waterproof plastic bags or other suitable container - for holding bacteria sample bottles during transport.
10. Filtration Apparatus (either Peristaltic or Vacuum type) – for sample preservation.

Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.*, glass, metal, wire, etc.). They should also be worn during sample preservation or at any time while handling the fixatives, which are concentrated acids. Bottles containing fixatives should be stably seated inside a lidded container to prevent breakage and leakage.

WARNING!! SOME FIXATIVES ARE CORROSIVE AND MAY EMIT TOXIC FUMES. BE SURE TO USE THE APPROPRIATE SAFETY GEAR AND PRESEVE SAMPLES IN A WELL VENTALIATED AREA. DO NOT FIX SAMPLES IN THE VEHICLE, AS ACCIDENTAL SPILLS CAN AND WILL OCCUR.

Do not place liquid acid or base into sample bottles prior to sample collection. Always add fixatives to sample. **NEVER ADD SAMPLE WATER TO LIQUID ACIDS OR BASES, AS A STRONG CHEMICAL REACTION CAN OCCUR.**

Part 1. Procedures for Collecting Water Quality Samples

Labeling Sample Containers

Label each sample container with a sharpie. The following information must be included: Agency Name (WVDEP), Project Name (*e.g*., WAB, TMDL, Deploy, AWQN, LTMS, Lakes, etc.), WQ Sample ID (this is a unique number for each water sample that is taken from the Water Quality Sample Labels), Stream Name, AN-code and Mile Point, Date/Time (Military) collected, Random # (if applicable) and type of fixative/preservation used. In addition, distinct sub-sample descriptions should be indicated somewhere on the form (*e.g*., Top vs. Bottom, Left Bank vs. Right Bank, etc,). Duplicate or replicate samples should be distinguished by a Dup #1 vs. Dup #2 or Rep #1 vs. Rep #2. It is recommended that some additional identifying mark be put on the lids as the sides of the containers can be abraded pretty easy and lose their labels. This additional identifying mark can be the WQ Sample ID, Random #, or something as simple as the time of collection.

NOTE: The Water Quality Sample Labels do not stick well to any surface that is not smooth. Therefore, if a fecal sample is being collected, use the label on the fecal bottle. If no fecal sample (or other sample that has a container with a smooth surface) is being collected, then copy the WQ Sample ID to other containers and discard the unused label.

Direct Dip/Grab Method

1. At the selected water quality sampling location, (X-site for random sites), attempt to locate a good sampling location with adequate depth and flow near mid-stream. If mid-stream is not available due to high flows or deep water, you may take the sample from the bank if you are sure that there is no plumes from pollution sources or tributaries that may be flowing along either bank. Be sure to document where you sampled on the habitat form. **Be sure to not disturb the substrate above this point until all water sampling is completed.**

2. Place the water quality sonde downstream in the same flow vector as your sample point and turn it on so that it can begin to take readings (***see Section A. Part 2. Field Procedures starting on page 116 for more information on how to use the water quality sonde in the field***).

3. Collect the water samples upstream as follows:
   A. Fecal Coliform Sample:
   - Use pre-sterilized bottle with Sodium thiosulfate tablet. Keep the bottle closed until you are ready to collect the sample.

- Open bottle and handle carefully to avoid contamination. DO NOT TOUCH THE INSIDE OF THE LID OR BOTTLE.
- Using a quick dipping motion, submerge and fill the bottle to the 100 ml mark. DO NOT RINSE OR REFILL THE BOTTLE. If the bottle is too full, slowly pour a little out. *The head-space is necessary in a fecal sample to provide oxygen to the bacteria until the sample can be analyzed.*
- Place cap tightly on bottle and secure cap lock.

B. Other Water Samples (Cubitainer Samples):

- All remaining water quality samples are collected in plastic Cubitainers.
- Rinse the Cubitainer twice with stream water
- Submerge and fill the Cubitainer with sample water and expunge as much of the airspace as possible.
  Note: When collecting a sample to be analyzed for Alkalinity (unfixed sample) as much air as possible should be expunged from the sample container to avoid contamination (*i.e.*, no head-space).

Remember: Take water samples at lower end of reach for Non- Random targeted sites. Take water samples at X-site for random sites regardless of the location of the lower end of 100 m assessment reach.

Indirect Methods

In some cases, water levels or flows will not permit direct water sampling. In these cases, it is necessary to use special equipment in order to get the sample. Such indirect methods are better explained in ***Chapter X. Section A. Part 2. Bridge Crane Method starting on page 227 and Chapter XI. Section D. Part 3. Van Dorn Sampler Method for Depth Profiles on page 279.***

Part 2. Sample Preservation (Filtration, Fixation, & Holding)

Preserve the sample as indicated on the Analysis Request Form. ***See Figure 38 on page 130 for an example of a Analysis Request Form with COC***. **Preservation must occur within 15 minutes of sample collection, even if you must pack bags of wet ice and the filtration equipment in on a 6 mile hike. NO EXCEPTIONS!** ***The preservation methods and holding times are summarized in Table 7 in the Holding section on page 128 below.***

Samples should be preserved in the following order:

1) Unfixed or Iced (Wet Ice) Samples (*e.g.*, Fecal Coliform, Unfixed Cubitainer)
2) Filtered Samples (*e.g.*, Dissolved Metals or Nutrients)
3) Fixed Samples (*e.g.*, Total Metals or Nutrients).

The fecal coliform sample should be double bagged before being put on wet ice to prevent accidental contamination of other samples should the sample container become compromised. Do not submerge the fecal sample in ice water! The samples that only need to be cooled on ice (commonly referred to as Unfixed or No Fix) can also be placed on wet ice at this time.

Remember: If Alkalinity is being analyzed, 100% of the air must be expunged from the unfixed cubitainer to avoid contamination.

Filtration

A net minimum of 200 mL of filtered sample should be turned in for dissolved metal or nutrient analysis at most labs we deal with.

Protocols for Sample Filtration with Peristaltic Pump/Drill Apparatus (Dissolved Metals & Dissolved Nutrients)

The components of the filtering apparatus are:

1. Peristaltic Pump mounted on Stabilizing Board
2. Power Drill with Pump Adaptor Bit
3. Tygon Tubing
4. Filters (50 mm cellulose acetate membranes with a 0.45 micron pore size); two varieties: Flat Disc or Cartridge.
5. Two sample containers (one for the stream sample and one to receive the filtered water).

It is important to keep the filtering equipment and area around the equipment clean. Try to handle all parts by the exterior components. Fingerprints and other dirt can contaminate samples. The tubing and filters should be kept in their sealed plastic bags until time of use to reduce exposure to dust and other contaminants.

Ideally, the filtering process would occur at streamside by taking the filtered samples (*e.g.*, dissolved metals and dissolved orthophosphate) directly from the water column. However, this is dependent upon there being a flat, streamside surface to work on and no precipitation that could short the drill. If filtering cannot occur directly from the water column, the sample water to be filtered should be collected in a clean container that is rinsed twice with stream water and transported to a suitable area for filtration and preservation within 15 minutes of collection. **This container should be the only one that will be exposed to the Tygon tubing and not reused from site to site. Do not filter from the Total Metals sample container as the insertion of the Tygon tubing may contaminate the sample.**

Procedure:

1. Assemble the filtration unit:
   - Place the drill upside down on stabilizing board and carefully insert the bit into the peristaltic pump.
     - The bit may need to be rotated slightly in order to line up with the receiving shaft and engage fully.
   - Place the unfiltered stream sample container near the pump and remove the cap.
   - Open the pump clamp by lifting the lever.
   - Without directly touching the tubing, open the sealed tubing bag and remove about 8 inches of tubing. Place this end into the unfiltered stream sample container. The rest of the tubing can now be manipulated directly with the hands, but avoid touching the other end of the tubing if at all possible. Thread the tubing through the pump and close clamp.
     - If filtering directly from stream, the tubing can be touched with the hands.
     - Place the stream end of the tubing so that sediment is not being collected from streambed.
   - Attach the filter to one end of tubing
     - Handle filter by edges only, with the pressure valve facing toward the pump and stream sample. The cartridge filters should have an arrow indicating the direction of flow.
     - Make sure not to touch the end of the filter that will be discharging into the dissolved sample container.

2. Flush the filter and tubing briefly with sample water by engaging drill slowly for several seconds.
     - Do not collect the flushed water in the filtered container. Discard elsewhere.

3. Rinse the filtered container:
   - Hold the filter at an angle above the mouth of the receiving (filtered water) container at the point of where the tubing is attached.
     - This will allow the user to feel if pressure is building up too quickly in the tube and prevent the tube from explosively detaching from the filter and potentially contaminating the filtered sample. **Do not hold the tube too tightly as this could also cause leakage around the attachment point.**
   - Engage the drill slowly and fill the receiving container with about 50 mL of water. **DO NOT OPEN THE DRILL FULL THROTTLE AS IT WILL RUPTURE THE FILTER AND CONTAMINATE THE SAMPLE!!!**
   - Cap the container, shake vigorously and discard filtrate.
   - Repeat.

4. Filtering the sample:
   - Engage the drill and fill the receiving container with at least 200 mL, unless otherwise directed. On one liter cubitainers, this location is near the first character on the long diagonal bar on the side of the cubitainer.
     - Use slow drill speeds (never full throttle) to filter the sample, especially when approaching the desired sample about. This method is supposed to be cleaner, not necessarily faster.
     - If you are close to being done and the pressure is building to fast in the tubing, try using a pulsation with the drill speed. This will often get you to the end without having to change the filter.

5. Changing filters:
   Sometimes it becomes necessary to change the filter while in the middle of processing a water sample. This is usually due to the filter membrane becoming overwhelmed with small particles of silt, which causes the sample to filter extremely slowly. The filter can also become clogged with seemingly clear water due to unseen periphyton. This will also cause the filter to be changed. If the field personnel feel that it is necessary to change the filter to achieve the minimum amount of sample necessary, the following steps should be taken:
   - Cap the receiving container, relieve the pressure in the tube by reversing the drill momentarily or unlocking the clamp, and remove the clogged filter from the end of the tubing.
   - Replace with a clean filter as before in Step 1 being careful not to touch ends of filter.
   - Flush the new filter as in Step 2 and resume filtering.

   Repeat these steps until a sufficient sample is collected. Record on the lab analysis form how many filters were used. This would give the lab an idea about how high the total suspended solids in the sample should be.

6. You must discard and restart the sample if:
   - The filter is cracked or split during use.
   - The filter is dislodged from tubing while filtering and the unfiltered water contaminates the filtered sample during use. This would be typical if this happens explosively.
   - Sediment is collected directly from bottom of stream.

NOTE: The tubing and filters are disposable, and should only be used once. Discard the each filter after one use and discard the tubing after each sample. Obtain a clean set for the next sampling event.

Protocols for Sample Filtration using a Vacuum Pump (Dissolved Metals & Dissolved Nutrients)

The components of the filtering apparatus are:

1. Filter Flask – Receptacle for the filtered sample
2. Filter Funnel – Consists of two parts: A cup to hold the unfiltered sample and the funnel itself.
3. Filters – Cellulose Nitrate membranes with a 0.45 micron pore size.
4. Vacuum Pump – A variety of hand operated pumps are available.

It is important to keep the filtering apparatus clean. Try to handle all parts by the exterior components or by the stopper. Fingerprints and other dirt can contaminate samples. The Filter Funnel & Filter Flask should be stored in a Zip Loc bag or other container (even when driving from one site to another) to reduce exposure to dust and other contaminants.

Procedure:

The water for the filtered sample must be taken from a portion of the total metals sample.

1. Rinse off the filter apparatus (cup, funnel and flask) with deionized water.
   - Be careful not to get water into the nipple on the flask.
   - Rinse each part separately. Do let rinse water from cup drip into either the funnel or flask and do not let rinse water from the funnel drip into the flask.

2. Assemble the filtration unit:
   - Attach the funnel to the flask.
   - Place a filter on the funnel.
     - Handle the filter by the edges only.
     - Make sure the filter is centered on the funnel's screen.
   - Attach cup, be sure to get a good seal.

3. Initial Rinse:
   - Pour a small amount of sample into cup.
   - Filter sample, making sure all the water has passed through.
   - Depressurize the pump.
   - Wipe drips from exterior of cup & funnel and remove from flask without disassembling cup from funnel.
   - Rinse the flask with a swirling motion and discard filtrate (be careful to avoid getting filtrate in the flask nipple).

4. Filtering the sample:
    - Place cup & funnel assembly back into flask.
    - Pour a larger amount of the sample into the cup.
        - If water is turbid, use small amounts; filter may clog and need to be changed.
        - Do not put too much sample into the cup since this may exceed the capacity of the flask, causing water to be sucked into the pump.
        - Wipe off any spills outside of the cup.
    - Filter sample using full strokes on the pump.
    - Depressurize pump after sample has been filtered and before changing filters.

5. Changing filters:

Sometimes it becomes necessary to change the filter while in the middle of processing a water sample. This is usually due to the filter membrane becoming overwhelmed with small particles of silt which causes filtering to become extremely slow. If the field personnel feel that it is necessary to change the filter to achieve the minimum amount of sample necessary the following steps should be taken:

- If there is any left, pour off the excess water out of the cup by turning the filter apparatus on its side with the siphon arm up so that no filtered water can escape from the flask or enter the vacuum tube. One should support both the cup and the lower funnel so that the two do not break the magnetic seal and separate.
- Filter off any excess water until the filter is dry.
- Remove the cup from the funnel.
- Holding funnel sideways, remove old filter. Start from the top of the filter and pull downward.
- If there is any question that unfiltered water may have dripped into the funnel or into the flask, assume that the sample has been contaminated and the filtering process must be reinitiated from the beginning.
- Install a fresh filter handling only by edges.
- Replace the cup and continue filtering.
- Repeat these steps until sufficient sample (usually a net of 200ml of sample after rinsing the cubitainer 1-2 times, but check with lab beforehand). It is also a good idea to put on the lab analysis form how may filters were used if greater than 1. This would give the lab an idea about how high the Total Suspended Solids in the sample should be.

7. You must discard and restart the sample if:
    - Filter is cracked or split during use.
    - Sediment on filter is off-center (no white ring around entire edge).

8. End of week cleaning:
    - Rinse cup, funnel and flask with tap water; wipe off scum.

- Use a brush to lightly clean the funnel's screen.
- Rinse cup, funnel and flask thoroughly with deionized water and shake off excess droplets.
- Place a filter on the funnel's screen and store cup/funnel assembled in a zip loc bag.
- Rinse only the glass flask with 10% HCl. The plastic portions (funnel and cup) may only be rinsed with deionized water and lightly rubbed with a paper towel.
- Do not touch inside surfaces of filtration apparatus.

Fixation

As outlined in ***Table 7 below in the Holding section***, some samples will need to be fixed with acids before being stored. Samples that are preserved with Sulfuric Acid should always be preserved before samples that are preserved with Nitric Acid. This is because the volatile Nitric Acid vapors may contaminate Nutrient samples and give false Nitrogen results. If you do accidentally preserve the Nitric Acid sample first, then move away from that area when fixing the Sulfuric Acid (*e.g*., the opposite end of the vehicle or 20 feet away).

When fixing a sample with acids, careful consideration must be given to the ambient chemistry of the stream (*i.e*., pH and conductivity) and volume of sample being preserved. Any given ampoule of acid is designed to preserve 1 liter of normal water (*i.e*., pH near neutral and normal conductivities (approximately 200 μmhos/cm). If a stream has a low pH and/or low conductivity, one ample of acid may over preserve the sample. Conversely, if a stream has a high pH and/or high conductivity, one ample of acid may not be enough to adequately preserve the sample. A larger volume of sample water would also require more acid; a smaller volume less. Less experienced individuals should use pH test strips in order to gage how much acid to add to adequately fix sample.

<u>Testing a sample with a pH test strip</u>

1) First add a small amount of acid to the sample (maybe half of an ampoule).
2) Seal the sample and shake it to mix in the acid.
3) Open the sample and pour a small amount onto a pH test strip. **Never dip the pH test strips into the sample!**
4) Compare the pH test strip color to the color key on the pH test strip package. The target pH is just below 2.
5) If more acid needs to be added, then add more accordingly. Otherwise, seal the sample and put it on wet ice if necessary.

Holding

With the exception of fecal coliform, all samples should be delivered to the lab within the holding times specified in "Standard Methods for the Examination of Water and Wastewater", 18th Edition and as outlined above in ***Table 7 Preservation Methods and Holding Times below***.

The holding time for fecal coliform sample has been expanded by the WAB from 6 hours to 24 hours because the six-hour holding time places severe limitations on the amount of time a crew can spend in the field and the majority of these samples are not collected for enforcement purposes. **However, fecal samples collected for the TMDL program may need to comply with the six-hour holding time depending on the specific instructions given for that watershed.**

Table 7. Preservation Methods and Holding Times

Parameter	Preservation	Max. Holding Time
Fecal Coliform	Cool <10 °C, 0.0008% Na2S2O3	6 hours. (24 hours for TMDL/WAB samples)
Acidity	Cool ≤6 °C	14 days.
Alkalinity	Cool ≤6 °C	14 days.
Ammonia	Cool ≤6 °C, H2SO4 to pH<2	28 days.
Chloride	None required	28 days.
Kjeldahl (TKN) and Organic N	Cool ≤6 °C, H2SO4 to pH<2	28 days.
Chromium VI	Cool ≤6 °C, pH = 9.3–9.7	28 days.
Mercury (CVAA)	HNO3 to pH<2	28 days.
Mercury (CVAFS)	5 mL/L 12N HCl or 5 mL/L BrCl	90 days.
Total Metals (except Boron, Chromium VI, and Mercury)	HNO3 to pH<2	6 months
Dissolved Metals (except Boron, Chromium VI, and Mercury)	Filtered, HNO3 to pH<2	6 months
Nitrate	Cool ≤6 °C	48 hours.
Nitrite	Cool ≤6 °C	48 hours.
Nitrate-Nitrite (NO2-NO3-N)	Cool ≤6 °C, H2SO4 to pH<2	28 days.
Total Orthophosphate	Cool ≤6 °C	48 hours.
Dissolved Orthophosphate	Filtered, Cool ≤6 °C	Filter within 15 minutes; 48 hours.
Phosphorous, Total	Cool ≤6 °C, H2SO4 to pH<2	28 days.
Total Solids, Total Suspended Solids (TSS), Total Dissolved Solids (TDS)	Cool ≤6 °C	7 days.
Sulfate	Cool ≤6 °C	28 days.

Documentation

Using a black or blue permanent ink pen, fill out an Analysis Request Form for each water sample. If multiple samples are taken at a particular site during the sampling event, you will need a separate form for each distinct sub-sample (*e.g.*, Top, Middle, and Bottom Lake samples; Left Bank, Middle, and Right Bank cross-section samples). In addition, you will need to fill out an Analysis Request Form for each laboratory. For example, if the fecal sample will be delivered to one laboratory and the other samples to another, complete a request form for each lab unique to the parameters that they will be analyzing. The person who actually collected the sample must be the person indicated on the form and the one who signs the chain-of-custody (COC).

A completed Analysis Request Form includes: Project, Laboratory Name, Stream Name, WQ Sample ID (Use the Station Number line if this is not available on the form), Watershed Name, AN-Code, Random # (if applicable), # of Containers, Sampled By, Filtered By, Sample Type, Acid Lot #s, Field Meter # (*i.e.*, Sonde ID), Date and Time (Military), Field Values, Parameters Requested, # of Filters Used, Type of Filters Used, and Relinquished By. In addition, distinct sub-sample descriptions should be indicated somewhere on the form (*e.g.*, Top vs. Bottom, Left Bank vs. Right Bank, etc,). Duplicate or replicate samples should be distinguished by a Dup #1 vs. Dup #2 or Rep #1 vs. Rep #2.

REMEMBER: Until the Analysis Request Form can be updated, put the WQ Sample ID in the spot labeled Station Number.

After the sample has been turned over to the laboratory, the Date and Military Time, Received By, and Lab name must be filled out on the COC portion at the bottom of the Analysis Request Form. ***See Figure 38 below for an example of a fully completed Analysis Request Form with COC***. Keep the white copy for WAB records and give the yellow copy to the lab.

WV DEPARTMENT OF ENVIRONMENTAL PROTECTION - WATERSHED BRANCH

General Analysis Request Form, Rev. 03/09

TMDL WAS (AWQN) LAKES RANDOM

Laboratory Name: Biochem Circle Activity

Stream Name: Gauley River Watershed Name: Gauley

AN-Code: KG-(813) Station Number: 45681 Random #: _____ # of Containers 5

Sampled By: KDS Filtered By: KDS

Sample Type: Water X Sediment _____ Other _____ Specified Method: **40 CFR 136**

Acid Lot #, Nitric: 926007 Sulfuric: 928803 HCl: _____ Field Meter #25 Flow Meter #_____

Grab: Date-Time: 7/12/10 1300 Lat: _____ Long: _____

Positioning Method (Circle One) Map GPS GIS

Field Values[1]: Temp. 26.52 pH 7.38 D.O. 6.98 Cond. 129 Flow: _____

Pres.	Analysis	Pres.	Analysis	Pres.	Analysis	Tot	Diss[1]
(3)	Acidity (Hot)	3	Tot. Solids	(5)	Sodium		
(3)	Alkalinity	(3)	Tot. Diss. Solids	(5)	Aluminum	(H)	(L)
(5)	Hardness	(3)	Tot. Susp. Solids	(5)	Cadmium	L	(L)
(3)	Sulfate	(4)	T. Phosphorus-P	5	Chromium	H L	H L
3	Turbidity	4	T. Phosphate	(5)	Copper	L	(L)
(2)	Chloride	3	Tot. Ortho PO_4-P	(5)	Iron	(H)	(H)
3	BOD5	8	Diss. Ortho PO_4-P	(5)	Lead	L	(L)
4	COD	(4)	TKN	5	Magnesium		
4	TOC	(4)	Ammonia-N	(5)	Manganese	(H)	
(7)	Fecal Coli., MF	4	Unionized NH3	5	Mercury	H	
	24 hour holding	4	Org-N	(5)	Nickel	L	(L)
7	Fecal Coli., MF	3	NO_3-N (Nitrate)	(5)	Zinc	L	(L)
	6 hour holding	3	NO_2-N (Nitrite)	5	Calcium	H	
7	E. Coli, Numeric	(4)	NO_2-NO_3-N	(5)	Selenium	(L)	
7	Fecal Strep.			(5)	Arsenic	(L)	L
3	pH (lab)	10	Chlorophyl a	(5)	Silver		(L)
3	Cond. (lab)			5	Potassium		
3	Acidity (Cold)	3	Semi-Vol. Organics	(5)	Boron		
		9	Volitile Organics	(5)	Barium		

Preservation Code

1. None - Determined on-site
2. None
3. Iced immediately
4. H_2SO_4 to pH <2, iced immediately (Phenols in glass container)
5. HNO_3 to pH<2
6. (Cyanide) NaOH to pH>12, iced immediately (0.6 g ascorbic acid used on samples with residual chlorine)
7. Sterile + 0.008% $Na_2S_2O_3$, iced immediately
8. Filtered immediately, iced immediately
9. HCl to pH<2, iced immediately
10. $MgCO_3$ & Ice, _____ml sample
11. Other (Specify)

H=High (e.g. 5ppb) L=Low (e.g. 0.05 ppb)

REMARKS:

1 # of Filters used

Filter type (circle): Disc (Cartridge)

[1]Filtered immediately, nitric acid added to pH <2

Relinquished by:	Date & Time	Received by:	Relinquished by:	Date & Time	Received by:
Kevin Seagle	7/12/10 2200	Lab:			Lab:

Mail Results to: ATTN: Janice Smithson (Lab Instructions: On invoice bill to Organization Unit 9480),
WVDEP, DWWM, Watershed Branch, 601 57th Street SE, Charleston, WV 25304 Phone (304) 926-0499 ex. 1051, Fax. 926-0496

WHITE - Sample Collector Copy CANARY -Laboratory Copy

Figure 38. Example of a fully completed Analysis Request Form with Chain-of-Custody (COC) at bottom

Part 3. Common Water Quality Parameter Suites

Take Hydrolab readings & Fecal coliform at every site!

***Random & Potential Reference Sites*:**
- Acidity (Hot), Alkalinity, Sulfate, Chloride, Fecal coli., TSS, TDS, Tot. Phos., TKN, NO_2-NO_3-N, Mg, K, Na, Al (Tot. & Dis.), Cu (Dis.), Fe (Tot. & Dis.), Mn, Zn (Dis.), Ca, Se (Tot.). (Note: Order Low Level Detection on Tot. & Dis. Cu, Zn, & Se.)

4 cubitainers (wet iced, HNO_3, filtered HNO_3, & H_2SO_4) & fecal

***Acid Rain Parameters*:**
Take when: 1) pH <6.0 & conductivity is <50, 2) if stream is on the 303(d) list for pH unrelated to mining, or 2) if for any reason you suspect acid rain deposition impacting the stream:
- Acidity (Hot), Alkalinity, Sulfate, Fecal coli., Acidity (Cold), TSS, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn, & Ca (Tot.).

3 cubitainers (wet iced, HNO_3, & filtered HNO_3) & fecal

***AMD Parameters*:**
Take when: 1) conductivity alone is >500, 2) pH <6.0 & conductivity is >200, 3) if stream is on the 303(d) list for AMD, or 4) if for any reason you suspect mine drainage:
- Acidity (Hot), Alkalinity, Sulfate, Fecal coli., TSS, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn, & Se (Tot. & Dis.). Take Ammonia-N (NH_3) if it is suspected that Ammonia is being used to treat the stream water.

3 cubitainers (wet iced, HNO_3, & filtered HNO_3) & fecal

***Nutrient Enrichment*:**
Take within 24 hours of a significant rain or when animal waste, straight pipes, STP outfalls, etc., may be impacting the stream:
- TSS, Tot. Phos., TKN, NO_2-NO_3-N, & Fecal coli. Take Ammonia-N (NH_3) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia input.

2 cubitainers (wet iced, H_2SO_4) & fecal

***TDS Ions*:**
Take anywhere in Monongahela Basin (Dunkard, Monongahela, West Fork, Tygart, Youghiogheny, & Cheat):
- Sulfate, Chloride, Fecal coli, TDS, Mg, K, Na, & Ca.

2 cubitainer (wet iced) & fecal

***Oil & Gas*:**
Take if oil or gas activities are evident & cond. >200 in absence of other sources like AMD:
- Chloride, & Fecal coli.

1 cubitainer (wet iced) & fecal

Water Sample Collection Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with water quality sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of water quality samples is included. In the field, biological sampling teams will consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in collecting water quality will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to collect water quality solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Lot numbers of all preservatives are recorded on the Analysis Request Form for each sample submitted and entered into the database to allow for easy tracking. Sample transfer to the lab shall also be documented using the Chain-of-Custody (COC) portion of the Analysis Request Form.

Duplicate sampling and field blanks must be performed at a minimum of 2.5% of our sites. To assure we meet these requirements, each team list will have a designated duplicate and field blank. The field blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented below. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Field Blanks

Overview

To evaluate sample containers for contamination, each team will prepare field blanks weekly. Distilled, deionized water is used as the blank "sample". This water should be carried in an unused, well-sealed, one-gallon cubitainer. During the designated sampling event, an extra set of cubitainers are prepared as field blanks, one container for each type of preservation method. The blanks are labeled according to the protocols. These containers are filled with the distilled/deionized water and are preserved and stored in the same manner as the actual samples. A separate Analysis Request Form with Chain-of-Custody (COC) is completed for the field blanks and the samples are submitted to the laboratory.

Field blanks are simply samples of deionized water that are preserved in the field. The purpose of the field blank is to detect onsite contamination and verify the purity of the sample fixatives.

Obtaining the Field Blank Water

Before leaving the office, obtain the deionized water by collecting it directly from the laboratory supplied containers.

Procedures for obtaining water from the laboratory supplied containers are as follows:
1. Fill up an unused, one-gallon cubitainer with some water (approximately 100 mL).
2. Screw on the lid, shake the rinse water, and dump. Repeat.
3. After two rinses, completely fill up the one-gallon cubitainer, expunge any remaining air, and place in the vehicle to be used in the field as a source for the field blank water.

Field blanks are to be prepared in the field only and not in the laboratory or garage. A stream location is sometimes designated on the sample list for a field blank. If you miss the exact location indicated on the sheet, prepare a field blank at the next location. The reason why field blanks are indicated on your list is to remind you to do it AND to assure that field blanks are prepared at random locations and times.

A field blank will consist of any parameters that are or may be analyzed during the work week. This may include:
- 1 full cubitainer for Unfixed Samples (Chlorides, Hot Acidity, Alkalinity, TSS, Sulfates, Lab pH, Lab Cond., Cold Acidity, Total Orthophosphate, etc.)
- 1 full cubitainer for Sulfuric Acid Preserved Samples (Total Phosphorous, TKN, NO_2-NO_3-N, Unionized NH_3)
- ½ full cubitainer for Nitric Acid Preserved Samples (All Total Metals)

- ½ full cubitainer for Filtered Nitric Acid Preserved Samples (All Dissolved Metals)
- ½ full cubitainer for Filtered Unfixed Samples (Dissolved Orthophosphate)

Do not prepare a field blank for fecal samples, as the deionized water is not sterile.

Field Procedures

1. To prepare a field blank, retrieve your pre-filled one-gallon cubitainer with DI water from storage in the vehicle.
2. Label an appropriate number of one liter cubitainers in a manner that it will appear to be an actual water sample to the lab, but will also be recognizable as a field blank to WAB employees.
3. Fix and handle the samples as you would do for a stream sample by substituting the DI water in the one-gallon cubitainer for actual stream water (including filtering for dissolved parameters if that was or will be done during the week).
4. After the sample has been submitted to the lab, write "FIELD BLANK" at the top of the DEP copy (white) of the Analysis Request Form with Chain-of-Custody (COC) before turning it in with the other forms.

Duplicate Samples

Both duplicates are collected at the same date and time and literally side by side by different individuals. If the sampling team consists of one person, as is often the case during a TMDL assessment, the duplicate is still performed by the one sampler. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been disturbed by the first duplicate. TMDL replicates are collected at any TMDL site with the full potential of parameters on the TMDL list. TMDL replicate sites are not specifically assigned; however, field crews should not repeatedly duplicate the same site.

Duplication will be limited to the water quality parameters assigned to that site; *i.e.*, if the site is fecal only, just do fecal. Duplicates for lists that have varying water analysis suites should be conducted at sites where the most parameters on the list are collected (if such sites exist on the list) and, if repeated, should be rotated to different sites each sampling event.

Results of the duplicates are compared and any samples not falling within an acceptable range are examined for sampling error. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual

techniques will be documented and used in future training sessions or individual re-training.

Note: If two people are involved in collecting a duplicate, each person should filter his or her own sample and not filter the other person's sample.

Chapter IV. STREAM FLOW MEASUREMENT PROTOCOLS

Section A. Sum of Partial Discharges Method

Most discharge measurements of stream flow are made by the **Sum of Partial Discharges Method** using a velocity-meter because it is adaptable to a wide range of velocities and is practically unlimited as to the total discharge that can be measured. Essentially, the method consists of: 1) measuring the velocity of flow in and the area of each of several parts of a cross-sectional transect; 2) computing the discharge in each part as the product of the velocity and area; and 3) summing the partial discharges to obtain the total.

The usual method of making a discharge measurement is explained in ***Figure 39***, which shows the cross-section of a channel.

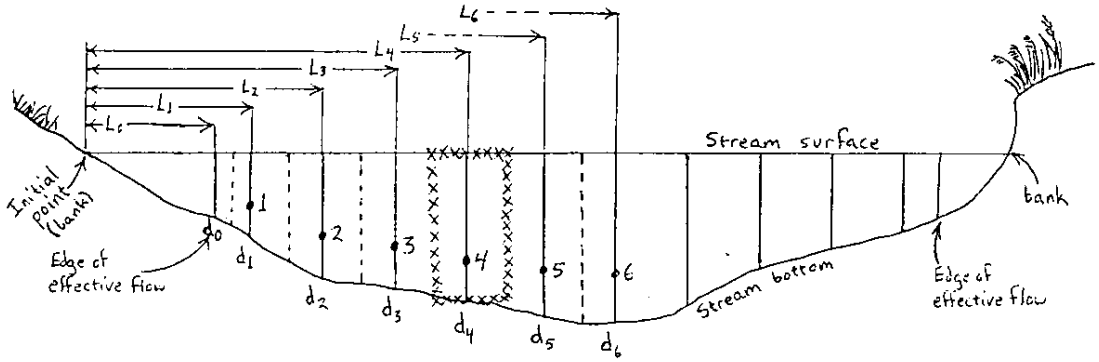


Figure 39. Cross section of stream channel

Where:

1, 2, 3, 4, 5, 6, - Velocity measurement points or observation points for each of six consecutive partial sections.

L_1, L_2, L_3, L_4, L_5, L_6 – Distances in feet from the initial point to each observation point's vertical intersect with the stream surface for each of six consecutive partial sections.

d_1, d_2, d_3, d_4, d_5, d_6 – Depths of water in feet at the observation points in each of six consecutive partial sections.

Dotted lines indicate the boundaries of partial sections.

The depth of water is measured by rod at observation points 1, 2, 3, 4, and so forth. The velocity of the water is measured by velocity meter at each of these locations at such position(s) in the vertical that the mean velocity in the vertical is obtained.

The discharge past a partial section is computed by the following equation:

Equation 2. Calculation of Partial Stream Flow or Discharge

$$\mathbf{Q_4 = (V_4)(d_4)\ \frac{[\ (L_5\ -\ L_3)\ ]}{2}}$$

Where:

Q_4 = discharge or flow in cubic feet per second through partial section 4 (***see Figure 39 above***)

V_4 = mean velocity in feet per second at location 4.

d_4 = depth of water in feet and tenths of a foot (not inches) at location 4.

L_3, L_4, L_5 = distances in feet and tenths of a foot (not inches) from the initial point to locations 3, 4, and 5, respectively (***see Figure 39 above***).

The area defined by this formula is that shown by the X-line highlight around location 4 in ***see Figure 39 above***.

The summation of the discharges for all the partial sections is the total discharge of the stream. It is calculated by computer program once all the necessary data are plugged into the program (***See Table 8 on page 144 for an example of the data used to calculate the total discharge or flow***).

Materials and Supplies

1. Wading Rod – for measuring stream depth and setting depth of flow measuring device.
2. Marsh-McBirney Flo-Mate – for measuring water velocity.
3. Tape Measure in feet and tenths – for determining the distance between velocity readings.
4. Flow Record Sheets (***see Chapter II. Section C. Part 2. APPENDIX #1 - Stream Discharge (Flow) on page 78***) – for recording data collected along the flow transect and for final computation of flow.
5. Pencils.
6. Lightweight clipboard with string to drape around neck - for carrying flow sheet while keeping both hands free to manipulate wading rod and Flo-mate.

Part 1. Operation and Maintenance of Flo-Mate

For a more complete description of the Care and Operation of the Model 2000 Marsh-McBirney Flo-Mate, consult the instruction manual provided by the manufacturer (***see Figure 40 below***).

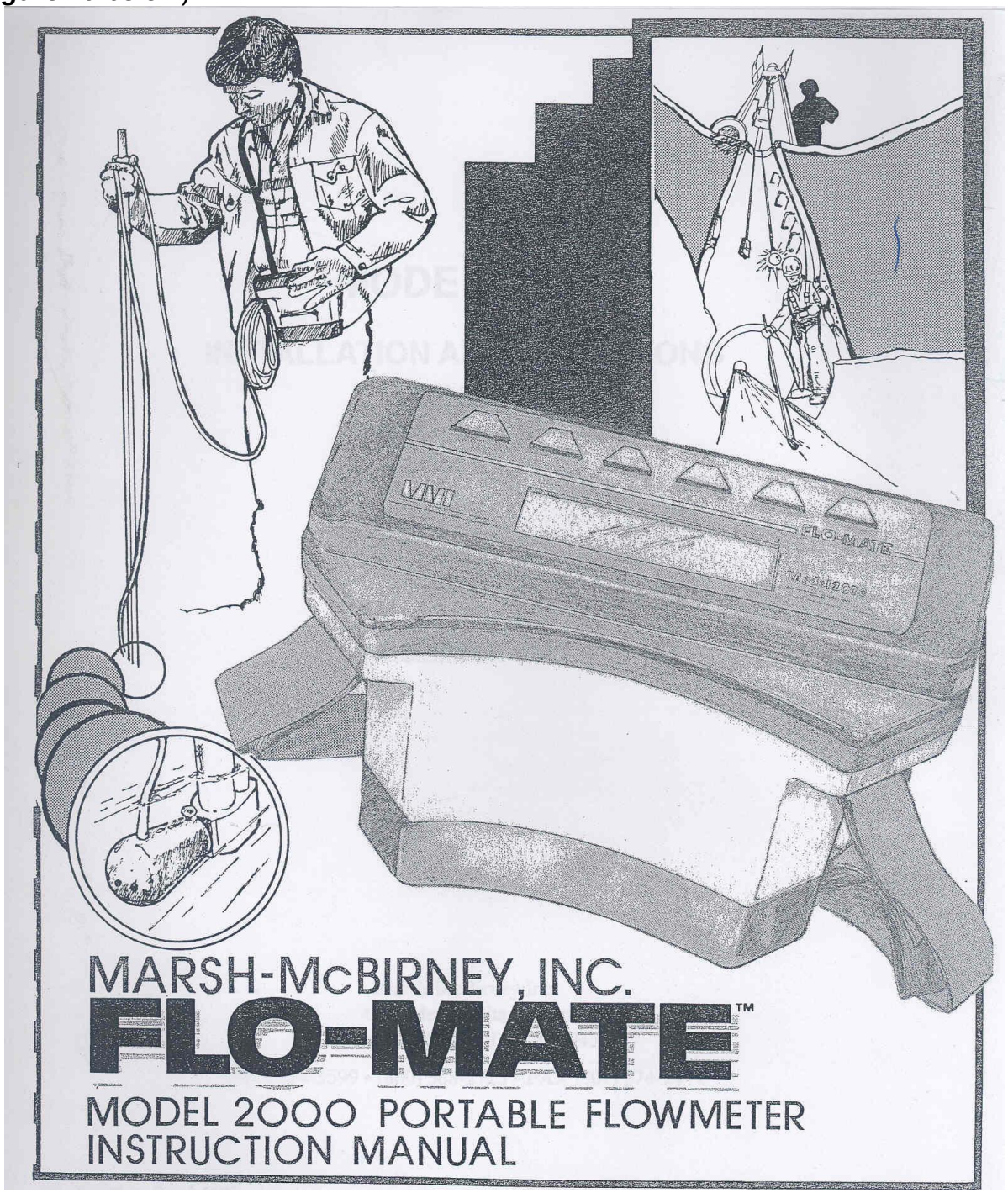


Figure 40. Cover of Model 2000 Marsh-McBirney Flo-Mate Instruction Manual

Theory of Operation (From the Marsh-McBirney Flo-Mate Manual, 1990)

The Flo-Mate measures velocity using the Faraday law of electromagnetic induction. This law states that as a conductor moves through a magnetic field, a voltage is produced. The magnitude of this voltage is directly proportional to the velocity at which the conductor moves through the magnetic field.

When the velocity approaches the sensor from directly in front, then the direction of the flow, the magnetic field, and the sensed voltage are mutually perpendicular to each other. Hence, the voltage output will represent the velocity of the flow at the electrodes.

The sensor is equipped with an electromagnetic coil that produces the magnetic field. A pair of carbon electrodes measure the voltage produced by the velocity of the conductor, which in this case is the flowing liquid. The measured voltage is processed by the electronics and output as a linear measurement of velocity.

Flo-Mate Settings

The use of the function keys are described in Figure 41 below.

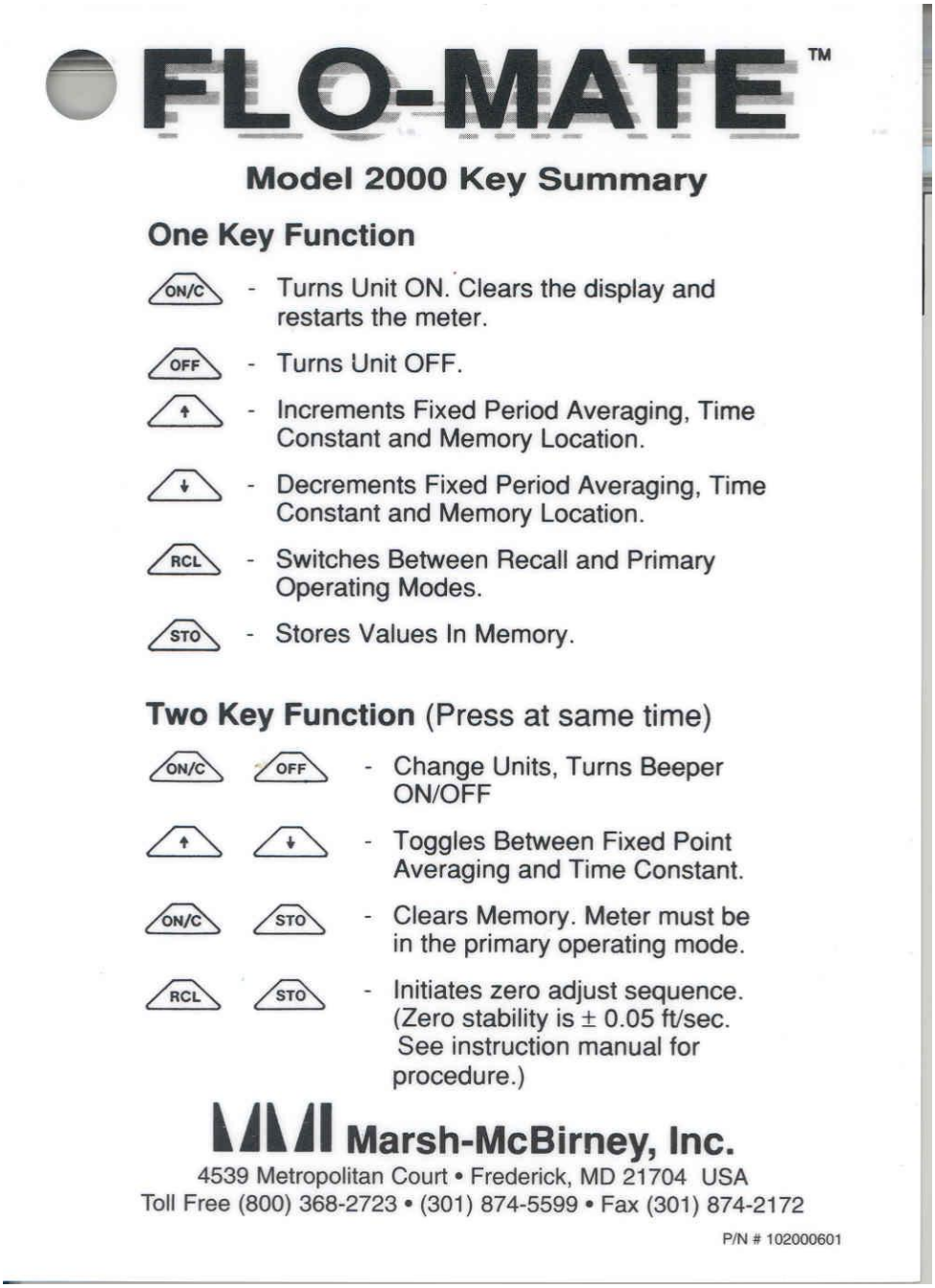
Units-You can check the unit to see that it is reading in FT/S by pressing ON/C and OFF keys simultaneously. Press these two keys until FT/S is displayed. You can choose to have the beeper on or off (watch for the little speaker symbol in the lower right-hand corner) by toggling between:

- FT/S no beeper
- M/S no beeper
- FT/S with beeper
- M/S with beeper

Filtering-The fluid dynamics around the sensor electrodes may cause the readings to bounce around. To stabilize the readings, the output to the display is dampened. The display can be dampened by Fixed Point Averaging (FPA) or by time constant filtering (rC). Fixed Point Averaging is an average of velocities over a fixed period of time. Time constant filtering is a software algorithm that mimics an RC analog circuit.

To check the unit and see what filtering method is being used, press the Up and Down arrow keys at the same time until the meter displays FPA (fixed point average). The display will show the letters rC when you first switch to the time constant mode.

Except for the first period, the display is updated at the end of each averaging period. For example, if the FPA is set to 20 seconds, the display is updated once every twenty seconds. The FPA display will have a horizontal time bar under the velocity output. The time bar provides an indication as to the amount of time left until the display is updated.



FLO-MATE™

Model 2000 Key Summary

One Key Function

ON/C - Turns Unit ON. Clears the display and restarts the meter.

OFF - Turns Unit OFF.

↑ - Increments Fixed Period Averaging, Time Constant and Memory Location.

↓ - Decrements Fixed Period Averaging, Time Constant and Memory Location.

RCL - Switches Between Recall and Primary Operating Modes.

STO - Stores Values In Memory.

Two Key Function (Press at same time)

ON/C + OFF - Change Units, Turns Beeper ON/OFF

↑ + ↓ - Toggles Between Fixed Point Averaging and Time Constant.

ON/C + STO - Clears Memory. Meter must be in the primary operating mode.

RCL + STO - Initiates zero adjust sequence. (Zero stability is ± 0.05 ft/sec. See instruction manual for procedure.)

Marsh-McBirney, Inc.

4539 Metropolitan Court • Frederick, MD 21704 USA

Toll Free (800) 368-2723 • (301) 874-5599 • Fax (301) 874-2172

P/N # 102000601

Figure 41. Key Function descriptions for the Model 2000 Marsh-McBirney Flo-Mate

Time Increment-To set the increment, press the up arrow or down arrow to see if the unit is set to read in 20 second intervals. Note that fixed point averaging is limited to whole seconds in the range of 2-120 seconds.

The Watershed Assessment Branch (WAB) has a standard of collecting velocity measurements in feet per second using Fixed Point Averaging Filtering in 20 second intervals.

Maintenance of Marsh-McBirney Flo-Mate

According to the Marsh-McBirney Flo-Mate manual, the only routine maintenance of the unit is confined to cleaning the sensor, changing the batteries (two alkaline D Cell batteries), and zero-adjusting the instrument. Any instrument calibration or repair must be conducted by the manufacturer.

Cleaning

Non conductive coatings like oil and grease can cause errors or interfere with the velocity readings. This can be remedied by routinely cleaning the sensor head with soap and water. **Do not use any solvents to clean the sensor head!** If the problem persists, clean the electrodes with very fine grit (600) sandpaper. In error readings persist in the field or where you do not have immediate access to soap and water, you may try to use your fingers underwater to rub away oil and grease from the sensor electrodes. Fine clay (smaller than 600 grit) and pencil erasers may also be effective at removing oil and grease.

Zero Check and Adjust

Every month, the meter will need to undergo a zero check and possibly a zero adjust.

Zero Check Procedure

1. Clean the sensor with soap and water as stated above. Make sure the unit is set to operate in FT/S using fixed point averaging (FPA) filtering (***see Flo-Mate Settings above for more information***).
2. Place the sensor in a plastic five-gallon bucket of water. Sensor should be 3 inches away from the sides and bottom of the bucket. This could possibly be achieved by attaching the senor to the flow rod during the zero check and balancing the flow rod in a hands-free standing position in the bucket. Make sure the water is not moving and wait 10 to 15 minutes. DO NOT TAKE ANY READINGS WHILE WAITING.
   NOTE: When conducting the Zero Check or Zero Adjust procedure at the WVDEP Headquarters in Charleston, be sure that there is not a passing train during the Zero Check Procedure. It has been observed that passing trains create excessive vibrations in the laboratory end of the building to the extent that it may seriously affect the Zero Check.
3. Using a filter value of 5 seconds (*i.e.*, change the instrument's increment reading from 20 to 5 seconds using the up and down arrows) take a reading from the unit in the still bucket. BE SURE NOT TO CAUSE ANY EXCESS VIBRATIONS VIA THE SENSOR CORD OR FROM UNNECESSARY MOVEMENT. Zero Stability is a reading of +/- 0.05 ft/sec. Record the unit

number, the initial zero stability reading for entry into a database later. If the reading is out of this range, the unit will need to be Zero Adjusted (Steps 4-8). If the unit is within the acceptable range, restore the increment reading back to 20.

Zero Adjust Procedure

Note: Each key in the zero adjust sequence must be pressed within 5 seconds of the previous key. If the time between key entries is longer than 5 seconds or if a wrong key is pressed, the unit will display an ERR 3. Turn the unit OFF then back ON and try again.

4. Keep the position of the sensor as described above in step 2. Press STO and RCL keys at the same time. The unit will display a "3".
5. Use the "down" arrow to decrement to zero. The number "32" will be displayed.
6. Unit will decrement itself to zero and turn off. Zero adjust is complete. Return to step 1 above and repeat the zero check to make sure the instrument is within the acceptable range and record the final zero stability reading. If the unit is still out of range, you may attempt the zero adjust sequence again. If repeated attempts to zero adjust fail to correct the unit, it will need to be sent back to the manufacturer for recalibration and possibly repair. If the final zero check is acceptable, restore the increment reading back to 20 and turn the unit off.

Flow Meter Accuracy

The Flow Meter accuracy is +/- 2% of reading + zero stability (which is +/- 0.05 ft/sec). The range is -0.5 to +19.99 ft/sec.

Using the Flo-Mate

1. Insert the sensor peg on the bottom of the sliding flow rod shaft into the hole on the back of the sensor. Position the three electrical sensors horizontal to the plane of the bottom (the foot) of the flow rod. If the sensor is properly positioned, the cord should be coming out of the top of the sensor perfectly parallel to the flow rod shaft. Tighten the thumb screw so that the sensor will not slide off or rotate out of position.

2. AFTER you have set up your flow transect (***See Setting up the Transect below under Stream Flow Measurement Procedures on page 145***) and have the sensor in the water, turn the instrument by pressing the ON/C button.

3. Allow the instrument to run through two cycles. The first 20 second cycle is to allow the turbulence and eddies around the flow rod and sensor to reach equilibrium so that the final reading during the second cycle is as accurate as possible. Since the real time readings are only visible during the first cycle (***as stated above in Flo-***

Mate Settings on page 139), it is recommended that you initialize the second cycle by pressing the ON/C button. In the rare case that the readout during the first cycle stabilizes well before the end of the 20 second cycle, you may initialize the second cycle early by clearing the display. At the end of the second cycle, record the readout. This readout is the average velocity for the second 20 second cycle only. It does not consider the velocity data gathered during the first cycle.

4. Move the sensor to the second increment in the stream, and press the ON/C button to initiate a **new** cycle to begin (you don't want the 20 second cycle reading to include movement while you were moving the wading staff). Repeat Step 2. Repeat steps 2 & 3 until the flow transect is complete.

Notes about error readings

The purpose of displaying errors is to alert the user of possible problems with either the unit or application. Errors can be displayed as messages or numerical codes. There are three error messages and five numerical codes. With the exception of Err 2, error codes freeze the display. Turn the unit OFF then back ON to clear the display. If after corrective action the error still exists, call the factory. Descriptions of the meanings of each error message are as follows:

Low Bat – Indicates low batteries. Replace the batteries

Noise – Indicates excessive electrical noise is present in the velocity that will interfere with normal operation. This will cause the display to blank out.
Note: The noise flag usually comes on for few a seconds after the sensor is submerged even though there is no noise present. This is normal.

Con Lost – Indicates that either the sensor electrodes are out of the water or they have become coated with oil or grease. After 5 minutes, the unit will turn itself OFF. If the electrodes are coated, clean them (see *Cleaning* above).

Error#1 – There is a problem with sensor drive circuit. Check sensor disconnect.

Error#2 – Memory full error. Memory must be cleared before another reading can be stored.

Error#3 – Incorrect zero-adjust-start sequence. Reinitiate zero-adjust-start sequence.

Error#4 – Zero offset is greater than the zero adjust range. Repeat the zero-adjust procedure. If the error is still displayed, the unit needs servicing.

Error#5 – Conductivity lost or noise detected during zero adjust. This is usually caused by the sensor being out of the water.

Note: The sensor on the flow meter has an operating temperature of 0^0C to 72^0C (32^0F to 160^0F). The electronics on the flow meter have an operating temperature of 0^0C to 50^0C (32^0F to 122^0F).

Part 2. Stream Flow Measurement Procedures

Table 8. Example of Flow Measurement Form: Recording field data.

STREAM DISCHARGE MEASUREMENT (Calculated in cubic feet per second – cfs)					
Measurer	*Doug Wood*	*Meter ID*	*2*	Time	*12 noon*
Location	Distance	Depth	Velocity	Discharge cfs	
Bank	1.3				
EEF	2.6	This is the Edge of Effective Flow			
1	2.93	0.64			
2	3.59	0.90			
3	4.25	0.93			
4	4.91	0.97			
5	5.57	1.01			
6	6.23	1.12			
7	6.89	1.06			
8	7.55	0.92			
9	8.21	0.88			
10	8.87	0.63			
11	9.2	0.60			
EEF	9.9	This is the Edge of Effective Flow			
Bank	10.5				
Notes:	Some turbulence from rocky substrate. Slightly skewed flow. Drizzling rain.				

9.2 ← Edges of effective
-2.6 ←
6.6 ← Effective flow width

6.6 ÷ 10 = 0.66 maximum distance between each of the ten measuring points.

Note: ALL MEASUREMENTS TAKEN IN INCHES MUST BE CONVERTED TO FEET AND TENTHS/HUNDREDTHS IN ORDER TO GIVE A WIDTH MEASUREMENT THAT CAN BE USED IN CALCULATING FLOW/DISCHARGE IN UNITS OF CUBIC FEET PER SECOND (cfs).

Setting up the Transect

1. Select a stream reach having the following characteristics:
   a. A straight stretch of water with the horizontal velocity vectors running parallel to the stream bank
   b. A stable, even streambed without large rocks, weeds and protruding obstructions that create turbulence.
   c. A level streambed configuration to reduce variation in the vertical components of velocity.

All of these conditions are seldom satisfied. Nevertheless, select the best possible reach using these criteria.

2. Next, select a flow transect. An ideal transect:
   a. Is perpendicular to the direction of flow (velocity vectors). It is often very hard to find an area of stream where 100% of the flow is perpendicular to the flow transect. If there is no better place to take the velocity readings, then the number of varying velocity vectors on a flow transect should be kept to a minimum.
   b. Has uniform bed and stream banks
   c. Has a minimum velocity of 0.05 feet/second. Avoid transects with eddies or areas of "dead" water. **(However, you should include positive number readings of less than 0.05 feet/second on the flow sheet should you encounter them.)**
   d. Adequate depth for the meter to function. Typically, this means the entire velocity probe bulb should be immersed. **Please note if readings were taken with part of the bulb exposed to air. If the bulb was removed from the flow rod and placed on the substrate, please note this as well.**

   Note: You may alter the channel to help meet these requirements at the selected site <u>before</u> you begin making any measurements, but <u>NEVER AFTER</u> measurement has begun.

3. Determine the width of the stream by placing a tape measure perpendicular to the stream flow.

4. Determine the width of "effective flow". The effective flow is the segment of the transect having measurable downstream velocity and exclusive of "dead" areas or reverse flows (eddies) beside the stream banks. Do not exclude similar anomalous flow areas that do not touch the banks from the width measurement (bank to bank). For simplicity, utilize this effective flow width in establishing endpoints for measurement of velocity and depth (***see Table 8 above***).

5. Determine spacing of velocity measurements. There is no set rule about how to space the measurements other than there is a required minimum number of measurements depending on the width of the effective flow (see arrow bullets below this paragraph). **Velocity measurements should be spaced to document and define areas of turbulence, extreme changes in velocity, and sudden changes in depth. For example, if the there is a large obstacle to the flow like an unmovable boulder directly in, in front of, or behind the flow transect, a few extra measurements should be taken on each side of the boulder and spaced closer than the average increment. The velocity measurements can also be spaced farther apart in areas where the velocity and depth are more uniform.**

   - In streams with effective flow width greater than 3 feet, take no fewer than ten nearly even-spaced measurements within the effective flow transect. For example, if the effective flow is 3.5 feet wide, the minimum number of ten measurements could be taken every 4.2 inches (this is 10% of the effective flow width, 42 inches).
   - If the effective flow width is less than 3 feet, take as many measurements as possible using an increment no smaller than 0.3 ft using best professional judgment.
   - If the effective flow is >10 feet, a minimum of 20 measurements should be obtained.

6. Record the following information on the ***APPENDIX #1 -*** Stream Discharge (Flow) ***form (see Figure 27 above on page 79)***:
   a. Record the flow measurer.
   b. The time of the flow measurement.
   c. The assigned number of the flow meter used.
   d. Any conditions that might affect the flow measurement (*i.e.,* wind, rain, skewed bottom configuration, ice and leaf packs in the water, necessity of removing the probe bulb from the rod due to extremely shallow water depth).

Taking Flow Measurements

7. Begin Flow measurement (***See Figure 39 above on page 136***):

   a. For simplicity, establish the bank as the initial point. Then record the distance (L_0) from the initial point to the edge of effective flow. Record the depth at the edge of effective flow (d_0).

   b. Record the distance (L_1) from the edge of effective flow to the first velocity measuring point along the tape. (This point is called a “vertical”). This first vertical should be established at a distance very close to the edge of effective flow (about 0.3-0.5 feet). Record water depth (d_1) at this vertical.

c. If the depth is less than 2 feet, use the one-point 0.6-depth method for measuring velocity: Adjust the rod so that the sensor is at the depth that is six-tenths below the water surface (indicated by "1" in ***Figure 27 above on page 79)*** by lining up the rod's sliding "foot" with the tenth scale vernier on the top of the flow rod (*e.g.*, if total depth is 0.9 foot, then line up the "0" line on the rod's sliding foot scale with "9" on the tenth vernier. If depth is 1.2 feet, then line up the "1" sliding foot scale line with the "2" vernier line). If the depth is greater than 2 feet, two readings are taken: 0.2 and 0.8 from the surface.

   - To set the sensor at 0.2 of the depth, multiply the total depth by two and repeat the above procedure. For a depth of 2.7 feet, this would be 5.4 feet. Line up the 5 on the foot scale with 4 on the tenth scale.
   - To set the sensor at 0.8 of the depth, divide the total depth by two and repeat the above procedure. For a depth of 2.7 feet, this would be 1.35 feet. Line the 1 on the foot scale with 0.35 on the tenth scale.

   Note: To obtain a discharge figure for entry in the computer program for a two-reading measurement, calculate the average of the two velocity readings, and use this average velocity for calculating the discharge in the two-reading transect increment.

d. Place the sensor at the proper depth and allow it to adjust to the velocity before starting the observation. The following precautions should be taken:
   - Hold the rod perfectly straight up with the sensor pointing upstream into the velocity vector (parallel). The bow wave produced by the wire at the top of the sensor will be symmetrical if the sensor is pointing directly into the flow. A ribbon attached to the bottom of the flow rod also helps visualize the flow direction.
     Note: The manufacturer says the sensor shape produces a cosine response that greatly reduces errors due to sensor positioning. For example, if the front of the sensor is pointed away from the flow at a 10° angle, the cosine of 10° is 0.98480. This is only 1.5% lower than the actual velocity. What this means is that even though you may not have the flow reading positioned 100% parallel to the flow vector, a few degrees off of center will yield pretty accurate results. Nevertheless, you should still try to position the sensor as close to parallel to the flow vector as possible! Experience shows the manufacturer's rhetoric is just that. In the real world, slight angles away from parallel with the velocity vector, produce noticeably different velocity readings.
   - Stand downstream from the meter and at a distance so that you do not create turbulence that will impact the reading. In very small streams, attempt to straddle the wetted area to decrease your feet's contributions to altering the flow.

e. Allow the Flo-Mate to go through two complete cycles (40 seconds total) as described in ***Using the Flo-Mate above on page 142***.

f. Record the second readout on the "velocity" column on the form.

8. Repeat Step 7 at each vertical, recording the distance, depth, and velocity. Be sure to record the distance to and depth of the far bank and vertical point of effective flow.

Calculating Flow Using a Spreadsheet

1. Open Excel, and then open the file in Q:\WATER RESOURCES\WAB\TOOLS\Flow Template.xls.
2. At the bottom of the spreadsheet select the tab for the number of actual flow readings obtained. If there is no tab for the number of measurements you have taken, you will need to insert a new worksheet, copy contents of another worksheet into the new worksheet, and add or delete rows as needed.
3. Type in the left and right edge-of-effective-flow values in the "no flow" rows of the "Distance" column.
4. Enter the distance, depth, and velocity in the appropriate columns.
5. The flow or discharge in CFS is automatically calculated in the lower right-hand corner of the spreadsheet.

Section B. Measuring Flow Using a Bucket and Stop Watch

On some rare occasions, it may be possible or more practical to measure flow using a bucket of known volume and a stop watch. Such instances include measuring flows coming out of a pipe where there is adequate room to place the bucket underneath the pipe. The procedure to measure a flow in this manner is to measure the seconds it takes to fill up the bucket. It is recommended that you measure this at least three times and take the average time as the final reading. Converting this measurement into cubic feet per second (CFS) units may require some research into conversion units (*e.g.*, gallons or liters into cubic feet). However, the flow template sheet mentioned above (located at Q:\WATER RESOURCES\WAB\Flow Template.xls) contains a worksheet already calibrated to convert measurements using gallons per second into cubic feet per second. Simply enter the seconds it took the fill up the bucket, the volume of the bucket in gallons and the resultant CFS will be calculated at the end of the row. Document on the field sheet the flow method and the individual and final average CFS.

Section C. Measuring Flow Using a USGS Gauging Station

In some instances, a sampling station on a larger stream may coincide or be very near a USGS Gauging Station. If this is the case, then flow readings could be read from the USGS gage. USGS maintains a website to access current and historical stream

discharge and stream stage data from the gages. The web addresses for West Virginia daily stream gage data are:
http://waterdata.usgs.gov/wv/nwis/current?type=dailystagedischarge
http://wv.usgs.gov/
http://waterwatch.usgs.gov/?m=real&r=wv
See Figure 77 and Figure 78 in Chapter X. Section A. Part 4. Measuring Stream Flow starting on page 230 for examples of USGS website data displays.

Once you have accessed a specific gage, you will need to use the real time and table options to view hourly gage data. Record the USGS gage number, discharge and/or stage readings, for the date and time sampled, onto the analysis request form. Hourly data are available for up to 60 days, from the date a site is visited. Daily averages are available up to two years.

Flow Measurement Quality Assurance/Quality Control

Before use, each Flo-mate velocity-meter should be examined for wear and fouling, and adjustments should be made as required.

Zero check and adjust logbooks are maintained for each instrument and entered into a database. Any instrument failing to meet zero check requirements is zero adjusted or shipped to the manufacturer for diagnosis and repair. Flow meters are checked monthly and may be zero checked and adjusted in the field if necessary.

Each flow meter has an identification number, which is recorded on the habitat assessment sheet each time it is used. If any instrument fails a zero check, readings taken prior to the failed zero check will be examined for reliability and accuracy. Documentation of the instrument used at each site will help to keep data loss to a minimum. All repair logs to flow meters are documented and maintained by the manufacturer and noted in a repair log.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the measurement and collection of flow data is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. Individual training will occur simultaneously on the same stream so the results can be compared to the group average. Readings that deviate exceptionally from the norm will be examined for errors. In the field, individuals who are more experienced in determining flows will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to measure flows solo. This document is also provided to all program personnel for review and use in the field.

Chapter V. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS

Overview

Definitions

MACROINVERTEBRATES - Animals that are large enough to be seen with the naked eye and do not have a backbone.

BENTHIC ORGANISMS (or BENTHOS) - Living organisms that reside on the bottom of streams, rivers, or lakes. Benthos may include vertebrates, invertebrates, or plants.

KICK - One method for collecting benthos. A hand-held net is held in the stream. The stream bed upstream of the net is disturbed using a kicking motion to dislodge the organisms, which then float into the net.

Benthic Macroinvertebrates as Environmental Indicators

Benthic macroinvertebrates are small animals living among the sediments and stones on the bottom of streams, rivers, and lakes. Insects comprise the largest diversity of these organisms and include mayflies, stoneflies, caddisflies, beetles, midges, crane flies, dragonflies, and others. Other members of the benthic macroinvertebrate community are snails, clams, aquatic worms, and crayfish. These organisms are extremely important in the food chain of aquatic environments. They are extremely important in the food chain of aquatic environments as they are important players in the processing and cycling of nutrient and are major food sources for fish and other aquatic animals.

Benthic macroinvertebrates have been used for many years to assess water quality. Currently, they are utilized throughout the world in water quality assessments, as environmental indicators of biological integrity, to describe water quality conditions or health of aquatic ecosystems, and to identify causes of impairment. Benthic macroinvertebrate communities are known to respond to a wide array of environmental stressors, and in different ways. This response will often make it possible to determine the type of stress that has affected the community. Many macroinvertebrate taxa have relatively long life cycles. Thus, community structure is a function of past water quality conditions.

Basis of Sampling Method

The sampling methods to be used in the WVDEP Watershed Assessment Branch (WAB) are qualitative in nature and are outlined in "Rapid Bioassessment Protocols for Use in Wadeable Rivers and Streams, Second Edition" - U.S. Environmental Protection

Agency, July 1999 (EPA 841-B-99-002) (***see Figure 11. Cover of EPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Second Edition) in Chapter II. Section C. Part 1. PAGES 5, 6, 5a, and 6a starting on page 52***). This protocol has been adopted for use by many states and organizations. The WAB will utilize the Single Habitat Approach when possible, using a rectangular dip net (0.5 m wide) or smaller (0.3 m wide) D-net with 595 µm mesh size to sample riffle/run habitats. The Multi-habitat Approach (also called MACS, which stands for Mid-Atlantic Coastal Streams) may be used in slow-moving wetland type streams, using the smaller D-net**. It is important to note that the following protocols were established for use by the Watershed Assessment Branch monitoring programs and were intended to provide cost-effective techniques with comparable data across the state. Special projects outside of the Watershed Assessment Branch monitoring agenda (*i.e.*, point source surveys, spills, large river monitoring) may not allow strict adherence to these protocols.**

The sampling protocols are listed and prioritized below:

1. Rectangular Dip Net - for riffle habitats $\geq$ 0.5 meter wide
2. D-Frame Net - for riffle habitats < 0.5 meter wide
3. D-Frame Net - used in the absence of moving water (for use in low-gradient streams and glide/pool habitat – MACS or Multi-habitat Approach)
4. Hand Picking - used in very small streams where other sampling apparatus cannot be used.

These methods are described in detail in the subsequent sections.

Selecting Sampling Sites

Predominantly, streams in West Virginia are high gradient with coarse substrate materials such as boulder, cobble, and gravel. These physical conditions are responsible for the typical riffle/run habitats commonly found in most areas of the state. WAB establishes sample sites and assessment reaches on streams based on the best available riffle/run habitat (random sites excluded). There should be at least one square meter of riffle/run habitat in the assessment reach to obtain a complete benthic macroinvertebrate sample.

It is important that the sampling method be selected based on the availability of the reference condition (riffle/run predominant for most of WV) and not of potentially impaired streams. For example, sampling decisions should not be altered for situations where the amount of cobble/gravel substrate in streams influenced by heavy sediment deposition may be substantially reduced from the amount of cobble/gravel substrate expected for the region. That is, sample sites on streams with heavy deposits of fine sediments should not be avoided if it is determined that the sedimentation is not typical of the area and has resulted from poor land-use practices. Occasionally, low gradient streams are encountered that have heavy deposits of fine sediments as a result of naturally high sedimentation rates. In this case, the Multi-habitat Approach should be employed. Currently, WAB does not conduct benthic assessments on low gradient

streams unless there is a special interest for the resultant data. The decision to sample a particular stream site is field based and should be made after corroboration by WAB team members or by the most experienced person. In any event, detailed notes describing the situation should be recorded on the field form.

Another concern when locating a benthic sampling site is tributaries or sources that enter the stream within the reach and may significantly alter the water quality. It is extremely important that the benthic data collected always match the water chemistry observed and collected at the X-Site. During the site selection and planning that occurs in the office, every effort is made to try to avoid such situations by locating the site above tributaries and known sources. However, occasionally sources are unknown or moving the site is not possible (*e.g.*, randomly selected sites). The most important thing to do is to always inspect the sample area as thoroughly as possible prior to beginning the benthic collection. Some things to look for are:

1) Significant change in water chemistry (*i.e.*, pH, conductivity, DO, Temperature) from above the source to below the source.
2) Visual indicators that the tributary or source has a significant impact on the mainstem area downstream (*e.g.*, sudden appearance of hydroxides, oils, grease, etc. below the tributary or source).
3) In larger streams, pluming of water chemistry along one bank due to an inadequate mixing zone in the mainstem.

In such cases, the entirety of the benthic kicks should be located either above or below the source. Unfortunately, outside of specific directions on the field list, there is little in the way of guidelines on picking one or the other and the samplers must rely on best professional judgment. In the case of a randomly selected site where the X-site is located below a source or tributary with a significant water quality impact to the stream and there is inadequate room to collect benthos in the area below the source, it would be best to treat the source or tributary with significant water chemistry issues using the same rules as sliding the reach downstream around the X-site to avoid crossing stream orders (***see Chapter II. Section A. Part 2. Sliding the Reach starting on page 11***) so that the X-site and benthic collection area are in similar water quality.

Before sampling begins, a 100-meter assessment reach is established containing the X-site (usually located at the downstream terminus of the reach). All assessment activities are conducted within this designated reach including the collection of water samples, benthic macroinvertebrate samples, and habitat assessments. The benthic collector should select sampling points with the intent to make collections throughout the entire 100 meters in a diversity of the best available habitats. For example, look for varying conditions within the reach such as fast and slow riffle/runs, deep and shallow riffle/runs, shaded and exposed riffle/runs, and sample from the best available in each observed. In some instances, the best available habitat (*e.g.*, riffle) may be limited to a small area within the reach. In this case, collections should be made within those areas only. However, if riffle areas occur throughout the 100-meter reach, an effort should be

made to collect from as many different points within the reach as possible. It is important to sample the diversity of riffle/run conditions if they exist.

The various habitat types that may be encountered are defined as follows:

Pool - Still water with low velocity. Water surface is smooth and glassy. Usually deep compared to other parts of the channel.

Glide - Slow moving water with a smooth, unbroken surface. Turbulence is low. Usually shallow compared to other parts of the channel.

Run – Similar to glide but water is moving slightly faster. Turbulence is low and the surface is without ripples that produce gurgling sounds. Runs may have small waves.

Riffle - Water moving with small ripples, waves and eddies. Produced a babbling or gurgling sound.

Snag - Submerged woody debris (logs, root wads, etc.).

Submerged Macrophytes - Aquatic vegetation growing beneath the water surface.

Vegetated and Undercut Banks - Stream banks having submerged vegetation (shrubs, etc.) and/or root wads.

Section A. Benthic Macroinvertebrate Sampling

Materials and Reagents

See Figure 42 & Figure 43 for Diagrams & Picture of most of these materials.

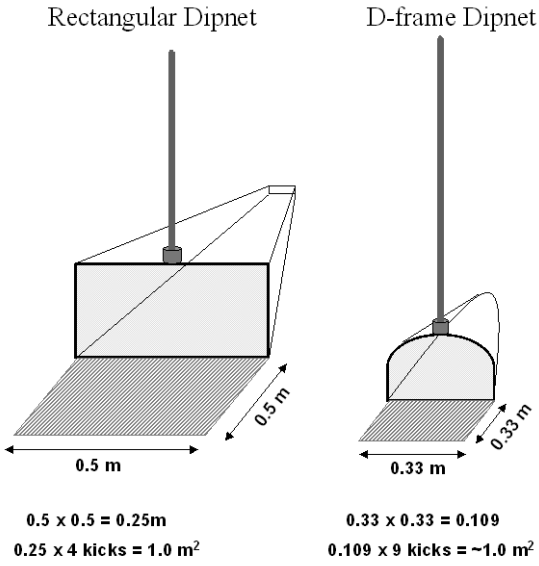


Figure 42. Diagram comparing the dimensions and number of kicks necessary to sample 1 m2 of a Rectangular Frame Dip Net versus a D-Frame Dip Net

Figure 43. Photo of Materials used in Benthic Macroinvertebrate Sampling

1. Rectangular Frame Dip Net – A net with a 0.5 m wide and 0.3 m high frame with 595 µm mesh openings and 0.5 m nylon bag attached to a four foot pole will be used to collect benthic macroinvertebrates in riffles and runs.
2. D-Frame Dip Net - A D-frame (D-net) aquatic dip net with 595 µm mesh openings and 1 ft. nylon bag will be used to sample streams that are too small to be sampled using the rectangular frame dip net.
3. Five-gallon bucket - to composite kick samples in the field.
4. 30 mesh sieve (600 µm) - to remove small particulates and water from samples.
5. Small dish washing scrub brush – aid in removing macroinvertebrates from stream substrate particles such as cobble and cleaning the net.
6. Small plastic container or tray – to temporarily hold the organic materials and elutriate.
7. Gallon-sized sample jars - containers to hold benthic sample and associated debris.
8. Inside and outside labels - for sample identification and tracking.
9. Fine-tipped forceps – for removing organisms from net or sieve.
10. One liter squirt bottle – for washing benthic organisms from the bucket, sieve, and elutriate container.
11. 95% Denatured ethanol - for preservation of benthic macroinvertebrates.
12. Ice chest / cooler - for the storage of samples during transport.
13. Sample log book - for tracking the locations of the biological samples.

14. Scientific collecting permit – Obtained yearly by Watershed Assessment Branch from the WVDNR.

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.*, glass, metal, wire, etc.). They should also be worn during sample preservation or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes.

Part 1. Sample Collection Methods

Before any benthic sampling event:

- Fill out a pre-printed sample label with a No. 2 pencil. Attach to the outside of the sample jar using clear, waterproof tape. Fill out a pre-printed sample label made of waterproof paper for the inside of the sample jar. Some place the inside label inside the jar before the sample is collected; others do so after the sample is collected. **Just make sure that the inside label gets inside the jar.**
- Fill the sample jar ½ full with 95% denatured ethanol.
- If using a net, check the net to ensure there are no holes or benthic remnants of previous samples. If there are holes or tears in the net, it should be repaired immediately before the next sample is collected and/or replaced as soon as possible.
- Wash the net in the stream to ensure that there are no benthic remnants of previous samples.

A. Rectangular Dip Net (Riffle/Run Habitats = Comparable)

This method is used in streams having riffle/run habitat and a width ≥ 0.5 meter. This method is to be used even when there is no cobble substrate in the riffle/run area. If the stream has enough flow to wash benthic macroinvertebrates into the net this is the method to use.

1. Select a riffle/run area to sample. Position the net on the stream bottom so as to eliminate gaps under the frame with the net opening upstream. Large rocks or logs that prevent the net from seating properly should be avoided (***see Figure 44 on right***).

Figure 44. Photo of Rectangular Frame Dip Net being placed on stream bottom

2. Hold the sampler in position on the substrate while checking for heavy organisms such as clams and snails in an area of about 0.25 m^2 (0.5m wide net x 0.5m upstream) in front of the net. Hand-pick these organisms and place them in the net or the bucket if placed nearby. Do not collect large freshwater mussels! Some mussel species are endangered and should not be disturbed. Record their presence on the field form and identify them if possible.

Figure 45. Photograph of the Brushing process in front of the net.

3. Brush the surfaces of all coarse gravel, cobble, boulder, and bedrock substrate (***see Figure 45***). If the substrate is removable, pull it up and hold it underwater in front of the center of the net while brushing all surfaces so that dislodged organisms flow into the net. Cleaned substrate should then be set aside. In low flow situations, these rocks can be placed at the edge of the net in a manner that increases the amount of water flowing through the net. Large substrate that is partially in the kick sample area should only be brushed on that portion which resides in the 0.25 m^2 kick area.

4. Hold the net handle securely while kicking the substrate vigorously for 20 seconds to a depth of 10 cm in an area of about 0.25 m^2 (0.5m wide net x 0.5m upstream) in front of the net (***see Figure 46***). At this time it may be possible to remove large objects (*e.g.*, cobble, large gravel) from the net while the water is still sweeping through the net.

Figure 46. Photograph of the Kicking process in front of the net.

Figure 47. Photographs showing the removal of the net from the water with an upstream motion.

5. Remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net (***see Figure 47***). Empty the contents of the net into a five gallon bucket that is partially filled with water (***see Figure 48***). Emptying the net after each kick sample is recommended because debris can clog the net mesh causing reduced flow- through and back eddies, both of which can result in the loss of organisms. It is not necessary to fine pick every last item from the net at this point, just the get the bulk of the sample into the bucket.

Figure 48. Photograph of Emptying the net into a 5-Gallon bucket partially filled with stream water.

6. Repeat this process until 4 riffle/run habitats have been sampled. This will result in 4 individual kick samples that cover approximately 1 m^2 (4 x 0.25 m^2) of stream substrate. The 4 kick samples will be composited into 1 sample. If a diversity (fast and slow – stacked and flat, etc.) of riffle/run types is not present, collect the 4 samples from the best available habitat. It is important to obtain 4 kick samples for the composite. Always record the type and number of each riffle/run sampled on the field assessment form.

Note: The RBP protocol (EPA 841-B-99-002) suggests that 2 square meters of substrate should be sampled and composited at a given site. WAB determined through analysis of duplicate data (2 m^2 versus 1 m^2) and consultation with EPA Region III biologists that a 1 square meter sample is

adequate for characterizing riffle/run streams in West Virginia where the West Virginia Stream Condition Index is to be used for impairment classification.

7. Inspect the net for clinging organisms. Using a pair of small forceps, remove all the remaining organisms and place them in the bucket.

8. After compositing all four kicks into the bucket, all large objects (rocks, sticks, leaves, etc.) should be carefully washed, inspected for organisms, and discarded (***see Figure 49***). It is very important to remove as much rough material as possible without losing organisms. This will reduce laboratory sorting time and limit the crushing and grinding that damages benthic specimens. However, if there is an excess of leaves in the sample, this step may become too time intensive to pursue beyond a cursory sorting and removal of the leaves. You can base the amount of time to spend with this by estimating how much longer your partner needs to finish the habitat assessment.

Figure 49. Photograph of Biologist inspecting benthic sample and removing rough material (rocks, sticks, and leaves)

9. Elutriate the bucket's soft, organic material (bugs, leaves, CPOM) by using a stirring or swirling motion. Begin pouring some of the elutriated organic material into U.S. Standard 30 sieve. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample. Transfer this material from the sieve into a temporary container (*e. g.*, another bucket, a tray, another sample jar) (***see Figure 50***). Repeat this process until almost all of the organic material is removed from the bucket. If possible, release any fish and/or salamanders and document the species and number released in the Wildlife Observations section of the Habitat Form. Set the container of

Figure 50. Photograph of soft, organic material placed and stored in a temporary container.

elutriated material aside.

10. Begin the elutriation process again with the inorganic material (gravel, sand, silt). Pour some of the contents of the bucket through a U.S. Standard 30 sieve. Too much material in the sieve may result in accidental spillage.

11. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample. **DO NOT IMMERSE THE SIEVE ENTIRELY AS THIS WILL RESULT IN THE LOSS OF ORGANISMS.** If possible, release all fish and salamanders and document the species and number released in the Wildlife Observations section of the Habitat Form.

12. Pour the hard, inorganic material such as fine gravel and sand from the sieve into a sample jar already 1/2 filled with denatured ethanol (***see Figure 51***). Repeat Steps 9-11 until all of the inorganic material is sieved and placed into the sample jar. Using a squirt bottle filled with stream water, rinse any remaining material from the bucket onto the sieve.

Figure 51. Photograph of Biologist transferring the hard, inorganic material (e.g., fine gravel, sand, and silt) to a sample jar ½ filled with alcohol.

13. Use the squirt bottle to aid in removing remnants of the sample from the sieve, but avoid getting large amounts of water in the sample jar, as this will dilute the preservative. Inspect the sieve carefully for any remaining organisms and place them in the sample jar.

Figure 52. Photograph of Biologist inspecting transferring the soft, organic material (e.g., shredded leaves and benthic organisms) to the sample jar.

14. Return to the elutriated soft, organic material (bugs, leaves, CPOM) that was set aside earlier from Step 9. Using a quiet area of the stream or fresh water in the bucket, gently touch the bottom of the sieve to the water surface and rotate it in a circular motion. This will aid in removing fine sediments from the sample. Once all of the fine sediments are

thoroughly removed, place the elutriated organic contents in the sieve on top of the inorganic material (gravel, sand, silt) previously in the sample jar as in Step 12 (***see Figure 52 above***). Placing the elutriated material on top in the sample jar will protect the often fragile benthic organisms from damage due to grinding and compaction during transport to the laboratory. Do not invert or shake the sample jar after the elutriated materials are placed inside.

B. D-net (Riffle/Run Habitat = Comparable)

In some situations the stream may be too narrow or shallow to sample using a Rectangular Dip Net. In this case, a D-net will be substituted for sample collection. The methods outlined for the Rectangular Dip Net are applicable when using the D-net in riffle/run streams. The only modification is an increase in the number of kick samples to be collected. This change is necessary to sample approximately the same area (1 square meter). Since the D-net is ≈ 0.33 m wide, we will sample a square area in front of the net of 0.1108 m^2 (0.333m x 0.333m). In order to sample 1 m^2, we need to collect from 9 locations (0.1108 m^2 x 9 = 0.9972 m^2).

C. D-net – Multi-habitat Approach (Low Gradient Streams, Glide/Pool Habitat=Non-Comparable)

The RBP procedures described above are only applicable to flowing, wadeable streams. The Multi-habitat Approach is based on protocols developed by the Mid-Atlantic Coastal Streams (MACS) Workgroup, which are employed in low gradient, slow moving streams. **This method is to be used only in wetland type habitat where flow is insufficient to move suspended materials into a net.**

Note: This type of sampling is considered non-comparable at this time as the majority of other samples taken by the Watershed Assessment Branch and analyzed using the WVSCI (West Virginia Stream Condition Index). Therefore, it should only be used for special surveys/projects or if specifically specified in the sampling plan/instructions.

1. Determine the types of productive habitat to be sampled and the percentage of each habitat within the sample station. Productive habitats are snags, vegetated banks, and submerged macrophytes. A total of 20 jab-sweeps (see next step) are collected based on the proportion of productive habitats available in the 100-meter assessment area. For example, if 50% of the habitat is snag material and 50% is submerged macrophytes, then 10 jab-sweeps (50%) are taken in snags and 10 jab-sweeps (50%) are taken in submerged macrophytes. If a particular type of habitat is rare (<5%), it is not sampled.

2. Collect macroinvertebrates by jab-sweeping the net into productive and stable habitat. A "jab-sweep" is an aggressive thrusting and sweeping of the net into productive habitat for a distance of one half meter. **Make only one jab-sweep;**

resist the urge to re-sweep! A total of 20 jab-sweeps will be combined to complete the sample. The precise jab-sweep technique will vary with the type of habitat being sampled.

A. **Snags** –Disturb the snag area first by kicking it to dislodge the organisms. Then quickly jab-sweep the net into small sticks and branches or scrape the net along the lower surface of logs. Medium sized snag material is best –sticks and branches. Large logs should be avoided because they are generally difficult to sample adequately.

B. **Submerged Macrophytes** - In deep water, drag the net through the vegetation from the bottom to the water surface (maximum of 0.5 m each jab). In shallow water, bump the net along the stream bottom within the macrophyte bed, avoiding sediments where possible.

C. **Vegetated and Undercut Banks** - Use the snag collection method for collecting from roots and emergent plants that are on the lower banks of streams. Submerged areas of undercut banks are included here. Sample unvegetated banks by bumping the net along the substrate.

3. After five jab-sweeps have been collected, empty the net into a 5-gallon bucket containing stream water. (The net may be emptied more frequently, depending on the amount of material.) Repeat until 20 jab-sweeps have been collected.

   The remaining procedure is the same as for the Rectangular Dip Net. Follow steps 8 through 14 under Sample Collection Methods – I. Rectangular Dip Net (Riffle/Run Habitats = Comparable) to complete field processing and preservation.

D. Hand Picking (Small narrow streams with minimal/interstitial flow = Non-Comparable)

<u>This sampling method should only be used for special surveys/projects or if specifically specified in the sampling plan/instructions as it is considered non-comparable to other samples.</u> This method should be used in very shallow low-flow situations where there is not enough water to flow over the lip of the Rectangular Dip Net or D-net. Do not collect a sample if there is no interstitial flow in the areas between pools.

1. Sample in areas that would be considered riffles in higher flows. Do not sample in pool habitat. Pick up rocks (small gravel to small boulder) from about 0.25 m^2 (same area as that would be sampled by the Rectangular Dip Net) of substrate. Rub and rinse the rocks into a 5 gallon bucket partially filled with water. Repeat this procedure at four different areas - looking for the best habitats (highest interstitial water flow and most cobble sized rocks).

2. Use the rocks sampled to complete the benthic substrate section of the Habitat Assessment Form.

3. Pour the entire contents of the bucket through a U.S. Standard 30 sieve. Using a squirt bottle, rinse any remaining organisms from the bucket onto the sieve. Using forceps, remove any remaining organisms and transfer to jar. Place sample jar in cooler or other air-tight container designated for benthic macroinvertebrates.

The remaining procedure is the same as for the Rectangular Dip Net. Follow steps 8 through 14 under Sample Collection Methods – I. Rectangular Dip Net (Riffle/Run Habitats = Comparable) to complete field processing and preservation.

Part 2. Sample Preservation Methods

1. Fill a gallon sized sample jar about 75% full with 95% denatured ethanol. The goal is to reach a concentration of ethanol near 70% after the sample and some water has been added. If there is a small amount of water and organic material in the sample, it may not be necessary to fill the jar to 75% capacity to reach a 70% concentration. It is important that sufficient ethanol be used to reach 70% concentration. In addition, enough alcohol should be added to at least immerse all of the material in the jar. If more ethanol is needed, it can be added after the sample is received at the laboratory.

2. Make sure that there is a waterproof label filled out with pencil inside the jar and a label affixed to the outside of the jar using clear packing tape. Include stream name, AN-Code, and date on both labels. Place the jar in a cooler or other container designated for the storage and transport of benthic macroinvertebrate samples to the laboratory.

3. Avoid agitating the sample jars as much as possible. Do not invert the jars.

Part 3. Laboratory Documentation or Check-In

Upon return to the office, all samples are to be logged into a Benthic Macroinvertebrate Sample Logbook. Each entry is to include: Date of Collection, date received by office, stream name, Random number (if applicable), AN-Code, and collector's initials. If a sample is in multiple jars, each jar is entered individually and designated as "1 of 2" or "2 of 2", as appropriate.

Benthic Sampling Quality Assurance/Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sample equipment will be checked for residual benthic material,

rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Benthic macroinvertebrates will be collected along with other activities at the designated duplicate WAB sites. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been depleted by the first duplicate. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training. In addition the duplicate data is looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of benthic macroinvertebrate samples is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, biological sampling teams will consist of two people. Individuals who are more experienced in collecting benthic macroinvertebrates will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Section B. Laboratory Processing of Benthic Macroinvertebrate Samples

Benthic macroinvertebrate sample sorting is performed utilizing a modification of U.S EPA's RBP II 200-count sub-sampling method. It is described in more detail in subsequent sections.

Sorting macroinvertebrates (a procedure often referred to as "bug picking") is an extremely important step in the biological research performed by the Watershed

Assessment Branch. The quality of the work performed by the "picker" influences the quality of subsequent processes, such as identification and data analysis. A competent "picker" must be able to recognize the morphological diversity of aquatic organisms, as well as the various methods these organisms may use to hide themselves from predators. The outcome of the final study may be affected if only a few organisms are overlooked during the picking process.

The biologists in the Watershed Assessment Branch acknowledge the fact that the sorting process can be tedious at times. The picker is advised to discuss alternate sorting techniques that may be applied to difficult samples with senior biologists. All types of aquatic macroinvertebrates should be picked including insects, snails, clams, crustaceans (including crayfish), and worms.

Materials and Supplies

1. Sample jar - contains the unprocessed sample.
2. Sample vial - for storage of processed sample.
3. Enamel pans - contains sample during the sorting process.
4. Denatured ethanol - preservative used in unprocessed and processed samples.
5. # 30 sieve - used to separate alcohol and fine debris from the sample prior to picking.
6. Gridded sorting tray – (***See Figure 53 for an example***) a Plexiglas framed sorting tray is used to evenly distribute the washed sample and for randomly selecting the 200 organism subsample. The internal dimension of the tray is 20 inches by 5 inches. There are 100 grids in the tray and each is 1 inch by 1 inch in dimension.

Figure 53. Photograph of a Home-Made Gridded Sorting Tray featuring a random number matrix on the bottom.

7. Cookie cutter - a homemade cookie cutter, 1 inch by 1 inch is used in conjunction with the sorting tray to isolate each of the subsamples.
8. Labels - Self-adhesive labels are used to identify the contents of the sample bottle (*i.e.*, the picked sample).
9. Tape - used on label as additional adhesive.
10. Pencil - used to label sample bottle.
11. Crucible - or other small container, is used for short term, intermediate storage of the sample during the picking process.
12. Forceps - Fine tipped forceps are used to remove the organisms from the debris.

13. Illuminated magnifier - an optical aid to illuminate and magnify the sample during the picking process. Alternatively, magnifying visors and a desk lamp can be used.
14. Squirt bottle - filled with alcohol, used to rinse organisms into sample bottle.
15. Plexiglas - used to cover sample overnight to prevent evaporation.
16. Counter – used to count the number of organism removed from the sample.

Laboratory Safety Precautions

Protective eyewear should be worn during sample processing to prevent contact with the residual alcohol in the specimens and debris or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes. All sample processing should occur in a well ventilated area to reduce inhalation of alcohol fumes.

Benthic Sample Processing Methods

1. Select the sample to be sorted. A supervising biologist may provide the picker with a particular sample to be sorted. Be sure that the sample information (*e.g.*, date of collection, collector, stream name, county, AN-Code, etc.) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also mark the sign-out date for processing and your initials in the logbook.

2. Select a small bottle/vial that will hold the organisms after sorting is completed. Usually 10 mL bottle or 4 dram Vial is adequate for a 200-organisms sub-sample. A larger bottle or vial may be needed if the sample contains large organisms such as crayfish. In some cases, it may be necessary to split the sample into multiple bottles or vials.

3. Prepare a label for the sample bottle/vial(s):
    - It may be necessary to prepare a second label for the outside of the bottle/vial. If so, avoid using self-adhesive labels as the adhesive tends to lose its stickiness after exposure to alcohol.
    - Use a pencil or an archival quality ink pen on the labels (*e.g.*, Pigma Pens). Most inks will run if alcohol is spilled on the label.
    - Be sure to copy all information on the sample jar label onto the self-adhesive label. The label must include the following information:
        - ✓ Stream Name
        - ✓ Station Number (Random Number and/or AN-Code)
        - ✓ Sample Date
        - ✓ County
        - ✓ Collection Method
        - ✓ Initials of Sample Collector
        - ✓ Initials of Sample Processor
        - ✓ # of grids picked (to be added after the sample picking is done)

- ✓ # of organisms in final sample (to be added after the sample picking is done)
- ✓ Vial # out of Total Vials (to be added after the sample picking is done)

If any of this information is missing from the original sample jar label, notify the supervising biologist so that the error can be corrected.

4. Prepare the sample for sorting. This step is performed in a sink and should be done under a fume hood or in a well ventilated area.

   a. Under a fume hood, open sample jar and pour contents into the # 30 mesh sieve. Capture the ethanol and transfer it to a long-term holding container for later disposal.

   b. Rinse sample jar into sieve with water and examine jar to make sure all detritus has been removed.

   c. Rinse the contents of the sieve in tap water to remove remaining alcohol and to rinse out fine sand and sediment.

   d. Carefully rinse any large detritus (*i.e.* leaves) or stones, making sure that all organisms on these items are returned to the sieve. Discard the leaves and rocks after rinsing.

   e. Place the contents of the sieve in the gridded sorting tray. Fill the tray 1/3 full with water and gently swirl it until the contents are evenly distributed (***See Figure 54***). If the sample was divided into more than one jar, wash the contents of the additional sample jars and combine them with the first jar's contents in the sorting tray at this point.

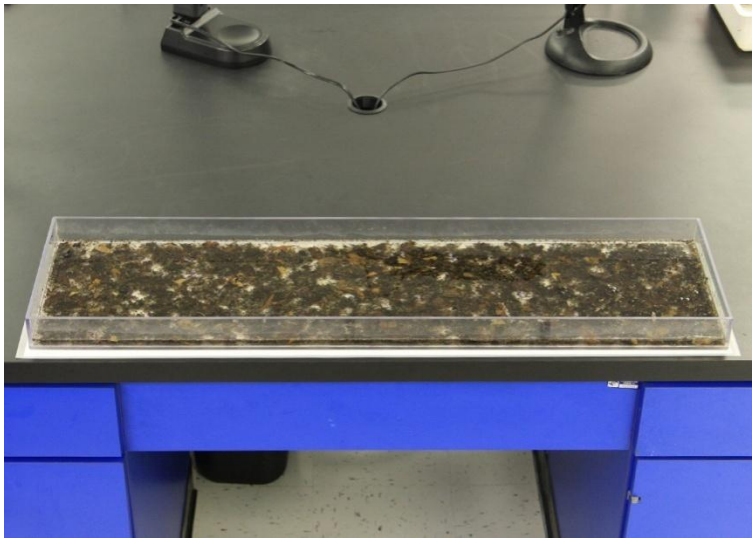


Figure 54. Photograph of a Gridded Sorting Tray with sample contents evenly distributed in water.

f. Using a random number generator, select the first grid to be picked (***see Figure 55***). Using the "cookie cutter", isolate the organisms within the chosen grid and scoop the contents of the grid into a white enamel pan with just enough water in the bottom to easily maneuver the organisms. Be careful not to destroy any organisms during this step. Organisms with their head inside the grid are to be included within the grid. If you can't distinguish which end is the head, then the organism belongs in the grid that contains the largest portion of the body.

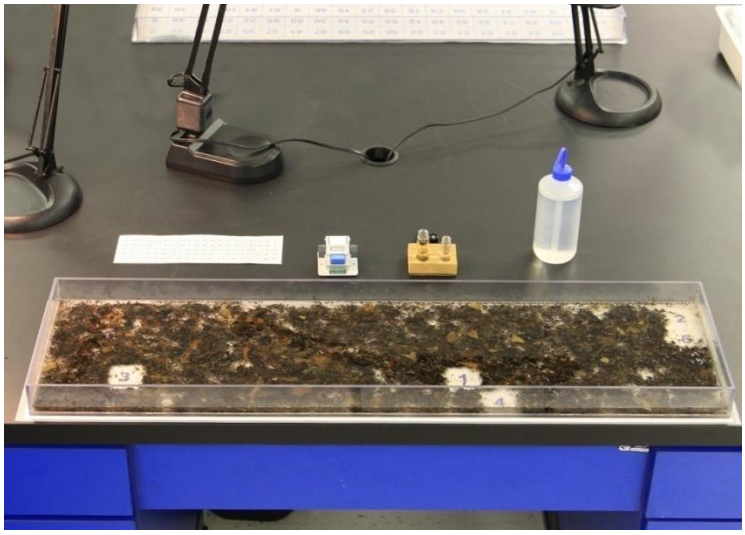


Figure 55. Photograph of a Gridded Sorting Tray with 5 grids randomly removed. Note that he sequence of numbers on the bottom of the tray known by referencing a piece of paper that has the locations of each grid mapped out.

5. Sorting (Picking)

a. Fill a crucible or temporary storage vial with 75% ethanol. If preferred, another small wide-mouth container may be substituted for the crucible.

Note: A small piece of tape, rolled into a ring so the adhesive is exposed, may be attached to the bottom of the crucible to prevent tipping.

b. Using fine-tipped forceps and illuminated magnifier or magni-visor (***see Figure 56***), remove all invertebrates from the sub-sample and transfer to the alcohol filled crucible or labeled storage vial. Keep track of the number of organisms that have been picked.

Figure 56. Photograph of Biologist sorting a benthic sample under an illuminated magnifier. Note the enamel pan filled with some water and the temporary sample container.

c. If leaves are present, be sure to examine both surfaces. Examine the debris for unusual clumps of twigs, leaves, or sand, which may be protective cases for some organisms. If cases are found, both the case and the organism should be picked. If the organism is in the case, the case and organism should be kept together. If an empty case is found, it should also be removed, but not counted towards the final number of organisms picked.

d. If there is any doubt to the identity of an object (is it a seed or a bug?), it should be picked, but not counted. A senior biologist should be notified if a large number of questionable objects are present.

e. When all the organisms appear to have been removed from the pan, agitate the contents of the pan and look again. Often the agitation will reorient an organism that was previously overlooked.

f. Have a senior biologist inspect the pan after picking has been completed. The biologist will point out any organisms that have been overlooked or misidentified as detritus. As the picker becomes more proficient at his/her task, this step will be reduced in frequency.

g. Discard the contents of the enamel pan by pouring the contents through a "waste sieve" in the sink. The contents of the waste sieve may be emptied into the trash as necessary.

h. Continue the Sorting process repeatedly (steps 4-f through 5-e) until a subsample of 200 (+/- 20% is reached) (***see Figure 55 above***). Several rules must be observed in order to get a subsample that is both random and representative of the whole sample.

    1. The total organisms in the sample must be between 160 and 240 organisms. If fewer than 160 organisms have been collected, another grid is randomly chosen and steps 4-f through 5-e are repeated until at least 160 organisms are obtained or until the entire sample has been picked. Every attempt should be made to get the final subsample as close to 200 as possible. Therefore, the person conducting the sub-sampling should keep track of the approximate number or organisms per grid in order to know if one more grid will get the subsample number as close to 200 as possible.
    2. If subsampling should result in significantly more than 240 organisms in the subsample, then the subsample should be re-subsampled to bring the number of organisms down to the 200 (+/- 20%) organism goal.
    3. Should the 200 (+/- 20%) organism goal be reached in less than 4 grids, then picking should continue until 4 total grids have been picked and then that subsample should be re-subsampled to reach the 200 (+/- 20%)

organism goal. This step will ensure representativeness of the subsample compared to the total sample.

For further information about subsampling rules, refer to the EPA Rapid Bioassessment Protocol References listed in Chapter II. Section C. Part 1. PAGES 5, 6, 5a, and 6a EPA's Rapid Habitat Assessment Form starting on page 52.

Note: Based on WVDEP's experience, >90% of the time, 4 or more grids out of 100 will need to be picked in order to reach the target 200 organism subsample for a 1m^2 kick area.

i. Place the label made earlier inside the bottle/vial(s). If a second label is prepared for the outside of the bottle/vial, then affix it using tape. Be sure to write down the # of grids picked, # of organisms in final subsample, and if applicable, the Bottle/Vial # out of the Total Bottles/Vials for the subsample before you place the label inside the bottle/vial(s)

j. Pour the subsample contents of the crucible (or temporary container) into the final storage bottle/vial(s). Use a squirt bottle containing alcohol to rinse the organisms from the crucible. Make sure that all organisms in the bottle/vial are fully submerged in the alcohol and that none are clinging to the sides of the bottle. Use the squirt bottle to rinse the sides of the bottle/vial, if necessary.

k. If required, return the remainder of the unpicked sample to the original sample jar and preserve with alcohol. These samples may be processed later to determine picking efficiency.

l. After a sample has been picked, record the date or return and your initials in the Benthic Macroinvertebrate Sample Logbook to indicate that the sample was returned from processing. Be sure that the sample information (*e.g.*, date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the bottle/vial(s) matches the Benthic Macroinvertebrate Sample Logbook.

Benthic Laboratory Processing Quality Assurance/Quality Control

Sorting efficiency is evaluated for 2.5% of the samples. These samples are randomly selected after they are received by the laboratory, but before they are sent to the pickers. Pickers conduct processing of the sample as normal, but each time they are done picking a subsample grid in the enamel pan, a second picker (usually a senior biologist) will review the pan for any missed organisms. The missed organisms for the entire sample are totaled.

Percent Sorting Efficiency (PSE)

The Percent Sorting Efficiency (PSE) (AKA Bias) can then be calculated by the following formula:

Equation 3. Percent Sorting Efficiency (PSE)

$$\frac{\#\ \textbf{Organisms Originally Sorted}}{\#\ \textbf{Organisms Recovered by Checker} + \#\ \textbf{Organisms Originally Sorted}} = \textbf{PSE}$$

A PSE >= 90% is considered passing.

Pickers may also be instructed to retain the unpicked portion. The unpicked portion can then be checked by a senior biologist to determine if the number of grids that need to be picked to get a second subsample is comparable to the original pick. This will indicate if the sample was evenly distributed in the tray.

Section C. Identification of Benthic Macroinvertebrates

Ultimately, the WAB uses benthic macroinvertebrates to bioassess the condition of wadeable streams in WV. To accomplish this, the WAB uses a multi-metric index called the West Virginia Stream Condition Index (WVSCI). The WVSCI summarizes six biological metrics that represent elements of the structure and function of benthic macroinvertebrate communities. Taxonomic resolution for the WVSCI is family level except for Nematoda and Collembola. However, all taxa should be identified to the genus level or lowest practical taxon. All aquatic macroinvertebrates should be identified including insects, snails, clams, crustaceans (including crayfish), and worms.

Materials and Supplies

1. Dissecting microscope - for examination of gross features.
2. Compound microscope - for examining minute features.
3. Fine-tipped forceps - for manipulating specimens.
4. Fine-tipped probes - for manipulating specimens.
5. Petri dishes - hold specimens during identification.
6. Alcohol - 75% ethanol is used to preserve the samples and to prevent desiccation during identification.
7. Wash bottle - used for alcohol storage.
8. Microscope slides, cover slips, and mounting media - for examination of tiny specimens and/or body parts under a compound microscope.
9. Benthic macroinvertebrate lab sheet - standard for recording results of identification and enumeration (***see Figure 57 below on page 174***).
10. Taxonomic Keys - (***see List of Taxonomic References below***)

List of Taxonomic References

The taxonomic references most frequently used by the WAB biologists for identification of macroinvertebrates include, but are not limited to:

Brigham, A.R. 1982a. Coleoptera. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Brigham, A.R. 1982b. Megaloptera. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Brown, H.P. 1972. Aquatic Dryopoid Beetles (Coleoptera) of the United States. U. S. Government Printing Office.

Burch, J.B. 1982. Freshwater Snails (Mollusca: Gastropoda) of North America. EPA-600-3-82-026.

Edmunds, G.F., Jr., S.L. Jensen, and L. Berner. 1976. Mayflies of North and Central America. University of Minnesota Press.

Epler, J.H. 1995. Identification Manual for the Larval Chironomidae (Diptera) of Florida. Revised Edition. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, Florida.

Epler, J.H. 1996. Identification Manual for the Water Beetles of Florida (Coleoptera: Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Hydraenidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, Scirtidae). Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, Florida.

Epler, J.H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. North Carolina Department of Environmental and Natural Resources, Division of Water Quality, Raleigh, North Carolina.

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Jezerinac, R.F., G.W. Stocker, and D.C. Tarter. 1995. The Crayfishes (Decapoda: Cambaridae) of West Virginia. Ohio Biological Survey Bulletin. New Series. Vol. 10, No.1.

Lugo-Ortiz, C.R., and W.P. McCafferty. 1998. A New North American Genus of Baetidae (Ephemeroptera) and Key to *Baetis* Complex Genera. *Entomological News* **109**: 345-353.

Merritt, R.W., and K.W. Cummins *(editors).* 1995. An Introduction to the Aquatic Insects of North America. 3rd edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Merritt, R.W., K.W. Cummins, and M.B. Berg *(editors).* 2008. An Introduction to the Aquatic Insects of North America. 4th edition/revised edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Peckarsky, B.L., P.R. Fraissinet, M.A. Penton, and D.J. Conklin, Jr. 1990. Freshwater Macroinvertebrates of Northeastern North America. Cornell University Press, Ithaca, New York.

Pennack, R.W. 1978. Fresh-water Invertebrates of the United States. 2nd edition. John Wiley & Sons, New York.

Ross, H.H. 1944. The Caddisflies, or Trichoptera, of Illinois. *Bulletin of the Illinois Natural History Survey* **23**: 1-326.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea. 4th edition. John Wiley & Sons, New York.

Stewart, K.W. and B.P. Stark. 1988. Nymphs of North American Stonefly Genera (Plecoptera). Entomological Society of America.

Unzicker, J.D. and P.H. Carlson. 1982. Ephemeroptera. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Unzicker, J.D. and V.H. McCaskill. 1982. Plecoptera. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Unzicker, J.D.; V.H. Resh; and J. C. Morse. 1982. Trichoptera. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Wiggins, G.B. 1977. Larvae of the North American Caddisfly Genera (Trichoptera). University of Toronto Press, Toronto, Canada.

Wiggins, G.B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). 2nd edition. University of Toronto Press, Toronto, Canada.

White, D.S. 1982. Elmidae. *In* Brigham, A.R., W.U. Brigham, and A. Gnilka *(editors).* Aquatic Insects and Oligochaetes of North and South Carolina. Midwest Aquatic Enterprises. Mahomet, IL.

Safety Precautions

Protective eyewear should be worn during sample identification to prevent contact with the residual alcohol in the specimens and debris or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes. All sample identification should occur in a well ventilated area to reduce inhalation of alcohol fumes.

Macroinvertebrate Identification Procedures

1. Check out the sample in the Benthic Macroinvertebrate Sample Logbook. The laboratory manager may pre-assign which taxonomist gets which sample and if that sample will be subject to a QA check. Be sure that the sample information (*e.g*., date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also mark the sign-out date for identification and your initials in the logbook.

2. Complete the top portion of a "Benthic Macroinvertebrate Lab Sheet" with the sample information (*e.g*., date of collection, collector, stream name, county, AN-Code, etc.) (***see Figure 57 belo*w**).

3. Using the taxonomic keys listed above (***see List of Taxonomic References above***); identify the contents of the sample to the family or genus level, depending on the specifications of the project. Use the reference collection as additional confirmation, if necessary. **IF YOU HAVE ANY UNCERTAINTY ABOUT THE IDENTIFICATION OF A SPECIMEN, CONSULT A FELLOW BIOLOGIST FOR CONFIRMATION.** If an organism is too small or damaged and cannot be identified to the designated taxonomic level, identify it to the lowest positively-identified taxon and document why the identification was not complete (*e.g*., immature or damaged specimens).

4. Record results of the identification and enumeration on a "Benthic Macroinvertebrate Lab Sheet" (***see Figure 57 belo*w**). Be sure to include notes for each taxa about immature or damaged specimens, life stages other than larvae (*i.e*., Adults and Pupae), terrestrial specimens that were picked inadvertently, numbers of specimens pulled for reference collections, and likely characters that would place the specimen in a lower level taxon if you are unfamiliar with the organism.

WVDEP-WAB BENTHIC MACROINVERTEBRATE LAB SHEET

Stream Name: _____ AN-Code: WV_____ R#: _____

Sample ID: _____ Collection Date (mm/dd/yy): _____ County, State: _____

Sorted by: _____ Number of Grids Picked: _____ Number of Organisms Picked: _____

ID By: _____ Collected By: _____

Taxon ID/Taxon	Count	Taxon ID/Taxon	Count	Taxon ID/Taxon	Count
Annelida		**Plecoptera**		**Diptera (Chronomidae)**	
Amphipoda					
Isopoda					
				Diptera (other)	
Decapoda		**Trichoptera**			
Ephemeroptera					
		Megaloptera			
				Mollusca	
Odonata					
		Coleoptera			
				Other Taxa	

Figure 57. Example of a Benthic Macroinvertebrate Lab Sheet.

5. Return the specimens to the original sample bottle and mark the label with an "X" to indicate the sample has been identified.

6. Return the identified sample bottle/vial(s) and corresponding lab sheet. Be sure that the sample information (*e.g.*, date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the vial matches the Benthic Macroinvertebrate Sample Logbook. Also mark the date of return from identification and your initials in the logbook.

Benthic Laboratory Identification Quality Assurance/Quality Control

The precision of the identification process is evaluated for 2.5% of the samples. These samples are randomly selected after they are received by the laboratory, but before they are sent to the taxonomists. Taxonomists conduct the identification and enumeration of the sample as normal. After they are done, if the sample is designated for a QA/QC check, then all of the specimens (mounted or loose) are passed on to the second taxonomist. The second taxonomist will identify and enumerate the sample in the same fashion as the first. From these two sets of data, two evaluations of precision can be calculated:

Percent Difference in Enumeration (PDE)

The Percent Difference in Enumeration (PDE) is calculated by the following formula:

Equation 4. Percent Difference in Enumeration (PDE)

$$\frac{(\mathbf{n_1} - \mathbf{n_2})}{(\mathbf{n_1} + \mathbf{n_2})} \times \mathbf{100} = \mathbf{PDE}$$

Where:

n_1 = # of organisms counted by taxonomist 1

n_2 = # of organisms counted by taxonomist 2

A PDE <=10% is considered passing.

Percent Taxonomic Difference (PTD)

Percent Taxonomic Difference is a comparison of the accuracy in identifications from one taxonomist to another. This begins thru the use of a Taxonomic Comparison Form. On this form, the identifications by both taxonomists are matched up to each other and then difference in enumerations between the two taxonomists is compared. The number of agreements is defined as the lower of the two numbers for the given taxon being compared.

The Percent Taxonomic Difference (PTD) is calculated by the following formula:

Equation 5. Percent Taxonomic Difference (PTD)

$$\left[1 - \frac{(\mathbf{comp_{pos}})}{(\mathbf{N})}\right] \times \mathbf{100} = \mathbf{PTD}$$

Where:

N = Highest count of organisms from taxonomist 1or 2

$comp_{pos}$ = Total # of taxonomic agreements from the Taxonomic Comparison Form

A PTD <=10% is considered passing for Family Level taxonomy.
A PTD <=15% is considered passing for Genus Level taxonomy.

PTD is not an evaluation of which taxonomist is correct. However, the process does include a method by which conflicts in taxonomic identification are reconciled. After the PTD is calculated, both taxonomists and a third party sit down and attempt to ascertain where the differences in identifications and enumerations are coming from. Reasons for the differences include:

1. **Misidentification of the Taxon**.
   - Example 1. One of the taxonomists may not be as familiar with a particular taxon as the other and keyed it wrong. This may be a consistent error in all of the QA samples involving the taxonomists.
   - Example 2. One taxonomist is using an outdated key that refers to a taxon that has been lumped with or is synonymous with another taxon.
   - Example 3. One of the taxonomists accidentally included a terrestrial specimen from a taxon that is very similar to an aquatic taxon.
2. **Taxonomic Resolution**.
   - Example 1. The first taxonomist may have inadvertently damaged a key feature of a specimen that prevented it from being identified by the second taxonomist to the same taxonomic level.
   - Example 2. One of the taxonomists may be better experienced and familiar with that particular taxon and be able to identify it the lower taxonomic level where the other taxonomist cannot.
3. **Specimens Lost Between Taxonomists**. This should be kept to a minimum if the two taxonomists view the sample before it is put back into the bottle/vial(s).
   - Example 1. Specimens may have been pulled from the sample (*e.g.*, Reference Collection or Slide Mounting) and not viewed by the second taxonomist.
   - Example 2. Specimens stuck to the bodies of larger organisms (*e.g.*, an Elmidae beetle stuck in the “armpit” of a large *Corydalus* specimen) are missed by one taxonomist.
   - Example 3. One taxonomist was including pupae, body parts, or empty shells/cases in the count while the other was not.

Example 4. One taxonomist may have counted partial organisms as whole organisms. This is most common with Oligochaeta as the head are difficult to find and they often get broken up into pieces easily.

4. **Transcription, Translation, and Typographic (TTT) Errors**.
   Example 1. One taxonomist meant to write down an 11 and accidentally wrote down a 1.
   Example 2. The person who calculated the PTD mistook an 11 for a 2.
   Example 3. The taxonomist wrote down a very similarly spelled taxon (*e.g*., *Thienemannimyia* vs. *Thienemanniella* vs. *Thienemannia*)

After this reconciliation, the PTD can be recalculated correcting for these most of these errors (called a corrected PTD).

Section D. Benthic Macroinvertebrate Data Analysis

Part 1. West Virginia Stream Condition Index (WVSCI)

WVSCI Reference

A detailed description of the procedures used to develop the WVSCI as well as the steps necessary to calculate final WVSCI scores can be found in the following document:

Gerritson, J., J. Burton, and M.T. Barbour. 2000. *A Stream Condition Index for West Virginia Wadeable Streams.* Tetra Tech, Inc. Owing Mills, MD.

Or on the web at:
http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI.pdf
and an addendum document at:
http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI Addendum.doc

WVSCI Overview

All organisms identified for analysis using the WVSCI (including all Oligochaeta, Hirudinea, Acari, Mollusca, and Crustacea) should be identified to at least the Family level except for Nematoda and Collembola.

The following metrics are applied to the benthic data:

1. Family Level Taxa Richness
2. Family Level Ephemeroptera, Plecoptera, Trichoptera (EPT) Taxa Richness
3. Percent EPT
4. Percent Contribution of Dominant 2 Family Level Taxa
5. Percent Chironomidae
6. Modified Family Level HBI (Hilsenhoff's Biotic Index)

The individual metric scores are then standardized on a 100 point scale based on best standard values for a set of reference sites or conditions. The scores are then averaged to give the WVSCI (West Virginia Stream Condition Index).

Restrictions for Calculating the WVSCI

A. Sample methodology – Identical sampling area (4 – 0.25m^2) and gear (0.5 m rectangular kicknet with **595µm mesh**) should be used in **riffle/run habitat**. In limited circumstances, 0.3 m d-frame nets with comparable mesh size can be used as long as **1 m^2 total area** is sampled.

B. Comparable samples – The following scenarios should be considered before collecting benthic macroinvertebrate samples for biological health assessments because they are not necessarily associated with human perturbations:
   1) **low flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net,
   2) collecting samples following **drought** may result in reduced organism numbers and diversity,
   3) **high flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net,
   4) collecting samples following a **scour or flood event** may result in reduced organism numbers and diversity.

C. Laboratory subsampling – samples in which more than the target subsample size was picked **(200 ±20%)** should be re-sorted to obtain the preferred number of organisms. **As a rule-of thumb, samples containing less than 100 organisms should be scrutinized for comparability before calculating a WVSCI score.** These sites may be heavily impacted, or were recently subjected to drought or scour events.

D. Taxonomic resolution – Taxonomic resolution for the WVSCI is **family level except for Nematoda and Collembola. This includes the non-insect groups like Oligochaeta, Hirudinea, Acari, Mollusca, and Crustacea.** If higher taxonomy is necessary (*e.g.*, early instar or damaged specimens), then these taxa should not be counted in richness metrics unless they are believed to be distinct from other taxa identified in the sample. WVDEP WAB should be consulted for exact taxonomic resolution of some groups.

E. Seasonality – Acceptable collection dates are from **April 15 to October 15**.

F. Tolerance values – WVSCI metrics that rely on **tolerance values** (HBI) are **specifically calibrated** to those used by WAB and these specific tolerance values should be used for valid final WVSCI scores.

G. WVSCI Calculations — Use only those best standard values (BSVs) and component metrics found in the WVSCI development document. Component metrics used for calculating WVSCI scores are restricted to those listed above. **Exclusion of any one of these metrics or the inclusion of additional metrics will result in an invalid final WVSCI score.**

Using the WVSCI for Data Analysis

Macroinvertebrate data is evaluated through the preparation of a stream assessment chart (***see Figure 58 below***). This chart considers the biological and habitat conditions of each stream and compares them to those of the reference sites. Reference sites are those stations having optimal habitat (as defined by the RBP/EMAP matrix scores) and no obvious impairments in water quality. The number of reference sites selected depends on such variables as stream order and ecoregions. The framework for these assessments is the West Virginia Stream Characterization Index (WVSCI). Tetra Tech, Inc. developed this index specifically for use in West Virginia. Stream scores are plotted within this chart and the results are used for overall watershed assessments, 305(b) reporting and 303(d) listing. Streams falling in the green area are considered fully supporting (for 305(b) reporting) or non-impaired (for WAB reporting). The condition of streams in the gray area may be unclear and are considered "Insufficient Data" (305(b)) and non-impaired (for WAB reporting). Water quality data must be evaluated to determine if a stream in the gray area is threatened or fully supporting. Often best professional judgment cannot be avoided. The yellow, orange, and red areas contain streams that are not supporting (305(b)) or impaired (WAB reporting). All streams falling in the yellow, orange and red sections are subject to inclusion on the 303(d) list.

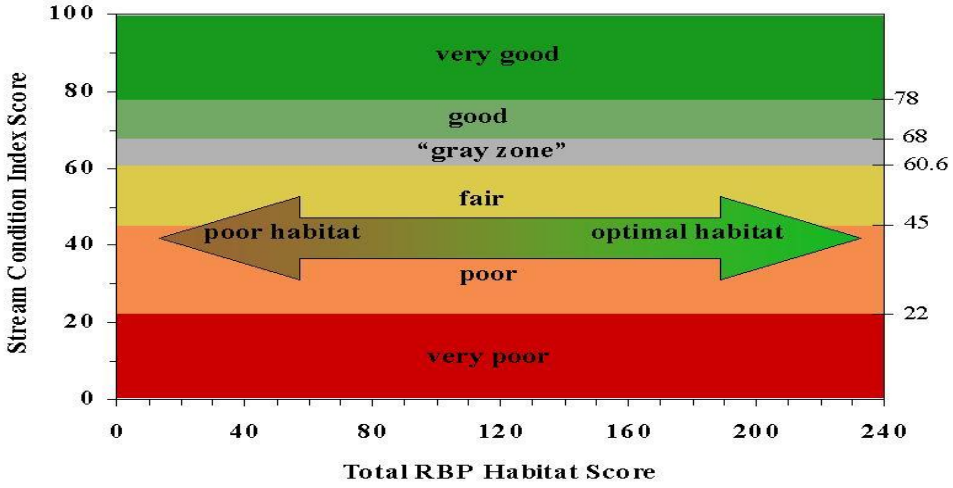

Figure 58. WVSCI vs. RBP Habitat Scoring Categories

Part 2. Dirty Null Stressor Identification Model

The benthic data is also imported into an analysis model that compares each sample's community structure to that a set of "reference" data with well known and established stressor types (Metals, Sediment, Ionic Stress, and Reference Condition), also known as "Dirty Nulls". The data that results from the Dirty Null Stressor Identification Model is a set of similarity indexes and probability percentages that help identify potential stressor or stressors to the stream community.

Chapter VI. FISH COLLECTION PROTOCOLS – WADEABLE STREAMS

Overview

Fish as Environmental Indicators

Fish community assessments are an important component of many water quality management programs. These assessments are useful for making decisions in regard to aquatic life use-support designations, biological integrity, consumption advisories, and overall stream health. There are several advantages of using fish as indicators of biological integrity:

- Fish are long-lived and mobile, thus they serve as good indicators of long-term effects and broad habitat conditions.
- Fish assemblages generally represent a variety of trophic levels (omnivores, herbivores, insectivores, planktivores, and piscivores) and are reflective of overall stream health.
- Life history and distribution information of most fish are well known.
- Fish are relatively easy to collect and identify to the species level.
- Fish are at the top of the aquatic food web and are consumed by humans, making them important for assessing contamination.

Basis of Sampling Method

Sampling methods used in the WVDEP-Watershed Assessment Branch (WAB) are qualitative in nature and essentially derived from USEPA – EMAP protocols with some deviations (Lazorchak, J.M., Klemm, D.J., and D.V. Peck (editors) 1998. *Environmental Monitoring and Assessment Program – Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams*. EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, D.C.). These methods are widely accepted and used by many states and agencies, each usually with their own specific alterations to better meet their individual needs. However, it is important to note that consistency in regards to methods, time/effort expended, and overall sample collection is critical for obtaining comparable assessments. In general, the methods involve the use of a device capable of generating an electric current, usually a backpack electrofishing unit.

Currently, the main objective of fish community assessments for WVDEP-WAB is to collect data from random (probabilistic) and targeted sites (***refer to Chapter II. Section A. Accessing the Site starting on page 7 for a description***) that can be assessed with a fish based Index of Biotic Integrity (IBI). Primarily, sampling is focused on randomly selected sites chosen each year as part of a five year rotating statewide cycle.

Ten sites are selected from each of three ecoregions (Western Allegheny Plateau, Central Appalachians, Ridge and Valley) within the state. Five of the ten sites are new (not previously visited) and five are repeat sites that were previously sampled in the last cycle. WAB began collecting fish community data in 2006, therefore no fish sample sites will be true repeats until 2011.

Beginning in 2011, additional fish samples will be collected from LTMS (Long Term Monitoring Stations) and statewide AWQN (Ambient Water Quality Network) locations. Approximately ten sites will be sampled each year in a three year cycle and then re-sampled in the next three year cycle. Long-term sampling of these sites is important in establishing trend data and in making observations on variability in the fish community. Data from sites that meet sampling suitability criteria (explained below) will be assessed with an IBI. Currently (July 2010), WAB does not have a fish IBI developed for use with fish community data. However, data collection is the first step in the IBI development process.

Selecting Sampling Sites

Sites will be selected and sampled based on the following criteria that will produce comparable data that can be assessed using an IBI.

1. The stream reach must be wadeable. Wadeable streams are those that can be safely waded while electrofishing with either a backpack electrofisher or tote barge and allow the shocker and netter to reach all available habitats. Exceptionally deep pools or deep/fast runs may be omitted or sampled with alternative methods. Ultimately, a careful, concerted effort must be made to sample as much of the reach as possible using comparable methods.

2. Watershed size for a selected site is between 2,000 and 100,000 acres which encompasses some first order up through fourth order streams. The minimum size was selected to exclude the smaller streams which may be limited to one or two species or no fish at all and the maximum size corresponds to streams sites exceeding 100,000 acres, which are typically too large to be considered wadeable due to morphology and /or ecoregion characteristics (long- deep pools, water turbidity, etc.). Additionally, this maximum size has been used by researchers as the upper size limit for some fish IBI development projects.

3. Assessments will be conducted from mid-May through the end of October which will be considered the Index Period. Initial focus will be on small (1^{st}, 2^{nd} order) streams and progress to larger streams later in the year when lower flows allow for easier sampling. In general, 3^{rd} and 4^{th} order streams should not be sampled until mid-June or later. Most importantly, all sampling should occur during normal flow conditions.

Other types of sites or sampling related to special projects (fish kills, stream restoration, trout surveys, etc.) may or may not allow strict adherence to these criteria due to needs of the project.

Determining Site Suitability

Many of the sites selected (primarily randoms) for fish community assessment will be visited by a sample team to collect benthic macroinvertebrates prior to the fish collection visit. This team should make observations and record notes regarding the suitability of the site for fish collection. The notes should contain information pertaining to flow status (e.g., too deep, possibly dry later in the summer, etc.), site access (e.g., landowner issues, limiting physical barriers), and stream morphology that could influence the sampling effort (e.g., large pools or falls). Notes should be given to the fish crew prior to the site visit. Thus, if conditions exist that would prevent a comparable fish collection the fish crew could avoid a costly trip to the site.

Some sites will be selected for long term temperature monitoring in order to determine their summer maximum. Ultimately, the temperature information may be used to assess whether the fish community at a particular site is representative of warm, cool, or cold water conditions. This may also be important in the development of a fish IBI. These sites will be visited by members of the fish crew in the spring for placement of deployable temperature units. At this time, crew members can also make observations on the suitability of the site for fish collection.

In order to determine if a site can be sampled, the fish collection crew leader should examine the entire proposed reach upon arrival before any sampling occurs. The primary factors to consider when determining if a reach can be effectively electrofished are safety, available habitat, and flow status. In addition, the crew leader should consider abnormal or unnatural features (e.g., bridges, culverts, etc.) that may be present in the reach. Some features may not prohibit sampling if adjustments are made properly. For example, if a small, short culvert is present within the reach, the culvert length should be measured and that distance added to the upper end of the reach. Subsequently, the culvert can be simply omitted from the sample area. Possible conditions that would prevent sampling are a dry stream channel, dense overhanging vegetation that prohibits efficient movement and/or collection, and above normal flows. Under no circumstances should a stream be sampled if dangerous conditions are present.

Establishing the Sample Reach

The length of the sample reach will be 40 times the average wetted width of the stream, with a minimum length of 160 m and maximum length of 500 m. The average wetted width is determined by taking three to five measurements (based on variability) within a reasonable distance (~100 m) from the x-site. This should be done in an upstream

direction if the X-site is at the downstream terminus of the reach. It should be done both upstream and downstream if the X-site will not be used as the downstream terminus during the fish collection.

The sample reach should include all available habitat types. The various habitat types that may be encountered are defined as follows:

Pool - Still water with low velocity. Water surface is smooth and glassy. Usually deep compared to other parts of the channel.
Glide - Slow moving water with a smooth, unbroken surface. Turbulence is low. Usually shallow compared to other parts of the channel.
Run – Similar to glide but water is moving slightly faster. Turbulence is low and the surface is without ripples that produce gurgling sounds. Runs may have small waves.
Riffle - Water moving with small ripples, waves and eddies. Produced a babbling or gurgling sound.
Snag - Submerged woody debris (dead logs, root wads, etc.).
Submerged Macrophytes - Aquatic vegetation growing beneath the water surface.
Vegetated and Undercut Banks - Stream banks having submerged vegetation (shrubs, etc.) and/or root wads.

If possible, the lower and upper end of the reach should be located at or near some type of hydraulic feature (e.g., riffle, plunge pool, etc.) which will serve as a barrier to fish movement. If no barrier is located at the ends of the reach, then block nets or seines should be used to corral and contain fish during electrofishing process.

All sample locations should be chosen based on these criteria. Any deviations should be thoroughly documented so that a determination can be made as to whether the sample is comparable for IBI purposes.

Section A. Fish Sampling

The number(s) and type(s) of sample gear will be determined based on stream width and morphology. Experienced professional judgment is critical in this determination. The goal is to be confident that the fish community is being adequately and thoroughly assessed.

Materials and Reagents

1. Electrofishers - Smith-Root Model 24LR or Model 12 backpack electrofisher
2. Electrofisher batteries and chargers – Spare batteries should be handy and available to ensure that a site can be electrofished quickly
3. Electrofisher cathode and anode

4. Tow barge – Includes a generator, anode pole and cable, GPP electrofisher, and cooler, and fuel.
5. Dipnets - 1/4" mesh; assorted frame sizes
6. Seines/blocknets - 1/4 in. mesh; 4'x20' or 4'x30' dimensions
7. 1 gal. Nalgene jars
8. 37% Formaldehyde
9. Assorted plastic buckets with lids – Used to hold fish between capture and field processing
10. Sample Jar Identification Labels - For both inside and outside of the jar
11. Chest Waders – Waders should not be breathable in order to prevent accidental electrical shock
12. Rubber Gloves – To be worn at all times by electroshockers and netters
13. Measuring board and digital scales
14. 100 meter tape measures – At least 5
15. Polarized sunglasses
16. Hearing protection – Used when using the tow barge/generator
17. Fish collection form and WAB field assessment form
18. Digital camera – Used to document large fish that will be released (e.g., large game fish or rare, threatened, or endangered species) and fish health anomalies
19. GPS
20. Scientific collecting permit – Obtained yearly by Watershed Assessment Branch from the WVDNR.

Field Safety Precautions

Formaldehyde is a known human carcinogen! The vapors and solution may cause severe irritation upon contact with skin and eyes. Use caution when handling and wear nitrile gloves and eye protection. If indoors, always work in a well ventilated area.

Safety methods and protocols can be referred to in the following documents:

Professional Safety Committee. 2008. *Fisheries safety handbook*. American Fisheries Society, Bethesda, Maryland.

User's Manual. *GPP 2.5, 5.0, 7.5, and 9.0 Portable Electrofishers*. Smith-Root, Inc. Electrofishing Safety and Principles section, pgs. 14 -18.

All electrofishing crew members must read and be familiar with these safety protocols. In addition, anyone participating in an electrofishing activity will be required to read and sign the "Acknowledgement of Electrofishing Orientation" form found on page 19 of the American Fisheries Society safety handbook.

Part 1. Sample Collection Methods

Before sampling event:

- Fill out sample labels with a No. 2 pencil. Attach to the outside of the sample jar using clear, waterproof tape. Fill out a pre-printed sample label made of waterproof paper for the inside of the sample jar.
- Fill the sample jar 1/5 full with 37% formaldehyde.
- Check all of the nets to ensure there are no holes. If there are holes or tears in the net, it should be repaired immediately before the next sample is collected and/or replaced as soon as possible.

Electrofishing

Figure 59. An electrofishing crew consisting of two backpack shockers and three netters.

Electrofishing is the primary method of fish collection used by the Watershed Assessment Branch (WAB) in wadeable streams and rivers. It is usually the most efficient and effective method, however other methods such as seining, gill netting, and

angling are also utilized. The electrofishing crew consists of one crew leader and a minimum of one other experienced crew member. Normally, there are at least three crew members at each site. If there are additional crew members, they do not have to be experienced, but they must be knowledgeable of the safety and principles of electrofishing. ***See Table 9 below for guidance on the number of electrofishers and netters required for different stream sizes and depths***.

Table 9. Personnel and equipment required to effectively electroshock various types of streams.

Stream Width	Stream Depth	Number/ Type of Electrofishers	Number of Netters
≤ 4m	Shallow (< 2')	1 backpack	2
≤ 4m	Deep (> 2')	2 backpacks	2 – 3
4 – 8 m	Shallow	2 backpacks	3 – 4
4 – 8 m	Deep	2 backpacks or Barge	2 – 4
> 8 m	Shallow	2 backpacks	3 – 4
> 8 m	Deep	Barge	2 – 4

In general, where conditions allow, one backpack electroshocker will be used in streams up to four meters wide. In shallow streams with little or no area for fish escape, one electrofisher working upstream in a side to side motion can adequately shock the majority of the habitat present. In streams four to eight meters wide and in deeper, trough-like streams less than four meters wide (often associated with reduced visibility), two backpack electroshockers will be used (***see Figure 59 above***). The two electrofishers will parallel each other working side to side in an upstream direction (***see Figure 60 below***). It's important to keep the two anodes from getting too close to one another. If the anodes are too close it will tax the system, reduce the effective range, and produce a much larger voltage gradient near the anodes which could be lethal to the fish (refer to the backpack electrofisher manual for further explanation). However, don't allow an excessive distance between the anodes which would create potential for fish to escape.

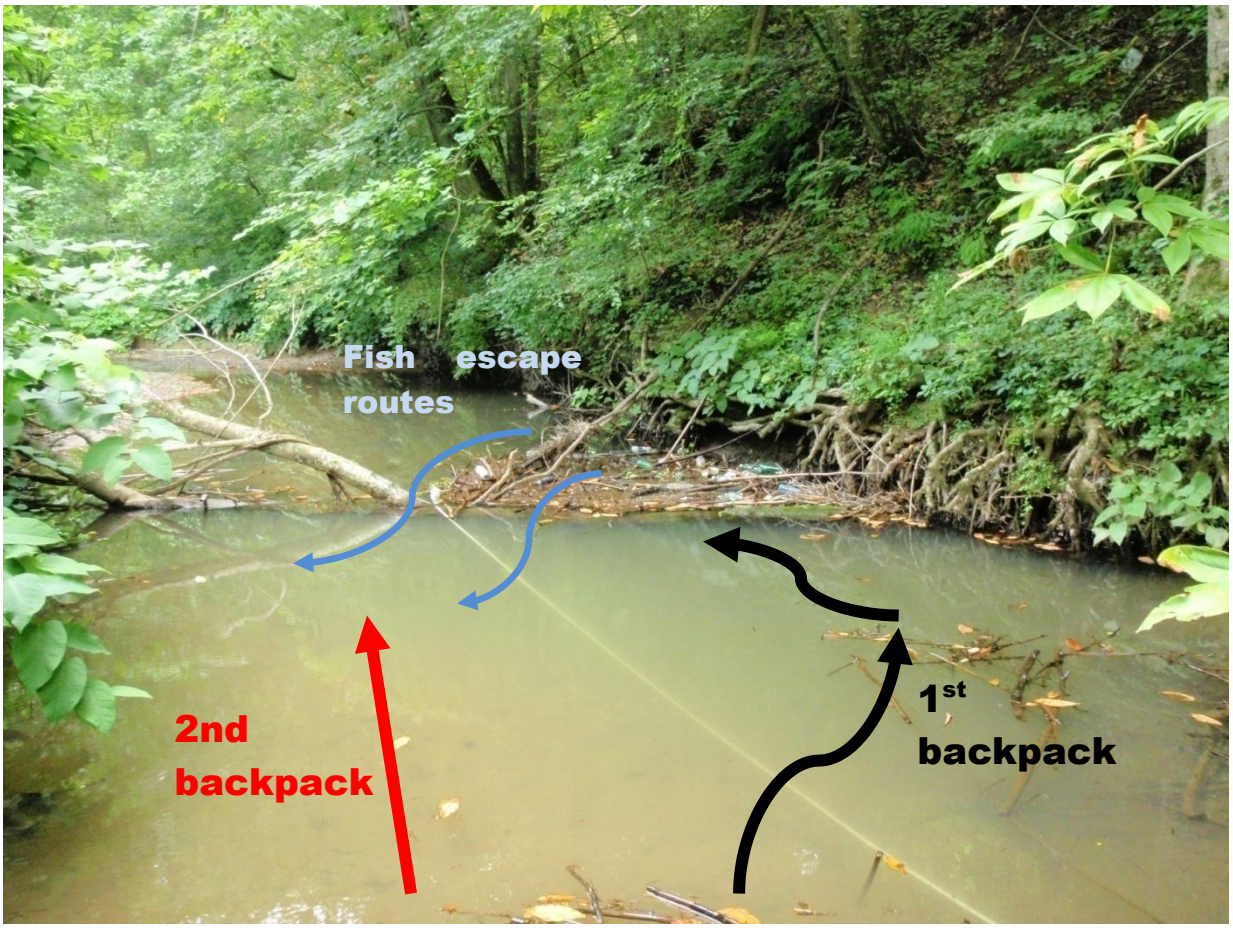

Figure 60. Technique for sampling a deep, narrow stream with two backpacks, looking upstream.

A tow barge ***(see Figure 61 below)*** will be used in streams eight meters wide or greater (assuming that water depth is adequate to move the barge) (***see Figure 62 on next page***). The tow barge offers more power to a much larger sampling area than a backpack electrofishing unit. The barge should also be used whenever possible in water with higher specific conductance (>1,000 µmhos/cm) because a backpack unit is often not capable of producing enough power to adequately stun fish in these conditions. If a stream greater than 8m wide is too shallow to use the barge, then two backpacks should be used. The decision should be made by the crew leader as to whether the backpacks are adequate to obtain a representative sample. If not, then the stream should not be sampled as it would result in a non-comparable sample.

Figure 61. Tow barge with generator, live well, and shocking wand.

Figure 62. Reach type requiring barge electrofisher. Note the large width and lack of constraining features or habitat.

In a stream with multiple or braided channels (***see Figure 63 below***), each channel should be electrofished. Using one backpack, select a channel and shock it upstream to the point where the main channel splits. Walk back down that channel to the starting point and then begin working up the next channel. Continue this pattern until all available channels have been sampled. When sampling a stream with substantial quantities of a specific cover type (e.g. boulders, logs, undercut banks, overhanging vegetation), focus your effort on that habitat. Most species of fish tend to hide as a first response to disturbance. Use the anode to draw fish out of the cover where they can easily be netted. If the proper settings are used, the anode will actually work like a magnet and the fish will swim to the anode.

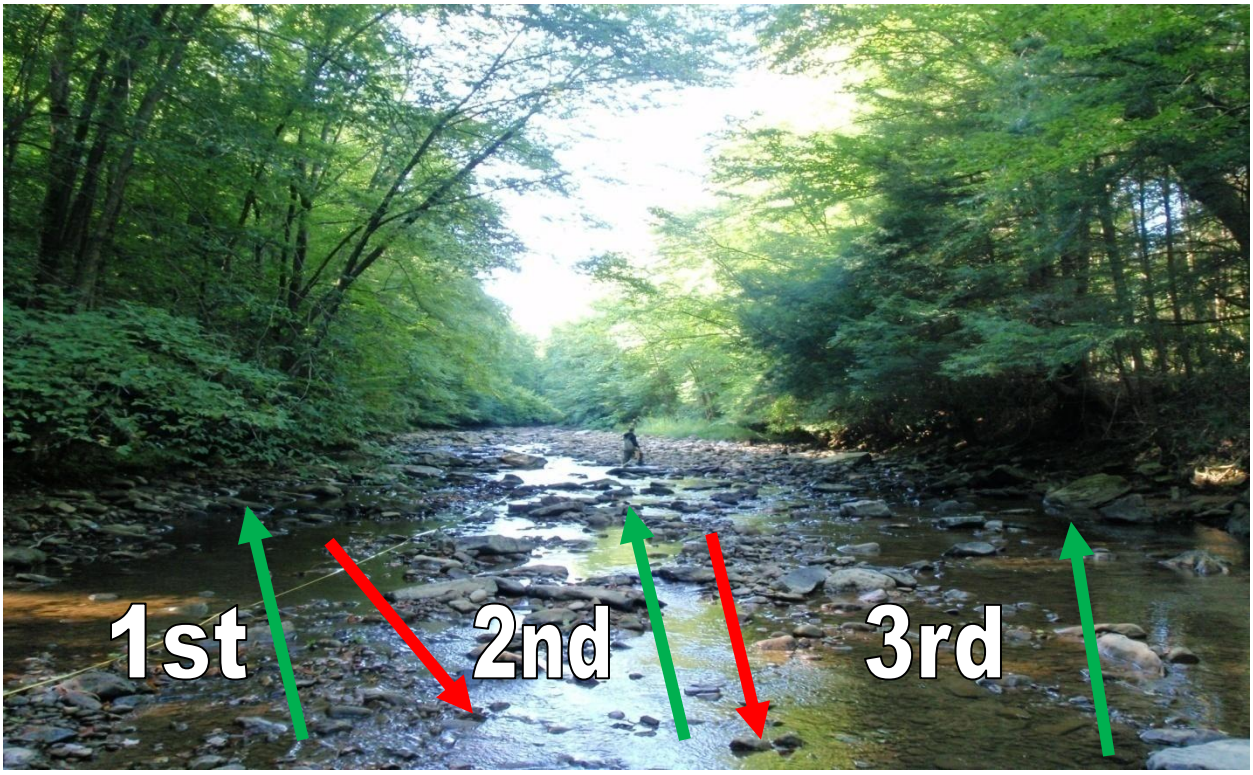

Figure 63. Proper technique for sampling a stream with multiple channels.

One to two netters should accompany each backpack electrofisher and a minimum of two netters (one on each side) must accompany the tow barge. Therefore, a minimum of three crewmembers are required for backpack electrofishing and at least four crewmembers are required for tow barge electrofishing (one shocker, one barge mover, and two netters). In most cases, one or more of the netters can carry a bucket for fish transport and aeration. If an additional crewmember is available, that person can carry the bucket and maintain the fish. This results in added convenience for the netters. When using the tow barge, a cooler or large plastic tub is placed in the barge for fish transport. *IMPORTANT: Focus should be placed on transferring collected fish as often as possible from the nets to the bucket or cooler to reduce mortality rates. Also, any*

fish determined to be voucher specimens need to be placed in sample containers with preservative as often as necessary. Ideally, fish should be preserved while they are still alive to maintain as many distinguishing characters as possible. Dead fish will lose their color and markings very quickly.

Netting

Initially, netting fish would seem like a very simple procedure. However, certain guidelines must be followed for the electrofishing survey to be carried out properly and efficiently. These guidelines are as follows:

1. Netters should remain adjacent or slightly behind the backpack shocker. Netters moving ahead of the shocker will not only frighten fish away, but will be out of position to net fish as they float behind the shocker. Equally as important, the netter(s) should not trail too far behind the shocker to allow the fish to recover and swim away. The goal is to stun the fish temporarily so they can be netted quickly. All netters should be aware of the effective electrofishing field so they can anticipate where fish will float to the surface of the water.

2. Keep the net at or just below the surface of the water while moving upstream. Fish can often float by very quickly or dart around the net if they are not completely stunned. The closer the net is to the fish and shocking area, the more likely the fish will be captured. Do not carry the net on your shoulder or use it as a leaning post. Be ready at all times.

3. Do not use the net as a shovel; we are not collecting gravel. Fish that are trapped between substrate features can easily be obtained using a simple technique. Place the front of the frame of the net just over the trapped fish. Then quickly pull the net up and away from the fish creating a surge of water toward the surface which should draw the stunned fish up from the bottom (Note: this may take a few attempts) allowing the fish be netted. With practice, the technique can be perfected and proves very effective.

4. If an additional person is available to carry the bucket, that person can also carry a small net to collect any stragglers that the netters have missed. This person should stay a few feet behind the shocker and primary netters.

Seining

When sampling larger streams with deep pools, there are times when electrofishing is not possible or at least not productive (e.g. the pool is too deep to wade without submerging the backpack shocker, the stream bottom is unstable, the water is too turbid to see fish, etc.). In these conditions, the sampling crew should attempt a seine haul. One crewmember stands with one end of the seine on one bank. The seine is then

stretched, perpendicular to flow, across the stream and held by another crew member on the opposite end. Either crew member (only one) should then walk upstream or downstream in an arc to the opposite bank keeping the seine moderately taught at all times, but with some slack to form a pocket. After reaching the bank, the seine should be lifted quickly and carefully out of the water and placed on the bank. Fish are then removed and placed in buckets. Based on success of the first haul, a second may be performed, but no more than two sweeps should be performed for consistency. The success of the seine haul is determined by the crew leader or most experienced crew member.

Fish Collection Procedure

1. Prior to or upon arrival at the site, the crew leader should review all available information so decisions regarding time and effort needed to sample the site can be made. Also, all equipment should be checked to make sure it is present and in working condition.

2. For random, AWQN, and LTMS sites, locate the x-site for proper reach confirmation. For non-random sites, simply determine where the reach will begin. Obtain GPS coordinates and verify correct location (***see Chapter II. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) starting on page 20***). If a previous visit was made to the site for macroinvertebrate collection, be sure the coordinates match.

3. Collect appropriate water quality samples (e.g., fecal, metals, nutrients, etc.) and field meter parameters. It is important to note that no one should enter the stream, above the x-site or bottom of reach, except for the water sampler until after the samples have been collected (***see Chapter III. WATER COLLECTION PROTOCOLS starting on page 95***). If necessary, a block net can be placed at the bottom of the reach to prevent fish from moving downstream during water collection.

4. Measure the stream at three to five locations, based on variability, to obtain an average width. Multiply the average width by forty to calculate the length of the sample reach.

5. Using two or more 100 meter measuring tapes, lay out the sample reach. When walking the reach, try to stay on or near the bank to minimize fish and habitat disturbance. Remember, minimum reach length is 160 m and maximum is 500 m. If the site has been sampled previously for macroinvertebrates, the original 100 m reach must be included in the fish reach. It is not critical that the lower end of the reach matches the original reach. The fish reach may extend downstream of the macroinvertebrate reach if accessibility/sampleability issues require.

6. Examine the lower and upper ends of the reach to determine if hydrological features (e.g., riffles, plunge pools, etc.) exist to prevent fish passage. If not, place block nets as needed to trap fish within the reach. Be sure the bottom (weighted rope) of the block net is firmly attached to the stream bottom. The net should be upright and the top should be at or above the surface of the stream.

7. Place buckets or plastic tubs on the bank at one or more locations throughout the reach for fish holding. Holding containers (with lids, if necessary, to prohibit fish escape) should be placed in shaded areas if possible to prevent excessive temperatures. Sample jars with formalin may also be placed at these locations for fish preservation.

8. Determine how many and what types of electrofishing equipment will be used. Set the unit voltage according to water conductivity and then shock a small test zone downstream of the reach to evaluate the effectiveness of the unit. Adjust settings according to fish reactions. Further explanation on using the electroshockers can be found in the user's manual. Record the voltage settings on the fish collection form and reset the timer to zero.

9. Before electrofishing begins, ensure that all members of the electrofishing crew are wearing polarized sunglasses, rubber gloves, and appropriate waders for respective stream depth.

10. Begin at the downstream end and electrofish in an upstream pattern going from bank to bank, including all side channels and backwater pools. Thoroughly sample all available habitats and net all fish observed. Keep nets positioned lower in the water in faster current and anywhere turbidity limits fish spotting. Extra attention should be paid to collecting benthic fishes such as darters, sculpins, and catfish. These fish are often missed by crew members holding their nets too high in the water column.

11. Continue working upstream stopping as often as necessary to process fish (***see Field Sample Processing below***). Game fish, especially trout, should be measured, photographed, and released regularly to reduce the chance of mortality. Check buckets often to observe fish behavior. If fish are swimming erratically or belly up, it is either time to change the water or process/preserve. Fish that are being retained as vouchers should be placed in formalin jars as necessary while they are alive. Larger fish that can easily be identified in the field may be released after a maximum length for each species is obtained and a photograph is taken. **Be sure that all fish released are counted on the fish collection form. Also, make sure fish are released somewhere downstream of the processing point so that they are not recaptured.**

12. Electrofish to the upper end of the reach, making sure to thoroughly collect around the block net (if used). At this point, process the remaining fish and be sure to record the total shock time on the fish collection form. This is normally a good time to record a rough taxa list from for the stream on the collection form (can be useful later when identifying preserved fish). As the crew is walking back down the stream to the original starting point, they should net any dead fish observed along the way and add them to the specimen collection jars.

13. Review fish collection form (***see Field Data Collection below***) to ensure that all necessary information is completed.

Part 2. Field Sample Processing

Field Identification

All fish that can be positively identified in the field will be processed, enumerated, and released if they are in suitable condition (i.e., not dead) except those that are retained for voucher or reference collection reasons (see ***Voucher/Reference Preservation Method below***). Fish that are too large to fit in the sample container should be photographed and released. All RTE (Rare, Threatened, Endangered) and game fish will be released as soon as possible (ideally just after netting and subsequent documentation) to minimize mortality. Released fish will be measured for maximum length of largest specimen and minimum length of smallest specimen for each species. Photograph any specimen if deemed necessary.

Voucher/Reference Preservation Method

At a minimum, at least one fish of each non-RTE species should be vouchered (either preserved in the container or by photograph). It is preferred to voucher at least five individuals of each species. More vouchers are preferred if it is a difficult species to identify or unknown. Minnows, darters, and any other fish that can be difficult to identify should be photographed while still alive and very colorful to help when lab identifications are performed.

Fish retained for voucher or reference collections will be placed in a one gallon Nalgene container approximately 20% (or 1/5th) filled with 37% formalin. Add stream water to the container until it is about half full, and then begin adding fish. Fill the container approximately 70% full with fish. This will reduce bending and distorting of the fish specimens as well as poor preservation. Also, any fish greater than 6” long should have a small incision made in the abdominal wall for proper preservation. Once the jar is full, seal the lid with electrical tape to prevent leakage or spillage. For reference, only jars containing formalin should have a taped lid. Also, make sure the container has an inside and outside label. Fish should remain in formalin for a minimum of two weeks for

proper preservation. Normally the fish remain in formalin for several weeks until the end of the field season.

WARNING: Formaldehyde is a known human carcinogen! The vapors and solution may cause severe irritation upon contact with skin and eyes. Use caution when handling and wear nitrile gloves and eye protection. If indoors, always work in a well ventilated area.

Part 3. Field Data Collection

The following list includes the type of data that will be collected and recorded at each fish community assessment site:

- ✓ Stream name, an-code, and reach length.
- ✓ Date and time of collection.
- ✓ The name and number of each fish species.
- ✓ The minimum and maximum length of each species.
- ✓ DELT (deformity, erosion, lesion, and tumor) anomaly information for any and all fish collected.
- ✓ Photographs of game fish, larger non-game fish, and any RTE (rare, threatened, and endangered) species.
- ✓ Voucher counts. A voucher collection of five or more individuals (if available) of each species (except RTE's) will be retained for later verification.
- ✓ Total shock time, voltage, and the number and type of gear used, the number of netters, and whether block nets were used.

Part 4. Laboratory Documentation or Check-In

Upon return to the office, all samples are to be logged into a Fish Sample Logbook located in the WAB Water laboratory. Each entry is to include: Date of Collection, date received by office, stream name, Random number (if applicable), AN-Code, and collector's initials. If a sample is in multiple jars, each jar is entered individually and designated as "1 of 2" or "2 of 2", as appropriate.

Fish Sampling Quality Assurance/Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sample equipment will be checked, rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of fish samples is included. Any persons unable to attend the annual training

session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, fish sampling teams will consist of three or more people. Individuals who are more experienced in collecting fish will be charged with overseeing the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Section B. Laboratory Processing of Fish Samples

Materials and Supplies

1. Fume Hood – to vent fumes while processing fish
2. Water – to remove the formaldehyde
3. 20% ethanol – first ethanol wash
4. 50% ethanol – second ethanol wash
5. 70% ethanol – third ethanol wash

Laboratory Safety Precautions

Protective eyewear should be worn during sample processing to prevent contact with the residual formaldehyde or alcohol in the specimens. Formaldehyde is a known carcinogen and alcohol can be a skin irritant and can cause damage to the eyes. All sample processing should occur in a well ventilated area to reduce inhalation of fumes.

Fish Sample Lab Processing Methods

1. After at least two weeks of preservation in formaldehyde, remove the fish from the container and properly dispose of the waste formaldehyde. Also, mark the date that processing began and your initials in the Fish Sample Logbook. Be sure that the sample information (*e.g.*, date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the container matches the Fish Sample Logbook.

2. Place the fish back in the container, fill with water, and allow the specimens to soak overnight. Repeat this step three to five times depending on the number of fish in the jar. The more fish specimens that are in the container, the more formalin there is in the fish tissues which takes longer to remove. Add new water each day. The subsequent washings do not need to be disposed of in the same manner as the original waste formalin and may be poured down the drain.

3. For long term preservation, the fish need to be transferred to ethanol. Begin by placing the fish in 20% ethanol and allow them to soak overnight. Then proceed to 50% ethanol (overnight), and finally 70% ethanol for long term. Do not transfer fish directly to 70% ethanol as this will cause hardening, shrinking, and bending of the fish which makes identifications more difficult or impossible.

4. Document the date and your initials in the Fish Sample Logbook when the sample has been fully processed into 70% ethanol.

Fish Laboratory Processing Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. While a hands-on session concerning the laboratory processing of fish samples is not included, any persons involved with this task will be instructed and evaluated by an experienced, senior biologist before being allowed to conduct this task unsupervised. Individuals who are more experienced in laboratory processing of fish samples will be charged with overseeing the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the lab.

Section C. Identification of Fish

Ultimately, the WAB intends to use fish to bioassess the condition of streams in WV. To accomplish this, the WAB hopes to develop a multi-metric index for assessing fish community data. If developed, the fish IBI will summarize elements of the structure and function of fish communities. Taxonomic resolution for the fish IBI will be to the species level.

Materials and Supplies

1. Dissecting microscope - for examination of gross features.
2. Compound microscope - for examining minute features.
3. Fine-tipped forceps - for manipulating specimens.
4. Fine-tipped probes - for manipulating specimens
5. Scalpel – for light dissection of specimens
6. Petri dishes - hold specimens during identification.
7. Alcohol - 70% ethanol is used to preserve the samples and to prevent desiccation during identification.
8. Wash bottle - used for alcohol storage.
9. Fish collection form – add voucher identifications to those done in the field.
10. Fish Measuring Board/Digital Scales – for lab measurements of specimens
11. Taxonomic Keys - (***see List of Taxonomic References below***)

List of Taxonomic References

The taxonomic references most frequently used by the WAB biologists for identification of fish include, but are not limited to:

Eddy, S. and J.C. Underhill. 1978. How to Know the Freshwater Fishes. Third Edition. The Pictured Key Nature Series. Wm. C. Brown Co., Dubuque, Iowa.

Jenkins, R.E. and N.M. Burkhead. 1993. Freshwater Fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Page, L.M and B.M. Burr. 1991. A Field Guide to Freshwater Fishes, North America North of Mexico. Petersons Field Guide Series. Houghton Mifflin Co., New York.

Pflieger, W.L. 1975. The Fishes of Missouri. Missouri Department of Conservation, Columbia, Missouri.

Stauffer, J.R. Jr., and J.M. Boltz, and L.R. White. 1995. Fishes of West Virginia. Academy of Natural Sciences of Philadelphia, Philadelphia, Pennsylvania.

Trautman, M.B. 1981. The Fishes of Ohio with Illustrated Keys. Revised Edition. Ohio State University Press, Columbus, Ohio.

Safety Precautions

Protective eyewear should be worn during sample identification to prevent contact with the residual alcohol in the specimens and debris or at any time while handling alcohol, which can be a skin irritant and can cause damage to the eyes. All sample identification should occur in a well ventilated area to reduce inhalation of alcohol fumes.

Fish Identification Procedures

Check out the sample in the Fish Sample Logbook by marking the date and your initials. Be sure that the sample information (*e.g*., date of collection, collector, stream name, county, AN-Code, # of bottle/vial(s), etc.) on the container matches the Fish Sample Logbook. Identify all of the fish in the container and add those records to those identified in the field.

Fish Identification Quality Assurance/Quality Control

The precision of the identification process is evaluated for 2.5% of the samples. These samples are randomly selected after they are received by the laboratory, but before they are sent to the taxonomists for identification. Taxonomists conduct the identification and enumeration of the sample as normal. After they are done, if the

sample is designated for a QA/QC check, then all of the specimens are returned to the sample container and passed on to the second taxonomist. The second taxonomist will identify and enumerate the sample in the same fashion as the first. From these two sets of data, two evaluations of precision can be calculated. In addition, voucher and reference specimen identifications will be confirmed by WVDNR fishery biologist expert Dan Cincotta.

Chapter VII. PERIPHYTON COLLECTION PROTOCOLS

Periphyton Overview

Periphyton is attached algae, *i.e.*, algae that grow on the exposed surfaces of rocks and other submerged objects. Phytobenthic (bottom-dwelling) algae are usually the dominant component of a periphyton community. Phytobenthic algae, the primary producers in the stream ecosystem, are sensitive indicators of change in lotic waters. Because it is attached to the substrate, the periphyton community integrates physical and chemical disturbances to a stream. Another advantage of using periphyton in water quality assessments is that the periphyton community contains a naturally high number of species, making data useful for statistical and numerical applications to assess water quality. Response time of the periphyton is rapid, as is recovery time, with recolonization after a disturbance often more rapid than for other organisms. Diatoms, in particular, are useful indicators of biological integrity because they are ubiquitous; at least a few can be found under almost any conditions. Most diatoms can be identified to species by experienced biologists and tolerances or sensitivities to specific changes in environmental conditions are known for many species. By using algal data in association with macroinvertebrate data, the biological integrity of stream ecosystems can be better ascertained.

Materials and Supplies

1. Support Ring – A piece of PVC pipe (1 cm long & 4 cm inside diameter) to delimit the sample area on rocks (12.56 cm^2 of area inside ring
2. Scraping Tool - microspatula
3. Small brush - toothbrush that is replaced at least weekly
4. Sample container - 4 oz “specimen jar”
5. 10 % Formalin - for sample fixing/preservation
6. Cooler w/ Wet Ice - for sample storage/preservation
7. Electrical Tape -for sealing lids of sample jars
8. Labels - labels are to be placed inside sample container & on outside
9. Clear Tape - to affix label to container
10. Squirt Bottle

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.*, glass, metal, wire, etc.). They should also be worn during sample preservation or at any time while handling formalin, a known carcinogen.

Part 1. Field Sampling Procedures

Collect periphyton at benthic sampling sites (*e.g.*, reference, random, TMDL Bio Sites, targeted sites) or as directed on the stream list. Periphyton may also be collected at big streams (large rivers where WVSCI is not applicable – Elk River near mouth), and at streams that are too deep for benthos collection (*i.e.*, water over the net) but not too deep to reach in and grab cobble to sample periphyton as a biological indicator for the site.

Ideally, samples should only be collected during stable flow conditions. After extremes of flooding or drought, a two-week period is required for adequate recolonization. Because sampling tends to be conducted within a short index period (random sites), periphyton will be collected when streams are not turbid (*i.e.*, the substrate is visible).

1. Label sample container with Stream Name, AN-Code, date, collector, and "w/ formalin".

2. To be consistent, samples will only be collected from rocks (epilithic habitat) from riffle/run areas of the streams. Collect five separate cobble-sized rocks that are exposed to varying light conditions and contain varying periphyton communities (brown vs. green) and intensities from throughout the reach. This includes rocks with just green or brown algae, rocks with both intermixed, rocks with long stringy algae, and rocks with a layer of periphyton growing on top of a thick layer of silt or sand (which tend to be motile species). Even a seemingly clean rock will have an unseen or undetectable community of periphyton that can be quantified. If there are no rocks available from the reach, collect periphyton from removable wood (same technique as for rocks), documenting on the field sheet exactly what was sampled. These riffle/run areas should roughly coincide with the areas where benthic macroinvertebrates are collected (if benthos collected) to avoid sampling above and below a source. **The most important thing is to be representative of the site when picking the five rocks for the sample!!!**

3. Rinse the PVC ring, toothbrush, microspatula, and squirt bottle thoroughly with stream water at the site before each sampling event to avoid contamination from prior sampling of subsequent collections.

4. Using the PVC ring to delimit the sample area (12.56 cm^2), use the microspatula to scrape **all algae** from **upper surface** of rocks into the sample jar. Use the toothbrush to loosen any remaining periphyton. In some cases, if the mineral content of the rock is just right, you may notice that you are removing a significant layer of the rock material along with the periphyton. In such a case, it is probably safe not to use the toothbrush after scraping since it is doubtful that any periphyton remains in the scraped area unless the rock is excessively fissured and rough.

5. Remove sampler and rinse loosened algae into the sample jar using clear stream water collected from that site in the squirt bottle. Repeat Step 5 until all of the periphyton from the five rocks (representing 62.8 cm^2 of sampled area) is composited into one sample jar.

6. Rinse the microspatula, toothbrush, and PVC ring into the sample, removing as much of the lingering periphyton as possible. Snap the labeled lid onto the container.

7. Rinse the PVC ring, toothbrush, microspatula, and squirt bottle thoroughly with stream water at the site after each sampling event to avoid contamination of subsequent collections.

8. A guideline for preservation is as follows: Assuming the sample jar is about 3/4 (120 ml) full, preserve with an adequate amount (a "plop") of 10% formalin from the squeeze bottle) for sparse to normal periphyton amounts. Add more for samples with heavy amounts of green algae. **The specimens cannot be over preserved.** The specimen cups are graduated (ml) so adding the proper amount of formalin can be measured. **Take extra care when preserving, as formalin is a known carcinogen.** Note: Samples do not need to be preserved immediately. It may be easier to preserve all periphyton samples collected in a given day at one time – upon returning to office or hotel parking lot. Whether samples are fixed immediately or not, they should be placed in a cooler with wet ice. Sample jars should be taped by sealing the rim of the lid with electrical tape to minimize the chance of spillage or cross-contamination.

9. Record the number of rocks "scraped" from each of the varying habitats (riffle vs. run and sunlight exposure classes). For example, 2 rocks in riffle, 3 in run/3 rocks with full exposure, 1 with partial shade, 1 with partial exposure. The yes/no questions and comments box on the Habitat Assessment Form (***see Chapter II. Section C. Part 1. PAGE 9-Periphyton Collection Information starting on page 73***) will be used to aid in interpreting data from scoured or drought affected reaches.

Part 2. Laboratory Methods

Periphyton identification and biomass determinations are performed by a private contractor. The contractor is required to have a degreed biologist on staff that performs the actual identifications. The contractor must adhere to the following protocols.

A. **"Soft" Algae (Non-Diatoms) –** Relative and abundance are to be determined as follows:

Homogenize sample in a blender and pipette a subsample into a Palmer counting cell. Dilute the sample if cells overlap too much for accurate counting. Identify and count 300 non-diatom algal units to the lowest taxonomic level at 400X magnification. Colonial species are to be counted as individual cells, when appropriate. Filamentous species should not be counted as individual cells, but as cell units of 10 micrometers in length. The number of "live" diatoms observed should also be recorded (identification will be done under a separate procedure). Record the numbers and species of "soft" algae on the bench sheet.

B. **Diatoms** – Diatoms are to be analyzed after the "soft" algal identifications are complete, as the clearing process will destroy soft tissue. Procedures are as follows:

1) Clear the diatom frustules of organic material using either nitric acid or hydrogen peroxide/potassium dichromate oxidation.
2) Prepare slides and identify diatoms to species or lowest taxonomic level possible.
3) Record all taxa encountered on the bench sheet to create a species list prior to enumeration. Continue identification until no new taxa are found after a 2-3 minute scan. To obtain quantitative data, count a minimum of 600 valves and record the taxa and number encountered on the bench sheet.

Part 3. Periphyton Data Assessment

An assessment of biological integrity can be made based on the periphyton data. The goal is to categorize water quality as excellent, good, fair, or poor and to determine the degree and cause of aquatic life use impairments in fair or poor streams.

Biological indices represent mathematical models of community changes. Changes in water quality will affect resident biota, and indices that reflect these changes in a particular community are useful biological indicators of water quality. The periphyton community, especially diatoms, is a useful biological indicator because:

- They are attached to the substrate and, therefore, subjected to any immediate or prolonged disturbances;
- Diatoms are ubiquitous, with at least a few individuals found under almost any aquatic conditions;
- Total number of taxa at any given site is usually high enough for use in calculating various metrics;
- Diatoms, especially the most abundant species, are identifiable to species by trained professionals;
- Tolerance of or sensitivity to changes (autecological requirements) is known or suspected for many species or assemblages of diatoms; and

- Periphyton communities, especially diatoms, have a rapid response and recovery time because of their relatively short lifecycle (as compared to fish or macroinvertebrates) and their ability to quickly recolonize formerly disturbed (impacted) sites.

Several metrics have been used successfully to assess water quality conditions using periphyton. Some have the diagnostic ability to indicate the type of impact (nutrient enrichment, toxicity, acidity, salinity, sewage (organic) pollution, and siltation).

Periphyton Quality Assurance and Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for residual algal material, rubbed clean and thoroughly rinsed with stream water before and after each sampling event.

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Periphyton will be collected along with other activities at the designated duplicate WAB sites. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been depleted by the first duplicate. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of periphyton samples is included. In the field, biological sampling teams will consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. Individuals who are more experienced in collecting periphyton will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Chapter VIII. GOLDEN ALGAE COLLECTION PROTOCOLS

Golden Algae Overview

Golden Algae (*Prymnesium parvum*) is a common saltwater toxin-producing microscopic algae first identified in WV in the fall of 2009 on Dunkard Creek, a large stream that straddles the border between Pennsylvania and West Virginia. In the first week of September 2009, the first reports of an aquatic life kill (*i.e*., fish, freshwater mussels, and amphibians) began to come into various state and federal regulatory agencies. By the end of the month, the aquatic life kill had exploded both in degree of severity and range within Dunkard Creek. The cause of the fish kill was eventually determined to be due to Golden Algae, specifically the toxin produced by the algae.

Golden Algae has become an invasive saltwater algae that is now being found in brackish (both natural and anthropogenic) inland waters in several states. The effects of the toxin produced by Golden Algae are dependent on several factors including: algal cell densities, availability of cations, temperature, pH, nutrient availability, and native freshwater algae which compete with Golden Algae for resources. In some cases, the algae can persist in streams completely unnoticed until one or more of the factors above change and force the release of toxin and consequently cause an aquatic life kill. The only viable means of controlling Golden Algae toxicity once the algae has become established in a water body is to control the factors that cause the release of the toxin.

Due to the great concern with the spread of Golden Algae to other waterbodies in the state, we have begun monitoring for the algae in target waters (*e.g.,* streams with conductivities greater than 1000) across the state that may be suitable for the algae to survive and act as a source for further spread. In addition, we continue to monitor the water chemistry and algae in Dunkard Creek in order to prevent the algae from causing further aquatic life kills.

Materials and Supplies

The following materials are required for filtering Golden Algae samples for qPCR analysis (***see Figure 64 on right***):

1. 1-liter cubitainer – Used as a sample container.
2. Filter Flask – Receptacle for the filtered water (at least 500 mL in size.)
3. Filter Funnel – Consists of three parts: A sterile Nalgene plastic 250 mL cup to hold the unfiltered sample, a small disposable plastic funnel that attaches below cup, and rubber stopper that affixes the filter cup to the flask.

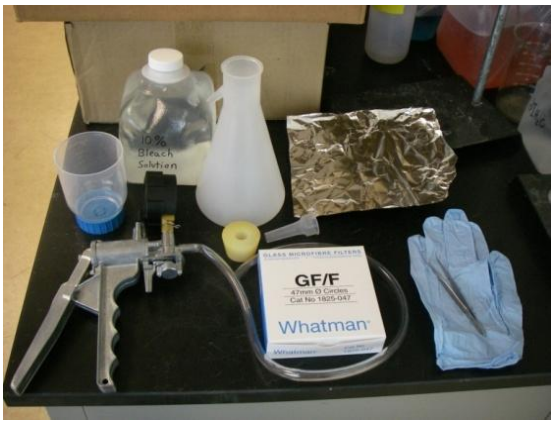

Figure 64. Photo of the materials used to filter a Golden Algae sample.

4. Filters – *Whatman* Glass microfiber filters GF/F 47 mm diameter with 0.45 µm pore size.
5. Vacuum Pump – A variety of hand operated pumps are available. **Do not use Peristaltic Pump/Drill Apparatus.**
6. Vacuum Tubing – usually already attached to vacuum pump.
7. 10% Bleach Solution - for sterilization.
8. Distilled drinking water and industrial grade deionized (DI) water.
9. Sterilized stainless steel forceps or sterile plastic forceps.
10. 4" squares of clean aluminum foil (1 square of foil per sample).
11. Zip Lock Baggies – 1 sandwich size per sample and 1 gallon size to hold all samples per filtering session.
12. Sample Labels (***see Figure 65 on right***) – 1 paper label to be included with each foil pouch. Label should have:
    a. Volume filtered
    b. Number of filters
    c. Stream name
    d. AN-code
    e. Date collected
    f. Date filtered
    g. Collector
    h. Filtered by
13. Golden Algae Analysis Request forms with Chain-of-Custody (COC).

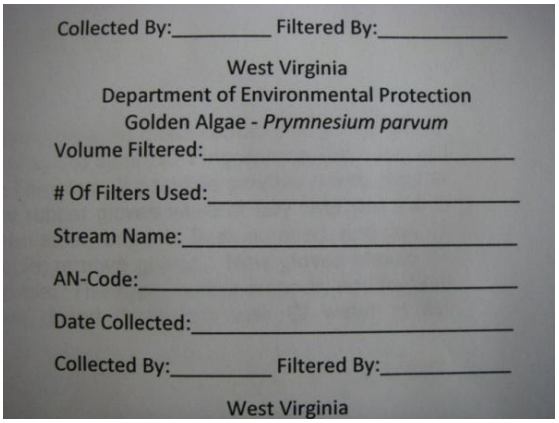

Figure 65. Photo of a Golden Algae label.

Field Safety Precautions

Rubber gloves and protective eyewear should be worn during sample collection to avoid bacterial contamination and for personal health protection as many streams may have sharp objects embedded in the substrate (*e.g.*, glass, metal, wire, etc.).

Part 1. Field Sampling Procedures

Golden algae samples are collected in a 1 liter cubitainer from a slow run or preferably a pool at approximately elbow depth (12-18 inches). To achieve a completely subsurface sample, the sample container must be capped until it is at the desired depth. The main concern is to avoid the upper surface of the water since the *P. parvum* cells may be sensitive to direct UV-light.

1. Rinse the 1-liter cubitainer and lid twice with stream water.

2. Cap the cubitainer and submerge to the desired depth.

3. Uncap the cubitainer and fill completely with no air space (*i.e.*, no head-space).

Remove capped/sealed cubitainer from water, quickly shake off external water and place inside a black or dark disposable plastic bag to prevent exposure to UV-light. Ideally the sample can now be placed in gallon zip-loc bag labeled with AN-code and date.

4. Samples are then placed on wet ice inside a cooler (maintained at 4° C) to keep them in the dark until filtering is started. Ideally, samples should be filtered immediately. If this is not possible, unfiltered samples should be maintained at 4° C and kept in the dark until they can be filtered.

Part 2. Sample Preservation (Filtration & Holding)

If possible, samples should be filtered and frozen by the end of the day they were collected. If this is not possible, unfiltered samples should be maintained at 4° C and kept in the dark until they can be filtered.

It is very important to keep the filtering process **as clean as possible** to prevent contamination. Therefore, filtration should be done in as controlled of an environment as possible. This may range anywhere from the back of a clean enclosed vehicle, to a hotel room, to the laboratory at WVDEP depending on the needs of the project.

Be sure to handle all materials by their exterior or by the stopper. Fingerprints, dirt/dust, and liquid from other samples can contaminate samples. Store all filtration equipment, aluminum foil, and sample labels in sealed plastic baggies when not in use. While handling aluminum foil at any time (cutting, folding, or removing from plastic bag); always be sure your hands and any surfaces it comes in contact with are clean and sanitized. If possible sanitize hands prior to handling any of the above materials and/or wear disposable laboratory gloves any time you are in contact with the sample. New gloves should be used for handling each individual sample. The flask, rubber stopper, and steel forceps should be soaked in 10% bleach solution for one minute and rinsed thoroughly with DI water at the beginning of and following each filtering session. Additionally, steel forceps and rubber stopper should be rinsed in bleach solution between each sample.

Inspect all sterilized materials for traces of liquid. Any liquid should be removed from the flask vacuum nozzle or the vacuum tube.

Sample blanks are generally prepared and processed as below using either distilled drinking water from freshly opened gallon containers or industrial grade deionized water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described below. Two blanks should be prepared for each batch of samples. One of the blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered.

Filtration

1. Place plastic funnel snuggly into rubber stopper, and insert stopper snuggly into empty flask (***see* Error! Reference source not found. *n right***).

2. Attach vacuum tube and hand pump to flask.

3. Snap bottom of filtration cup onto plastic funnel then carefully remove top portion of the cup from its base revealing prepackaged nitrate seal/filter (***see Figure 67*Error! Reference ource not found. *below***).

Figure 66. Photo of the insertion of rubber stopper into flask with plastic funnel already inserted through rubber stopper.

Figure 67. Photo of cup being removed to access the prepackaged filter for removal.

4. Remove prepackaged nitrate seal/filter from cup base using sterilized forceps and place in a clean zip loc bag for potential use in other water quality monitoring projects.

Figure 68. Photo of the GF/F filter being placed on top of support screen with forceps.

5. Using sterilized forceps, place a new glass GF/F 47 mm filter in center on top of support screen found on the cup-base (***see Figure 68 right***). The cross-hatch pattern side of the disk should be placed facing down against the support screen (***see Figure 69 below***). Note: *Whatman brand filter disks are packaged with the cross hatch facing up – so, reversing them before placing on the support screen puts them in the*

proper position for filtering.

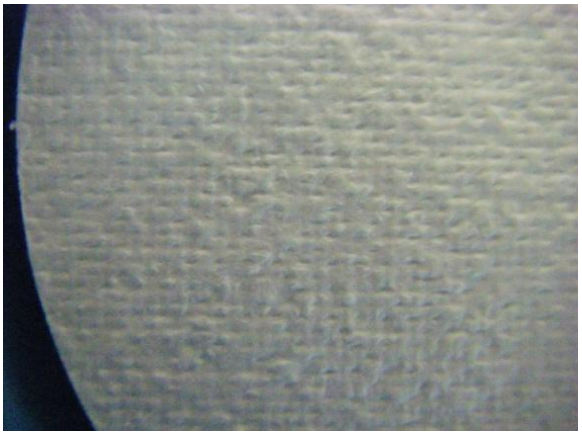

Figure 69. Photo of the gridded pattern of GF/F filter. This side placed down when filtering Golden Algae sample.

Figure 70. Photo of the pouring of a 250 mL sample into filter cup.

6. Replace top portion of cup straight down onto base with a snap to ensure seal. Make sure the filter disk is still centered.

7. Remove plastic lid from cup. Apply disposable laboratory gloves now before touching sample bag or bottle.

8. Mix sample thoroughly by inverting the cubitainer/sample bottle three times. Pour 250 mL of sample into filter cup and replace cup lid to ensure proper vacuum (***see Figure 70 above***). Place remaining sample back into bag to prevent further UV exposure.

Figure 71. Photo of monitoring the pump pressure while filtering sample – keep pressure under 5 psi.

9. Filter 250 mL into the flask. Agitate the remaining sample in cubitainer again, remove cup lid, and pour an additional 250 mL into filter cup. This should be done without disassembly so that a total of 500 mL is filtered through a single filter. If more or less than 500 mL is filtered, be sure to record the actual amount as precisely as possible.

10. Minimal pressure/suction (<u>not to exceed 5 psi</u>) is used via the hand pump to pull sample through filter (***see Figure 71 above***). This is important because of the potential to break the filter and lose a portion of the algal sample into the flask. Turbid samples may take many minutes to filter so remain patient.

11. After full sample volume has been filtered, carefully remove filter cup from its base and discard. Filter disk will usually remain on support screen, but be prepared to remove from top portion of cup with sterile forceps if necessary. Save the remainder of unfiltered sample in the cubitainer by putting it a dark 4° C cooler or refrigerator. This unfiltered sample will be sent to the identifying laboratory to use for QA/QC purposes. Now remove disposable plastic gloves carefully and dispose gloves in trash.

12. The filter disk is removed with sterile forceps, folded into a half-circle (still using only forceps) with sample-side in (grid side out for Whatman filters), and wrapped loosely in one 4"X4" aluminum foil square to prevent loss of algal cells via compression and smearing (***see Figure 72 on right***). The sample is then tagged with a completed sample label on outside of foil, placed in an individual sandwich-sized zip lock bag, and then placed in a freezer before shipping. If multiple samples are filtered, all of the individually packaged and bagged filters should be placed in a gallon-sized zip lock bag for storage and shipping to laboratory.

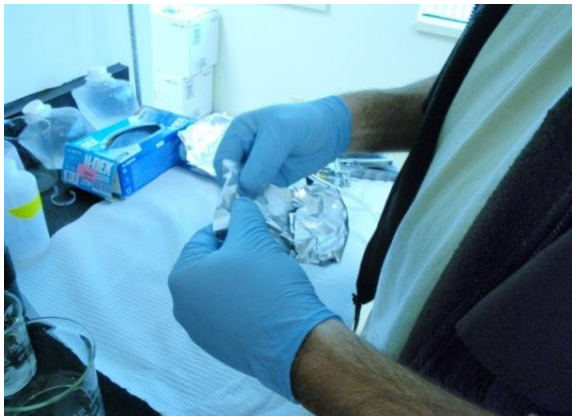

Figure 72. Photo of the filter disk being wrapped loosely in one 4"X4" aluminum foil square to prevent loss of algal cells via compression and smearing

13. After filter disk is properly packaged, labeled, and frozen, discard all water in flask by removing rubber stopper, and pouring out the remainder. Be sure not to allow water into flask nozzle or vacuum tube. If enough plastic funnels are available you can now dispose of used plastic funnel. If not replacements are available see directions below.

14. Between filtering samples, rinse the small plastic funnel and forceps with tap water, soak in a 10% bleach solution for 1 minute (***see Figure 73 on right***), and rinse with distilled drinking water (***see Figure 75 below***). A final rinse with industrial grade deionized water completes the cleaning process between samples (***see Figure 74 above***).

Figure 73. Photo of Forceps and small plastic funnel soaking in 10% bleach solution for 1 minute.

Figure 75. Photo of forceps being rinsed with deionized (DI) water.

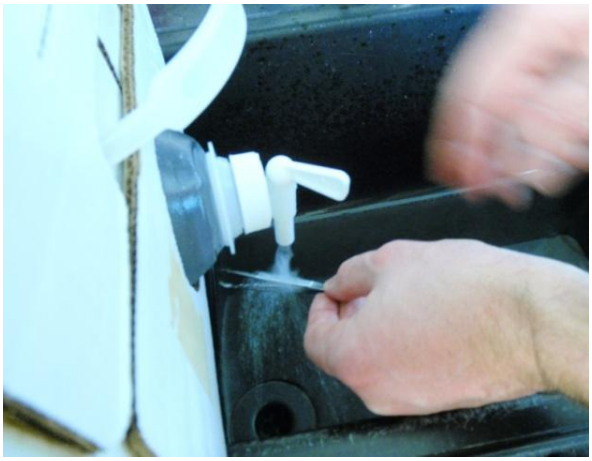

Figure 74. Photo of small plastic funnel being rinsed with distilled water.

NOTE: If desired volume of water (500 mL) will not go through filter due to clogging, start procedure from the beginning with a new cup and new filter with reduced sample volume in the cup. Do not remove any volume from the cup once the sample has been added or filtering has started, as this may cause contamination or loss of sample. The desired volume may be obtained by reducing the sample into smaller aliquots and filtering through a separate filter per aliquot. For example, it may be necessary to filter 250 mL through one filter and 250 mL through a second filter in order to obtain the desired 500 mL sample. If this is necessary, both filters should be folded and placed in separate sample foil packets. Both foil packets can be placed in 1 small zip-lock bag. However; when using the two filter method, a new cup must be used for each half and the funnel/forceps must be cleaned using step 14 above.

<u>Remember</u>: Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade deionized water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described above. Two blanks should be prepared for each batch of samples. One of the blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered.

Holding

If possible, samples should be filtered and the filter samples frozen by the end of the day on which they were collected. Freezing can be accomplished by placing the filter samples in a freezer or on dry ice (during field processing). Frozen samples are shipped overnight in batches or individually on dry ice to appropriate contract identification lab. An Analysis Request Form with Chain-of-Custody (COC) designed for Golden Algae samples should be completed which includes all samples (field and blanks). ***See Figure 76 below for an example of a Golden Algae Analysis Request Form with COC***.

WV DEPARTMENT OF ENVIRONMENTAL PROTECTION - WATERSHED ASSESSMENT BRANCH

Golden Algae Analysis Chain of Custody Form, Rev. 2/10

Laboratory Name: _____

Preservation Method:
Unfiltered Sample: Store on ice at 4° C
Filtered Sample: Frozen

Stream Name	AN-Code	Collection Information			Filtration Information			
		By:	Date:	Time:	By:	Date:	Vol. (ml)	# of Filters

Relinquished by:	Date & Time:	Received By:	Relinquished by:	Date & Time:	Received By:

Mail Results to: ATTN: Janice Smithson, WVDEP, DWWM, Watershed Branch 601 57th Street SE, Charleston, WV 25304
Phone: (304) 926-0499 Ext. 1051, Fax. (304) 926-0496
Mail Invoice to: ATTN: Susan Rose, WVDEP, DWWM, Watershed Branch 601 57th Street SE, Charleston, WV 25304
Phone: (304) 926-0499 Ext. 1056, Fax. (304) 926-0496
On invoice, bill to Organization Unit 9481
Original COC Mailed to Laboratory, Photocopy retained by WVDEP

Figure 76. Example of a Golden Algae Analysis Request Form with COC

Golden Algae Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of Golden Algae samples is included. In the field, sampling teams will often consist of two people. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in collecting water quality will be teamed up with the less experienced to assure reinforcement of training and accurate results before they are allowed to collect Golden Algae samples solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Sample transfer to the lab shall be documented using the Chain-of-Custody (COC) portion of the Golden Algae Analysis Request Form.

The unfiltered portion of the original sample water is to be retained after filtering and may be sent to the contract identification laboratory with the filter samples. If the original filter sample appears to be contaminated, the contract lab may elect to replicate the filter sample using the original sample water as a mean of investigation.

Duplicate sampling and sample blanks must be performed at a minimum of 2.5% of our sites. To assure we meet these requirements, each team list will have a designated duplicate and sample blank. The sample blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented below. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Sample Blanks

Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade deionized water. A 500 mL sample is filtered and all prep, handling, and rinsing is the same as described above. Two blanks should be prepared for each batch of samples. One of the

blanks should be processed before any stream samples are filtered. The second blank should be processed after all stream samples have been filtered. Laboratory analysis of the sample blanks should indicate if there is any contamination originating from the filtering process.

Duplicate Samples

Both duplicates are collected at the same date and time and literally side by side by different individuals. If the sampling team consists of one person, as is often the case during a TMDL assessment, the duplicate is still performed by the one sampler. Extreme care is taken to assure that the second duplicate is not taken from an area that may have been disturbed by the first duplicate. TMDL replicates are collected at any TMDL site with the full potential of parameters on the TMDL list. TMDL replicate sites are not specifically assigned; however, field crews should not repeatedly duplicate the same site.

Duplicates should be rotated to different sites each sampling event.

Results of the duplicates are compared and any samples not falling within an acceptable range are examined for sampling error. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that different teams perform the duplicate sampling throughout the sampling season to ensure that all variability is being captured. The variances between individual techniques will be documented and used in future training sessions or individual re-training.

Note: If two people are involved in collecting a duplicate, each person should filter his or her own sample and not filter the other person's sample.

Chapter IX. RELATIVE BED STABILITY/SUBSTRATE CHARACTERIZATION PROTOCOLS (INCLUDING GRADIENT)

Materials and Supplies

1. 100 meter Measuring Tape – used to delineate the length of the substrate characterization reach and to demarcate the data points along this reach.
2. Flagging Tape – used to mark the eleven data point intervals in the substrate transect.
3. Survey Extension Pole – used to determine stream width and data points along each transect. Also used in conjunction with the clear plastic tubing to measure the rise in the stream between the two ends of the reach.
4. Thalweg Pole – used to measure the thalweg and to determine the substrate character at each transect data point. Also used in conjunction with the clear plastic tubing to measure the rise in the stream between the two ends of the reach.
5. Handheld Eye Level – used as an alternative method to measure the rise in the stream between the two ends of the reach.
6. Water Level– made of clear plastic tubing with valves on each end; used to measure the rise in the stream between the two ends of the reach.
7. Relative Bed Stability Form – Forms for substrate characterization are a not a normal component of the WAB habitat sheet and are documented on appendix sheets (***See Chapter II. Section C. Part 2. APPENDIX #5 – Substrate Characterization (Pebble Count) including Gradient on page 91)***

Procedures

Part 1. Establishing Reach and Transects

1. Determine the substrate characterization reach by multiplying the average stream width (as determined during the Rapid Bioassessment Protocol survey) by 40. The minimum and maximum widths are 100 and 500 meters, respectively. Record the reach length on the Habitat Assessment Form (***See Chapter II. Section C. Part 2. APPENDIX #5 – Substrate Characterization (Pebble Count) including Gradient starting on page 91***).

2. Determine the transect intervals by dividing the total reach length by 10. Measurements are taken at each of these transects including the upstream and downstream endpoints for a total of 11 transects. Each transect is assigned a letter, with the first (downstream) transect identified as Transect A and the upstream terminus being Transect K.

Part 2. Substrate Measurement (AKA Pebble Count), Thalweg Profile, and Bankfull Height

1. Begin at Transect A and work upstream. Mark the measurer and recorder of the data.

2. Using the survey extension pole, determine the wetted stream width. Divide the stream with by four to determine the measurement points. Measurements will be taken at the right descending bank (0% of the wetted-width), right-center (25% of the wetted-width), center (50% of the wetted-width), left-center (75% of the wetted-width), and at the left descending bank (100% of the wetted-width). **Note: If a split channel is encountered one of two things can occur:**
   - **A) If the split channel features a bar** (**bar definition: a channel feature below the bankfull height that is dry during baseflow conditions)** then conduct the measurements at that transect as if there was only one channel and note the presence of the bar. Any measurements that fall on the bar should be treated just as if it was inundated with water, but noted as being taken on a bar.
   - **B) If the split channel features an island (island definition: a channel feature even with the surrounding flood plain or above the bankfull height that remains dry even at bankfull flow)** then conduct a separate transect in each channel for the length of the island. The situation should be documented and the second transect information is recorded continuing the transect letters down the alphabet starting with J.

3. To take a substrate measurement, hold the thalweg pole vertically at the transect point and lower it straight down to the bottom. Pick up the particle at the tip of the pole (if it is not a boulder or bedrock). Using the markings on the thalweg pole, measure the particle at its median diameter. Each particle will have three dimensions: width, depth, and height. Measure the "middle" dimension, *i.e*., the dimension that is neither the largest nor smallest. Record the size class the particle falls into based on the following table (***see Table 10 below***).

   NOTE: In cases where there is a deposit of fine material (silt or sand) on top of another substrate type, you must use the THUNK test to determine which layer to count. The THUNK test consists of slowly lowering your thalweg pole straight down to the bottom as normal. If your pole hits the particle abruptly and makes a sort of “THUNK” sound, then the deposit of fine material is not considered and you count the underlying material. If your pole hits the bottom and can continue down to some degree with minimal resistance, then you record the fine material on top. Much of this determination relies upon experience and best professional judgment. Be sure to confer with your team partner and if in doubt, write notes.

Table 10. Substrate Size Classes for Substrate Characterization (Pebble Counts)

Class	Code	Size	Description
Bedrock	BR	>4000 mm	Bigger than car
Boulder	BL	>250-4000 mm	Basketball to car
Cobble	CB	>64-250 mm	Tennis ball to Basketball
Coarse Gravel	CG	>16-64 mm	Marble to Tennis ball
Fine Gravel	FG	>2-16 mm	Ladybug to marble
Sand	SA	>0.06-2 mm	Gritty between fingers
Silt & Fines	ST	<0.06 mm	Smooth, not gritty (silt & muck)
Clay	CL	>4000 mm	Slick/ hard clay or hard-pan clay bottom
Leaves	LD	Regardless of size	Leaf packs
Wood	WD	Regardless of size	Root wads, snags, logs, sticks

4. Repeat this step for each of the five measurement points.

5. Determine the thalweg of the transect. The thalweg is the deepest part of the stream channel at the transect. Use the thalweg pole to determine water depth at the thalweg. **Read the depth on the <u>side</u> of the thalweg pole to avoid the wave produced by turbulence.**

6. The bankfull height is defined as the channel height that is filled by moderate-sized flood events that occur every one or two years. Look for a variety of bank characteristics to determine the bankfull height. First, determine the location of the active floodplain. Next, look for an obvious slope break in the banks that differentiates the channel from a flat floodplain higher than the channel. A transition zone often exists between exposed substrate and vegetation, which marks the bankfull height. Look for a change from water-loving and scour-tolerant vegetation to more drought-tolerant vegetation. Also, it may be determined by moss or vegetation growing on rocks along the banks. A change from well-sorted stream sediments to unsorted soil materials is also a good indicator. In addition, indicators from the previous season's flooding are may be used if there have been no recent large floods or prolonged droughts: the presence of drift material (*e.g.*, leaves, trash) along the bank or on overhanging branches from the previous seasons flooding, the level where deciduous leaf-fall is absent on the ground because it was swept into the stream by flooding since the last leaf-fall, and unvegetated sand, gravel or mud deposits from previous seasonal flooding. In cases where the channel is cutting a valley sideslope and has oversteepened and destabilized that slope, the bare "cutbank" against the steep hillside is not necessarily an indication of recent incision. In such a case, the opposite bank may be lower and a more obvious indicator of bankfull and channel heights and widths.
   In streams in deep V-shaped valleys, the difference between the bankfull and channel depth may be indistinguishable due to a lack of stream incision.

7. **Be sure to record a minimum of three bankfull height measurements throughout the sample reach. These measurements can occur anywhere along the reach, but should be spaced out along the reach.**

8. Repeat these steps for Transects B through K. The next transect can be located by moving upstream the transect interval as calculated above using the 100m measuring tape as a guide. Be sure the data from each transect is recorded in the appropriate space on the data sheet.

Part 3. Gradient Measurement

IMPORTANT: Gradient measurements must be taken along the full length of the reach. If the full reach is not measured with the tape measure, the gradient calculation will be incorrect and the time taken to record this data will be wasted.

There are two options for devices to measure slope: the Handheld Eye Level and the Water Level. Each device has its positives and negatives and each should be considered when selecting a device.

The Handheld Eye Level is much smaller than the Water Level in both weight and volume, so it may be more ideal in situations where a lengthy hike is necessary. It also is possible to measure longer distances with the Handheld Eye Level if the stream is straight enough and there are not major line-of-sight issues (*i.e.,* overhanging vegetation, houses, bends, etc.). Larger reaches (*e.g.,* >250 m) may benefit from the use of the Handheld Eye Level in both ease of use and reduction in the amount of time to obtain the Gradient Measured. On the negative side, the Handheld Eye Level is less accurate than the water level. It can also be problematic in raining conditions as the lenses can fog up. In addition, if two shorter people are working together, it may become necessary to use shorter distances between readings so that the person at the downstream end can point out with a stick or even read the level mark.

The Water Level is definitely more accurate. But the distance between readings is limited (usually to 20 m) by the length of tubing. In addition, high gradient streams may require one to shorten the length of the tube in order to capture the reading on the downstream end. The Water Level is ideal for situations with dense overhanging vegetation that prevent the use of the Handheld Eye Level. Because of its weight (the tubing and water inside the tubing) may be more useful when the sample site is immediately near the roadside and jeep.

Measurement Methodology

The primary method is to use a handheld eye level. In the Handheld Eye Level Method, the slope is measured by “backsiting” or “backshooting” downstream between the two reach ends. If a situation occurs where using the handheld eye level is not feasible

(*e.g.*, the stream is too sinuous or there is too much overhanging vegetation) then the Water Level Method may be used instead to cover that distance.

The secondary method is to use a water level in the form of clear plastic tubing with some sort of length measuring device on each end (*e.g.*, the Thalweg and Survey Extension Poles). If a situation occurs where using the water level is not feasible (*e.g.*, the stream goes under a road or culvert) then the handheld eye level may be used instead to cover that distance.

Handheld Eye Level

Note: Each individual should determine and remember their eye level height (the point on the survey pole that their eye is level with) before doing any slope measurements using the handheld eye level. Also, keep in mind that this point can change when wearing different wading boots or footwear.

1. One individual stands at the water surface along the bank with the handheld eye level while the other holds the survey extension pole at the water surface downstream (as far as the individual can see accurately with the handheld eye level).

2. Looking through the eye level, the upstream individual determines where along the vertical surface of the survey extension pole their eye is level. The upstream individual will instruct the downstream individual to move a horizontal marker (*e.g.*, finger, stick, pencil, thalweg pole) up or down to the same spot. The upstream individual's eye level height is then subtracted from the measured height to determine the rise of the stream for that distance. This value is recorded on the field form.

3. The upstream individual must now move to the position of the downstream individual (which can be marked by stacking rocks or with placement of an object or flagging) and the downstream individual moves down as far as the upstream individual can see accurately with the handheld eye level). Repeat the measurements as described until the downstream end has been reached.

Water Level Method

1. Fill the tubing by holding both ends level and pouring stream water collected in a cubitainer into the tubing until full. An alternative method to fill the tubing is to put stretch the tubing along the stream and submerge the upstream end under the water surface while siphoning the downstream end until enough of a draw is created to fill the tube.

2. Each partner secures an end by placing a rubber stopper or thumb into the end of the tubing, and then stretching the tubing to length along the contour of the stream starting at the upstream end of the reach.

3. Place the surveyor pole at water level at the downstream end. Stretch the tubing along the surveyor pole with the end of the tube at least to the 1 m mark (or higher if necessary). To help hold the tube against the pole, you may use your foot to help hold the tube at water level. Perform the same steps at the upstream end with the exception of using the thalweg pole.

4. When both ends are in position, the upstream individual must remove the stopper or thumb from the end of the tubing, followed by the downstream individual. The water level will oscillate until equilibrium is reached. Once the water level in the tube is stable, record the approximate location of the meniscus on each end, then subtract the upstream from the downstream measurement. Record this as the change in elevation or rise of that stream segment.

5. The upstream individual must now move to the position of the downstream individual (which can be marked by stacking rocks or with placement of an object or flagging). The tubing is again stretched to length and the method is repeated. Repeat the measurements as described until the downstream end has been reached

NOTE: If you encounter a high waterfall in the reach, measure the rise of the waterfall from the edge (if safe) of the fall to the splash-zone below using the surveyor pole and record it in one of the extra blanks on the field form (***See Chapter II. Section C. Part 2. APPENDIX #5 – Substrate Characterization (Pebble Count) including Gradient starting on page 91***). Also describe the reading and include what it is (*i.e.* waterfall) and where in the reach the waterfall was located (transect location). Then continue measurements past the waterfall as normal.

The final gradient measurement (% Gradient) is calculated after data entry via a query calculation:

Equation 6. Calculation of Percent Gradient

$$\%\ \textbf{Gradient} = \frac{\textbf{Sum of Rises}}{\textbf{Reach Length}} \textbf{x } 100$$

Where:

Sum of Rises = the summation of all the measured rises within the reach in meters

Reach Length = the total length of the reach in meters

Substrate Characterization Data Analysis

All of this data (Pebble Count, Thalweg Profile, Bankfull Height, and Gradient) are entered into the WAB database and numerous values and statistics are calculated via a series of queries. These values define the approximate characteristics of the stream's substrate (D_{50} or average particle size) and the relative extent of impairment by sedimentation that is occurring (D_{84} or bankfull particle size).

Substrate Characterization Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and recording of Substrate Characterization data is included. Individual training will occur simultaneously on the same stream so the results can be compared to the group average. Readings that deviate exceptionally from the norm will be examined for errors. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, Substrate Characterization teams will consist of two people. Individuals who are more experienced in measuring Substrate Characterization data will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

CHAPTER X. AMBIENT WATER QUALITY NETWORK PROTOCOLS

Overview

The Division of Water and Waste Management's predecessor, the Water Resources Division of the Department of Natural Resources, began the Ambient Water Quality Monitoring Program in the 1960's. Many changes have occurred to the program since then, but the basic goal remains the same: to monitor water quality at a set of West Virginia's streams over long periods of time. Natural resource managers have divided the state into 32 watersheds. Each of these watersheds is viewed as a unit for managing various environmental programs. The Ambient Water Quality Network consists primarily of sites on the main stem streams of these watersheds, mostly focusing near the downstream ends of the watersheds. A listing of the Ambient Water Quality Network stations is as follows:

1. **BST-(0.15)** Tug Fork At Fort Gay
2. **OG-(2.8)** Guyandotte River At Huntington
3. **OG-(74.1)** Guyandotte River At Pecks Mill
4. **K-(31.7)** Kanawha River At Winfield
5. **K-(76.9)** Kanawha River At Chelyan
6. **KC-(11.6)** Coal River At Tornado
7. **KE-(4.3)** Elk River At Charleston
8. **KG-(8.3)** Gauley River At Beech Glen
9. **KN-(1.55)** New River At Gauley Bridge
10. **KN-(67.4)** New River At Hinton
11. **KN-(96.2)** New River at Glen Lyn
12. **KNG-(1.6)** Greenbrier River At Hinton
13. **LK-(28.9)** Little Kanawha River At Elizabeth
14. **LKH-(1.5)** Hughes River West Of Freeport
15. **M-(99.4)** Monongahela River At Star City
16. **M-1-(20.6)** Dunkard Creek East Of Pentress
17. **MT-(6.2)** Tygart Valley River At Colfax
18. **MW-(12.0)** West Fork River At Enterprise
19. **MC-(3.5)** Cheat River Below Lake Lynn, Pa
20. **MC-(30.0)** Cheat River At Albright
21. **OMI-(12.3)** Middle Island Creek At Arvilla
22. **O-2-(8.8)** Twelvepole Creek South Of Ceredo
23. **P-4-(2.2)** Opequon Creek East Of Bedington
24. **PC-(6.1)** Cacapon River South Of Great Cacapon
25. **PSB-(13.4)** South Branch Of Potomac River
26. **S-(0.9)** Shenandoah River At Harpers Ferry

The sites in the Network are currently visited every two months or six times per year. Sites (or nearby proxy sites) that are wadeable or partially wadeable during summer low flows are sampled for benthic macroinvertebrates and full habitat assessment once a year as a part of the Long Term Monitoring Stations program (***see Chapter I. INTRODUCTION TO WATERSHED ASSESSMENT BRANCH SAMPLING ACTIVITIES Sampling Programs of the Watershed Assessment Branch on page 1 for a description***).

Generally, one person will collect the samples and utilize commercial laboratories for analysis. The data are then entered into the Watershed Assessment Branch database, where they are made available for trend analysis, general water quality assessments, pollutant loading calculations, and other tasks necessary for various agencies to fulfill their commitments to environmental management.

In general, the Ambient Water Quality Monitoring Program utilizes the same sampling techniques other Watershed Assessment Branch programs. Specifically, the SOP sections that apply to this program are as follows:

Chapter II. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) starting on page 6

Chapter III. WATER COLLECTION PROTOCOLS starting on page 95

Chapter IV. STREAM FLOW MEASUREMENT starting on page 136

Chapter V. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS starting on page 150

Chapter XII. Section A. Field Blanks and Duplicates starting on page 285

Since only a few people routinely sample a the Ambient Water Quality Network stations, the main purpose for the inclusion of this section in the SOP is to give any person in the Watershed Assessment Branch the ability to locate and take a sample from these stations should the need arise.

Section A. METHODS AND PROCEDURES

Brief descriptions about some of the specific sampling techniques and sample handling as they apply to the Ambient Water Quality Monitoring Program are provided below. ***See Chapter III. Section B. Water Quality Sample Collection and Preservation starting on page 119 for more detailed information about these techniques.***

Part 1. Ambient Water Quality Network Water Parameters

Table 11. The current list of Ambient Water Quality Network Water Parameters, MDLs, Analysis Methods, and Holding Times

Parameter	MDL or Instrument Accuracy*	EPA Method	Holding Time
Acidity	5 mg/l	305.1	14 Days
Alkalinity	5 mg/l	310.1	14 Days
Aluminum, Dissolved	0.08 mg/l	202.1 / 200.7	6 Months
Aluminum, Total	0.08 mg/l	202.1 / 200.7	6 Months
Ammonia Nitrogen	0.10 mg/l	350.1	28 Days
Arsenic, Total	0.05 mg/l	200.9 / 206.2	6 Months
Barium	0.002 mg/l	200.7	6 Months
Boron	0.003 mg/l	200.7	6 Months
Cadmium, Dissolved	0.0003 mg/l	200.9	6 Months
Chloride	1 mg/l	325.2	28 Days
Copper, Dissolved	0.003 mg/l	200.9	6 Months
Fecal Coliform	N/A	SM9222D	24 Hours
Hardness	0.01 mg/l	SM2340B	6 Months
Iron, Dissolved	0.05 mg/l	236.1	6 Months
Iron, Total	0.05 mg/l	236.1	6 Months
Lead, Dissolved	0.0005 mg/l	200.9	6 Months
Manganese, Total	0.01 mg/l	243.1	6 Months
Mercury, Total	0.0001 mg/l	SM3112B	6 Months
Nickel, Dissolved	0.07 mg/l	200.9	6 Months
Nitrate + Nitrite	0.2 mg/l	353.2	28 Days
Phosphorus, Total	0.1 mg/l	365.1	28 Days
Selenium, Total	0.001 mg/l	200.8	6 Months
Sodium	0.5 mg/l	200.7	6 Months
Silver, Dissolved	0.0003 mg/l	272.2	6 Months
Sulfate	5 mg/l	375.2	28 Days
Total Kjeldahl Nitrogen	1 mg/l	351.2	28 Days
Total Dissolved Solids	5 mg/l	SM2540C	7 Days
Total Suspended Solids	1 mg/l	160.2	7 Days
Zinc, Dissolved	0.03 mg/l	289.1	6 Months
Field Sp Conductivity	+/- 0.5% of range*	YSI	Instant
Field Dissolved Oxygen	+/- 0.2 mg/l*	YSI	Instant
Field pH	+/- 0.2 units*	YSI	Instant
Field Temperature	+/- 0.15° C*	YSI	Instant

Part 2. Water Sampling Techniques

Since the Ambient Water Quality Network stations vary in size from large wadeable streams to fully boatable rivers, different methods of water collection must be employed from site to site. Factors to consider when selecting a method are water depth, proximity to upstream tributaries that may not be fully mixed into the main channel, and safety.

Direct Dip/Grab Method

See Chapter III. Section B. Part 1. Procedures for Collecting Water Quality Samples Direct Dip/Grab Method starting on page 120 for more details.

The direct dip or grab method is the preferred method to obtain a sample as it eliminates the need for extra equipment that may introduce contamination into the sample and allows the multiprobe sonde direct contact with the water column in the same flow vector as the lab water. This method may be employed if:

1. *The stream is wadeable.* Generally, this is common at some of the sites, especially during the low flow summer months.
2. *The stream is boatable and you have access to a boat.* This is rarely employed as it is often difficult to control the boat so that you can obtain all of the samples in the same spot, especially when one is working solo. In addition, the Watershed Assessment Branch boats may not be available due to use or maintenance.
3. *Circumstances force you to sample from the bank*. This is the least preferred means of obtaining a sample as it may not characterize the main channel, especially if there is a tributary upstream that is not adequately mixing into the main channel water. You should only employ this method if you are sure that there are no such tributaries upstream. If there is a bridge nearby, it may be preferable to sample from the bridge using one of the methods discussed below.

Bridge Crane Method

This method will likely not allow the direct contact of the multiprobe sonde to the water column due to the shortness of the cord between the sonde and the display unit. There are some longer cables available that could potentially allow the sonde to reach the water column from a bridge, but they are often scarce and in use with other sampling efforts (*e.g.*, Lake Sampling). In any case, it may be more comparable to keep all water measurements limited to the same sampling method (*i.e*., get lab water and sonde readings both from a direct grab or bridge method, but don't mix and match).

In the absence of the bridge crane, a simple rope with a latching hook can be used. However, this presents its own issues with safety and potential contamination as it is more difficult to control the rope, especially on a tall bridge.

When sampling at bridge sites, the use of specialized equipment is required.

Van Dorn Horizontal Sampler

The Van Dorn Horizontal Sampler (VDHS) is used to collect water samples, by being lowered from a bridge into a stream, via a bridge crane device. The bridge crane can be adjusted to allow compatibility with the height of the bridge railing/berm (*i.e*., prevent the rope from touching the side of the bridge and potentially knocking contaminants into the sampling device or immediate sampling area).

1. Before sampling, rinse the VDHS with DI or distilled water.

2. From the selected bridge sampling location, securely attach the VDHS to the rope from the bridge crane winch. Secure the ends of the VHDS to the trigger mechanism.

3. Lower the VDHS over the bridge and allow the VDHS to be rinsed in the stream.

4. After thoroughly rinsing the VDHS, attach a messenger (sliding weight) to the rope, and drop, to the trigger, to close the ends of the VDHS.

5. Raise the VDHS and release a small amount of water from each valve.

6. Discard the first fill and repeat Steps 3 thru 5 gathering enough sample water to rinse collection bottles twice. Use the remaining water to gather physicochemical water quality parameters using a multiprobe sonde placed directly into the sampler or a large container.

7. Rinse each sampling container twice then fill.

Stainless Steel Bucket

In select sampling situations, the direct dip and VDHS techniques are not practical or applicable. Alternately a stainless steel bucket (SSB) may be used along with the bridge crane to retrieve water samples.

1. Before sampling, rinse the SSB with DI or distilled water.

2. From the bridge location, securely attach the SSB to the rope from the bridge crane winch and lower.

3. Allow the bucket's bottom to touch the stream surface, then gently tilt the bucket's mouth upstream, allowing at least one gallon of rinse water to enter the bucket.

4. Retrieve the bucket and thoroughly agitate the water inside, rinsing the inside of the bucket with stream water.

5. Repeat Steps 3 and 4 and gathering enough sample water to rinse collection bottles twice. Use the remaining water to gather physicochemical water quality parameters using a multiprobe sonde placed directly into the bucket. Extra care must be taken to prevent the SSB from heating up quickly in the summer months due to contact with hot concrete or steel (*i.e*. try to keep the bucket in the shade).

6. Lower the bucket again allowing it to fill with water. Raise the SSB. Fill sample bottles and cap quickly to avoid road contamination.

Fecal Coliform Bacteria Sampler

It is not possible to use a VDHS or SSB to collect a Fecal Coliform Bacteria sample because the sampling apparatus needs to be 100% sterile and clean. To get around this, one must lower the sterile fecal coliform bottle from the bridge directly into the water column using a special metal apparatus that holds the bottle securely and in the open position.

Bridge Sampling Safety

Taking a water sample from a bridge is an inherently dangerous activity. Hazards are abundant and change with time of day, season and local weather conditions. They include boats, jet skis, passing cars and trucks, bridge height, power lines, strong winds, ice, rain, unsteady footing, and pedestrians. Wearing a safety vest and PFD is highly recommended when sampling from any bridge location. Always be aware of your surroundings, and any potential hazards in the area. Avoiding falls from the bridge and contact with traffic must be the sampler's primary focus when taking this type of sample. Check for/be aware of anything below when on the bridge and anything above you when under it. Anything placed on the berm is subject to falling off the bridge and becoming a projectile, so avoid this if at all possible. Traffic cones should be used when parking on a bridge or when a sufficient walkway or emergency lane does not exist at the sampling site. Failure to consider potential hazardous situations while bridge sampling could lead to a serious injury of either the sampler(s) and/or passersby.

Part 3. Water Sample Preservation/Filtering & Handling

All water sample preservation/filtering and handling techniques are identical to those presented in ***Chapter III. Section B. Part 2. Sample Preservation (Filtration, Fixation, & Holding) on page 121***.

Part 4. Measuring Stream Flow

Small Streams

Stream flow can be measured at some of the smaller Ambient Water Quality Network sites by hand held flow meter. Measurements should be made during low flow periods, typically in the summer months at the following sites:

O-2-(8.8) Twelvepole Creek
LKH-(1.5) Hughes River
OMI-(12.3) Middle Island Creek
M-1-(20.6) Dunkard Creek
P-4-(2.2) Opequon Creek
PC-(6.1) Cacapon River

Flow measurements should be made whenever the water depth and velocity allow it and according to techniques described in ***Chapter IV. STREAM FLOW MEASUREMENT starting on page 136***. Be sure to wear a personal flotation device when measuring flow. Calculate the total discharge and record on the appropriate form (***see Chapter II. Section B. Part 2. APPENDIX #1 - Stream Discharge (Flow) on page 78***).

Large Streams

Most of the larger Ambient streams have been purposefully stationed at or very close to a United States Geological Survey (USGS) gauging station. USGS maintains a website to access current and historical stream discharge and stream stage data from these stream gages. The web addresses for West Virginia daily stream gage data are:
http://waterdata.usgs.gov/wv/nwis/current?type=dailystagedischarge
http://wv.usgs.gov/
http://waterwatch.usgs.gov/?m=real&r=wv
See Figure 77 and Figure 78 below for examples of USGS website data displays.

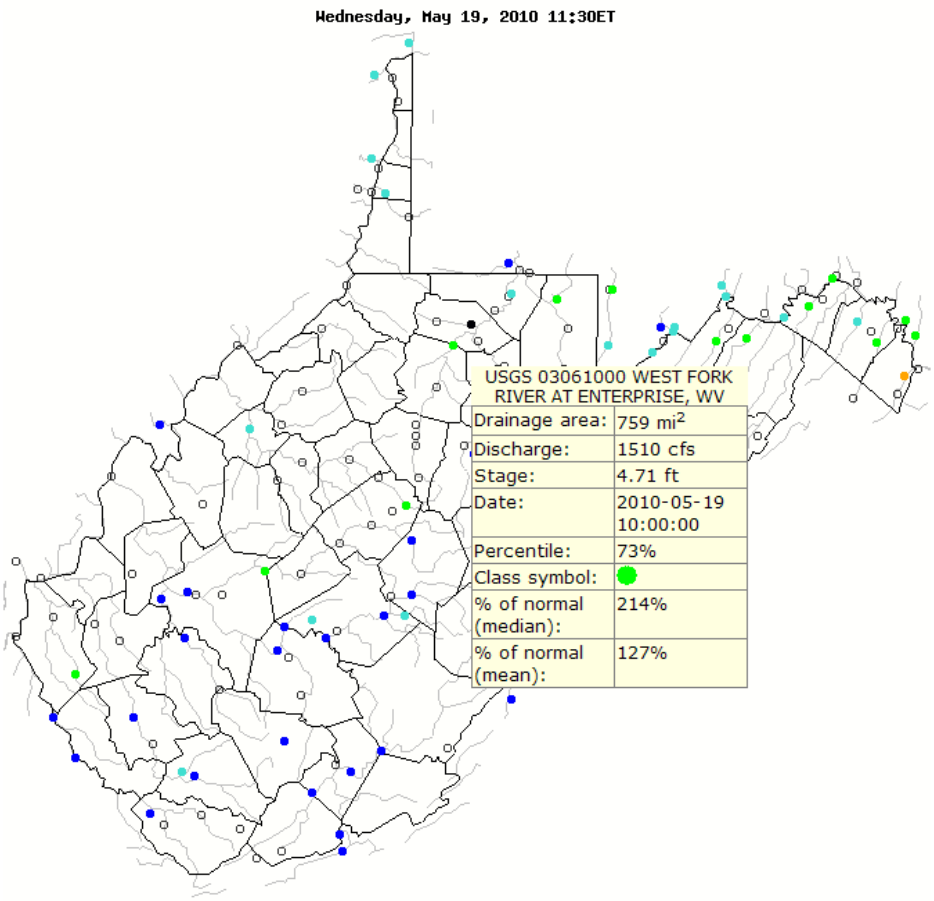

Figure 77. Example of USGS stream flow website (http://waterwatch.usgs.gov/?m=real&r=wv)

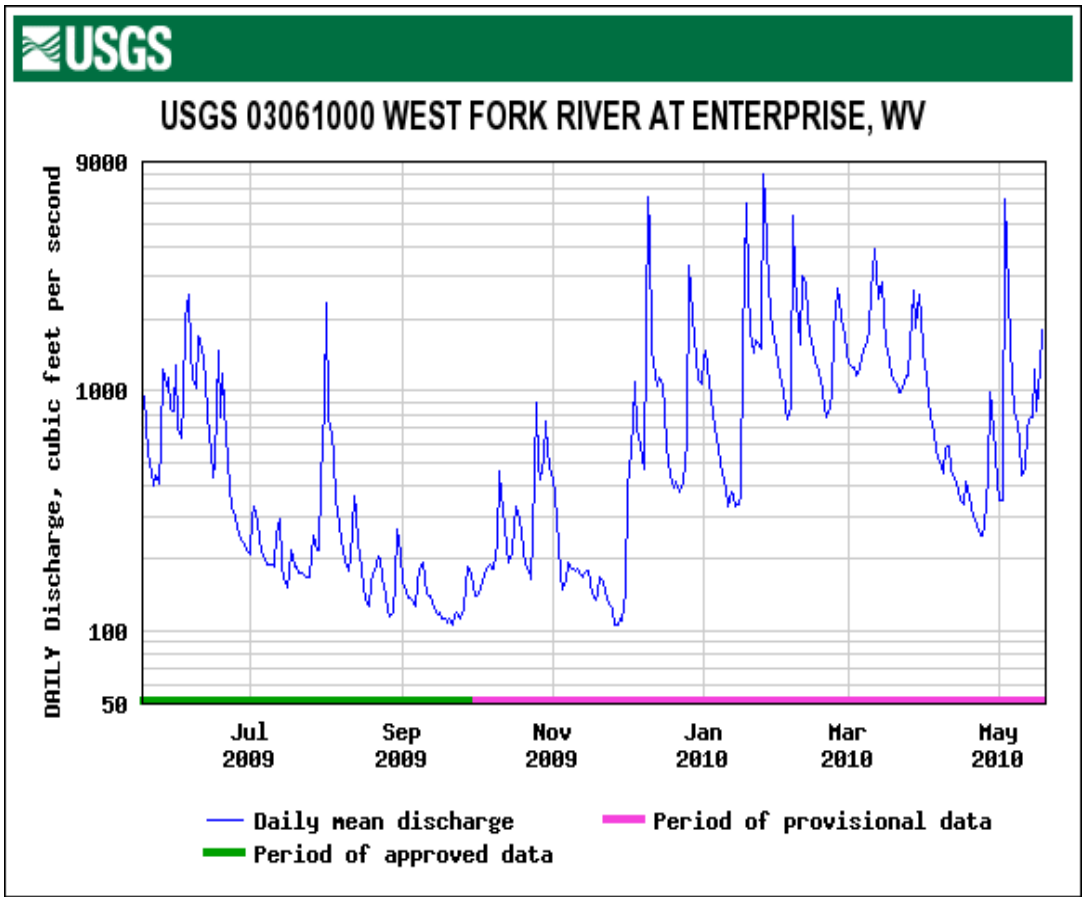

Figure 78. Example of USGS Stream Gage Output Graph

Refer to the specific station monitoring information (***see Section C. AMBIENT SAMPLING STATION DESCRIPTIONS below for the location and USGS ID number***). Once you have accessed a specific gage, you will need to use the real time and table options to view hourly gage data. Record the USGS gage number, discharge and/or stage readings, for the date and time sampled, onto the form (***see Chapter II. Section B. Part 2. APPENDIX #1 - Stream Discharge (Flow) on page 78***). Hourly data are available for up to 60 days, from the date a site is visited. Daily averages are available up to two years.

Section B. DATA REVIEW & HANDLING

When the Ambient Water Quality results are received from the laboratory, all of the sampling data is entered into the Watershed Assessment Branch database. During this process, all water quality results are compared to the analysis request form and field habitat forms to make sure all site and sampling information is correct and all requested analyses were performed Minimum detection limits of each are checked for compliance with current water quality criteria. Next, the results are reviewed for violations of water quality criteria and notes of these are made for future reference. Any unusual numbers should be confirmed with the laboratory and data entry mistakes corrected.

Section C. AMBIENT SAMPLING STATION DESCRIPTIONS

BST-(0.15) Tug Fork River

USGS Quadrangle: Louisa, KY **Basin:** Tug Fork **County:** Wayne
Coordinates: Latitude – 38° 07' 1.12" N Longitude – 82° 35' 56.07" W

Figure 79. 2003 Aerial Photo of the BST-(0.15) Tug Fork Ambient Sample Site in Fort Gay, WV. Channel on Right is Tug Fork; Left is Levisa Fork. Note that there is a boat ramp into the Levisa Fork just north of the bridge (Middle Left Edge of Photo).

Directions to Sample Site

Sample site is located at Fort Gay in Wayne County on the WV Route 37 Bridge, which crosses into Louisa, KY. Parking for this site is located along KY Route 3, between the Tug Fork Bridge and the Levisa Fork Bridge, at the end of the bridge sidewalk.

Description of Sampling Point

Sample is collected midstream, from the bridge sidewalk, on the upstream side. Be aware of the permitted discharge on right descending bank.

Sampling Technique

This sample can only be obtained using the Bridge Crane Method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Kermit, WV / USGS #03214500

Special Instructions

Wear orange safety vest and PFD!!!

O-2-(8.8) Twelvepole Creek

USGS Quadrangle: Burnaugh, KY **Basin**: Twelvepole **County**: Wayne
Coordinates: Latitude – 38° 21' 20.31" N Longitude – 82° 30' 30.56" W

Figure 80. 2003 Aerial Photo of the O-2-(8.8) Twelvepole Creek Ambient Sample Site in Wayne Co., WV.

Directions to Sample Site

Sample site is located on WV Route 75 Bridge just west of the intersection with Wayne County Route 7. Refer to map for additional information. Parking is available at a church just east of the bridge.

Description of Sampling Point

During low flow a grab sample can be taken at riffle approximately 150 meters downstream of bridge. During high flow sample is collected midstream, from the bridge, on the downstream side.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained in summer low flows at same riffle as described above.
High Flow: Bridge Crane Method.

Flow

Low Flow: Measure at riffle downstream of bridge. The substrate of the creek is deep sandy silt.
High Flow: During normal to high flows access USGS website – Gage site: Wayne, WV / USGS #03207020

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended. Be careful of steep, slick banks when accessing the low flow sampling location.

OG-(2.8) Guyandotte River

USGS Quadrangle: Barboursville, WV **Basin:** Lower Guyandotte **County:** Cabell
Coordinates: Latitude – 38° 24' 48.4 N" Longitude – 82° 21' 39.83" W

Figure 81. 2003 Aerial Photo of the OG-(2.8) Guyandotte River Ambient Sample Site in Huntington, WV.

Directions to Sample Site

Sample site is located on the Cabell County Route 26 Bridge, which is accessed from I-64 via the Huntington 29th St. East exit (#15). Parking is available at the used auto sales business at the south east end of the bridge.

Description of Sampling Point

Sample is collected midstream, from the bridge sidewalk, on the upstream side.

Sampling Technique

This sample can only be obtained using the Bridge Crane Method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Branchland, WV / USGS #03204000

Special Instructions

Wear orange safety vest and PFD!!!

OG-(74.1) Guyandotte River

USGS Quadrangle: Henlawson, WV **Basin**: Lower Guyandotte **County**: Logan
Coordinates: Latitude – 37° 55 35.48 N Longitude – 81° 58 54.0 W

Figure 82. 2003 Aerial Photo of the OG-(74.1) Guyandotte River Ambient Sample Site in Pecks Mill, WV.

Directions to Sample Site

Sample site is located upstream of the WV Route 10 Bridge and Mill Creek at Pecks Mill in Logan County. Access to the sample point is as follows: from WV Route 10 in Pecks Mill turn onto County Route 8, travel approximately 0.1 mile and turn right onto County Route 12, follow County Route 12 till you see a pull off area on the right, from this point walk over the bank and follow Mill Creek across RR tracks to the Guyandotte River.

Description of Sampling Point

Sample is collected at midstream of Guyandotte River 15 meters upstream of Mill Creek.

Sampling Technique

Direct dip/grab method. Wadeable benthic sample can be obtained in summer low flows at same riffle.

Flow

Access USGS website for flow information – Gage site: Logan, WV / USGS #03203600

Special Instructions

Wear orange safety vest and PFD!!!

K-(31.7) Kanawha River

USGS Quadrangle: Winfield, WV
Basin: Lower Kanawha
County: Putnam
Coordinates: Latitude – 38° 31' 28.3" N Longitude – 81° 54' 42.79" W

Directions to Sample Site

Sample site is located, on US Route 35 at the AEP Winfield Hydropower Plant on the south east side of the Locks & Dam structure, at Winfield in Putnam County. Parking is allowed inside the fenced area.

Description of Sampling Point

Sample is collected at the midpoint of the power plant intake.

Sampling Technique

Direct dip/grab method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Winfield, WV / #03201305

Figure 83. 2003 Aerial Photo of the K-(31.7) Kanawha River Ambient Sample Site at Winfield Locks & Dam, WV.

Special Instructions

Call the Winfield Power Plant at (304) 586-3006 to arrange access. Safety training is required for each visit and provided inside plant. ***Safety equipment required: Hardhat, PFD, & Safety glasses.***

Figure 84. Photo of the Winfield Locks & Dam.

Figure 85. Photo of the Winfield Locks & Dam Intake Sample Area.

K-(76.9) Kanawha River

USGS Quadrangle: Cedar Grove, WV **Basin**: Upper Kanawha **County**: Kanawha
Coordinates: Latitude – 38° 11' 50.69" N Longitude – 81° 29' 49.02" W

Figure 86. 2003 Aerial Photo of the K-(76.9) Kanawha River Ambient Sample Site at Chelyan, WV.

Figure 87. Photo of the Kanawha River Sample Site from Boat Dock in Chelyan, WV.

Directions to Sample Site

Sample site is located at Chelyan, downstream of the Kanawha River Bridge, along WV Route 61 in Kanawha County.

Description of Sampling Point

Sample is collected midstream utilizing watercraft, launching from the old boat ramp adjacent to the WV Department of Highways office when feasible. When sampling conditions don't permit boat sampling, a grab sample may be taken upstream of the boat ramp on left descending bank.

Sampling Technique

Direct dip/grab method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Kanawha Falls, WV / USGS #03193000

Special Instructions

Follow watercraft safety guidelines. Wear PFD!!!

KC-(11.6) Coal River

USGS Quadrangle: Alum Creek, WV **Basin**: Coal **County**: Kanawha
Coordinates: Latitude – 38° 20’ 21.03” N Longitude – 81° 50’ 27.96” W

Figure 88. 2003 Aerial Photo of the KC-(11.6) Coal River Ambient Sample Site at Tornado, WV.

Directions to Sample Site
Sample site is located on the County Route 9 Bridge, near Tornado in Kanawha County.
Description of Sampling Point
Sample is collected midstream, from the bridge sidewalk, on the downstream side.
Sampling Technique
This sample is primarily obtained using the Bridge Crane Method. A proxy wadeable benthic sample can be obtained from bottom of old lock channel downstream of site on right descending bank next to Upper Falls of Coal River during summer low flows.
Flow
Access USGS website for flow information – Gage site: Tornado, WV / #03200500
Special Instructions
Wear orange safety vest and PFD!!! The Upper Falls area is notorious for downing accidents due to heavy deposits of sand below the falls and an extremely fast and deep pool in the old lock channel on the right descending bank.

KE-(4.3) Elk River

USGS Quadrangle: Big Chimney, WV
Basin: Elk
County: Kanawha
Coordinates: Latitude – 38° 23' 10.96" N Longitude – 81° 35' 3.36" W

Directions to Sample Site

Sample site is located at Coonskin Park, which is accessed from WV Route 114 in Kanawha County. Park at the boat ramp and walk the Elk River Trail downstream on left descending bank. This point is located in a slight bend in the river.

Figure 89. 2003 Aerial Photo of the KE-(4.3) Elk River Ambient Sample Site at Charleston, WV.

Description of Sampling Point

Sample is collected from the furthest boulder out from the left bank that you can safely sample from.

Sampling Technique

Direct dip/grab method. A proxy wadeable benthic sample can be obtained from shoals just below Mink Shoals Run approximately 0.6 miles upstream during summer low flows.

Figure 90. Photo of the Elk River Sampling Site from Upstream at Coonskin Park.

Figure 91. Photo of the Elk River Sampling Site Looking Upstream.

Flow

Access USGS website for flow information – Gage site: Queen Shoals, WV / #03197000

Special Instructions

Wear PFD!!!

KG-(8.3) Gauley River

USGS Quadrangle: Gauley Bridge, WV **Basin**: Gauley **County**: Fayette
Coordinates: Latitude – 38° 13' 35.57" N Longitude – 81° 09' 10.19" W

Figure 92. 2003 Aerial Photo of the KG-(8.3) Gauley River Ambient Sample Site at Beech Glen, WV.

Directions to Sample Site

Sample site is located west of Jodie in Beech Glen at the Fayette/Nicholas County line, under the CR 60/3 bridge. Alternately the site can be sampled from the CR 60/3 bridge during high flows.

Description of Sampling Point

Sample is collected midstream, upstream of the mouth of Rich Creek on the upstream side of the bridge.

Sampling Technique

Low Flow: Direct dip/grab method.
High Flow: Bridge Crane Method.
A proxy wadeable benthic sample can be obtained from riffle downstream approximately 0.1 miles at top of island during summer low flows.

Flow

Access USGS website for flow information – Gage site: Belva, WV / USGS #03192000

Special Instructions

The bridge to access the sampling point experiences a large volume of coal truck traffic.
Wear orange safety vest and PFD!!!

KN-(1.55) New River

USGS Quadrangle: Gauley Bridge, WV **Basin**: Lower New **County**: Fayette
Coordinates: Latitude – 38° 08’ 53.5” N Longitude – 81° 10’ 33.9” W

Figure 93. 2003 Aerial Photo of the KN-(1.55) New River Ambient Sample Site near Gauley Bridge, WV.

Directions to Sample Site

Sample site is located at the Elkem Power Station, which is 1.5 miles upstream of Gauley Bridge in Fayette County. Parking to access the Station is the first right following Cathedral Falls roadside park, along US Route 60.

Description of Sampling Point

Sample is collected from the right descending bank, just downstream Elkem Power Station East of Gauley Bridge.

Sampling Technique

Direct dip method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Thurmond, WV / USGS #03185400

Special Instructions

Wear PFD!!! This location may experience a rapid increase in flow and depth at any time due to a release from the aqueduct. Heed all warnings posted at the parking area.

KN-(67.4) New River

USGS Quadrangle: Hinton, WV **Basin**: Upper New **County**: Summers
Coordinates: Latitude – 37° 39' 4.53" N Longitude – 80° 53' 11.9" W

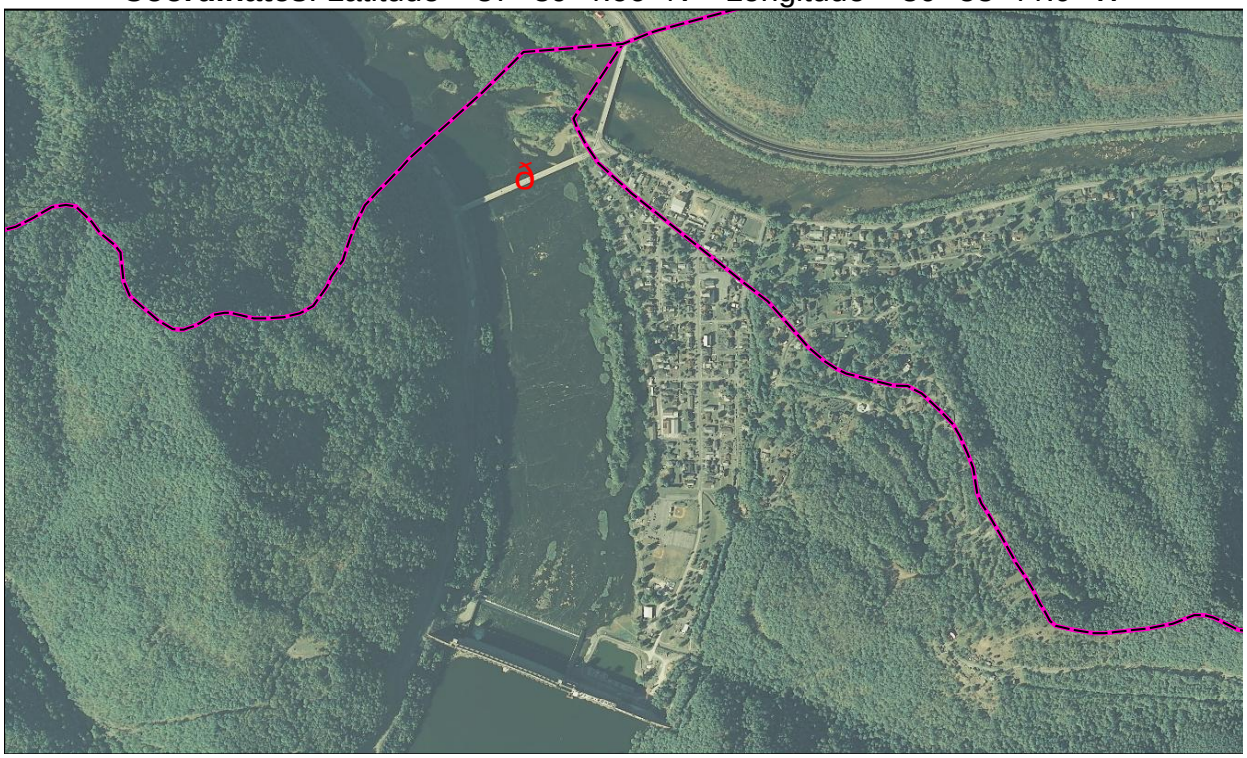

Figure 94. 2003 Aerial Photo of the KN-(67.4) New River Ambient Sample Site in Hinton, WV.

Directions to Sample Site

Sample site is located on the WV Route 3 Bridge at Hinton in Summers County. Parking for this site is at the old USGS Gage on the east side of the bridge.

Description of Sampling Point

Sample is collected from the bridge sidewalk, at midstream on the downstream side. During low flows, sample can be collected from the same location by wading out from right descending bank. Be aware of CSO outfall on Right Descending Bank near bridge.

Sampling Technique

Low Flow: Direct dip/grab method.
High Flow: Bridge Crane Method.
Wadeable benthic sample can be obtained from riffles immediately below bridge in right descending half of channel during summer low flows.

Flow

Access USGS website for flow information – Gage site: Hinton, WV / USGS #03184500

Special Instructions

Wear orange safety vest and PFD!!! This location may experience a rapid increase in flow and depth at any time due to a release from the dam. Heed all warnings posted at the area.

KNG-(1.6) Greenbrier River

USGS Quadrangle: Talcott, WV **Basin**: Greenbrier **County**: Summers
Coordinates: Latitude – 37° 39' 08.24" N Longitude – 81° 51' 40.31" W

Figure 95. 2003 Aerial Photo of the KNG-(1.6) Greenbrier River Ambient Sample Site in Hinton, WV.

Directions to Sample Site

Sample site is located at Wiggins Bridge, on County Route 13, which connects with WV Route 3 approximately 1.5 miles east of Hinton in Summers County. Parking is located on the south side of the bridge at a gravel pit beside a fenced road to the river.

Description of Sampling Point

Sample is collected midstream from the bridge, on the downstream side. During low flows, sample can be collected from the same location by wading.

Sampling Technique

Low Flow: Direct dip/grab method.
High Flow: Bridge Crane Method.
A proxy wadeable benthic sample can be obtained at cobble riffle upstream approximately 1.0 miles during summer low flows.

Flow

Access USGS website for flow information – Gage site: Hilldale, WV / USGS #03184000

Special Instructions

Wear your orange safety vest and PFD!!! Traffic cones recommended.

KN-(96.2) New River

USGS Quadrangle: Peterstown
Basin: Upper New
County: Giles, VA
Coordinates: Latitude – 37° 23' 15.2" N Longitude – 80° 52' 5.8" W

Directions to Sample Site

Sample site is located upstream of Smith Branch north of Glen Lyn, VA. Directions are as follows: Just after crossing the WV/VA state line on Rt. 460 E, take the next left, before crossing bridge over New River. Go 0.9 miles and go right onto gravel road going alongside the river. Go 1.3 miles and pull into campsite on right.

Description of Sampling Point

Sample is collected close to midstream by wading along the large fractured bedrock slabs. Ensure sample is taken upstream of Smith Branch and as close to mid-stream as flow levels allow.

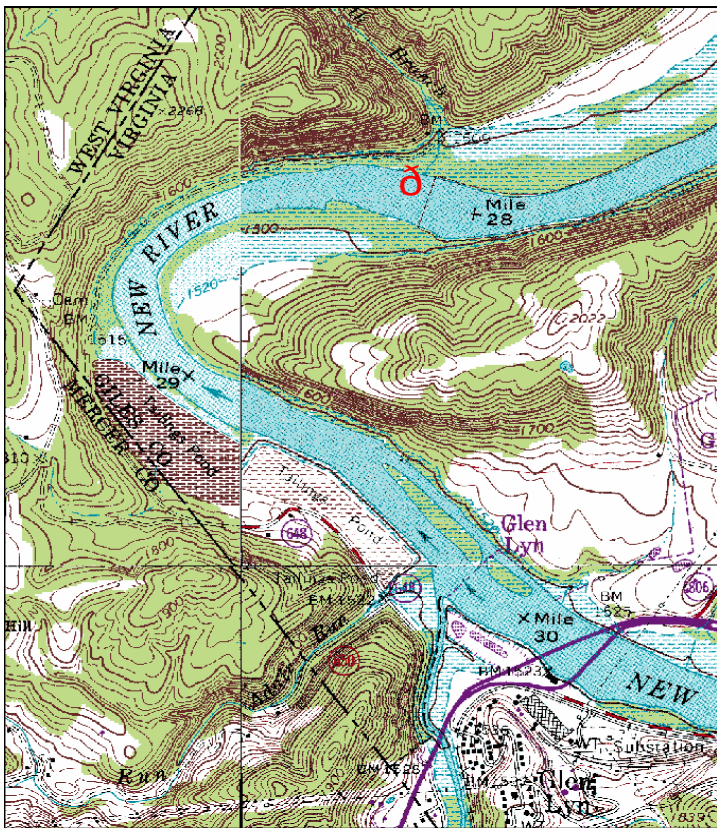

Figure 96. USGS 24k Topographic Map of the KN-(96.2) New River Ambient Sample Site north of Glen Lyn, VA.

Figure 97. Photo from X-Site looking toward Left Descending Bank at Mouth of Smith Branch and Parking Area.

Sampling Technique

Direct dip/grab method. Wadeable benthic sample can be obtained from riffle below Smith Branch on left descending bank during summer low flows.

Flow

Access USGS website for flow information – Gage site: Glen Lyn, VA / USGS #03176500

Special Instructions

Wear your PFD!!! VA Scientific Collection Permit with 24 hour notification is required to sample at this location.

LK-(28.9) Little Kanawha River

USGS Quadrangle: Elizabeth, WV **Basin**: Little Kanawha **County**: Wirt
Coordinates: Latitude – 39° 03’ 18.4” N Longitude – 81° 23’ 25.84” W

Figure 98. 2003 Aerial Photo of the LK-(28.9) Little Kanawha River Ambient Sample Site near Elizabeth, WV.

Directions to Sample Site
Sample site is located on the WV Route 5 Bridge, southeast of Elizabeth in Wirt County. Parking is located on the east side of the bridge, at a small convenience store.

Description of Sampling Point
Sample is collected at midstream, from the bridge sidewalk, on the upstream side.

Sampling Technique
Bridge Crane Method. Wadeable benthic sample cannot be obtained.

Flow
Access USGS website for flow information – Gage site: Palestine, WV / USGS #03155000

Special Instructions
Wear your orange safety vest and PFD!!!

LKH-(1.5) Hughes River

USGS Quadrangle: Kanawha, WV **Basin**: Little Kanawha **County**: Wirt
Coordinates: Latitude – 39° 07’ 54.29” N Longitude – 81° 22’ 38.21” W

Figure 99. 2003 Aerial Photo of the KLH-(1.5) Hughes River Ambient Sample Site near Greencastle, WV.

Directions to Sample Site
Sample site is located on Wirt County Route 6 Bridge East of Greencastle.

Description of Sampling Point
Sample is collected at midstream, from the bridge, on the downstream side. During low flows, sample may be collected at the riffle 120 m downstream of the bridge.

Sampling Technique
Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from same riffle during summer low flows.
High Flow: Bridge Crane Method.

Flow
Flow is measured only during low flow at the riffle described above.

Special Instructions
The sampling point is a one-lane bridge, be very careful!!! Parking for the sampling site is at the church parking lot on the east side of the bridge. Walk to the flow measurement site on the gravel road between the church and the river. ***Wear your orange safety vest and PFD!!! Traffic cones recommended. Banks can be slick!***

OMI-(12.3) Middle Island Creek

USGS Quadrangle: Bens Run, WV **Basin**: Middle Ohio North **County**: Pleasants
Coordinates: Latitude – 39° 26’ 08.18” N Longitude – 81° 04’ 16.91” W

Figure 100. 2003 Aerial Photo of the OMI-(12.3) Middle Island Creek Ambient Sample Site in Arvilla, WV.

Directions to Sample Site

Sample site is located on Pleasants County Route 7 Bridge at Arvilla. Parking for sample collection is available on the west end of the bridge along County Route 7/2.

Description of Sampling Point

Sample is collected at midstream, from the bridge, on the upstream side.

Sampling Technique

Bridge Crane Method. A proxy wadeable benthic sample can be obtained at riffle downstream approximately 0.5 miles during summer low flows.

Flow

Flow is measured during low flows at riffle described above. Access to site is by Pleasants County Route 34.

Access USGS website for flow information – Gage site: Little, WV/ USGS # 03114500

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended. Bank at proxy site is steep.

M-(99.4) Monongahela River

USGS Quad: Morgantown North, WV **Basin**: Monongahela **County**: Monongalia
Coordinates: Latitude – 39° 39’ 28.88” N Longitude – 79° 59’ 35.33” W

Figure 101. 2003 Aerial Photo of the M-(99.4) Monongahela River Ambient Sample Site in Star City, WV.

Directions to Sample Site

Sample site is located on the WV Route 7 Bridge at Star City.

Description of Sampling Point

Sample is collected at midstream, from the bridge sidewalk, on the upstream side.

Sampling Technique

Bridge Crane Method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Morgantown Lock & Dam, WV / USGS #03062450

Special Instructions

Wear orange safety vest and PFD!!! Bridge is very high. Although there is a large sidewalk on the bridge, traffic volume is large and fast. Also be aware of any boating activity below.

M-1-(20.6) Dunkard Creek

USGS Quadrangle: Osage, WV **Basin**: Dunkard **County**: Monongalia
Coordinates: Latitude – 39° 42’ 55.47” N Longitude – 80° 06’ 39.96” W

Figure 102. 2003 Aerial Photo of the M-1-(20.6) Dunkard Creek Ambient Sample Site at Mason-Dixon Historical Park, WV.

Directions to Sample Site

Sample site is located just downstream or on Monongalia County Route 39 Bridge, near the Mason-Dixon Historical Park east of Pentress. This will be the second bridge you encounter after turning off WV Route 7. Parking is available at the Historical Park at a site above the playground.

Description of Sampling Point

Sampling occurs primarily at the riffle approximately 75 meters below bridge. During high flows the sample can be collected at midstream, from the bridge, on the upstream side.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from same riffle during summer low flows.
High Flow: Bridge Crane Method.

Flow

Flow is measured, during low flow, at riffle described above.

Special Instructions

Wear your orange safety vest and PFD!!! Traffic cones recommended if sampling from the bridge.

MT-(6.2) Tygart Valley River

USGS Quadrangle: Fairmont West, WV **Basin**: Tygart Valley **County**: Marion
Coordinates: Latitude – 39° 26’ 16.2” N Longitude – 80° 07’ 56.4” W

Figure 103. 2003 Aerial Photo of the MT-(6.2) Tygart Valley River Ambient Sample Site at Colfax, WV.

Directions to Sample Site

Sample site is located on Marion County Route 62 Bridge at Colfax, which is accessed via US Route 250 south of Fairmont. Parking is available on the west side of the bridge. A low flow direct dip/grab site is at riffle downstream approximately 0.1 miles from bridge. Continue down road alongside the river to small pull off on right. Walk down bank and wade out as far as flow level permits.

Description of Sampling Point

Sample is collected at midstream, from the bridge, on the downstream side during high flows. During low flows, go to the downstream site and wade as far as flow level permits.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from same riffle during summer low flows.

High Flow: Bridge Crane Method.

Flow

Access USGS website for flow information – Gage site: Colfax, WV / USGS #03057000

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended.

MW-(12.0) West Fork River

USGS Quadrangle: Shinnston, WV **Basin**: West Fork **County**: Harrison
Coordinates: Latitude – 39° 25’ 25.02” N Longitude – 80° 16’ 32.91” W

Figure 104. 2003 Aerial Photo of the MW-(12.0) West Fork River Ambient Sample Site at Enterprise,

Directions to Sample Site

Sample site is located on a bridge that intersects US Route 19 at Enterprise in Harrison County. Parking is available on the bridge berm. Turn on vehicle emergency flashers.

Description of Sampling Point

Sample is collected at midstream, from the bridge, on the downstream side.

Sampling Technique

Bridge Crane Method. A proxy wadeable benthic sample can be obtained at riffle upstream approximately 0.1 miles on opposite side of confluence of Laurel Run on right descending bank during summer low flows.

Flow

Access USGS website for flow information – Gage site: Enterprise, WV / USGS #03061000

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended. Traffic volume is not high, but speeders are common.

MC-(3.5) Cheat River

USGS Quadrangle: Lake Lynn, WV **Basin**: Cheat **County**: Fayette, PA
Coordinates: Latitude – 39° 43’ 17.18” N Longitude – 79° 51’ 27.79 W

Figure 105. 2003 Aerial Photo of the MC-(3.5) Cheat River Ambient Sample Site in Lake Lynn, PA.

Directions to Sample Site

Sample site is located at the tail waters of Lake Lynn, which is best accessed from, Point Marion, PA, via US Route 119. From US Route 119 in Point Marion, turn onto River Road and follow Cheat River upstream to the tail water access parking area.

Description of Sampling Point

Sample is collected off of the right descending bank below the parking area.

Sampling Technique

Direct dip/grab method. Wadeable benthic sample cannot be obtained.

Flow

Access USGS website for flow information – Gage site: Lake Lynn, PA / USGS #03071600

Special Instructions

This location may experience a rapid increase in flow and depth at any time due to a release from the dam. Heed all warnings posted at the parking area. Wear orange safety vest and PFD!!!

MC-(30.0) Cheat River

USGS Quadrangle: Kingwood, WV **Basin**: Cheat **County**: Preston
Coordinates: Latitude – 39° 29' 41.13" N Longitude – 79° 38' 42.99" W

Figure 106. 2003 Aerial Photo of the MC-(30.0) Cheat River Ambient Sample Site in Albright, WV.

Directions to Sample Site

Sample site is located on the WV Route 26 Bridge, at Albright in Preston County. Parking is available at the east end of the bridge.

Description of Sampling Point

Sample is collected at midstream, from the bridge, on the upstream side. During low flows, direct dip/grab method may be used in run upstream of bridge.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from riffle upstream of bridge approximately 100 meters during summer low flows.

High Flow: Bridge Crane Method.

Flow

Access USGS website for flow information – Gage site: Albright, WV / USGS #03070260

Special Instructions

Wear orange safety vest and PFD!!!

P-4-(2.2) Opequon Creek

USGS Quadrangle: Hedgesville, WV **Basin**: Potomac Direct Drain **County**: Berkeley
Coordinates: Latitude – 39° 31' 02.96" N Longitude – 77° 53' 21.87" W

Figure 107. 2003 Aerial Photo of the P-4-(3.5) Opequon Creek Ambient Sample Site near Bedington,

Directions to Sample Site

Sample site is located on Berkeley County Route 12 Bridge east of Bedington. Parking is available on the west end of the bridge along County Route 12.

Description of Sampling Point

Sample is collected at the thalweg, from the bridge, on the upstream side or by wading out to same point during low flows.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from riffle under bridge during summer low flows.
High Flow: Bridge Crane Method.

Flow

Flow is measured, during low flows, at a shallow point upstream of the bridge.
Access USGS website for flow information during normal to high flows – Gage site: Martinsburg, WV / USGS #01616500

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended when sampling from bridge. Note the permitted discharge below the bridge on the left descending bank.

PC-(6.1) Cacapon River

USGS Quadrangle: **Great Cacapon, WV** Basin: **Cacapon** County: **Morgan**
Coordinates: **Latitude – 39° 34' 55.43" N Longitude – 78° 18' 31.72" W**

Directions to Sample Site

Sample site is located on Morgan County Route 7 Bridge south of Great Cacapon. Parking is available on the north end of the bridge.

Description of Sampling Point

Sample is collected midstream, from the bridge, on the downstream side.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained from riffle under bridge during summer low flows.

High Flow: Bridge is low, without railing. Direct dip/grab method may be used by lying down and reaching over edge of bridge. Otherwise use a VDHS or SSB with rope.

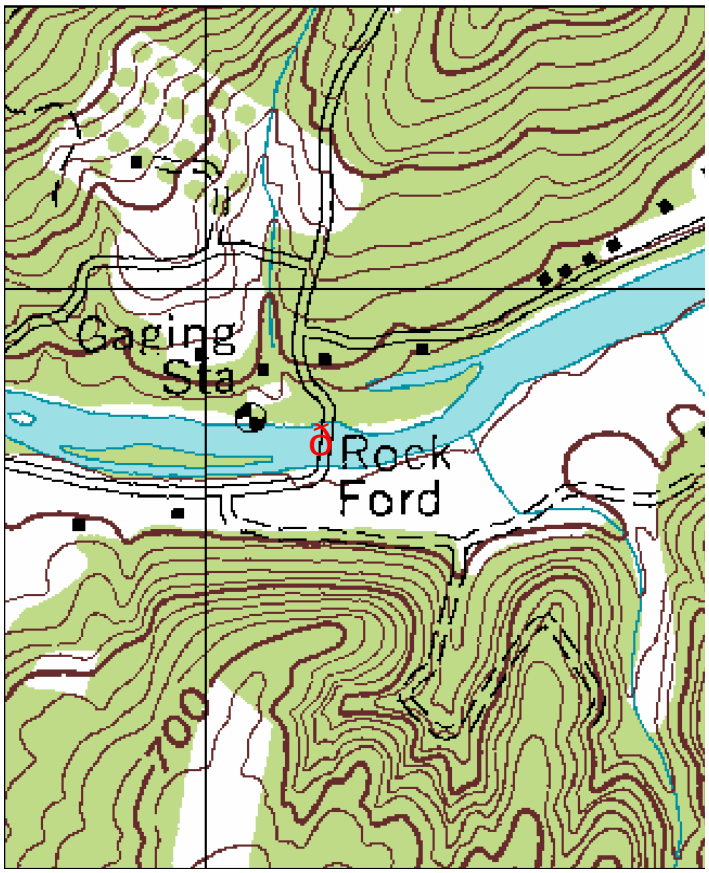

Figure 108. USGS 24k Topographic Map of the PC-(6.1) Cacapon River Ambient Sample Site south of Great Cacapon, WV.

Flow

Flow is measured, during low flows, at a riffle immediately downstream of the bridge.

Access USGS website for flow information during normal to high flows – Gage site: Great Cacapon, WV / USGS #01611500

Special Instructions

Wear orange safety vest and PFD!!! Be aware of traffic while sampling from bridge.

Figure 109. Photo from CR 7 bridge at the PC-(6.1) Cacapon River Ambient Sample Site south of Great Cacapon, WV.

PSB-(13.4) South Branch Potomac River

USGS Quad: Springfield, WV **Basin**: South Branch Potomac **County**: Hampshire
Coordinates: Latitude – 39° 26' 51.74" N Longitude – 78° 39' 15.25" W

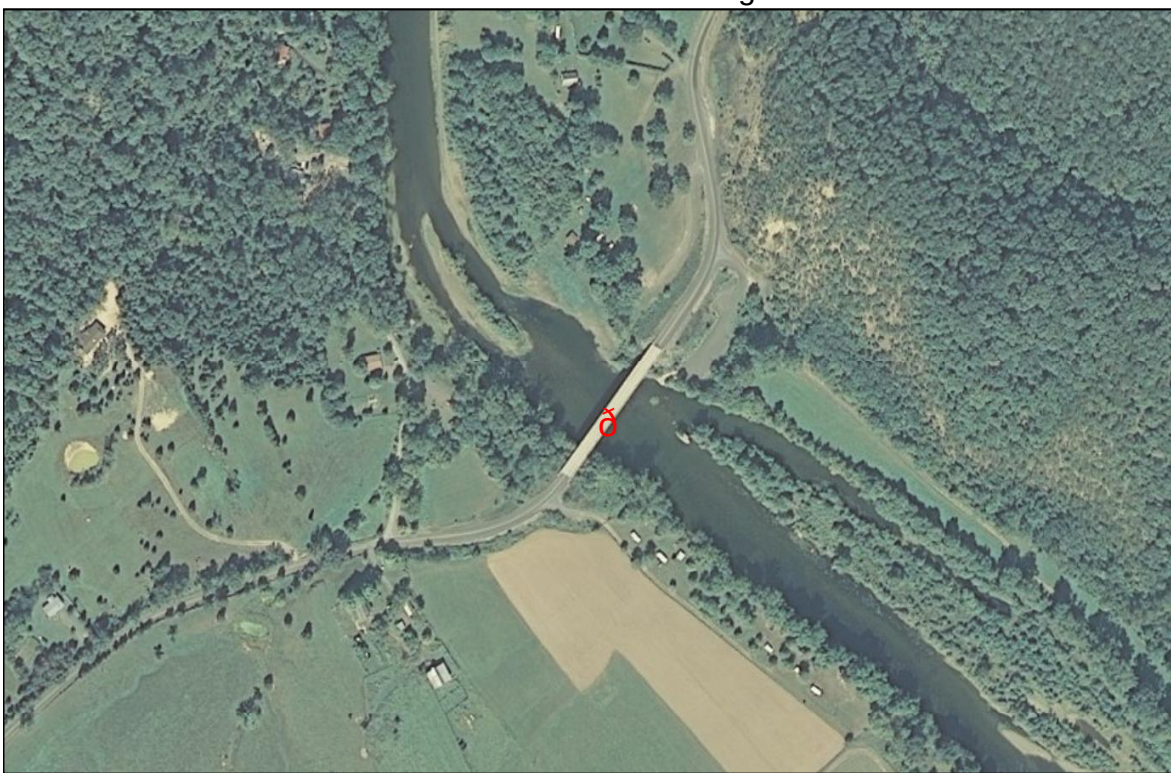

Figure 110. 2003 Aerial Photo of the PSB-(13.4) South Branch Potomac River Ambient Sample Site near Springfield, WV.

Directions to Sample Site

Sample site is located on or immediately below Hampshire County Route 3 Bridge, east of Springfield.

Description of Sampling Point

Sample is collected at midstream, from the bridge, on the downstream side or by wading out as far as the flow will allow during low flows.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained at riffle immediately downstream of bridge at top end of island during summer low flows.
High Flow: Bridge Crane Method.

Flow

Access USGS website for flow information – Gage site: Springfield, WV / USGS #01608500

Special Instructions

Wear orange safety vest and PFD!!! Traffic cones recommended when sampling from bridge.

S-(0.9) Shenandoah River

USGS Quadrangle: Harpers Ferry, WV **Basin**: Shenandoah **County**: Jefferson
Coordinates: Latitude – 39° 19' 12.36" N Longitude – 77° 44' 31.97" W

Figure 111. 2003 Aerial Photo of the S-(0.9) Shenandoah River Ambient Sample Site near Harpers Ferry, WV.

Directions to Sample Site

Sample site is located on US Route 340 Bridge at Harpers Ferry in Jefferson County. Parking is available at the west end of the bridge.

Description of Sampling Point

Sample is collected at midstream, from the bridge sidewalk, on the downstream side or by wading out to midstream above the bridge during low flow.

Sampling Technique

Low Flow: Direct dip/grab method. Wadeable benthic sample can be obtained at riffle approximately 100 meters upstream of bridge off of the left descending bank during summer low flows.

High Flow: Bridge Crane Method.

Flow

Access USGS website for flow information – Gage site: Millville, WV / USGS #01636500

Special Instructions

Wear orange safety vest and PFD!!! Be aware of high volume of traffic on bridge. Riffles above bridge also have a high volume of recreational users during warm months. Be careful to try and sample undisturbed substrate.

Ambient Water Quality Network Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. Whilst a specific session on Ambient Water Quality Network sampling is not covered, other sessions (*e.g.,* site documentation and completing the stream assessment forms, water collection protocols, stream flow measurement, field blanks and duplicates, etc.) are covered. In the field, individuals who are more experienced in sampling the Ambient Water Quality Network will be teamed up to give hands-on training to less experienced to assure reinforcement of training and accurate results before they are allowed to sample these stations solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the pertinent information. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Lot numbers of all preservatives are recorded on the Analysis Request Form for each sample submitted and entered into the database to allow for easy tracking. Sample transfer to the lab shall also be documented using the Chain-of-Custody (COC) portion of the Analysis Request Form.

Duplicate sampling and field blanks must be performed at a minimum of 2.5% of Ambient Water Quality Network sites for each sampling round. The field blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented below. ***See Chapter XII. Section A. Field Blanks and Duplicates starting on page 285 for additional information.***

Chapter XI. LAKE SAMPLING PROTOCOL

Overview of Lake Monitoring Program

In 2006, the Watershed Assessment Branch resumed sampling lake, reservoir, and pond waterbodies after an absence of activity since 1996. Using the rotating Watershed Basin Schedule much like TMDL sampling and the targeted Wadeable Stream Monitoring sampling occurs on targeted lakes (within the watershed group for that year) four times during the summer months (June - September or May - August). The number of stations per lake varies and is generally proportional to the size of the lake or the number of major branches or arms of the lake. The components of sampling include a vertical water chemistry profile (including the physiochemical properties, nutrients, and turbidity measurements), chlorophyll-*a* and fecal coliform sampling, Secchi depth, and some limited habitat and disturbance observations.

One of most unique aspects of Lake Sampling is the chlorophyll-*a* sampling, which is an indirect measure of phytoplankton.

Instructions for Assessing the Lake Site (Including Setting up the Site, Site Documentation, and Guidelines for Completing the Lake Assessment Forms)

The following is an instruction on how to establish lake sampling sites, document them, and use the Lake Assessment Form to evaluate various lake assessment parameters. Many of the procedures required to conduct this assessment have already been described above in the following Chapters:

Chapter II. Section C. Guidelines for Completing the Stream Assessment Forms on page 29

Chapter III. WATER COLLECTION PROTOCOLS starting on page 95

Chapter XII. Section A. Field Blanks and Duplicates starting on page 285

Section A. Setting up the Site

A field crew typically consists of two individuals charged with collecting habitat and biological/physicochemical data (*i.e.*, water quality). In some cases, smaller lakes and can be accessed with a one-man craft (*e.g.,* a canoe) and the field crew may consist of just one individual operating on a solo basis. This usually only occurs after the sampling stations have been thoroughly established, after the initial visit.

Throughout the following discussions, the term "Geomorph" will be used to describe the crewmember in charge of collecting habitat information and usually the water samples. "Biomorph" is the term used to describe the crewmember in charge of collecting Secchi and physicochemical data. In the case of a solo sampler, these roles are both played out by the same individual.

USGS topographic maps with a 1:24,000 scale will be used to navigate to lakes and sampling sites on the lakes (GIS or Geographic Information System maps on laptop, county maps, or Gazetteer maps are supplemental). The map coordinator generally will not mark sites or sampling stations prior to begin of sampling since the index sites are established during the initial visit (unless a revisit of a previously established site is designated).

After the location of the lake has been navigated to and confirmed, the Geomorph is responsible for establishing the index site by traversing the lake from the access point to the dam (where the deepest point is usually located) taking note of lake depths, and pertinent disturbance information. The index site should be placed at the deepest point in the lake, provided this location poses no danger to the field crew. This deepest point is the most lake-like part of the reservoir. Larger lakes or lakes with multiple arms may require additional sampling stations. If a thorough reconnaissance is conducted during the initial visit, it is not necessary to travel throughout the entire lake during every visit as this may require more time than is available for secondary visits. An attempt to recon the lake via a motor vehicle during every visit should be done if there is a road that parallels the lake shore. **THERE SHOULD BE NO DEVIATION FROM THE ABOVE PROTOCOL. THE GEOMORPH MUST THOROUGHLY COVER THE LAKE TO ACCURATELY COMPLETE THE ACTIVITIES/DISTURBANCES FORM.** The Geomorph will perform other duties concurrent with the establishment of the index site ***(outlined in Section C*. *Guidelines for Completing the Lake Assessment Forms on page 266)***. Procedures specific to each sample type are discussed below.

Part 1. Accessing the Lake

Since the WAB Lakes Sampling Program is limited to targeted lakes, the majority of the lakes should be easily accessible with fully operational boat ramps. However, in some cases, due to the remoteness of some sites or access points (usually on very small lakes or reference impoundments), traversing to the sample site may require strenuous hikes carrying the watercraft over difficult terrain; NOT DANGEROUS TERRAIN! If a difficult hike is necessary to get to a site, carefully consider the terrain and your personal ability and health to access the lake. If you feel it is too difficult (*e.g.,* too far to hike) or dangerous (*e.g.,* steep banks) to get to the lake or assess it, do not attempt it. Discuss it with other sampling teams who may be willing to try to get the site later**. DO NOT NAVIGATE TO ANY ASSESSMENT SITE THAT PRESENTS A DANGEROUS SITUATION TO YOU OR ANOTHER TEAM MEMBER!**

Target Lakes

Beginning in 2006, the Lakes Sampling Program has covered a representative portion of the lakes found in each hydrologic year's watersheds according to the rotating Watershed Basin Schedule. On the order of a 9-10 lakes will be sampled each year, with a greater or less number of lakes being sampled as data needs dictate and to avoid duplication of efforts, as some lakes are often sampled by other agencies.

Target sites are defined as natural and man-made lakes, ponds, and reservoirs greater than 5 acres. If there are a large number of waterbodies that meet this description in the given hydrologic year, the cutoff size may be elevated to 10 acres. To be considered a lake or reservoir for criterion purposes, summer residence times must be greater than 14 days. Limnologists usually agree that phytoplankton only accumulate in lake-like bodies of water with retention times greater than 7 days. Retention time is the most indicative factor making a body of water lake-like in behavior. Hence, only reservoirs with longer retention times are worth treating as lake-like for management purposes. Generally speaking, the number of sites per lake will follow this schedule:

Size (acres)	Number of Sites
Area ≤20	3
20 < Area ≤ 50	4
50 < Area ≤ 200	5
200 < Area ≤ 1000	6
1000 < Area	7

Sampling personnel will distribute points to sample a lake's largest arms or branches if there are any.

The target lakes and number of sites per lake are generally pre-determined and put on a site list (***See Table 12 below for an example of a lake site list***). Since you know you will be visiting all of the lakes on the list, they may be sampled in any order. This will allow you to work more efficiently, as some lakes may be adjacent on the list but not necessarily in geographical order. For example (***referring to Table 12 below***): If you were working this lake list, you might sample Mountwood Park Lake, North Bend State Park Lake, Tracy Lake, and Pennsboro Water Supply Reservoir all on the same day or couple of days, since they are all close to each other, and along U.S. Route 50. The date and initials columns on the list should be filled in as the sites are completed. The bottom of the list may be used for any notes to the lab data coordinator, including but not limited to: the temporary site name and details used for the duplicate and field blank samples, accessibility notes, and landowner phone numbers.

Coordinates for the sampling sites are included in the lake list after being established during the initial visit. In addition, GIS data of the sites will be available for use on the field laptops after they are incorporated.

Table 12. An example of a typical lake site list.

Lake	An-Code	Site_ID	Latdeg	latmin	latsec	londeg	lonmin	lonsec	Date	Initials
Mountwood Park Lake	LK-10-L-1	MP-1	39	14	21.00	81	18	47.00		
Miletree Lake	LK-31-X-1-(L1)	ML-1	38	48	20.60	81	22	1.70		
Cedar Creek Lake	LK-72-(L1)	CC-1	38	53	0.50	80	51	12.80		
Saltlick Pond 9	LK-95-(L1)	SP-1	38	43	53.60	80	35	40.30		
Tracy Lake	LKH-10-R-(L1)	TL-1	39	19	1.40	80	58	57.50		
North Bend State Park Lake	LKH-10-R.3-(L1)	NB-1	39	13	14.30	81	5	50.70		
North Bend State Park Lake	LKH-10-R.3-(L1)	NB-2	39	13	34.2	81	6	5.8		
Pennsboro Wat. Sup. Resv.	LKH-10-FF.5-(L1)	PR-1	39	16	55.10	80	55	29.50		
Big Run Lake (Ubwc-22)	M-23-O-8-(L1)	BR-1	39	36	9.5	80	23	1.6		
Huey Run Lake (Ubcw-2)	M-23-V-(L1)	HR-1	39	31	2.62	80	24	36.03		

An attempt should be made to access all lake sites unless it appears dangerous or too difficult to do so. The map coordinator should be notified and consulted about all sites which were not accessed due to dangerous or difficult conditions as a visit to that site may be attempted by another sampling team that may be better able to reach the site.

Locating the X-Site or Index Site on the Lake

Initial Visit

Unlike many other sampling programs, sampling stations for lake sites are not marked before the initial visit because index stations must be determined on-location. The sampling location is referred to as the **X-site** or **Index site** and is the deepest point in the particular section of the lake that is to be assessed. The **maximum** lake depth is observed via depth finder or other method to the nearest tenth of a foot. Traverse the lake thoroughly to locate this location. It is usually in the vicinity of the dam, or especially near drain structures, though not in all cases. While the index station should be located as closely as possible to this location, some drifting may occur during sampling. Do not set the index station near any potentially dangerous features. Some situations require creation of additional index sites when a large-sized or multi-lobed lake is involved.

Revisits

When returning to a lake site (either during the same sampling season or to an established site from years ago), GPS units should be used primarily to confirm the index latitude and longitude that is provided on the list for each index station. Using your GPS, if you can get one half of the coordinates to match almost exactly and the other half within a reasonable distance (no more than a couple of seconds), then you have adequately located the index site. If the GPS coordinates and the given index coordinates differ by more than a couple of seconds, re-check your position. **You should make an attempt to get an exact match if possible. Let the GPS run for several minutes (5-10) before matching the latitude and longitude.** Sampling teams should also consult other materials to ensure that they are at the correct location including: Laptop GIS programs, topographic, county, and/or gazetteer maps, or previous visit photocopies which include directions to the site, hand-drawn maps, and photos. **Note that topographic maps are recycled and older sites may appear on the topographic maps. Take extra care to make sure that you are targeting the correct site.**

⇒ **NOTE: Use the coordinates provided on the site list only as the coordinates on the previous visit photocopy may be in a different datum. Nevertheless, the hand-drawn map from the previous visit photocopy will be very useful in locating the exact same X-site that was established during the previous visit. You should make an attempt to get an exact match to the previous visit's X-site.**

There may be sites where the GPS unit will not track satellites and thus confirmation of the X-site coordinates may not be immediately possible. If you are certain from the other materials provided that you are in the correct location, you may begin sampling and try getting the GPS unit to lock onto satellites 10-15 minutes later. Team members should collaborate in these instances and utilize their best professional judgment (BPJ) to decide where the X-site is located. In such a case, finely tuned map reading skills are important.

After the X-site has been confirmed (or located via best professional judgment), the Geomorph will establish the index station. **Note: With the exception of fecal coliform samples, collect all physicochemical, water samples, and GPS coordinates at the index site.**

Duplicate Sites

In order to fulfill quality assurance and quality control or QA/QC requirements (***see Chapter XII. Section A. Field Blanks and Duplicates on page 285***), one duplicate water chemistry sample should be taken during each round of sampling. The sampler in charge will determine where to conduct a duplicate sample. However, the site used to collect the duplicate sample should be selected at random. Do not wait until the end of a week or list to sample for a duplicate stream.

During a duplicate, only the top water chemistry sample needs to be duplicated, not the bottom sample. Sufficient cubitainers should be filled, from the same depth in the water column, to have all nutrients analyzed. A second transfer bottle should also be filled so that a duplicate chlorophyll-a sample can be filtered. A duplicate fecal coliform sample should also be collected from the shoreline at the same location and conditions as the primary sample. However, do not allow any disturbances caused in the process of collecting the primary sample (*e.g*. stirred up sediment) interfere with the duplicate sample. The habitat form does not need to be duplicated, just the water sampling activities.

Section B. Site Documentation

Part 1. Lake Coordinates and Global Positioning Systems (GPS)

Basic guidance on how to use a GPS to document a site can be found in ***Chapter II. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) on page 20***.

Because each lake is visited only 4 times in a season coordinates should be recorded at every lake visit. ***Table 2 on page 22*** outlines some typical frequency of GPS readings for various sample types.

Part 2. Lake Photographic Documentation

Basic guidance on how to use a camera to document a site can be found in ***Chapter II. Section B. Part 2. Photographic Documentation on page 25***.

Specifically for lakes, we need a minimum of the following from each site to aid in relocating the site if necessary:

- View upstream from index site
- View downstream (usually of dam) from index site
- Typical lakeshore riparian cover

In addition, pictures of such items as the following may be useful:

- Lake alteration or management practices (boat ramps, campgrounds)
- Lake disturbances
- Waterfowl or other wildlife in or near lakes
- Silt laden streams flowing into clear lakes
- Scenic Views
- Field crews at work
- Distinctive views of lakes, buildings along lakes, industry along lakes, dams, boats or barges or other water related pictures.
- Pollution sources and features (*e.g.*, point and non-point sources, metal hydroxides, poorly constructed roads, feedlots, etc.)

All pertinent information about a photo should be recorded on the field sheet under the photography log section (***see Section C. Part 1. PAGE 4-Photography Log on page 274 below).***

Section C. Guidelines for Completing the Lake Assessment Forms

This section is intended to provide information on interpreting each parameter as well as identifying the value(s) of resultant data. Most of the parameters and values on the Lake Assessment Form have already been addressed in ***Chapter II. Section C. Guidelines for Completing the Stream Assessment Forms found on page 29***. The parameters that have already been addressed above will not be described here unless they vary in some way on the Lake Assessment Form.

What is presented here explains what is found on the Lake Assessment Form that is unique to Lake Sampling and not found on other forms (*i.e.*, WAB, AWQN, TMDL-Initial Visit, TMDL-Secondary Visit, TMDL-Final Visit, TMDL-Source, and General WQ). The instructions on how to fill out the sections are the same unless otherwise stated.

Part 1. Description of Lake Assessment Form

The quality and quantity of habitat is a major determinant of aquatic community potential. Consequently, a thorough habitat characterization is essential for proper interpretation of biological (chlorophyll-*a* and fecal coliform) assessment results.

> Important Note
> If a lake is considered "not target" (*e.g*., too small or inaccessible), Page 1 of this form must be completed. Also take photographs of the lake that display the reason why it was not considered target.

Front Side of All Pages

Like the other forms, the front sides of all pages have spaces to indicate the AN-Code of the site, Date of Collection, and Reviewer's Initials. ***See Chapter II. Section C. Part 1. Front Side of All Pages on page 29 for an example***.

PAGE 1

ALWAYS FILL OUT THE FIRST PAGE OF THE LAKE ASSESSMENT FORM, GET COORDINATES OF THE SITE, AND TAKE PHOTOGRAPHS, REGARDLESS OF WHETHER ANY TYPE OF SAMPLING WAS CONDUCTED (EVEN IF LAKE IS NON-TARGET)! THIS IS IMPORTANT INFORMATION AND ASSISTS IN DATABASE MANAGEMENT. ***See Figure 112 below for an example of PAGE 1 of the Lake Assessment Form*.**

For a description of parameters and values not seen below, ***See Chapter II. Section C. Part 1. PAGE 1-Site Verification on page 30 for an example***.

Lake Site Verification

Visit Type: Check which type of visit that this sample event represents: **Initial Visit** (First visit of the sampling year), **Secondary Visit** (Return visit during sampling year), Final Visit (Last Visit during sampling year), or **Other**.

Lake Name and Location Description: Make sure the lake name on the map corresponds with the assigned AN-Code from your printed lake list. If they do not match, make a note of it on the habitat sheet and printed list. Include a detailed description of the location such as: Summersville Lake near dam, Woodrum Lake DS (abbreviation for Downstream) of boat ramp, Summersville Lake US (abbreviation for Upstream) of Muddlety Creek, etc.

Lake Assessment Form

Visit Type:	☐ Initial ☐ Secondary ☐ Final ☐ Other:								
LOCATION VERIFICATION>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>						Reviewers Initials			
Lake Name (with Temporary Site Code & map location)								Site ID	
AN-Code		Date		Start Time		Geo		Bio	
Basin		County		Quad					
GPS Type		EPE		XY's Proofed				By	
Field Lat X-site	N	Field Lon X-site	W						
Corrected Lat	N	Corrected Lon	W						
Sampled	☐ Yes ☐ No	If not, why?	☐ No Access-Physical Barrier (☐ *Permanent* / ☐ *Temporary*) ☐ No Access-Landowner Denial (☐ *Verbal Denial* / ☐ *Posted* / ☐ *Fenced* / ☐ *Private*) ☐ Dry ☐ Filled ☐ Other:						
Sample Type	☐ YSI ☐ Fecal ☐ Lake ☐ AMD ☐ Nutrients ☐ Acid Rain ☐ Orthophosphate ☐ Other:								
Duplicate type	☐ None ☐ Lab ☐ Fecal	Duplicate #		Was site moved?	☐ Yes ☐ No				
Explanation?									
Directions To Site									
Sketch of Lake Area and Comments: Indicate North with (↑), indicate flow direction from head of lake toward dam, indicate shore fecal sample (F), water sample (wq), indicate lat and long site with (X). Draw the sketch with a coarse resolution to give an overall idea of the lake layout relative to the sample location. If possible, include roads, structures, and landmarks on the shore.									
Notes									

Page 1 WVDEP WAB Lake Assessment Form (4/12/2010)

Figure 112. Example of the Site Verification section (Page 1) of the Lake Assessment Form

Site ID: Temporary site codes that help distinguish multiple stations on any one lake. Usually pre-assigned by the sampling coordinator and found on the sampling list (see above). If you are going to add a sample locations add, just continue the numbering sequence given on the site list.

AN-Code: It is extremely important that the **correct** AN-Code (Alpha-Numeric Code) be recorded for each lake site. Mistakes in translation from the printed stream list to the habitat sheet must be avoided. Mistakes in this step create mass confusion and plenty of extra work during data entry. All lakes will have an AN-Code with the lake code designated between brackets (*e.g.,* - {L1}, -{L2}, etc.).

Date: Use mm/dd/yyyy format: *e.g.,* 04/29/1999

Start Time: Use military time (*e.g.,* 1315). For lake sampling, this time represents the general start of sampling activities.

Geomorph: Initials of the team member completing the habitat form and usually the lab water samples.

Biomorph: Initials of the team member collecting Secchi depth and YSI readings.

Sampled?: Answer appropriately; **YES** or **NO.** This must be answered. In some instances you may be sampling some aspect (*e.g.*, WQ only) even if the site is declared to be inappropriate for a full season's assessment.

Sample Type: Indicate which of the data types were collected (1) **YSI** (represents any type of water quality sonde), (2) **Fecal**, (3) **Lake (includes nutrients and chlorophyll-a)**, (4) **AMD**, (5) **Nutrients**, (6) **Acid Rain**, (7) **Orthophosphate**, (8) **Other**. **Do not include Hydrolab/YSI sonde readings as part of the lab water data. This refers to laboratory-analyzed samples only.**

Was site moved?: If for any reason the lake site is moved, indicate here. This is most likely to be used during a Secondary Visit. Answer **YES** or **NO.**

Explanation?: Explain why the site was moved and where the site was moved to. **If the site is moved, it is important to identify and mark the location of the new assessment site on a topographic map with date and initials of team and fill out a form for both sites.**

Directions to Lake Site: Give a detailed description on how the lake site was accessed. Include highway names & numbers, distances from prominent landmarks (manmade and/or natural), proximity to towns, etc. Indicate if contact with landowner/stakeholder/groundskeepers, etc., are necessary and note where, when, and why they should be contacted. Addresses of and other specifics about the

landowner/stakeholder/groundskeepers can be written down on PAGE 4 of the form under the section called Landowner/Stakeholder Information.

Bird's-eye-view Sketch of Lake Assessment Area and General Comments: Provide a detailed sketch of the area and include reservoir flow direction, land use on left and right bank, upstream activities (if possible), proximity to permanent land marks, indicate direction by drawing a North arrow **(↑)**, and any observations which may provide pertinent information to the assessment and location of the lake area. Indicate where GPS coordinates are collected by marking the spot in the lake with an (**X**). Indicate direction of flow with an arrow (↑). Mark the lakeshore areas where fecal coliform samples are collected with an (**F**)**,** and mark water sample collection areas with a (**wq**), though these should be the same as where coordinates are taken at the index site. **Keep in mind that a different field crew may be revisiting the site in 5 years and will rely heavily on your description/drawing to get back to the same location. In other instances, it may be necessary to determine the location using GIS programs.** General comments can be very important when interpreting sample data. Therefore, any anomalies or outstanding attributes should be noted. On larger lakes, a printed aerial image or topographic map from GIS programs may be submitted in addition to the drawing since larger lakes may not all be viewable in an individual visit. When doing this, ensure the aerial image or topographic map is of the exact same location you visited (confirm by plotting the coordinates you acquired at the site in your GIS program over the image you will use). Print the image and attach it the lake assessment form BEFORE turning it in. Be sure to annotate the map with any features observed during your visit, especially the direction of north, the ramp or point used to access the lake, the index site, and the fecal coliform site. The location of dams or any other landmarks should also be marked whether the map is drawn or printed.

PAGE 2

Site Activities and Disturbances (Including Roads)

This section is generally the same as presented as above except it concentrates on what is seen near the index site. ***See Chapter II. Section C. Part 1. PAGE 2-Site Activities and Disturbances (Including Roads) on page 36 for an example***.

See ***Figure 113*** below for an example of the unique section of PAGE 2 on the Lake Assessment Form.

Human Activities: Look for signs of human activities specific to lakes around the immediate index site including fishing, swimming, etc. Answer **Yes** or **No**.

Boating Activities: Look for signs of boating activity around the immediate index site. Answer **Yes** or **No**.

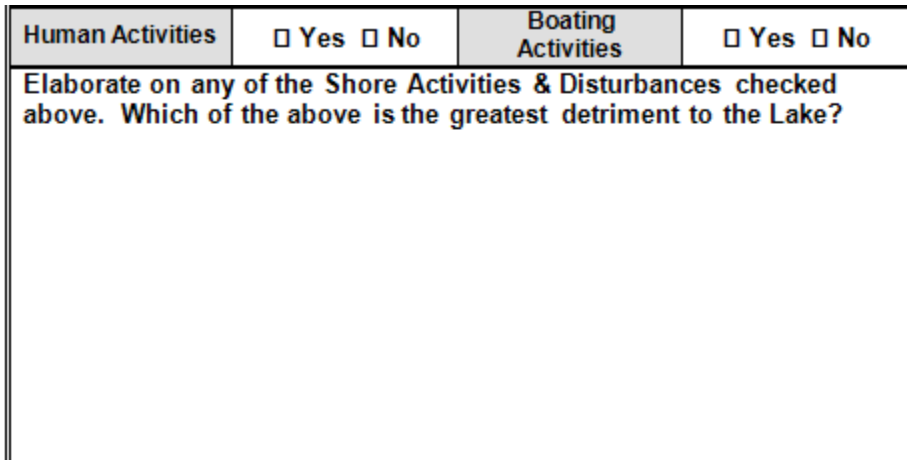

Figure 113. Example of the Human & Boating Activities (Center Left Page 2) of the Lake Assessment Form

Elaborate on any of the Shore Activities & Disturbances checked above. Which of the above is the greatest detriment to the lake?: This area is provided for notes about any of the Lake Assessment Area Activities & Disturbances checked above. If you checked boating activities, what type of boats are allowed, or feasible to use on this lake (electric only, 10 hp limit, any gas motors allowed, etc.). When known, record the type of drain controlling the discharge of the lake water to the downstream water body. At a minimum, you should note if the drain structure draws water from the top, bottom, or intermediate depth of the lake as this is important in assessing downstream impacts of the lake discharge.

This box should also be used to briefly describe the quality of the riparian zone around the lakeshore. What kind of trees dominates this zone? Are there any disturbances to the zone?

Comments Box: "If known, what is the predominant land use(s) in this lake's drainage? Is it mostly forested, agriculture, mining, logging, houses, urban? If mining present, is it active or abandoned, deep or strip, valley fills, etc. What is the predominant NPS pollution? Are there point sources above the assessment area? Indicate if you used maps (GIS) or field verified comments. **DO NOT LEAVE THIS BOX BLANK!"**

⇒ **Note: This area is a good place to put comments about the land use observed from recon trips, conversations with local residents, or gleaned from the GIS land use or topographic maps. If comments are based on the map, note them as such. Landowner comments about the upstream activities should also go here. The source of each bit of information should also be noted (*e.g.,* GIS, Topo, Recon, or Local or Landowner).**

PAGE 3

Field Water Quality Measures

For a description of parameters and values not seen below in ***Figure 114***, ***see Chapter II. Section C. Part 1. PAGE 4 Field Water Quality Measures on page 45 for an example***.

FIELD WATER»»		Reviewers Initials	
ANCode		Date	

WQ Sample Location	☐ Vertical ☐ Other:	WQ Type	☐ Profile ☐ Other:
Sonde Method	☐ Van-Dorn Bottle ☐ Grab	Lab Water Method	☐ Van-Dorn Bottle ☐ Grab

Sonde I.D. #:________	Seasonal Water Level		Water Odors		Surface "Oils"		Turbidity	
If any problems occur with the Water Meter or any readings are suspect, record notes in the space below.		Below Normal		Normal		None		Clear
		Normal		Sewage (Not Septic)		Flecks		Slightly Turbid
		Above Normal		Petroleum		Sheen		Moderately Turbid
		Flooding		Chemical		Globs		Highly Turbid
Notes:				Anaerobic (septic)		Slick	Water color:	
				Other:				
			Foam/Suds (Rate 0-4 or NR)					

ABOVE: Record readings in box for corresponding physicochemical parameter. Insert a √ in the box for other categories.

Precipitation Status and History					
Current		Past 24 Hours (If Known)		Major Rain Event in past week?	☐ Yes ☐ No
Field Water Notes and Precipitation Comments:					

Figure 114. Example of the Field Water Quality Measures (Top of Page 3) of the Lake Assessment Form

WQ Sample Location: Indicate the cross-sectional location of the water quality sampling: 1) **Vertical** (*i.e.*, vertical profiles done on lakes where samples are taken at multiple depths – these are the most common), 2) **Other** (please describe).

WQ Type: Indicate type of water quality sampling: 1) **Profile** (*i.e.*, samples are taken at multiple locations, but kept separate as distinct samples) – most common on lakes, 2) **Other** if an integrated sampler is used to composite a sample over a range of depths, or another type of sample is taken (*i.e.*, a single sample is taken at the surface) -please describe here.

Sonde Method: Indicate the type of collection method used with the water quality sonde: 1) **Van-Dorn Bottle** (note – the Van-Dorn sampler is rarely used to collect water for the sonde. **2) Grab** (*i.e.*, direct stream or water column measurement, including using the long cable for vertical profiles – this is most common on lakes),

Lab Water Method: Indicate the type of collection method used to obtain the lab water: 1) **Van-Dorn Bottle** this is the most common method used in lakes, 2) **Grab** (*i.e.*, direct water column sample – if only a surface sample is necessary, this may be appropriate.)

Lake Info

Lake Info							
Lake Depth	ft	Secchi Depth	ft	Depth of Top WQ Sample	ft	Depth of Bottom WQ Sample	ft
				Time of Top Sample		Time of Bottom Sample	
mL Filtered for Chlorophyll A Sample:				(Please transcribe the above depths and times to corresponding Lab Analysis Request forms)			
Lake Profile Notes:							

Figure 115. Example of Lake Info section (Bottom of Page 3) of the Lake Assessment Form

Lake Depth: The **maximum** lake depth observed via depth finder or other method should be recorded here to the nearest tenth of a foot.

Secchi depth: Record the single depth at which the Secchi disc disappears and reappears to the nearest tenth of a foot.

Depth and Time of Top and Bottom WQ samples: Record the time (military) of the Top and Bottom WQ samples. These times are to be transcribed to the Analysis Request Form later.

mL Filtered for Chlorophyll A Sample: Record the amount of water filtered to produce the chlorophyll-a filter sample.

Lake Profile Notes: Any other comments on lake dimensions and features can be recorded here, including wildlife observed.

PAGE 4

Landowner/Stakeholder Information

Photography Log

Page 4 includes information about landowner/stakeholders, site accessibility issues, and a photography log. This page is identical to that presented above in ***Chapter II. Section C. Part 1. PAGE 10-Photography Log on page 74.***

PAGES 5 & 6

Sonde Lake Profile Readings

Record the parameters that are available, along with the depth and time of each reading on the rows of this page, beginning with the top sample. (Starting at the bottom increases the risk of contamination of the probes by bottom sediments, plants, or structures.) Allow the readings to equilibrate (sometimes requires up to 1 min) at each depth before recording the data. Any suspect readings (fluctuating greatly or apparently out of calibration) should be flagged and noted in the Lake Profile Notes on the previous page.

WQ Sample ID: This ID is unique and comes pre-printed on labels. It is used whenever a lab water sample is collected. If multiple water quality samples are taken during the sampling event (*i.e*., a waterbody profile), then this information will be documented on another page with the specific collection information (*i.e*., depth, distance, transect, etc.).

Depth Description: A one word description of the depth at which the water parameters were measured (*e.g.,* Top, Middle Bottom, Thermocline, etc.).

Depth (in feet): Depth in feet of the measurement. **This is mandatory for each reading.**

Time: The time (military) at which the water parameters were measured at the given depth. **This is mandatory for each reading.**

Physicochemical Parameters: Record any flags and the values for each of the physicochemical parameters indicated from the water probe:

1. Temp (^{O}C)
2. pH (Standard Units)
3. D.O. (mg/l)

4. Conductivity (μmhos/cm)
5. Chlorophyll-*a* (μg/L)
6. Turbidity (NTU).

Reviewers Initials		ANCode					Date				SONDE LAKE PROFILE READINGS PART 1>>>					
Measurement	WQ Sample ID	Depth Description (e.g., Top, Middle Bottom, Thermocline, etc.)	Depth (in feet) (Mandatory for each reading)	Time (Mandatory for each reading)	Temperature Flag	Temp (°C)	pH Flag	pH (S.U.)	Dissolved Oxygen Flag	Dis. Oxygen (mg/L)	Conductivity Flag	Specific Conduct (umhos/cm)	Chlorophyll A Flag	Chlorophyll A (ug/L)	Turbidity Flag	Turbidity (NTU)
1																
2																
3																
4																
5																
6																
7																
8																
9																
10																
11																
12																
13																
14																
15																
16																
17																
18																
19																
20																

Page 5 WVDEP WAB Lake Assessment Form (4/12/2010)

Figure 116. Example of Sonde lake Profile Readings section (Pages 5 & 6) of the Lake Assessment Form

Section D. WATER COLLECTION PROTOCOLS

The water collection protocols listed below are to be conducted at every site established on a lake.

Part 1. Depth Classification

Lake Depth

The **maximum** lake depth is observed via depth finder or other method to the nearest tenth of a foot. Traverse the lake thoroughly to locate this location. It is usually in the vicinity of the dam, or especially near drain structures, though not in all cases. While the index station should be located as closely as possible to this location, some drifting may occur during sampling. Do not set the index station near any potentially dangerous features. This depth will be used later to help determine the intervals of sonde measurements in the lake profile.

Secchi Depth

A Secchi disk tethered to a rope or measuring tape demarcated by tenths of a foot is used to comparably measure the turbidity, or visibility through the upper fraction of the lake's water column. Readings should be taken using the naked (unimpeded/unaided) eye, without any hat or polarized glasses on, etc. Drop the disk down into the water and maintain visual contact with it by looking through a shaded surface of the water (*i.e*. in the boat's shadow, or a hill's shadow) when possible. If the sun is directly overhead or otherwise creating extra glare, note this in the profile notes section. Lower the disk in the water column until its pattern is no longer visible. Then raise the disk again until it is visible. This may take several back-and-forth attempts to finely determine the depth at which the disk disappears. If the depth at which the disk disappears is not the same as that at which it reappears, then the Secchi depth is the average of these two readings. Otherwise, record the single depth at which the depth disappears and reappears to the nearest tenth of a foot.

Part 2. Sonde Procedures

See Chapter III. Section A. Water Quality Sondes: Calibration, Maintenance, & Use on page 95 for information about how to calibrate and care for a sonde.

Before going to the field, make sure you know what the marks on your sonde cable mean, and check that they are accurate at least once at the beginning of the season. If your cable is not marked, a measuring tape can be attached to the sonde, but be sure

to account for the additional distance between the end of the tape and the actual probes on the sonde (usually about 1.5 ft).

Please note that a functioning Chlorophyll-*a* probe is not available on every lakes sonde as they are very expensive probes and provide redundant data when lab samples are also collected and analyzed. Make sure you know if your sonde has a functioning, well calibrated probe, and if not, do not record Chlorophyll-*a* data.

After the water quality sampling location (index site) has been located, place the water quality sonde in the water and turn it on so that it can begin to equilibrate its readings. The sonde will be lowered from the subsurface sample to the bottom sample at the index site. Make sure the sonde will not encounter any entanglement hazards, or intakes that may suck the sonde in. If the sonde profile must be moved slightly from the deepest point because of these hazards, do so. In any case, be sure to document where and how you sampled on the habitat form. **IF YOU ARE COLLECTING WATER FOR ANALYSIS AT A LAB, DIRECTLY FROM THE LAKE, YOU MUST PLACE THE SONDE IN THE SAME GENERAL LOCATION AS THE WATER SAMPLE COLLECTION.**

1. Remove the calibration cup from the end of the sonde, screw on the deployment guard, and deploy the sonde into the water column. **Be sure to not disturb the substrate at this point until all water data collection is completed.**

   Note: When deploying a sonde into the water, give it a little tap or shake once submerged. This will help dislodge any air bubbles inside the conductivity probe that will bias a reading. Make sure that all probes are submerged adequately.

2. Once fully submerged (*i.e*., the very top of the sonde is submerged-a depth of approximately 1.5 feet for the probe end) in the water turn the unit on.

3. Let the readings stabilize for a few minutes. This time could be used to fill out parts of the habitat form, collect water samples, or check on the GPS coordinates.

4. Determining the reading intervals: Approximately 10 readings should be recorded as you progress vertically through the water column. The lake depth is determined by using a depth finder (***see Part 1. Depth Classification Lake Depth above***).
   - For depths greater than 11 feet (determined by the depth finder), the interval is found by dividing the total depth by 10.
   - For depths of less than or equal to 11 feet, the minimum interval of 1 foot should be used, starting at 1.5 feet and ending at 10.5 feet. Use this same minimum interval for depths less than 11 ft, even though this will produce less than 10 total readings.
   - Finally, in deeper lakes where the sampling interval is greater, make an attempt to more precisely define the thermocline (the depth at which temperature, and

often dissolved oxygen, makes a sharp change). For example, if the sampling interval on a deep lake is 13 feet, and you notice that the temperature or DO drop significantly between the current reading and the previous reading, go back and take samples every 1-3 feet until you locate the thermocline.

5. Record each of the parameters, along with the depth (based on the marks on the cable or an attached tape measure) and time, beginning with the top subsurface sample (1.5 feet deep). Do not start at the bottom as it increases the risk of contamination of the probes by bottom sediments, plants, or structures. Allow the readings to equilibrate (sometimes requires up to 1 min) at each depth before recording the data. Any suspect readings (fluctuating greatly or apparently out of calibration) should be flagged next to the parameter and noted in the Notes Box on the previous page. Do not attempt to go to the absolute bottom for the last reading as this will stir up bottom sediments, result in inaccurate readings, and leave you with a dirty sonde. Rather, the deepest reading should be taken somewhere between 0.5 and 1.5 feet above the bottom, depending on how uneven the bottom appears when accessed via the depth finder (leave more room between the sonde and the bottom when it is very uneven to avoid collisions).

6. After recording all readings, turn the sonde off and retrieve from the lake. Take off the deployment guard and replace the calibration cup. Always make sure sand and other particles are kept clear of the threads on the sampling weight, cap, storage cup, and sonde itself. These threads are plastic and will strip if sand is caught in the treads while screwing these parts on and off.

7. Store the sonde securely for future use. When storing the sonde between sites or sampling events, only a small amount of 4.0 pH buffer inside the cup is necessary to keep the air (and membranes) moist. If the pH buffer is spilled at the site, you can get away with a few drops of water inside the cup until you can replace it back at a vehicle or the lab. **DO NOT STORE THE SONDE WITH A FULL CUP OF WATER, AS THIS WILL LESSEN THE LIFE OF THE pH PROBE.**

Part 3. Water Quality Sample Collection and Preservation

Materials and Reagents

In addition to the Materials and Reagents described ***in Chapter III. Section B. Materials and Reagents on page 119***, the following are required:

1. Chlorophyll-a transfer bottle – this can be the amber plastic 2L Nalgene bottle or simply a 1 L cubitainer surrounded by foil. In either case, the ***container should not be transparent*** as chlorophyll-*a* is light-sensitive. This transfer container may be reused from site to site, but should always be rinsed thoroughly with deionized water before sampling.
2. Van Dorn sampler – for retrieving top and bottom samples from lake (should be pre-rinsed before leaving the vehicle).
3. Fixatives (nitric acid, sulfuric acid, and sodium hydroxide) - for sample preservation.
4. Waterproof plastic bags or other suitable container - for holding bacteria sample bottles during transport.
5. Filtration Apparatus (Vacuum type) – for chlorophyll sample processing.
6. Glass fiber filters – these are GF/F filters (glass fiber – fine) from manufacturers such as Whatman.
7. Aluminum foil – for wrapping chlorophyll filter samples.

Safety Precautions

See ***Safety Precautions on page 119*** for information about how to handle water samples and fixatives.

Procedures for Collecting Water Quality Samples

See ***Labeling Sample Containers on page 120*** for information about how to label water sample containers.

Van Dorn Sampler Method for Depth Profiles

Make sure the Van Dorn sampling rope accurately reflects the distance to the middle of the sample tube at the beginning of each season.

At the selected water quality sampling location (index site), locate a good sampling location. Be sure to document where you sampled on the habitat form. **Be sure to not disturb the substrate around this point until all water sampling is completed.**

The top sample should be taken at a depth of approximately 1.5 ft, as this corresponds to the length of the lakes sondes (when the top of the sonde is at the water's surface, the probes are usually about 1.5 ft underwater). Taking the sample just below the surface also prevents interference from surface films or scrums. The time of the top and bottom samples should be the same as the time noted on ***PAGE 3-Lake Info on page 272 above*** of the Lake Assessment Form of this form.

Care should be taken when lowering Van Dorn sampler for bottom sample. Do not disturb lake sediments as mobilized (soluble) phosphorus will be analyzed in these samples and phosphorus-containing sediment should be considered contamination that will lead to inaccurately high results.

Collect the water samples as follows:

1. Set the Van Dorn sampler for collection by attaching the cable loops from each end cap to the trigger stub. The trigger is a sensitive mechanism and is easy to activate, so a demonstration by experienced personnel may be helpful. Also, make sure the valves at the spigot on each end are closed. (Note: the Van Dorn sampler should have been rinsed with deionized water prior to each use.)

2. Lower the Van Dorn sampler to the desired depth as measured by the marked rope attached (make sure this rope accurately reflects the distance to the middle of the sample tube at the beginning of each season). Make sure to hold the messenger at the surface.

3. When the sampler is at the desired depth, pause for up to a minute to allow water at that depth to circulate through the sampler, and then release the messenger. A clicking sound will indicate that the sampler has been closed.

4. Retrieve sampler and set it on stand in boat.

5. The spigot on the pressure-relief end of the sampler should be rotated upward and opened to allow air to replace the water which will be drawn from the sampler.

6. The spigot on the opposite end is where the water will be drawn from. It should be pointed downward.

7. Water quality samples are collected in plastic Cubitainers. At lake sites, typically 2 are needed for nutrient analysis: 1 unfixed, and 1 sulfuric-fixed.

8. Rinse the Cubitainer twice with water from the Van Dorn sampler by opening the downward-pointing spigot.

9. Fill the Cubitainer with sample water and expunge as much of the airspace as possible. The sulfuric-fixed nutrient sample should be preserved at the index station if there is a long travel time back to the vehicle.
   Note: When collecting a sample to be analyzed for Alkalinity (unfixed sample) as much air as possible should be expunged from the sample container to avoid contamination

10. Finally, fill the Chlorophyll-*a* transfer bottle (see Materials and Reagents above for a

definition) with water from the top sample only (no bottom sample).

11. Repeat Steps 1-9 for the bottom sample.

Direct Dip/Grab Method (Fecal coliform Sample)

1. Use pre-sterilized bottle with Sodium thiosulfate tablet. Keep the bottle closed until you are ready to collect the sample.

2. Open bottle and handle carefully to avoid contamination. DO NOT TOUCH THE INSIDE OF THE LID OR BOTTLE.

3. The fecal sample site should be the closest shoreline to the index station. Shoreline stations are used because they are the most representative of the contact a wading or bathing human would have with the lake water. Take care not to disturb, or muddy, the water at the shoreline when approaching via boat.

4. Dunk the inverted bottle to approximately 1-1.5 feet below the surface and turn right-side-up to fill. DO NOT RINSE OR REFILL THE BOTTLE. If the bottle is too full, slowly pour a little out. Avoid areas with lots of scum or surface debris as any solid matter will contain disproportionately high amounts of bacteria, skewing results.

5. Place cap tightly on bottle and secure cap lock.

Sample Preservation (Fixation, Filtration, & Holding)

Filtration

Protocols for Sample Filtration with Peristaltic Pump/Drill Apparatus (Dissolved Metals and Nutrients)

See ***Chapter III. Section B. Part 2. Sample Preservation (Filtration, Fixation, & Holding) Filtration on page 122*** for details on how to handle the filtration of any dissolved metals or nutrients.

Protocols for Sample Filtration with Hand Pump Apparatus (Chlorophyll-a and Water column algae)

The Chlorophyll-*a* is collected from the top sample (1.5 foot depth) because healthy phytoplankton are generally obtained from the photic zone (depth at which the illumination level is 1% of surface illumination). Filtering should be performed in subdued light as soon as possible after sampling since algal populations, thus chlorophyll *a* concentration, can change in a relatively short period of time. Aboard ship filtration is highly recommended.

The components of the Chlorophyll-a filtering are:

1. Nalgene filter cup apparatus
2. Hand pump and tubing to connect to filter cup
3. Whatman GF/F glass fiber filters
4. Forceps
5. Aluminum foil
6. Squirt bottle with deionized water
7. Ziploc bags
8. Cooler with Ice (Dry Ice preferred)

Procedure:

1. Nalgene filtration apparatus should be rinsed with deionized water before use at each site.

2. With hand pump attached to bottom of filter cup, place filter support screen on top, ensuring there is an o-ring between bottom half of filter cup and filter support. Lay glass fiber filter on top of support (With some filter types, there is an embossed grid-pattern on one side. Place this side facing down for consistency.) Now attach the top half of filter cup by threading collar over filter. Again ensure there is an o-ring between the filter support and the top of the filter cup.

3. Use a separate, higher accuracy graduated cylinder (also rinsed with deionized water) to measure the desired sample volume. Sample should be thoroughly mixed (via inversion) before pouring into cylinder so that solids are equally suspended.

4. Pour desired sample amount into cup (up to 500mL) and screw on top of filter cup apparatus to prevent contamination. Make sure at least one of the ports in the top of the filter cup apparatus is uncovered for pressure relief.

5. Pump the water from the top of the apparatus, through the filter, to the bottom collection cup.
   - **Do not exceed 7 psi when pumping; this will prevent breaking the filter and/or damage to the phytoplankton cells, causing a loss of chlorophyll. Excessive filtration time (>10 minutes) can also damage the cells. Do not increase the vacuum pressure once filtration has begun.**

6. If more than 500mL will be filtered, the bottom cup must be emptied by disassembling the apparatus. Otherwise, water will inundate the filter from underneath and ruin the process.

7. Repeat until steps 4-6 until a sufficient sample has been filtered to produce visible color on the filter. At least 500 mL should be filtered. If there is so much

suspended solids in the sample that the filter becomes clogged before 500 mL is reached, filter as much as possible. There should be some color on the filter when complete. If the water is clear, larger volumes than 500 mL may be filtered, up to the maximum container size of 2 L.

8. Squirt a small amount of deionized water from a squirt bottle into the top of the filter cup, washing all the sides down. This will transfer any residual solids to the filter itself. Pump the deionized water through.

9. Disassemble the apparatus and, using clean forceps, fold the filter in half so that the side containing the chlorophyll is folded on itself.

10. Place the folded filter onto a clean sheet of aluminum foil and fold it gently so that filter is held securely in place.

11. Place foil containing filter into a plastic bag and label the bag just as you would label the cubitainers containing the other samples. The sample type is "Chl-a" and also record the volume filtered on the baggie.

12. Place the bagged filter sample onto ice (dry ice is preferable, but wet ice may be only available) immediately and transfer it to a freezer as soon as possible. Filter should be kept frozen until analysis, with the exception of storage on wet ice during transfer to the lab. Make sure to keep the bagged filter from being submerged in ice water if using wet ice as the bag may leak and ruin the sample.

Fixation

See ***Chapter III. Section B. Part 2. Fixation on page 127*** for details on how to fix and preserve the other samples collected (*i.e.*, metals, nutrients, unfixed containers, fecal).

Holding

See ***Chapter III. Section B. Part 2. Holding on page 128*** for details on parameter holding times (*i.e.*, metals, nutrients, unfixed containers, fecal).

Documentation

See ***Chapter III. Section B. Part 2. Documentation on page 129*** for details on documentation of the sample using the Analysis Request Form with COC.

Lake Sampling Quality Assurance/Quality Control

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with sampling protocols and calibrated to sampling standards. Whilst a specific session on Lake Sampling is not

covered, other sessions (*e.g.,* site documentation and completing the stream assessment forms, water collection protocols, field blanks and duplicates, etc.) are covered. In the field, individuals who are more experienced with Lake Sampling will be teamed up to give hands-on training to less experienced to assure reinforcement of training and accurate results before they are allowed to sample these stations solo. This document is also provided to all program personnel for review and use in the field.

Sample labels are to be accurate and complete and contain all the pertinent information. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Lot numbers of all preservatives are recorded on the Analysis Request Form for each sample submitted and entered into the database to allow for easy tracking. Sample transfer to the lab shall also be documented using the Chain-of-Custody (COC) portion of the Analysis Request Form.

Duplicate sampling and field blanks must be performed at a minimum of 2.5% of Lake Sample sites for each sampling round. The field blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis. Procedures for performing duplicates and field blanks are presented below. ***See Chapter XII. Section A. Field Blanks and Duplicates on page 285 for additional information.***

Chapter XII. MISCELLANEOUS SAMPLING

Section A. Field Blanks and Duplicates

Overview

Duplicate sampling and field blanks must be performed at a minimum of 2.5% of our sites. To assure we meet these requirements, each team list will have a designated duplicate and field blank. Procedures for performing duplicates and field blanks are presented below.

Part 1. Field Blanks

Field blanks are simply samples of deionized water that are preserved in the field. The purpose of the field blank is to detect onsite contamination and verify the purity of the sample fixatives.

Obtaining the Field Blank Water

Before leaving the office, obtain the deionized water by collecting it directly from the laboratory supplied containers.

Procedures for obtaining water from the laboratory supplied containers are as follows:
1) Fill up an unused, one-gallon cubitainer with some water (approximately 100 mL).
2) Screw on the lid, shake the rinse water, and dump. Repeat.
3) After two rinses, completely fill up the one-gallon cubitainer, expunge any remaining air, and place in the vehicle to be used in the field as a source for the field blank water.

Field blanks are to be prepared in the field only and not in the laboratory or garage. A stream location is sometimes designated on the sample list for a field blank. If you miss the exact location indicated on the sheet, prepare a field blank at the next location. The reason why field blanks are indicated on your list is to remind you to do it AND to assure that field blanks are prepared at random locations and times.

A field blank will consist of any parameters that are or may be analyzed during the work week. This may include:

- 1 full cubitainer for Unfixed Samples (Chlorides, Hot Acidity, Alkalinity, TSS, Sulfates, Lab pH, Lab Cond., Cold Acidity, Total Orthophosphate, etc.)
- 1 full cubitainer for Sulfuric Acid Preserved Samples (Total Phosphorous, TKN, NO_2-NO_3-N, Unionized NH_3)
- ½ full cubitainer for Nitric Acid Preserved Samples (All Total Metals)

- ½ full cubitainer for Filtered Nitric Acid Preserved Samples (All Dissolved Metals)
- ½ full cubitainer for Filtered Unfixed Samples (Dissolved Orthophosphate)

Do not prepare a field blank for fecal samples, as the deionized water is not sterile.

Field Blank Field Procedures

1. To prepare a field blank, retrieve your pre-filled one-gallon cubitainer with DI water from storage in the vehicle.
2. Label an appropriate number of one liter cubitainers in a manner that it will appear to be an actual water sample to the lab, but will also be recognizable as a field blank to WAB employees.
3. Fix and handle the samples as you would do for a stream sample by substituting the DI water in the one-gallon cubitainer for actual stream water (including filtering for dissolved parameters if that was or will be done during the week).
4. After the sample has been submitted to the lab, write "FIELD BLANK" at the top of the DEP copy (white) of the Analysis Request Form with Chain-of-Custody (COC) before turning it in with the other forms.

Part 2. Duplicate Samples

Wadeable Benthic Sites (Random, Targeted, and TMDL Bio)

With the exception of GPS and Water Quality Sonde readings, a Wadeable Benthic site is to be duplicated in its entirety. Each team member should treat the site as though he/she is sampling alone: Do your own habitat, water quality and benthic and periphyton collection. The two sets of forms and benthic and water samples should be clearly marked with Dup #1 and Dup #2. On-site water quality data (*i.e.*, pH, Conductivity, DO, Temperature) should only be recorded on the first duplicate form (Dup #1).

Sites to be duplicated are indicated on the team lists. These sites are randomly selected and the main purpose of indicating these sites is to remind you to perform duplicate sampling and to assure that duplicates are performed at random locations and times. It is possible that the site selected is unsuitable for benthic sampling or has insufficient habitat to conduct duplicate benthos collections. If this is the case, the duplicate can be performed at an alternate site. Additionally, if you encounter a site that is ideal for duplicate sampling before you get to your designated site, you may conduct the duplicate at that site and drop the designated one. The important thing is that duplicate sampling is performed for the given group of samples or team list.

TMDL (Water Quality and Limited Habitat)

Duplicate samples for non-biological TMDL samples are limited to water quality only. There is no need to submit a duplicate TMDL-Initial or TMDL-Secondary habitat form, as most field personnel will be working solo and unable to replicate this portion. Duplication will be limited to the water quality parameters assigned to that site; *i.e.*, if the site is fecal only, just do fecal.

Duplicates for TMDL samples should be conducted at sites where the most parameters on the list are collected (if such sites exist on the list) and should be rotated to different sites each sampling event.

Field Blanks and Duplicates Quality Assurance/Quality Control

Sample labels are to be accurate and complete and contain all the information discussed above. Sampling equipment will be checked for contaminants and excess dirt or moisture cleaned before and after each sampling event. Lot numbers of all preservatives are recorded on the Analysis Request Form with Chain-of-Custody (COC) for each sample submitted and entered into the database to allow for easy tracking.

The field blank and duplicate data are looked at by Watershed Assessment Branch staff and scrutinized to find any possible discrepancies, contamination, or faults in the sampling methods and techniques. Any problems are brought to the attention of the program management and steps are made to immediately correct the problem. Data that is related to the problem are flagged with notes concerning the details of the situation so that decisions can be made whether or not to include the data in any further assessments or analysis.

Once a year, all field participants in the WAB attend mandatory training sessions in March-April prior to the initiation of the major sampling season. The purpose of these sessions is to ensure that all field personnel are familiar with habitat sampling protocols and calibrated to sampling standards. A hands-on session concerning the collection and handling of water quality samples is included. Any persons unable to attend the annual training session will be instructed and evaluated on the job in the following month by one of the WAB training instructors. In the field, individuals who are more experienced in collecting water quality field blanks and duplicates will be teamed up with the less experienced to assure reinforcement of training and accurate results. This document is also provided to all program personnel for review and use in the field.

Section B. Source Sampling Procedures for TMDL Monitoring

Source Sampling Overview

This section covers some of the techniques used to document sources during the Total Maximum Daily Load (TMDL) sampling and modeling process. This is a very important component of TMDL sampling as a good understanding of the location and nature of various pollutant sources will result in better TMDL models. It is necessary to document all sources of importance and relevance encountered and forward this information to the TMDL Source Tracker, whose primary task is to catalogue, document, and quantify as much pollutant source information as possible for the ongoing TMDL.

The following instructions are from a memo from the TMDL source tracker concerning various TMDL sources:

Part 1. AMD/AML

Some AMD/AML sources are selected to be included on the TMDL sample lists and will be sampled quarterly for AMD parameters and flow. One may encounter other AMD/AML sources on these and other streams that are not on this sample list while sampling. A source form can be filled out for these other sources as it is deemed appropriate. Include at least field readings, estimated flow, GPS coordinates, pictures, and the visible impact on stream. A sample is not needed unless it is suspected that this is a one-time event or intermittent source. The Field Supervisor will forward a copy to the Source Tracker and they will let you know if any follow-up is needed. Generally, the Source Tracker will follow up with additional source sampling.

Here are some tips about how to address AMD Sources previously published from a January 2007 Memo:

- ✓ AMD sources can discharge via pipes and culverts or simply as seeps from the hillside/stream bank, narrow channel-ways, and/or an artesian upwelling.
- ✓ A good strategy for locating AMD sources is to hike up the receiving stream towards the potential site looking closely for pour points, precipitates on the bottom (orange, white, black), water discoloration (opaque blue/greenish). It is also useful to routinely check YSI field readings of the receiving stream for changes as you move up the watershed. Increasing conductivity and decreasing pH values in the receiving stream as you move up <u>may</u> indicate that you are getting closer to a source. Underground mine discharges are typically 54 degrees F (12.2 degrees C). You can use stream temperatures to help guide you to sources.
- ✓ If you locate a cluster of AMD sources that collect into a common channel before entering the receiving stream, it is acceptable to sample the combined sources/channel below them to get the cumulative concentrations/impacts of the discharge. Also, it would be beneficial to get GPS coordinates for each source

portal even though you won't be sampling them individually; however, be certain to capture coordinates at the location of a grab sample and flow.

- ✓ If you discover a new source that has never been identified before (*i.e*., not currently on your TMDL list) and it appears substantial/significant it should be sampled. The terms substantial and/or significant can vary in their meaning among individuals. Examples of sources that should be considered substantial/significant are: 1) A small source volumetrically but with high concentrations of dissolved ions flowing into a stream causing discoloration due to precipitates and/or causing noticeable changes to field readings, 2) A large source volumetrically that constitutes a large proportion of the total flow in the receiving stream and substantially alters field readings – this type may not be as concentrated with dissolved ions or have very low pH values like the smaller source but because of its volume should be considered for sampling. Small trickles and seeps that ooze out of the bank and do not appear to influence the receiving stream are typically not sampled (in some areas these are numerous and would quickly overwhelm the budget anyway). Check YSI field readings in stream below the discharge if you are unsure – slowly moving away from the source to evaluate its potential impact. In most cases, it comes down to BPJ (best professional judgment).
- ✓ Photos are extremely important for source sampling since many of them are only visited one time. Several photos of the source should be taken along with its pour point into the receiving stream – describing and detailing its impact, etc.
- ✓ GPS coordinates and directions to the site are also critical. Driving directions and hiking directions should be clear and concise. Check GPS's to be sure they have been switched to NAD 83. All of the source tracking sites we will be doing in the Cheat in January will have coordinates associated with them. <u>However, these coordinates were derived using GIS – not GPS field readings.</u> Therefore they should be considered as approximate locations. In some cases the coordinates will probably be fairly accurate. Other locations are very approximate as they are reported locations that have not yet been confirmed, so you may need to search harder. Be prepared to collect grab samples and flow measurements at longer distances from the vehicle, which may require backpacking with the filtering apparatus, preservatives, and flow equipment.
- ✓ TMDL topographic maps will have the new source tracking sites marked in pink. In general, the source sites were assigned to a particular sample list based on their proximity to their monthly TMDL list of sites.
- ✓ Drawings, maps and good written descriptions of the source and what it does to the receiving stream are beneficial. As always document the local land use and any other pertinent info about the site.
- ✓ The Cheat Sheet has some good tips on recognizing sources, etc.
- ✓ If you arrive at a suspected source location and cannot locate a source, check the field water chemistry (conductivity and pH) of the receiving stream/waterbody. If the stream does not appear to be influenced by a source based on the field readings, and sources cannot be located after a sufficient search, the source tracking obligations are fulfilled.

- ✓ Remember, the absolute data requirements for source tracking are GPS locations, grab samples (indicating chemical properties, *i.e*., YSI), flow measurements, and photographs. If a stream is too small to sample for flow using the Marsh-McBirney flow meter, you may perform a timed-fill technique to estimate time required for a source flow to fill a volumetrically known container. Small flexible (smashable into the substrate) buckets and gallon or liter size cubes with upper side cut off, can be used as flow gathering devices – simply record the filling time and repeat as many times as deemed necessary. This should be done at least three times, using the average time among measurements as the recorded data. For example, 3.2 seconds to fill a 1-liter container. You may also use a small container (fecal bottle) to fill a larger container (1 liter cube) to desired level – keeping track of the time all the while. It is also common to have to measure part of a flow and estimate the total flow on this partial measurement; or even do a visual estimate based on a visual comparison of the source flow to another known/measured flow.

Part 2. Permitted Sources

If it is suspected that a permitted discharge is not within permit limits and is having a negative impact on the stream sample on a particular day, it would be very helpful to the TMDL model to have a sample of the permitted discharge (and its flow) on the same day that the stream sample is taken.

Part 3. Other Sources

It is not necessary to collect samples from “common occurrence” pollutant sources like pastures or log jobs. Reserve source sampling for the rare or severe instances that are impacting the stream samples on a particular day. If it is suspected that runoff from a specific source (*e.g.,* problem log job or dairy manure pond overflow) is impacting the stream sample, go ahead and get a source sample if it is practical to do so. If it is not practical to get a sample, any documentation that can be provided will be helpful (*e.g*., GPS coordinates, directions, pictures). Any general source information that may need to be passed on to the Source Tracker can be sent via email.

Chapter XIII. FIELD EQUIPMENT CHECKLIST

Personal Equipment		
	Waders	Essential…our signature piece of equipment
	Rain Gear	Stay Dry…or mostly Dry
	Personal Gear	Luggage, etc.
	Water Bottle	Stay Hydrated!
	Personal First Aid Kit	Small Red Fanny Bags
	Polarized sunglasses	Especially helpful to reduce glare and see under water surface
Weekly Needs		
	GPS Units	Various Garmin Models assigned per jeep (Keep in Camera Bag, Backpack, or Jeep)
	Water Quality Sonde	Various models assigned per jeep; Take inside at end of week; Calibrate at beginning of week or day; Cross-checked Monthly; Keep in Water Lab Jeep Stalls or Sonde Cabinet
	Camera	Various Models assigned per jeep; Take inside at end of week and Keep in Water Lab Jeep Stalls
	Wet Ice	From Ice Machine in the general garage area or buy at store
	95% EtOH	In Cylinder Room
Paperwork and Clipboard Stuff		
	Stream List	Get Assignment from Janice
	Maps	Topographic, WV Gazetteer, WV County Roads
	Habitat Forms	Main WAB Form, Write-In the Rain Version, Front Page only version (for Dry or Access denied sites), Flow Appendix Sheet, Glide/Pool RBP Appendix Sheet, TMDL Forms, Fish Hobo Form; all on the G: drive
	Analysis Request Form with Chain-of-Custody	In the Water Lab Cabinets; Extras in Janice's Office
	WQ Sample Labels	10 Unique WQ Sample Lab IDs per sheet; In the Water Lab Cabinets
	SOP	Get a copy from Janice or the G: drive
	Personal Field Books/Notepads	For the biomorph to write notes on while the Geomorph is away. Extras in Water Lab
	WAB Brochures/Pamphlets	To give to interested parties while in the field
	Data Request Forms	To give to interested parties while in the field; Get from Karen Light
	Clipboards	Assigned to each person
	Pencils and Pens	Get out of our Cabinet in the Mail Room or See Karen Light for Office Supplies
	Compass	To get your North Bearing
	Laptop	Update w/ Maps, WCMS Projects, Shapefiles, DOH County Maps and Database as needed
	Scientific Collection Permit	Obtained yearly by Watershed Assessment Branch from the WVDNR. Janice has original copy.

Jeep Maintenance		
	Fix-A-Flat	Action Packer or under backseat.
	Come-Along	Often stored under backseat.
	Tow Straps	Often stored under backseat.
	Collapsible Shovel	Often stored under backseat.
	Axe	Often stored under backseat.
	Tools	Screwdrivers, wrenches, ratchets, etc.
	Power Inverter	12 Volt DC to AC used to charge Laptops, Drill, etc.
	Flashlight	**MANDATORY!** In Action Packer?
	Car Phone	Hide in vehicle in console or glove box.
	Lab & Vehicle Phone Number Sheet	Should always stay in vehicle in glove box, middle console, or under sun visor.
Miscellaneous Jeep Stuff (Safety and Backup Supplies)		
	Action Packer	To store seldom used equipment
	First Aid Kit	**MANDATORY!** Should be checked regularly
	Fire Extinguisher	**MANDATORY!** Should be checked regularly
	Latex Gloves	To protect hands in nasty looking streams or keep contamination out of samples; Refills kept in Water Lab
	Handi-Wipes	To approximate cleaning of the hands. Refills in Water Lab
	Insect Repellant	DEET works the best; spray clothing only and keep off skin and out of eyes and mouth
	Blaze Orange Vest	Extras in Water Lab
	Life Vest/PFD	
	Machete	To help clear a path thru brush and briars
	Toilet Paper	“Acquire” from bathroom or hotel ☺
	AA Batteries	For GPS and Sonde Units; New ones in Water Lab
	C Cell Batteries	For Sondes; New ones in Water Lab
	D cell Batteries	For Flow Meters; New ones in Water Lab
	pH Standards	To recalibrate or check pH probe in field; In Water Lab
	Conductivity Standards	To recalibrate or check Conductivity probe in field; In Water Lab
	DO Maintenance Kits	To replace a DO membrane in field; Kept with YSI/Hydrolab; Extras In Water Lab
	pH Strips	To test sample fixation; Refills in Water Lab
	Flagging	To mark Transects and sites for easy identification; Extras in Water Lab

Water Quality Sampling (TMDL and WAB)		
	Fecal Coliform Bottles	Stored in Cage #1 in room 1193
	Cubitainers	Stored in Cage #1 in room 1193
	Lids for Cubitainers	Used if Cubitainers do not come with lids; Stored in Cage #1 in room 1193
	HNO_3	Refills in Water Lab under sinks; One extra in Action packer?
	H_2SO_4	Refills in Water Lab under sinks; One extra in Action packer?
	New Filter Apparatus (Primary)	Drill (w/Rechargeable Batteries and Recharger), Peristaltic Pump Board, Tubing, Disc and/or Cartridge Filters; Replacement Tubing and Filters are in the Water Lab
	Plastic File Case	To keep the filter apparatus or supplies clean and dry
	Sharpie	New ones in Water Lab drawers
	Small Zip-Lock Bags	To store individual fecal samples; Refills in Water Lab cabinets; One extra in Action packer?
	Large Zip-Lock Bags	To store multiple fecal samples; Refills in Water Lab cabinets; One extra in Action packer?
	Paper towels	To dab away excess water; “acquire” from janitor’s closet ☺.
	Stainless Steel Bucket or Sample Tube	To sample from bridges
	Rope	To sample from bridges; Also part of Jeep Maintenance
	Large Ice Chest	To store samples in field; In Water’s Garage Area
	Field Blanks (DI Water)	Get from sealed laboratory boxes, put into 1 gallon cubitainer, and mark with a check
Flow Measurement (TMDL and WAB)		
	Measuring Tape	Marked in tenths of a foot; Do not use metric; New ones in Water Lab
	Flow Rod w/Vector Ribbon	Marked in tenths of a foot; Do not use metric.
	Flow Meter	Numbered per unit
Secchi Depth & Chlorophyll-*a* (Lakes)		
	Secchi Disk	
	Chlorophyll-a transfer bottle	this can be the amber plastic 2L Nalgene bottle or simply a 1 L cubitainer surrounded by foil. In either case, the ***container should not be transparent*** as chlorophyll-*a* is light-sensitive. This transfer container may be reused from site to site, but should always be rinsed thoroughly with deionized water before sampling
	Van Dorn sampler	for retrieving top and bottom samples from lake (should be pre-rinsed before leaving the vehicle).
	Glass fiber filters	these are GF/F filters (glass fiber – fine) from manufacturers such as Whatman.
	Filtration Apparatus (Vacuum type)	Funnel, Filter Sieve, Flask, Hand Pump, DI Bottle; store in large Zip-Lock; Old extra ones in Water Lab; Make sure DI Bottle is not contaminated-Wash regularly
	Aluminum foil	for wrapping chlorophyll filter samples

Macroinvertebrate Sampling (Mainly Summer WAB)		
	Bug Jar Labels	Inner (on waterproof paper) and Outer; on the G: drive
	Surber-on-a-Stick	Remember to check for holes; handle marked with depth increments
	D-Net	Remember to check for holes; handle marked with depth increments
	Brush	To scrape at cobble and boulders; One extra in Action Packer would be a good idea
	Forceps	To pick macroinvertebrates off nets; One extra in Action packer?
	Sieve	To sift through benthic material; Extras in Water Lab; One extra in Action packer?
	Plastic tray or container	To temporarily store organic material until sand is put in bottom of bug jar first
	Wash Bottle	To rinse benthic material; doesn't need to be sterile; also used for Periphyton Sampling; new ones in Water Lab
	Plastic Bucket	To temporarily store benthic material during kicks
	Bug Jars	Large sizes for more permanent storage; In Cylinder Room off main garage
	Old Ice Chest or Box	To store Bug Jars
	Clear Packing Tape & Dispenser	To adhere a label on sample jars; Also used for Periphyton labels; Refills in Storage Cabinet in Water Lab
	Macroinvertebrate Book	Keep in the jeep for field bug reference
	100 m Measuring Tape	Extras stored in Water Lab
	Thalweg Pole with centimeter increments	To measure stream depths for stream reach characterization; also used for Pebble Count Measurement
Fish Sampling (Mainly Summer WAB)		
	Fish Jar Labels	Inner (on waterproof paper) and Outer; on the G: drive
	Backpack Electroshocker	Smith-Root Model 24LR or Model 12 backpack electrofisher; includes the cathode and anode
	Tow barge Electroshocker	Includes a generator, anode pole and cable, GPP electrofisher, and cooler.
	Electrofisher batteries and chargers	Spare batteries should be handy and available to ensure that a site can be electrofished quickly
	Dipnets	1/4" mesh; assorted frame sizes
	Seines/blocknets	1/4 in. mesh; 4'x20' or 4'x30' dimensions
	1 gal. Nalgene jars	
	37% Formaldehyde	
	Assorted plastic buckets with lids	Used to hold fish between capture and field processing.
	Rubber Gloves	To be worn at all times by electroshockers and netters.
	Measuring board and digital scales	
	Hearing protection	Used when using the tow barge/generator.

Periphyton Sampling (Special Sampling Occasions)		
	Periphyton Labels	Same label for inside and out; on the G: drive
	Scraping Tool	To scrape at the rocks; A microspatula or spoon-type instrument; Extras in Water Lab drawer; One extra in Action Packer?
	Small Brush (Toothbrush)	To brush at the rocks; One extra in Action Packer? Change once a week; Refills in Water Lab drawers
	Sample Container	4 oz “specimen jar”; Refills in Water Lab cabinets
	10 % Formalin	In a labeled dropper style bottle: WATCH YOUR EYES! Stored under hooded sink in Water Lab; Recommend double sealing this in a larger container
	Electrical Tape	To seal the periphyton container; Refills in Water Lab drawer
	PVC Ring	To delineate the periphyton sample area on a rock
	Small Ice Chest	To separate periphyton samples (nasty formalin) from the other water samples.
Golden Algae Sampling (Special Sampling Occasions)		
	1-liter cubitainer	Used as a sample container.
	Filtering Equipment	Filter Flask, Filter Funnel, Vacuum Pump, and Vacuum Tubing
	Filters	*Whatman* Glass microfiber filters GF/F 47 mm diameter with 0.45 µm pore size
	10% Bleach Solution	Stored in lab- for sterilization
	Distilled drinking water and industrial grade deionized (DI) water.	Stored in lab.
	Sterilized stainless steel forceps or sterile plastic forceps	
	4” squares of clean aluminum foil	1 square of foil per sample
	Golden Algae Sample Labels	
	Golden Algae Analysis Request forms with Chain-of-Custody	Can be obtained from Janice Smithson
	Dry Ice in cooler	Used only if filtering away from lab.

Chapter XIV. CHEAT SHEET

The Cheat Sheet can be used as a quick field guide to this SOP and can be found on the network at:

Q:\WATER RESOURCES\WAB\SOP'S\2010.zip

Chapter XV. AQUATIC NUISANCE SPECIES (ANS) AND DISEASE CONTROL PROTOCOLS

Reprinted from the WVDNR Wildlife Resources Section ANS Disinfect Policy- July 2007

Overview

Recent concerns over the introduction of aquatic nuisance species, fish-related diseases, and non-endemic genotypes of native aquatic species has lead to the development of procedures and guidelines to aid in the control of these potentially deleterious organisms. These guidelines follow accepted protocols currently implemented by many state and federal natural resources agencies. The goal of these procedures and guidelines is to protect the aquatic species inhabiting all of West Virginia public waters. It is the public policy of the State of West Virginia that the wildlife resources as defined as wild birds, wild animals, game and fur-bearing animals, fish (including minnows), reptiles, amphibians, mollusks, crustaceans, and all forms of aquatic life used fish bait whether dead or alive (20-1-2)) shall be protected for the use and enjoyment of all citizens of this State (20-2-1). These procedures and guidelines are intended to be an integral component of the management of fish and other aquatic resources as established in the agency's mission.

Required Disinfectant Tasks of All Equipment

These tasks are required for all WVDNR staff, as well as any governmental agency, individual, or private company operating in West Virginia public waters under the authority of a WVDNR issued scientific collecting permit (20-2-50) or as a WDVNR cooperator.

Part 1. All Boats including Electrofishing Boats

All WV Water Bodies: Bilge areas and live wells must be drained before leaving water body, as well as all trash (plastics, woody, etc) removed from boat. Props and trailers must be inspected and all mud, aquatic plants, and animals must be removed. Prior to next use, live wells must be disinfected using the appropriate techniques.

Water Bodies of Special Interest: Bilge areas and live wells must be drained before leaving water body, as well as all trash (plastics, woody, etc) removed from boat. Props and trailers must be inspected and all mud, aquatic plants, and animals must be removed. Prior to leaving the immediate area of the water body, live wells, bilge areas, and other exposed boat and trailer surfaces must be disinfected using the appropriate techniques.

Part 2. Field Sampling and Laboratory Equipment

Gill, hoop, and other nets must be cleaned and allowed to air dry in an area not in proximity to a public water body. Dip nets, boots, measuring boards, etc must be disinfected between uses. Balances, counter tops, cutting tools, and other laboratory equipment must be disinfected between uses or if moved within or between a facilities.

Part 3. Fish or Other Aquatic Species Stocking Equipment

All hauling tanks must be disinfected following each transportation event. Disinfection of stocking tanks will not be allowed at a WVDNR hatchery unless a disinfecting area has been developed. No non-disinfected equipment will be allowed on any WVDNR warmwater hatchery and hatchery personnel will deny entry to any who did not disinfect their equipment. Only hatchery dip nets are allowed to be used on any WVDNR warmwater hatchery. All non-hatchery dip nets must remain in the vehicle.

Water used for hauling tanks must be acquired at the individual WVDNR warmwater hatchery, or as assigned by WVDNR warmwater hatchery staff. If broodstock are being transported into a WVDNR warmwater hatchery, fish will be removed from the stock tank and treated by WVDNR warmwater hatchery staff. Water will be treated with a chlorine solution and disposed at a developed disinfecting area. No untreated water will be disposed at WVDNR warmwater hatchery facility. All broodstock must be treated while in transport from collecting water body to a WVDNR warmwater hatchery facility.

Part 4. Other Equipment

Care must be given to the cleaning and disinfection of other equipment, outerwear (*i.e*., waders and wading boots), etc. Thus, it is dependent on individual staff to be cognitive of their responsibility.

Part 5. Record Keeping

A log of the use and disinfection of boats and fish stocking equipment must be maintained.

Water Bodies of Special Interest

A. All Great Lakes, Potomac River sub-basin water bodies, as well as the Ohio and Kanawha Rivers and any water body within a Great Lake bordering state
B. Any water body within a state experiencing VHS or other fish disease problem
C. Any water body experiencing a fish kill
D. Any water body known to be inhabited by zebra mussels, silver and bighead carp, rough goby, and other recognized injurious species

Selection of Surface Disinfectants

Reprinted from CDFA-Biosecurity-Selection and Use of Surface Disinfectants

Selection of an appropriate surface disinfectant is governed by several factors including the type of surface to be disinfected, temperature, weather conditions, effectiveness against specific disease causing organisms, and time required for the disinfectant to inactivate the agent. The efficacy of most disinfectants is impaired by the presence of organic material and thorough cleaning prior to their application is critical.

Precautions

When using surface disinfectants, always:

- Follow label directions regarding use and safety precautions.
- Take proper precautions to protect the environment and ensure that no one is injured
- Devices and coverings for protecting the hands, skin, nose, mouth, and eyes should be worn when indicated by the product label

Glossary of Biosecurity Terminology

Disinfectant: a substance that destroys harmful microorganisms. According to the Environmental Protection Agency (EPA), a disinfectant destroys 100% of the vegetative (actually growing) bacteria of a certain species under specified conditions. However, disinfectant does not include efficacy against fungi, viruses, *Mycobacterium tuberculosis* or bacterial spores (unless specifically tested against those organisms with EPA approved methods).

Sanitizer: reduces vegetative cells, but not the spores of, bacteria to a safe level as may be judged by public health requirements (by reduction of 99.9% of vegetative bacteria).

Virucide: kills or inactivates viruses. For EPA label claims, EPA accepted protocols must be used in testing specific viruses.

Sporicide: kills all microorganisms including bacterial endospores, a very resistant form of certain microorganisms, which develop as a means of survival under adverse conditions.

Fungicide: kills or inactivates fungi. For EPA label claims, EPA accepted protocols must be used in testing specific fungi.

Bactericide: kills or inactivates bacteria. For EPA label claims, EPA accepted protocols must be used in testing specific bacteria.

Detergent: Cleansing agents that assist in the removal of soils by emulsifying grease and suspending dirt particles.

Disinfectant detergent: Combination product for one-step cleaning, disinfecting, and deodorizing.

Tuberculocidal: kills *Mycobacterium tuberculosi*s, an acid fast bacterium which is generally more difficult to kill than most bacteria. Making label claims for tuberculocidal activity requires testing under specific EPA

Material Safety Data Sheet (MSDS): Informational sheet describing properties, usages, and safety concerns of a material or product.

Selected Surface Disinfectants

Potassium peroxymonosulfate – Virkon S®

Instructions for use: Use a 1% solution (1.3 oz./ gallon water)

Advantages: Bactericidal, virucidal, fungicidal. Solution stable for 7 days. This is a U.S. Environmental Protection Agency Registered Farm disinfectant with label claims against FMD virus.

Disadvantages: Powder is irritating to eyes, mucous membranes and respiratory tract. Do not ingest. Do not immerse metal for longer than 10 minutes

Sodium hypochlorite (household bleach, 6% of sodium hypochlorite)

Instructions for use:

1) Clean before disinfecting hard surfaces.
2) Allow a mixture of 3/4 cup bleach/gallon of water (higher concentrations should be used when high levels of organic matter are present) to contact surface for 10 minutes, and then rinse with water.
3) Once mixed with water, bleach breaks down quickly – replace disinfecting solutions daily.
4) Mix in well ventilated area and wear gloves.

Advantages: Bactericidal, virucidal.

Disadvantages: Wear gloves when applying; skin, eye, nose and throat irritant when concentrate inhaled; ingestion can cause esophageal injury, stomach irritation,

prolonged nausea, and vomiting. Household bleach forms toxic gas when mixed with ammonia or vinegar - Do not mix with other cleaners

Alcohol (ethanol and isopropyl 70-95%)

Instructions for use: Disinfect hard surfaces by direct application.

Advantages: Bactericidal, tuberculocidal, fungicidal.

Disadvantages: No action against spores or nonenveloped viruses; no detergency; flammable (store in closed container away from sources of ignition); eye irritation and damage; irritating if vapor inhaled; prolonged skin contact will cause irritation.

Quaternary ammoniums with bis-n-tributyltin oxide - Roccal®- D Plus

Instructions for use:

1) Use to clean and disinfect hard surfaces on farms, veterinary clinics, animal facilities, and vehicles. Useful for boot baths.
2) Apply diluted Roccal® mixture (½ounce/gallon water) by immersion or flushing solution over surfaces, allow to stand 10 minutes prior to rinsing. To clean heavily soiled areas, use up to 1½ ounce Roccal®/gallon water.
3) Boot baths use 1 ounce Roccal®/gallon water.
4) Change daily and anytime bath is visibly soiled.

Advantages: Bactericidal, fungicidal; one-step soapless disinfectant detergent; effective in the presence of organic soil; non- corrosive to many surfaces; safe to use in immediate vicinity of animals.

Disadvantages: Concentrate is corrosive to tissues; causes eye damage and skin irritation; do not get in eyes, on skin, or on clothing; harmful/fatal if swallowed.

Chlorhexidine diacetate - Nolvasan®-S

Instructions for use:

1) Disinfection of veterinary and farm premises; *some formulations appropriate for hand washing (Nolvasan® Skin and Wound Cleanser or Surgical Scrub).*
2) For inanimate objects: dilute 3 ounces/gallon of water; for farm and veterinary premises dilute 1 ounce/gallon of water.

Advantages: Bactericidal, virucidal.

Disadvantages: Not effective against spore-forming bacteria; do not contaminate water or food with disinfectant; harmful if swallowed; irritating to eye and mucous membranes.

Quaternary ammonium chloride - Spectrasol®

Instructions for use: Use on hard, nonporous surfaces at a dilution of 1 ounce Spectrasol®/gallon of water

Advantages: Bactericidal, virucidal, fungicidal. One-step cleaning and disinfectant; hard water or organic soil (5% load) does not affect efficacy of disinfectant.

Part 6. Suggested Disinfectant Concentrations

Table 13. Suggested Bio-disinfectant Concentrations

	Disinfectant		
Quantity of Water	**5.25% Chlorine (*Bleach*)**	***Virkon®S***	***Net-Dip™***
Gallons	***Fluid Ounces***	***Ounces***	***Fluid Ounces***
1	2	2	1
5	3	7	5
10	5	14	10
25	11	34	25
50	22	67	50
100	44	134	100
Contact Time	10 minutes	30 minutes	10 minutes
		Metals-10 minutes	
Application	Soaking	Soaking/Misting	Cleaning

West
Virginia
Department of
Environmental
Protection

Watershed Branch
2010 Standard Operating Procedures

Front Cover Photo: Otter Creek in Otter Creek Wilderness Area, Monongahela National Forest (photo by Mike Whitman)

Back Cover Photo: North Fork of South Branch of Potomac River near Cabins, WV (photo by Kevin Seagle)