



# Watershed Assessment Branch 2015 Field Sampling Standard Operating Procedures



West Virginia  
Department of Environmental Protection  
Division of Water and Waste Management  
Watershed Assessment Branch  
601 57th Street S.E.  
Charleston, WV 25304  
(304) 926-0495  
[www.dep.wv.gov](http://www.dep.wv.gov)

## Acknowledgements

This document is the result of over a decade of contributions by several staff members: Jeff Bailey, Chris Barry, Ben Lowman, Jason Morgan, Nicholas Murray, Kevin Seagle, Janice Smithson, James Summers, Michael Whitman, John Wirts, and Doug Wood. It was edited and formatted by Michael Whitman, Nicholas Murray, and Jeff Bailey.

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 (e.g., 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:

West Virginia Department of Environmental Protection  
Division of Water and Waste Management  
Watershed Assessment Branch  
601 57<sup>th</sup> Street S.E.  
Charleston, WV 25304  
(304) 926-0495  
Attention: Michael Whitman  
Email: [Michael.J.Whitman@wv.gov](mailto:Michael.J.Whitman@wv.gov)

### ***Preferred Citation***

WVDEP (West Virginia Department of Environmental Protection). 2015. Watershed Assessment Branch 2015 Field Sampling Standard Operating Procedures. Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV.

### ***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.

## Table of Contents

<b>ACKNOWLEDGEMENTS</b> .....	<b>I</b>
PREFERRED CITATION .....	I
DISCLAIMER .....	I
<b>TABLE OF CONTENTS</b> .....	<b>II</b>
<b>LIST OF FIGURES</b> .....	<b>XIII</b>
<b>LIST OF TABLES</b> .....	<b>XIX</b>
<b>LIST OF EQUATIONS</b> .....	<b>XIX</b>
<b>LIST OF ABBREVIATIONS AND ACRONYMS</b> .....	<b>XX</b>
<b>NEW IN THE 2015 EDITION</b> .....	<b>XXI</b>
<b>ERRATA FROM PREVIOUS VERSIONS</b> .....	<b>XXI</b>
<b>CHAPTER 1. INTRODUCTION TO WATERSHED ASSESSMENT BRANCH SAMPLING ACTIVITIES</b> .....	<b>1-1</b>
FUNCTION OF THE WATERSHED ASSESSMENT BRANCH .....	1-1
SAMPLING PROGRAMS OF THE WATERSHED ASSESSMENT BRANCH .....	1-2
SCOPE OF SOP FOR WATERSHED ASSESSMENT BRANCH SAMPLING PROGRAMS .....	1-5
OTHER DOCUMENTS TO CONSIDER .....	1-5
GENERAL QUALITY ASSURANCE/QUALITY CONTROL .....	1-6
<i>Quality Assurance Project Plan</i> .....	1-6
<i>Training</i> .....	1-6
<i>Standard Operating Procedures Document</i> .....	1-7
<i>Data Retention &amp; Storage</i> .....	1-7
<b>CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS)</b> .....	<b>2-1</b>
OVERVIEW .....	2-1
SECTION A. SETTING UP THE SITE .....	2-1
<i>Part 1. Initial Site Survey</i> .....	2-1
<i>Part 2. Accessing the Site</i> .....	2-2
Random Sites (Probabilistic Sites) .....	2-2
Alternate Sites .....	2-4
Locating the X-Site .....	2-6
Site Verification .....	2-6
Sliding the Reach .....	2-9
Targeted Sites .....	2-11
Duplicate Sites .....	2-13
Reference Sites and Potential Reference Sites .....	2-14
Reference Site Criteria .....	2-14
Determining Candidate Reference Sites While In the Field .....	2-18
SECTION B. SITE DOCUMENTATION .....	2-20
<i>Part 1. Coordinates and Global Positioning Systems (GPS)</i> .....	2-20
GPS Overview .....	2-20
Quick Operation of the Garmin III+ or V GPS Unit .....	2-21
Procedures for obtaining coordinates with a GARMIN GPS III+ or V .....	2-22
Procedures for checking/changing the datum with a GARMIN GPS III+ or V .....	2-22
GPS Quality Assurance/Quality Control .....	2-23
<i>Part 2. Photographic Documentation</i> .....	2-24

Photography Overview .....	2-24
Procedures for In the Field .....	2-24
Procedures for In the Office .....	2-26
Downloading the Photos for tagging .....	2-26
Tagging the Photos with a Photo ID .....	2-26
Photos that are taken at sampling sites .....	2-26
Photos that are not taken at sampling sites .....	2-27
Photography Quality Assurance/Quality Control .....	2-28
SECTION C. GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS .....	2-28
<i>Important Notice about Personally Identifiable Information</i> .....	2-29
<i>Part 1. Wadeable Benthic Stream Assessment Form</i> .....	2-29
<i>Front Side of All Pages</i> .....	2-29
<i>PAGE 1</i> .....	2-30
Site Verification .....	2-30
<i>PAGE 2</i> .....	2-37
Site Activities and Disturbances (Including Roads) .....	2-37
<i>PAGE 3</i> .....	2-43
Physical Characterization .....	2-43
Sediment Characterization .....	2-44
Substrate Particle Layer Profile .....	2-50
Dominant Substrate Type and Reach Characterization .....	2-51
<i>PAGE 4</i> .....	2-53
Stream Debris (Not found on the Wadeable Benthic Assessment Form) .....	2-53
Field Water Quality Measures .....	2-54
Flow Information (Not found on the Wadeable Benthic Assessment Form) .....	2-59
Stream Bank/Riparian Buffer Zone Vegetation/Cover Type .....	2-60
Riparian Vegetation Classification .....	2-60
<i>PAGES 5, 6, 5a, and 6a</i> .....	2-66
USEPA's Rapid Visual-Based Habitat Assessment .....	2-66
USEPA's Rapid Visual-Based Habitat Assessment Form .....	2-68
Riffle/Run Prevalence .....	2-69
Glide/Pool Prevalence Form (Low Gradient Macs-Type Sites Only) .....	2-81
<i>PAGE 7</i> .....	2-84
Non-RBP Parameters .....	2-84
PRS and Stressor Info .....	2-86
<i>PAGE 8</i> .....	2-87
Wildlife & Freshwater Mussel Observations .....	2-87
Trout Observations (For Sites that are not actively being sampled for Fish!) .....	2-89
<i>PAGE 9</i> .....	2-91
Benthic Macroinvertebrate Collection Information .....	2-91
Benthic Substrate Sample Composition .....	2-92
Visual Estimation of Periphyton and Aquatic Plant Density .....	2-93
Periphyton Collection Information .....	2-103
<i>PAGE 10 &amp; 11</i> .....	2-103
A Visual Guide to Stream Macroinvertebrates .....	2-103
<i>PAGE 12</i> .....	2-106
Landowner/Stakeholder Information, Recon, & Photos .....	2-106
Recon/Accessibility Information .....	2-107
Photography Log .....	2-108
<i>Part 2. APPENDIX FORMS</i> .....	2-110
APPENDIX #1 - Stream Discharge (Flow) .....	2-110
APPENDIX #2 - Stream Bank Erodibility and Channel Profile Measurements .....	2-112
Stream Bank Erodibility Factors .....	2-113
Estimated Channel Profile (Width to Depth Ratio) .....	2-115
APPENDIX #3 – TMDL/Wadeable Benthic Appendix Form .....	2-118
Sketch of Assessment Reach and Comments .....	2-118



APPENDIX #4 – Water Quality Profile ..... 2-120

APPENDIX #5 – Relative Bed Stability (Pebble Count) including Gradient ..... 2-122

Assessment Form Quality Assurance/Quality Control..... 2-124

Forms Used In the Watershed Assessment Branch ..... 2-125

**CHAPTER 3. WATER COLLECTION PROTOCOLS..... 3-1**

SECTION A. WATER QUALITY SONDES & SENSORS: CALIBRATION, ROUTINE MAINTENANCE, & USE ..... 3-1

Overview ..... 3-1

Part 1. Sonde/Sensor Calibration, Maintenance, Diagnostics, and Accuracy ..... 3-3

Display Unit Overview ..... 3-6

    YSI 600XL or 600XLM Sonde/650 MDS Display ..... 3-6

    Maintenance of YSI Display Unit ..... 3-6

Hydrolab Quanta G ..... 3-6

    Maintenance of Quanta G Display..... 3-7

Dissolved Oxygen Sensor ..... 3-7

DO Sensor Overview ..... 3-7

DO Sensor Calibration..... 3-7

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-8

    Hydrolab Quanta G ..... 3-10

    Hydrolab LDO Sensor..... 3-11

DO Sensor Maintenance ..... 3-11

    Clark-cell DO Sensor ..... 3-11

    LDO Sensor ..... 3-12

DO Sensor Diagnostic..... 3-13

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-13

DO Sensor Accuracy ..... 3-14

    YSI Clark-cell ..... 3-14

    Hydrolab Quanta Clark-cell..... 3-14

    Hydrolab (Hach) LDO ..... 3-14

Conductivity/Specific Conductance Sensor..... 3-14

Conductivity Sensor Overview ..... 3-14

Conductivity Sensor Calibration..... 3-15

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-15

    Hydrolab Quanta G ..... 3-16

Conductivity Sensor Maintenance..... 3-17

Conductivity Sensor Diagnostic ..... 3-18

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-18

Conductivity Sensor Accuracy..... 3-18

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-18

    Hydrolab Quanta G ..... 3-18

pH Sensor ..... 3-18

pH Sensor Overview..... 3-18

pH Sensor Calibration ..... 3-18

    YSI 600XL or 600XLM Sonde/650 MDS Display (Three-Point calibration example) ..... 3-19

    Hydrolab Quanta G (a Two-Point calibration) ..... 3-22

pH Sensor Maintenance and Troubleshooting ..... 3-22

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-22

    Hydrolab Quanta G ..... 3-24

pH Sensor Diagnostic (Nernst Equation)..... 3-25

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-25

pH Sensor Accuracy ..... 3-26

    YSI 600XL or 600XLM Sonde/650 MDS Display..... 3-26

    Hydrolab Quanta G ..... 3-26

Temperature Sensor ..... 3-26

    Temperature Sensor Maintenance and Troubleshooting..... 3-26

    Temperature Sensor Accuracy..... 3-27

Additional Sensors ..... 3-27

<i>Part 2. Sonde Routine Maintenance &amp; Storage</i> .....	3-28
Maintenance Kit Contents .....	3-28
Maintenance Schedule.....	3-29
Miscellaneous Sonde Maintenance .....	3-30
Sonde Storage .....	3-30
<i>Part 3. Sonde Use (Field Procedures)</i> .....	3-30
Setting up the Water Quality Sample Site.....	3-31
<i>Sonde Quality Assurance/Quality Control</i> .....	3-32
SECTION B. WATER QUALITY SAMPLE COLLECTION AND PRESERVATION .....	3-33
<i>Materials and Reagents</i> .....	3-33
<i>Safety Precautions</i> .....	3-34
<i>Part 1. Procedures for Collecting Water Quality Samples</i> .....	3-34
Labeling Sample Containers.....	3-34
Direct Dip/Grab Method .....	3-35
Indirect Methods .....	3-37
<i>Part 2. Sample Preservation (Filtration, Fixation, &amp; Holding)</i> .....	3-38
Filtration.....	3-38
Protocols for Sample Filtration with Peristaltic Pump/Drill Apparatus (Dissolved Metals & Dissolved Nutrients) 3-38	
Protocols for Sample Filtration using a Vacuum Pump (Dissolved Metals & Dissolved Nutrients).....	3-42
Fixation .....	3-44
Testing a sample with a pH test strip.....	3-45
Holding.....	3-45
Documentation .....	3-46
<i>Part 3. Common Water Quality Parameter Suites &amp; Detection Limits</i> .....	3-49
Random & Potential Reference Sites: .....	3-49
Acid Rain Parameters.....	3-49
AMD Parameters.....	3-50
Nutrient Enrichment .....	3-50
Large River Algae/Nutrient Enrichment .....	3-50
TDS Ions .....	3-50
Oil & Gas .....	3-50
Sediment.....	3-50
Current Water Quality Analyte Method Detection Limits.....	3-51
<i>Water Sample Collection Quality Assurance/Quality Control</i> .....	3-52
Field Blanks .....	3-52
Duplicate Samples (Blind Duplicates).....	3-54
<i>WVDEP Laboratory Quality Assurance Program</i> .....	3-55
<b>CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS .....</b>	<b>4-1</b>
OVERVIEW.....	4-1
SECTION A. SUM OF PARTIAL DISCHARGES METHOD .....	4-1
<i>Materials and Supplies</i> .....	4-3
<i>Part 1. Operation and Maintenance of Marsh-McBirney Flo-Mate</i> .....	4-4
Theory of Operation (From the Marsh-McBirney Flo-Mate Manual, 1990) .....	4-5
Flo-Mate Settings.....	4-5
Maintenance of Marsh-McBirney Flo-Mate.....	4-7
Cleaning .....	4-7
Zero Check and Adjust .....	4-7
Zero Check Procedure .....	4-7
Zero Adjust Procedure.....	4-8
Flow Meter Accuracy .....	4-8
Using the Flo-Mate.....	4-8
Notes about error readings .....	4-9
<i>Part 2. Wadeable Stream Flow Measurement Procedures Using Marsh-McBirney Flo-Mate</i> .....	4-10
Setting up the Transect .....	4-11
Starting the Transect.....	4-12

Taking Flow Measurements or Verticals ..... 4-13

Calculating Flow ..... 4-17

SECTION B. MEASURING FLOW USING A BUCKET AND STOP WATCH ..... 4-17

SECTION C. MEASURING FLOW USING A USGS GAUGING STATION..... 4-17

*Flow Measurement Quality Assurance/Quality Control*..... 4-18

**CHAPTER 5. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS ..... 5-1**

OVERVIEW..... 5-1

*Definitions* ..... 5-1

*Benthic Macroinvertebrates as Environmental Indicators*..... 5-1

*Basis of Sampling Method*..... 5-2

*Selecting Sampling Method and Device* ..... 5-2

*Selecting Sampling Sites and Kick Locations* ..... 5-4

SECTION A. BENTHIC MACROINVERTEBRATE SAMPLING ..... 5-6

*Materials and Reagents* ..... 5-6

*Field Safety Precautions* ..... 5-7

*Part 1. Field Sample Collection Methods*..... 5-7

        Single Habitat-Riffle/Run Habitats using a Rectangular Frame Dip-Net/Kick-Net..... 5-7

        Single Habitat-Riffle/Run Habitat using a D-Frame Dip-Net/Kick-Net..... 5-12

        Hand Picking (Small narrow streams with minimal/interstitial flow)..... 5-12

        MACS (Multi-habitat Approach for Low Gradient Streams, Glide/Pool Habitat) ..... 5-13

*Part 2. Field Sample Preservation Methods* ..... 5-14

*Part 3. Laboratory Documentation or Check-In* ..... 5-14

*Benthic Sampling Quality Assurance/Quality Control* ..... 5-14

SECTION B. LABORATORY PROCESSING OF BENTHIC MACROINVERTEBRATE SAMPLES ..... 5-16

*Materials and Supplies* ..... 5-16

*Laboratory Safety Precautions* ..... 5-17

*Benthic Sample Processing Methods*..... 5-17

*Benthic Laboratory Processing Quality Assurance/Quality Control* ..... 5-21

        Percent Sorting Efficiency (PSE) ..... 5-22

SECTION C. IDENTIFICATION OF BENTHIC MACROINVERTEBRATES ..... 5-22

*Materials and Supplies* ..... 5-22

*List of Taxonomic References* ..... 5-23

        General Keys ..... 5-23

        Annelida ..... 5-23

        Crustacea ..... 5-24

        Acarina ..... 5-25

        Ephemeroptera ..... 5-25

        Odonata ..... 5-26

        Plecoptera ..... 5-26

        Heteroptera ..... 5-27

        Coleoptera ..... 5-27

        Megaloptera and Neuroptera ..... 5-27

        Trichoptera ..... 5-27

        Diptera ..... 5-28

        Mollusca ..... 5-29

*Safety Precautions*..... 5-30

*Macroinvertebrate Identification Procedures* ..... 5-30

*Benthic Laboratory Identification Quality Assurance/Quality Control* ..... 5-33

*Part 1. SFS Taxonomic Certification Program* ..... 5-33

        Program Background and Justification ..... 5-33

*Part 2. Benthic Identification QA/QC Metrics*..... 5-34

        Percent Difference in Enumeration (PDE) ..... 5-34

        Percent Taxonomic Difference (PTD) ..... 5-35

SECTION D. BENTHIC MACROINVERTEBRATE DATA ANALYSIS ..... 5-36

*Part 1. Genus-Level Index of Most Probable Stream Status (GLIMPSS)* ..... 5-36

        GLIMPSS Reference ..... 5-36

        GLIMPSS Overview ..... 5-37

        Restrictions for Calculating the GLIMPSS ..... 5-39

        Using the GLIMPSS for Data Analysis ..... 5-41

*Part 2. West Virginia Stream Condition Index (WVSCI)* ..... 5-41

        WVSCI Reference ..... 5-41

        WVSCI Overview ..... 5-41

        Restrictions for Calculating the WVSCI ..... 5-42

        Using the WVSCI for Data Analysis ..... 5-43

*Part 3. Dirty Null Stressor Identification Model* ..... 5-44

SECTION E. BENTHIC MACROINVERTEBRATE SAMPLE COMPARABILITY ..... 5-44

*Benthic Macroinvertebrate Data Analysis Quality Assurance/Quality Control* ..... 5-46

        Comparison of Index Result (CIR) ..... 5-46

**CHAPTER 6. FISH COLLECTION PROTOCOLS ..... 6-1**

OVERVIEW ..... 6-1

*Fish as Environmental Indicators* ..... 6-1

SECTION A. WADEABLE STREAM FISH COMMUNITY ASSESSMENT PROTOCOL ..... 6-1

*Basis of Sampling Method* ..... 6-1

*Part 1. Selecting Sampling Sites* ..... 6-2

*Part 2. Determining Site Suitability* ..... 6-3

*Part 3. Establishing the Sample Reach* ..... 6-4

*Part 4. Field Sampling Methodology* ..... 6-4

            Materials and Reagents ..... 6-5

            Field Safety Precautions ..... 6-5

            Selecting the Collection Methods ..... 6-6

                Electrofishing ..... 6-7

                Netting ..... 6-13

                Seining ..... 6-13

                Fish Collection Procedure ..... 6-14

            Field Sample Processing ..... 6-16

                Field Identification ..... 6-16

                Voucher/Reference Preservation Method ..... 6-16

            Field Data Collection ..... 6-17

            Laboratory Documentation or Check-In ..... 6-19

            Fish Sampling Quality Assurance/Quality Control ..... 6-19

*Part 5. Laboratory Processing of Fish Samples* ..... 6-19

            Materials and Supplies ..... 6-19

            Laboratory Safety Precautions ..... 6-19

            Fish Sample Lab Processing Methods ..... 6-20

            Fish Laboratory Processing Quality Assurance/Quality Control ..... 6-20

*Part 6. Identification of Fish* ..... 6-20

            Materials and Supplies ..... 6-21

            List of Taxonomic References ..... 6-21

            Safety Precautions ..... 6-22

            Fish Identification Procedures ..... 6-22

            Fish Identification Quality Assurance/Quality Control ..... 6-22

                Taxonomic Certification ..... 6-22

                Fish Identification QA/QC Metrics ..... 6-23

                    Percent Difference in Enumeration (PDE) ..... 6-23

                    Percent Taxonomic Difference (PTD) ..... 6-23

SECTION B. TROUT STREAM VERIFICATION PROTOCOL ..... 6-24

*Basis of Sampling Method* ..... 6-24

*Part 1. Selecting Sampling Sites* ..... 6-25



<i>Part 2. Field Sampling Methodology</i> .....	6-25
Deviations from other Fish Protocols .....	6-26
<b>CHAPTER 7. PERIPHYTON COLLECTION PROTOCOLS.....</b>	<b>7-1</b>
PERIPHYTON OVERVIEW.....	7-1
<i>Materials and Supplies</i> .....	7-1
<i>Field Safety Precautions</i> .....	7-1
<i>Part 1. Field Sampling Procedures</i> .....	7-2
<i>Part 2. Laboratory Methods</i> .....	7-4
<i>Part 3. Periphyton Data Assessment</i> .....	7-5
<i>Periphyton Quality Assurance and Quality Control</i> .....	7-5
<b>CHAPTER 8. FILAMENTOUS ALGAE MONITORING.....</b>	<b>8-1</b>
FILAMENTOUS ALGAE OVERVIEW.....	8-1
<i>Materials and Supplies</i> .....	8-3
<i>Field Safety Precautions</i> .....	8-3
<i>Part 1. Field Sampling Procedures</i> .....	8-3
Site Selection.....	8-3
Transect Selection.....	8-4
Establishing the Transect.....	8-4
Documentation using the Filamentous Algae Monitoring Form.....	8-5
Taking Filamentous Algae Measurements.....	8-8
Measuring Canopy Density with a Densiometer.....	8-16
<i>Part 2. Filamentous Algae Data Analysis</i> .....	8-18
Calculating Percent Algae Cover.....	8-18
Calculating Percent Water Column Filled.....	8-19
<i>Filamentous Algae Measurement Quality Assurance and Quality Control</i> .....	8-20
<b>CHAPTER 9. GOLDEN ALGAE COLLECTION PROTOCOLS.....</b>	<b>9-1</b>
GOLDEN ALGAE OVERVIEW.....	9-1
<i>Materials and Supplies</i> .....	9-1
<i>Field Safety Precautions</i> .....	9-2
<i>Part 1. Field Sampling Procedures</i> .....	9-2
<i>Part 2. Sample Preservation (Filtration &amp; Holding)</i> .....	9-3
Filtration.....	9-4
Holding.....	9-7
<i>Golden Algae Quality Assurance/Quality Control</i> .....	9-9
Sample Blanks.....	9-9
Duplicate Samples.....	9-10
<b>CHAPTER 10. RELATIVE BED STABILITY/SUBSTRATE CHARACTERIZATION PROTOCOLS (INCLUDING GRADIENT)</b>	<b>10-1</b>
MATERIALS AND SUPPLIES.....	10-1
SECTION A. PROCEDURES.....	10-1
<i>Part 1. Establishing Reach and Transects</i> .....	10-1
<i>Part 2. Substrate Measurement (AKA Pebble Count), Thalweg Profile, and Bankfull Height</i> .....	10-2
<i>Part 3. Gradient Measurement</i> .....	10-4
Measurement Methodology.....	10-4
Handheld Eye Level.....	10-5
Water Level Method.....	10-5
SECTION B. SUBSTRATE CHARACTERIZATION DATA ANALYSIS.....	10-6
<i>Substrate Characterization Quality Assurance/Quality Control</i> .....	10-7
<b>CHAPTER 11. AMBIENT WATER QUALITY NETWORK PROTOCOLS.....</b>	<b>11-1</b>

OVERVIEW..... 11-1

SECTION A. METHODS AND PROCEDURES..... 11-2

*Part 1. Ambient Water Quality Network Water Parameters* ..... 11-3

*Part 2. Water Sampling Techniques* ..... 11-5

        Direct Dip/Grab Method ..... 11-5

        Bridge Crane Method..... 11-5

            Van Dorn Horizontal Sampler ..... 11-6

            Stainless Steel Bucket ..... 11-6

            Fecal Coliform Bacteria Sampler..... 11-7

            Bridge Sampling Safety ..... 11-7

*Part 3. Water Sample Preservation/Filtering & Handling* ..... 11-7

*Part 4. Measuring Stream Flow*..... 11-8

        Small Streams..... 11-8

        Large Streams ..... 11-8

SECTION B. DATA REVIEW & HANDLING ..... 11-10

SECTION C. AMBIENT SAMPLING STATION DESCRIPTIONS ..... 11-11

*BST-(0.15) Tug Fork River* ..... 11-11

*O-2-(8.8) Twelvepole Creek* ..... 11-12

*OG-(2.8) Guyandotte River*..... 11-13

*OG-(74.1) Guyandotte River*..... 11-14

*K-(31.7) Kanawha River*..... 11-15

*K-(74.1) Kanawha River*..... 11-16

*KC-(11.6) Coal River*..... 11-17

*KE-(4.3) Elk River* ..... 11-18

*KG-(8.25) Gauley River* ..... 11-18

*KN-(1.2) New River* ..... 11-20

*KN-(67.4) New River*..... 11-21

*KNG-(1.6) Greenbrier River*..... 11-22

*KN-(96.2) New River*..... 11-23

*LK-(28.9) Little Kanawha River* ..... 11-24

*LKH-(1.5) Hughes River*..... 11-25

*OMI-(12.3) Middle Island Creek* ..... 11-26

*M-(99.4) Monongahela River*..... 11-27

*M-1-(20.6) Dunkard Creek*..... 11-28

*MT-(6.2) Tygart Valley River* ..... 11-29

*MW-(12.0) West Fork River*..... 11-30

*MC-(3.5) Cheat River* ..... 11-31

*MC-(30.0) Cheat River* ..... 11-32

*P-4-(2.2) Opequon Creek* ..... 11-33

*PC-(6.1) Cacapon River*..... 11-34

*PSB-(13.4) South Branch Potomac River* ..... 11-34

*S-(0.9) Shenandoah River* ..... 11-36

AMBIENT WATER QUALITY NETWORK QUALITY ASSURANCE/QUALITY CONTROL ..... 11-37

**CHAPTER 12. LAKE SAMPLING PROTOCOL ..... 12-1**

    OVERVIEW OF LAKE MONITORING PROGRAM..... 12-1

    INSTRUCTIONS FOR ASSESSING THE LAKE SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE LAKE ASSESSMENT FORMS) ..... 12-1

    SECTION A. SETTING UP THE SITE ..... 12-1

*Accessing the Lake* ..... 12-2

            Target Lakes ..... 12-3

            Locating the X-Site or Index Site on the Lake..... 12-5

            Initial Visit..... 12-5

Revisits.....	12-5
Duplicate Sites .....	12-6
SECTION B. SITE DOCUMENTATION .....	12-7
<i>Part 1. Lake Coordinates and Global Positioning Systems (GPS)</i> .....	12-7
<i>Part 2. Lake Photographic Documentation</i> .....	12-7
SECTION C. GUIDELINES FOR COMPLETING THE LAKE ASSESSMENT FORMS .....	12-7
<i>Description of Lake Water Quality Assessment Form</i> .....	12-8
<i>Front Side of All Pages</i> .....	12-8
PAGE 1 .....	12-8
Lake Site Verification.....	12-8
PAGE 2 .....	12-11
Site Activities and Disturbances (Including Roads) .....	12-11
PAGE 3 .....	12-13
Field Water Quality Measures .....	12-13
Lake Info.....	12-15
PAGE 4 .....	12-16
Landowner/Stakeholder Information .....	12-16
Photography Log .....	12-16
PAGES 5 & 6 .....	12-16
Sonde Lake Profile Readings .....	12-16
SECTION D. WATER COLLECTION PROTOCOLS.....	12-17
<i>Part 1. Depth Classification</i> .....	12-17
Lake Depth .....	12-17
Secchi Depth .....	12-18
<i>Part 2. Sonde Procedures</i> .....	12-18
<i>Part 3. Water Quality Sample Collection and Preservation</i> .....	12-20
Lake Water Quality Parameters .....	12-20
Materials and Reagents .....	12-20
Safety Precautions .....	12-21
Procedures for Collecting Water Quality Samples .....	12-21
Van Dorn Sampler Method for Depth Profiles.....	12-21
Direct Dip/Grab Method (Fecal coliform Sample) .....	12-22
Sample Preservation (Fixation, Filtration, & Holding) .....	12-23
Filtration .....	12-23
Protocols for Sample Filtration with Peristaltic Pump/Drill Apparatus (Dissolved Metals and Nutrients) .....	12-23
Protocols for Sample Filtration with Hand Pump Apparatus (Chlorophyll-a and Water column algae) .....	12-23
Fixation .....	12-25
Holding.....	12-25
Documentation.....	12-25
LAKE SAMPLING QUALITY ASSURANCE/QUALITY CONTROL .....	12-25
<b>CHAPTER 13. CONTINUOUS WATER QUALITY MONITORING PROCEDURES .....</b>	<b>13-1</b>
OVERVIEW OF CONTINUOUS WATER QUALITY MONITORING PROGRAM.....	13-1
INSTRUCTIONS FOR ESTABLISHING/MAINTAINING THE DEPLOYABLE SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE FORMS) .....	13-1
METHODS AND PROCEDURES .....	13-2
SECTION A. ESTABLISHING A SITE .....	13-2
<i>Part 1. Accessing Site</i> .....	13-2
<i>Part 2. Locating Established X-Site</i> .....	13-2
<i>Part 3. Equipment</i> .....	13-4
SECTION B. SITE DOCUMENTATION .....	13-5
<i>Part 1. Deployable Coordinates and Global Positioning Systems (GPS)</i> .....	13-5
<i>Part 2. Deployable Photographic Documentation</i> .....	13-5
SECTION C. FORM COMPLETION .....	13-5
<i>Description of Deployable Sonde Form</i> .....	13-6

*PAGE 1* ..... 13-6  
 Deployable Site Verification ..... 13-6

*PAGE 2* ..... 13-7  
 Discrete Sonde Info ..... 13-7  
 Deployment Sonde Info ..... 13-8

SECTION D. DEPLOYABLE SONDE OPERATION (CALIBRATION, PROGRAMING, AND DOWNLOADING PROTOCOLS)..... 13-9

*Part 1. Sonde Calibration Procedures*..... 13-9  
 YSI Deployables ..... 13-9  
 Hydrolab Deployables ..... 13-10  
 Eureka Manta Deployables ..... 13-11  
 HOBO - Temperature/Light Intensity & Temperature/Conductivity Loggers..... 13-13

*Part 2. Sonde Programming Procedures* ..... 13-14  
 General ..... 13-14  
 YSI Deployables ..... 13-14  
 Hydrolab Deployables ..... 13-15  
 Eureka Manta Deployables ..... 13-17  
 HOBO Temperature/Conductivity & Temperature/Light Intensity Loggers ..... 13-18

*Part 3. Sonde Downloading Procedures* ..... 13-19  
 General ..... 13-19  
 YSI Deployables ..... 13-19  
 Hydrolab Deployables ..... 13-20  
 Eureka Manta Deployables ..... 13-20  
 HOBO Conductivity Loggers ..... 13-21

SECTION E. SONDE ANCHORING AND CONCEALMENT PROCEDURES ..... 13-21

*Part 1. Anchor Techniques*..... 13-22  
 Rootwads ..... 13-24  
 Instream Boulder ..... 13-25  
 Large Stream Debris ..... 13-26  
 Anchor Maintenance..... 13-26

*Part 2. Concealment*..... 13-27

SECTION F. WATER QUALITY SAMPLING PROCEDURES ..... 13-31  
*Side-By-Side Measurement* ..... 13-31

SECTION G. SPECIAL CONCERNS..... 13-31

*Part 1. Sediment*..... 13-31  
*Part 2. Heavily Populated Sites* ..... 13-32  
*Part 3. Missing Sondes* ..... 13-32  
*Part 4. Low Conductivity* ..... 13-32  
*Part 5. Large Rivers* ..... 13-33

SECTION H. TROUBLESHOOTING AND MANUFACTURERS..... 13-34

*Part 1. Troubleshooting by Brand* ..... 13-34  
 YSI ..... 13-34  
 Hydrolab..... 13-36  
 Eureka ..... 13-38

*Part 2. Service Contacts*..... 13-39

SECTION I. CONTINUOUS MONITORING DATA ANALYSIS..... 13-39  
 CONTINUOUS MONITORING QUALITY ASSURANCE/QUALITY CONTROL ..... 13-39

**CHAPTER 14. MISCELLANEOUS SAMPLING..... 14-1**

SECTION A. BLANKS AND DUPLICATES ..... 14-1  
*Overview* ..... 14-1  
*Part 1. Field Blanks*..... 14-1  
 Obtaining the Field Blank Water ..... 14-1  
 Field Blank Field Procedures ..... 14-3  
*Part 2. Duplicate Samples (Blind Duplicates)* ..... 14-3  
 Wadeable Benthic Sites (Random, Targeted, and TMDL Bio) ..... 14-3



TMDL (Water Quality and Limited Habitat) ..... 14-4  
*Blanks and Duplicates Quality Assurance/Quality Control* ..... 14-4  
 SECTION B. SOURCE SAMPLING PROCEDURES FOR TMDL MONITORING ..... 14-5  
     *Part 1. Source Sampling Overview* ..... 14-5  
     *Part 2. AMD/AML*..... 14-5  
     *Part 3. Permitted Sources*..... 14-7  
     *Part 4. Other Sources* ..... 14-7  
**CHAPTER 15. FIELD EQUIPMENT CHECKLIST..... 15-1**  
**CHAPTER 16. QUICK REFERENCE GUIDE ..... 16-1**  
**CHAPTER 17. AQUATIC NUISANCE SPECIES (ANS) AND DISEASE CONTROL PROTOCOLS ..... 17-1**  
 OVERVIEW..... 17-1  
 SECTION A. REQUIRED DISINFECTANT TASKS OF ALL EQUIPMENT ..... 17-1  
     *Part 1. All Boats including Electrofishing Boats*..... 17-1  
     *Part 2. Field Sampling and Laboratory Equipment*..... 17-2  
     *Part 3. Fish or Other Aquatic Species Stocking Equipment* ..... 17-2  
     *Part 4. Other Equipment* ..... 17-2  
     *Part 5. Record Keeping*..... 17-2  
 SECTION B. WATER BODIES OF SPECIAL INTEREST ..... 17-2  
 SECTION C. SELECTION OF SURFACE DISINFECTANTS ..... 17-3  
     *Precautions*..... 17-3  
     *Glossary of Biosecurity Terminology* ..... 17-3  
     *Selected Surface Disinfectants* ..... 17-4  
         Potassium peroxymonosulfate – Virkon S® ..... 17-4  
         Sodium hypochlorite (household bleach, 6% of sodium hypochlorite) ..... 17-4  
         Alcohol (ethanol and isopropyl 70-95%) ..... 17-5  
         Quaternary ammoniums with bis-n-tributyltin oxide - Roccal®- D Plus ..... 17-5  
         Chlorhexidine diacetate - Nolvasan®-S ..... 17-5  
         Quaternary ammonium chloride - Spectrasol® ..... 17-6  
     *Suggested Disinfectant Concentrations* ..... 17-6  
**CHAPTER 18. FREQUENT TERMINOLOGY ..... 18-1**  
 CLEAN WATER ACT BASIC TERMS ..... 18-1  
 OIL/GAS RELATED TERMS ..... 18-3  
 MINING RELATED TERMS ..... 18-6  
     *Types of Mining*..... 18-7  
     *Coal Mining Structure and Waste Terms* ..... 18-8  
     *Coal Processing Terms*..... 18-8  
     *Coal Combustion Waste Terms* ..... 18-9  
 WATER TREATMENT RELATED TERMS ..... 18-10  
**CHAPTER 19. DOCUMENT REVIEW SIGNATURES..... 19-1**

## List of Figures

FIGURE 1-1. EXAMPLE OF WV ROTATING WATERSHED BASIN SCHEDULE. ....	1-1
FIGURE 1-2. WAB DATA FLOW .....	1-8
FIGURE 2-1. AN EXAMPLE OF SLIDING THE REACH TO AVOID LARGER/SMALL CONFLUENCES, LAKES, PONDS, ETC. (USED FOR RANDOM SITES ONLY). ....	2-10
FIGURE 2-2. EXAMPLE OF GPS BEING USED TO LOCATE AND DOCUMENT THE X-SITE. ....	2-20
FIGURE 2-3. EXAMPLE OF IDENTIFICATION FIELDS ON FRONT TOP OF EACH FIELD FORM.....	2-29
FIGURE 2-4. EXAMPLE OF THE SITE VERIFICATION SECTION (PAGE 1) OF THE FIELD FORM.....	2-31
FIGURE 2-5. EXAMPLE OF THE SITE ACTIVITIES & DISTURBANCES SECTION (PAGE 2) OF THE FIELD FORM.....	2-38
FIGURE 2-6. EXAMPLE OF A POINT SOURCE DISCHARGE .....	2-40
FIGURE 2-7. EXAMPLE OF LIVESTOCK ACCESS TO A STREAM. ....	2-42
FIGURE 2-8. EXAMPLE OF THE PHYSICAL CHARACTERIZATION SECTION (TOP OF PAGE 3) OF THE FIELD FORM .....	2-43
FIGURE 2-9. EXAMPLE OF THE SEDIMENT CHARACTERIZATION SECTION (MIDDLE OF PAGE 3) OF THE FIELD FORM.....	2-44
FIGURE 2-10. EXAMPLE OF MANGANESE SHEEN THAT RESEMBLES A TRUE OIL SHEEN IN ITS RAINBOW COLORATION. ....	2-45
FIGURE 2-11. EXAMPLE OF A LAYER OF MARL UNDER A LAYER OF SOIL IN A STREAM BANK. ....	2-47
FIGURE 2-12. EXAMPLE OF RED DOG AND COAL CHUNK SEDIMENT DEPOSITS. ....	2-47
FIGURE 2-13. LEFT: AN IRON COATED ROCK ON LEFT AND A PIECE OF RED DOG ON RIGHT. RIGHT: A CROSS SECTION OF THE SAME ROCKS (IRON COATED ROCK ON LEFT AND A PIECE OF RED DOG ON RIGHT). ....	2-48
FIGURE 2-14. EXAMPLES OF STREAMS COATED WITH IRON HYDROXIDES. ....	2-48
FIGURE 2-15. EXAMPLE OF ROCKS STAINED BLACK WITH MANGANESE HYDROXIDES. ....	2-49
FIGURE 2-16. EXAMPLES OF MANGANESE HYDROXIDES RUBBING OFF OF A PIECE OF SUBSTRATE.....	2-49
FIGURE 2-17. EXAMPLE OF THE SUBSTRATE PARTICLE LAYER PROFILE SECTION (MIDDLE OF PAGE 3) OF THE FIELD FORM.....	2-50
FIGURE 2-18. EXAMPLE OF THE DOMINANT SUBSTRATE TYPE AND REACH CHARACTERIZATION SECTION (BOTTOM OF PAGE 3) OF THE FIELD FORM.....	2-51
FIGURE 2-19. AN EXAMPLE OF HEAVY TO EXTREME IRON (ORANGE/YELLOW/RED) METAL HYDROXIDES COATING THE SUBSTRATE... ..	2-52
FIGURE 2-20. EXAMPLE OF SURFACE DEBRIS SECTION (TMDL AND AMBIENT NETWORK FORMS) .....	2-53
FIGURE 2-21. EXAMPLE OF THE FIELD WATER QUALITY MEASURES SECTION (TOP OF PAGE 4) OF THE FIELD FORM.....	2-54
FIGURE 2-22. EXAMPLE OF FOAM. ....	2-56
FIGURE 2-23. EXAMPLE OF A HIGHLY TURBID STREAM WITH A BROWNISH COLOR. ....	2-57
FIGURE 2-24. EXAMPLE OF A MODERATELY TURBID STREAM. NOTE THE PRESENCE OF FeOH ON THE ROCKS AND MILKY WHITE COLOR OF THE WATER CAUSED BY ALOH. ....	2-58
FIGURE 2-25. EXAMPLE OF FLOW INFORMATION SECTION (TMDL AND AMBIENT NETWORK FORMS).....	2-59
FIGURE 2-26. EXAMPLE OF THE STREAM BANK/RIPARIAN BUFFER ZONE VEGETATION/COVER TYPE SECTION (BOTTOM OF PAGE 4) OF THE FIELD FORM.....	2-61
FIGURE 2-27. COVER OF USEPA'S RAPID BIOASSESSMENT PROTOCOLS FOR USE IN WADEABLE STREAMS AND RIVERS (SECOND EDITION) .....	2-67
FIGURE 2-28. EXAMPLE OF THE RIFFLE/RUN RAPID HABITAT ASSESSMENT PART 1 (PAGE 5) OF THE FIELD FORM.....	2-69
FIGURE 2-29. EXAMPLES OF GOOD FISH HABITAT. LEFT SHOWS TREE ROOTS HANGING OVER THE BANK THAT PROVIDE GOOD COVER FOR FISH IN THE STREAM. RIGHT SHOWS A POOL WITH SUBMERGED LOGS AND OVER-HANGING BRANCHES.....	2-70
FIGURE 2-30. VISUALIZATION OF EMBEDDEDNESS. ....	2-71
FIGURE 2-31. EXAMPLE OF COBBLE THAT IS PARTIALLY EMBEDDED. ....	2-71
FIGURE 2-32. EXAMPLES OF VARYING DEGREES OF CHANNEL ALTERATION (CONCRETE CHANNEL ON LEFT AND RIP/RAP CHANNEL ON RIGHT). ....	2-72
FIGURE 2-33. EXAMPLE OF CHANNEL ALTERATION ACTIVELY OCCURRING DUE TO AN INSTALLATION OF SEWAGE PIPES ACROSS A STREAM CHANNEL. ....	2-73
FIGURE 2-34. EXAMPLES OF SEDIMENT DEPOSITION. ....	2-74
FIGURE 2-35. EXAMPLE OF SEDIMENT DEPOSITION. NOTE THE LARGE AMOUNT OF SAND ON THE BANK. ....	2-74
FIGURE 2-36. EXAMPLE OF THE RIFFLE/RUN RAPID HABITAT ASSESSMENT PART 2 (PAGE 6) OF THE FIELD FORM.....	2-75
FIGURE 2-37. EXAMPLES OF STREAMS WITH POOR CHANNEL FLOW STATUS.....	2-76
FIGURE 2-38. EROSION IS SLIGHT TO MODERATE BECAUSE ROOTS ARE HOLDING THE BANK INTACT.....	2-77

FIGURE 2-39. EXAMPLE OF AN UNSTABLE BANK. NOTE THE LACK OF TREES (AND ROOTS) TO PROVIDE SOIL STABILIZATION. ....	2-77
FIGURE 2-40. EXAMPLE OF HEAVY EROSION (PHOTO RIGHT). ....	2-78
FIGURE 2-41. EXAMPLES OF POOR BANK VEGETATIVE PROTECTION. ....	2-79
FIGURE 2-42. EXAMPLES OF GOOD WIDTH OF UNDISTURBED VEGETATION ZONE ON LEFT AND POOR ON THE RIGHT. NOTICE THE RIP-RAP IN THE LEFT PHOTO THAT WOULD INCREASE THE BANK STABILITY SCORE BUT DECREASE THE CHANNEL ALTERATION SCORE. ....	2-79
FIGURE 2-43. EXAMPLE OF THE GLIDE/POOL RAPID HABITAT ASSESSMENT PART 1 (PAGE 5A) OF THE FIELD FORM ....	2-81
FIGURE 2-44. EXAMPLE OF THE GLIDE/POOL RAPID HABITAT ASSESSMENT PART 1 (PAGE 6A) OF THE FIELD FORM ....	2-83
FIGURE 2-45. EXAMPLE OF THE NON-RBP PARAMETER SECTION (TOP OF PAGE 7) OF THE FIELD FORM.....	2-84
FIGURE 2-46. EXAMPLE OF THE PRS AND STRESSOR INFO SECTION (MIDDLE OF PAGE 7) OF THE FIELD FORM .....	2-86
FIGURE 2-47. EXAMPLE OF THE EXTRA SPACE FOR COMMENTS AND NOTES SECTION (BOTTOM OF PAGE 7) OF THE FIELD FORM .....	2-87
FIGURE 2-48. EXAMPLE OF THE WILDLIFE & FRESHWATER MUSSEL OBSERVATIONS SECTION (TOP OF PAGE 8) OF THE FIELD FORM. ....	2-88
FIGURE 2-49. EXAMPLE OF THE TROUT OBSERVATIONS (NON-FISH SITES) SECTION (BOTTOM OF PAGE 8) OF THE FIELD FORM .....	2-89
FIGURE 2-50. EXAMPLE OF THE BENTHIC MACROINVERTEBRATE COLLECTION INFORMATION SECTION (TOP OF PAGE 9) OF THE FIELD FORM .....	2-91
FIGURE 2-51. EXAMPLE OF THE BENTHIC SUBSTRATE SAMPLE COMPOSITION SECTION (MIDDLE OF PAGE 9) OF THE FIELD FORM ....	2-93
FIGURE 2-52. EXAMPLE OF THE VISUAL ESTIMATION OF PERIPHYTON & AQUATIC PLANT DENSITY SECTION (MIDDLE OF PAGE 9) OF THE FIELD FORM.....	2-93
FIGURE 2-53. EXAMPLE OF HEAVY PERIPHYTON COATING SUBSTRATE.....	2-95
FIGURE 2-54. EXAMPLE OF PERIPHYTON (DIATOMS) ON ROCKS REMOVED FROM THE WATER COLUMN FOR SAMPLING (DARK CIRCLES ARE WHERE THE PERIPHYTON HAS BEEN SAMPLED). ....	2-95
FIGURE 2-55. EXAMPLE OF PERIPHYTON GROWING ON ROCK. ....	2-96
FIGURE 2-56. UNDERWATER VIEW OF PERIPHYTON GROWING ON SUBSTRATE. NOTE THAT SOME OF MATERIAL IS SILT THAT IS TRAPPED BY THE PERIPHYTON. ....	2-96
FIGURE 2-57. FERRAPHYLIC (MEANING IRON-LOVING) FILAMENTOUS ALGAE ARE FOUND IN LOW PH STREAMS WITH HIGH IRON CONCENTRATIONS. ....	2-97
FIGURE 2-58. CLOSE-UP OF BLUE-GREEN ALGAE GROWING ON ROCK. NOTICE THE ABSENCE OF LONG FILAMENTS.....	2-97
FIGURE 2-59. EXAMPLE OF BLUE-GREEN ALGAE (EDGES) AND PERIPHYTON GROWING ON ROCK.....	2-98
FIGURE 2-60. UNDERWATER VIEW OF SUBMERGED AQUATIC VEGETATION. NOTE THE FILAMENTOUS ALGAE GROWING AMONG THE SUBMERGED VEGETATION. ....	2-99
FIGURE 2-61. EXAMPLE OF SUBMERGED (DARK GREEN IS A <i>HYDRILLA SP.</i> ) AND FLOATING (LIGHT GREEN IS DUCKWEED) AQUATIC VEGETATION. ....	2-99
FIGURE 2-62. EXAMPLE OF EMERGENT AQUATIC PLANTS (WATER WILLOW) GROWING IN BACKWATER AND GRAVEL BARS. ....	2-100
FIGURE 2-63. EXAMPLE OF EMERGENT AQUATIC PLANTS (CATTAILS) ADJACENT TO THE STREAM CHANNEL. ....	2-101
FIGURE 2-64. EMERGENT AQUATIC PLANTS (CATTAILS) SURROUNDING A MINING DISCHARGE.....	2-101
FIGURE 2-65. EXAMPLES OF FLOATING AQUATIC PLANTS ( <i>I.E.</i> , LILLY PADS) AND EMERGENT AQUATIC PLANTS ( <i>I.E.</i> , CATTAILS) ON A LAKE. .....	2-102
FIGURE 2-66. EXAMPLE OF THE PERIPHYTON COLLECTION INFORMATION SECTION (BOTTOM OF PAGE 9) OF THE FIELD FORM .....	2-103
FIGURE 2-67. EXAMPLE OF THE VISUAL GUIDE TO STREAM MACROINVERTEBRATES SECTION (PAGE 10) OF THE FIELD FORM .....	2-104
FIGURE 2-68. EXAMPLE OF THE VISUAL GUIDE TO STREAM MACROINVERTEBRATES SECTION (PAGE 11) OF THE FIELD FORM .....	2-105
FIGURE 2-69. EXAMPLE OF THE LANDOWNER/STAKEHOLDER INFORMATION SECTION (TOP OF PAGE 12) OF THE FIELD FORM.....	2-106
FIGURE 2-70. EXAMPLE OF THE RECON/ACCESSIBILITY INFORMATION SECTION (TOP OF PAGE 12) OF THE FIELD FORM .....	2-107
FIGURE 2-71. EXAMPLE OF THE PHOTOGRAPHY LOG SECTION (BOTTOM OF PAGE 12) OF THE FIELD FORM.....	2-108
FIGURE 2-72. EXAMPLE OF THE FLOW MEASUREMENTS APPENDIX PAGE 1 FIELD FORM .....	2-111
FIGURE 2-73. EXAMPLE OF THE STREAM BANK ERODIBILITY APPENDIX FIELD FORM.....	2-113
FIGURE 2-74. EXAMPLE OF THE CHANNEL PROFILE MEASUREMENTS APPENDIX FIELD FORM .....	2-116
FIGURE 2-75. EXAMPLE OF THE TMDL-WADEABLE BENTHIC APPENDIX FIELD FORM .....	2-119
FIGURE 2-76. EXAMPLE OF THE WQ PROFILE APPENDIX FIELD FORM.....	2-120
FIGURE 2-77. EXAMPLE OF THE SUBSTRATE CHARACTERIZATION (PEBBLE COUNT) INCLUDING GRADIENT APPENDIX FIELD FORM ...	2-123
FIGURE 3-1. EXAMPLE OF A YSI 660 XL SONDE (RIGHT) AND A 650 MDS DISPLAY UNIT (LEFT). ....	3-1
FIGURE 3-2. EXAMPLE OF SONDE CALIBRATION LOG SHEET (PAGE 1) .....	3-4
FIGURE 3-3. EXAMPLE OF SONDE CALIBRATION LOG SHEET (PAGE 2).....	3-5
FIGURE 3-4. TEMPERATURE/PH CURVE FOR PH 7 BUFFER SOLUTION .....	3-19

FIGURE 3-5. TEMPERATURE/PH CURVE FOR PH 10 BUFFER SOLUTION .....	3-20
FIGURE 3-6. TEMPERATURE/PH CURVE FOR PH 4 BUFFER SOLUTION .....	3-21
FIGURE 3-7. THE NERNST EQUATION .....	3-25
FIGURE 3-8. EXAMPLE OF SAMPLE CONTAINERS LABELED WITH SAMPLE INFORMATION.....	3-35
FIGURE 3-9. EXAMPLE OF A FECAL SAMPLE BEING TAKEN FROM A STREAM.....	3-36
FIGURE 3-10. EXAMPLE OF CUBITAINER BEING FILLED AND EMPTIED (I.E., RINSED WITH STREAM WATER). .....	3-37
FIGURE 3-11. EXAMPLE OF PERISTALTIC PUMP/DRILL APPARATUS FOR SAMPLE FILTRATION BEING USED UNDER THE TAILGATE OF A VEHICLE.....	3-39
FIGURE 3-12. EXAMPLE OF SAMPLE BEING PRESERVED WITH NITRIC ACID.....	3-45
FIGURE 3-13. EXAMPLE OF A FULLY COMPLETED ANALYSIS REQUEST FORM WITH CHAIN-OF-CUSTODY (COC) AT BOTTOM .....	3-48
FIGURE 4-1. CROSS SECTION OF STREAM CHANNEL.....	4-2
FIGURE 4-2. COVER OF MODEL 2000 MARSH-McBIRNEY FLO-MATE INSTRUCTION MANUAL.....	4-4
FIGURE 4-3. KEY FUNCTION DESCRIPTIONS FOR THE MODEL 2000 MARSH-McBIRNEY FLO-MATE.....	4-6
FIGURE 4-4. A) TOP-SETTING WADING ROD AND B) CLOSE-UP OF HANDLE OR TOP-SETTING WADING ROD.....	4-14
FIGURE 4-5. EXAMPLE OF TAKING FLOW MEASUREMENTS AT A TRANSECT. ....	4-15
FIGURE 5-1. DIAGRAM COMPARING THE DIMENSIONS AND NUMBER OF KICKS NECESSARY TO SAMPLE 1 M <sup>2</sup> OF A RECTANGULAR FRAME DIP NET VERSUS A D-FRAME DIP NET.....	5-6
FIGURE 5-2. PHOTO OF MATERIALS USED IN BENTHIC MACROINVERTEBRATE SAMPLING.....	5-6
FIGURE 5-3. PHOTO OF RECTANGULAR FRAME DIP NET BEING PLACED ON STREAM BOTTOM .....	5-7
FIGURE 5-4. PHOTOGRAPH OF THE BRUSHING PROCESS IN FRONT OF THE NET. ....	5-8
FIGURE 5-5. PHOTOGRAPH OF THE KICKING PROCESS IN FRONT OF THE NET. ....	5-8
FIGURE 5-6. PHOTOGRAPHS SHOWING THE REMOVAL OF THE NET FROM THE WATER WITH AN UPSTREAM MOTION. ....	5-9
FIGURE 5-7. PHOTOGRAPH OF EMPTYING THE NET INTO A 5- GALLON BUCKET PARTIALLY FILLED WITH STREAM WATER. ....	5-9
FIGURE 5-8. PHOTOGRAPH OF BIOLOGIST INSPECTING BENTHIC SAMPLE AND REMOVING ROUGH MATERIAL (ROCKS, STICKS, AND LEAVES) .....	5-10
FIGURE 5-9. PHOTOGRAPH OF SOFT, ORGANIC MATERIAL PLACED AND STORED IN A TEMPORARY CONTAINER.....	5-10
FIGURE 5-10. PHOTOGRAPH OF BIOLOGIST TRANSFERRING THE HARD, INORGANIC MATERIAL (E.G., FINE GRAVEL, SAND, AND SILT) TO A SAMPLE JAR ½ FILLED WITH ALCOHOL. ....	5-11
FIGURE 5-11. PHOTOGRAPH OF BIOLOGIST INSPECTING TRANSFERRING THE SOFT, ORGANIC MATERIAL (E.G., SHREDDED LEAVES AND BENTHIC ORGANISMS) TO THE SAMPLE JAR. ....	5-11
FIGURE 5-12. PHOTOGRAPH OF A HOME-MADE GRIDDED SORTING TRAY FEATURING A RANDOM NUMBER MATRIX ON THE BOTTOM.....	5-16
FIGURE 5-13. PHOTOGRAPH OF A GRIDDED SORTING TRAY WITH SAMPLE CONTENTS EVENLY DISTRIBUTED IN WATER. ....	5-18
FIGURE 5-14. PHOTOGRAPH OF A GRIDDED SORTING TRAY WITH 5 GRIDS RANDOMLY REMOVED.....	5-19
FIGURE 5-15. PHOTOGRAPH OF BIOLOGIST SORTING A BENTHIC SAMPLE UNDER AN ILLUMINATED MAGNIFIER. NOTE THE ENAMEL PAN FILLED WITH SOME WATER AND THE TEMPORARY SAMPLE CONTAINER.....	5-19
FIGURE 5-16. EXAMPLE OF A BENTHIC MACROINVERTEBRATE LAB SHEET.....	5-32
FIGURE 5-17. BENTHIC COMPARABILITY FLOW CHART.....	5-45
FIGURE 6-1. TWO BACKPACK ELECTROFISHERS (SMITH-ROOT MODELS LR-24 AND LR-20B) USED BY WAB PERSONNEL. ....	6-7
FIGURE 6-2. COMPONENTS OF A BACKPACK ELECTROFISHING SYSTEM. ....	6-8
FIGURE 6-3. AN ELECTROFISHING CREW CONSISTING OF TWO BACKPACK SHOCKERS (ELECTROFISHERS) AND THREE NETTERS. ....	6-9
FIGURE 6-4. TECHNIQUE FOR SAMPLING A DEEP, NARROW STREAM WITH TWO BACKPACKS, LOOKING UPSTREAM. ....	6-10
FIGURE 6-5. TOW BARGE WITH GENERATOR, LIVE WELL, AND SHOCKING WAND. ....	6-11
FIGURE 6-6. REACH TYPE REQUIRING BARGE ELECTROFISHER. NOTE THE LARGE WIDTH AND LACK OF CONSTRAINING FEATURES OR HABITAT. ....	6-11
FIGURE 6-7. PROPER TECHNIQUE FOR SAMPLING A STREAM WITH MULTIPLE CHANNELS.....	6-12
FIGURE 6-8. EXAMPLE OF FISH COLLECTION FORM .....	6-18
FIGURE 7-1. MICROSPATULA BEING USED TO SCRAPE ALGAE AND PERIPHYTON FROM UPPER SURFACE OF ROCK.....	7-3
FIGURE 7-2. TOOTHBRUSH BEING USED TO SCRUB REMAINING ALGAE AND PERIPHYTON FROM UPPER SURFACE OF ROCK.....	7-3
FIGURE 7-3. LOOSENED ALGAE AND PERIPHYTON BEING RINSED INTO THE SAMPLE JAR. ....	7-3
FIGURE 8-1. A FILAMENTOUS ALGAE BLOOM ON THE TYGART RIVER UPSTREAM OF NORTON, JUNE 2012. ....	8-1
FIGURE 8-2. HYPOTHETICAL EXAMPLE OF STREAM SEGMENTS ACROSS A FILAMENTOUS ALGAE TRANSECT .....	8-5
FIGURE 8-3. EXAMPLE OF ADDITIONAL TRANSECT LDB AND RDB COORDINATE BOXES ON PAGE 1 .....	8-6



FIGURE 8-4. EXAMPLE OF THE ADDITIONAL CANOPY DENSITY (DENSIOMETER) READINGS SECTION ON PAGE 2 ..... 8-6

FIGURE 8-5. EXAMPLE OF THE EXPANDED RECON/ACCESSIBILITY SECTION ON PAGE 4 ..... 8-6

FIGURE 8-6. EXAMPLE OF PAGE 3 OF THE FILAMENTOUS ALGAE MONITORING FORM ..... 8-7

FIGURE 8-7. 3% FILAMENTOUS ALGAE COVER ..... 8-9

FIGURE 8-8. 4 % FILAMENTOUS ALGAE COVER ..... 8-10

FIGURE 8-9. 12% FILAMENTOUS ALGAE COVER ..... 8-10

FIGURE 8-10. 15% FILAMENTOUS ALGAE COVER ..... 8-11

FIGURE 8-11. 20% FILAMENTOUS ALGAE COVER (SHORT STRANDS)..... 8-11

FIGURE 8-12. 20% FILAMENTOUS ALGAE COVER (LONG STRANDS) ..... 8-12

FIGURE 8-13. 26% FILAMENTOUS ALGAE COVER ..... 8-12

FIGURE 8-14. 35% FILAMENTOUS ALGAE COVER (SHORT STRANDS). NOTE THAT THIS IS THE SAME LOCATION AS FIGURE 8-15. .... 8-13

FIGURE 8-15. 38% FILAMENTOUS ALGAE COVER (LONG STRANDS). NOTE THAT THIS IS THE SAME LOCATION AS FIGURE 8-14. .... 8-13

FIGURE 8-16. 39% FILAMENTOUS ALGAE COVER ..... 8-14

FIGURE 8-17. 47% FILAMENTOUS ALGAE COVER ..... 8-14

FIGURE 8-18. 65% FILAMENTOUS ALGAE COVER ..... 8-15

FIGURE 8-19. 98% FILAMENTOUS ALGAE COVER ..... 8-15

FIGURE 8-20. EXAMPLES OF MODIFIED CONVEX SPHERICAL CANOPY DENSIMETER (FROM MULVEY ET AL. 1992) ..... 8-17

FIGURE 9-1. PHOTO OF THE MATERIALS USED TO FILTER A GOLDEN ALGAE SAMPLE. .... 9-1

FIGURE 9-2. PHOTO OF A GOLDEN ALGAE LABEL..... 9-2

FIGURE 9-3. PHOTO OF THE INSERTION OF RUBBER STOPPER INTO FLASK WITH PLASTIC FUNNEL ALREADY INSERTED THROUGH RUBBER STOPPER..... 9-3

FIGURE 9-4. PHOTO OF CUP BEING REMOVED TO ACCESS THE PREPACKAGED FILTER FOR REMOVAL ..... 9-4

FIGURE 9-5. PHOTO OF THE GF/F FILTER BEING PLACED ON TOP OF SUPPORT SCREEN WITH FORCEPS..... 9-4

FIGURE 9-6. PHOTO OF THE GRIDDED PATTERN OF GF/F FILTER. THIS SIDE PLACED DOWN WHEN FILTERING GOLDEN ALGAE SAMPLE. . 9-5

FIGURE 9-7. PHOTO OF THE POURING OF A 250 ML SAMPLE INTO FILTER CUP. .... 9-5

FIGURE 9-8. PHOTO OF MONITORING THE PUMP PRESSURE WHILE FILTERING SAMPLE – KEEP THE PRESSURE UNDER 5 PSI. .... 9-5

FIGURE 9-9. 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 ..... 9-6

FIGURE 9-10. PHOTO OF FORCEPS AND SMALL PLASTIC FUNNEL SOAKING IN 10% BLEACH SOLUTION FOR 1 MINUTE..... 9-6

FIGURE 9-11. LEFT: PHOTO OF SMALL PLASTIC FUNNEL BEING RINSED WITH DISTILLED WATER. RIGHT: PHOTO OF FORCEPS BEING RINSED WITH INDUSTRIAL GRADE DEIONIZED WATER. .... 9-7

FIGURE 9-12. EXAMPLE OF A GOLDEN ALGAE ANALYSIS REQUEST FORM WITH COC ..... 9-8

FIGURE 13. EXAMPLE OF COC WITH AMBIENT PARAMETERS SELECTED..... 11-4

FIGURE 11-2. EXAMPLE OF USGS STREAM FLOW WEBSITE ([HTTP://WATERWATCH.USGS.GOV/?M=REAL&R=WV](http://waterwatch.usgs.gov/?M=REAL&R=WV)) ..... 11-9

FIGURE 11-3. EXAMPLE OF USGS STREAM GAGE OUTPUT GRAPH ..... 11-10

FIGURE 11-4. 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). .... 11-11

FIGURE 11-5. 2003 AERIAL PHOTO OF THE O-2-(8.8) TWELVEPOLE CREEK AMBIENT SAMPLE SITE IN WAYNE CO., WV. .... 11-12

FIGURE 11-6. 2003 AERIAL PHOTO OF THE OG-(2.8) GUYANDOTTE RIVER AMBIENT SAMPLE SITE IN HUNTINGTON, WV. .... 11-13

FIGURE 11-7. 2003 AERIAL PHOTO OF THE OG-(74.1) GUYANDOTTE RIVER AMBIENT SAMPLE SITE IN PECKS MILL, WV..... 11-14

FIGURE 11-8. 2003 AERIAL PHOTO OF THE K-(31.7) KANAWHA RIVER AMBIENT SAMPLE SITE AT WINFIELD LOCKS & DAM, WV. 11-15

FIGURE 11-9. PHOTO OF THE WINFIELD LOCKS & DAM. .... 11-15

FIGURE 11-10. PHOTO OF THE WINFIELD LOCKS & DAM INTAKE SAMPLE AREA. .... 11-15

FIGURE 11-11. 2003 AERIAL PHOTO OF THE K-(74.1) KANAWHA RIVER AMBIENT SAMPLE SITE AT CHELYAN, WV. .... 11-16

FIGURE 11-12. PHOTO OF THE KANAWHA RIVER SAMPLE SITE FROM BOAT DOCK IN CHELYAN, WV. .... 11-16

FIGURE 11-13. 2003 AERIAL PHOTO OF THE KC-(11.6) COAL RIVER AMBIENT SAMPLE SITE AT TORNADO, WV..... 11-17

FIGURE 11-14. 2003 AERIAL PHOTO OF THE KE-(4.3) ELK RIVER AMBIENT SAMPLE SITE AT CHARLESTON, WV..... 11-18

FIGURE 11-15. PHOTO OF THE ELK RIVER SAMPLING SITE FROM UPSTREAM AT COONSKIN PARK. .... 11-18

FIGURE 11-16. PHOTO OF THE ELK RIVER SAMPLING SITE LOOKING UPSTREAM. .... 11-18

FIGURE 11-17. 2003 AERIAL PHOTO OF THE KG-(8.25) GAULEY RIVER AMBIENT SAMPLE SITE AT BEECH GLEN, WV..... 11-19

FIGURE 11-18. 2010 AERIAL PHOTO OF THE KN-(1.2) NEW RIVER AMBIENT SAMPLE SITE NEAR GAULEY BRIDGE, WV. NOTE THE

PREVIOUS LOCATION UPSTREAM AT THE ELKEM POWER STATION AT 1.55 .....	11-20
FIGURE 11-19. 2003 AERIAL PHOTO OF THE KN-(67.4) NEW RIVER AMBIENT SAMPLE SITE IN HINTON, WV. ....	11-21
FIGURE 11-20. 2003 AERIAL PHOTO OF THE KNG-(1.6) GREENBRIER RIVER AMBIENT SAMPLE SITE IN HINTON, WV.....	11-22
FIGURE 11-21. USGS 24K TOPOGRAPHIC MAP OF THE KN-(96.2) NEW RIVER AMBIENT SAMPLE SITE NORTH OF GLEN LYN, VA. ....	11-23
FIGURE 11-22. PHOTO FROM X-SITE LOOKING TOWARD LEFT DESCENDING BANK AT MOUTH OF SMITH BRANCH AND PARKING AREA. 11-23	
FIGURE 11-23. 2003 AERIAL PHOTO OF THE LK-(28.9) LITTLE KANAWHA RIVER AMBIENT SAMPLE SITE NEAR ELIZABETH, WV. ....	11-24
FIGURE 11-24. 2003 AERIAL PHOTO OF THE KLH-(1.5) HUGHES RIVER AMBIENT SAMPLE SITE NEAR GREENCASTLE, WV. ....	11-25
FIGURE 11-25. 2003 AERIAL PHOTO OF THE OMI-(12.3) MIDDLE ISLAND CREEK AMBIENT SAMPLE SITE IN ARVILLA, WV.....	11-26
FIGURE 11-26. 2003 AERIAL PHOTO OF THE M-(99.4) MONONGAHELA RIVER AMBIENT SAMPLE SITE IN STAR CITY, WV. ....	11-27
FIGURE 11-27. 2003 AERIAL PHOTO OF THE M-1-(20.6) DUNKARD CREEK AMBIENT SAMPLE SITE AT MASON-DIXON HISTORICAL PARK, WV.....	11-28
FIGURE 11-28. 2003 AERIAL PHOTO OF THE MT-(6.2) TYGART VALLEY RIVER AMBIENT SAMPLE SITE AT COLFAX, WV. ....	11-29
FIGURE 11-29. 2003 AERIAL PHOTO OF THE MW-(12.0) WEST FORK RIVER AMBIENT SAMPLE SITE AT ENTERPRISE, WV. ....	11-30
FIGURE 11-30. 2003 AERIAL PHOTO OF THE MC-(3.5) CHEAT RIVER AMBIENT SAMPLE SITE IN LAKE LYNN, PA.....	11-31
FIGURE 11-31. 2003 AERIAL PHOTO OF THE MC-(30.0) CHEAT RIVER AMBIENT SAMPLE SITE IN ALBRIGHT, WV.....	11-32
FIGURE 11-32. 2003 AERIAL PHOTO OF THE P-4-(3.5) OPEQUON CREEK AMBIENT SAMPLE SITE NEAR BEDINGTON, WV. ....	11-33
FIGURE 11-33. USGS 24K TOPOGRAPHIC MAP OF THE PC-(6.1) CACAPON RIVER AMBIENT SAMPLE SITE SOUTH OF GREAT CACAPON, WV.....	11-34
FIGURE 11-34. PHOTO FROM CR 7 BRIDGE AT THE PC-(6.1) CACAPON RIVER AMBIENT SAMPLE SITE SOUTH OF GREAT CACAPON, WV. ....	11-34
FIGURE 11-35. 2003 AERIAL PHOTO OF THE PSB-(13.4) SOUTH BRANCH POTOMAC RIVER AMBIENT SAMPLE SITE NEAR SPRINGFIELD, WV.....	11-35
FIGURE 11-36. 2003 AERIAL PHOTO OF THE S-(0.9) SHENANDOAH RIVER AMBIENT SAMPLE SITE NEAR HARPERS FERRY, WV. ....	11-36
FIGURE 12-1. EXAMPLE OF THE SITE VERIFICATION SECTION (PAGE 1) OF THE LAKE WATER QUALITY ASSESSMENT FORM .....	12-9
FIGURE 12-2. EXAMPLE OF THE HUMAN & BOATING ACTIVITIES (CENTER LEFT PAGE 2) OF THE LAKE ASSESSMENT FORM.....	12-12
FIGURE 12-3. EXAMPLE OF THE FIELD WATER QUALITY MEASURES (TOP OF PAGE 3) OF THE LAKE ASSESSMENT FORM .....	12-14
FIGURE 12-4. EXAMPLE OF LAKE INFO SECTION (BOTTOM OF PAGE 3) OF THE LAKE ASSESSMENT FORM .....	12-15
FIGURE 12-5. EXAMPLE OF SONDE LAKE PROFILE READINGS SECTION (PAGES 5 & 6) OF THE LAKE ASSESSMENT FORM .....	12-17
FIGURE 13-1. ITEMS REQUIRED FOR DEPLOYING THE CONTINUOUS MONITOR .....	13-4
FIGURE 13-2. EXAMPLE OF THE DEPLOYABLE VISIT TYPE ON PAGE 1 OF THE DEPLOYABLE SONDE FORM.....	13-6
FIGURE 13-3. EXAMPLE OF THE DISCRETE SONDE INFO SECTION ON THE TOP OF PAGE 2 OF THE DEPLOYABLE SONDE FORM.....	13-7
FIGURE 13-4. EXAMPLE OF THE DEPLOYMENT INFO SECTION ON THE BOTTOM OF PAGE 2 OF THE DEPLOYABLE SONDE FORM.....	13-8
FIGURE 13-5. PICTURE OF TYPICAL ANCHOR LOOP.....	13-22
FIGURE 13-6. PICTURE OF ANCHOR CABLE LOOPED DIRECTLY THRU THE PVC SONDE CASE.....	13-22
FIGURE 13-7. PICTURE OF CABLE LOOPED THRU PVC SONDE CASE AND SONDE. ....	13-22
FIGURE 13-8. PICTURE OF CABLE THREADED THRU PVC SONDE CASE AND THEN LOOPED THRU SCREW CAP. ....	13-23
FIGURE 13-9. PICTURE OF A LOOP USED AS A SEMI-PERMANENT ANCHOR INTERLOCKED WITH A TEMPORARY LOOP ATTACHED TO THE END OF THE SONDE CASE. ....	13-23
FIGURE 13-10. PICTURE OF SEMI-PERMANENT ANCHOR OF FIXED LENGTH IN FIELD.....	13-23
FIGURE 13-11. PICTURE OF FIELD DEPLOYED SONDE WITH ADEQUATE ANCHOR CABLE LENGTH TO ENSURE PROPER PLACEMENT IN STREAM WATER COLUMN. ....	13-24
FIGURE 13-12. PICTURE OF TYPICAL ROOTWAD USED FOR ANCHORING A SONDE. ARROW POINTS TO ACTUAL SONDE DEPLOYMENT LOCATION. ....	13-25
FIGURE 13-13. PICTURE OF TYPICAL INSTREAM BOULDER USED FOR ANCHORING A SONDE. ARROW POINTS TO ACTUAL SONDE DEPLOYMENT LOCATION ON THE DOWNSTREAM SIDE OF BOULDER. ....	13-25
FIGURE 13-14. PICTURES OF TYPICAL LARGE STREAM DEBRIS USED FOR ANCHORING A SONDE. LEFT PICTURE IS A LARGE PIECE OF METAL TRASH/DEBRIS (E.G., AUTOMOBILE PARTS, LARGE APPLIANCES, ETC.). RIGHT PICTURE IS A DOWNED TREE THAT IS PARTIALLY BRIDGING AND MOSTLY OUT OF THE STREAM CHANNEL. ....	13-26
FIGURE 13-15. PICTURE OF SONDE CONCEALED BY LARGE SUBSTRATE.....	13-27
FIGURE 13-16. PICTURE OF SONDE ANCHOR WIRE BEFORE CONCEALMENT WITH COBBLE.....	13-28
FIGURE 13-17. PICTURE OF SONDE ANCHOR WIRE AFTER CONCEALMENT WITH COBBLE. NOTE PLACEMENT OF THE PROBE END OF THE	

SONDE INTO THE CHANNEL ..... 13-28

FIGURE 13-18. PICTURE OF SONDE ANCHOR WIRE BEFORE CONCEALMENT WITH COBBLE ..... 13-29

FIGURE 13-19. PICTURE OF SONDE ANCHOR WIRE AFTER CONCEALMENT WITH COBBLE. NOTE PLACEMENT OF THE PROBE END OF THE  
SONDE INTO THE CHANNEL. .... 13-29

FIGURE 13-20. PICTURE OF SONDE BEFORE (LEFT) AND AFTER (RIGHT CLOSE-UP) CONCEALMENT WITH COBBLE. ARROW POINTS TO SONDE  
PLACED IN STREAM CHANNEL. .... 13-30

FIGURE 13-21. PICTURE OF SONDE CONCEALED BY COBBLE IN POOL CREATED BY ROOTWAD. .... 13-30

FIGURE 14-1. EXAMPLE OF QA BLANK FORM ..... 14-2

## List of Tables

TABLE 2-1. AN EXAMPLE OF A TYPICAL RANDOM SITE LIST .....	2-5
TABLE 2-2. TYPICAL FREQUENCY OF GPS READINGS FOR VARIOUS WATERSHED ASSESSMENT BRANCH ACTIVITIES .....	2-21
TABLE 2-3. SUBSTRATE SIZE CLASSIFICATION FOR SUBSTRATE LAYER PROFILE AND DOMINANT SUBSTRATE TYPE AND REACH CHARACTERIZATION .....	2-51
TABLE 2-4. EXAMPLES OF FLAG VALUES USED ON THE FIELD FORMS .....	2-55
TABLE 2-5. A BRIEF LIST OF INVASIVE PLANT SPECIES IN WV .....	2-65
TABLE 2-6. TOTAL RBP SCORE CATEGORIES .....	2-80
TABLE 2-7. SOME EXAMPLES OF INVASIVE FAUNA IN WV .....	2-89
TABLE 3-1. FROM APPENDIX D TABLE 2 OF THE YSI OPERATING MANUAL (PAGE 227).....	3-10
TABLE 3-2. SONDE MAINTENANCE KIT BASIC CONTENTS.....	3-28
TABLE 3-3. ROUTINE SONDE MAINTENANCE SCHEDULES FOR THE BASIC SENSORS .....	3-29
TABLE 3-4. PRESERVATION METHODS AND HOLDING TIMES .....	3-46
TABLE 3-5. CURRENT WATER QUALITY ANALYTE MDLS .....	3-51
TABLE 4-1. EXAMPLE OF FLOW MEASUREMENT FORM: RECORDING FIELD DATA.....	4-10
TABLE 4-2. RELATIONSHIP BETWEEN USGS AND WAB VELOCITY MEASUREMENT METHODS .....	4-13
TABLE 6-1. PERSONNEL AND EQUIPMENT REQUIRED TO EFFECTIVELY ELECTROFISH VARIOUS TYPES OF STREAMS. ....	6-7
TABLE 10-1. SUBSTRATE SIZE CLASSES FOR SUBSTRATE CHARACTERIZATION (PEBBLE COUNTS) .....	10-3
TABLE 11-1. THE CURRENT LIST OF AMBIENT WATER QUALITY NETWORK WATER PARAMETERS, MDLS, ANALYSIS METHODS, AND HOLDING TIMES.....	11-3
TABLE 12-1. AN EXAMPLE OF A TYPICAL LAKE SITE LIST.....	12-4
TABLE 17-1. SUGGESTED BIO-DISINFECTANT CONCENTRATIONS .....	17-6

## List of Equations

EQUATION 1. CALCULATION OF PARTIAL STREAM FLOW OR DISCHARGE .....	4-2
EQUATION 2. PERCENT SORTING EFFICIENCY (PSE).....	5-22
EQUATION 3. PERCENT DIFFERENCE IN ENUMERATION (PDE) .....	5-34
EQUATION 4. PERCENT TAXONOMIC DIFFERENCE (PTD) .....	5-35
EQUATION 5. PERCENT DIFFERENCE IN ENUMERATION (PDE) .....	6-23
EQUATION 6. PERCENT TAXONOMIC DIFFERENCE (PTD) .....	6-23
EQUATION 7. CALCULATION OF PERCENT GRADIENT .....	10-6



## List of Abbreviations and Acronyms

AMD - Acid Mine Drainage or sometimes Alkaline Mine Drainage  
AML - Abandoned Mine Lands  
AWQN - Ambient Water Quality Network (monitored by WVDEP-WAB)  
COC - Chain-of-Custody  
CWA - Clean Water Act  
DI - Deionized Water  
DO - Dissolved Oxygen  
DS - Downstream  
EEF - Edge of Effective Flow  
EMAP - Environmental Monitoring and Assessment Program (managed by USEPA)  
GIS - Geographic Information Systems  
GLIMPSS - Genus-Level Index of Most Probable Stream Status (developed by the WVDEP)  
GPS - Geographic Positioning System  
HUC - Hydrologic Unit Code (managed by USGS)  
IBI - Index of Biotic (or Biologic) Integrity  
LTMS - Long-Term Monitoring Station; aka: LiTMuS (monitored by WVDEP-WAB)  
MAHA - Mid-Atlantic Highlands Assessment (conducted by USEPA)  
MAIA - Mid-Atlantic Integrated Assessment (conducted by USEPA)  
MMI - Multi-metric Index  
NARS - National Aquatic Resources Survey (conducted by USEPA)  
NHD - National Hydrographic Dataset (maintained by USGS)  
NPS - Nonpoint Source  
NRSA - National Rivers and Streams Assessment (USEPA-NARS)  
PS - Point Source  
QA/QC - Quality Assurance/Quality Control  
QAPP - Quality Assurance Project Plan  
RBP - Rapid Bioassessment Protocol (developed by USEPA)  
RTE - Rare, Threatened, or Endangered (managed by USFWS & WVDNR)  
SAV - Submerged Aquatic Vegetation  
SOP - Standard Operating Procedure  
TMDL - Total Maximum Daily Load  
US - Upstream  
USFS - United States Forestry Service  
USFWS - United States Fish and Wildlife Service  
USEPA - United States Environmental Protection Agency  
USGS - United States Geological Survey  
USNPS - United States National Park Service  
VBHA - Visual-Based Habitat Assessment (developed by USEPA for use in RBP)  
WAB - Watershed Assessment Branch (of the WVDEP)  
WCMS - Watershed Characterization and Modeling System (developed for the WVDEP)  
WQ - General shorthand for water quality  
WV - West Virginia  
WVBPH - West Virginia Bureau for Public Health  
WVDEP - West Virginia Department of Environmental Protection  
WVDHHR - West Virginia Department of Health and Human Resources  
WVDNR - West Virginia Division of Natural Resources  
WVEQB - West Virginia Environmental Quality Board  
WVSCI - West Virginia Stream Condition Index (developed for the WVDEP)

## New in the 2015 Edition

- **All Pages.** Page numbering has been altered to include the Chapter number followed by the page number within that chapter (e.g., 1-2, 10-18, etc.). Figure and Table numbering has been altered in a similar fashion. In addition to the page number at the bottom of the page, the general contents of the page have been tagged at the bottom of the page. The left page has been tagged with the Chapter Title and the right page has been tagged with the general topic of the two open pages.
- **CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS.** Starting on page 4-1. Has been reorganized to begin to accommodate new flow meter model (OTT MF Pro) and new specifications.
- **CHAPTER 18. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS**  
**Section C Identification of Benthic Macroinvertebrates Benthic Laboratory Identification Quality Assurance/Quality Control (starting at page 5-33).** Expanded to include **SFS Taxonomic Certification Program** details.
- **CHAPTER 18. FREQUENT TERMINOLOGY (starting at page 18-1).** Expanded to include **Oil/Gas Related Terms.**

## Errata from Previous Versions

- **CHAPTER 3. Section A. Part 1. DO Sensor Calibration. Hydrolab Quanta G (page 3-11).** Corrected the last line of Step 7 from “DO” to “DO%”.
- **CHAPTER 5. Section A. Benthic Macroinvertebrate Sampling Part 1. Field Sample Collection Methods Single Habitat-Riffle/Run Habitat using a D-Frame Dip-Net/Kick-Net**
- **Comparable for use with current MMI/IBIs (starting on page 5-12).** Corrected the dimensions of the D-Frame Dip-Net and the number of kicks required (from 9 to 11) to approximate 1.0 m<sup>2</sup> sampling area.

# CHAPTER 1. 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 (e.g., 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.

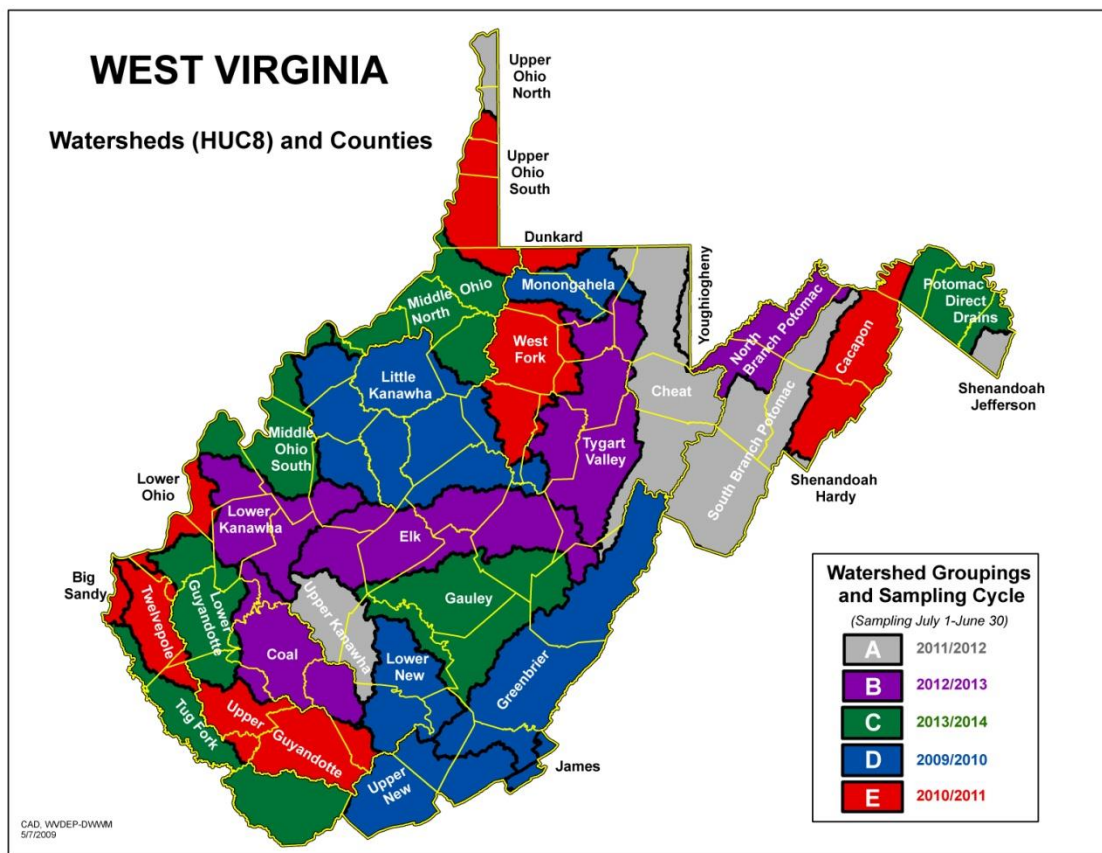


Figure 1-1. Example of WV rotating Watershed Basin Schedule.

All waterbodies (i.e., streams, rivers, lakes, reservoirs, ponds, navigable waters, wetlands, etc.) in the state are grouped into 32 watersheds (see Figure 1-1 above) 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.

The following document further describes the Watershed Assessment Branch's duties and functions:

WVDEP (West Virginia Department of Environmental Protection). December, 2007. West Virginia's Water Quality Monitoring Strategy. Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV. Available online at:

[http://www.dep.wv.gov/WWE/watershed/wqmonitoring/Documents/2007\\_WV\\_Monitoring\\_Strategy.pdf](http://www.dep.wv.gov/WWE/watershed/wqmonitoring/Documents/2007_WV_Monitoring_Strategy.pdf)

### ***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<sup>st</sup>-4<sup>th</sup> order streams, but may include some smaller 5<sup>th</sup> and 6<sup>th</sup> 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 MMI (Multi-Metric Index) or IBI (Index of Biotic Integrity) scores that are uncertain or “gray” or streams with prior collections),
4. Streams that have known impairments (e.g., 303(d) listed streams, AMD or Acid Mine Drainage, Biological impairments),
5. Streams of particular public interest (e.g., 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 mid-1940s. The bimonthly components of sampling include water quality samples and limited habitat observations. These sampling activities are covered mainly in **CHAPTER 11. AMBIENT WATER QUALITY NETWORK PROTOCOLS starting on page 11-1.**

**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 12. LAKE SAMPLING PROTOCOL starting on page 12-1.**

**Continuous (Time-Series) Monitoring** is often used to provide more detailed and frequent water quality data in support of other sampling programs (e.g., TMDL, Special Projects). Deployment and retrieval of the deployable sondes may be accompanied by a water quality sample and habitat observations at targeted locations. These sampling activities are covered mainly in **CHAPTER 13. CONTINUOUS WATER QUALITY MONITORING PROCEDURES starting on page 13-1.**

**Fish Tissue Monitoring and Consumption Advisories** are conducted and published thru a cooperative effort between WVDEP and WV Division of Natural Resources (WVDNR) and WV Bureau of Public Health (a section of the WV Department of Health and Human Resources or WV DHHR). Information about fish contamination in West Virginia has been collected since the late 1970s. Beginning in September 2000, an Executive Order formalized the cooperative effort between the agencies. More information on fish consumption advisories can be found at:

<http://www.wvdhhr.org/fish/>

and information on specific contaminant values can be found in the WV Sport Fish Consumption Advisory Guide at:

[http://www.wvdhhr.org/fish/Current\\_Advisories.asp](http://www.wvdhhr.org/fish/Current_Advisories.asp)

**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, continuous (time-series) monitoring, biological samples, limited habitat assessments, or intensive habitat assessments.

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, Lake Monitoring** and **Continuous 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.

### ***Other Documents to Consider***

At its inception in 1996, the Watershed Assessment Branch adapted methodologies already commonly in use by USEPA and other surrounding states. Specifically, WVDEP WAB staff members who participated in USEPA's EMAP sampling for the MAHA and MAIA pilot projects in 1993-1997 in West Virginia were instrumental in setting up the WAB sampling methodologies. For more insight into these other methodologies consult the following documents (listed in chronological order):

- 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. United States Environmental Protection Agency. EPA/444/4-89-00.
- Klemm, D.J., P.A. Lewis, F. Fulk, and J.M. Lazorchak. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA 600-4-90-030. U.S. Environmental Protection Agency; Office of Research and Development; Washington, D.C.
- Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1997. Revision to Rapid Bioassessment Protocols for Use in Streams and Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Draft. EPA 841-D-97-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.
- 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.



Barbour, M.T., J. Gerritsen, B.D. Snyder, J.B. Stribling. 1999. Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, benthic macroinvertebrates, and fish. Second Edition. United States Environmental Protection Agency. EPA/841/B-99-002.

Davis, W, and J. Scott (editors). 2000. Mid-Atlantic Highlands Streams Assessment: Technical Support Document. EPA 903-B-00-004. U.S. Environmental Protection Agency Region 3; Philadelphia, PA.

These documents are available for download at:

<http://www.epa.gov/ncepihom/index.html>

## ***General Quality Assurance/Quality Control***

### **Quality Assurance Project Plan**

The Watershed Assessment Branch Quality Assurance Project Plan (QAPP), which is submitted and approved by EPA on a regular basis, can be viewed and downloaded at:

[http://www.dep.wv.gov/WWE/watershed/Documents/2005\\_06\\_SOP\\_All.pdf](http://www.dep.wv.gov/WWE/watershed/Documents/2005_06_SOP_All.pdf)

### **Training**

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 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.

Specialized training sessions (e.g., WVDNR Boating Safety, Red Cross First Aid & CPR/AED training) as well as thematic training seminars (e.g., AMD/Acid Rain Training, Fish Taxonomy) are also scheduled as needed.



In supplement, WAB personnel may undergo additional education in the form of Workshops, Seminars, and Societal Meetings (e.g., Association of Mid-Atlantic Aquatic Biologists, American Fisheries Society, or Society for Freshwater Science) and often trained and participate in other projects with similar methodologies to those in this document (e.g., USEPA's National Aquatic Resource Surveys for Streams and Rivers, Lakes, and Wetlands).

## Standard Operating Procedures Document

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\SOP2015

and externally via the web at:

<http://www.dep.wv.gov/WWE/watershed/Pages/WBSOPs.aspx>

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.

## Data Retention & Storage

All data generated by WAB are stored in a networked Oracle-platform database (Back-end) and accessed by users with multiple Microsoft Access databases (Front-ends). Combined, these are referred to as WABbase (**see Figure 1-2 on next page**). The major exception to this is any Fish Tissue Data generated for Health Consumption Advisories, which is stored in an independent Microsoft Access database.

All paper field forms, COCs, and laboratory analytical results and bench sheets are scanned into an electronic format (.pdf) and stored on the network server which is backed up nightly. The original paper forms (if available) are filed and stored in a file room for future reference.

Any electronic deliverables from contract laboratories (e.g., water quality analysis, bench sheets), raw files generated by a device (e.g., deployable or flow meter), or photographs are also stored for future reference on the network server.

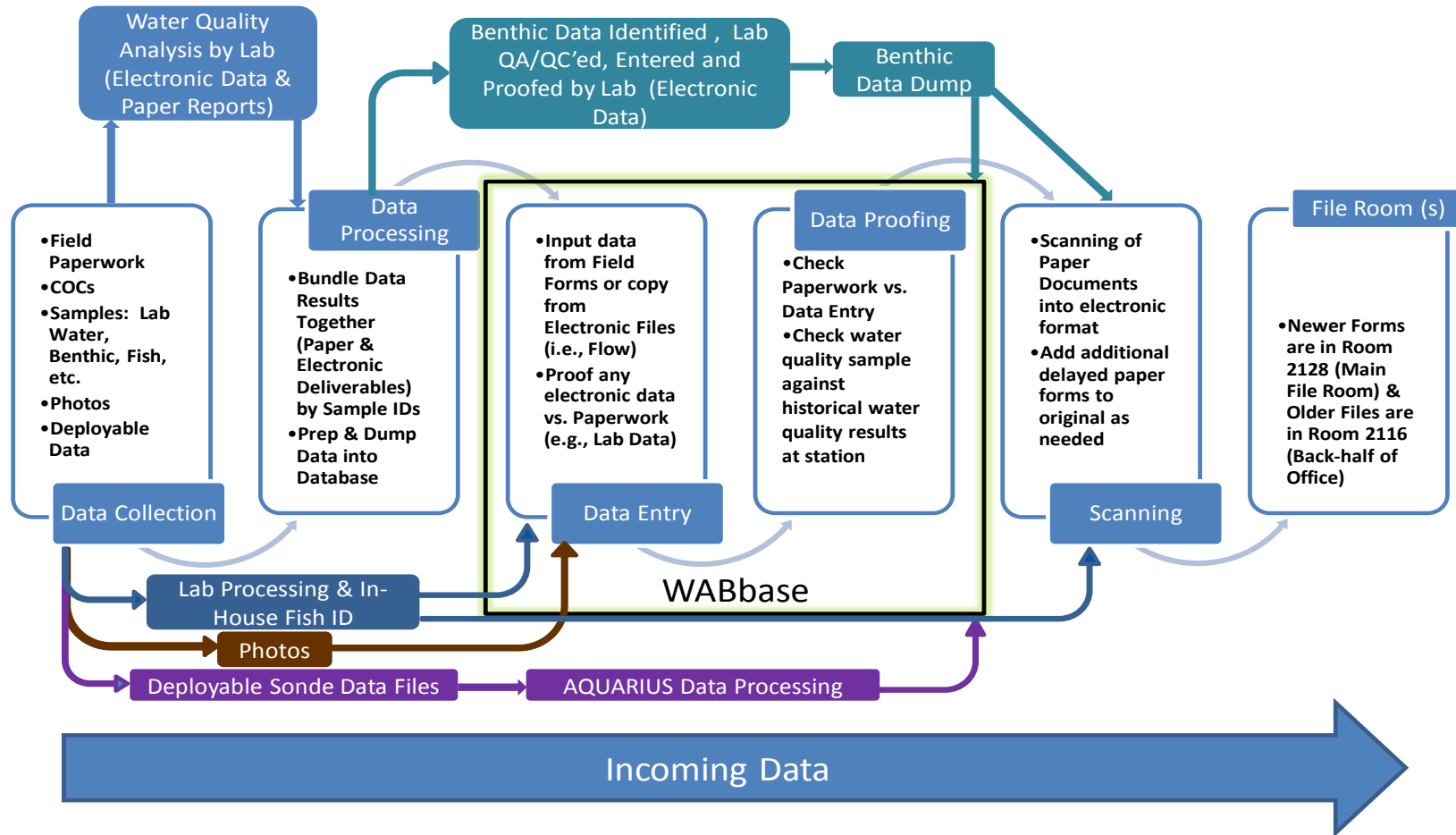


Figure 1-2. WAB Data Flow

## **CHAPTER 2. 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 (e.g., 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 physicochemical 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 targeted 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:** 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.

***IMPORTANT:*** *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 2. Section C. Part 1. Wadeable Benthic Stream Assessment Form starting on page 2-29.*** 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.

**WARNING:** **DO NOT NAVIGATE TO ANY ASSESSMENT SITE THAT PRESENTS A DANGEROUS SITUATION TO YOU OR ANOTHER TEAM MEMBER!**

### **Random Sites (Probabilistic Sites)**

Beginning in 2007 the Random Sampling Program switched from a statewide watershed based 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:

70-Western Allegheny Plateau  
69-Central Appalachians  
67-Ridge and Valley

Note: Due to the limited size of 66-Blue Ridge Mountains in WV, it has been combined with Ecoregion 67.

The **Target Population** is all flowing wadeable streams and rivers (1<sup>st</sup>-4<sup>th</sup> Strahler orders at 100k scale) within West Virginia. Samples are selected by computer using USGS's NHD Plus (a stream network coverage at the 100k scale) as a **Sample Frame**. A Generalized Random Tessellation Stratified (GRTS) **Survey Design** for a linear resource was used. **Multi-Density Categories** were set up by Ecoregion and Strahler orders 1-4. **Unequal weighting** was used on Strahler order. Specifically Strahler order 1 was weighted by 1.3 to make approximate sample proportions (by order) of 71%, 15%, 9%, and 5%, which approximates the true abundance by stream order found in WV and yet compensates for the estimated 30% of 1<sup>st</sup> order sites that are too small to be sampled using WAB methodology (e.g., Non-Target ephemeral and non-flowing intermittent stream reaches). Sites were deselected automatically as Non-Target if the watershed area above the sites was less than 100 acres (based on best professional judgment and past probabilistic sampling efforts). Sites were sampled in order so that there was equal sampling effort amongst the three ecoregions. Five **Panels** were setup; one for each year of sampling round. The **Expected Sample Size** for the 2013-2017 5-year cycle is 195. Since not all sites that are selected turn out to be target, an **Oversample** of 195 sites are selected to serve as alternate sites to replace those on the main list found to be non-target.

Before the field season begins each year, the map coordinator will review the proposed sampling sites for the year and attempt to eliminate any that do not match the target population.

***IMPORTANT: For random (probabilistic) sites, our target stream sites must have riffle/run habitat, be wadeable, and can be sampled using kick protocols that result in comparable data.***

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 will consist of about 5 to 8 samples. **See Table 2-1 on page 2-5 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 2-1 on page 2-5**): 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* on page 2-6 for more information.**

***IMPORTANT:*** Every 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 so that a visit may be attempted by another sampling team that may be better physically able to reach the site.

### 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 (*e.g.*, 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.

***NOTE:*** 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 2-1. An example of a typical Random Site List

**Random List C**

E-Reg	R#	ANCODE	STREAM NAME	Latitude			Longitude			TOPONAME	Date	Initials
<b>R &amp; V</b>	<b>6003</b>	<b>PSB-16-B-2-{0.2}</b>	<b>UNT/Brushy Run RM 0.46</b>	<b>39</b>	<b>12</b>	<b>13.02</b>	<b>78</b>	<b>51</b>	<b>0.38</b>	<b>Sector</b>		
			Shows as an intermittent stream on topo, should be done early (Late April early May)									
<b>R &amp; V</b>	<b>6019</b>	<b>PSB-21-0.21A-{0.7}</b>	<b>UNT/So Fork RM 3.39/South Br</b>	<b>39</b>	<b>1</b>	<b>23.51</b>	<b>78</b>	<b>57</b>	<b>41.74</b>	<b>Moorefield</b>		
			Shows as an intermittent stream on topo, should be done early (Late April early May)									
R & V	6015	PC-20-{4.4}	Moores Run	39	7	32.20	78	35	41.32	Yellow Spring		
			Wardensville topo also included to aid in navigation. May require a hike. Fish will be collected here later.									
R & V	6031	PC-16-A-{2.9}	Dry Run	39	8	57.91	78	28	33.78	Capon Springs		
R & V	2051-R2	P-19-D-{4.9}	Crooked Run	39	22	22.30	78	35	20.40	Hanging Rock		
R & V	2077-R2	P-19-E-2-{0.1}	UNT/Graybill Hollow RM 1.21	39	25	36.60	78	35	30.90	Levels		
<i>Perform Duplicate Sampling Each Week! Duplicate Name: Elm Creek PC-76-X-{8.6}</i>												
<i>Perform Dfield Blanks Weekly! Field Blank Name: Buzzard Fork PC-76-Y-{4.6}</i>												
<b>Four Cubitainers and one Fecal bottle:</b>												
<b>Hot Acidity, Alkalinity, Sulfate, TDS, TSS, Chloride, Bromide, Total P, TKN, NO2-NO3-N, Fecal Coliform</b>												
<b>[Total Mg, K, Na, Al, Fe, Mn, Ca, Se Be] and [Diss. Al, Cu, Fe, Zn]</b>												
<b>Note: Two new parameters: Beryllium (Be) a metal, and Bromide (Unfixed) You'll have to write in Be on the COC</b>												

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. This spot is referred to as the **X-site** and is the downstream end of a 100 m reach that is to be assessed.

**NOTE:** 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.

## Site Verification

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:** 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.

**IMPORTANT:** For revisit sites, use the coordinates provided on the site list only. 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 electronic 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.

Some situations require sliding the reach and thus the X is not at the downstream end (**see *Sliding the Reach* on next page for details**).



Sometimes it is possible a stream site will not be physically visited. 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.

**IMPORTANT: If you get coordinates at your current location (e.g., a locked gate) 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!**

Several questions must be answered for site verification of a probabilistic site:

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 trespass to 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 or a house). **Physical Barriers** are those that may be **temporary** (e.g., a water flooded road) or **permanent** (e.g., high cliffs).

**NOTE: There have only been one or two instances in 15 years of probabilistic sampling where a physical barrier prevented access to a site. 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**.

***IMPORTANT: This field 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! Photos of non-target sites (or evidence of non-target status) must also be included!!***

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

- **Low Flow**
  - **Permanent** (*i.e.*, non-drought, *e.g.*, subsidence) or
  - **Low Flow-Temporary** (*i.e.*, 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
  - **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/low gradient)
- **Wetland** (*e.g.*, 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; this is more common in the Eastern Panhandle of WV),
  - **Urban/Residential** (stream is culverted to make room for houses/yards/residential roads/airports),
  - **Road** (stream is culverted or filled for a major road like and interstate or 4 lane expressway), or
  - **Industry** (*e.g.*, landfills, fly ash dumps, culverted or filled under an industrial facility)
- **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; *applies to Ohio, Kanawha, and Monongahela River backwaters only*), 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 also 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.

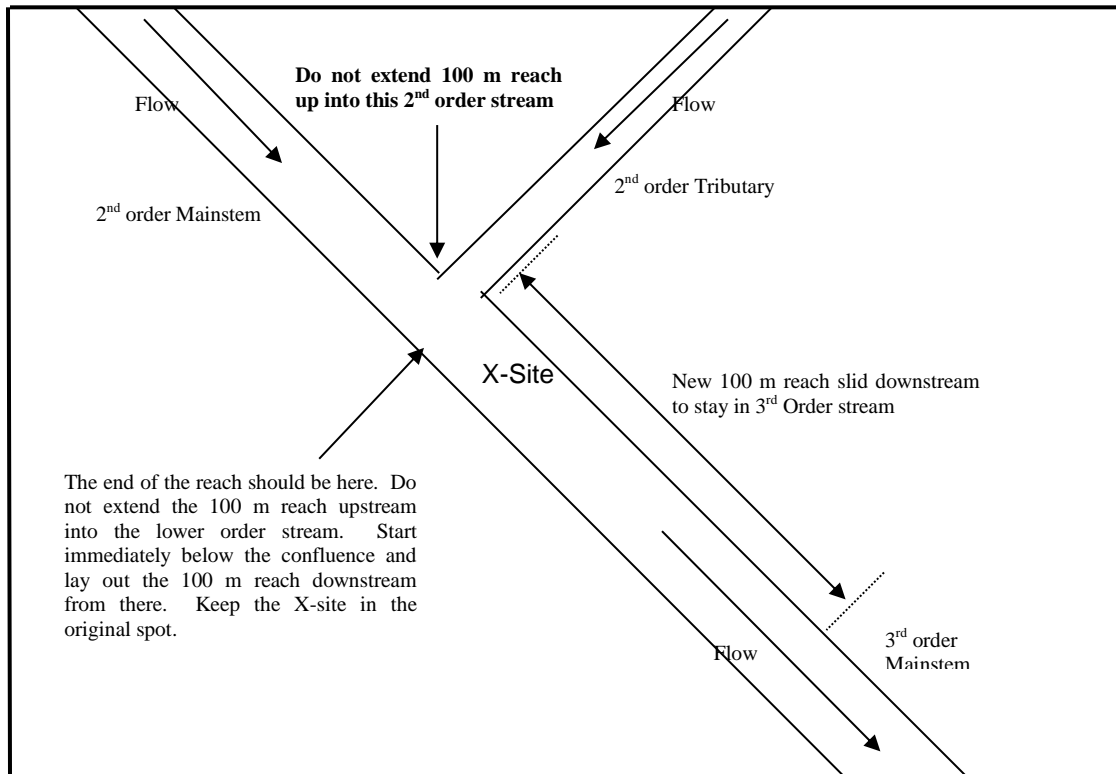
After the X-site has been confirmed (or located via best professional judgment), the Geomorph will establish a 100-meter assessment area based on the X-site. If there are no riffle/run habitats within 100 m reach, the site is considered non-target for random sites and should not be sampled.

***IMPORTANT: For random (probabilistic) sites, our target stream sites must have riffle/run habitat, be wadeable, and can be sampled using kick protocols that result in comparable data.***

### 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 2-1 on the next page.**

**NOTE:** The confluence must be within the proposed 100 m reach for this sliding to apply. Confluences above or below the proposed 100 m reach do not require sliding, but may require some considerations when sampling within in the reach (e.g., not sampling the benthos at the upstream end of the reach where the water has not yet adequately mixed).



**Figure 2-1. An example of sliding the reach to avoid larger/small confluences, lakes, ponds, etc. (used FOR RANDOM SITES ONLY).**

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 2-1 above**). 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.

***IMPORTANT: Take photographs of the situation necessitating the sliding of the reach and the areas above and below the new reach.***

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 Figure 2-1 on page 2-10**) so that the X-site and benthic collection area are in similar water quality.

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 need to actively traverse the stream from one end to the other taking note of pertinent habitat information and measuring the 100 m area.

**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).**

The Geomorph will perform other duties concurrent with the establishment of the 100 m assessment area (***outlined in Section B. Site Documentation and Section C. Guidelines for Completing the Stream Assessment Forms***). 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 3. Section B. Part 3. Common Water Quality Parameter Suites starting on page 3-49 and on the Error! Reference source not found..***

### **Targeted Sites**

*Targeted sites are preselected in the office either from a pool of known sampleable locations or from staff knowledge of the stream. They are usually located at the mouth of tributaries, on the mainstem above the confluence of a tributary, or bracketing pollution sources or areas of interest. Targeted sites allow more latitude with relocating the site for better sampling conditions and stream access, as long as any directions provided on the stream list are observed. Targeted 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.*

Targeted sampling stations are marked with an **X (highlighted in yellow)** and with the sample year on USGS 1:24,000 scale topographic maps.

**NOTE: 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 for targeted sites should be established above bridges. Additionally, bridges should not be included in the assessment reach, if possible. Targeted 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 benthic MMI/IBI scores. For example, if a riffle/run site can be found a ¼ mile further upstream without going above a significant tributary or changing land use (agriculture, etc.), 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.**

***IMPORTANT: 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. Part 1. PAGE 1-Site Verification starting on page 2-30 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 targeted 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 (e.g., 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 on next page***).

### **Duplicate Sites**

In order to fulfill quality assurance and quality control or QA/QC requirements (**see *CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1***), a select number of sites will be assessed in each watershed in duplicate. 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 (e.g., 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, collect water samples, and obtain appropriate physicochemical samples as if they are the only person there.

***IMPORTANT: Do not put your benthic sampling data on the other person's form when conducting duplicate sampling!***

Water quality sonde and flow readings should be recorded on the DUP 1 assessment form only. GPS coordinates can be shared between the two duplicates. **All Water samples and COCs are marked as blind duplicates (with a false stream name and AN-Code and no indication that it is a duplicate sampling event occurring).** The field habitat forms and sampling list will contain the true identities of the **Blind Duplicates based on the WQ ID, Date/Time of the sample, and name of the sampler.** If for some reason the designated duplicate is not sampleable, the team should replace the duplicate site with another stream in the same week.

### **Reference Sites and Potential Reference Sites**

**WARNING: 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 2-37**).
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.  $\geq 5.0$  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  $< 500$   $\mu\text{mhos/cm}$  - 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/100 mL is double the maximum set by the WV Environmental Quality Board (WV EQB) which states that fecal coliform shall not exceed 400/100 mL in more than 10 percent of all samples taken during the month. This value was raised to 800/100 mL 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 800/100 mL would decrease the possibility of excluding some anthropogenically undisturbed 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. Because of their toxicity, metals are the primary consideration when evaluating data for violations.

**NOTE: Note: This violation rule does not include fecal coliform bacteria for the reasons described above.**

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-USEPA's Rapid Visual-Based Habitat Assessment starting on page 2-64**). 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, (*e.g.*, 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 targeted 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.

**IMPORTANT: 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.

**WARNING: 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.



Figure 2-2. Example of GPS being used to locate and document the X-site.

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 (*see Figure 2-2 above*). 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 on page 2-23*).

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 2-30*). 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 2-2 on the next page** 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 2-2. 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	1 <sup>st</sup> , 2 <sup>nd</sup> , and Final Visits
Special Projects	1 <sup>st</sup> , 2 <sup>nd</sup> , and Final Visits
Deployable Sondes	1 <sup>st</sup> , 2 <sup>nd</sup> , and Final Visits ( <i>i.e.</i> , Installation, 1 <sup>st</sup> Retrieval, Last Retrieval)
Ambient Network	Old Sites-Only when the site is moved (e.g., moved us 30 m because of a new bridge) New Sites-1 <sup>st</sup> and 2 <sup>nd</sup> 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.

### **Procedures for obtaining coordinates with a GARMIN GPS III+ or V**

- 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 an application called WCMS (Watershed Characterization and Modeling System), 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 used NAD 1927 CONUS for a datum. The newer WCMS version (9.x and above) uses NAD 1983 CONUS. Watershed Assessment transitioned to NAD 1983 CONUS 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<sup>0</sup> 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 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 (*e.g.*, 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 2. Section C. Part 1. PAGE 12-Photography Log on page 2-108*). 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**

### **Downloading the Photos for tagging**

2015 Photos should be downloaded to the following location:

Q:\WATER RESOURCES\WAB\PHOTOS\Unclaimed Photos\2015

Upon opening the folder you will see a subfolder with each person's name. It has been found that the **Photographers Name** works best for naming the subfolder. If your name is on the field form as the photographer, then store the photos in your folder. For example – if person John Doe is in charge of taking photos while doing an assessment then the photos should be stored in the **Unclaimed Photos\2015\Doe** folder. This applies even if the person is not using a camera that has assigned them. If there is more than one photographer for a group of photos, then you can use either name.

The procedure for creating a date subfolder under your name is as follows

- 1. Find the oldest date for the list of photo's you are downloading**
- 2. Create a directory corresponding to that date using this format YYYY DD MM under your name. For example: if the oldest date in a list of photos to download is April 29, 2015 – then the subfolder would look like this – 2015 4 29. If the photos were taken by John Doe the final folder would look like this: Q:\WATER RESOURCES\WAB\PHOTOS\Unclaimed Photos\2015\John Doe\2015 4 29. There are single spaces between the 2015 4 29.**

The camera can be brought to the photo coordinator for download or be downloaded directly by the photographer.

**IMPORTANT: Do not put the photos in any other location on the network!**

### **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. The 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 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**

This section explains the various data components found on the Wadeable Benthic Stream Assessment Form and its appendices. Other forms (e.g., TMDL-Initial Visit, TMDL-Secondary Visit, TMDL-Final Visit, TMDL-Source, Ambient Network, Lake, and General WQ) share many of the same components (e.g., Site Verification, Field Water, and Photography) and have some unique components not found on the Wadeable Benthic Assessment Form (indicated as such). Nevertheless, the following instructions on how to fill out the sections are the same as presented here unless specifically stated/presented otherwise in subsequent chapters.



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 on 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

### Site Verification

#### **IMPORTANT INSTRUCTIONS**

**Always** fill out the PAGE 1 (Site Verification) of the habitat assessment form, regardless of whether any type of sampling was conducted (i.e., even if the stream site is deemed "Not Target", dry, impounded, or inaccessible)! This includes:

- 1) Coordinates of the site or location near the site (e.g., bridge downstream of site, locked gate, landowner's house),
- 2) A detailed explanation of why it was not sampled,
- 3) Photographs that display the reason why it was not considered target,
- 4) Precise directions to the site

This is important information and assists in database management. See *Figure 2-4 on the next page for an example of this page.*

If water quality only is collected, you must at least complete PAGES 1-4, 8 and 10, as best as you can and take plenty of photographs.





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.

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 Garmin 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 #: 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 2-23 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

Provided/Prev. Latitude and Longitude at X-site: Either the coordinates provided on the stream list for Probabilistic sites (random), LTMS, TMDL, WAB, *etc.* or enter the coordinates from a photocopy of a previous visit to this sampling station.

**NOTE: This field set is only on the Wadeable Benthic Stream Assessment Form.**

Field Latitude and Longitude: Enter for all sites after obtaining readings in the field using Garmin or Trimble GPS units (*see CHAPTER 2. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) starting on page 2-20*)

Corrected Lat: Enter the corrected versions of the coordinates here. Correction is done in the office after they are proofed (see XY's Proofed above).

**NOTE: This field set is only on the Wadeable Benthic Stream Assessment Form.**

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 trespass to 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 or a house). **Physical Barriers** are those that may be **temporary** (e.g., a water flooded road) or **permanent** (e.g., high cliffs).

**NOTE:** There have only been one or two instances in 15 years of probabilistic sampling where a physical barrier prevented access to a site. **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**.

***IMPORTANT:*** *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! Photos of non-target sites (or evidence of non-target status) must also be included!!*

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

- **Low Flow**
  - **Permanent** (i.e., non-drought, e.g., subsidence) or
  - **Low Flow-Temporary** (i.e., 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
  - **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/low gradient)
- **Wetland** (e.g., 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; this is more common in the Eastern Panhandle of WV),
  - **Urban/Residential** (stream is culverted to make room for houses/yards/residential roads/airports),
  - **Road** (stream is culverted or filled for a major road like and interstate or 4 lane expressway), or
  - **Industry** (e.g., landfills, fly ash dumps, culverted or filled under an industrial facility)
- **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; *applies to Ohio, Kanawha, and Monongahela River backwaters only*), 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 also 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) **Fish**, (7) **Flow**, (8) **BE/CP** (i.e., the Stream Bank Erodibility Factors/Estimated Channel Profile Form), (9) **Other**. **Do not include sonde readings as part of the lab water data. This refers to laboratory-analyzed samples only.**

**NOTES:**

- ⇒ **Other forms may have specific lab water suites as options (e.g., AMD, Acid Rain, Nutrients, Orthophosphate, etc.). Yet others may have a place to put a suite number. Please fill out accordingly.**
- ⇒ **Periphyton has been removed as an option until it again becomes a common Sample Type.**

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) **YSI**, 3) **Lab Water**, 4) **Fecal**, 5) **Habitat**, 6) **Bugs**. Water quality sonde readings should be recorded on the DUP 1 assessment form only **UNLESS two different sondes are present with the sampler(s)**. 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 WQ ID: The WQ ID number of the duplicate sample, if applicable.

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 2. Section A. Part 2. Sliding the Reach starting on page 2-9**). 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.

***IMPORTANT: 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 (e.g., 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 (N←), 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).

***IMPORTANT: 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) are collected. If periphyton samples are collected, mark the locations (p). 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.

***IMPORTANT: 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 comment/notations on the map 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.

Some common map/note abbreviations that can be used include:

<b>RF</b> = Riffle	<b>AV</b> = Aquatic Vegetation
<b>RN</b> = Run	<b>SAV</b> = Submerged Aquatic Vegetation
<b>PL</b> = Pool	<b>ROW</b> = Right-of-Way
<b>CMP</b> = Corrugated Metal Pipe (e.g. Culvert-type pipe)	Any of the Substrate Abbreviations on <i>PAGE 3 (page 2-50)</i> .

**NOTES:**

- ⇒ **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 bad IBI site, etc.). If this is a duplicate sample, this is the location where Dup #1 vs. Dup #2 can be documented. 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 (e.g., a waterbody profile), then this information will be documented on another page with the specific collection information (e.g., 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 2-5 on the next page 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 scoured 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.*

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. **See CHAPTER 18. FREQUENT TERMINOLOGY starting on page 18-1 for a description and examples of Nonpoint Sources of pollution.**

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**. **See CHAPTER 18. FREQUENT TERMINOLOGY starting on page 18-1 for a description and examples of Point Sources of pollution.**



**Figure 2-6. Example of a Point Source Discharge**

**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.

**NOTE:** If you are unsure about if a source is Nonpoint vs. Point, 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 (e.g., 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 (e.g., pesticide applications). The presence and extent of livestock access to the stream is very important to document (**see Figure 2-7 on next page**) as it contributes to bank erosion and localized sedimentation as well as nutrient runoff. In most cases, the livestock will not be directly observed in the stream, but rather the signs of livestock (hoof prints, wallows, droppings, lack of fence keeping the livestock from the stream, etc.). In general, agriculture activity can also be considered a Nonpoint Source since it is not explicitly covered under a permit and can occur over a broad landscape (**see Local NPS Pollution above**).

**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.





Figure 2-7. Example of Livestock Access to a stream.

**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 **A=Single**, **B=Double**, or **C=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



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 as described on page 2-51.**

**Sediment Characterization**

Sediment Odors		Sediment "Oils"	Sediment Deposits	
Normal		Absent	Overall Silt	Relic Shells
Sewage		Slight	Overall Sand	Marl (See Note at Left)
Petroleum		Moderate	Overall Fine Gravel	Limestone Chunks or Fines
Chemical		Profuse	Coal Chunks and Fines	Paper Fiber
Anaerobic (Septic)		Rate Sediment Deposits: 0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, NR-Not Rated	Red Dog	Sludge
Other:			Iron (Orange Hydroxide)	Probable Source: <input type="checkbox"/> Mining <input type="checkbox"/> Natural <input type="checkbox"/> Other
Note: Marl (crumbly, grayish, lightweight) *** Potomac Direct Drains Only*** Place Marl In Proper Size Class Under "Inorganic Substrate" on Page 9			Aluminum (White Hydroxide)	Probable Source: <input type="checkbox"/> Mining <input type="checkbox"/> Natural <input type="checkbox"/> Other
			Manganese (Black Hydroxide)	Probable Source: <input type="checkbox"/> Mining <input type="checkbox"/> Natural <input type="checkbox"/> Other
			Other:	
Sediment Notes & Comments (Describe other or intensity):				

Figure 2-9. 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.



**NOTE:** It should be noted that Manganese compounds will often form sheens on the surface of waters and in the sediment that can resemble oil (see *Figure 2-10 on next page*). This is why this category has oils in quotation marks.



Figure 2-10. Example of Manganese sheen that resembles a true oil sheen in its rainbow coloration.

Sediment Deposits: Note the presence of and rate the following sediment deposits:

- Overall Silt,
- Overall Sand,
- Overall Fine Gravel,
- Coal Chunks or Fines (*see Figure 2-12 on page 2-47*),
- Red Dog (*see Figure 2-12 on page 2-47 and Figure 2-13 on page 2-48*),
- Relic Shells,
- Marl (*see box on next page and Figure 2-11 on page 2-47*),
- Limestone Chunks or Fines (fines to boulders),
- Paper Fiber,
- Sludge,
- Iron Hydroxides or FeOH (*see Figure 2-13 and Figure 2-14 on page 2-48, Figure 2-19 on page 2-52, and Figure 2-24 on page 2-58*),
- Aluminum Hydroxides or AlOH (*see Figure 2-24 on page 2-58*),
- Manganese Hydroxides or MnOH and other Manganese compounds (*see Figure 2-10 on previous page and Figure 2-15 & Figure 2-16 on page 2-49*),
- Any other deposits not listed which are present in the sampling area.

**NOTE:** Sediment deposits may be counted more than once. For example, Limestone Fines may be rated as Overall Sand as Limestone Chunks or Fines.

Rate each 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)**. Limestone Chunks and Fines should include any non-native limestone (e.g., road gravel, rip-rap, etc.) that is found in the stream.

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 **Mining, Natural or Other**. If the probable source it is not known, do not guess natural. If both Mining and Natural seem likely, indicate both.

If other is indicated as a sediment deposit, then describe further under Sediment Notes & Comments. Collaborate with the Biomorph before making final rating decisions.

#### **WHAT IS MARL?**

Marl is a lime-rich mud or mudstone material that is considered transitional between Lime/Limestone (which is >75% Calcium carbonate) and Mud/Mudstone (which is >75% clay). Specifically, it is 65- 35% Calcium carbonate and 35-65% clay. It is rather light in color (greyish, like limestone) and soft/crumby & lightweight (like clay). In West Virginia, it is found almost exclusively in certain streams in the extreme Eastern Panhandle (Potomac Direct Drains Watershed). This area is underlain by large limestone deposits. It often appears as sand or fine gravel size pieces, but can also be apparent as a clay-like deposit on the stream bank.





**Figure 2-11. Example of a layer of Marl under a layer of soil in a stream bank.**



**Figure 2-12. Example of Red Dog and Coal Chunk Sediment Deposits.**



**HOW TO IDENTIFY RED DOG:**

An intern or new field worker may not be familiar with what exactly Red Dog is. Red Dog is a material created from the inadvertent slow burning of small bits of rock and coal in a refuse pile. It is characterized by the red color from minerals containing iron, which is brought out by the burning in a low oxygen environment. Sometimes hard to distinguish from crushed red brick or iron hydroxide coated rocks by a novice. The first photo below (*left*) shows the similarity (iron coated rock on left, Red Dog on right). The second (*right*) shows the striking difference in cross section. To a new field worker these phenomena are often novel and interesting. Also see **Figure 2-19** on **page 2-52** for an illustration of heavy to extreme iron (orange/yellow/red) metal hydroxides coating the substrate of a stream.



Figure 2-13. Left: An iron coated rock on left and a piece of red dog on right. Right: A cross section of the same rocks (iron coated rock on left and a piece of red dog on right).



Figure 2-14. Examples of streams coated with Iron Hydroxides.





**Figure 2-15. Example of rocks stained black with Manganese Hydroxides.**



**Figure 2-16. Examples of Manganese Hydroxides rubbing off of a piece of substrate.**

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	Substrate Particle Layer Profile			
			Location <sup>1</sup>	Habitat Type <sup>2</sup>	Substrate Particle <sup>3</sup>	Sand & Silt Thickness (cm) <sup>4</sup>
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)	<i>Special Instructions:</i> 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).			
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)	<i>Notes:</i>			
Clay	CL	Slick/ hard clay or hard-pan clay				
Metal Hydroxide	MH	Any Metal Hydroxide Deposits (Use only in the Substrate Layer Profile)				
			R	M	L	
			RDB		LDB	

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 2-17. Example of the Substrate Particle Layer Profile section (Middle of PAGE 3) of the field form

***IMPORTANT:*** *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.*

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 2-17 on the previous page or Table 2-3 below**) one layer at a time. If any sand or silt is documented, record the depth of that layer in cm.

***IMPORTANT: 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.

**Table 2-3. 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

**Figure 2-18. Example of the Dominant Substrate Type and Reach Characterization section (Bottom of PAGE 3) of the field form**

**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 USEPA's Rapid Visual-Based Habitat Assessment parameters Epifaunal Substrate, Sediment Deposition, and Riffle Frequency).

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 1<sup>st</sup> dominant substrate type (**using the Substrate Size Classification in Table 2-3 on the previous page**) and its percent aerial coverage (what you see on top in your “cone of vision”), and then the 2<sup>nd</sup> 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).**

***IMPORTANT:*** Do not use Metal Hydroxides as a class when evaluating the dominant substrate type; only use the functional size classes (i.e., those that have size ranges). Instead evaluate how the metal hydroxide is functioning structurally in the stream. Does the metal hydroxide just coat some cobble or gravel and does not prevent them from functioning structurally as cobble or gravel; or does the metal hydroxide fuse the substrate to the point of being structurally more like bedrock? See Figure 2-19 on next page for an illustration.

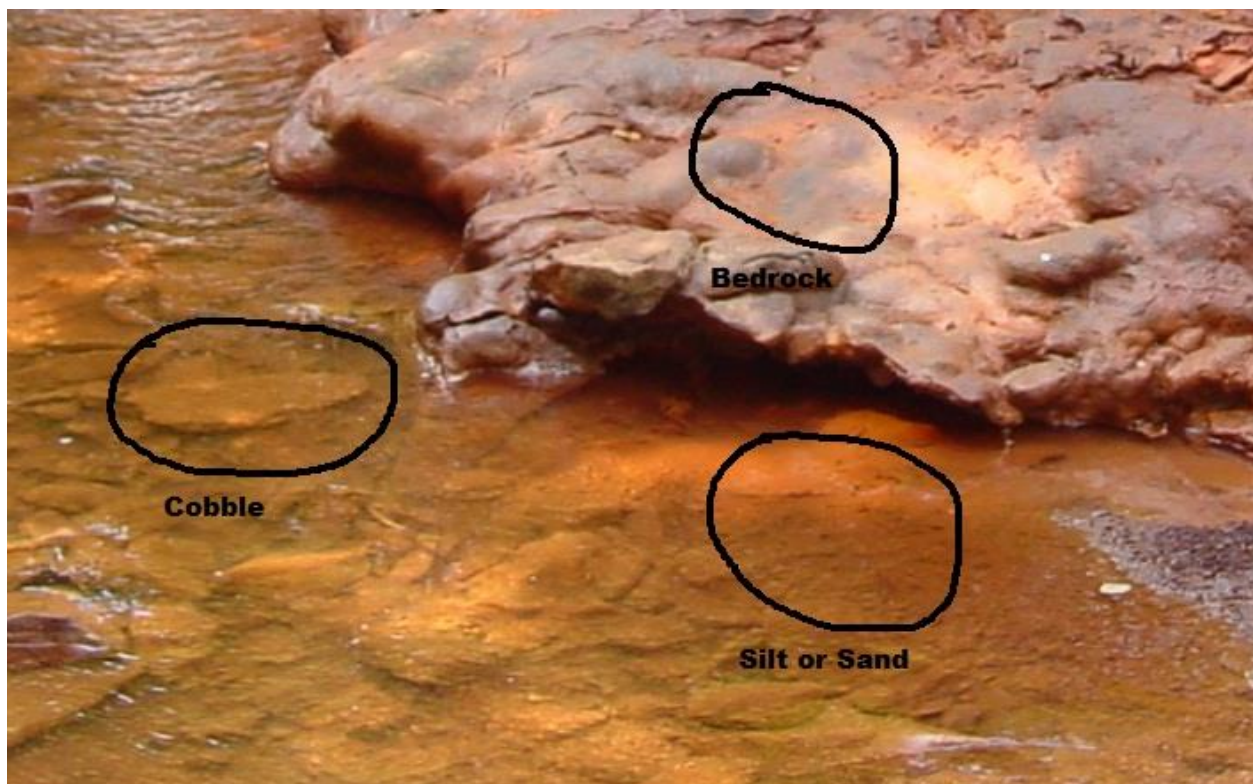


Figure 2-19. An example of Heavy to Extreme Iron (Orange/Yellow/Red) Metal Hydroxides coating the substrate

The area labeled cobble is just cobble coated with the metal hydroxide, but still functions like cobble. The area labeled silt or sand is metal hydroxide that has coated or settled out like silt or sand; it is still loose. However, the area in the upper right functions like bedrock due to fusing/armoring of the substrate by the metal hydroxides.







samples with unique time stamps = > 1 WQ IDs), 3) **Composite** (*i.e.*, discrete samples are taken at multiple locations, based on varying depth, width, or length of the reach locations, but combined into one sample = one WQ ID), 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 2-4 below.**

**Table 2-4. Examples of Flag values used on the field forms**

<b>I</b>	Parameter not recorded or deleted due to instrument problems or maintenance issues
<b>L</b>	Parameter recorded but suspected to be incorrect value; There is a low probability that the value is incorrect
<b>M</b>	There is a moderate probability that the value is incorrect
<b>H</b>	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**-°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 old jeep number (often marked on the camera in white ink).

**IMPORTANT: Do not record the number written on the display unit as this unit does not store calibration information.**

If for some reason the instrument identification number of the sonde 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 (**see *Figure 2-22 on right***). Foams formed in the presence of acid mine drainage will often take on the color



**Figure 2-22. Example of Foam.**

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** – Little to no apparent material suspended and does not obscure visibility through in the water column.

- 2) **Slightly Turbid** – Some material suspended in the water column that which to a minor degree obscures visibility & may prevent seeing the bottom substrate.
- 3) **Moderately Turbid** – Material suspended in the water column which noticeably obscures visibility & mostly prevents seeing the bottom substrate.
- 4) **Highly Turbid** (or Turbid) – Material suspended in the water column which highly obscures visibility & totally prevents seeing the bottom substrate.

**NOTE:** There can often be situations in which riffle/run areas appear to have drastically different turbidities than deeper pool areas. If this is the case, base the rating on the average turbidity found across the reach.

See *Figure 2-23 below and Figure 2-24 on next page for examples of turbidity.* Also, you can look at the water samples collected to help gage the turbidity extent of low turbidity water (*i.e.*, the difference between Clear and Slightly Turbid).



**Figure 2-23. Example of a Highly Turbid Stream with a brownish color.**

**Water Color:** Indicate whether the water color is normal (clear) or colored (*e.g.*, orange for iron impacted streams, milky white in aluminum impacted streams). **See Figure 2-23 above and Figure 2-24 on below for examples of water color.** The color of the water will often indicate the types and origin of the suspended material in the stream (*e.g.*, brownish water is likely sediment, red/orange may be from FeOH in mine drainage, white may be from AlOH in mine drainage, etc.).



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.



**Figure 2-24. Example of a Moderately Turbid stream. Note the presence of FeOH on the rocks and milky white color of the water caused by AlOH.**

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 1<sup>st</sup> 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) **N/A**, 2) **<1 hour**, 3) **1 to 4 hours**, 4) **4 to 12 hours**, 5) **12 to 24 hours**, 6) **1 to 2 Days**, 7) **2 to 4 Days**, 8) **4 to 7 Days**, or 9) **Unknown**. Unless you have monitored the rainfall prior to arriving, the most likely answer is **Unknown** during your first day in the area. If you are experiencing a drought period, then **N/A** may be the most appropriate answer.

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.

**NOTE:** This box is not on the Wadeable Benthic form. 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.

***Flow Information (Not found on the Wadeable Benthic Assessment Form)***

<b>FLOW INFORMATION</b>	If using an OTT MF Pro meter, then record information here. If using a Marsh-McBirney meter or conducting a gage reading, put info on a Flow Appendix sheet.						
<b>No Flow?:</b> If a flow was scheduled for the site and not performed, then indicate if one of the following applies			<input type="checkbox"/> Dry <input type="checkbox"/> Low Flow <input type="checkbox"/> Too Deep/Too Fast <input type="checkbox"/> Instrument Failure <input type="checkbox"/> Frozen/Ice <input type="checkbox"/> Safety <input type="checkbox"/> Substrate				
<b>Profile Name</b>		<b>Measurer</b>		<b>Flow Method</b>	<input type="checkbox"/> Flow Meter <input type="checkbox"/> Timed-Bucket <input type="checkbox"/> Gage <input type="checkbox"/> Estimate		<b>Flow Meter ID</b>
<b>File Name</b>		<b>Time</b>					
<b>Do you think that this flow measurement is comparable?</b>		<input type="checkbox"/> Yes <input type="checkbox"/> No	<b>If not comparable, Why? (Write Below)</b>		<b>Final Discharge Reading (cfs or ft<sup>3</sup>/s)=</b>		
<b>Flow Notes:</b>							

**Figure 2-25. Example of Flow Information Section (TMDL and Ambient Network Forms)**

This data component is not found on the Wadeable Benthic Assessment Form, but is found on the TMDL and Ambient Network forms. The main purpose of this section is to allow for a place for flow measurement metadata to be documented on paper when using the OTT MF Pro Flow Meter, which stores the individual stream flow measurement information (distance, depth, velocity, etc.) electronically to a file.

**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**.

**Profile Name:** The name of the flow profile entered into the OTT MF Pro Flow Meter. **This should be the same as the WQ ID.**

**File Name:** The name of the file (.csv) for the profile entered into the OTT MF Pro Flow Meter. **This should be the same as the WQ ID.**

**Measurer:** Record the flow measurer.

**Time:** The time of the flow measurement.

Flow Method: The method used to assess the flow: 1) **Flow Meter**, 2) **Timed Bucket**, 3) **Gage**, or 4) **Estimate**.

Flow Meter I.D.: The assigned number of the flow meter used. **Do not confuse this with the old 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.

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; elaborate under Flow Notes below.

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.

Flow Notes: Any notes about the flow measurement in general (e.g., general substrate type, reasons why not comparable, etc.).

### **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 Stream Bank / Riparian Buffer Zone Vegetation / Cover Type section on PAGE 4 of the Stream Assessment Form. See Figure 2-26 above 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? <input type="checkbox"/> Deciduous <input type="checkbox"/> Coniferous (i.e., Spruce, Pine, Hemlock, Rhododendron) <input type="checkbox"/> Mixed Deciduous (>10-49% Coniferous) <input type="checkbox"/> 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 1 <sup>st</sup> 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
		<i>Big Trees such as Sycamore, Oaks, Maples, Box Elder, River Birch, Hemlock</i>	<i>Small trees and shrubby Vegetation such as Willow, Alder, Knotweed (blue devil), Rhododendron, Wingstem</i>	<i>Ferns, Grasses, Mosses, Wildflowers</i>	<i>Exposed soil surface, Readily erodible – not rock faces or asphalt roads</i>
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? <input type="checkbox"/> Vernal <input type="checkbox"/> Mud Puddle <input type="checkbox"/> Sed. Pond <input type="checkbox"/> Farm Pond <input type="checkbox"/> Ditch <input type="checkbox"/> Lake <input type="checkbox"/> Cattail Wetland <input type="checkbox"/> Other					
Comments:					
Invasive Species in the reach riparian area?	Trees: <input type="checkbox"/> Tree-of-Heaven   Vines: <input type="checkbox"/> Kudzu <input type="checkbox"/> Japan. Honeysuckle		<input type="checkbox"/> Other:		
	Bushes: <input type="checkbox"/> Autumn Olive <input type="checkbox"/> Multi-flora Rose <input type="checkbox"/> Japan. Barberry				
	Herbaceous/Tall GC: <input type="checkbox"/> Japan. Knotweed <input type="checkbox"/> Bamboo <input type="checkbox"/> Mile-A-Minute				
Grass/Low GC: <input type="checkbox"/> Japan. Stilt Grass <input type="checkbox"/> Crown Vetch <input type="checkbox"/> Purple Loosestrife <input type="checkbox"/> Garlic Mustard					

**Figure 2-26. 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.

***IMPORTANT: 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** (e.g., Oak, Maple, Sycamore, Birch, Beech, etc. >90%), 2) **Coniferous** (e.g., 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.

***IMPORTANT: 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. **See CHAPTER 18. FREQUENT TERMINOLOGY starting on page 18-1 for examples of terms that can be used to describe the human activities in the immediate riparian area and beyond.**

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 (e.g., Greenbottom Swamp or Canaan Valley).
- 8) **Other** – Include comments in the area provided to elaborate on any of these.

Invasive Plant Species in reach riparian area?: Indicate if any of the following invasive species were present in the riparian area of the stream assessment reach:

**Trees:**

*Tree-of-Heaven*

**Vines:**

*Kudzu*

*Japanese Honeysuckle*

**Bushes:**

*Autumn Olive*

*Multi-flora Rose*

*Japanese Barberry*

**Herbaceous/Tall GC (Ground Cover):**

*Japanese Knotweed*

*Bamboo*

*Mile-A-Minute*

**Grass/Low GC (Ground Cover):**

*Japanese Stiltgrass*

*Crown Vetch*

*Purple Loosestrife*

*Garlic Mustard*

**Other:** Check this box and write in the name of any other less common invasive species (***see examples Table 2-5 on the next page***).

The following references are to be used in the field to assist in identifying Invasive Plant Species:

Swearingen, J., B. Slattery, K. Reshetiloff, and S. Zwicker. 2010. Plant Invaders of Mid-Atlantic Natural Areas, Fourth Edition. National Park Service and U.S. Fish and Wildlife Service. Washington, D.C. Available online at: <http://www.nps.gov/plants/alien/pubs/midatlantic/midatlantic.pdf>

Pennsylvania's Field Guide to Aquatic Invasive Species. 2013. Pennsylvania State University, Pennsylvania Sea Grant. Available online at: [http://www.anstaskforce.gov/Documents/AIS\\_Field\\_Guide\\_Finalweb.pdf](http://www.anstaskforce.gov/Documents/AIS_Field_Guide_Finalweb.pdf)

If there is any doubt or concerns about the identification, the plant should be either photographed, sampled, or both for confirmation by staff experts.

Table 2-5. A brief list of Invasive Plant Species in WV.

Scientific Name	Common Name	Type
<i>Achillea millefolium</i>	Common Yarrow	Flower
<b><i>Ailanthus altissima</i></b>	<b>Tree Of Heaven</b>	<b>Tree</b>
<i>Albizia julibrissin</i>	Mimosa, Silk-Tree	Tree
<b><i>Alliaria petiolata</i></b>	<b>Garlic Mustard</b>	<b>Ground Cover</b>
<b><i>Berberis thunbergii</i></b>	<b>Japanese Barberry</b>	<b>Bush</b>
<i>Cichorium intybus</i>	Chicory	Flower
<i>Cleome hassleriana</i>	Spiderflower	Flower
<i>Commelina communis</i>	Asiatic Day-Flower	Flower
<b><i>Coronilla varia</i></b>	<b>Crown Vetch</b>	<b>Ground Cover</b>
<i>Daucus carota</i>	Queen Anne's Lace, Wild Carrot	Flower
<b><i>Elaeagnus umbellata</i></b>	<b>Autumn Olive</b>	<b>Bush</b>
<i>Glechoma hederacea</i>	Ground-Ivy	Vine
<i>Hedera helix</i>	English Ivy	Vine
<i>Hemerocallis fulva</i>	Common Day Lily	Flower
<b><i>Lonicera japonica</i></b>	<b>Japanese Honeysuckle Vine</b>	<b>Vine</b>
<i>Lotus corniculatus</i>	Birdsfoot Trefoil	Flower/Herb
<i>Lysimachia nummularia</i>	Moneywort	Vine/Ground Cover
<b><i>Lythrum salicaria</i></b>	<b>Purple Loosestrife</b>	<b>Tall Herb/Wildflower</b>
<b><i>Microstegium vimineum</i></b>	<b>Japanese Stiltgrass</b>	<b>Grass</b>
<i>Myriophyllum aquaticum</i>	Parrot's Feather	Aquatic/Submerged
<i>Myriophyllum spicatum</i>	European Water-Milfoil	Aquatic/Submerged
<i>Najas minor</i>	Eutrophic Water-Nymph	Aquatic/Submerged
<i>Paulownia tomentosa</i>	Princess-Tree	Tree
<b><i>Phyllostachys aurea</i></b>	<b>Bamboo</b>	<b>Grass</b>
<b><i>Polygonum cuspidatum</i></b>	<b>Japanese Knotweed</b>	<b>Tall Herb</b>
<b><i>Polygonum perfoliatum</i></b>	<b>Mile-A-Minute</b>	<b>Vine</b>
<i>Potamogeton crispus</i>	Curly Pondweed	Aquatic/Submerged
<b><i>Pueraria lobata</i></b>	<b>Kudzu Vine</b>	<b>Vine</b>
<b><i>Rosa multiflora</i></b>	<b>Multiflora Rose</b>	<b>Bush</b>
<i>Rubus phoenicolasius</i>	Wineberry	Bush
<i>Trifolium pratense</i>	Red Clover	Herb/Flower
<i>Urtica dioica</i>	Stinging Nettle, Common Nettle	Herb
<i>Verbascum blattaria</i>	Moth Mullein	Flower
<i>Verbascum thapsus</i>	Great Mullein	Flower

Note: The plants of major concern are highlighted in Bold and already given as options on the form.

**PAGES 5, 6, 5a, and 6a****USEPA's Rapid Visual-Based Habitat Assessment**

The Visual-Based Habitat Assessment approach (VBHA) used in this protocol is adapted from USEPA's Rapid Bioassessment approach and refined from various applications across the country (*see Figure 2-27 on next page*). 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.

Refer to the following references for further information about the Rapid Bioassessment Protocols and Visual-Based Habitat Assessment:

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. <http://water.epa.gov/scitech/monitoring/rsi/bioassessment/download.cfm>

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. <http://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=9100LGCA.txt>

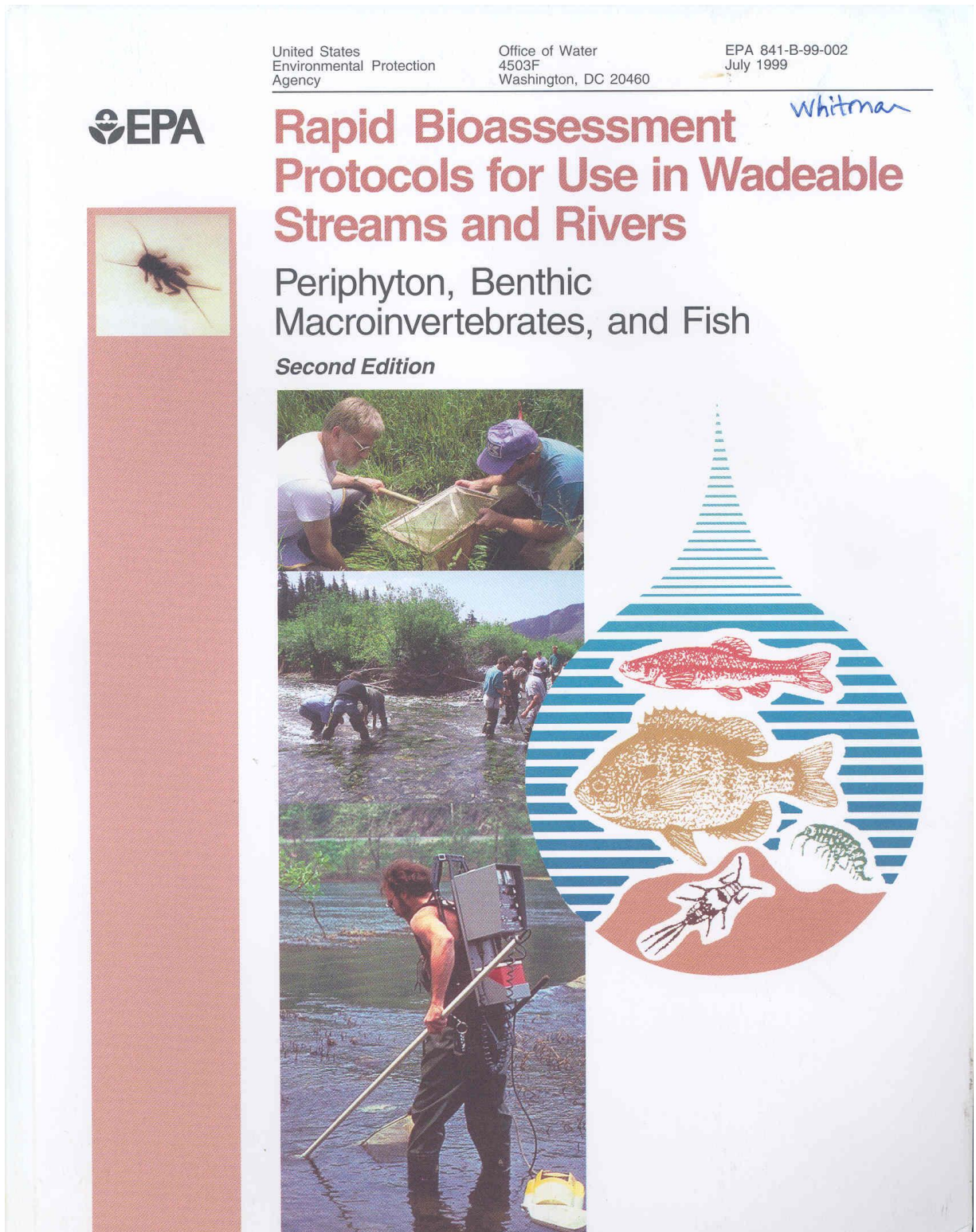


Figure 2-27. Cover of USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Second Edition)

**USEPA's Rapid Visual-Based Habitat Assessment Form**

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<sup>2</sup> of substrate. Accordingly, fill out the **riffle/run** Rapid Habitat assessment form (see **Figure 2-28 and Figure 2-36 on pages 2-69 & 2-75**). A glide/pool habitat form should only be used when the MACS sampling method is used (see **Figure 2-43 and Figure 2-44 on pages 2-81 & 2-83**).

**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 not be sampled by MACS methods. Riffle/run protocols should be followed (i.e., benthic samples should be collected by kicking the sand). Also, MACS methods should only be used if there are enough good habitats to collect all 20 jabs/sweeps.*

**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.



Riffle/Run Prevalence

<b>RAPID HABITAT ASSESSMENT: RIFFLE/RUN</b>						Reviewers Initials																										
AN-Code			Date																													
HABITAT PARAMETER	CATEGORY																															
	Optimal		Sub-optimal		Marginal		Poor																									
<b>1. EPIFAUNAL SUBSTRATE/ AVAILABLE FISH COVER</b>	<b>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.)</b>								<b>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).</b>								<b>20 to 40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.</b>								<b>Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.</b>							
SCORE:	20 19 18 17 16		15 14 13 12 11		10 9 8 7 6		5 4 3 2 1 0		<b>Consider Benthic Macroinvertebrate Substrate and Fish Habitat on Page 7 before rating here.</b>																							
<b>2. EMBEDDEDNESS</b>	<b>Gravel, cobble and boulder particles are between 0 and 25% surrounded by fine sediment; layering of cobble provides a diversity of niche space.</b>								<b>Gravel, cobble, and boulder particles are between 25 and 50% surrounded by fine sediment.</b>								<b>Gravel, cobble and boulder particles are between 50 and 75% surrounded by fine sediment.</b>								<b>Gravel, cobble and boulder particles are over 75% surrounded by fine sediment.</b>							
SCORE:	20 19 18 17 16		15 14 13 12 11		10 9 8 7 6		5 4 3 2 1 0		<b>Rate in Riffles or Runs Remember to consult with Biomorph</b>																							
<b>3. VELOCITY/ DEPTH REGIMES</b>	<b>Slow (&lt;0.3 m/S or ≈1 FT/S); deep (&gt;0.5 m or ≈1.5 FT); slow, shallow (&lt;0.5 m); fast (&gt;0.3 m/S); deep; fast, shallow habitats all present.</b>								<b>Only 3 of 4 habitat types present (if fast-shallow is missing, score lower than if other types are missing).</b>								<b>Only 2 of the 4 habitat types are present (if fast-shallow or slow-shallow are missing, score low).</b>								<b>Dominated by one velocity/depth regime (usually slow-deep).</b>							
SCORE:	20 19 18 17 16		15 14 13 12 11		10 9 8 7 6		5 4 3 2 1 0																									
<b>4. CHANNEL ALTERATION</b>	<b>Channelization or dredging absent or minimal; stream with normal pattern.</b>								<b>Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e. dredging, (&gt; than past 20 years) may be present, but no evidence of recent channelization.</b>								<b>Channelization may be extensive; embankments or shoring structures present on both banks; and 40% to 80% of the stream reach channelized and disrupted.</b>								<b>Banks shored with gabion or cement; over 80% of the stream reach is channelized and disrupted. Instream habitat greatly altered or entirely removed.</b>							
SCORE:	20 19 18 17 16		15 14 13 12 11		10 9 8 7 6		5 4 3 2 1 0																									
<b>5. SEDIMENT DEPOSITION</b>	<b>Little or no enlargement of island or point bars and less than 5% of the bottom is affected by sediment deposition.</b>								<b>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.</b>								<b>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.</b>								<b>Heavy deposits of fine material; increased bar development; more than 50% of bottom is changing frequently; pools almost absent due to substantial sediment deposition.</b>							
Upstream Watershed Total																																
Score:																																
SCORE:	20 19 18 17 16		15 14 13 12 11		10 9 8 7 6		5 4 3 2 1 0		<b>Rate in Pools &amp; Depositional Areas</b>																							

Page 5 WVDEP WAB Wadeable Stream Assessment Form (4/18/2012)

Figure 2-28. 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 (*see Figure 2-29 below*).



Figure 2-29. Examples of good fish habitat. Left shows tree roots hanging over the bank that provide good cover for fish in the stream. Right shows a pool with submerged logs and overhanging branches

**NOTE:** The Benthic Macroinvertebrate Substrate and Fish Habitat parameters at the top of *PAGE 7-Non-RBP Parameters* should be considered when rating this parameter as the combination of the two is essentially the same as this 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 (*see Figure 2-30 and Figure 2-31 on next page*). 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.



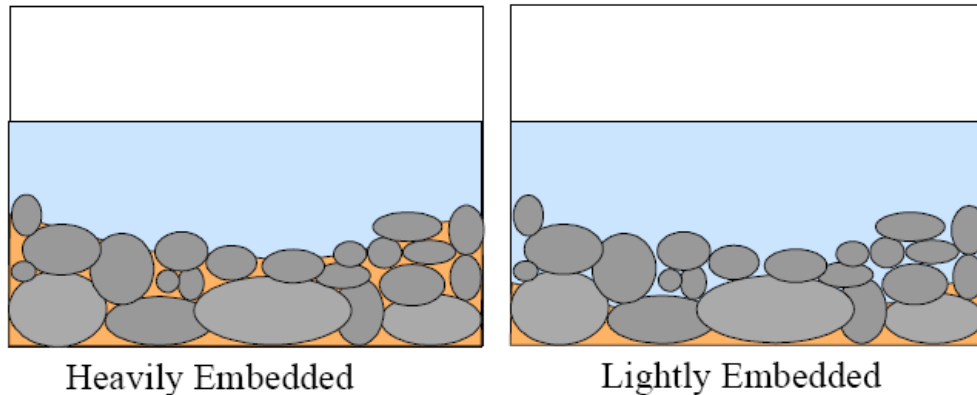


Figure 2-30. Visualization of Embeddedness.



Figure 2-31. Example of cobble that is partially embedded.

**NOTE:** 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. Clean Bedrock is not considered embedded!

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 for significant distances; when dams and bridges are present; and when other such changes have occurred (*see Figure 2-32 below and Figure 2-33 on the next page*). 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.



Figure 2-32. Examples of varying degrees of Channel Alteration (concrete channel on left and rip/rap channel on right).

**NOTE:** 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.





Figure 2-33. Example of Channel Alteration actively occurring due to an installation of sewage pipes across a stream channel.

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 (*see Figure 2-34 and Figure 2-35 on next page*); shoals; or results 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.

**NOTE:** To avoid confusion with **EMBEDDEDNESS** (habitat parameter number 2), observations of sediment deposition should be taken in pools and slow water depositional areas.





**Figure 2-34. Examples of Sediment Deposition.**



**Figure 2-35. Example of sediment deposition. Note the large amount of sand on the bank.**





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 (**see Figure 2-37 below**). 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.



Figure 2-37. Examples of streams with poor Channel Flow Status.

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 (**see Figure 2-38, Figure 2-39 and Figure 2-40 on the following pages**). 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.

***IMPORTANT:*** 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.





**Figure 2-38. Erosion is slight to moderate because roots are holding the bank intact.**



**Figure 2-39. Example of an unstable bank. Note the lack of trees (and roots) to provide soil stabilization.**





Figure 2-40. Example of heavy erosion (photo right).

9. **BANK VEGETATIVE PROTECTION**: measures the amount of the stream bank that is covered by ***native 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 (see Figure 2-41 on next page).** 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. **This parameter does not evaluate the same area as the Width of Undisturbed Vegetation Zone parameter below!** 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 ungrazed/un-mowed). *The native/non-native/invasive status of the vegetation must also be considered since non-native plants generally do not perform as well as native vegetation in erosion control or uptake of nutrients and should be rated lower than an equivalent coverage of native vegetation.* 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.





Figure 2-41. Examples of poor bank vegetative protection.

***IMPORTANT: This parameter is scored by considering right and left banks separately throughout the entire 100 m assessment area.***

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) (see **Figure 2-42 below**).



Figure 2-42. Examples of good width of undisturbed vegetation zone on left and poor on the right. Notice the rip-rap in the left photo that would increase the bank stability score but decrease the channel alteration score.

***IMPORTANT: This parameter does not evaluate the same area as the Bank Vegetative Protection parameter! For WAB assessments, this parameter is measured from the crest-over at top of bank out into the flood plain and adjacent areas.***

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. 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.

***IMPORTANT: This parameter is scored by considering right and left banks separately throughout the entire 100 m assessment area.***

***TOTAL:*** Total all of the scores for a final RBP score from 0-200. ***See Table 2-6 below for the Total RBP Score Categories.***

Table 2-6. Total RBP Score Categories

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



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.**
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 2-6 on page 2-80 for the Total RBP Score Categories.**

**PAGE 7**

**Non-RBP Parameters**

BENTHIC & FISH HABITAT, AESTHETIC, & REMOTENESS RATINGS & EXTRA SPACE >>>					Reviewers Initials																
AN-Code				Date																	
PARAMETER	Optimal					Sub-optimal					Marginal					Poor					
BENTHIC MACRO-INVERTEBRATE SUBSTRATE	Preferred substrate <b>abundant</b> , stable, & at full colonization potential (riffles well developed & <b>dominated by cobble</b> ; substrate <b>not</b> new or <b>transient</b> ).					Substrate <b>adequate</b> for maintenance of populations; <b>abundance of cobble</b> with coarse gravel &/or boulders <b>common</b> ; small areas of new &/or <b>transient</b> substrate particles (sand and fine gravel) <b>may be present</b> .					Preferred substrate <b>uncommon</b> ; <b>some cobble</b> present but gravel or large boulders & bedrock <b>prevalent</b> ; <b>transient</b> substrate areas may be <b>frequent</b> .					Preferred substrate <b>virtually absent</b> ; gravel or large boulders & bedrock <b>dominant</b> ; <b>transient</b> areas may be <b>dominant</b> .					
	<b>Remember to consult with Biomorph Rate for entire reach even if the reach is not representative of benthic sample area</b>																				
SCORE:	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
FISH HABITAT	Variety of <b>stable fish habitat</b> is available in <b>≥ 75%</b> of the reach: boulders, undercut banks, woody debris, submerged roots and trees, macrophytes, overhanging veg. (<1m from water surface), filamentous algae, and artificial structures					Fish habitat available in <b>40 to 75%</b> of reach; adequate for maintenance of populations; small, <b>unstable or transient</b> areas <b>present</b>					Preferred habitat less common, available in <b>10 to 40%</b> of reach; <b>featureless</b> and/or <b>unstable</b> areas <b>more common</b>					<b>Less than 10%</b> of reach with stable, usable habitat; <b>dominated by featureless</b> and/or <b>transient</b> areas					
	<b>Score lower if dominated by filamentous algae or artificial structures</b>																				
SCORE:	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	0
TRASH INDEX	<b>Little or no</b> evidence of <b>human refuse</b> present.					Human refuse present in <b>small amounts</b> .					Human refuse present in <b>Moderate amounts</b> .					Human refuse <b>abundant and unsightly</b> .					
	SCORE:	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
REMOTENESS RATING	Stream assessment site <b>more than ¼ mile</b> from nearest Road; access difficult and little or <b>no</b> evidence of <b>human disturbance</b> .					Stream assessment site <b>within ¼ mile</b> of roadside; site with <b>moderately wild</b> character.					Stream <b>within ¼ mile</b> of roadside; <b>development</b> activities <b>evident</b> .					Segment <b>immediately adjacent</b> to roadside access; visual, olfactory, and/or auditory <b>displeasure experienced</b> .					
	SCORE:	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

Figure 2-45. 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 similar to 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 (e.g., 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 not 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.

***IMPORTANT: 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.***

***FISH HABITAT:*** This parameter measures the quality of the fish habitat throughout the assessment reach. ***This measure is similar to the Available Fish Cover half of the Epifaunal Substrate/Available Fish Cover PAGES 5, 6, 5a, and 6a. Only the fish habitat quality should be considered with this parameter.*** The fish habitat is essentially the amount of stable habitat (e.g., boulders, undercut banks, woody debris, submerged roots and trees, macrophytes, overhanging vegetation, filamentous algae, and artificial structures) available for fish to use as refuge from predators, breeding and feeding grounds. The greater the diversity and abundance of available habitats translates into a greater diversity and abundance of fish that the stream could support.

***IMPORTANT: Rate this parameter for the entire reach which is 100m in most cases, but may be longer if an actual fish sampling event is occurring (see CHAPTER 6. FISH COLLECTION PROTOCOLS Establishing the Sample Reach on page 6-4).***

***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?	<input type="checkbox"/> Yes <input type="checkbox"/> 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).	<input type="checkbox"/> Sediment	<input type="checkbox"/> Fecal	<input type="checkbox"/> Nutrients
	<input type="checkbox"/> Metals	<input type="checkbox"/> pH	<input type="checkbox"/> Sulfate
	<input type="checkbox"/> Conductivity <input type="checkbox"/> 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.	<input type="checkbox"/> Impoundment: <input type="checkbox"/> Lake <input type="checkbox"/> Ag Pond <input type="checkbox"/> Mining Pond		
	<input type="checkbox"/> Flood Control <input type="checkbox"/> Beaver <input type="checkbox"/> Instream Pool		
	<input type="checkbox"/> Concrete Low Water Ford/Bridge		
	Impoundment Release Type: <input type="checkbox"/> Bottom <input type="checkbox"/> Spillover		
	<input type="checkbox"/> Valley Fill: <input type="checkbox"/> Mining		
	<input type="checkbox"/> 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 2-46. Example of the PRS and Stressor Info section (Middle of PAGE 7) of the field form

**Is Site a Potential Reference?:** Answer **Yes** or **No**. Consider the Water Chemistry, Benthos, Habitat, Human Disturbance, Location (e.g., Ecoregion), Level I vs. Level II Reference Condition, etc. **Refer to Determining Candidate Reference Sites While In the Field under CHAPTER 2. Section A. Part 2. Determining Candidate Reference Sites While In the Field starting on page 2-18 for more information.**

**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:** A yes answer here 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 CHAPTER 2. Section A. Part 2. Determining Candidate Reference Sites While In the Field starting on page 2-18 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 2-47 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”).”

<p><b>EXTRA SPACE FOR SPILL-OVER COMMENTS AND NOTES BELOW.</b> 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 &amp; 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”).</p>

Figure 2-47. 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. INVASIVE PLANT SPECIES SHOULD BE NOTED ON PAGE 4 IN THE RIPARIAN VEGETATION SECTION, REMEMBER TO DOCUMENT ANY SPECIMENS COLLECTED FOR OTHER GROUPS HERE! See Figure 2-48 below for an example of this section of the form.**





Table 2-7. Some examples of Invasive fauna in WV.

Scientific Name	Common Name	Type
<i>Corbicula fluminea</i>	Asiatic Clam	Mollusca
<i>Dreissena polymorpha</i>	Zebra Mussel	Mollusca
<i>Lymantria dispar</i>	Gypsy Moth	Caterpillar
<i>Agilus planipennis</i>	Emerald Ash Borer	Beetle
<i>Adelges tsugae</i>	Hemlock woolly adelgid	Aphid-like

Observed: The initials of the observer.

Did you see fresh water mussels?: Answer **Yes** or **No**.

Alive or Dead?: Answer **Alive** or **Dead**.

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

**Trout Observations (For Sites that are not actively being sampled for Fish!)**

Trout Observations (For Sites that are not actively being sampled for Fish!)						
Did you see trout?	<input type="checkbox"/> Yes <input type="checkbox"/> 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 2-49. Example of the Trout Observations (Non-Fish Sites) section (Bottom of PAGE 8) of the field form

***IMPORTANT: This section is only for documenting passive observations of trout (e.g., casual sightings, trout that were inadvertently caught in the benthic net, etc.) and not for use with any sort of active fish sampling activities (e.g., electrofishing, netting, etc.). All data from active fish sampling activities are documented via a different protocol and set of forms. See Figure 2-49 on the next page for an example of this section of the form.***

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.

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.

***IMPORTANT: All photo description information should still be entered on PAGE 12-Photography Log (see page 2-108). This field just links a photo to an individual or group of specimens.***



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, overabundance 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. **Use PAGES 10 & 11 to document the benthic macroinvertebrates found in the sample!!! Please note any fish, trout, or salamanders released from the benthic sample on PAGE 8-Wildlife & Freshwater Mussel Observations!!!!** Comment on the abundances and diversity of the organisms captured/observed and the overall benthic quality of the site.

### **Benthic Substrate Sample Composition**

Inorganic Substrate Components: Provide a visual estimate of the relative proportion of each of the seven particle types listed in **Figure 2-51 below**.

***IMPORTANT: 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<sup>2</sup> riffle/run area that was sampled using the following scale provided in **Figure 2-51 below**.

Inorganic Substrate (1m <sup>2</sup> 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 <u>relative sizes</u> (e.g., small-sized vs. large-sized cobble or boulders), <u>shapes</u> (globular vs. flat vs. angular), <u>texture</u> (e.g., rough vs. smooth bedrock), <u>layering</u> (i.e., was the cobble stacked) and <u>embeddedness</u> (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 2-51. Example of the Benthic Substrate Sample Composition section (Middle of PAGE 9) of the field form

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

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 (e.g., 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: 0=None, 1=Low, 2=Moderate, 3=High, 4=Extreme, NR=Not Rated	Periphyton ( <i>Brown-slick; Diatoms</i> )	Filamentous Algae ( <i>Green; Long</i> )	Blue-Green Algae ( <i>Blueish-Green Slime; Not Long</i> )	Aquatic Mosses
	Submerged Aquatic Plants (e.g., <i>Stargrass, Hydrilla</i> )	Emergent Aquatic Plants (e.g., <i>Water Willow</i> )	Floating Aquatic Plants (e.g., <i>Lily Pads, Duckweed</i> )	Total Aquatic Plants
Periphyton/Algae/Aquatic Plants & Mosses Notes:				

Figure 2-52. Example of the Visual Estimation of Periphyton & Aquatic Plant Density section (Middle of PAGE 9) of the field form



Periphyton are 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. Although generally included in the broad class of periphyton, filamentous algae (macroalgae) and blue-green algae (cyanobacteria) will be rated separately from the (microalgae) in this section. Note: during Periphyton collection, all three of these groups are collected together (see CHAPTER 7. PERIPHYTON COLLECTION PROTOCOLS. starting on page 7-1).

Aquatic plants are generally associated with larger streams such as the New River and Cacapon River. Stargrass and Water Willow are examples that would be included in the aquatic plant category. Aquatic plants provide stream habitat for both macroinvertebrates and fish as well as being a sink for nutrients found in the water column (*i.e.*, the aquatic plants can absorb and fix excess nutrients). The roots of aquatic plants can also provide some level of stabilization to fine sediments.

Indicate Abundance of each Periphyton and Aquatic Plant category:

Indicate the abundance of each in the stream assessment area as **0-None, 1- Low, 2-Moderate, 3-High, 4-Extreme, and NR-Not Rated. See Figure 2-52 above for an example of this section of the form.**

**Periphyton (Brown-slick; Diatoms):** In this section of the WAB assessment, “periphyton” will include only the microalgae (*e.g.*, diatoms). These are the microscopic organisms that make the substrate slick and slimy. They usually leave a brownish-yellow stain on your hand when rubbed. **See Figure 2-53, Figure 2-54, Figure 2-55, and Figure 2-56 on the following pages and on page 2-96 for examples of Periphyton.**



**Figure 2-53. Example of heavv periphvton coating substrate.**

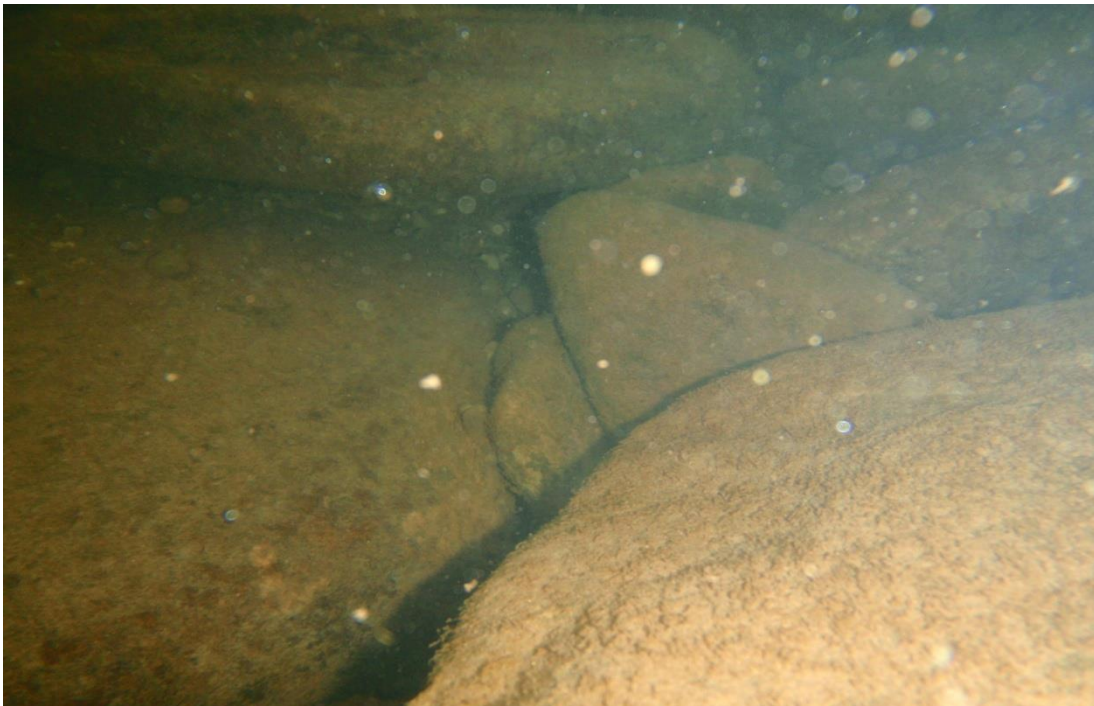


**Figure 2-54. Example of Periphyton (Diatoms) on rocks removed from the water column for sampling (Dark Circles are where the Periphyton has been sampled).**





**Figure 2-55. Example of Periphyton growing on rock.**



**Figure 2-56. Underwater view of periphyton growing on substrate. Note that some of material is silt that is trapped by the periphyton.**

### **Filamentous Algae (Green; Long):**

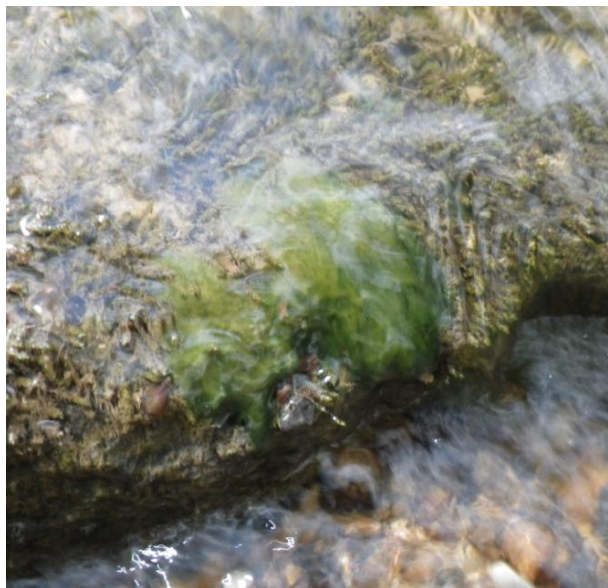
Filamentous algae include the long stringy algae that are green in coloration and exhibit wavy undulations in stream currents. Sometimes filamentous algae are associated with low pH, high iron mine seeps and discharges (**See Figure 2-57 on right**). Other times, it is found in larger streams with a more open canopy and sun exposure that allows for its growth. In the case of larger rivers,



**Figure 2-57. Ferraphylic (meaning Iron-loving) filamentous algae are found in low pH streams with high iron concentrations.**

filamentous algae may coincide with an abundance of submerged aquatic vegetation (**See Figure 2-60 on page 2-99 for an example**). Excessive Filamentous Algae can become a nuisance and impede recreational use like fishing, swimming, and boating. **See CHAPTER 8. FILAMENTOUS ALGAE MONITORING and Figure 8-7 thru Figure 8-19 on pages 8-9 thru 8-15 for further examples of varying degrees of Filamentous Algae coverage.**

**Blue-Green Algae (Blueish-Green “Slime”; Not Long):** Also known as Cyanobacteria; a photosynthesizing group of bacteria (**see Figure 2-58 on right & Figure 2-59 on next page**). Colonies can be found as short filaments, sheets or hollow balls.



**Figure 2-58. Close-up of Blue-Green Algae growing on rock. Notice the absence of long**





Figure 2-59. Example of Blue-Green Algae (edges) and Periphyton growing on rock.

**Aquatic Mosses:** 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.

**Submerged Aquatic Plants (e.g., Stargrass, *Hydrilla* or Waterweed):** Aquatic vegetation that has stems and leaves that grow entirely underwater. These plants are adapted to be permanently submerged. However, in some cases of drought, what may be typically called submerged aquatic plants appear to be emergent or floating due to the lower than normal flows. **See Figure 2-60 and Figure 2-61 on the next page for examples of Submerged Aquatic Plants.**





**Figure 2-60. Underwater view of Submerged Aquatic Vegetation. Note the Filamentous Algae growing among the submerged vegetation.**



**Figure 2-61. Example of Submerged (Dark Green is a *Hydrilla* sp.) and Floating (Light Green is Duckweed) Aquatic Vegetation.**



**Emergent Aquatic Plants (e.g., Water Willow):** Aquatic vegetation that is rooted in the stream or lake bottom, but the leaves and stem extends out of the water. Cattails and, in some cases, purple loosestrife (an invasive aquatic plant) are examples. **See Figure 2-62 below, Figure 2-63 & Figure 2-64 on the next page and Figure 2-65 on page 2-102 for examples of Emergent Aquatic Plants.**



**Figure 2-62. Example of Emergent Aquatic Plants (Water Willow) growing in backwater and gravel bars.**



**Figure 2-63. Example of Emergent Aquatic Plants (Cattails) adjacent to the stream channel.**



**Figure 2-64. Emergent Aquatic Plants (Cattails) surrounding a mining discharge.**



**Floating Aquatic Plants (e.g., Lily Pads, Duckweed):** Aquatic vegetation that are rooted in the stream or lake bottom, but their leaves and flowers float on the water's surface. *See Figure 2-60 on page 2-99 and Figure 2-65 below for examples of floating aquatic vegetation.*



Figure 2-65. Examples of Floating Aquatic Plants (i.e., Lilly pads) and Emergent Aquatic Plants (i.e., Cattails) on a lake.

**Total Aquatic Vascular Plants:** Combine the three subtypes of aquatic plants into an overall rating.

***IMPORTANT:*** *This should not be done in an additive fashion (i.e., a 2 rating for emergent and submerged aquatic vegetation does not equal a 4 rating for Total Aquatic Vascular Plants.*

**Periphyton Collection Information**

Periphyton sample collected?		<input type="checkbox"/> Yes <input type="checkbox"/> 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)					<input type="checkbox"/> Yes <input type="checkbox"/> 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 2-66. Example of the Periphyton Collection Information section (Bottom of PAGE 9) of the field form

**NOTE:** This section has been removed from PAGE 9 starting April 2011 due to a lack of Periphyton Collection in the last few years. Should Periphyton Collection resume, this section will be added back to 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**.

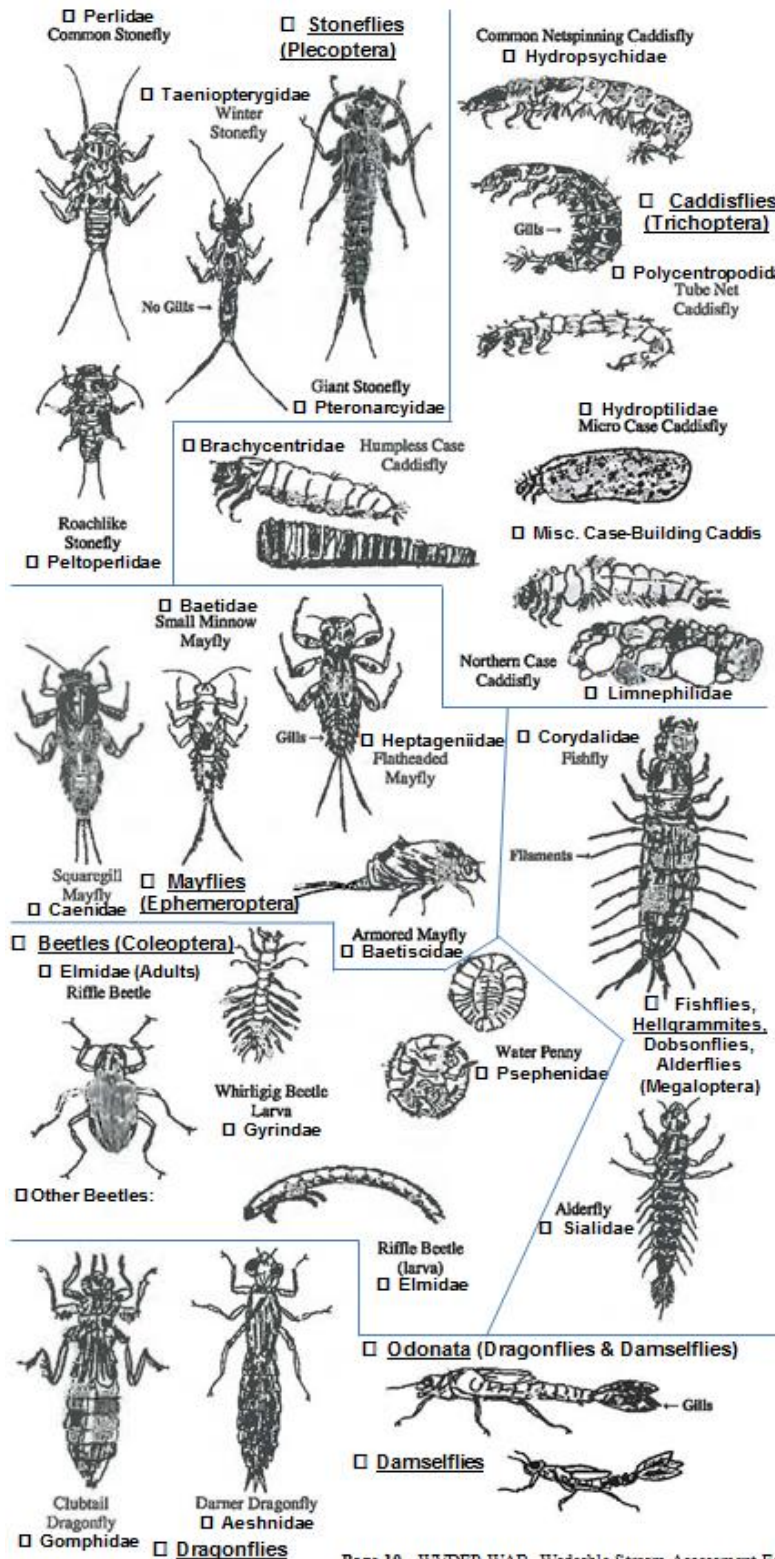
Periphyton Sample Notes: Use the space below to describe the Periphyton sample. Explain any variances from the collection protocol that may affect comparability. Was the substrate stable and undisturbed? Could the substrate have been scoured? Dry?

**PAGE 10 & 11**

**A Visual Guide to Stream Macroinvertebrates**

Document the benthic macroinvertebrates found in the sample by putting check marks next to the names/drawings of the organisms (see **Figure 2-67 & Figure 2-68** below).





## A Visual Guide to Stream Macroinvertebrates

### Insect Groups

**Stoneflies (order Plecoptera)** "very sensitive": Two pairs of legs (6); 2 hooked claws at the end of each leg; no gills on the abdomen but many gills on the legs and thorax; 2 tails and 2 long antennae.

**Mayflies (order Ephemeroptera)** "sensitive": Two pairs of legs (6); one hooked claw at the end of each leg; gills on the abdomen (may be covered by plates); 2 or 3 tails and 2 short antennae.

**Caddisflies (order Trichoptera)** "sensitive": Two pairs of legs (6); segmented grub-like body; may have gills along lower and upper portions of the abdomen; small hair-like tails or hooks. Some kinds may be enclosed in a case that they construct using stream bottom materials such as pebbles, sand grains, woody debris, pieces of plant material or some combination; others construct nets, and others do neither (free-living caddisfly). The case builders often construct a specific case that can sometimes be used in their identification. The common netspinning caddisfly (family *Hydropsychidae*) is more tolerant than most of the group so they are placed into the less sensitive category on the stream survey. The family *Hydropsychidae* can be distinguished from other caddisflies by the abundant gills on the underside of their body.

**Beetles (order Coleoptera)** "less sensitive": Two pairs of legs (6); mainly rounded or oval shape as adults; a few kinds have tails hooks or filaments, hard bodies and visible wing-pads. The most commonly encountered beetles are the riffle beetle (family *Elmidae*), which is a small dark beetle and water penny (family *Psephenidae*), which looks like a penny. The whirligig beetle larva (family *Gyrinidae*) may have many filaments along their bodies similar to fishflies.

**Fishflies, Dobsonflies, Alderflies (order Megaloptera)** "less sensitive": Two pairs of legs (6); filaments along the body starting just below the legs; variable tails at the end of the abdomen.

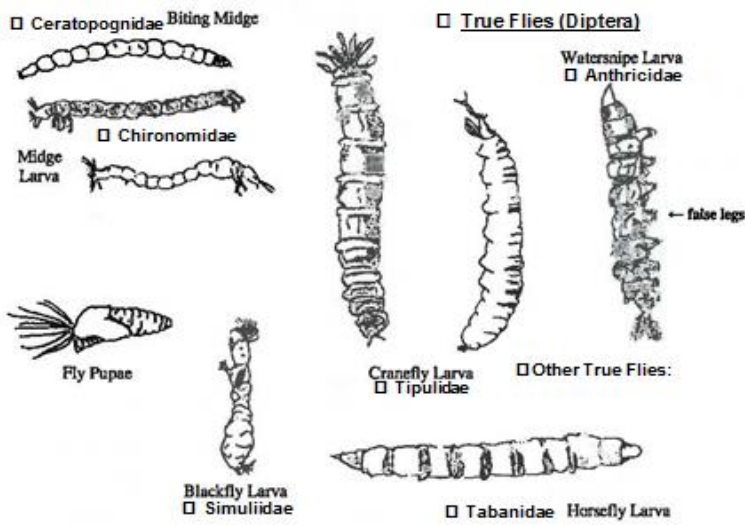
Alderflies (family *Sialidae*) have a long tapered tail; dobsonflies "also called hellgrammites" and fishflies have hooked-tails (family *Corydalidae*); dobsonflies have gill-tufts under each of their filaments, fishflies and alderflies do not. All members of the group have large pinching jaws on the head.

**Damselflies (sub order Zygoptera)** "tolerant": Two pairs of legs (6); long, thin abdomen; large eyes; extended lower lip; 3 fan like structures, which are actually their gills, at the end of the abdomen.

**Dragonflies (sub order Anisoptera)** "less sensitive": Two pairs of legs (6); extended lower lip; large eyes; rounded or extended abdomen; no gills on the abdomen; no tails but may have knobs or points on the abdomen that resemble tails.

Illustrations by Tim Craddock

Figure 2-67. Example of the Visual Guide to Stream Macroinvertebrates section (PAGE 10) of the field form



**Insect Groups Continued**

**True Flies (order Diptera)** "less sensitive to tolerant": No legs or may have structures that look like legs (false-legs); mainly segmented grub-like or worm-like bodies; tiny hair-like tails, lobes, tentacles or other structures at the end of their abdomen (or no tails); often a distinct head can be seen, but on others no head is visible. Many different kinds of flies are encountered, the more common kinds include the crane flies (family *Tipulidae*), which have no legs, a plump segmented body and numerous tentacles; watersnipes (family *Athericidae*) have false legs and look like a caterpillar with a forked tail; midge fly larva (family *Chironomidae*) are very small with a thread-like body (some are red in color) and a very erratic wriggling motion; blackfly larva (family *Simuliidae*) have a bowling pin shape and fan-like structure on their head. There are many other kinds of Diptera larva that are sometimes collected, but only a few more examples are illustrated here.

**Non-Insect Groups**

**Operculate Snails (class Gastropoda)** "less sensitive": Single coiled shell that mostly opens to the right when the point is held facing towards you. These snails have an operculum "a door that shuts the shell" and are commonly known as gilled snails.

**Non-Operculate Snails (class Gastropoda)** "tolerant": Single shell that on some kinds opens to the left when the point is held facing towards you; shells also may be rounded flat or coiled. These snails do not have an operculum and are commonly known as pouch or pond snails.

**Clams and Mussels "less sensitive"** (class *Pelecypoda*): Two cup-shaped shells connected by a hinged structure; the shell is made of calcium carbonate and is usually very strong and hard to open. Mussels (family *Uenoidae*) have an oblong rough, often dark color shell. Most clams are smaller and have a rounded shell. The Asian clam can be distinguished from the native pea clam (family *Sphaeriidae*) by the raised ridges; pea clams are often smaller and its shell feels smooth to the touch.

**Crustaceans "less sensitive to tolerant"**: More than two pairs (more than 6) of legs; claws on the first several pairs of legs may be enlarged; long antenna. This group includes the crayfish (family *Cambaridae*), which looks like a small lobster, scuds "also called sideswimmer" (order *Amphipoda*) resemble a shrimp, and the aquatic sowbugs (order *Isopoda*) resemble a pill bug. Freshwater shrimp (family *Palaemonidae*) are rarely encountered.

**Annelids "tolerant"**: Worm-like appearance; no legs and many segments along the entire length of the body. This group includes the aquatic worms (class *Oligochaeta*) and leeches (class *Hirudinea*). The suckers on both ends of their body distinguish the leeches from other annelids.

Illustrations by Tim Craddock

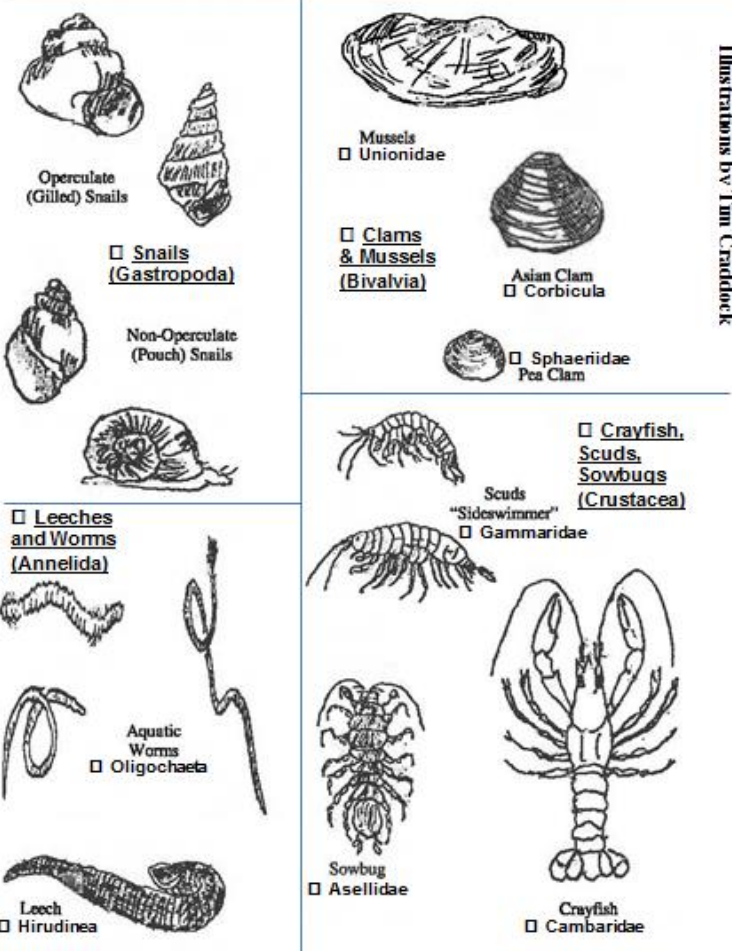


Figure 2-68. Example of the Visual Guide to Stream Macroinvertebrates section (PAGE 11) of the field form





phone).

Stream Data Requested?: Were the results from this sample requested by the landowner/stakeholder(s)? Check if **Yes**. Again, checking this box will not ensure prompt delivery of the stream data, so also use the **Landowner Data Request Card**.

Watershed Report Requested?: Was a future watershed report from the watershed being sampled requested by the landowner/stakeholder(s)? Check if **Yes**.

Other Pertinent Landowner Information (e.g., email) & Comments: A blank field is provided to document other pertinent landowner info (e.g., how to find the landowner/stakeholder). 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.

**Recon/Accessibility Information**

Discuss the accessibility to the site including accessibility, posted property, fenced, beside road, long walk over treacherous terrain, hike length, 4 x 4 needed, get key from landowner, etc.	
Check all that apply:	<input type="checkbox"/> Easy Access <input type="checkbox"/> Difficult Access <input type="checkbox"/> Private Property <input type="checkbox"/> Posted <input type="checkbox"/> Fenced <input type="checkbox"/> Gated <input type="checkbox"/> Get Key from Landowner <input type="checkbox"/> Beside Road <input type="checkbox"/> Short Hike <input type="checkbox"/> Long Hike <input type="checkbox"/> 4x4 Needed <input type="checkbox"/> Other (explain)
Recon/Accessibility Notes:	

Figure 2-70. Example of the Recon/Accessibility Information section (Top of PAGE 12) of the field form

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)**.

Recon/Accessibility Notes: A blank field is provided to discuss the accessibility of the site including elaborations on the Site Accessibility check boxes discussed above.

***IMPORTANT:*** 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".





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. If known, write down the AN-Code of the waterbody featured in the photo.

**NOTE: 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.**

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.

**NOTE: 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)

**NOTE:** 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 4. STREAM FLOW MEASUREMENT. See Figure 2-72 on the next page for an example of this section of the form.**

WQ Sample ID: The pre-assigned Water Quality Sample ID used with this sample (**see PAGE 1 Site Verification**). This may be used as a profile and file name if using a meter that records the measurement data electronically.

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 old 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.

Measurement: The number of the measurement.

Location Description: Record the Left and Right Descending Banks (LDB and RDB) and EEF (Left vs. Right) designations here. Also document if different channels were measured (Left vs. Middle vs. Right) for braided streams.

Distance: Record distance from one bank along the flow transect (using a measuring tape) where the flow measurement is occurring. Also indicate which ends of the measuring tape are the Left and Right Descending Banks (LDB and RDB) by checking the appropriate box.



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

Velocity: Record the velocity(s) at the point of the flow measurement. When the total depth is < or = 1.5 feet, then only one velocity measurement is taken at 0.4 of the depth from the stream bottom. When the depth is greater than 1.5 feet, two velocity measurements are taken at 0.2 and 0.8 of the depth from the stream bottom. In rare cases (>1.5 feet depth and obstructions on bottom or top of water column), all three may be taken.

Measurement Notes: Any information (e.g., an obstruction, soft substrates) regarding the individual flow readings at the give vertical (measurement).

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.

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? Flow Notes: Why it is believed the flow measurement is considered not comparable or any general information about the flow documentation as a whole.

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.

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

## **APPENDIX #2 - Stream Bank Erodibility and Channel Profile Measurements**

**NOTE:** 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 to twice during the course of 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 2-73 on the next page**) 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 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.

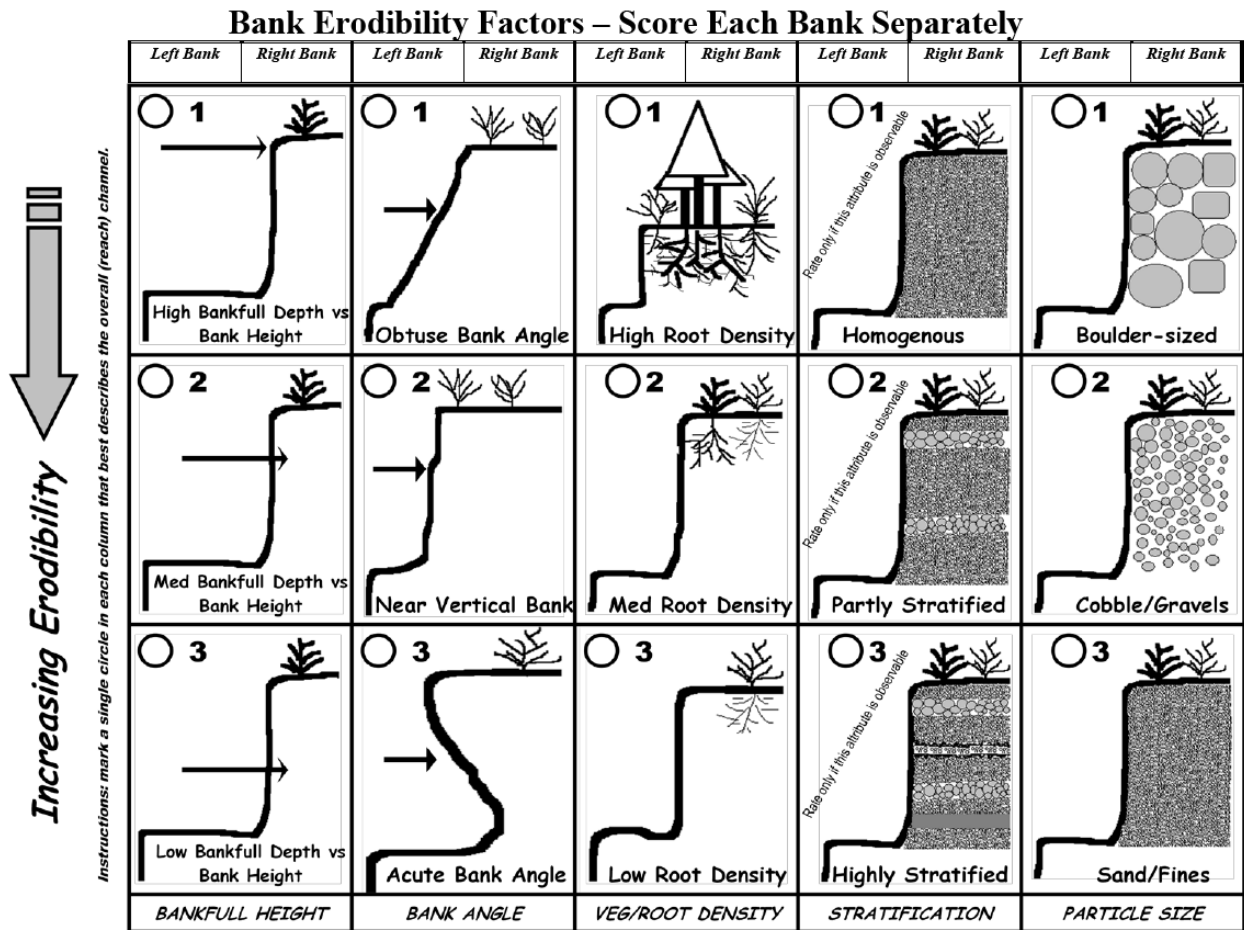


Figure 2-73. Example of the Stream Bank Erodibility Appendix field form

**IMPORTANT:** 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.

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 if 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 2-74 on page 2-116*).

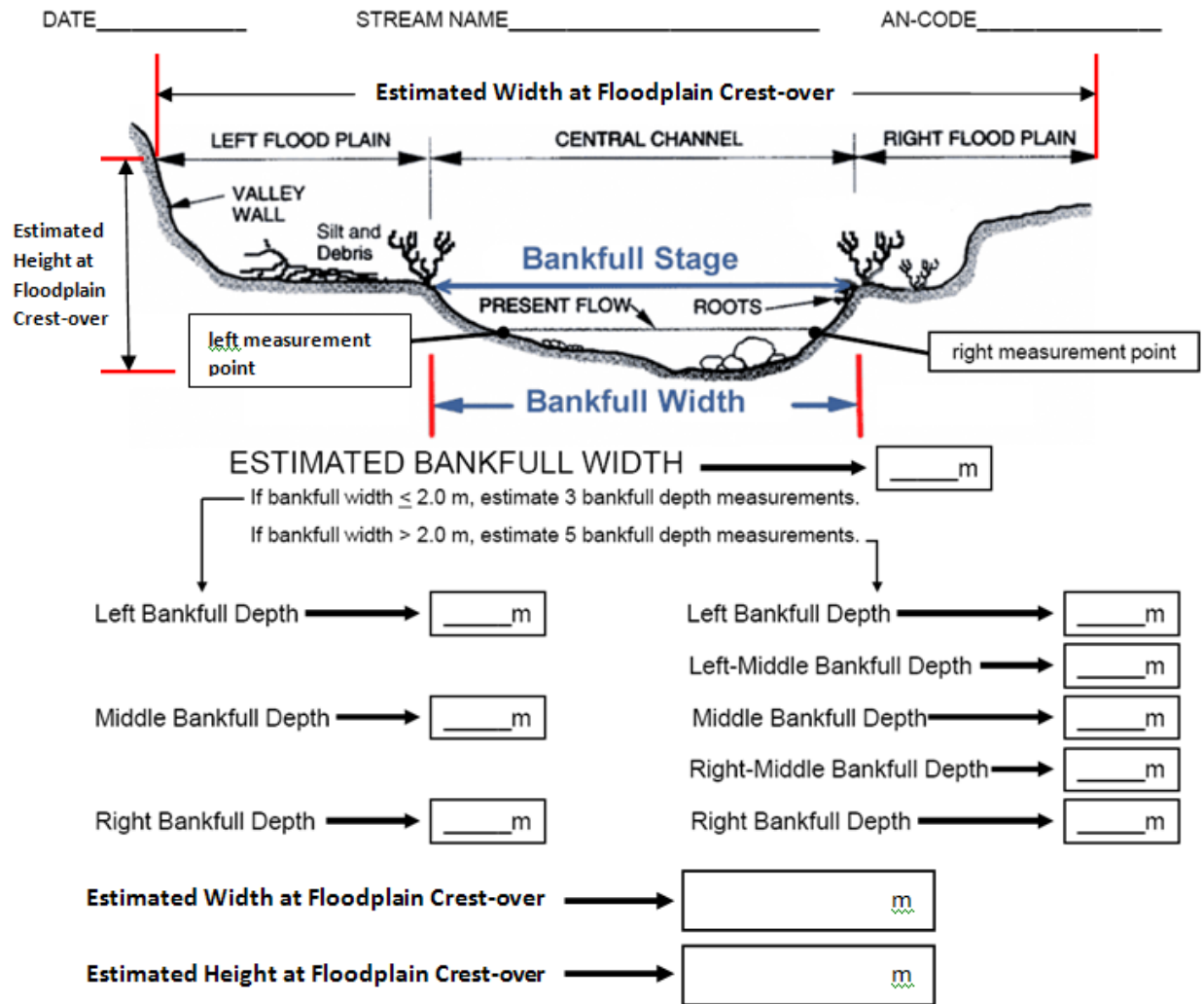


Figure 2-74. Example of the Channel Profile Measurements Appendix Field Form

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 down cutting 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 over steepened 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.

***IMPORTANT: Remember that the channel depth may be equal to the bankfull depth, (an indication that the stream channel is not incised or down cutting) but it should never be less than the bankfull height.***

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.

***IMPORTANT: 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  $\leq 2.0$  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**

**NOTE:** 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 2-75 on the next page 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.

**NOTE:** You only need to do this sketch if you are conducting a TMDL-Initial assessment concurrently with a Wadeable Benthic Assessment. See *CHAPTER 2. Section C. Part 1. PAGE 1-Site Verification on page 2-30 to contrast the needs of this coarse resolution sketch versus the detailed sketch for the Wadeable Benthic Assessment form.*





**APPENDIX #4 – Water Quality Profile**

**NOTE:** 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 2-76* 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 2-76. 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 °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  $\mu\text{mhos/cm}$ .

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

**APPENDIX #5 – Relative Bed Stability (Pebble Count) including Gradient**

**NOTE:** 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 2-77 on page 2-123 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 10. RELATIVE BED STABILITY/SUBSTRATE CHARACTERIZATION PROTOCOLS (INCLUDING GRADIENT)** on page 10-1 for instructions on completing this section.

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 2-77 on the next page.***

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

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





## 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 at wadeable benthic sites along with other activities at the designated duplicate sites **and must include the PAGE 1-Site Verification (minus the stream reach sketch), PAGES 5, 6, 5a, and 6a-USEPA's Rapid Visual-Based Habitat Assessment Form, PAGE 7-Non-RBP Parameters, and PAGE 9-Benthic Macroinvertebrate Collection Information and Benthic Substrate Sample Composition.** 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 14. Section A. Blanks and Duplicates starting on page 14-1 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\2015 Forms.zip

and externally via the web at:

<http://www.dep.wv.gov/WWE/watershed/Pages/WBSOPs.aspx>

**This page intentionally left blank.**

## CHAPTER 3. WATER COLLECTION PROTOCOLS

### Section A. *Water Quality Sondes & Sensors: Calibration, Routine Maintenance, & Use*

#### Overview

A sonde is a collection of multiple sensors and probes housed into one casing and designed to give readings on multiple parameters simultaneously (often referred to as a Multi-Parameter Sonde). Sondes can vary in length, diameter, and housing construction. The most common four sensors on sondes are Temperature, pH, Dissolved Oxygen, and Specific Conductance (typically housed in a 2 inch diameter casing). Larger sondes (e.g., 3.5 – 4 inch diameter casing) can be equipped with additional, larger sensors and probes (e.g., Depth, Chlorophyll-a, and Turbidity). On some sondes (e.g., YSI models) the sensors and probes can easily be changed or replaced by the user. On other sondes (e.g., Hydrolab models), the sensors and probes cannot be easily changed without opening the sonde housing and electronics (either by a very experienced user or by sending the instrument back to the manufacturer's service department).



Figure 3-1. Example of a YSI 660 XL Sonde (right) and a 650 MDS Display Unit (left).

Sensors and probes may utilize drastically contrasting technologies to measure a parameter. For example, one sonde may have a Dissolved Oxygen sensor that utilizes the smaller Clark-cell technology (it has a membrane) with a stirrer; another sonde may use a similar Clark-cell technology and not have a stirrer; yet another sonde may use the larger Luminescence (LDO) technology (no membrane). In this case, the different sensor/probe technologies respectively offer pros and cons based on size the accuracy of the reading and size of the sonde casing required to house the sensor.

Many sensors are reliant on other sensors to take accurate readings. For example, the pH, Dissolved Oxygen, and Specific Conductance sensors require the Temperature sensor to be working and accurately reading in order to make temperature dependent corrections. The Dissolved Oxygen sensor is also dependent on the Specific Conductance sensor. Some manufacturers have the multiple sensors bundled into one probe (e.g., YSI's Temperature/Conductivity Probes).

The Watershed Assessment Branch utilizes multiple brands (e.g., YSI, Hydrolab, Eureka, Onset, etc.) and models (e.g., 600XL, DS5x, Quanta, Manta, Tidbit) of sondes. Not all of the procedures described below will work or be applicable for all sondes (e.g., brand and/or model specific order in which buttons are pressed during calibration or operation). However, most of the procedures for calibration and maintenance (e.g., rinsing steps, calibration reagents and endpoints, QA/QC checks, and maintenance schedules) are universal across brands, models, and probes.

In addition, the Watershed Assessment Branch utilizes sondes that are only capable of discrete, instantaneous readings as well as sondes/data loggers that are equipped to programmed, deployed, and take time-series incremental or continuous readings. The Calibration and Maintenance procedures of both types are basically the same with the exception of some data loggers (e.g., Temperature, Temperature/Light, and Temperature/Conductivity Data Loggers). For more specific information about the Calibration, Maintenance, and Programing of these deployable sondes/data loggers refer to **CHAPTER 13. CONTINUOUS WATER QUALITY MONITORING PROCEDURES starting on page 13-1.**



## **Part 1. Sonde/Sensor Calibration, Maintenance, Diagnostics, and Accuracy**

The following are a basic overview of the procedures for the calibration, maintenance, diagnostics and accuracy of for an YSI 600XL or 600XLM Sonde/650 MDS display combination and a 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. Additional resources may be available on the internet (e.g., [HachHydromet.com](http://HachHydromet.com) has calibration instructional videos for the Hydrolab models of sondes).

**NOTE: The Hydrolab Quanta G directions are very similar to the older Scout 2 Hydrolab directions.**

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 as well as maintenance performed are documented on a permanent Sonde Calibration Sheet. 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 3-2 & Figure 3-3 on the following pages**).

## Full Calibration Log Sheet

Sonde ID: \_\_\_\_\_ Date: \_\_\_\_\_ Time: \_\_\_\_\_ Calibrator's Initials: \_\_\_\_\_  
 Battery Check (V): \_\_\_\_\_ Field User's Initials (if different from Calibrator): \_\_\_\_\_  
 Temperature (°C): \_\_\_\_\_ *Temperature must be recorded for all Calibrations  
 (even single probe calibrations)!*

### DO Probe Calibration

#### Routine Maintenance Checklist:

1. *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 it too old?*  Yes or  No
2. *Is the D.O. Membrane > 30 days old?*  Yes or  No *If so, it needs to be changed regardless of the amount of use it has seen.*
3. *For those sondes that have it, is the D.O. charge <100?*  Yes or  No *If not, then resurface the probe's electrodes with sandpaper.*

Atmospheric Pressure (mm Hg): \_\_\_\_\_  New DO Membrane Installed  
 Initial % Sat: \_\_\_\_\_ Final % Sat: \_\_\_\_\_  
 Initial DO (mg/L): \_\_\_\_\_ Final DO (mg/L): \_\_\_\_\_ DO Charge: \_\_\_\_\_

### Specific Conductance Probe Calibration

#### Routine Maintenance Checklist:

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  $\mu\text{mhos/cm}$ ). Was Specific Conductance calibrated accordingly?*  Yes or  No
2. *Be sure to check the age of the Specific Conductivity probe. It should be no more than 3 years old. Is it too old?*  Yes or  No

Conductivity Solution ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_  
 Initial Sp Cond ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_ Final Sp Cond ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_  
 Low-End Sp Cond Check (i.e., <5  $\mu\text{mhos/cm}$ )  
 Solution:  Deionized or  Distilled Water Sp Cond ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_  
 Monthly Mid-Range Sp Cond Check (e.g., 500 or 1000  $\mu\text{mhos/cm}$ )  
 Conductivity Solution ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_ Sp Cond ( $\mu\text{mhos/cm}$ ): \_\_\_\_\_

**pH Probe Calibration**

**Routine Maintenance Checklist:**

1. *Be sure to check the age of the pH probe. It should be no more than 12 to 18 months old. Is it too old?*  Yes or  No
2. *If the pH probe has electrodes that can be refreshed with new Reference Solutions and Tablets, check them to see if they need refreshed. Was new pH Reference Solution & Tablet Installed?*  Yes or  No

**IMPORTANT:** *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 on-site recalibration is necessary. If expectations are unknown, use a three-point calibration.*

Initial pH (7): \_\_\_\_\_ Final pH (7): \_\_\_\_\_ mV (7): \_\_\_\_\_ (0 +/- 30 mV)

3. *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 with the ranges of mV on the 10 and/or 4 buffer solutions. After +/- 50mV, the probe should no longer be used. Is the pH 7 calibration showing an acceptable range?*  Yes or  No

Initial pH (10): \_\_\_\_\_ Final pH (10): \_\_\_\_\_ mV (10): \_\_\_\_\_ (-180 +/- 30 mV)

Initial pH (4): \_\_\_\_\_ Final pH (4): \_\_\_\_\_ mV (4): \_\_\_\_\_ (+180 +/- 30 mV)

4. *Is the Nernst Equation (mV ranges) OK for all the calibration points?*  Yes or  No

**Probe Maintenance Info**

Probe Installed:  New or  Used      Probe Type:  pH  DO  Temp/Sp. Cond.

Probe Model #: \_\_\_\_\_ Probe S/N: \_\_\_\_\_

Calibration/Maintenance Notes: \_\_\_\_\_

---



---



---



---



---



---



---



---



---



---

Figure 3-3. Example of Sonde Calibration Log Sheet (PAGE 2)

**NOTES ABOUT CALIBRATION:**

- ⇒ 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 (DI) or distilled 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 pointing upward and at temperatures as close to room temperature as possible (25°C). If calibration does not occur at room temperature (e.g., a field calibration) then every attempt should be made to temperature adjust the calibration solutions according to the manufacturers' guidelines presented in Figure 3-4, Figure 3-5, & Figure 3-6 on pages 3-19, 3-20, & 3-21 respectively.

**Display Unit Overview****YSI 600XL or 600XLM Sonde/650 MDS Display**

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.

**Hydrolab Quanta G**

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.

### Dissolved Oxygen Sensor

#### **DO Sensor Overview**

Two major technologies are used for DO Sensors: Clark-cell and LDO (Luminescence Dissolved Oxygen). Clark-cell sensors utilize a paired gold (Au) and silver (Ag) electrode immersed in a KCl electrolyte solution under a Teflon membrane. Dissolved oxygen diffuses across the membrane and measured by the electrical current between the two electrodes. One disadvantage to Clark-cell technology is that the process of measuring the dissolved oxygen actually consumes the oxygen. Therefore, Clark-cell sensors need to be used where there is adequate flow to replenish the oxygen. One way to get around this is the use of an external stirrer. For example, Hydrolab Clark-cell sensors have a stirrer that can be turned on if the water flow is less than 1ft/second. YSI Clark-cell sensors do not have a stirrer due to a slight variation; it is a pulse probe turns on and off rapidly so that oxygen depletion is prevented (whereas a Hydrolab Clark-cell is a steady-state probe). The main drawbacks with a Clark-cell are that the membrane is fragile and easily torn and can be fouled with biofilms and dirt quickly (especially during long-term deployment use).

LDO sensors utilize a luminescent material that is excited by light to measure the oxygen concentration. Specifically, a blue LED illuminates the chemical and it instantly becomes excited. As the material relaxes, it releases red light. The amount of red light is detected by a photodiode and the time it takes to relax is inversely proportional to the oxygen concentration in the water. LDO sensors have an advantage over Clark-cell sensors in that they give more stable and accurate readings, they do not consume oxygen, have longer sensor lives, and can be used in environments with little to no flow. Typically they come with a wiper that will remove biofilms and dirt from the sensor cap. Because of this, an LDO sensors are bulky and need to be mounted on larger sonde casings (3.5 – 4 inch diameter), which is the technology's biggest drawback at this time.

#### **DO Sensor Calibration**

***IMPORTANT: If a Clark-cell DO sensor will not calibrate, the membrane may be torn (including micro-tears not visible to the naked eye), wrinkled, dirty, damaged, or a bubble may be trapped in the probe. See DO Sensor Maintenance Clark-cell DO Sensor for information on how to change a membrane on a Clark-cell DO sensor.***



YSI 600XL or 600XLM Sonde/650 MDS Display

**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**. Also, check the membrane for wrinkles, tears, bubbles, dirt, etc. and replace membrane, if necessary.

**IMPORTANT:** YSI recommends against touching DO membranes when replacing or servicing them. There is a potential for oils or dirt to affect O<sub>2</sub> diffusion through the membrane.

2. Reattach the calibration cup to the sonde and add no more than 1/8-inch of DI (deionized) or distilled 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<sub>2</sub> 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 on the Sonde Calibration Log Sheet. 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 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 at different elevations or Appendix D (page 227) from the YSI operating manual (*see Table 3-1 on next page*). YSI probes may be calibrated at lower elevations and then brought to higher elevations and still be accurate (the percent air oxygen saturation should go down with an increase in elevation). 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 3-1. 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.

### Hydrolab Quanta G

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) on the Sonde Calibration Log Sheet. 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 on the Sonde Calibration Log Sheet.

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 then press the **enter** key.
8. Determine the barometric pressure for entry as the calibration standard and record on the Sonde Calibration Log Sheet. *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 on the Sonde Calibration Log Sheet. Press the **enter** key to toggle to the next screen and record the final or post-calibration % DO saturation on the Sonde Calibration Log Sheet.

#### Hydrolab LDO Sensor

See **CHAPTER 13. CONTINUOUS WATER QUALITY MONITORING PROCEDURES Section D. Deployable Sonde Operation (Calibration, Programming, and Downloading Protocols) Part 1. Sonde Calibration Procedures Hydrolab Deployables on page 13-10 for example instructions on how to calibrate an LDO sensor.**

### **DO Sensor Maintenance**

#### Clark-cell DO Sensor

The Teflon membrane on the DO probe should be examined for fouling and bubbles before calibration and during use. Look to see if the membrane is torn, dirty or wrinkled, or if there are bubbles under the membrane. Whenever these conditions are observed, the membrane must be replaced. YSI recommends the membrane be replaced at least every 30 days. Frequent replacement of membranes can also lengthen the life of the probe.

To replace the membrane, remove the O-ring and old membrane and shake the remaining electrolyte (KCl solution) out of the probe. Check the silver and gold electrodes for excess tarnish (caused by contaminants that cross the membrane with the dissolved oxygen). The silver electrode will get dark (especially without maintenance) with use. If tarnish is excessive, use GoJo and a cotton swab or YSI sanding disk to remove tarnish (move clockwise on gold electrode gently with light

pressure...remember that the silver and gold electrodes are very malleable). If the silver electrode remains dark despite cleaning, then the sensor has reached the end of its lifespan. New KCl is available as an undissolved solid pre-aliquoted in a bottle and provided with each new DO sensor or in an YSI maintenance kit. This bottle should be filled with DI or distilled water to the **neck** to provide the proper working concentration. Add a few drops of fresh KCl solution to the sensor. The tip of the sensor should be filled to create a positive meniscus (looks like an "outie"), and should be free of bubbles. If bubbles are present, tap the side of the sensor to get the surface tension to pop. Gently place the membrane onto the meniscus of the KCl solution and hold the new membrane between your fingers and the sensor body. Use your free hand to place the O-ring over the tip of the sensor on top of the membrane. Without overly touching the membrane with your fingers pull/push the O-ring down over the membrane and down the side of the sensor to the depression that holds the O-ring. Secure the loose ends with your forefinger and pull any wrinkles out. If any bubbles are present, you must start again. Cut off excess membrane. Document any membrane replacement on the Sonde Calibration Log Sheet.

***WARNING: THE KCL SOLUTION USED UNDER THE DO MEMBRANE IS ESPECIALLY CORROSIVE TO THE ELECTRICAL CONTACT PINS ON THE SENSORS AND SHOULD NOT BE ALLOWED TO CONTACT THESE ELECTRODES OR COME IN CONTACT ANYWHERE NEAR OPEN SENSOR PORTS WHEN A SENSOR IS BEING REMOVED OR INSTALLED.***

***IMPORTANT: The membrane should be replaced 24 hours before use or calibration to allow time for the new membrane to relax. If, it is an emergency and the DO sensor 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 sensor 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 sensor before every site.***

### LDO Sensor

Clean the exterior of the sensor with mild soap and a toothbrush or cotton swab. Inspect the LDO cap for excessive wear. This cap should be replaced every 1-2 years.

***IMPORTANT: Do not expose the interior of the cap to sunlight as this will adversely affect the performance of the sensor. Exposure of the exterior of the cap to sunlight is to be expected and OK.***



With the cap removed, examine the sensor lens. If water is visible under the lens, the sensor will need to be replaced. Be careful not to confuse water with oils that belong under the sensor lens.

**ADDITIONAL NOTES ABOUT LDO SENSORS:**

- ⇒ **The Wiper Arm on Luminescence (e.g., LDO) or Fluorometric (e.g., Chlorophyll-a) sensors should be positioned over the temperature probe when installed and at rest on Hydrolab models. If they are showing excessive wear in one direction, you can remove the bristles and turn them the other direction. You can also replace the clip that attaches the bristles to the wiper arm.**
- ⇒ **The Hydrolab LDO probe has two generations of caps. The first generation cap is more tapered and was prone to water leakage. It needs to be upgraded to a second generation cap (less tapered) that does not leak and the internal sonde software upgraded as well.**
- ⇒ **Hydrolab LDO probes had a four-digit time stamp during manufacture. The first digit is the last digit of the year. The remaining three digits is the Julian Day. For example, 1074 is the 74<sup>th</sup> day of 2011 and 8329 is the 329<sup>th</sup> day of 2008.**

## **DO Sensor Diagnostic**

### YSI 600XL or 600XLM Sonde/650 MDS Display

To check the quality of the calibration or diagnose a potential problem with the DO sensor, 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 sensor is in adequate condition and calibrated successfully, the number should range from 25 - 75 with a score of 50 being optimum.
6. If the sensor 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 gold 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 sensor surface. It may be necessary to consult with an YSI representative before attempting this action.* **YSI recommends running newly-buffed sensors 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 sensor may need to be replaced.

YSI's 6562 Clark-cell DO sensors have an expected lifetime of 3-5 years from the date of manufacture. The date of manufacture is stamped on the side of the sensor 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 sensor has passed its expected lifetime, it may simply be too old to give proper calibration and readings.

## **DO Sensor Accuracy**

### YSI Clark-cell

The DO sensor accuracy is +/- 0.2 mg/L (or ppm) O<sub>2</sub> or 2% of the reading (whichever is greater). The range for % saturation is or +/- 2 % or the reading or Air Saturation (whichever is greater).

### Hydrolab Quanta Clark-cell

The DO sensor accuracy is +/- 0.2 mg/L (or ppm) O<sub>2</sub> at ≤ 20 mg/L or +/- 0.6 mg/L (or ppm) O<sub>2</sub> at >20 mg/L.

### Hydrolab (Hach) LDO

The DO sensor accuracy is +/- 0.1 mg/L (or ppm) O<sub>2</sub> at ≤ 1 mg/L or +/- 0.2 mg/L (or ppm) O<sub>2</sub> at >1 mg/L.

## **Conductivity/Specific Conductance Sensor**

### **Conductivity Sensor Overview**

The Conductivity Sensor typically consists of a sensor with at least two or more holes that allows the flow water into an area of known volume with two electrodes. An

electrical charge is applied between the electrodes and the conductivity of the known volume of water is measured. The temperature reading is applied to correct the conductivity reading into a Specific Conductance reading.

### Conductivity Sensor Calibration

#### YSI 600XL or 600XLM Sonde/650 MDS Display

1. Remove the lid on the calibration cup and use the special brush designed to fit inside the Conductivity Sensor's 2 end ports, vigorously scrub each port 5-10 times.
2. Rinse the sensor 3 times with DI or distilled water.
3. Dry the Conductivity Sensor using a paper towel. This will reduce the chances of diluting the conductivity standard.
4. Rinse the sensor 2 times with a small amount of **fresh** conductivity standard in the **1000-5000** microSiemens or  $\mu\text{S}$  (also known as micromhos or  $\mu\text{mhos/cm}$ ) range. The exact concentration of the standard will be written on the bottle. The concentration of solution used should be dictated by the conductivities expected to be encountered during the use of the instrument during the week.
5. 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 on the Sonde Calibration Log Sheet.
6. Press **Escape**. Scroll to **conductivity**; press **Enter**.
7. Scroll to **SpCond**; press **Enter**.
8. Type in concentration of standard in milliSiemens (not microSiemens). For example, 1000 microSiemens = 1.000 milliSiemens. Press **Enter**.
9. Allow the reading to stabilize, (a maximum of one minute, though the conductance sensor response time is usually the fastest of all sensors). Record the initial or pre-calibration readout on the Sonde Calibration Log Sheet. Press **Enter** to calibrate and record the final or calibrated readout on the Sonde Calibration Log Sheet. Press **Enter** again to continue back to the conductivity menu.
10. Now, after at least 3 rinses with DI or distilled water, exit from the calibration menu
11. Enter the discrete sampling mode (Sonde Run from the Main Menu), and conduct a **Low-End Specific Conductance Check** using either DI or distilled

water (expected conductance  $\leq 5$  microSiemens). This Low-End Specific Conductance Check should be conducted at least once a week during calibration. Indicate the solution used and the Low-End conductivity solution reading on the Sonde Calibration Log Sheet.

12. At least once a month, conduct a **Mid-Range Specific Conductance Check** using either a known 500 or 1000 microSiemens solution, depending on the solution used to calibrate the Conductivity Sensor. Indicate the conductivity of the solution used and the Mid-Range conductivity solution reading on the Sonde Calibration Log Sheet.

### Hydrolab Quanta G

1. Remove the lid on the calibration cup and rinse the sensor 3 times with DI or distilled water.
2. Dry the Conductivity Sensor using a paper towel. This will reduce the chances of diluting the conductivity standard.
3. Rinse the sensor 2 times with a small amount of **fresh** conductivity standard in the **1000-5000** microSiemens or  $\mu\text{S}$  (also known as micromhos or  $\mu\text{mhos/cm}$ ) range. The exact concentration of the standard will be written on the bottle. The concentration of solution used should be dictated by the conductivities expected to be encountered during the use of the instrument during the week.
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. Wait for the readings to stabilize. Record the concentration of calibration standard used and the initial or pre-calibration specific conductance readings on the Sonde Calibration Log Sheet.
5. 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 then press the **enter** key.
6. Press the **arrow** keys to raise or lower the specific conductance to match the calibration standard in mS/cm.
7. 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.
8. Press the **Esc**  $\infty$  key to return to the real-time data screen. Record the final or post-calibration specific conductance readings on the Sonde Calibration Log Sheet.

9. Rinse at least 3 times with DI or distilled water.
10. Conduct a **Low-End Specific Conductance Check** using either DI or distilled water (expected conductance  $\leq 5$  microSiemens). This Low-End Specific Conductance Check should be conducted at least once a week during calibration. Indicate the solution used and the Low-End conductivity solution reading on the Sonde Calibration Log Sheet.
11. At least once a month, conduct a **Mid-Range Specific Conductance Check** using either a known 500 or 1000 microSiemens solution, depending on the solution used to calibrate the Conductivity Sensor. This is also known as a Linearity Test. Indicate the conductivity of the solution used and the Mid-Range conductivity solution reading on the Sonde Calibration Log Sheet. The Mid-range check should vary less than 10% from the Mid-Range solution.

**NOTE: The Hydrolab deployable sondes (e.g., DS5x, DS5, or MS5 sondes) have the ability to do a 0-point calibration. The Quanta sonde models do not have this ability.**

### Conductivity Sensor Maintenance

Air bubbles in the Conductivity sensor's openings will affect the accuracy of the reading (*i.e.*, air bubbles will change the volume of water in the sensor's void. Biofilms and dirt often help trap air bubbles and contribute to inaccuracy as well. Because of this, the openings that allow fluids to access the conductivity electrodes should be cleaned regularly (once a month at least) using mild soap and the small acrylic brush included in the YSI calibration kit, a soft toothbrush, or cotton swabs. Dip the brush in mild soap and water and insert it into each hole 20-30 times. The electrodes should also be inspected and cleaned. Rinse with plenty of water to remove the debris and soap.

Excessive scratches Conductivity sensor's openings will also affect the accuracy of the reading and are an indication that the sensor should be replaced. YSI's Temperature/Conductivity Sensors have an expected lifetime of 2-3 years from the date of manufacture. The date of manufacture is stamped on the side of the sensor 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 sensor has passed its expected lifetime, it may simply be too old to give proper calibration and readings.

**NOTE: YSI 556 sondes have Conductivity Sensors that are not interchangeable with the YSI 600 series.**



## Conductivity Sensor Diagnostic

### YSI 600XL or 600XLM Sonde/650 MDS Display

The Conductivity Sensor 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.

## Conductivity Sensor Accuracy

### YSI 600XL or 600XLM Sonde/650 MDS Display

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

### Hydrolab Quanta G

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

## pH Sensor

### pH Sensor Overview

A basic pH sensor generally has two components: the pH bulb or “voltage provider” and the reference junction or “ground” that create a complete circuit via a salt bridge in the water. YSI pH sensors house both of these components into one self-contained probe. By contrast Hydrolab sensors house both of these components in two separate probes.

### pH Sensor Calibration

The pH Sensor on a sonde can usually be calibrated using one three methods:

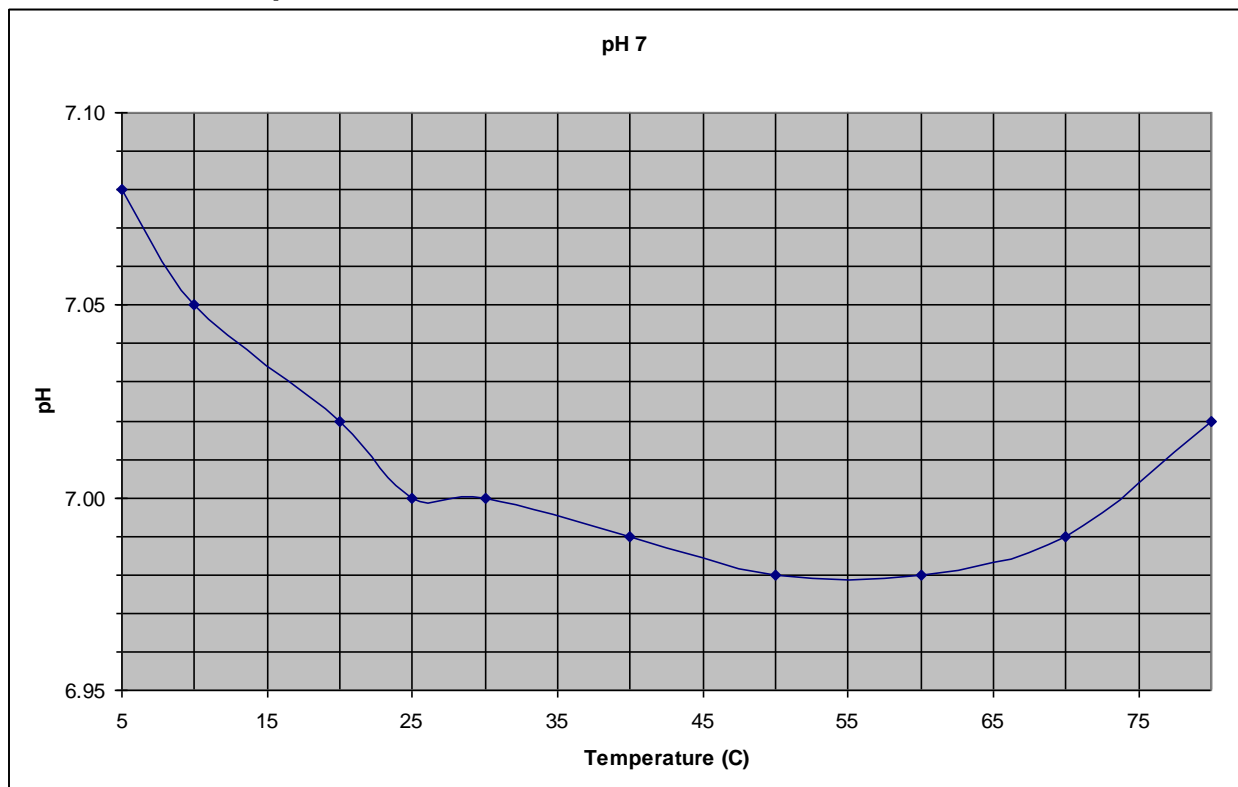
- 1) One-Point
- 2) Two-Point=Preferred Calibration Method
- 3) Three-Point

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.

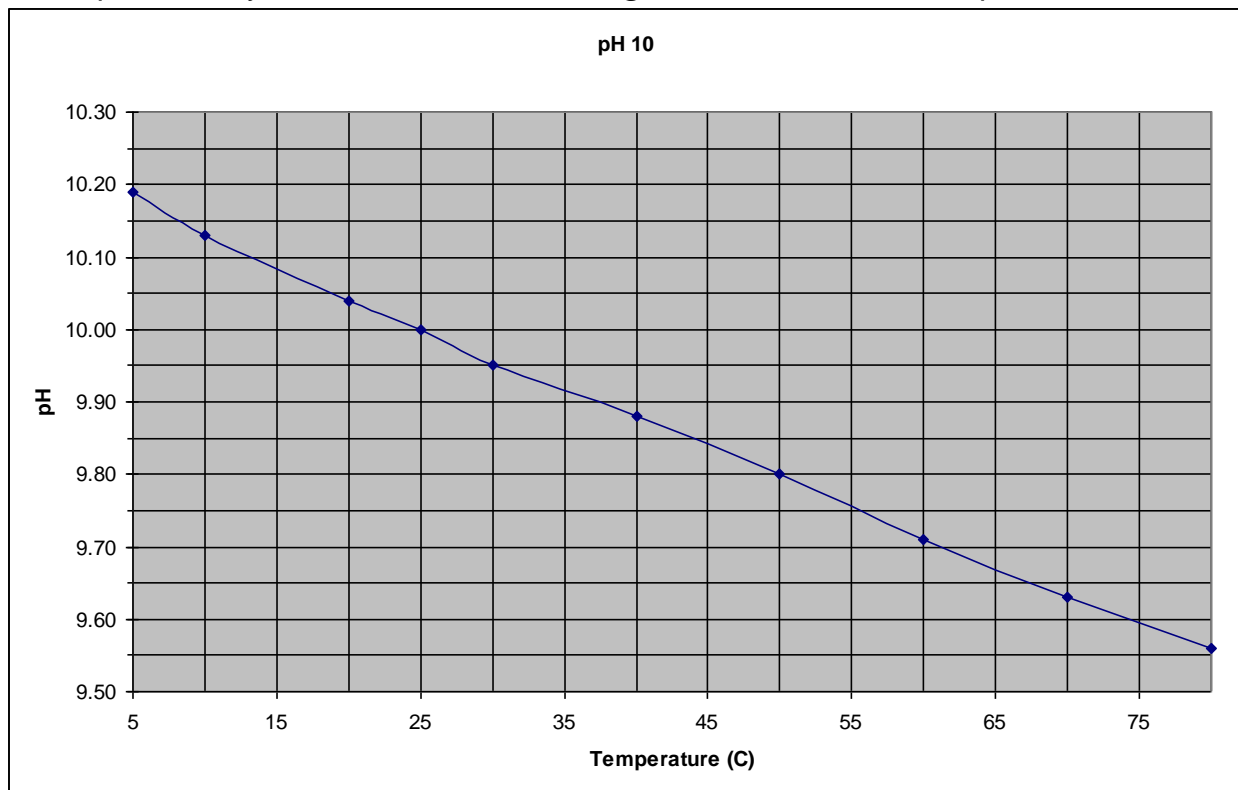
*YSI 600XL or 600XLM Sonde/650 MDS Display (Three-Point calibration example)*

1. Press **Escape** to get to Calibration mode.
2. Rinse probe three times with DI or distilled water.
3. Scroll down to **ISE1 pH**; press **Enter**.
4. Scroll down to **3 Point**; press **Enter**.
5. Rinse probe twice with DI or distilled 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 3-4 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 3-4. Temperature/pH curve for pH 7 Buffer Solution**

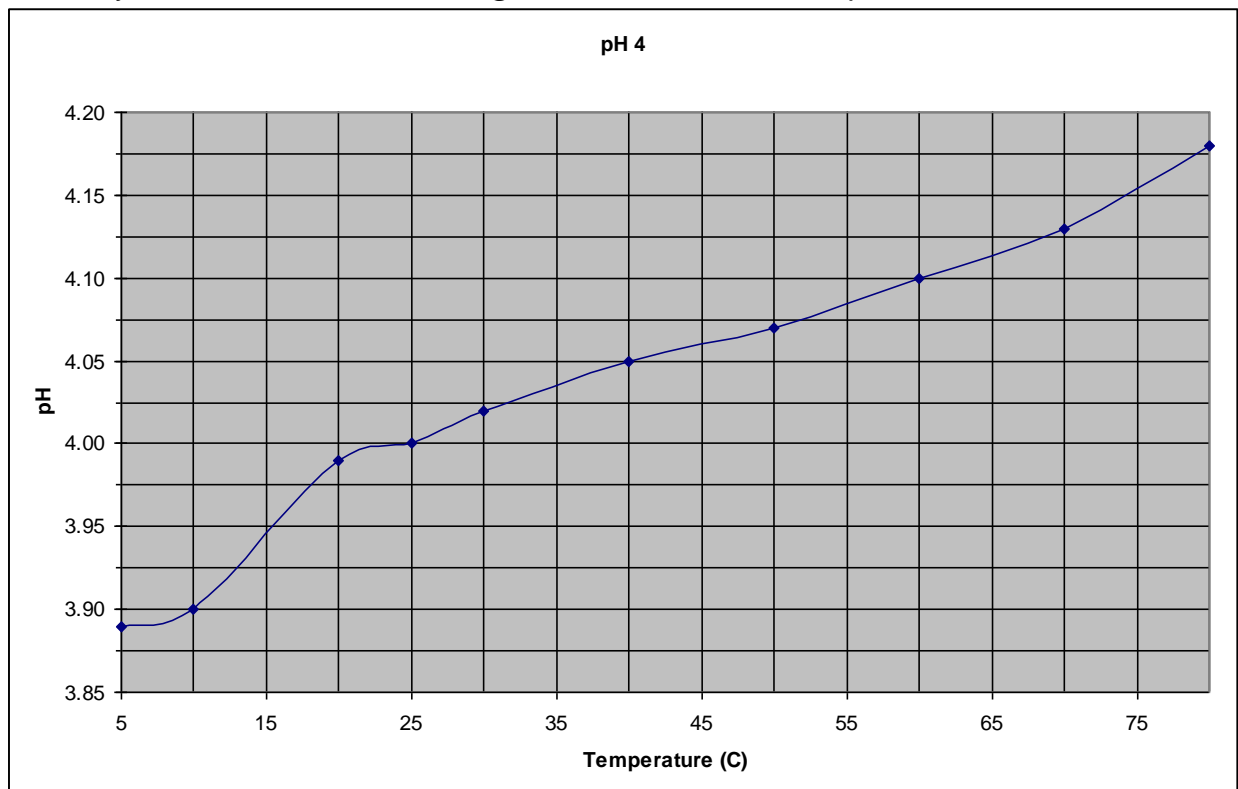
8. Type in the exact pH for the 7.00 buffer solution for the current temperature; press **Enter**. Allow readout to stabilize (approximately one minute).
9. Record the initial or pre-calibration readout. Press **Enter** to calibrate.
10. Record the final or calibration readout and press **Enter** again.
11. Rinse probe 2 times with DI or distilled 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 3-5 below** as in Step 7 above.



**Figure 3-5. 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 DI or distilled 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 3-6 below** as in Step 7 above.



**Figure 3-6. 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 Sensor 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 Sensor.

Hydrolab Quanta G (a Two-Point calibration)

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

1. Rinse the probe 3 times with DI or distilled 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 on the Sonde Calibration Log Sheet.
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 then 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 3-4 on page 3-19 under YSI 600XL or 600XLM Sonde/650 MDS pH**).
6. Press the **enter** key to finish the 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 on the Sonde Calibration Log Sheet.
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 3-5 and Figure 3-6 (See pages 3-20 & 3-21 under YSI 600XL or 600XLM Sonde/650 MDS 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.

### **pH Sensor Maintenance and Troubleshooting**

YSI 600XL or 600XLM Sonde/650 MDS Display

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 Sensors have an expected use lifetime of about 18 months. This is mainly because the pH Sensor on an YSI sonde operates using a polypropylene wick from the water (or sampling) side to a concentrated KCl side sealed inside the probe. The reference electrolyte solution (KCl) in the probe is sealed in and cannot be recharged (unlike a Hydrolab pH Sensor). Therefore, depending on the amount and types of usage over time (*i.e.*, long-term vs. short-term submersion in water and dilute vs. concentrated dissolved ions in the water column), the reference electrolyte will eventually be leached out of the probe. 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 pH by the sonde is a temperature dependent calculation. If the temperature probe is malfunctioning, then it may appear as if the pH Sensor 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 sensor 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 Sensor, 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 Sensor 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.

pH Sensor Cleaning is required should be done monthly or when response becomes slow or when deposits build up on the surfaces. To clean the glass bulb, remove the probe and use a mild soap, soft cloth, tissue, soft toothbrush, or cotton swab 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 mild soap. 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 fouling 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 representative.*

### Hydrolab Quanta G

Two electrodes are used to measure pH: one inside of a glass pH probe and a reference electrode enclosed in a reference sleeve. If the response time for pH seems slow then the probes may need to be cleaned.

Glass pH probe: The surface of the glass pH bulb acts as the voltage provider in a circuit with the reference electrode. 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 mild soap (a cotton swab or soft toothbrush will also do). Rinse with tap water.

Reference electrode: The reference electrode acts as a ground for the salt bridge to the glass pH bulb. 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. Clean the metal electrode at the bottom with mild soap and a cotton swab. Refill the sleeve to the top with a reference electrolyte tablet and solution. 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. A sure sign that it is clogged is that the readings look good in buffer (not a dilute solution, therefore a good salt bridge is established) but do not look good in the field (a more dilute solution, and a salt bridge is not established). Another sign is if the inside of the Teflon junction looks clean (white) and the outside look dirty (gray). If these are the case, replace it. Since this Teflon Reference Junction cannot be cleaned, it should be replaced once every 1-2 years. When seating the reference sleeve, trapped air and excess electrolyte is purged. This purging flushes the porous Teflon Reference Junction.

## pH Sensor Diagnostic (Nernst Equation)

pH Sensors operate using the Nernst Equation (*see Figure 3-7 below*). Simply put, it is 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 as in the illustration below. If this slope flattens, the pH Sensor will lose resolution. This slope is a result of the probe condition as well as the quality of the calibration. If it the sonde is capable, a parameter called pH mV may be used to check this slope.

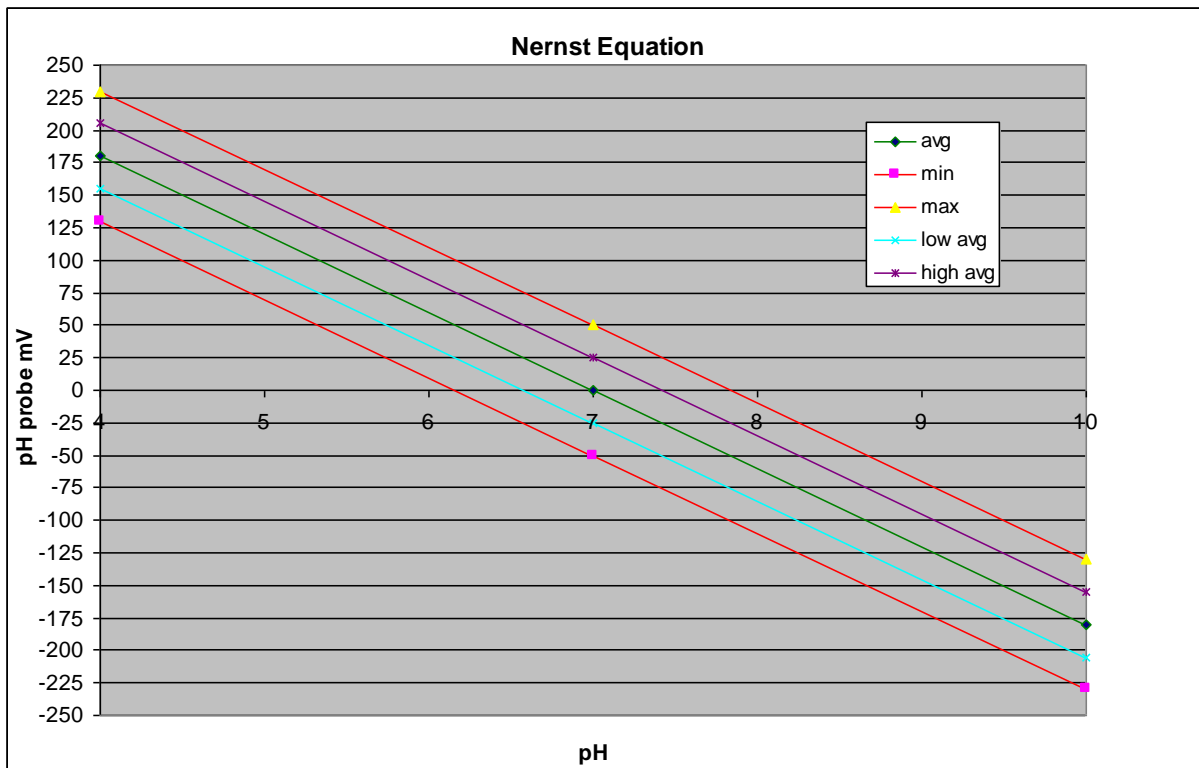


Figure 3-7. The Nernst Equation

### YSI 600XL or 600XLM Sonde/650 MDS Display

The Nernst Equation may be checked and calculated on an YSI sonde by following the steps below. Alternatively, it can be checked during calibration if the pH mV parameter on the display is turned on.

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 –30 and 30, 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 (+/- 30 )or, if using 10.00, the second reading should be around –200 (+/- 30).

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 *Sonde Storage on page 3-30 for prevention of contamination of the KCl solution inside the pH Sensor.***

### **pH Sensor Accuracy**

#### YSI 600XL or 600XLM Sonde/650 MDS Display

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

#### Hydrolab Quanta G

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

### **Temperature Sensor**

#### **Temperature Sensor Maintenance and Troubleshooting**

The Temperature probe needs to be cleaned with mild soap and a toothbrush or cotton swab monthly. After cleaning, the metal should be checked for signs of rust as this is a warning sign that the probe is potentially compromised and needs to be replaced.

The temperature reading should be checked against a NIST certified thermometer yearly for accuracy in water baths set at several temperatures. If the difference in the readings is outside of the probe's accuracy rating, then the probe should be replaced. YSI has suggested that their Temperature probe's lifespan can be up to 5 years before it has drifted out of range.

Note: Some brands (e.g., YSI) have the Temperature and Conductivity Sensors bundled into one probe.

### **Temperature Sensor Accuracy**

The Temperature probe accuracy is +/- 0.15<sup>0</sup> C.

### **Additional Sensors**

Additional sensors that may be installed on certain models of sondes include:

Depth Sensor or Pressure Transducer  
Ion Probes (e.g., Chloride, Ammonium, Nitrate)  
Fluorometers (e.g., Chlorophyll-a, Rotamine, Blue-Green Algae)  
Total Dissolved Gasses  
Light Penetration  
Turbidity  
Oxidization Reduction Potential (ORP)

## Part 2. Sonde Routine Maintenance & Storage

### Maintenance Kit Contents

At all times, each field crew should have with them in the field a Sonde Maintenance Kit consisting of items necessary to perform routine/basic maintenance and cleaning of the sonde. **See Table 3-2 below** for the contents of a maintenance kit and each item's function.

Table 3-2. Sonde Maintenance Kit Basic Contents

Item	Function
Soft Toothbrush	Light cleaning of all probe surfaces
Mild Soap (e.g., Simple Green Pro 3)	Light cleaning of all probe surfaces; note that the Simple Green contains an anti-bacterial component that will help prevent the growth of biofilms on the sensors. <b>Never clean sensors with alcohol or other organic solvents. This will degrade the probe materials.</b>
Long Cotton Swabs	Cleaning in small tight spaces (e.g., conductivity ports)
YSI Acrylic Cleaning Brush	Used to clean probe surfaces (especially the conductivity ports); Provided in an YSI maintenance kit.
GoJo & Cotton Swab	Pumice helps clean tarnished electrodes on Clark-cell DO sensor.
YSI Electrode Sanding Pad	Used to clean tarnished electrodes; Provided in an YSI maintenance kit.
KCl Electrolyte Solution	Required for Clark-cell DO membrane replacement; Provided in an YSI maintenance kit.
Teflon DO Membranes	Required for Clark-cell DO membrane replacement; Provided in an YSI maintenance kit.
pH KCl Reference Tablets/Solution	To recharge the reference pH probe on Hydrolab or other capable sondes.
Mini-Allen Wrench	To remove the wiper arm on Luminescence (e.g., LDO) or Fluorometric (e.g., Chlorophyll-a) sensors for cleaning.



**Maintenance Schedule**

Provided **below in Table 3-3** is a brief outline of the regular maintenance schedule (beyond calibration) that needs to be performed on the sondes. Each of these actions are more thoroughly discussed **above in Part 1. Sonde/Sensor Calibration, Maintenance, Diagnostics, and Accuracy on pages 3-3 to 3-28.**

**Table 3-3. Routine Sonde Maintenance Schedules for the Basic Sensors**

<b>Probe</b>	<b>Action</b>	<b>Frequency</b>
All Probes	Clean all probe surfaces with mild soap, toothbrush, and cotton swabs.	Monthly
Temperature	Clean and check Metal Temperature Probe for Rust (Not applicable on Data Loggers).	Monthly
	Check Temperature against a NIST Certified Thermometer in several water baths.	Yearly
pH	Clean glass bulb on pH probe.	Monthly
	Replace Reference pH Tablet/Solution (Hydrolab models).	
	Replace Reference pH Teflon Junction Cap every 1-2 years (Hydrolab models).	Yearly
DO	If Clark-cell type probe, clean electrodes with GoJo and cotton swab (Hydrolab) or sanding disc (YSI) to remove tarnish; Add new KCl electrolyte solution and Teflon membrane.	Monthly
	If LDO, check the Cap for material loss/wear and look under the LDO lens for leakage; if so replace.	
	Replace LDO Cap every 1-2 years (Hydrolab models only)	Yearly
Conductivity	Clean Conductivity Ports and electrodes with cotton swab or YSI acrylic cleaning brush. Check for scratches.	Monthly

## **Miscellaneous Sonde Maintenance**

### **Sonde Storage**

With any sonde, certain storage procedures (both short and long-term) must be observed in order to prevent damage to the sensors and probes.

- 1) Never store the sensors in DI water. This will deplete the reference pH electrolytes and the surface of the pH bulb. To increase the life of a probe, proper storage of the probe must be implemented.

For **short-term storage** (e.g., between sites and over the weekend), the sensors only need to remain moist. Place only a minute amount of water (1/8<sup>th</sup> of an inch is probably too much) in the cup for storage making sure that no water will inundate the pH Sensor. 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 **longer-term storage** (e.g., over winter), use pH 4 solution or clean tap water (between 1 Tablespoon and ¼ cup depending on the size of the sonde cup). YSI recommends that the cup be filled with a concentrated KCl solution. Alternatively, the pH probe can be uninstalled and put into its original shipping container with the KCl solution. This will lengthen the life of the probe and help maintain the concentration of KCl inside of the pH Sensor. *To obtain the concentration of KCl solution, consult the YSI operating manual or an YSI representative.*

- 2) Remove all batteries for long-term storage. If the batteries should leak, the acid may destroy the sonde housing. The exception to this are any Lithium Ion batteries found connected the sonde electronics that maintain the internal clock; these types of batteries are not supposed to leak and typically last 2-5 years.
- 3) Install all connector covers and cable plugs to protect the sonde's electronic connection pins.
- 4) Do not store sonde in freezing temperatures. Even though the sondes may have just a small amount of liquid in the storage cup to keep the sensors moist, many sondes have sensors that contain liquid inside the probe (e.g., pH reference probe). Additionally, the freezing action of just the small amount of liquid inside of the storage cup can cause damage to the housing and probe connections.

## **Part 3. Sonde Use (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 (e.g., 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.

***IMPORTANT: 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 Sensor 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 sonde run for at least ten minutes to stabilize.

Reading stabilization is typically based on the sonde's chemical parameter sensors (i.e., pH, DO, and Specific Conductance). If the individual readings do not change over the course of three reading intervals (minutes) by more than the sensor's accuracy (pH = +/- 0.2 pH units, DO = +/- 0.2 mg/L (or ppm), Specific Conductance = +/- 5  $\mu$ S), then the sensors are assumed to have stabilized to the water.

Note that it has been observed that the pH readings take as long as 20-30 minutes to stabilize under extremely low temperature or low conductivity situations. In addition, the age of the sonde/probes also can play a role in how long it takes for the readings to stabilize. 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 liquid (preferable pH 4 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.

***IMPORTANT: Do not store the sonde with a full cup of water, as this will lessen the life of the pH sensor.***

**TIPS FOR USAGE OF SONDES IN THE FIELD:**

- ⇒ If taking readings from an intermediate water container (e.g., a bucket), make sure to keep the hole on the Conductivity Sensor 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, if equipped, 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.

Sonde Calibration Log Sheets are used for any calibration or maintenance performed on an instrument and entered into the database weekly and examined for patterns that indicate potential or imminent probe failure. 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. Dissolved Oxygen is calibrated daily. In addition, all sondes and probes are frequently cross-checked against each other in variable water bath solutions 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.

**Duplication of sonde data is only possible if two different sondes are present during the sampling event. If this is the case, then the sampler should be all means document the duplication of sonde data.**

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. Additional training provided by the Sonde manufacturer may also occur as available.

## **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 Containers (e.g., 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.

**WARNING: NEVER ADD SAMPLE WATER TO LIQUID ACIDS OR BASES, AS A STRONG CHEMICAL EXOTHERMIC 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 (**see Figure 3-8 below**). In addition, distinct sub-sample descriptions should be indicated somewhere on the form (e.g., Top vs. Bottom, Left Bank vs. Right Bank, etc.). Blind Duplicate samples (usually Dup #2 on the Habitat form) should be labeled with false Stream Names and AN-Code to disguise the fact that it is a duplicate. Replicate samples should be distinguished by a 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.**



Figure 3-8. Example of Sample Containers Labeled with sample information.

**IMPORTANT:** 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.

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. Water Quality Sondes & Sensors: Calibration, Routine Maintenance, & Use. Part 3. Sonde Use (Field Procedures) starting on page 3-28 for more information on how to use the water quality sonde in the field*).

**NOTE:** The definitive order of sample container filling is as follows:

1. Fecal Coliform Sample
2. Unfixed Cubitainer
3. Nutrient (H<sub>2</sub>SO<sub>4</sub>) Cubitainer
4. Total Metals (HNO<sub>3</sub>) Cubitainer
5. X-Bottle/Dissolved Metals (to be Filtered/HNO<sub>3</sub>) Cubitainer

3. Collect the water samples upstream of the sonde 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 below the surface of the water (6 inches if possible) and fill the bottle to the 100 mL mark (*see Figure 3-9 on right*). 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.



Figure 3-9. Example of a Fecal Sample being taken from a stream.

B. Other Water Samples (e.g., Unfixed, Total Metals, Dissolved Metals, Nutrient cubitainer samples):

- All remaining water quality samples are collected in containers (e.g., cubitainers) provided by the analytical lab (per the state analytical contract).

**IMPORTANT:** *If using cubitainers or other collapsible container, do not blow into the containers to open. The sample can become contaminated by your breath (e.g., Phosphoric acid effervescing from your mouth after drinking a cola and contaminate the Phosphorus readings).*

**NOTE:** If water depth is limiting the ability to submerge the container lip without stirring up sediment and organic debris:

1. Cut the top off of open a fresh, unused cubitainer.
2. Thoroughly rinse with stream water as normal (three times).
3. Use the cut cubitainer to carefully collect water and pour into an intact cubitainer.
4. Discard the cut cubitainer after filling up all cubitainers for the site.



Figure 3-10. Example of Cubitainer being filled and emptied (i.e., rinsed with stream water).

- Rinse the sample container **three times** at least **one-half full** with sample water. During the rinse, secure the lid on the containers, shake for 5 seconds, and then empty (*see Figure 3-10 above*).
- Submerge below the surface of the water (6 inches if possible) and completely fill the containers with sample water (1000 mL).
- Expunge as much of the headspace as possible without losing sample water.
- Make sure to limit pieces of organic matter in the sample container as much as possible as these can cause contamination.
- When sealing the container, remove as much air as possible should be expunged from the sample container to avoid contamination (*i.e.*, no head-space).

### **Indirect Methods**

In some cases, water levels (high or low) or strong flows will not permit direct water sampling. In these cases, it is necessary to use special equipment in order to get the

sample. The use of such equipment is explained in **CHAPTER 11. Section A. Part 2. Bridge Crane Method starting on page 11-5 and CHAPTER 12. Section D. Part 3. Van Dorn Sampler Method for Depth Profiles on page 12-21.** In the case of low flows, the use of a surrogate container (e.g., a clean, unused fecal sample container) may be necessary to fill the sample container. When doing this, make sure to thoroughly rinse out any of the Sodium Thiosulfate that may be in the Fecal container before filling the sample containers.

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

Preserve the sample as indicated on the Analysis Request Form. **See Figure 3-13 on page 3-48 for an example of an Analysis Request Form with COC.**

***IMPORTANT: 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 3-4 in the Holding section on page 3-46.***

Samples should be preserved in the following order:

- 1) Unfixed or Iced (Wet Ice) Samples (e.g., Fecal Coliform, Unfixed container)
- 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.

***IMPORTANT: If Alkalinity is being analyzed, 100% of the air must be expunged from the unfixed containers to avoid contamination. If nutrients (i.e., Nitrogen or Phosphorus) are being analyzed, avoid water samples with visible organic debris in the container.***

### **Filtration**

A net minimum of 500 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).



**Figure 3-11. Example of Peristaltic Pump/Drill Apparatus for Sample Filtration being used under the tailgate of a vehicle.**

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 highly dependent upon there being a flat, streamside surface to work on and no precipitation/moisture that could short the drill. Because of these impracticalities, the sample water to be filtered is 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 (*see Figure 3-11 above*).

***IMPORTANT: 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.

**NOTE: This is done to remove any contaminants from the fresh filter that may bias the sample (positive bias).**

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.



***IMPORTANT: 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.

***IMPORTANT: 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 500 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 Lock 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 DI or distilled 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 500 mL of sample after rinsing the cubitainer 3 times, but check with lab beforehand). It is also a good idea to put on the lab analysis form how many 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 DI or distilled water and shake off excess droplets.
- Place a filter on the funnel's screen and store cup/funnel assembled in a zip lock bag.
- Rinse only the glass flask with 10% HCl (a desorbing agent). The plastic portions (funnel and cup) may only be rinsed with DI or distilled water and lightly rubbed with a paper towel.
- Do not touch inside surfaces of filtration apparatus.

### **Fixation**

As outlined in **Table 3-4 on page 3-46 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  $\mu\text{mhos/cm}$ ). If a stream has a low pH and/or low conductivity, one ampoule of acid may over preserve the sample. Conversely, if a stream has a high pH and/or high conductivity, one ampoule 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 gauge how much acid to add to adequately fix sample.



Figure 3-12. Example of sample being preserved with Nitric Acid.

### Testing a sample with a pH test strip

- 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.

**IMPORTANT: 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, remove the headspace, 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", 18<sup>th</sup> Edition and as outlined in **Table 3-4 Preservation Methods and Holding Times on the next page.**

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 3-4. Preservation Methods and Holding Times

Parameter	Preservation	Max. Holding Time
Fecal Coliform	Cool <10 °C, 0.0008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub>	6 hours. (24 hours for TMDL/WAB samples)
Acidity	Cool ≤4 °C	14 days.
Alkalinity	Cool ≤4 °C	14 days.
Ammonia	Cool ≤4 °C, H <sub>2</sub> SO <sub>4</sub> to pH<2	28 days.
Chloride	None required	28 days.
Kjeldahl (TKN) and Organic N	Cool ≤4 °C, H <sub>2</sub> SO <sub>4</sub> to pH<2	28 days.
Chromium VI	Cool ≤4 °C, pH = 9.3–9.7	28 days.
Mercury (CVAA)	HNO <sub>3</sub> 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)	HNO <sub>3</sub> to pH<2	6 months
Dissolved Metals (except Boron, Chromium VI, and Mercury)	Filtered, HNO <sub>3</sub> to pH<2	6 months
Nitrate	Cool ≤4 °C	48 hours.
Nitrite	Cool ≤4 °C	48 hours.
Nitrate-Nitrite (NO <sub>2</sub> -NO <sub>3</sub> -N)	Cool ≤4 °C, H <sub>2</sub> SO <sub>4</sub> to pH<2	28 days.
Total Orthophosphate	Cool ≤4 °C	48 hours.
Dissolved Orthophosphate	Filtered, Cool ≤4 °C	Filter within 15 minutes; 48 hours.
Phosphorous, Total	Cool ≤4 °C, H <sub>2</sub> SO <sub>4</sub> to pH<2	28 days.
Total Solids, Total Suspended Solids (TSS), Total Dissolved Solids (TDS)	Cool ≤4 °C	7 days.
Sulfate	Cool ≤4 °C	28 days.

### **Documentation**

**Using a black or blue permanent ink pen**, fill out an Analysis Request Form/COC 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).

***IMPORTANT: All samples COCs for a sample event must be filled out as soon as the sample is placed in the cooler.***

A completed Analysis Request Form includes: Project, Laboratory Name, Stream Name, WQ Lab (Sample) ID, 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.). Blind Duplicate samples (usually Dup #2 on the Habitat form) should be labeled with false Stream Names and An-Code to disguise the fact that it is a duplicate. Replicate samples should be distinguished by a Rep #1 vs. Rep #2.

***IMPORTANT: Make sure that vehicles containing samples are always locked when unattended!***

After the sample has been turned over to a WVDEP certified analytical laboratory (see **page 3-55 for more details about the WVDEP Laboratory Quality Assurance Program**), 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 3-13 on the next page 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. 11/12

TMDL LTMS WAB RANDOM AWON DEPLOY. LAKES FISH OTHER

Laboratory Name: Biochem Circle Activity

Stream Name: Tug Fork Watershed Name: Tug

AN-Code: WA BST-(0.15) WQ Lab ID: 63-388 Random #: \_\_\_\_\_

Sampled By: JDH Filtered By: JDH # of Containers 5

Sample Type: Water  Sediment \_\_\_\_\_ Other \_\_\_\_\_ Specified Method: 40 CFR 136

Acid Lot #, Nitric: 7697-37-2 Sulfuric: 7664-93-9 HCl: \_\_\_\_\_ Field Meter # 95 Flow Meter # \_\_\_\_\_

Grab: Date-Time: 4 / 16 / 13 1130 Lat: \_\_\_\_\_ Long: \_\_\_\_\_

Positioning Method (Circle One) Map  GPS  GIS

Field Values<sup>1</sup>: Temp. 16.81 pH 8.02 D.O. 8.60 Cond. 476 Flow: \_\_\_\_\_

**Bold type/Cell Border = Random Parameter Suite ( Al & Fe: Tot/Diss, Cu & Zn: Diss only)**

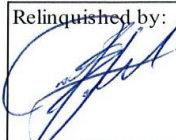
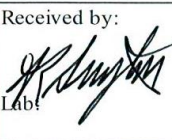
Pres.	Analysis / Item #*	Pres.	Analysis / Item #*	Pres.	Analysis / Item #	Tot	Diss <sup>+</sup>	Preservation Code
<u>3</u>	Acidity (Hot) / 2	<u>3</u>	Tot. Diss. Solids / 15	<u>5</u>	Aluminum / 53	<u>Ⓚ</u>	<u>Ⓚ</u>	1. None - Determined on-site 2. None 3. Iced immediately 4. H <sub>2</sub> SO <sub>4</sub> to pH <2, iced immediately 5. HNO <sub>3</sub> to pH<2 6. Sterile + 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> , iced immediately 7. Filtered immediately, iced immediately 8. HCl to pH<2, iced immediately 9. MgCO <sub>3</sub> & Ice, _____ ml sample 10. Other (Specify)  * Item # specifies Method and MDL in current WQ Analytical Contract (DEP15037)
<u>3</u>	Alkalinity / 3	<u>3</u>	Tot. Susp. Solids / 16	<u>5</u>	Iron / 64	<u>Ⓚ</u>	<u>Ⓚ</u>	
<u>3</u>	Sulfate / 6	<u>4</u>	T. Phosphorus-P / 26	<u>5</u>	Manganese / 67	<u>Ⓚ</u>	<u>Ⓚ</u>	
<u>2</u>	Chloride / 10	<u>4</u>	TKN / 20A	<u>5</u>	Selenium / 72	<u>Ⓚ</u>	<u>Ⓚ</u>	
<u>6</u>	Fecal Coli., MF / 12 24 hour holding	<u>4</u>	NO <sub>2</sub> -NO <sub>3</sub> -N / 25	<u>5</u>	Copper / 63A	<u>Ⓚ</u>	<u>Ⓚ</u>	
		4	Low level T. Phosphorus-P / 26A	<u>5</u>	Zinc / 78A	<u>Ⓚ</u>	<u>Ⓚ</u>	
6	Fecal Coli., MF/12 6 hour holding	4	Diss. Phosphorus	<u>5</u>	Magnesium/ 66	<u>Ⓚ</u>	<u>Ⓚ</u>	
<u>2</u>	Bromide / 9	4	TKN / 20	<u>5</u>	Calcium / 60	<u>Ⓚ</u>	<u>Ⓚ</u>	
5	Hardness / 4	<u>4</u>	Ammonia-N / 21	<u>5</u>	Potassium / 71	<u>Ⓚ</u>	<u>Ⓚ</u>	
3	Acidity (Cold)	3	Tot. Ortho PO <sub>4</sub> -P	<u>5</u>	Sodium / 74	<u>Ⓚ</u>	<u>Ⓚ</u>	
3	Bicarbonate / 48	7	Diss. Ortho PO <sub>4</sub> -P	<u>5</u>	Beryllium	<u>Ⓚ</u>	<u>Ⓚ</u>	
3	BOD / 29	9	Chlorophyl a / 38	<u>5</u>	Barium / 56	<u>Ⓚ</u>	<u>Ⓚ</u>	
4	COD	3	NO <sub>3</sub> -N (Nitrate) / 23	<u>5</u>	Cadmium/ 59A	<u>Ⓚ</u>	<u>Ⓚ</u>	
4	TOC / 32	3	NO <sub>2</sub> -N (Nitrite) / 24	<u>5</u>	Lead / 65	<u>Ⓚ</u>	<u>Ⓚ</u>	
3	pH (lab) / 1	3	Semi-Vol. Organics	<u>5</u>	Nickel / 70A	<u>Ⓚ</u>	<u>Ⓚ</u>	
3	Cond. (lab) / 5	8	Volatile Organics	<u>5</u>	Arsenic / 55	<u>Ⓚ</u>	<u>Ⓚ</u>	
				<u>5</u>	Silver / 73	<u>Ⓚ</u>	<u>Ⓚ</u>	
				<u>5</u>	Boron / 58	<u>Ⓚ</u>	<u>Ⓚ</u>	
				3	Hex. Chromium/36			
				5	Mercury / 68			
				5	LL Mercury / 68A			

REMARKS:

1 # of Filters used

Filter type (circle): Disc Cartridge

<sup>+</sup>Filtered immediately, nitric acid added to pH <2

Relinquished by: 	Date & Time <u>4/16/13</u> <u>2236</u>	Received by:  Lab:	Relinquished by:	Date & Time	Received by:
---	--	---	------------------	-------------	--------------

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 - DEP Copy CANARY -Laboratory Copy

Figure 3-13. Example of a fully completed Analysis Request Form with Chain-of-Custody (COC) at bottom

### Part 3. Common Water Quality Parameter Suites & Detection Limits

#### ***Take Hydrolab readings at every site!***

Because of the susceptibility of the pH and Conductivity Sensors to infrequent failure, it is important to have redundant information to corroborate the sonde readings. Therefore, always order Lab pH or Lab Specific Conductivity under either of the two following circumstances:

- 1) The site you are visiting is a single or infrequent visit site (e.g., Random or Target Sites, LTMS, Lakes) and the instantaneous pH reading (i.e., sonde) indicates a violation of the pH WQ standards (i.e., <6 or >9)

Or

- 2) The site you are visiting is a multiple visit site (e.g., TMDL, Deployable, Ambient, Special Projects) and the instantaneous pH reading (i.e., sonde) is outside of normal historic ranges as indicated on user maintained pH/Conductivity log sheets. For example if the site has a pH reading of 5.5 and has been visited X times in the past year and normally has a pH between 6.5 and 8.0.

Although Lab pH and Lab Specific Conductance readings are not the same as instantaneous readings due to a lag in analysis time, the lab readings should give us the ability to confirm if there is a failure with the sonde's probes.

***Target analytical method detection limits are displayed in Table 3-5 on page 3-51.***

***The Lab Parameter Suites below listed in order of appearance on the COC in Figure 3-13 on Page 3-48 above.***

#### **Random & Potential Reference Sites:**

- Acidity (Hot), Alkalinity, Sulfate, Chloride, Fecal coli., Bromide, TDS, TSS, Tot. Phos., TKN, NO<sub>2</sub>-NO<sub>3</sub>-N, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn (Tot.), Se (Tot.-Low Level), Cu (Dis.-Low Level), Zn (Dis.-Low Level), Mg (Tot.), Ca (Tot.), K (Tot.), Na (Tot.), Be (Tot.), Ba (Tot.), & Sr (Tot.).

**4 containers (wet iced, HNO<sub>3</sub>, filtered HNO<sub>3</sub>, & H<sub>2</sub>SO<sub>4</sub>) & fecal bottle**

#### **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 (Tot.), Mg (Tot.), & Ca (Tot.).

**3 containers (wet iced, HNO<sub>3</sub>, & filtered HNO<sub>3</sub>) & fecal bottle**

**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, Chloride, Fecal coli., TDS, TSS, Al (Tot. & Dis.), Fe (Tot. & Dis.), Mn (Tot.), Se (Tot. & Dis.), Mg (Tot.), & Ca (Tot.). Take Ammonia-N (NH<sub>3</sub>) if it is suspected that Ammonia is being used to treat the stream water.

**3 containers (wet iced, HNO<sub>3</sub>, & filtered HNO<sub>3</sub>) & fecal bottle**

**Nutrient Enrichment:**

**Take within 24 hours of a significant rain or when animal waste, straight pipes, STP outfalls, etc., may be impacting the stream:**

- Fecal coli, TSS, Tot.& Dis. Phos., TKN, & NO<sub>2</sub>-NO<sub>3</sub>-N, Total Orthophosphate (PO<sub>4</sub>-P) Take Ammonia-N (NH<sub>3</sub>) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia input.

**3 containers (wet iced, H<sub>2</sub>SO<sub>4</sub>) & fecal bottle**

**Large River Algae/Nutrient Enrichment:**

**Take in large rivers that are experiencing exceptionally excessive algal blooms (e.g., Greenbrier River, Tygart River):**

- Alkalinity, Fecal coli, TSS, Tot. & Dis. Phos., TKN, NO<sub>2</sub>-NO<sub>3</sub>-N, Total Orthophosphate (PO<sub>4</sub>-P), Mg (Tot.), & Ca (Tot.). Take Ammonia-N (NH<sub>3</sub>) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia input.

**4 containers (wet iced, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>) & fecal bottle**

**TDS Ions:**

**Take anywhere in Monongahela Basin (Dunkard, Monongahela, West Fork, Tygart, Youghiogheny, & Cheat):**

- Alkalinity, Sulfate, Chloride, Bromide, Fecal coli, TDS, TSS, Mg (Tot.), Ca (Tot.), K (Tot.), & Na (Tot.).

**2 containers (wet iced & HNO<sub>3</sub>) & fecal bottle**

**Oil & Gas:**

**Take if oil or gas activities are evident & cond. >200 in absence of other sources like AMD:**

- Alkalinity, Sulfate, Chloride, Fecal coli, TDS, TSS, Mg (Tot.), Ca (Tot.), K (Tot.), Na (Tot.), Ba (Tot.), Sr (Tot.).

**2 containers (wet iced & HNO<sub>3</sub>) & fecal bottle**

**Sediment:**

**Typically only used during WQ only sampling events (e.g., TMDL sampling). Take anywhere where it is suspected that Suspended Solids are contributing solely to the Iron load in the stream bed during precipitations events:**

- Fecal coli, TSS, & Fe (Tot. & Dis.).

**3 containers (wet iced & HNO<sub>3</sub>) & fecal bottle**

**Current Water Quality Analyte Method Detection Limits****Table 3-5. Current Water Quality Analyte MDLs**

Analyte	Method Detection Level	Analyte	Method Detection Level
Alkalinity	5 mg/L	Kjeldahl Nitrogen	0.1 mg/L
Aluminum	0.005 mg/L	Lead	0.001 mg/L
Ammonia Nitrogen	0.1 mg/L	Magnesium	0.05 mg/L
Antimony	0.005 mg/L	Manganese	0.005 mg/L
Arsenic	0.005 mg/L	MBAS	0.05 mg/L
Barium	0.005 mg/L	Mercury	0.0001 mg/L
Beryllium	0.001 mg/L	Mercury / Method 1631E	0.5 ng/L
Bicarbonate (Standard Methods)	1 mg/L	Mineral Acidity	1 mg/L
BOD	1 mg/L	Molybdenum	0.005 mg/L
BOD-carbonaceous	1 mg/L	Nickel	0.005 mg/L
Boron	0.02 mg/L	Nitrate-Nitrogen	0.05 mg/L
Bromide	1 mg/L	Nitrite-Nitrate	0.05 mg/L
Bromide Alt. Method	0.1 mg/L	Nitrite-Nitrogen	0.05 mg/L
Cadmium	0.0002 mg/L	Oil-Grease	2 mg/L
Calcium	0.02 mg/L	Organic Nitrogen	0.5 mg/L
Chloride	5 mg/L	Orthophosphate	0.01 mg/L
Chlorophyll A	0.5 mg/L	Percent Solids	1%
Chromium	0.001 mg/L	Phenolics	0.01 mg/L
Cobalt	0.001 mg/L	Potassium	0.05 mg/L
COD	0.5 mg/L	Selenium	0.001 mg/L
Color (ADMI)	10 ADMI value	Silver	0.0002 mg/L
Color (APHA)	5 color units	Sodium	0.05 mg/L
Copper	0.001 mg/L	Specific Conductance	3 uS/cm <sup>2</sup>
Cyanide, Amenable	0.005 mg/L	Sulfate	5 mg/L
Cyanide, Free (ASTM)	0.01 mg/L	Sulfide	1 mg/L
Dissolved Organic Carbon	1 mg/L	Suspended Solids (TSS)	3 mg/L
Dissolved Solids (TDS)	1 mg/L	Thallium	0.001 mg/L
Escherichia Coli (Numeric Result)	1 col/100 mL	Tin	0.02 mg/L
Fecal Coliform (MF)	4 col/100 mL	TOC	1 mg/L
Fecal Coliform (MPN)	4 col/100 mL	Tot Petroleum Hydrocarbons GRO/DRO (8015)	0.5 mg/L
Fecal Streptococci	4 col/100 mL	Total Acidity	1 mg/L
Ferrous Iron (Standard Methods)	0.05 mg/L	Total Cyanide	0.005 mg/L
Fluoride	0.2 mg/L	Total Phosphate	0.01 mg/L
Hardness	1 mg/L	Total Phosphorus	0.005 mg/L
Hexavalent Chromium	0.005 mg/L	Total Solids	1 mg/L
Hot Acidity	5 mg/l	Turbidity	1 NTU (higher OK if highly turbid)
Hot Acidity Alt. Method	*	Vanadium	0.005 mg/L
Iron	0.01 mg/L	Volatile Solids	1 mg/L
		Zinc	0.002 mg/L

## 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% and 5% of our sites respectively. 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 in **CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1.**

### Field Blanks

#### Overview

To evaluate sample containers for contamination, each team will prepare field blanks **weekly per team**. DI or distilled 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 sample containers 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 DI or distilled water and are preserved and stored in the same manner as the actual samples. A separate



Analysis Request Form with Chain-of-Custody (COC) and QA Blank Field Sheet is completed for the field blanks and the samples are submitted to the laboratory.

Field blanks are simply samples of DI or distilled 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.

**NOTE: Be aware that Distilled Water provided in plastic jugs (store bought jugs) may contain some Phthalates that will leach from the plastic. Do not use this water if the target parameter is a Phthalate.**

### Obtaining the Field Blank Water

Before leaving the office, obtain the DI or distilled 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<sub>2</sub>-NO<sub>3</sub>-N, Unionized NH<sub>3</sub>)
- 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)

***IMPORTANT: Do not prepare a field blank for fecal samples, as the DI or distilled water is not sterile.***

## Field Procedures

1. To prepare a field blank, retrieve your pre-filled one-gallon cubitainer with DI or distilled water from storage in the vehicle.
2. Label an appropriate number of one liter sample containers 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/distilled 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 (Blind Duplicates)**

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. Bottles for same analytes should be collected side by side.

Duplication must include the full list of parameters sampled during the week. 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.

During repeat sampling efforts, (e.g., TMDL and Ambient), field crews should not repeatedly duplicate the same site.

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.**

## WVDEP Laboratory Quality Assurance Program

All water quality samples must be analyzed by a laboratory that has been approved through the WVDEP Laboratory Quality Assurance Program. The Quality Assurance Program is responsible for certifying environmental laboratories to ensure that the DEP receives accurate and reliable analytical data. Laboratories are certified when they follow approved methods, employ well-trained capable staff, and use equipment and instrumentation suited to the work they perform.

Certified laboratories are grouped into **three** categories.

1. **Commercial** – stand-alone laboratories testing samples for a fee.
2. **Municipal** - laboratories associated with publicly owned treatment works operated by cities or public service districts.
3. **Industrial** - manufacturing company owned and operated laboratories.

The majority of water quality samples collected by the Watershed Assessment Branch will be analyzed by certified commercial laboratories. However, sometimes it may be necessary to use a certified municipal laboratory due to holding times combined with distance to a commercial lab (e.g., fecal coliform samples). In addition, industrial laboratories may be used when cooperating with industry partners in certain studies.

The WVDEP maintains an up-to-date list of approved laboratories on the web located at: <http://www.dep.wv.gov/WWE/Programs/lab/Pages/default.aspx>

**This page intentionally left blank.**

## CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS

### Overview

Refer to the following references for further information about the Measurement & Calculation of Stream Flow:

Turnipseed, D.P., and Sauer, V.B. 2010. Discharge measurements at gaging stations: U.S. Geological Survey Techniques and Methods book 3, chap. A8, 87 p. Available online at: <http://pubs.usgs.gov/tm/tm3-a8/>

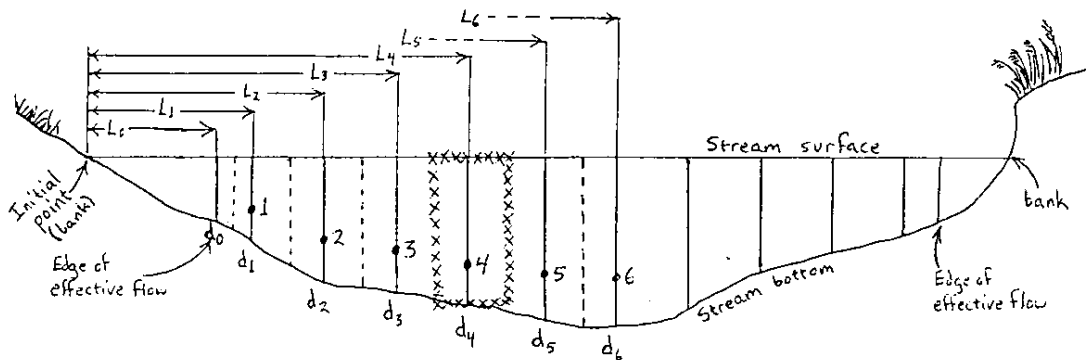
Rantz, S.E., and others, 1982, Measurement and computation of streamflow: Volume 1. U.S. Geological Survey Water-Supply Paper 2175, pages 1-284. Available online at: [http://pubs.usgs.gov/wsp/wsp2175/pdf/WSP2175\\_vol1a.pdf](http://pubs.usgs.gov/wsp/wsp2175/pdf/WSP2175_vol1a.pdf)

Rantz, S.E., and others, 1982, Measurement and computation of streamflow: Volume 2. Computation of Discharge. U.S. Geological Survey Water-Supply Paper 2175, pages 285-631. Available online at: [http://pubs.usgs.gov/wsp/wsp2175/pdf/WSP2175\\_vol2a.pdf](http://pubs.usgs.gov/wsp/wsp2175/pdf/WSP2175_vol2a.pdf)

### 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 4-1**, which shows the cross-section of a channel.



**Figure 4-1. 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 1. Calculation of Partial Stream Flow or Discharge**

$$Q_4 = (V_4)(d_4) \frac{[(L_5 - L_3)]}{2}$$

Where:

$Q_4$  = discharge or flow in cubic feet per second through partial section 4 (**see Figure 4-1 on the previous page**).

$V_4$  = 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 4-1 on the previous page**).

The area defined by this formula is that shown by the X-line highlight around location 4 in **see Figure 4-1 on the previous page**.

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 4-1 on page 4-10 for an example of the data used to calculate the total discharge or flow**).

## Materials and Supplies

1. Top-Setting Wading Rod – for measuring stream depth and setting depth of flow measuring device.
2. Marsh-McBirney Flo-Mate or OTT MF Pro – for measuring water velocity.
3. Tape Measure in feet and tenths – used as a tag line for determining the distance between velocity readings.
4. Flow Record Sheets (**see CHAPTER 2. Section C. APPENDIX #1 - Stream Discharge (Flow) on page 2-110**) – 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 Marsh-McBirney 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 4-2 below**).

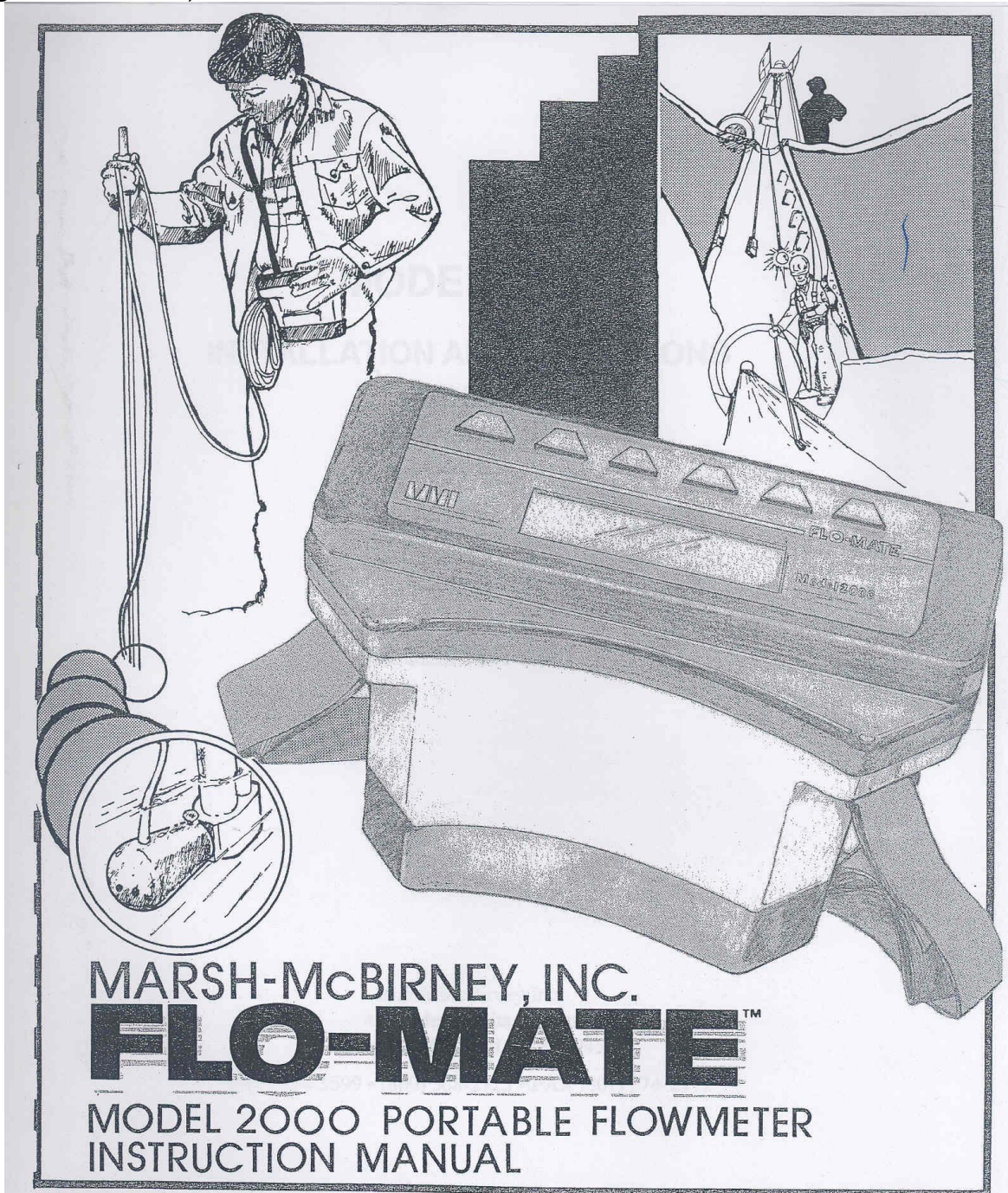


Figure 4-2. Cover of Model 2000 Marsh-McBirney Flo-Mate Instruction Manual

**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 4-3 on the next page.***

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.











### Model 2000 Key Summary

#### One Key Function

-  - Turns Unit ON. Clears the display and restarts the meter.
-  - Turns Unit OFF.
-  - Increments Fixed Period Averaging, Time Constant and Memory Location.
-  - Decrements Fixed Period Averaging, Time Constant and Memory Location.
-  - Switches Between Recall and Primary Operating Modes.
-  - Stores Values In Memory.

#### Two Key Function (Press at same time)

-   - Change Units, Turns Beeper ON/OFF
-   - Toggles Between Fixed Point Averaging and Time Constant.
-   - Clears Memory. Meter must be in the primary operating mode.
-   - Initiates zero adjust sequence. (Zero stability is  $\pm 0.05$  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 # 10200601

**Figure 4-3. 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**

Nonconductive 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 on page 4-5 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 sensor to the wading rod during the zero check and balancing the wading 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.**

- 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.

- 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".
- Use the "down" arrow to decrement to zero. The number "32" will be displayed.
- 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

- Insert the sensor peg on the bottom of the sliding wading 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 wading rod shaft. Tighten the thumb screw so that the sensor will not slide off or rotate out of position.
- AFTER you have set up your flow transect (**See *Setting up the Transect under Wadeable Stream Flow Measurement* Procedures on page 4-11**) 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 wading 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 in Flo-Mate Settings on page 4-5**), 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°C to 72°C (32°F to 160°F). The electronics on the flow meter have an operating temperature of 0°C to 50°C (32°F to 122°F).

## Part 2. Wadeable Stream Flow Measurement Procedures Using Marsh-McBirney Flo-Mate

Table 4-1. Example of Flow Measurement Form: Recording field data.

Stream Discharge Measurement (Calculated in cubic feet per second – cfs)							
ANCode		Date		WQ ID			
Measurer	Doug Wood	Time	1300	Meter ID	2		
Measurement	Description	Distance	Depth	Velocity			Notes
				0.2	0.4	0.8	
1	Left Bank	1.3	0	0	0	0	
2	Left EEF	2.6	0.3		0.01		
3		2.9	0.65		0.10		
4		3.6	0.9		0.45		
5		4.3	0.95		0.35		
6		4.9	0.95		0.51		
7		5.6	1.2		0.94		
8		6.2	1.6	0.97		0.84	
9		6.9	1.15		0.80		
10		7.5	0.9		0.69		
11		8.2	0.9		0.64		
12		8.9	0.65		0.35		
13		9.2	0.6		0.45		
14	Right EEF	9.9	0.4		0.02		
15	Right Bank	10.5					
16							
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 average increment between each of the targeted ten measuring points.

**IMPORTANT:** 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 or optimal 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 wading rod and placed on the substrate, please note this as well.**

***IMPORTANT: You may alter the channel to help meet these requirements at the selected site before you begin making any measurements, but NEVER AFTER measurement has begun. It is often possible in small streams to build dikes to cut off dead water and shallow flows within the transect. You can also improve the transect by removing rocks and debris within the section of water immediately upstream and downstream of the flow transect. ALWAYS allow the flow to stabilize after modifying the transect before starting the measurements.***

3. Determine the wetted width of the stream by placing a tape measure perpendicular to the stream flow to use as a tag line.
4. Determine the approximate 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 4-1 on page 4-10*).

5. Determine the approximate average 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 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.**
  - If the effective flow width is **less than 3 feet**, take **as many measurements as possible** no closer than 0.3 ft. apart using best professional judgment.
  - In streams with effective flow width **between 3 and 10 feet**, take **no fewer than ten 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 is **greater than 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 2-72 on page 2-111))**:
  - a. Record the ANCode, Date, and WQ Sample ID (used to designate measurement file names when using the OTT MF Pro meter).
  - b. The Measurer, Time of the Flow Measurement and assigned number of the flow meter used.
  - c. Any conditions that might affect the flow measurement (e.g., wind, rain, skewed bottom configuration, ice and leaf packs in the water, overhanging vegetation, necessity of removing the probe bulb from the rod due to extremely shallow water depth).

### **Starting the Transect**

7. Begin the Flow Measurement Process (*refer to Table 4-1 on page 4-10 and Figure 4-1 on page 4-2*):
  - a. Establish one wetted edge as the initial measurement point (Measurement 1). For simplicity, it is often easiest to start with the wetted that has the low end (closest to 0) of the tape measure tag line and then proceed along the tape measure. Record the Location Description (*i.e.*, Left Bank or Right Bank) and distance along the tag line.

- b. Determine the location of the edge of effective flow (EEF) along the tag line from the initial measurement point. This can be determined by using the meter to gage where the flow changes from 0 or negative to a slightly positive number. It can also be helpful to slightly disturb the sediments just upstream of the area observe where the water seems to be flowing and where there is dead water. Describe the location of the EEF (*e.g.*, Left EEF, Right EEF, Left Channel Left EEF, Left Channel Right EEF, *etc.*) under Measurement 2. This will be the location of the first flow measurement or vertical.

### **Taking Flow Measurements or Verticals**

8. Record the depth at the vertical. For a Marsh-McBirney Flo-Mate meter, the depth will be determined by observing the floating meniscus hitting the bottom of the wading rod. For at OTT MF Pro, the depth is determined by sensor readout on the display screen.

The depth of the vertical will determine how many velocity readings need to be taken at the vertical. There are three methods: the one-point, two-point, and three-point. Generally speaking, the more velocity measurements taken in a vertical, the more accurate the flow measurement will be. However, taking fewer velocity measurements may be necessary due to depth, time and stream conditions (*e.g.*, rapidly changing flow, ice cover, *etc.*).

Please note that the USGS refers to the depth locations in these methods in reference to the depth from the top of the water column while WAB refers to the depth locations in reference to the depth from the bottom of the water column. The table below shows the relationship between the two perspectives.

**Table 4-2. Relationship between USGS and WAB velocity measurement methods**

Agency			USGS	WAB
Reference Point			Top of Water Column	Bottom of Water Column
Method	One-Point	Middle	0.6 from Top	0.4 from Bottom
		Top	0.2 from Top	0.8 from Bottom
	Two-Point	Bottom	0.8 from Top	0.2 from Bottom
		Top	0.2 from Top	0.8 from Bottom
	Three-Point	Middle	0.6 from Top	0.4 from Bottom
		Bottom	0.8 from Top	0.2 from Bottom

The following conditions dictate which method should be used:

- a. If the depth is **less than or equal to 1.5 feet**, use the **One-Point** method for measuring velocity.

- b. If the depth is **greater than 1.5 feet**, use the **Two-Point** method for measuring velocity.
- c. The **Three-Point** method should be used only if the following conditions are met:
  - 1) If the depth is greater than 1.5 feet **and**
  - 2) The Top reading is greater than 2 times the Bottom velocity reading **or**
  - 3) The Bottom reading is greater than the Top reading **or**
  - 4) The Top reading is seriously affected by an obstruction (e.g., ice/ slush cover or overhanging vegetation dipping into the stream channel) **or**
  - 5) The Bottom reading is seriously affected by friction or turbulence produced by the streambed or an obstruction.



Figure 4-4. A) Top-Setting Wading Rod and B) Close-up of handle or Top-Setting Wading Rod.

9. Adjust the wading rod so that the sensor is at the depth appropriate for the method selected. By default, the wading rod is calibrated to set the sensor at 0.4 from the bottom of the water column.

a. To set the sensor at 0.4 from the Bottom of the Water Column line up the rod's sliding "foot" with the tenth scale Vernier on the wading rod handle (**see Figure 4-4 to the left**). For example, 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.

b. To set the sensor at 0.8 from the bottom, 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.

c. To set the sensor at 0.2 from the bottom, 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:** When more than one velocity reading is taken at a vertical, they are averaged together (by the spreadsheet or database programming) to come up with a final velocity measurement.



10. Place the sensor at the proper depth and allow it to adjust to the velocity using the following precautions before starting the observation:
  - a. Hold the rod perfectly straight up and vertical 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 wading rod also helps visualize the flow direction.

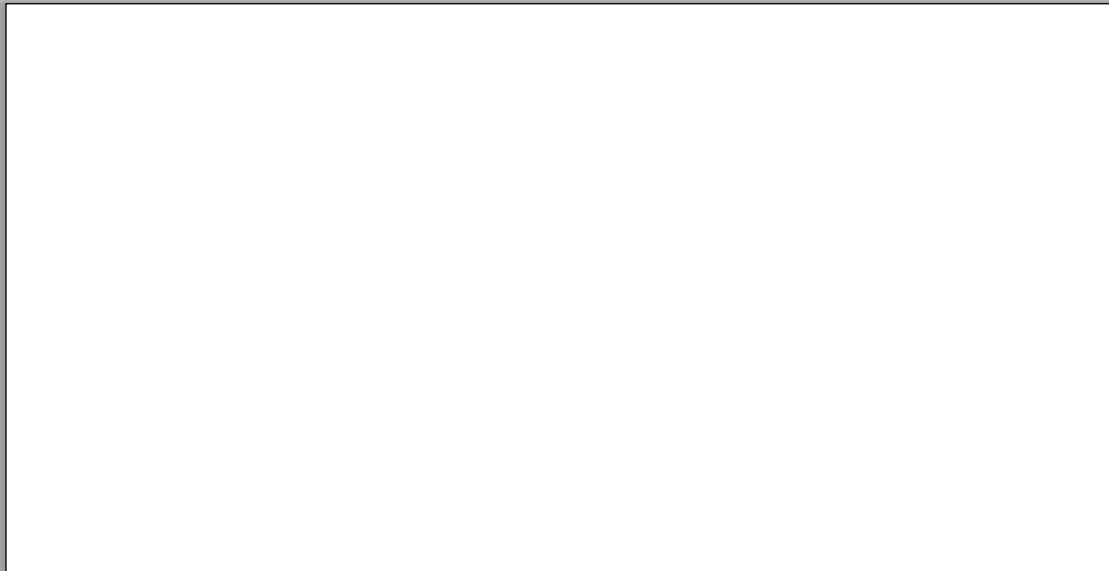
**NOTE:** The Marsh-McBirney manufacturer says the sensor shape produces a cosine response that greatly reduces errors due to sensor positioning. For example, if the front of the sensor is pointed away from the flow at a  $10^\circ$  angle, the cosine of  $10^\circ$  is 0.98480. This is only 1.5% lower 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.

- b. Stand downstream from the meter in a position that least affects the velocity the water passing the current meter by facing the bank with the water flowing against the side of the leg. This presents a narrower profile in the water than standing facing upstream (*see Figure 4-5 below*). In very small streams, attempt to straddle the wetted area to decrease your feet's contributions to altering the flow.



Figure 4-5. Example of taking flow measurements at a transect.

- c. Holding the wading rod at the tag line, stand 1 to 3 inches downstream of the tag line and 18 inches or more from the wading rod. This distance should create minimal turbulence that will impact the reading.
  - d. When measuring in streams with shifting beds, the scoured depressions left by your feet can affect the velocity readings. Make sure to always keep the meter as far upstream from the hydrographer body and feet in such situations and record notes describing the situation for each such measurement.
11. Begin recording the velocity for each depth necessary. Allow the Marsh-McBirney Flo-Mate meter to go through two complete cycles (40 seconds total) as described in ***Using the Flo-Mate on page 4-8***. Record the second readout on the appropriate “velocity” column on the form. Repeat for any other depths necessary at that vertical.
  12. Establish the next vertical (Measurement 3) at an appropriate distance from the previous one by observing the change in depth and velocity while moving across the stream (discussed in Step 5). Record the distance along the tag line, the depth, and appropriate velocities according to Steps 8 thru 12 at each vertical.
  13. When nearing the far wetted edge, be mindful of establishing a final vertical somewhere near the EEF. Be sure to record the distance and description of the far wetted edge along the tag line. When done, a vertical should be taken at all of the measurement points except the first one and last one (the wetted edges of the stream where depth and velocity is interpreted to be 0).



### **Calculating Flow**

The flow can be calculated in one of three ways:

- 1) Using a Spreadsheet
  - a. Open Excel, and then open the file in Q:\WATER RESOURCES\WAB\TOOLS\Flow Template.xls.
  - b. 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.
  - c. Type in the left and right edge-of-effective-flow values in the “no flow” rows of the “Distance” column.
  - d. Enter the distance, depth, and velocity in the appropriate columns.
  - e. The flow or discharge in CFS is automatically calculated in the lower right-hand corner of the spreadsheet.
- 2) Via data entry into the Database
- 3) Within the instrument (e.g., OTT MF Pro meter)

### **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 11-2 and Figure 11-3 in CHAPTER 11. Section A. Part 4. Measuring Stream Flow starting on page 11-8 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.

All measurements calculated within the instrument (*i.e.*, OTT MF Pro meter) are downloaded to a .csv file and stored permanently on the network drive. During data entry, the raw measurement value (distance, depth, velocities at different depths, *etc.*) is copied from the .csv to the database. The copied data is in turn run thru a separate calculation (identical to the spreadsheet calculation) within the database and the results are compared between the two for discrepancies (beyond differences in rounding). Any discrepancies are resolved and documented.

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 5. 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.

**MULTI-METRIC INDEX** – An index that incorporates several attributes (community richness, abundance, and tolerance metrics) reflecting biological integrity into one synthetic score.

### **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 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 used in the WVDEP Watershed Assessment Branch (WAB) are qualitative in nature and are adapted from "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 2-27. Cover of USEPA's Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Second Edition) in CHAPTER 2. Section C. PAGES 5, 6, 5a, and 6a starting on page 2-64*). Specifically, WAB uses a RBP III-type method, which consists of laboratory processing and identification of field samples (as opposed to RBP I or II methods which involves field identifications of the samples). The RBP III-type method has become the standard for the bioassessment of the benthic macroinvertebrate assemblage and has been adopted and modified for use by many states and organizations conducting such bioassessments.

## Selecting Sampling Method and Device

The sampling methods with device are outlined and prioritized below:

RBP III Single Habitat Methods and Devices:

- A. Rectangular Dip-Net/Modified Kick-Net – This method is a disturbance-removal sampling type used in riffle/run habitats  $\geq 0.5$  meter wide and refers to the use of a modified **dip-net/kick-net** or “surber-on-a-stick” type device that has a **rectangular frame** and net opening (0.5 m wide by  $\sim 0.3$  m tall) with an internal net space that tapers to a point. It is used as a one man kick-net and requires **four**  $0.25 \text{ m}^2$  (0.5 m wide by 0.5 m long in front of the net) **kicks** to get a  $1.0 \text{ m}^2$  total kick area. This is the most commonly used method for WAB benthic sampling (> 99% of the time).

**NOTE:** *This method is comparable with established MMI/IBIs (i.e., WVSCI & GLIMPSS) if you sample a  $1.0 \text{ m}^2$  total kick area and have an appropriate flow (i.e., not too high so that water is going over the net, not too fast so that benthos gets pushed out of the net by eddies, and not too little or low so that the benthos does not get pushed into the net).*

- B. D-Frame Dip-Net/Kick-Net – This method is a disturbance-removal sampling type used in riffle/run habitats  $< 0.5$  meter wide and refers to the much smaller, narrower **D-shaped frame** and net opening (1.0 ft. wide by  $\sim 0.6$  ft. tall) with an internal net space that tapers to a point much like the Rectangular Dip Net Kick-Net does. It should be noted that the length of the net may vary (i.e., some are longer, some are shorter). It takes **eleven**  $1.0 \text{ ft}^2$  (1.0 ft. wide by 1.0 ft. long in front of the net) **kicks** to approximate a  $1.0 \text{ m}^2$  total kick area. We only use this net when the wetted stream channel is too narrow across to fit the 0.5 m wide Rectangular Dip-Net/Kick-Net, but still has adequate flow to



push the benthos into the net. It is very rare to have such conditions in WV, so WAB rarely uses this method.

**NOTE: This method is comparable with established MMIs if you sample a 1.0 m<sup>2</sup> total kick area and have an appropriate flow (keeping in mind that this device is slightly shorter than the rectangular frame net).**

- C. Hand Picking – This method is a disturbance-removal sampling type used in riffle/run habitats in very small or low flow streams where other sampling apparatus cannot be used. It basically involves **hand picking** and washing the substrate into a bucket. It is only used in special surveys or when instructed to get a benthic sample even if it is too low to kick.

**NOTE: This method is not comparable with established MMI/IBIs.**

RBP III Multi-Habitat Methods and Devices:

- D. D-Frame Dip-Net/MACS – Also called the Mid-Atlantic Coastal Stream (**MACS**) method, this method is used in the absence of moving water (*i.e.*, in low-gradient streams, glide/pool habitat, or other wetland-like aquatic habitats where flow is insufficient to move suspended materials into a net). The device used is the same **D-shaped frame** and net opening (1.0 ft. wide by ~0.6 ft. tall) as described above. However, the net attached to the frame is different as it does not taper, but is flat along the back edge and sampling consists of taking 20 jabs in multiple habitats like submerged vegetation, logs, undercut banks, and snags. It is only used in special surveys or when instructed to get a benthic sample even if no riffle/run habitat is available.

**NOTE: This method is not comparable with established MMI/IBIs.**

It is important to note that the first two methods listed above were established for use by the Watershed Assessment Branch monitoring programs and intended to provide cost-effective techniques with comparable data across the state. The WAB will utilize the Single Habitat Approach whenever possible, using a rectangular dip net (0.5 m wide) or smaller (0.3 m wide) D-net with a **595-600 µm mesh size** (note that this is different than what is described in the RBP manual) to sample riffle/run habitats. Note that these two are the only sampling protocols that are currently supported by a benthic macroinvertebrate MMI or IBI in WV (**see Section E Benthic Macroinvertebrate Sample Comparability starting on page 5-44**). Special projects outside of the normal Watershed Assessment Branch monitoring agenda (*e.g.*, point source surveys, spills, large river monitoring) may not allow strict adherence to these protocols. These methods are described in detail in the subsequent sections.

## Selecting Sampling Sites and Kick Locations

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 2. Section A. Part 2. Sliding the Reach starting on page 2-9*) 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 kick sampling points with the intent to make collections throughout the entire 100 meters in a diversity of the best available habitats (also called richest-targeted habitat methodology). 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.

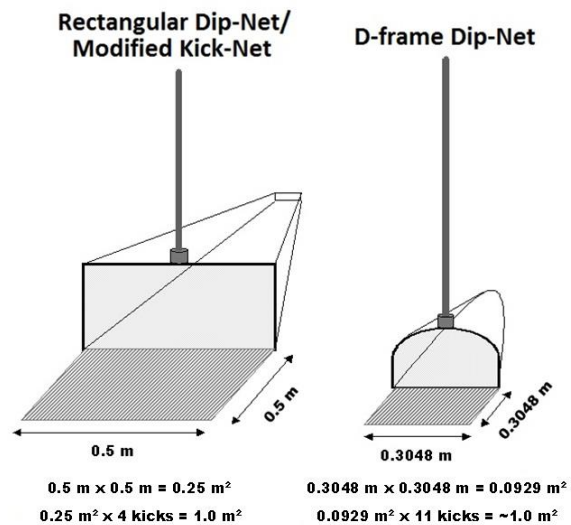
**Vegetated and Undercut Banks** - Stream banks having submerged vegetation (shrubs, etc.) and/or root wads.

## Section A. Benthic Macroinvertebrate Sampling

### Materials and Reagents

See *Figure 5-1* & *Figure 5-2* for *Diagrams & Picture of most of these materials.*

1. Rectangular Frame Dip-Net (AKA Modified Kick-Net) – A net with a 0.5 m wide and 0.3 m high frame with **595-600  $\mu$ m 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 **595-600  $\mu$ m 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  $\mu$ m) - 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.



**Figure 5-1. Diagram comparing the dimensions and number of kicks necessary to sample 1 m<sup>2</sup> of a Rectangular Frame Dip Net versus a D-Frame Dip Net**



**Figure 5-2. Photo of Materials used in Benthic Macroinvertebrate Sampling**

## 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. Field 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  $\frac{1}{2}$  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.

### Single Habitat-Riffle/Run Habitats using a Rectangular Frame Dip-Net/Kick-Net

#### ✓ Comparable for use with current MMI/IBIs

This method is used in streams having riffle/run habitat and a width  $\geq 0.5$  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 5-3 on right*).
2. Hold the sampler in position on the substrate while checking for heavy organisms such as clams and snails in an area of about  $0.25 \text{ m}^2$  (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.



**Figure 5-3. Photo of Rectangular Frame Dip Net being placed on stream bottom**



***IMPORTANT:*** 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.

3. Brush the surfaces of all coarse gravel, cobble, boulder, and bedrock substrate (**see Figure 5-4 on right**). 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<sup>2</sup> kick area.



Figure 5-4. Photograph of the Brushing process in front of the net.

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<sup>2</sup> (0.5m wide net x 0.5m upstream) in front of the net (**see Figure 5-5 on right**). 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 5-5. Photograph of the Kicking process in front of the net.





**Figure 5-6. 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 5-6 above*). Empty the contents of the net into a five gallon bucket that is partially filled with water (*see Figure 5-7 on right*). 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 5-7. 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<sup>2</sup> (4 x 0.25 m<sup>2</sup>) 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<sup>2</sup> versus 1 m<sup>2</sup>) and consultation with USEPA Region III biologists that a 1 square meter sample is adequate for characterizing riffle/run streams in West Virginia where the a MMI/IBI 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 5-8 on right**). 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 5-8. 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 5-9 on right**). 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 elutriated material aside.



**Figure 5-9. Photograph of soft, organic material placed and stored in a temporary container.**

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.

***IMPORTANT: 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 5-10 on right*). 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 5-10.** Photograph of Biologist transferring the hard, inorganic material (e.g., fine gravel, sand, and silt) to a sample jar 1/2 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 5-11.** 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 5-11 on previous page*). 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.

**Single Habitat-Riffle/Run Habitat using a D-Frame Dip-Net/Kick-Net**✓ **Comparable for use with current MMI/IBIs**

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 1 ft. wide (or  $\approx 0.3048$  m wide), we will sample a square area in front of the net of  $0.0929 \text{ m}^2$  ( $0.333\text{m} \times 0.333\text{m}$ ). In order to sample  $1 \text{ m}^2$ , we need to collect from 11 locations ( $0.0929 \text{ m}^2 \times 11 = 1.0219 \text{ m}^2$ ).

**Hand Picking (Small narrow streams with minimal/interstitial flow)**✓ **Non-Comparable for use with current MMI/IBIs**

***IMPORTANT: 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.***

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  $0.25 \text{ m}^2$  (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.

**MACS (Multi-habitat Approach for Low Gradient Streams, Glide/Pool Habitat)****✓ D-Frame Dip-Net=Non-Comparable for use with current MMI/IBIs**

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.

**IMPORTANT: This method is to be used only in wetland type habitat where flow is insufficient to move suspended materials into a net. This type of sampling is considered non-comparable. 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.

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. Field 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. The majority of these events will occur when there are at least two people on the sampling team (which is the norm). However, a new effort will be made to have duplicate samples conducted by one sampler to measure within-sampler variance in the collection methodology. Such single-sampler duplicates can occur whenever a sampler is working alone or in a team and the opportunity (both in time and available comparable habitat) is available to collect two sets of samples. In such cases, only the Benthic Collection Information portion of the field form must be filled out as one cannot have a within-sampler RBP habitat duplicate.

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 14. Section A. Blanks and Duplicates starting on page 14-1 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 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 5-12 on right**) 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 40 inches by 10 inches. There are 100 grids in the tray and each is 2 inch by 2 inch in dimension.
7. Cookie cutter - a homemade cookie cutter, 2 inch by 2 inch is used in conjunction with the sorting tray to isolate each of the subsamples.



Figure 5-12. Photograph of a Home-Made Gridded Sorting Tray featuring a random number matrix on the bottom.

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.

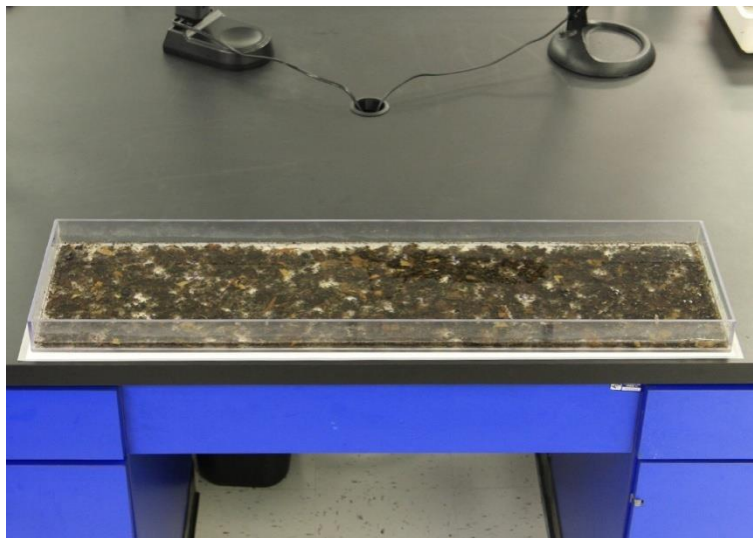
## Benthic Sample Processing Methods

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)

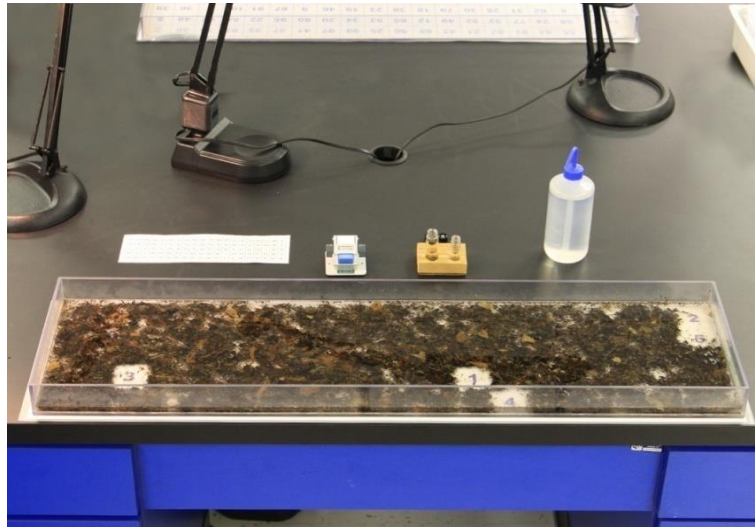
If any of this information is missing from the original sample jar label, notify the supervising biologist so that the error can be corrected.

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 (600  $\mu$ m) 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 5-13 on right**). 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 5-13. 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 5-14 on right**). 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 5-14. Photograph of a Gridded Sorting Tray with 5 grids randomly removed. Note that the 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 magnivisor (**see Figure 5-15 on right**), 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 5-15. 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 5-14 on page 5-19**). 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 of 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 **more than 240 organisms** in the subsample, then the subsample should be re-sampled 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-sampled to reach the 200 (+/- 20%) organism goal. This step will ensure representativeness of the subsample compared to the total sample.



**NOTE:** For further information about subsampling rules, refer to the *USEPA Rapid Bioassessment Protocol References listed in CHAPTER 2. Section C. Part 1. PAGES 5, 6, 5a, and 6a USEPA's Rapid Visual-Based Habitat Assessment starting on page 2-64.*

**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<sup>2</sup> 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 put 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 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 2. Percent Sorting Efficiency (PSE)**

$$\frac{\text{\# Organisms Originally Sorted}}{\text{\# Organisms Recovered by Checker} + \text{\# Organisms Originally Sorted}} \times 100 = \text{PSE}$$

A PSE  $\geq$  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 has multiple tools available (**see Section D Benthic Macroinvertebrate Data Analysis starting on page 5-36**), each with unique taxonomic resolution requirements (e.g., Family vs. Genus level taxonomy) that must be considered during the identification process. Ideally, all aquatic macroinvertebrate taxa should be identified to the genus level or lowest practical taxon 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 5-16 on page 5-32**).
10. Taxonomic Keys - (**see List of Taxonomic References below**).

## List of Taxonomic References

The taxonomic references frequently used by the WAB biologists for identification of macroinvertebrates include, but are not limited to:

### General Keys

Brigham, A.R., W.U. Brigham, and A. Gnilka (eds.). 1982. Aquatic Insects and Oligochaetes North and South Carolina. Midwest Aquatic Enterprises, Mahomet, IL.

Merritt, R.W., and K.W. Cummins (eds.). 1995. An Introduction to the Aquatic Insects of North America. 3<sup>rd</sup> edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Merritt, R.W., K.W. Cummins, and M.B. Berg (eds.). 2008. An Introduction to the Aquatic Insects of North America. 4<sup>th</sup> 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. 2<sup>nd</sup> edition. John Wiley & Sons, New York.

Pennack, R.W. 1989. Fresh-water Invertebrates of the United States – Protozoa to Mollusca. 3<sup>rd</sup> Edition. John Wiley and Sons, Inc., New York, New York. 628 pp.

Pfeiffer, J., Kosnicki, E., Bilger, M., Marshall, B.D. and W. Davis. 2008. Taxonomic Aids for Mid-Atlantic Benthic Macroinvertebrates (Ephemeroptera: Baetidae; Plecoptera: Capniidae/Leuctridae; Diptera: Simuliidae). EPA-260-R-08-014. United States Environmental Protection Agency, Office of Environmental Information, Environmental Analysis Division, Washington, DC. Available on-line at: <http://www.epa.gov/bioindicators/html/publications.html>

Smith, D.G. 2001. Pennack's Freshwater Invertebrates of the United States: Porifera to Crustacea. 4<sup>th</sup> edition. John Wiley & Sons, New York.

Thorp, J.H and A.P. Covich, Eds. 2001. Ecology and Classification of North American Freshwater Invertebrates. Second Edition. Academic Press.

### Annelida

Brinkhurst, R.O. 1986. Guide to the freshwater aquatic microdile oligochaetes of North America. *Canadian Special Publication of Fisheries and Aquatic Sciences* **84**: 259 pp.

Klemm, D.J. (ed.). 1985. A guide to the freshwater Annelida (Polychaeta, naidid and tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co., Dubuque, Iowa.

Klemm, D.J. 1997. Identification Guide to the Freshwater Leeches (Annelida: Hirudinea) of Florida and Other Southern States. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/leeches.pdf>

Milligan, M.R. 1997. Identification Manual for the Aquatic Oligochaeta of Florida: Volume I-Freshwater Oligochaetes. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/oligofw.pdf>

### **Crustacea**

Hobbs, H.H., Jr. 1972. Biota of Freshwater Ecosystems, Identification Manual no. 9. Crayfishes (Astacidae) of North and Middle America. EPA-WPCRS No. 18050, ELD05/72. Supt. Doc. No. 5501-0399, United States Environmental Protection Agency, Washington, D.C. 173 pp. Available on-line at:  
<http://www.epa.gov/nscep/index.html>

Holsinger, J.R. 1972. Biota of Freshwater Ecosystems, Identification Manual no. 5. Freshwater amphipod crustaceans (Gammaridae) of North America. WPCRS No. 18050, ELD04/72. Supt. Doc. No. 5501-0369, United States Environmental Protection Agency, Washington, D.C. 89 pp.

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.

Rogers, D.C. and M. Hill. 2008. Key to the Freshwater Malacostraca (Crustacea) of the Mid-Atlantic Region. EPA-230-R-08-017. United States Environmental Protection Agency, Office of Environmental Information, Environmental Analysis Division, Washington, DC. Available on-line at:  
<http://www.epa.gov/bioindicators/pdf/EPA-230-R-08-017KeystotheFreshwaterMalacostracaoftheMid-AtlanticRegion.pdf>

Taylor, C.A., and G.A. Schuster. 2004. The Crayfishes of Kentucky. Illinois Natural History Survey Special Publication No. 28. 219 pp.

Williams, W.D. 1972. Biota of Freshwater Ecosystems, Identification Manual no. 7. Freshwater isopods (Asellidae) of North America. WPCRS No. 18050, ELD05-72. Supt. Doc. No. 5501-0390, United States Environmental Protection Agency, Washington, D.C. 45 pp.

**Acarina**

Pluchino, E.S. 1984. Guide to the Common Water Mite Genera of Florida. Florida Department of Environmental Regulation, Technical Series Vol. 7 No. 1. Orlando, Florida. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/mites.pdf>

**Ephemeroptera**

Bednarik, A.F. and W.P. McCafferty. 1979. Biosystematic revision of the genus *Stenonoma* (Ephemeroptera: Heptageniidae). *Canadian Bulletin of Fisheries and Aquatic Sciences* **21**:1-73.

Berner, L. and M.L. Pescador. 1988. 2<sup>nd</sup> Ed. The mayflies of Florida. Univ. Florida Press, Gainesville, Florida. 352 pp.

Burks, B.D. 1953. The mayflies, or Ephemeroptera, of Illinois. Illinois Natural History Survey (Urbana) Bulletin 26, Part 1:1-211.

Edmunds, G.F., Jr., S.L. Jensen, and L. Berner. 1976. Mayflies of North and Central America. University of Minnesota Press.

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.

Lugo-Ortiz, C.R., W.P. McCafferty, and R.D. Waltz. 1999. Definition and reorganization of the genus *Pseudocloeon* (Ephemeroptera: Baetidae) with new species descriptions and combinations. *Transactions of the American Entomological Society* **125**: 1-37.

McCafferty, W.P. 1975. The burrowing mayflies (Ephemeroptera: Ephemeridae) of the United States. *Transactions of the American Entomological Society* **101**:447-504.

McCafferty, W.P. and R. D. Waltz. 1995. *Labiobaetis* (Ephemeroptera: Baetidae): New status, new North American species, and related new genus. *Entomological News* **106**(1):19-28.

McCafferty, W.P., M.L. Wagle, and R.D. Waltz. 1994. Systematics and biology of *Acentrella turbida* (McDunnough) (Ephemeroptera: Baetidae). *Pan-Pacific Entomologist* **70**(4):301-308.

Morihara, D.K. and W.P. McCafferty. 1979. The *Baetis* larvae of North America (Ephemeroptera: Baetidae). *Transactions of the American Entomological Society* **105**(2):129-221.

Pescador, M.L. and L. Berner. 1981. The mayfly Baetiscidae (Ephemeroptera). Part 2. Biosystematics of the genus *Baetisca*. *Transactions of the American Entomological Society* **107**:163-228.

Pescador, M.L. and B.A. Richard. 2004. Guide to the Mayfly (Ephemeroptera) Nymphs of Florida. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, Florida. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/mayflyguide.pdf>

Provonsha, A.V. 1990. A revision of the genus *Caenis* in North America (Ephemeroptera: Caenidae). *Transactions of the American Entomological Society* **116**(4):801-884.

Tarter, D.C. and R.F. Kirchner. 1978. A new species of *Baetisca* from West Virginia (Ephemeroptera: Baetiscidae). *Entomological News* **89**(9-10):209-213.

### **Odonata**

Needham, J.G. and M.J. Westfall, Jr., and M.L. May. 2000. Dragonflies of North America. Scientific Publishers, Gainesville, FL. 940 pp.

Richardson, J.S. 2003. Identification Manual for the Dragonfly Larvae (Anisoptera) of Florida. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/dragonflykey.pdf>

Richardson, J. 2010. Identification Manual for the Damselfly Larvae (Zygoptera) of Florida. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/zygoptera.pdf>

Westfall, M.J. and M.L. May. 1996. Damselflies of North America. Scientific Publishers, Inc., Gainesville, FL. 650 pp.

### **Plecoptera**

Frison, T.H. 1935. The stoneflies, or Plecoptera, of Illinois. *Illinois Natural Survey Bulletin* **20**: 280-471.

Pescador, M.L., B.A. Richard, and A.K. Rasmussen. 2000. A Guide to the Stoneflies (Plecoptera) of Florida. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/stonefly.pdf>

Stewart, K.W. and B.P. Stark. 1988. Nymphs of North American Stonefly Genera (Plecoptera). Entomological Society of America.



Stewart, K.W. and B.P. Stark. 2002. Nymphs of North American stonefly genera (Plecoptera). Second Edition. The Caddis Press, Columbus, OH. 510 pp.

### **Heteroptera**

Epler, J.H. 2006. Identification Manual for the Aquatic and Semi-Aquatic Heteroptera of Florida (Belostomatidae, Corixidae, Gelastocoridae, Gerridae, Hebridae, Hydrometridae, Mesoveliidae, Naucoridae, Nepidae, Notonectidae, Ochteridae, Pleidae, Saldidae, Veliidae). Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/heteroptera.pdf>

### **Coleoptera**

Brown, H.P. 1972. Aquatic Dryopoid Beetles (Coleoptera) of the United States. U. S. Government Printing Office. Available on-line:  
<http://www.archive.org/details/aquaticdryopoidb00brow>

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, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/beetles96.pdf>

Epler, J.H. 2010. Freshwater Beetles of Florida: An identification manual for the families Chrysomelidae, Curculionidae, Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae, Scirtidae). Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/beetles10.pdf>

### **Megaloptera and Neuroptera**

Rasmussen, A.K. and M.L. Pescador. 2002. A Guide to the Megaloptera and Aquatic Neuroptera of Florida. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, FL. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/megaloptera.pdf>

### **Trichoptera**

Chapin, J.W. 1978. Systematic of Nearctic *Micrasema* (Trichoptera: Brachycentridae). Ph.D. Dissertation, Clemson University, Clemson, South Carolina, 136 pp.

Flint, O.S. 1962. Larvae of the Genus *Rhyacophila* in eastern North America (Trichoptera: Rhyacophilidae). *Proceedings of the U.S. National Museum* (Washington, D. C.) **113**:465-493.

- Flint, O.S. 1984. The genus *Brachycentrus* in North America, with a proposed phylogeny of the genera of Brachycentridae (Trichoptera). Smithsonian Contributions to Zoology No. 398.
- Floyd, M.A. 1995. Larvae of the caddisfly genus *Oecetis* in North America. Bulletin of the Ohio Biological Survey, New Series, Vol. 10, No. 2, 85 pp.
- Glover, J.B. 1996. Larvae of the caddisfly genera *Triaenodes* and *Ylodes* (Trichoptera: Leptoceridae) in North America. Bulletin of the Ohio Biological Survey, New Series, Vol. 11, No. 2, vii+89 pp.
- Pescador, M.L., A.K. Rasmussen, and S.C. Harris 2004. Identification Manual for the Caddisfly (Trichoptera) Larvae of Florida. Revised Edition. Florida Department of Environmental Protection, Division of Water Facilities, Tallahassee, Florida. Available on-line at:  
<http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/caddisfly.pdf>
- Prather, A.L. and J.C. Morse. 2001. Easter Nearctic Rhyacophila species, with revision of the *Rhyacophila invaria* group (Trichoptera: Rhyacophilidae). *Transactions of the American Entomological Society* **127**:85-166.
- Ross, H.H. 1944. The Caddisflies, or Trichoptera, of Illinois. *Bulletin of the Illinois Natural History Survey* **23**: 1-326.
- Scheffer, P.W. and G.B. Wiggins. 1986. A systematic study of the Nearctic larvae of the *Hydropsyche morosa* group (Trichoptera: Hydropsychidae). Royal Ontario Museum, Toronto, Canada. 94 pp.
- Schuster, G.A. and D.A. Etnier. 1978. A manual for the identification of the larvae of the caddisfly genera *Hydropsyche* Pictet and *Symphitopsyche* Ulmer in eastern and central North America (Trichoptera: Hydropsychidae). EPA-600/4-78-060. United States Environmental Protection Agency, Washington, D.C. 129 pp. Available on-line at: <http://www.epa.gov/nscep/index.html>
- 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). 2<sup>nd</sup> edition. University of Toronto Press, Toronto, Canada.

### **Diptera**

- Adler, P.H. and K.C. Kim. 1986. The Black Flies of Pennsylvania (Simuliidae, Diptera). Bionomics, Taxonomy, and Distribution. The Pennsylvania State University Agricultural Experimental Station Bulletin. 856, 85 pp.

- Bode, R.W. 1983. Larvae of North American *Eukiefferiella* and *Tventenia* (Diptera: Chironomidae). *Bulletin of the New York State Museum* **452**:1-40.
- 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. Available on-line at: <http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/midges.pdf>
- 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. Available on-line at: [http://www.esb.enr.state.nc.us/BAUwww/Chiron\\_manual/intro.pdf](http://www.esb.enr.state.nc.us/BAUwww/Chiron_manual/intro.pdf)
- Gelhaus, J.K. 2002. Manual for the Identification of Aquatic Crane Fly Larvae for Southeastern United States. Unpublished.
- Gelhaus, J.K. 2008. Manual for the Identification of Aquatic Crane Fly Larvae for North America. North American Benthological Society Workshop. Unpublished Draft.
- McAlpine, J.F. (Ed.). 1989. Manual of Nearctic Diptera. Vols. 1-3. Research Branch Agriculture Canada. Monograph No. 32
- Pechuman, L.L., D.W. Webb, and H.J. Teskey. 1983. The Diptera, or true flies, of Illinois. I. Tabanidae. *Illinois Natural History Survey Bulletin* **33**(1):1-122.
- Simpson, K.W., R.W. Bode, and P. Albu. 1982. Keys for the genus *Cricotopus* adapted from "Revision der Gattung *Cricotopus* and der Wulp und ihrer Verwandten (Diptera, Chironomidae)" by M. Hirvenoha. Bull. 450. New York State Museum, Albany, NY.
- Webb, D.W. 1977. The Nearctic Athericidae. *Journal of the Kansas Entomological Society* **50**:473-495.

### **Mollusca**

- Branson, B.A. 1987. Keys to the aquatic gastropoda known from Kentucky. Transactions of the Kentucky Academy of Sciences. 48(1-2):11-19.
- Burch, J.B. 1972. Biota of Freshwater Ecosystems, Identification Manual no. 3. Freshwater Sphaeriacean Clams (Mollusca: Pelecypoda) of North America. WPCRS No. 18050, ELD03/72. Supt. Doc. No. 5501-0367, United States Environmental Protection Agency, Washington, D.C. 31 pp.

- Burch, J.B. 1973. Biota of Freshwater Ecosystems, Identification Manual no. 11. Freshwater Unionacean Clams (Mollusca: Pelecypoda) of North America. WPCRS No. 18050, ELD03/73. Supt. Doc. No. 5501-0367, United States Environmental Protection Agency, Washington, D.C. 181 pp. Available on-line at: <http://www.epa.gov/nscep/index.html>
- Burch, J.B. 1982. Freshwater Snails (Mollusca: Gastropoda) of North America. EPA-600-3-82-026. United States Environmental Protection Agency, Washington, D.C. Available on-line at: <http://www.epa.gov/nscep/index.html>
- Heard, W.H. 1979. Identification Manual of the Freshwater Clams of Florida. Florida Department of Environmental Regulation, Technical Series Vol. 4 No. 2. Orlando, Florida. Available on-line at: <http://publicfiles.dep.state.fl.us/dear/labs/biology/biokeys/clams.pdf>
- Parmalee, P.W. and A.E. Bogan. 1998. The Freshwater Mussels of Tennessee. University of Tennessee Press. Knoxville, Tennessee. 328 pp.

## 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 5-16 on page 5-32*).
3. Using the taxonomic keys listed above (*see List of Taxonomic References starting on page 5-23*); 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 5-16 on the next page***). 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.
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.

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
<b>Annelida</b>		<b>Plecoptera</b>		<b>Diptera (Chironomidae)</b>	
<b>Amphipoda</b>					
<b>Isopoda</b>					
				<b>Diptera (other)</b>	
<b>Decapoda</b>		<b>Trichoptera</b>			
<b>Ephemeroptera</b>					
		<b>Megaloptera</b>			
				<b>Mollusca</b>	
<b>Odonata</b>					
		<b>Coleoptera</b>			
				<b>Other Taxa</b>	

Figure 5-16. Example of a Benthic Macroinvertebrate Lab Sheet.



## Benthic Laboratory Identification Quality Assurance/Quality Control

### Part 1. SFS Taxonomic Certification Program

#### Program Background and Justification

The accurate and precise identification and classification of organisms provides the foundation for many ecological investigations of streams, rivers, and lakes, including biological assessment and monitoring programs aimed at evaluating the quality of habitat and water.

High quality taxonomy is crucial to credible ecological studies and reliable bioassessment programs. However, there is concern that:

- A) There are many errors and inaccuracies associated with the taxonomy of some on-going programs;
- B) There is no recognized protocol in North America for evaluating the taxonomic ability of people identifying invertebrates; and;
- C) Academic support for faculty positions and student training related to non-molecular, organismal taxonomy is declining.

These concerns have been expressed by a number of state and federal agencies (e.g., Kentucky, North Carolina; Environment Canada, US Environmental Protection Agency, US Geological Survey) involved in environmental regulation and monitoring as well as by individuals involved in both basic and applied scientific research.

Consequently, the Society for Freshwater Science (SFS, formerly North American Benthological Society or NABS) decided to implement a certification program for those involved with macroinvertebrate identification in North America. This program will certify that trained and skilled persons are providing aquatic invertebrate identifications. In addition, it is anticipated that this program will help promote undergraduate and graduate training of new taxonomic experts, the training of taxonomic technicians through workshops, the development of new manuals for identifying aquatic invertebrates, and taxonomic excellence in other disciplines and other parts of the world. The certification program will test a candidate's knowledge and skills in aquatic invertebrate taxonomy and will provide the successful applicant with a **certificate of proficiency that lasts five years**.

Genus level testing is conducted in one of two ways:

- 1) Specimen-based using actual whole or slide mounted specimens provided by SFS or
- 2) Online image-based (similar to the Family level test for Aquatic Insects).

In addition, Genus level testing is broken out into multiple taxonomic groups and by US geographic region (i.e., Eastern or *occurring east of the Rocky Mountains* vs. Western

or occurring in and west of the Rocky Mountains). The five Taxonomic Groups (eastern and western) are as follows:

- 1) Ephemeroptera, Plecoptera, and Trichoptera (EPT) genera (nymphs and larvae only) (**Image-Based test**),
- 2) Chironomidae genera (larvae only) (**Specimen-Based test**),
- 3) Genera of Crustacea and arthropods (including insects) other than EPT and Chironomidae (immatures and adults as appropriate) (**Specimen-Based test**),
- 4) Oligochaeta genera (**Specimen-Based test**),
- 5) Mollusca genera (field identifications are not included) (**Specimen-Based test**).

The WVDEP headquarters in Kanawha City is a certified testing center for all taxonomic identification tests offered by the SFS Taxonomic Certification Program (TCP). Individuals may be certified at either the Family or Genus-level. Details about testing can be found online at:

[http://www.dep.wv.gov/WWE/watershed/bio\\_fish/Documents/TaxonomicIdentificationCertification.docx](http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/TaxonomicIdentificationCertification.docx)

## Part 2. Benthic Identification QA/QC Metrics

The precision of the identification process is evaluated for at least 5% of the samples. The samples are randomly selected after they are received by the laboratory and picked, but before they are sent to the taxonomists. A proper sample for identification QA/QC must have a total count that falls within the 200 +/- 20% subsample size (*i.e.*, samples with counts below 160 should not be selected). 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, several evaluations of precision can be calculated:

### **Percent Difference in Enumeration (PDE)**

The Percent Difference in Enumeration (PDE) is calculated by the following formula:

#### **Equation 3. Percent Difference in Enumeration (PDE)**

$$\frac{(n_1 - n_2)}{(n_1 + n_2)} \times 100 = \text{PDE}$$

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 4. Percent Taxonomic Difference (PTD)**

$$\left[ 1 - \frac{(\text{comp}_{\text{pos}})}{(N)} \right] \times 100 = \text{PTD}$$

Where:

N = Highest count of organisms from taxonomist 1 or 2

comp<sub>pos</sub> = 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*).
  - Example 5. 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. Genus-Level Index of Most Probable Stream Status (GLIMPSS)**

#### **GLIMPSS Reference**

A detailed description of the procedures used to develop the Genus-Level Index of Most Probable Stream Status (GLIMPSS) MMI/IBI as well as the steps necessary to calculate final GLIMPSS scores can be found in the following document:

Pond, G.J., J.E. Bailey, B. Lowman, and M. J. Whitman. 2011. The West Virginia GLIMPSS (genus-level index of most probable stream status): a benthic macroinvertebrate index of biotic integrity for West Virginia’s wadeable streams. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Assessment Branch, Charleston, WV. Available online at:

[http://www.dep.wv.gov/WWE/watershed/bio\\_fish/Documents/20110829GLIMPSSFinalWVDEP.pdf](http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/20110829GLIMPSSFinalWVDEP.pdf)

A reduced version of the GLIMPSS (one that does not address the Winter season or drainages > 60 square miles) was also published in a peer reviewed journal:

Pond, G.J., J.E. Bailey, B.M. Lowman, and M.J. Whitman. 2013. Calibration and validation of a regionally and seasonally stratified macroinvertebrate index for West Virginia wadeable streams. *Environmental Monitoring and Assessment* **185**: 1515-1540 DOI 10.1007/s10661-012-2648-3. Or on the web at: [http://www.dep.wv.gov/WWE/watershed/bio\\_fish/Documents/20120513GLIMPSSPublicationEnvMonAssess.pdf](http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/20120513GLIMPSSPublicationEnvMonAssess.pdf)

### **GLIMPSS Overview**

WVDEP and USEPA developed this index specifically for use in West Virginia. It was stratified using Season, Region, and Size Class (based on catchment area of the stream). The following are the classifications:

- A. Season** – The seasonal periods are as follows: **Winter** (December-February), **Spring** (March-May), **Summer** (June-October 15). The boundaries between seasons are not hard fast and can vary between years. Because of this, it is recommended to apply a 2-3 week buffer between sampling Seasons to remove seasonal uncertainties. Sampling between October 16 and November 30 (Fall) should not occur due to sampling impracticalities (e.g., leaf fall, hydrology change).
- B. Region** – The state has been divided into two regions: the **Plateau** (corresponding to Ecoregion 70) and the **Mountains** (corresponding to Ecoregions 69, 67, and 66).

**NOTE:** For samples that are taken on streams that cross regional boundaries, deference may be given to the dominant region of the drainage area above the sample location and the distance to the region boundary over the region in which the sample station is located. For example, a sample taken at the Mouth (Mile 0.1) of Birch River (~38 miles in length) is located in Ecoregion 70 (Plateau). However, the watershed above this location is clearly dominated by Ecoregion 69 (Mountains). Additionally, the transition from Ecoregion 69 to 70 is only ~3-5 miles upstream of the mouth. In this situation, a Mountain designation for Region is more appropriate.

- C. Size Class** – Streams are divided into two main size classes: **>60 square miles** and **< 60 square miles**. This is only relevant when sampling in the Mountain region during the Summer season.

Combined, these three stratifications result in 7 different Season/Region/Size Class groupings, here forth referred to as **Seagions** (for Seasonal-Regions):

1. **Winter Plateau**
2. **Winter Mountains**
3. **Spring Plateau**
4. **Spring Mountains**
5. **Summer Plateau**
6. **Summer Mountains < 60 sq. mi.**
7. **Summer Mountains > 60 sq. mi.**

**NOTE:** Due to data restrictions (not a large dataset to work with) the Winter Seasons for both regions were developed using combined Best Standard Values & Worst Standard Values (BSV/WSVs) for both Winter and Spring. However, there was adequate data to set independent reference thresholds for the Winter Season. As more data becomes available, the Winter index will be adjusted using Winter only data to develop Winter specific BSV/WSVs.

Two general versions of the GLIMPSS are available depending on the extent of the Genus-Level taxonomy performed:

- A. GLIMPSS Chiro Genus or GLIMPSS CG:** All organisms (including all Oligochaeta, Hirudinea, Acari, Mollusca, and Crustacea) should be identified to at least the Genus level except for Hydracarina, Nematoda, and Turbellaria, which can be left at a higher level.
- B. GLIMPSS Chiro Family or GLIMPSS CF:** This version of the GLIMPSS was developed anticipating the potential limited availability of certified taxonomists who are capable of identifying Chironomidae to genera and associated increased costs for identification of samples. The GLIMPSS Chiro Family is identical to the Chiro Genus version except: 1) the identification of Chironomidae only to family is permitted, 2) Percent Orthoclaadiinae is replaced by Percent Chironomidae & Annelida in the applicable Seagions, and 3) Percent Tolerant Taxa (TV >6) is not used.

**NOTE:** If the level of taxonomy is restricted to Family for Chironomidae, then only the GLIMPSS Chiro Family can be utilized as the GLIMPSS Chiro Genus cannot be accurately calculated.

The following GLIMPSS metrics are applied to the benthic data depending the Seagion and version of GLIMPSS used:

1. **Genus Level Taxa Richness** or # Total Taxa
2. **Intolerant Genus Taxa Richness (TV <4)** or # Intolerant Taxa <4
3. **Intolerant Genus Taxa Richness (TV <3)** or # Intolerant Taxa <3
4. **EPT Taxa Richness** or # EPT Taxa
5. **Ephemeroptera Genus Taxa Richness** or # Ephemeroptera Taxa
6. **Plecoptera Genus Taxa Richness** or # Plecoptera Taxa



7. **Trichoptera Genus Taxa Richness** or # Trichoptera Taxa
  8. **Clinger Genus Taxa Richness** or # Clinger Taxa
  9. **Scraper Genus Taxa Richness** or # Scraper Taxa
  10. **Shredder Genus Taxa Richness** or # Shredder Taxa
  11. **Modified Genus-Level HBI (Hilsenhoff's Biotic Index)** or HBI
  12. **Percent Tolerant Taxa (TV >6)<sup>#</sup>** or % Tolerant Taxa >6<sup>#</sup>
  13. **Percent Contribution of Dominant 5 Genera Taxa** or % 5 Dominant Taxa
  14. **Percent Ephemeroptera, Plecoptera, Trichoptera (EPT) minus Cheumatopsyche** or % EPT minus Cheumatopsyche
  15. **Percent Ephemeroptera** or % Ephemeroptera
  16. **Percent Annelida & Chironomidae** or % Annelida & Chironomidae
  17. **Percent Chironomidae** or % Chironomidae
  18. **Percent Orthoclaadiinae<sup>#</sup>** or % Orthoclaadiinae<sup>#</sup>
- <sup>#</sup> Metric only used in Chiro Genus version

**NOTE:** The Richness and Tolerance (including HBI) metrics will vary between the two indices for any one sample since they will have different values depending on the identification level of the Chironomidae considered.

The Seagion relevant raw metric scores are then standardized on a 100 point scale based on the best standard values and worst standard values (BSV/WSVs) for the given metric. The applicable standardized metric scores are then averaged to give the **GLIMPSS Score** for the sample. Impairment thresholds are set based on the 5<sup>th</sup> percentile distribution of GLIMPSS scores for a set of reference condition sites in the given Seagion. To compare samples across Seagion, the GLIMPSS score is divided by the Impairment Threshold value for the Seagion resulting in a **Percent of the Threshold** (POT or %OT) for the sample. POT values that scored at or above the threshold value (*i.e.*, Unimpaired) are  $\geq 100\%$  and those that scored below the threshold value (*i.e.*, Impaired) are  $< 100\%$ .

### **Restrictions for Calculating the GLIMPSS**

- A. **Sample methodology** – Identical sampling area (4 x 0.25m<sup>2</sup> kicks = **Total of 1 m<sup>2</sup> area**) and gear (0.5 m rectangular kick-net with a **595-600  $\mu$ m mesh**) should be used in **rocky riffle/run substrate habitat** (*do not sample pools, undercut banks, or large woody debris*). Sampling should be restricted to the **Thalweg portions of the channel** (*i.e.*, the deeper portions of the channel that maintain flow the longest) and *should not occur in or near the shallow edges of the channel*. In limited circumstances, 0.3 m d-frame nets with comparable mesh size can be used as long as total of 1 m<sup>2</sup> 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) Collecting samples following a **drought** may result in reduced organism numbers and diversity. The benthic macroinvertebrates will either have

- drifted downstream or burrowed deep into the sediments beyond what is normally sampled in kick sampling. In many cases, a stream that has experienced extended dry periods or drought may respond to recent rainfall with a low flow, but the substrate will not have had time to be fully recolonized by benthic macroinvertebrates as it takes a prolonged flow event to break aestivation/diapause.
- 2) **Low flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net.
  - 3) **High flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net (*i.e.*, they may go over or around the net and the net may quickly become blocked by floating debris and cause back eddies directing material out of the net).
  - 4) **Turbid water** conditions should be avoided for sampling as it could affect sampling efficiency. As a general rule, if one cannot see the bottom of the sampling area enough to adequately estimate the substrate composition, one should not attempt a benthic sample and wait for the stream to clear.
  - 5) Collecting samples following a **scour or flood event** may result in reduced organism numbers and diversity.
- C. Seasonality – Acceptable collection dates are from **December 1 to October 15**. This is the time frame of the data that was used to develop the GLIMPSS and any sampling event outside of this window is considered not comparable. It is recommended to apply a 2-3 week buffer between sampling Seasons to remove seasonal uncertainties. Sampling between October 16 and November 30 (Fall) should not occur due to sampling impracticalities (*e.g.*, leaf fall, hydrology change).
- D. 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 carefully scrutinized for comparability before calculating a GLIMPSS score.** These sites may be heavily impacted by stressors (=impairment) or were recently subjected to drought or scour events (=non-impairment).
- E. Taxonomic resolution – Taxonomic resolution for the GLIMPSS is **genus-level except for Nematoda. This includes the non-insect groups like Turbellaria, Mollusca, Oligochaeta, Hirudinea, Acari, 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. Collembola is not used with the GLIMPSS.
- F. Tolerance values, Functional Feeding Groups, and Habits – GLIMPSS metrics that rely on **tolerance values** (*e.g.*, HBI), **Functional Feeding Groups** or FFGs

(e.g., # Shredder Taxa), or Habits (e.g. # Clinger Taxa) are **specifically designated** by WAB and only these designations should be used for a valid GLIMPSS scores calculation.

- G. **GLIMPSS Calculations** — Use only those **Best Standard Values/Worst Standard Values** (BSV/WSVs) and **component metrics** found in the GLIMPSS development document. Component metrics used for calculating GLIMPSS 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 GLIMPSS score.**

### **Using the GLIMPSS for Data Analysis**

Macroinvertebrate data is evaluated through the preparation of a stream assessment chart. 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 Visual-Based Habitat Assessment scores) and no obvious impairments in water quality. The condition quality of reference sites selected varies depending on such variables as stream size and region. In this case, the framework for these assessments is the GLIMPSS. Stream scores are plotted within a chart and the results are used for watershed assessments (e.g., pollution studies, spill response studies, etc.).

## **Part 2. West Virginia Stream Condition Index (WVSCI)**

### **WVSCI Reference**

A detailed description of the procedures used to develop the WVSCI MMI/IBI 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. Owning Mills, MD.

Or on the web at:

[http://www.dep.wv.gov/WWE/watershed/bio\\_fish/Documents/WVSCI.pdf](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](http://www.dep.wv.gov/WWE/watershed/bio_fish/Documents/WVSCI Addendum.doc)

### **WVSCI Overview**

Tetra Tech, Inc. developed this index specifically for use in West Virginia.

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** or # Total Taxa
2. **Family Level Ephemeroptera, Plecoptera, Trichoptera (EPT) Taxa Richness** or # EPT Taxa
3. **Percent EPT** or % EPT
4. **Percent Contribution of Dominant 2 Family Level Taxa** or % 2 Dominant Taxa
5. **Percent Chironomidae** or % Chironomidae
6. **Modified Family Level HBI (Hilsenhoff's Biotic Index)** or HBI

The individual metric scores are then standardized on a 100 point scale based on the best standard values (BSVs) for the given metric. The standardized metric scores are then averaged to give the WVSCI (West Virginia Stream Condition Index). A reference threshold is set based on the distribution of WVSCI scores for a set of reference condition sites.

### **Restrictions for Calculating the WVSCI**

- A. Sample methodology – Identical sampling area (4 x 0.25m<sup>2</sup> kicks = **Total of 1 m<sup>2</sup> area**) and gear (0.5 m rectangular kick-net with a **595-600 µm mesh**) should be used in **rocky riffle/run substrate habitat** (*do not sample pools, undercut banks, or large woody debris*). Sampling should be restricted to the **Thalweg portions of the channel** (*i.e.*, the deeper portions of the channel that maintain flow the longest) and *should not occur in or near the shallow edges of the channel*. In limited circumstances, 0.3 m d-frame nets with comparable mesh size can be used as long as total of 1 m<sup>2</sup> 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) Collecting samples following a **drought** may result in reduced organism numbers and diversity. The benthic macroinvertebrates will either have drifted downstream or burrowed deep into the sediments beyond what is normally sampled in kick sampling. In many cases, a stream that has experienced extended dry periods or drought may respond to recent rainfall with a low flow, but the substrate will not have had time to be fully recolonized by benthic macroinvertebrates as it takes a prolonged flow event to break aestivation/diapause.
  - 2) **Low flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being swept into the net.
  - 3) **High flow** conditions in riffle/runs may affect benthic sampling efficiency by reducing the number of organisms being captured in the net (*i.e.*, they may go over or around the net and the net may quickly become blocked by floating debris and cause back eddies directing material out of the net).
  - 4) **Turbid water** conditions should be avoided for sampling as it could affect sampling efficiency. As a general rule, if one cannot see the bottom of the

- sampling area enough to adequately estimate the substrate composition, one should not attempt a benthic sample and wait for the stream to clear.
- 5) Collecting samples following a **scour or flood event** may result in reduced organism numbers and diversity.
- C. Seasonality – Acceptable collection dates are from **April 15 to October 15**. This is the time frame of the data that was used to develop the WVSCI and any sampling event outside of this window is considered not comparable.
- D. 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 carefully scrutinized for comparability before calculating a WVSCI score.** These sites may be heavily impacted by stressors (=impairment) or were recently subjected to drought or scour events (=non-impairment).
- E. Taxonomic resolution – Taxonomic resolution for the WVSCI is **family level except for Turbellaria, Nematoda, and Collembola. This includes the non-insect groups like Mollusca, Oligochaeta, Hirudinea, Acari, 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.
- 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

**NOTE:** Because of the limitations of the WVSCI MMI/IBI compared to the GLIMPSS MMI/IBI, the WVSCI should only be used when benthic data is available, but further taxonomic identification is not viable (e.g., legacy data from older studies where the voucher specimens are not available for reidentification).

Macroinvertebrate data is evaluated through the preparation of a stream assessment chart. 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 Visual-Based Habitat Assessment scores) and no obvious impairments in water quality. The condition quality of reference sites selected varies depending on such variables as stream size and region. The framework for these assessments is the West Virginia Stream Characterization Index (WVSCI). Stream scores are plotted within a chart and the results are used for watershed assessments (e.g., pollution studies, spill response studies, etc.).

### **Part 3. Dirty Null Stressor Identification Model**

The benthic data is also imported into an analysis model that compares each sample's community structure to that of 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. The Dirty Null Stressor Identification Model is helpful during TMDL development during the Stressor Identification process.

### **Section E. Benthic Macroinvertebrate Sample Comparability**

Before a sample can go thru data analysis via an IBI or MMI, it must be checked for index comparability. Hopefully, all directions presented in the sections above followed. However, this is not always the case. Sometimes errors or mistakes are made when the sample is collected (e.g., the sampler did not know that the stream was dry the week before), processed (e.g., not enough or too many specimens were picked), or identified (e.g., the identifier got the counts reversed between two taxa). Other times, the sample is not comparable due to restrictions of the index. And in some rare instances, the sample is collected despite known problems with comparability (e.g., Limestone dominated stream, non-comparable method or equipment, after a scour event, etc.) because the goals of the project dictate the need to sample in those conditions (e.g., scour recovery survey) or to get any sort of benthic macroinvertebrate sample (e.g., spill response survey). The following flow chart (**Figure 5-17 on next page**) is designed to help make a decision about the comparability of the sample in the context of the WVSCI and GLIMPSS MMI/IBIs.



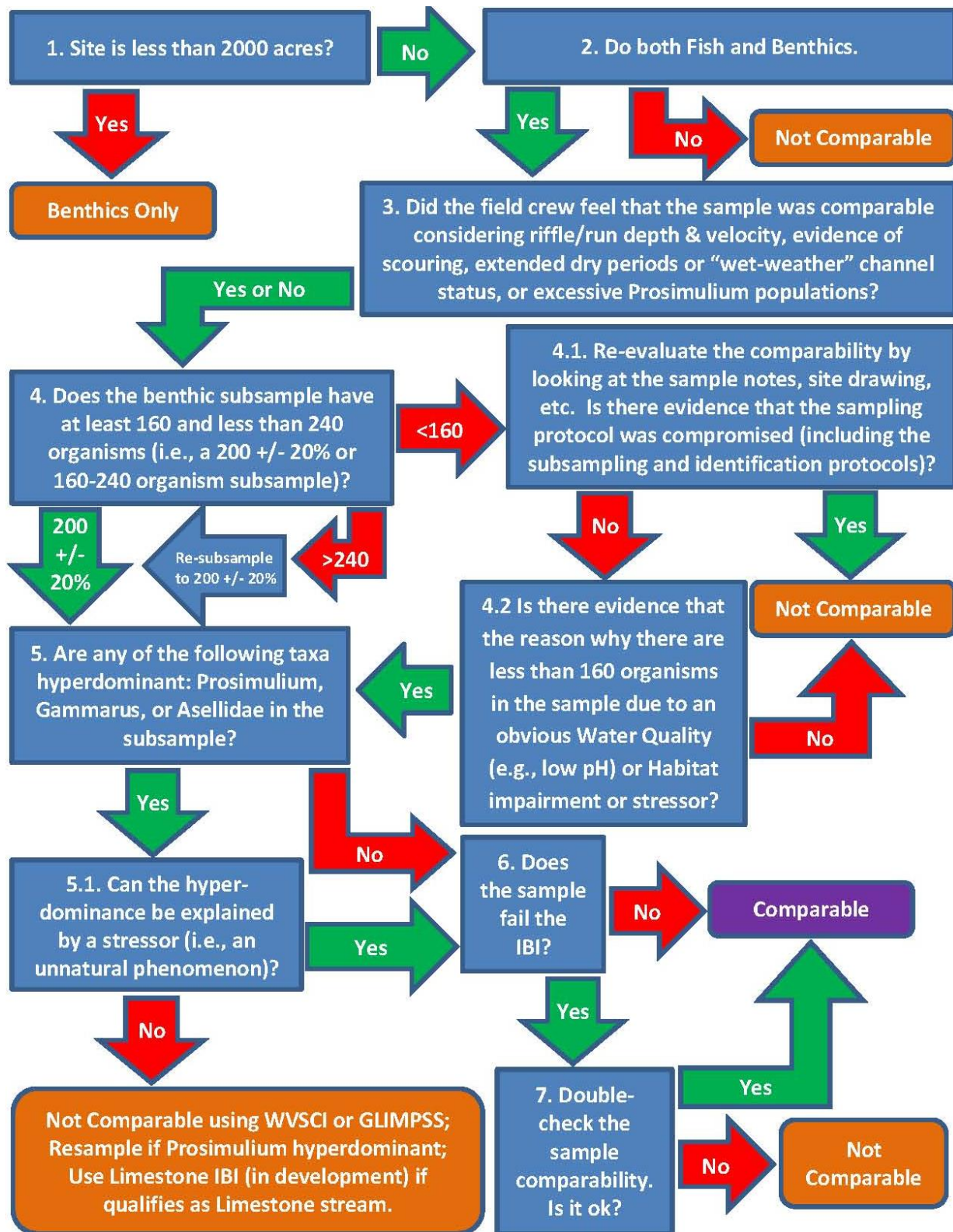


Figure 5-17. Benthic Comparability Flow Chart

## **Benthic Macroinvertebrate Data Analysis Quality Assurance/Quality Control**

The samples used in the ***Benthic Laboratory Identification Quality Assurance/Quality Control*** are also analyzed for index performance differences.

### **Comparison of Index Result (CIR)**

Comparison of Index Result is a simple comparison of how the identification differences between taxonomists would affect the MMI/IBI (Multi-Metric Index/Index of Biotic Integrity) score(s) and final impairment decision. To do this you would simply calculate the sample MMI/IBI score from each taxonomist's identification independently and then get the absolute value of the differences. Each MMI/IBI score can then be translated into the appropriate MMI/IBI narrative category (e.g., Unimpaired vs. Impaired, Unimpaired-Very Good vs. Unimpaired-Good, Slightly Impaired vs. Moderately Impaired) to see if the differences in identification between taxonomists result in conflicting decisions about the level of impairment. The absolute value of the differences of MMI/IBI scores can help qualify the extent of disagreement, especially when the MMI/IBI scores straddle a category threshold.

Additionally, duplicate samples taken by two different samplers at the same time (**see *Benthic Sampling Quality Assurance/Quality Control on page 5-14***) are also run thru a Comparison of Index Result analysis to see how two different samplers affect the performance of an index at a site. If the two duplicate samples leads to different narrative categories, then a more in-depth analysis of the field data and notes are performed to see if there was a reason (e.g., sampler error) why the two samples did not match.

## CHAPTER 6. FISH COLLECTION PROTOCOLS

### 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.

The WVDEP-Watershed Assessment Branch (WAB) currently sample fish using two differing protocols:

- 1) Wadeable Stream Fish Community Assessment
- 2) Trout Stream Verification

#### Section A. *Wadeable Stream Fish Community Assessment Protocol*

##### 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. Available online at: <http://www.epa.gov/emap/html/pubs/docs/groupdocs/surfwatr/field/MAHAWadeableStreams.pdf>

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 2. Section A. Accessing the Site starting on page 2-2 for a description*) that can be assessed with a fish based multi-metric index (MMI) or Index of Biotic Integrity (IBI).

Targeted sites include TMDL sites, LTMS (Long Term Monitoring Stations), and statewide AWQN (Ambient Water Quality Network) locations. The majority of targeted sites are TMDL stations. The fish community assessment is used, along with water quality samples and benthic macroinvertebrates, to assess the overall condition of the TMDL stream segment. A varying number of LTMS and AWQN sites are sampled each year as time permits. An effort is made to re-sample these sites every few years. 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 MMI/IBI. Currently (Feb 2015), WAB does not have a fish MMI/IBI developed for use with fish community data. However, data collection is the first step in the MMI/IBI development process.

## Part 1. Selecting Sampling Sites

Sites are selected and sampled based on the following criteria that will produce comparable data that can be assessed using an MMI/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 other fish MMI/IBI development projects.
3. Stream reaches that are not in close proximity to large stream or river confluences (i.e., have an adequate swim distance from drastically larger order streams that have a higher fish diversity and abundance).

4. 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<sup>st</sup>, 2<sup>nd</sup> order) streams and progress to larger streams later in the year when lower flows allow for easier sampling. In general, 3<sup>rd</sup> and 4<sup>th</sup> 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.

## **Part 2. Determining Site Suitability**

Many of the sites selected (primarily TMDL) for fish community assessment will be visited by a sample team to collect water samples and 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 MMI/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.

### Part 3. 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 us/ds) 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, if possible. 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.

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 MMI/IBI purposes.

### Part 4. Field Sampling Methodology

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 20B 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, 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 electrofisher operators 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 (i.e., Deformities, Erosions, Lesions, or Tumors, or DELTs)
19. GPS
20. Scientific collecting permit – Obtained yearly by Watershed Assessment Branch from the WVDNR.

### **Field Safety Precautions**

**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.**

Safety methods and protocols can be referred to in the following documents:

Professional Safety Committee. 2008. *Fisheries safety handbook*. American Fisheries Society, Bethesda, Maryland. [http://fisheries.org/docs/policy\\_safety.pdf](http://fisheries.org/docs/policy_safety.pdf)

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.

***WARNING: 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.***

Additionally, the US Fish and Wildlife Service (USFWS) offers a pair of online, self-study courses thru the National Conservation Training Center (NCTC) regarding electrofishing safety and techniques:

- a) **CSP2202A-OLT – Electrofishing Safety** (\$0.00 Fee) which is designed to be taken yearly by fish crew leaders and members
- b) **CSP2C01 - Principles and Techniques of Electrofishing** (\$50.00 Fee) which is designed to teach fish crew leaders and those who operate electrofishing equipment how to properly setup and use the equipment safely and efficiently.

### **Selecting the 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 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 6-1 on the next page for guidance on the number of electrofishers and netters required for different stream sizes and depths.***

Table 6-1. Personnel and equipment required to effectively electrofish 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	2-3 backpacks	3 – 4
4 – 8 m	Deep	2-3 backpacks or Barge	2 – 4
> 8 m	Shallow	3-4 backpacks	3 – 4
> 8 m	Deep	Barge	2 – 4

## Electrofishing

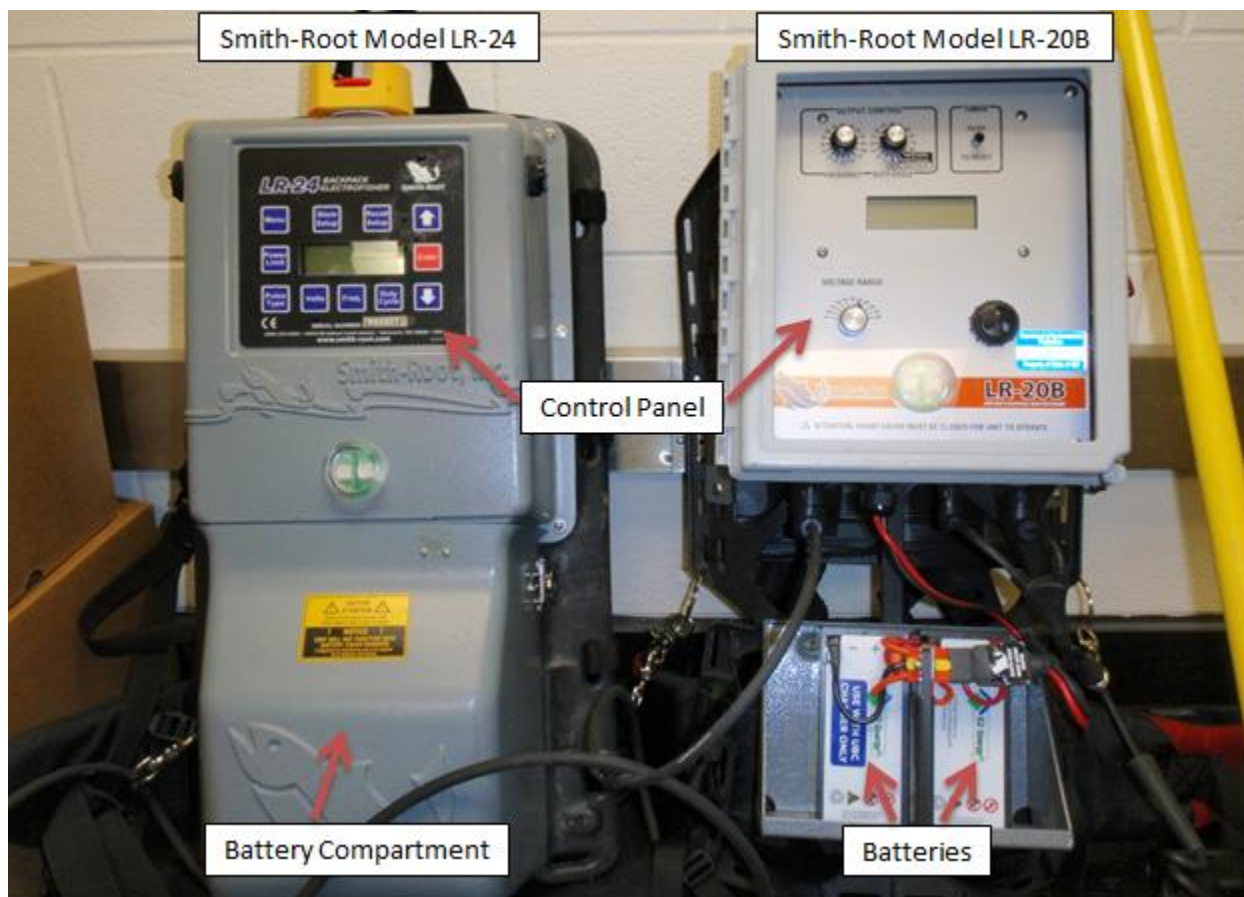


Figure 6-1. Two backpack electrofishers (Smith-Root Models LR-24 and LR-20B) used by WAB personnel.

In general, where conditions allow, one backpack electrofisher (*see Figure 6-1 and Figure 6-2 above and on next page*) 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.



Figure 6-2. Components of a backpack electrofishing system.





**Figure 6-3. An electrofishing crew consisting of two backpack shockers (electrofishers) and three netters.**

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 electrofishers will be used (**see Figure 6-3 above**). The two electrofishers will parallel each other working side to side in an upstream direction (**see Figure 6-4 on next page**).

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 6-4. Technique for sampling a deep, narrow stream with two backpacks, looking upstream.

A tow barge (*see Figure 6-5 on next page*) will be used in streams eight meters wide or greater (assuming that water depth is adequate to move the barge) (*see Figure 6-6 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 ( $>1,000 \mu\text{mhos/cm}$ ) 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 or more 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 6-5. Tow barge with generator, live well, and shocking wand.**



**Figure 6-6. 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 6-7 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. If additional backpacks and crewmembers are available, then all channels can be sampled simultaneously. 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 6-7. 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 to 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, TMDL, AWQN, and LTMS sites, locate the x-site for proper reach confirmation. For other sites, simply determine where the reach will begin. Obtain GPS coordinates and verify correct location (**see CHAPTER 2. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) starting on page 2-20**). 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 3. WATER COLLECTION PROTOCOLS starting on page 3-1**). 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 electrofishers 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 on next page***). 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.

***IMPORTANT: 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. Be sure not to “double-count” released game fish that did not recover/survive.

13. Review fish collection form (*see **Field Data Collection on next page***) to ensure that all necessary information is completed.

### **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 or dying) 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 1/5<sup>th</sup>) 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.**



**Field Data Collection**

The following list includes the type of data that will be collected and recorded at each fish community assessment site (**See Figure 6-8 on the next page for an example of what this form looks like**):

- ✓ 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, frequency (pulse rate), the number and type of gear used, the number of netters, and whether block nets were used.

<b>WVDEP    WATERSHED ASSESSMENT    FISH COLLECTION FORM</b>				PAGE <u>   </u> OF <u>   </u> .					
<b>ANCODE:</b>			<b>DATE:</b> /    /		<b>TIME:</b>	<b>FISH FIELD:</b> ID BY	<b>LAB:</b>	<b>ID</b> QA/QC:	
<b>STREAM NAME:</b>			<b>TOTAL SHOCK TIME</b> (1)        sec    (2)        sec (3)        sec    (4)        sec		<b>TOTAL FISHING</b> TIME        min		<b>VOLTAGE</b> (1)     v    (2)     v (3)     v    (4)     v		<b>FREQ.</b> ____ Hz
<b>REACH</b> <b>LENGTH</b> m	<input type="checkbox"/> NOT FISHED <input type="checkbox"/> NONE COLLECTED		<b>EQUIPMENT:</b> BACKPACKS-(1) LR-24 <input type="checkbox"/> (2) LR-20 <input type="checkbox"/> (3) LR-24 <input type="checkbox"/> (4) LR-20 <input type="checkbox"/> TOTE BARGE <input type="checkbox"/> BOAT <input type="checkbox"/>				<b>NO. OF</b> <b>NETTERS</b> ___		
<b>COLLECTION METHOD:</b> <input type="checkbox"/> ELECTROFISHING <input type="checkbox"/> SEINING <input type="checkbox"/> ANGLING <input type="checkbox"/> GILL NETTING									
<b>CREW MEMBERS:</b>									
COMMON NAME	FIELD TALLY	LAB TALLY	TOTAL COUNT	VOUCHER COUNT	LENGTH (mm)		WT. (g)	D.E.L.T ANOMALIES	
					MIN	MAX			

Figure 6-8. Example of Fish Collection Form

### **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.

## **Part 5. 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**

**WARNING: 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.

**Part 6. 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 MMI/IBI will summarize elements of the structure and function of fish communities. Taxonomic resolution for the fish MMI/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 on next page***).

### **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.
- Etnier, D.A. and W.C. Starnes. 1993. The Fishes of Tennessee. The University of Tennessee Press, Knoxville, Tennessee.
- 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**

***WARNING: 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 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****Taxonomic Certification**

Currently, there is no formal, nationally recognized certification for Fish Identification. Until such a certification is available, it is expected that fish identifications only be conducted by qualified personnel with one or more of the following qualifications:

- 1) MBSS Fish Taxonomy Certification: The border state of Maryland has a formal Biological Stream Survey Training and Certification program (MBSS) which has a Fish Taxonomy Certification component that is valid for 1 year only.
- 2) Completion of a Locally Relevant Fish Identification Course. For example:
  - a. The U.S. Fish and Wildlife Service (USFWS) Fish Identification course (CSP2220) is offered every other year at the National Conservation Training Center (NCTC) in Shepherdstown, WV for a tuition fee.  
Link:  
<http://nctc.fws.gov/NCTCWeb/catalog/CourseDetail.aspx?CourseCodeLong=FWS-CSP2220>
  - b. West Virginia University (WVU) semester-long Ichthyology course (BIOL 341) offered every spring.
  - c. Marshall University semester-long Ichthyology course (BSC 401).

Additionally, it is expected that those conducting fish identifications complete frequent continuing education opportunities (e.g., Fish Identification workshops) and identify samples on a regular basis to maintain identification skills.



## Fish Identification QA/QC Metrics

The precision of the identification process is evaluated for 10% of the samples identified by each taxonomist. These samples are randomly selected after all identifications are complete. The senior taxonomist will identify and enumerate the specimens in 10% of the total samples identified by any additional taxonomists. In addition, a subset the samples identified by the senior taxonomist will be re-identified and re-enumerated by WVDNR fishery biologist expert Dan Cincotta annually.

From each re-identification and re-enumeration effort, 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 5. Percent Difference in Enumeration (PDE)

$$\frac{(n_1 - n_2)}{(n_1 + n_2)} \times 100 = \text{PDE}$$

Where:

$n_1$  = # of organisms counted by taxonomist 1

$n_2$  = # of organisms counted by taxonomist 2

A PDE  $\leq$  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 6. Percent Taxonomic Difference (PTD)

$$\left[ 1 - \frac{(\text{comp}_{\text{pos}})}{(N)} \right] \times 100 = \text{PTD}$$

Where:

N = Highest count of organisms from taxonomist 1 or 2

$\text{comp}_{\text{pos}}$  = Total # of taxonomic agreements from the Taxonomic Comparison Form

A PTD  $\leq$  10% is considered passing for Family Level taxonomy.

A PTD  $\leq 15\%$  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, if necessary, 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. 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.

### **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 to the lower taxonomic level where the other taxonomist cannot.

### **3. Specimens Lost Between Taxonomists**

Example. Specimens may have been pulled from the sample (e.g., Reference Collection) and not viewed by the second taxonomist. This should be noted so the second taxonomist is aware of the missing specimen(s).

### **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.

After this reconciliation, the PTD can be recalculated correcting for these most of these errors (called a corrected PTD).

## **Section B. Trout Stream Verification Protocol**

### **Basis of Sampling Method**

As part of a cooperative effort with WVDNR, selected streams in West Virginia are sampled to determine the presence or absence of trout species. These verifications are used in the decision making process in relation to whether a stream should be considered a "trout water. A different, somewhat more protective, set of water quality standards are applied to trout waters since trout are considered to be less tolerant of disturbance and higher temperatures. When a permit related issue arises pertaining to

a stream or stream segment, it is critical to know if that stream or segment is considered trout waters so the appropriate water quality standards are used.

Streams must be able to support trout year round to be considered true trout waters; therefore, verification surveys should only be conducted from July 1<sup>st</sup> through September 30<sup>th</sup> when stream temperatures are their warmest. If trout are present within this time period, it is very likely the stream supports populations all year. This excludes streams that are stocked with trout (commonly known as “put and take” streams) in cooler times of the year (*e.g.* spring), but have temperatures that are too warm to support trout in the summer months.

Streams or stream segments (*e.g.* headwaters or upper parts of watersheds that are likely higher elevation and have cooler water temperatures) are chosen, most often, by one of two ways: (1) The stream historically supported trout, but has not been sampled to verify the presence of trout for many years, (2) The stream is expected to support trout based on current data (*e.g.* temperature data, good overall water quality, *etc.*), but has never been sampled to verify.

## **Part 1. Selecting Sampling Sites**

If a stream segment is pre-selected, then all sampling efforts should be within the specified segment. If no segment is selected, then sample sites should be located throughout the entire stream length. The locations of and number of sample sites can vary from stream to stream. For example, a stream that is three miles long may have three sample locations – one near the mouth, one near MP 1.5 (the mid-point of the stream), and one near the headwaters. A stream that is ten to fifteen miles long would likely have additional sampling locations throughout the length of it. There is no standard, set number of sampling locations; but a reasonable amount of effort should be applied to thoroughly assess the presence/absence of trout within a selected stream.

Available access is a major factor when selecting the number and location of sample sites. Dangerous terrain, landowner denial, stream morphology (deep pools), *etc.* may be factors that prohibit stream access. All attempts must be made to sample an adequate number of locations to establish a reasonable confidence level that trout are present or not.

## **Part 2. Field Sampling Methodology**

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 for the presence/absence of trout.

When sampling a selected location, focus efforts on the best available habitat (boulders, large woody debris, undercut banks, *etc.*) that trout are most likely utilizing. This will be more efficient and productive.

Streams are normally sampled by backpack electrofishing (the preferred methodology/device), seining, or angling. Characteristics such as flow, depth, available habitat type, *etc.*, should determine which type of sampling will be most effective.

At each sampling location, a minimum reach length of at least 100 meters is recommended. If no trout are observed within the first 100 meters, then continue sampling upstream (possibly up to 300-400 meters total). If no trout are observed within the sample reach and an adequate amount of suitable, productive habitat was sampled, it is likely no trout are present. If several trout are observed within the first 100 meters, it is not necessary to continue upstream.

In addition to sampling for trout, a trout verification field form must be completed and physicochemical parameters (temperature, pH, dissolved oxygen, and conductivity) must be collected (**see CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1**). Additional lab water quality parameters may be sampled to support the trout verification. Also, be sure to take several photos of the reach and habitat features, and any trout observed.

### **Deviations from other Fish Protocols**

1. Typically, a much smaller crew (2 people – one electrofisher operator and one netter) is needed because focus is only on trout species.
2. No fish are retained. Only photo documentation of any trout collected/observed is necessary, along with length measurements of each individual. Maintaining a list of all fish species observed (especially larger species) is recommended and could be useful for future reference.

## CHAPTER 7. PERIPHYTON COLLECTION PROTOCOLS

### *Periphyton Overview*

Periphyton are algae, diatoms, fungi, bacteria, protozoa, and associated organic matter associated with stream channel substrates (*i.e.*, they grow on the exposed surfaces of rocks and other submerged objects). Phytobenthic (photosynthesizing, bottom-dwelling) periphyton is usually the dominant primary producers in stream ecosystems (especially small stream systems). Because they are attached to the substrate, the phytobenthic periphyton community integrates physical and chemical disturbances to a stream. 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. 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<sup>2</sup> 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 benthic macroinvertebrate collection is not practical (e.g., 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.





**Figure 7-1.** Microspatula being used to scrape algae and periphyton from upper surface of



**Figure 7-2.** Toothbrush being used to scrub remaining algae and periphyton from upper surface of rock.

4. Using the PVC ring to delimit the sample area (12.56 cm<sup>2</sup>), use the microspatula to scrape **all algae** from **upper surface** of rocks into the sample jar (**see Figure 7-1 above**). Use the toothbrush to loosen any remaining periphyton (**see Figure 7-2 above**). 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 (**see Figure 7-3 below**). Repeat Step 5 until all of the periphyton from the five rocks (representing 62.8 cm<sup>2</sup> 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



**Figure 7-3.** Loosened algae and periphyton being rinsed into the sample jar.

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 2. Section C. Part 1. PAGE 9-Periphyton Collection Information starting on page 2-103**) 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:

- 3) Clear the diatom frustules of organic material using either nitric acid or hydrogen peroxide/potassium dichromate oxidation.
- 4) Prepare slides and identify diatoms to species or lowest taxonomic level possible.
- 5) 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 14. Section A. Blanks and Duplicates starting on page 14-1 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 8. FILAMENTOUS ALGAE MONITORING

### *Filamentous Algae Overview*

Since 2007, the Watershed Assessment Branch (WAB) has devoted much effort and resources to evaluating the causes, locations, and severity of filamentous algae blooms in West Virginia's streams and rivers (*see Figure 8-1 below*). As part of that effort, WAB has measured the development of filamentous algae blooms at various locations and reported the results as "percent algae cover" and occasionally "percent water column fill". Percent algae cover is the percent of the stream bottom covered by filamentous algae at a measured transect of the stream, and percent water column fill is the percent of the water column filled in a cross-sectional view of the stream at a given transect location. It should be noted that neither of these measurements have a longitudinal component; *i.e.*, these two measurements do not account for the length of stream reach impacted by filamentous algae.



Figure 8-1. A filamentous algae bloom on the Tygart River upstream of Norton, June 2012.



WAB has developed a method for measuring filamentous algae in streams based on the methods described in the following:

Morgan, A.M., T.J. Royer, M.B. David, and L. Gentry. 2006. Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. *Journal of Environmental Quality* **35**: 1110-1117.

Schaller, J.L., T.V. Royer, and M.B. David. 2004. Denitrification associated with plants and sediments in an agricultural stream. *Journal of the North American Benthological Society* **23**: 667-676.

At a given stream transect, a measuring tape is stretched across the stream; wetted width and portions (or segments) of the stream covered by filamentous algae are recorded. On occasion, WAB may also make an additional measurement of the filamentous algae thickness along the transect segments so that the amount of water column impact, not just stream cover, can be calculated.

This method was first used as a basis for filamentous algae measurement in West Virginia beginning in the fall 2008 in four priority river watersheds: Greenbrier River, Cacapon River, Tygart Valley River, and South Branch of Potomac River. In 2014, additional components to document submerged aquatic vegetation, canopy cover, and stream bearing were added.

Filamentous algae blooms tend to appear mostly during the summer months due to several factors including: flow induced high nutrient concentrations (e.g., low flow), higher air temperatures, and longer photoperiods (i.e., day lengths). Because of this, sampling has typically been constrained to this time period. However, filamentous algae blooms can appear under other conditions (e.g., in the Early Spring due to a lack of competition for light before full canopy leaf-out and increasing air temperatures).

The procedure below represents a summary and explanation of the method used by WAB to determine the “percent algae cover” and “percent water column filled” by filamentous algae. It is set forth both to serve as WVDEP’s recommended standard measuring method for future measurements of filamentous algae blooms, both by WAB and by others wishing to make measurements comparable to those made by WAB.

The Filamentous Algae Monitoring Program utilizes some of the same sampling protocols of other Watershed Assessment Branch programs. Specifically, the SOP sections that apply to this program are as follows:



**CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. Guidelines for Completing the Stream Assessment Forms on page 2-28**

**CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1**

**CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1**

## **Materials and Supplies**

**IMPORTANT:** *The unit of measurement can be either in feet (marked in tenths of a foot) or meters, but the units must be the same for both the distance and depth.*

1. 100 meter Tape Measure – Units can be in either tenths of a foot (rather than inches) on one side and meters on the other. It is recommended to use some sort of stakes or pins (e.g., steel rebar) to easily anchor each end of the tape.
2. Vertical Measurement Device – To be used only if calculating the “percent water column fill. A stiff vertical device such as a wading rod, thalweg pole, or surveyor rod marked in either tenths of a foot or meters. Other non-flexible measuring instruments (such as a yard stick with feet marked in **inches**) can be used if absolutely necessary, but the measurements will need to be converted to tenths of a foot before calculating any of the formulas below. Experience has shown that the use of more flexible metal measuring tapes are not suitable to measure water depth whenever there is significant water velocity in the stream.
3. Camera – to take photos of the transect area’s algae cover
4. Filamentous Algae Measurement Form

## **Field Safety Precautions**

Since this procedure often occurs on large streams and rivers, deep and swift water safety precautions (like the use of personal floatation devices) should be observed.

## **Part 1. Field Sampling Procedures**

### **Site Selection**

Basic guidelines for site selection.

**1) Site should be wadeable with some depth, but not too deep.**

If possible, select a stream measurement transect that may be waded comfortably and safely. Streams varying from one to three feet deep are ideal. Streams which are too deep to comfortably and safely wade may result in

measurement inaccuracy because so much effort is required to simply stay on one's feet. Also, deeper water decreases light penetration into the water, which tends to inhibit the development of algae growth. This may skew results if the measurer is looking for trends at different locations along a given stream.

**2) Site should have rocky substrate with minimal transient material (*i.e.*, fine sediments like silt, sand, and fine gravel).**

Growth of filamentous algae also tends to be inhibited by the lack of rocky substrate. So again, if possible, select measurement sites with little or no sediment accumulation to avoid skewing results in comparative studies.

**3) Sites in narrower streams should have a moderately open canopy.**

For narrow streams, shading from the tree canopy overhead can have an inhibitory effect on algae growth similar to that of streams that are too deep.

**4) Avoid sites that will potentially have a dry bar during low flow situations.**

This may not easily be determined during higher flows, but any attempt to avoid this will make the calculations easier later.

### **Transect Selection**

Basic guidelines for transect selection within a site.

**1) Level of algae growth.** Due to factors discussed above and other physical variables, the level of algae growth may vary considerably at a given location. When correlating the level of algae growth with stream chemistry, select a transect location that is representative of the general site location. If possible, avoid areas with fast currents or deep pools as this may interfere with algae growth.

**2) Return visits.** If the site is intended to monitor changes in algae growth over a period of time, the same transect location should be used for each visit. The exact transect location should be documented (drawing, photo, marked in the field or on the bank in some semi-permanent fashion) so that it can be consistently repeated for comparability.

### **Establishing the Transect**

Determine the wetted width of the stream by stretching the tape measure across the stream perpendicular to the stream banks. Secure the tape on each stream bank, pulling it tight enough to minimize sag (*i.e.*, a sag in the tape will give a wider wetted width measurement than really exists). Wide rivers may necessitate variations of measuring instrument and logistics, such as using an accurate laser range finder or pulling a measuring tape across the river in sections at a time. However, the basic concept will remain the same.

A minimum of five algae measurement segments should be recorded for each transect

across the stream channel. For example, a stream that is 200 feet wide would require at least five 40 foot ( $5 \times 40 = 200$ ) segments across the stream where the algae growth is measured. As you traverse the stream channel while setting up the tape measure, make some notes about where the minimum five segments should be placed. Segments should be placed to document and define areas with or without algae mats, significant changes ( $> 0.5$  feet or 0.15 meters) in amount of algae growth (both in percent cover and thickness) on the stream bed, and/or sudden changes in depth. For example, if the 200 foot stream in the previous example had a flow (or thalweg) channel that was only 10 or 15 feet wide and deeper than the rest of the stream, the flow channel should be treated as a separate segment in the stream transect as it is most likely conveying water all year long and will represent a fixed segment in trend measurements (*see Segment 3 in the Figure 8-2 below*). Conversely, segments can be lengthened in areas where the water depth and algae growth are more uniform. Additional segments may be added to the transect while taking measurements and the stream bottom is more thoroughly investigated.

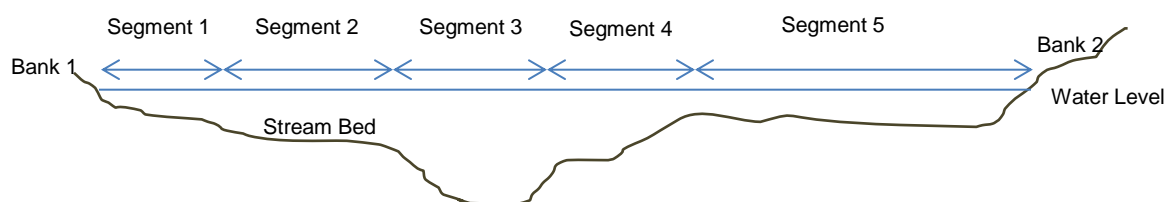


Figure 8-2. Hypothetical example of stream segments across a filamentous algae

**NOTE:** This concept of isolating differing sections laterally across a stream channel is used in the Flow measurement methodology in CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS starting on page 4-1 and longitudinally in the Dominant Substrate/Reach Characterization in CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. PAGE 3. Dominant Substrate Type and Reach Characterization on page 2-51.

### **Documentation using the Filamentous Algae Monitoring Form**

Record the site documentation information on the Filamentous Algae Monitoring Form as outlined in CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C.

**PAGE 1. Site Verification on page 2-30.**

Information includes:

- PAGE 1: Stream name, ANCode, and Location (e.g., Greenbrier River at I-64 East bridge.), Coordinates of the waters sample location (x-site) and, if the stream is excessively wide, coordinates where the transect ends (*see Figure 8-3 below*), Site Map, etc.

Field Lat X-site	N	Field Lon X-site	W
LDB Transect Lat	N	LDB Transect Lon	W
RDB Transect Lat	N	RDB Transect Lon	W

Figure 8-3. Example of additional Transect LDB and RDB Coordinate Boxes on PAGE 1

- PAGE 2: Field Water, Periphyton/Algae/Aq. Plant Info, Canopy Density. An additional section to document the canopy density along the stream transect (*see Figure 8-4 below*).

Canopy Density (Densiometer) Readings: 0-17 Possible per reading; Hold 1ft (0.3 m) above surface of water									
Left		Left Middle		Center		Right Middle		Right	
Up		Up		Up		Up		Up	
Left		Left		Left		Left		Left	
Down		Down		Down		Down		Down	
Right		Right		Right		Right		Right	

Figure 8-4. Example of the additional Canopy Density (Densiometer) Readings Section on PAGE 2

- PAGE 3: Filamentous Algae Measurements (*see Figure 8-6 on next page and Taking Filamentous Algae Measurements on page 8-8*)
- PAGE 4: Landowner/Stakeholder Information, Recon, & Photos. Additional options to document Houses, Campgrounds/Campsites, Public and Private Stream Access are provided under the Recon/Accessibility section in this version of the form (*see Figure 8-5 below*).

Discuss the accessibility to the site including accessibility, posted property, fenced, beside road, long walk over treacherous terrain, hike length, 4 x 4 needed, get key from landowner, etc.	
Check all that apply:	<input type="checkbox"/> Easy Access <input type="checkbox"/> Difficult Access <input type="checkbox"/> Public Property <input type="checkbox"/> Private Property <input type="checkbox"/> Posted <input type="checkbox"/> Fenced <input type="checkbox"/> Gated <input type="checkbox"/> Get Key from Landowner <input type="checkbox"/> Beside Road <input type="checkbox"/> Short Hike <input type="checkbox"/> Long Hike <input type="checkbox"/> 4x4 Needed <input type="checkbox"/> Houses <input type="checkbox"/> Campgrounds/Campsites <input type="checkbox"/> Public Stream Access <input type="checkbox"/> Private Stream Access <input type="checkbox"/> Other (explain)
Recon/Accessibility Notes:	

Figure 8-5. Example of the expanded Recon/Accessibility Section on PAGE 4



**Taking Filamentous Algae Measurements**

Fill out PAGE 3 of the Filamentous Algae Measurements Form using the following steps:

1. Determine the downstream channel orientation by taking a bearing measurement (e.g.,  $0^{\circ}$  being a stream flowing due North;  $90^{\circ}$  being a stream flowing due East;  $180^{\circ}$  being a stream flowing due South;  $270^{\circ}$  being a stream flowing due West;  $315^{\circ}$  being a stream flowing Northwest). Record this value near the bottom center of the page.
2. Indicate what the selected measurement units will be (i.e., Feet or Meters) near the bottom center of the page.
3. On the first or starting Bank (Bank<sub>1</sub>), record the measurement at the stream channel's or wetted edge as the initial point (Bank<sub>1</sub> D<sub>End</sub>). Check in the note field whether this is the left descending bank (LDB) or right descending bank (RDB). This same measurement for the bank (Bank<sub>1</sub> D<sub>End</sub>) should also be recorded as the beginning measurement for the first cross segment (1 D<sub>Begin</sub>). The D<sub>End</sub> measurement for each segment will be the D<sub>Begin</sub> measurement for the next segment.
4. Wade into the stream observing the water depth, submerged aquatic vegetation, filamentous algae percent cover or depth, and algae mats. If there are significant changes in any of these conditions before reaching the approximate predetermined segment width, define the end of the segment and record the distance on the tape measure that corresponds to the end of the first segment (1 D<sub>End</sub>). Again, the D<sub>End</sub> for each segment then becomes the D<sub>Begin</sub> for the next segment.
5. Within that segment, record the representative water depth (WD<sub>i</sub>), estimated percent stream bottom submerged aquatic vegetation (SAV) & filamentous algae cover (AC<sub>i</sub>), the representative algae thickness (AT<sub>i</sub>) (if necessary), and the Dominant Substrate Type.

**IMPORTANT: Remember that large variations in water depth (>0.5 feet or >0.15 m) or algae growth should cause the start of a new segment.**

- a. Water depth (WD<sub>i</sub>) is determined by evaluating a given transect segment for a representative depth measurement.
- b. The percent submerged aquatic vegetation (SAV) and percent filamentous algae cover (AC<sub>i</sub>) for the segment can only be visually estimated. Carefully observe the stream bottom (both the stream substrate and SAV/filamentous algae present) in a **one unit wide** area (centered on the transect tape measure either in feet or meters) for the entire length of the segment.



Estimate and record the percent of the stream bottom in the segment that is covered with submerged aquatic vegetation and filamentous algae growth respectively. "Covered" means that SAV or algae are present and visually obscuring the stream bottom. The SAV or algae may not necessarily be attached to the stream bottom within the one foot wide area. For example, algae may only be attached to 40% of the stream bottom in the one foot wide segment, but long strands of algae may extend down from upstream and cover 80% of the stream bottom within the transect area. **See Figure 8-7 to Figure 8-19 for photo examples of measured percent filamentous algae cover.**



**Figure 8-7. 3% Filamentous Algae Cover**





**Figure 8-8. 4 % Filamentous Algae Cover**



**Figure 8-9. 12% Filamentous Algae Cover**





**Figure 8-10. 15% Filamentous Algae Cover**



**Figure 8-11. 20% Filamentous Algae Cover (Short Strands)**





**Figure 8-12. 20% Filamentous Algae Cover (Long Strands)**



**Figure 8-13. 26% Filamentous Algae Cover**





**Figure 8-14. 35% Filamentous Algae Cover (Short Strands). Note that this is the same location as Figure 8-15.**



**Figure 8-15. 38% Filamentous Algae Cover (Long Strands). Note that this is the same location as Figure 8-14.**





**Figure 8-16. 39% Filamentous Algae Cover**



**Figure 8-17. 47% Filamentous Algae Cover**





**Figure 8-18. 65% Filamentous Algae Cover**



**Figure 8-19. 98% Filamentous Algae Cover**

- c. Algae Thickness ( $AT_i$ ) is determined by evaluating the given transect segment for a representative algae thickness measurement. Thickness measurements

are made to determine how much of the water column is filled by filamentous algae in relation to the water depth. Length of the filaments is not a primary concern here; only how much of the vertical water column is filled with algae. If algae mats are present, the recorded algae depth is determined by adding the depth of the algae mat to the depth of the algae growing on the stream bottom. Mats may need to be moved out of the way to observe the bottom layer of algae attached directly to the stream bottom. REMEMBER: Algae Thickness should never be more than the water depth.

- d. Dominant Substrate Type is determined by observing the predominant substrate size particle for the segment and using the classification table at the bottom of PAGE 3 (**Figure 8-6 above**).
6. Repeat steps two and three with each progressing segment of the transect.
  7. If you encounter a mid-stream dry bar (not underwater) then document the bar width as its own segment (using the wetted edges of the bar as  $D_{Begin}$  and  $D_{End}$  for the segment) and make a note on the form. This area will be excluded from the calculation later.
  8. When the far bank (Bank<sub>2</sub>) is reached, record the final tape measurement distance at the wetted edge (Bank<sub>2</sub>  $D_{End}$ ) and note LDB or RDB in the note field.

### **Measuring Canopy Density with a Densimeter**

Canopy Density is measured based on a method used in the USEPA's National Rivers and Streams Assessment (NRSA), which is an element of the National Aquatic Resources Survey (NARS). Measurements are made using a spherical densitometer (model A-convex type) that is modified with a permanent marker or tape **exactly as shown in Figure 8-20 on next page**.

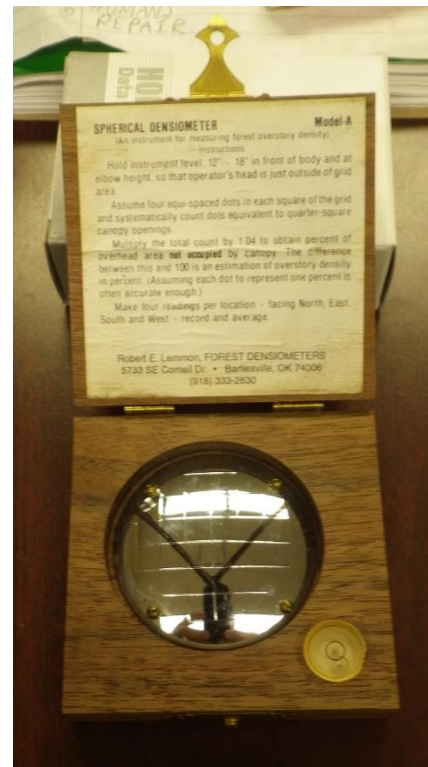
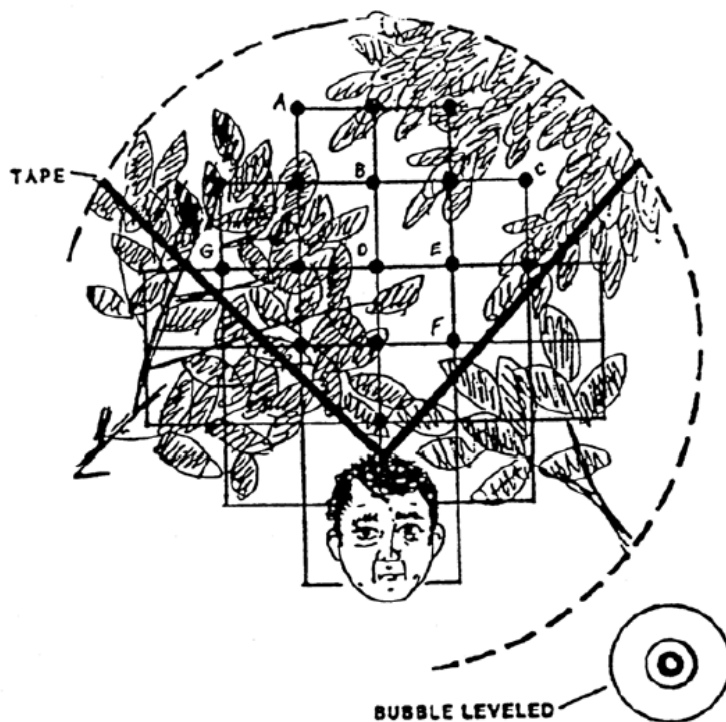


Figure 8-20. Examples of Modified Convex Spherical Canopy Densiometer (from Mulvey et al.

The markings or tape will limit the number of square grid intersections to 17 and densiometer readings can range from 0 (no canopy cover) to 17 (maximum canopy cover). Four measurements are obtained at each of 5 equidistant locations along the cross-section transect: Left (at the water's edge on the Left Descending Bank), Left Middle, Center, Right Middle, and Right (at the water's edge on the Right Descending Bank).

Fill out the bottom of PAGE 2 (see **Figure 8-4 on page 8-6**) of the Filamentous Algae Measurements Form using the following steps:

1. At the first location on the cross-section transect, stand in the stream and face upstream.
2. Hold the densiometer 0.3 m (1 ft.) above the surface of the stream. Level the densiometer using the bubble level. Move the densiometer in front of you so your face is just below the apex of the taped "V".
3. Count the number of grid intersection points within the "V" that are covered by a tree, a leaf, or a high branch. Record the value (0 to 17) in the Up field for the given location of the cross-section transect on the bottom of PAGE 2 under the Canopy Density section.
4. Face toward the left bank (left as you face downstream). Repeat Steps 2 and 3, recording the value in the Left field for the given location of the cross-section transect on the data form.

5. Repeat Steps 2 and 3 facing downstream and facing the right bank (right as you look downstream). Record the values in the Down and Right fields given location of the cross-section transect on the data form.
6. Repeat Steps 1 through 5 at for the remaining locations on the cross-section transect.

## Part 2. Filamentous Algae Data Analysis

Use the following equations to calculate the Percent Algae Cover and Percent Water Column Algae Fill. **See Figure 8-6 on page 8-7 for data used in the example calculations.**

### Calculating Percent Algae Cover

1. Calculate the transect wetted width (WW) by subtracting  $Bank_1D_{End}$  from  $Bank_2D_{End}$ .

$$WW = Bank_2D_{End} - Bank_1D_{End}$$

$$e.g., WW = 24.5 - 0.5 = 24.0$$

If any dry bars or islands were measured in the transect, add up their individual widths ( $Dry_i$ ) and subtract from WW calculated above to get an algal habitat width of the stream transect.

$$AHW = (Bank_2D_{End} - Bank_1D_{End}) - \sum (Dry_1, Dry_2, Dry_3, \dots, Dry_i)$$

$$e.g., AHW = (24.0) - 3.6 = 20.4$$

2. Calculate the width of each segment ( $W_i$ ) by subtracting  $D_{Begin i}$  from  $D_{End i}$ .

$$W_i = D_{End i} - D_{Begin i}$$

$$e.g., W_1 = 2.6 - 0.5 = 2.1$$

- Calculate the distance weighted algal cover of each segment ( $wAC_i$ ) by multiplying the width of each segment ( $W_i$ ) by the estimated percent bottom algae cover of that segment ( $AC_i$ ) and divide by 100.

$$wAC_i = W_i \times \frac{AC_i}{100}$$

$$e.g., wAC_1 = 2.1 \times \frac{25}{100} = 0.525$$

Any segment that was a dry bar ( $DB_i$ ) should be calculated with the Algae Cover ( $AC_i$ ) as 0.

$$e.g., wAC_4 = 3.6 \times \frac{0}{100} = 0$$

- Calculate the Estimated Percent Algae Cover (PAC) for this transect by dividing the sum of the distance weighted covers by the algal habitat width (AHW) and multiply by 100.

$$PAC = \frac{\sum(wAC_1, wAC_2, wAC_3, \dots, wAC_i)}{AHW} \times 100$$

$$e.g., PAC = \frac{0.525 + 1.15 + 2.0 + 0 + 1.275 + 3.0 + 1.98 + 0.665}{20.4} \times 100 = 51.9$$

### **Calculating Percent Water Column Filled**

- Calculate the volume of water in each segment ( $VW_i$ ) by multiplying the width of each segment ( $W_i$ ) (*from calculation 2 above*) by the water depth in segment ( $WD_i$ ). The third dimension of the volume is the one unit (foot or meter) wide area along the transect segment, effectively multiplying the equation by 1.

$$VW_i = W_i \times WD_i = W_i \times WD_i \times 1$$

$$e.g., VW_i = 2.1 \times 0.5 \times 1 = 1.05$$

**NOTE:** Any dry bar segments have a total volume ( $VW_i$ ) of 0 since they have a water depth ( $WD_i$ ) of 0 in this equation.

$$VW_i = W_i \times 0 = 0$$

$$e.g., W_4 = 3.6 \times 0 = 0$$

2. Calculate the volume of algae in each segment ( $VA_i$ ) by multiplying the width of each segment ( $W_i$ ) (*from calculation 2 above*) by the algae thickness in that segment ( $AT_i$ ) and by the estimated percent bottom algae cover of that segment ( $AC_i$ ). Alternatively, you can multiply  $wAC_i$  (*from calculation 3 above*) by the algae thickness in that segment ( $AT_i$ ).

$$VA_i = W_i \times \frac{AC_i}{100} \times AT_i = wAC_i \times AT_i$$

$$e.g., VA_1 = 2.1 \times \frac{25}{100} \times 0.2 = 0.525 \times 0.2 = 1.05$$

Any segment that was a dry bar ( $DB_i$ ) should be calculated with the volume of algae ( $VA_i$ ) as 0.

$$e.g., VA_4 = 3.6 \times \frac{0}{100} \times 0 = 0$$

3. Calculate the percent of the water column filled (PWCF) with algae by dividing the sum of the volumes of algae in each segment ( $VA_i$ ) by the sum of the volumes of water in each segment ( $VW_i$ ).

$$PWCF = \frac{\sum(VA_1, VA_2, VA_3, \dots, VA_i)}{\sum(VW_1, VW_2, VW_3, \dots, VW_i)} \times 100$$

$$e.g., PWCF = \frac{0.105 + 0.46 + 0.6 + 0 + 0.51 + 1.2 + 0.396 + 0.0665}{1.05 + 2.53 + 1.6 + 0 + 0.85 + 6.0 + 4.4 + 0.76} \times 100 = 19.4$$

## Filamentous Algae Measurement Quality Assurance and Quality Control

Duplicate samples will be collected from 2.5% of the sites sampled and only when at least two people are on a sampling team. Both duplicates are collected at the same date and approximate time (as equipment sharing will allow) by different individuals. The duplicate data will be analyzed to ensure precision and repeatability of the sampling technique. Every effort is made to assure that all of the personnel who perform the filamentous algae measurements participate in 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 14. Section A. Blanks and Duplicates starting on page 14-1 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. Whilst a specific session on Filamentous Algae



Monitoring is not covered, other sessions (*e.g.*, site documentation and completing the stream assessment forms, sonde calibration, water collection protocols, stream flow measurement, field blanks and duplicates, *etc.*) are covered. In the field, individuals who are more experienced in Filamentous Algae Monitoring 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 maintain these stations solo. This document is also provided to all program personnel for review and use in the field.

**This page intentionally left blank.**

## CHAPTER 9. 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 9-1 on right***):

1. 1-liter cubitainer – Used as a sample container.
2. Dark or Black Plastic Trash Bag – Used to isolate the collected sample from any light source.
3. Cooler with ice – Used to keep the samples cool.
4. Filter Flask – Receptacle for the filtered water (at least 500 mL in size.)



**Figure 9-1. Photo of the materials used to filter a Golden Algae sample.**

5. 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.
6. Filters – *Whatman* Glass microfibre filters GF/F 47 mm diameter with 0.45 µm pore size.
7. Vacuum Pump – A variety of hand operated pumps are available. **Do not use Peristaltic Pump/Drill Apparatus.**
8. Vacuum Tubing – usually already attached to vacuum pump.
9. 10% Bleach Solution - for sterilization.
10. Distilled water and industrial grade deionized (DI) water (if available).
11. Sterilized stainless steel forceps or sterile plastic forceps.
12. 4" squares of clean aluminum foil (1 square of foil per sample).
13. Zip Lock Baggies – 1 sandwich size per sample and 1 gallon size to hold all samples per filtering session.
14. Sample Labels (*see Figure 9-2 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
15. Golden Algae Analysis Request forms with Chain-of-Custody (COC).

Figure 9-2. 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 **three times** at least **one-half full** with sample water. During the rinse, secure the lid on the cubitainer, shake for 5 seconds, and then empty.

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-lock 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 or distilled 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.



**Figure 9-3.** Photo of the insertion of rubber stopper into flask with plastic funnel already inserted through rubber stopper.

Sample blanks are generally prepared and processed as below using either distilled drinking water from freshly opened gallon containers or industrial grade DI 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 snugly into rubber stopper, and insert stopper snugly into empty flask (*see Figure 9-3 on right*).



**Figure 9-4.** Photo of cup being removed to access the prepackaged filter for removal.

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 9-4 below*).



**Figure 9-5.** Photo of the GF/F filter being placed on top of support screen with forceps.

4. Remove prepackaged nitrate seal/filter from cup base using sterilized forceps and place in a clean zip lock bag for potential use in other water quality monitoring projects.
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 9-5 right*). The cross-hatch pattern side of the disk should be placed facing down against the support screen (*see Figure 9-6 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 9-6.** Photo of the gridded pattern of GF/F filter. This side placed down when filtering Golden Algae sample.



**Figure 9-7.** 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 9-7 above*). Place remaining sample back into bag to prevent further UV exposure.



**Figure 9-8.** Photo of monitoring the pump pressure while filtering sample – keep the 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 (not to exceed 5 psi) is used via the hand pump to pull sample through filter (*see Figure 9-8 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 9-9 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 9-9.** 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.



**Figure 9-10.** Photo of Forceps and small plastic funnel soaking in 10% bleach solution for 1 minute.

14. Between filtering samples, rinse the small plastic funnel and forceps with tap water, then soak in a 10% bleach solution for 1 minute (**see Figure 9-10 on right**).
15. A final rinse with industrial grade DI or distilled water completes the cleaning process between samples (**see Figure 9-11 below**).

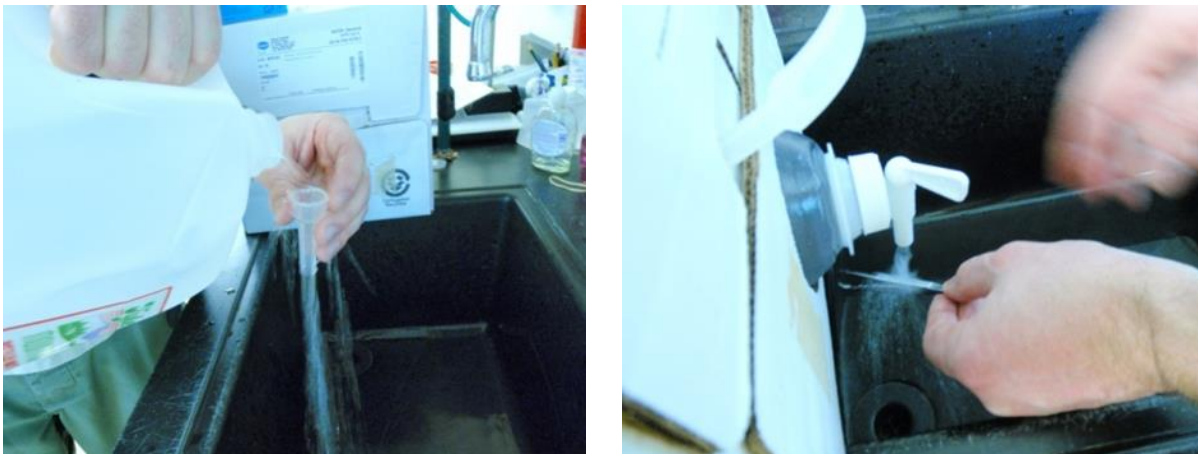


Figure 9-11. Left: Photo of small plastic funnel being rinsed with distilled water. Right: Photo of forceps being rinsed with industrial grade deionized water.



***IMPORTANT:*** Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade DI 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 9-12 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 9-12. 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 in **CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1.**

### **Sample Blanks**

Sample blanks are generally prepared and processed as above using either distilled drinking water from freshly opened gallon containers or industrial grade DI 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 10. 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 2. Section C. APPENDIX #5 – Relative Bed Stability (Pebble Count) including Gradient on page 2-122**)

### **Section A. 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 2. Section C. APPENDIX #5 – Relative Bed Stability (Pebble Count) including Gradient on page 2-122**).
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 width 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 10-1 on the next page*).



**Table 10-1. 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.

***IMPORTANT: Read the depth on the side 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 over steepened 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 (e.g., 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

***IMPORTANT: 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.

***IMPORTANT:*** *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 2. Section C. APPENDIX #5 – Relative Bed Stability (Pebble Count) including Gradient starting on page 2-122). 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 7. Calculation of Percent Gradient**

$$\% \text{ Gradient} = \frac{\text{Sum of Rises}}{\text{Reach Length}} \times 100$$

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

## **Section B. 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.

**This page intentionally left blank.**

## CHAPTER 11. 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-(74.1)** Kanawha River At Chelyan
6. **KC-(11.6)** Coal River At Tornado
7. **KE-(4.3)** Elk River At Charleston
8. **KG-(8.25)** Gauley River At Beech Glen
9. **KN-(1.2)** 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-(6.1)** 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 1. INTRODUCTION TO WATERSHED ASSESSMENT BRANCH SAMPLING ACTIVITIES. Sampling Programs of the Watershed Assessment Branch on page 1-2 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 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. Guidelines for Completing the Stream Assessment Forms on page 2-28**

**CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1**

**CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS starting on page 4-1**

**CHAPTER 5. BENTHIC MACROINVERTEBRATE COLLECTION PROTOCOLS starting on page 5-1**

**CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1**

Since only a few people routinely sample at 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 3. Section B. Water Quality Sample Collection and Preservation starting on page 3-33 for more detailed information about these techniques.**

## Part 1. Ambient Water Quality Network Water Parameters

Table 11-1. The current list of Ambient Water Quality Network Water Parameters, MDLs, Analysis Methods, and Holding Times

<b>Parameter</b>	<b>MDL or Instrument Accuracy*</b>	<b>EPA Method</b>	<b>Holding Time</b>
Acidity, Hot	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, Total	0.002 mg/L	200.7	6 Months
Beryllium, Total	0.00008 mg/L	200.7	6 Months
Boron, Total	0.003 mg/L	200.7	6 Months
Bromide, Total	0.1 mg/L	300.0	6 Months
Cadmium, Dissolved	0.0003 mg/L	200.9	6 Months
Calcium, Total	0.02 mg/L	200.7	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
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
Magnesium, Total	0.05 mg/L	200.7	6 Months
Manganese, Total	0.01 mg/L	243.1	6 Months
Nickel, Dissolved	0.07 mg/L	200.9	6 Months
Nitrate + Nitrite Nitrogen	0.2 mg/L	353.2	28 Days
Potassium, Total	0.05 mg/	200.7	6 Months
Phosphorus, Total	0.1 mg/L	365.1	28 Days
Selenium, Total	0.001 mg/L	200.8	6 Months
Sodium, Total	0.5 mg/L	200.7	6 Months
Silver, Dissolved	0.0003 mg/L	272.2	6 Months
Strontium, Total	0.001 mg/L	200.7	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

WV DEPARTMENT OF ENVIRONMENTAL PROTECTION - WATERSHED ASSESSMENT BRANCH  
General Analysis Request Form, Rev. 03/2015

Circle Project(s): TMDL LTMS WAB RANDOM **AWQN** DEPLOY. LAKES FISH OTHER \_\_\_\_\_

Stream Name: **Ambient Site (Including Ambient/LTMS)** Laboratory Name: \_\_\_\_\_  
Watershed Name: \_\_\_\_\_

AN-Code: \_\_\_\_\_ WQ Lab ID: \_\_\_\_\_ Random #: \_\_\_\_\_

Sampled By: \_\_\_\_\_ Filtered By: \_\_\_\_\_ # of Containers: \_\_\_\_\_

Sample Type: Water \_\_\_\_\_ Sediment \_\_\_\_\_ Other \_\_\_\_\_ Specified Method: **40 CFR 136**

Acid Lot #, Nitric: \_\_\_\_\_ Sulfuric: \_\_\_\_\_ HCl: \_\_\_\_\_

Sample Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ Time: \_\_\_\_\_

Field Values: Temp. \_\_\_\_\_ pH \_\_\_\_\_ D.O. \_\_\_\_\_ Cond. \_\_\_\_\_

**Bold type/Cell Border = Random Parameter Suite (Total & Dissolved Al & Fe, Diss. Only Cu & Zn)**

Pres.	Analysis / Item #*	Pres.	Analysis / Item #*	Pres.	Analysis / Item #	Tot	Diss*	Preservation Code
3	Acidity (Hot) / 2	3	Tot. Diss. Solids / 15	5	Aluminum Al/ 53	✓	✓	1. None - Determined on-site 2. None 3. Iced immediately 4. H <sub>2</sub> SO <sub>4</sub> to pH <2, iced immediately 5. HNO <sub>3</sub> to pH <2 6. Sterile + 0.008% Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> , iced immediately 7. Filtered immediately, iced immediately 8. HCl to pH <2, iced immediately 9. MgCO <sub>3</sub> & Ice, _____ ml sample 10. Other (Specify) 11. Filter, H <sub>2</sub> SO <sub>4</sub> to pH <2, iced immediately +Dissolved metals are to be filtered immediately, and nitric acid added to pH <2 * Item # specifies method, MDL, & PQL required in current WQ Analytical Contract  REMARKS:  _____ # of Filters used Filter type (circle): Disc Cartridge
3	Alkalinity / 3	3	Tot. Susp. Solids / 16	5	Iron Fe/ 64	✓	✓	
3	Sulfate / 6	4	T. Phosphorus-P / 26	5	Manganese Mn / 67	✓		
2	Chloride / 10	4	TKN / 20A	5	Selenium Se / 72	✓		
6	Fecal Coll., MF /12 24 hour holding	4	NO <sub>2</sub> -NO <sub>3</sub> -N / 25	5	Copper Cu / 63A		✓	
6	Fecal Coll., MF/12 6 hour holding	4	Low level T. Phosphorus-P / 26A	5	Zinc Zn/ 78A		✓	
2	Bromide / 9	11	Diss. Phosphorus	5	Magnesium Mg/ 66	✓		
5	Hardness / 4	4	TKN / 20	5	Calcium Ca / 60	✓		
3	Acidity (Cold)	4	Ammonia-N / 21	5	Potassium K/ 71	✓		
3	Bicarbonate / 48	3	Tot. Ortho PO <sub>4</sub> / 50	5	Sodium Na/ 74	✓		
3	BOD / 29	3	Diss. Ortho PO <sub>4</sub> / 50	5	Beryllium Be / 57	✓		
4	COD / 31	7	Chlorophyl a / 38	5	Barium Ba / 56	✓		
4	TOC / 32	9	NO <sub>3</sub> -N (Nitrate) / 23	5	Strontium Sr/77	✓		
3	pH (lab) / 1	3	NO <sub>2</sub> -N (Nitrite) / 24	5	Boron B/ 58	✓		
3	Cond. (lab) / 5	3	Semi-Vol. Organics	5	Cadmium Cd/ 59A		✓	
		8	Volatile Organics	5	Lead Pb/ 65		✓	
				5	Nickel Ni/ 70A		✓	
				5	Arsenic As/ 55	✓		
				5	Silver Ag / 73		✓	
				3	Hex. Chromium/36			
				5	Mercury Hg / 68			
				5	LL Mercury / 68A			

Relinquished by:	Date & Time	Received by:	Relinquished by:	Date & Time	Received by:
		Lab:			Lab:

Mail Results to: ATTN: Janice Smithson (Lab Instructions: On invoice bill to Organization Unit 9480),  
 WVDEP, DWWM, Watershed Assessment Branch, 601 57th Street SE, Charleston, WV 25304 Phone (304) 926-0499 ex. 1051, Fax. 926-0496  
 WHITE - DEP Copy CANARY -Laboratory Copy

**Figure 13. Example of COC with Ambient Parameters Selected**



## 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 3. Section B. Part 1. Procedures for Collecting Water Quality Samples Direct Dip/Grab Method starting on page 3-35 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:

2. The stream is wadeable. Generally, this is common at some of the sites, especially during the low flow summer months.
3. 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.
4. 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 (deionized) 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 VDHS 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 (*e.g.*, 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 called a **Sampling Iron** 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 3. Section B. Part 2. Sample Preservation (Filtration, Fixation, & Holding) on page 3-38.**

## 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  
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 4. STREAM FLOW MEASUREMENT starting on page 4-1**. Be sure to wear a personal flotation device when measuring flow. Calculate the total discharge and record on the appropriate form (see **CHAPTER 2. Section B. APPENDIX #1 - Stream Discharge (Flow) on page 2-110**).

### 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:

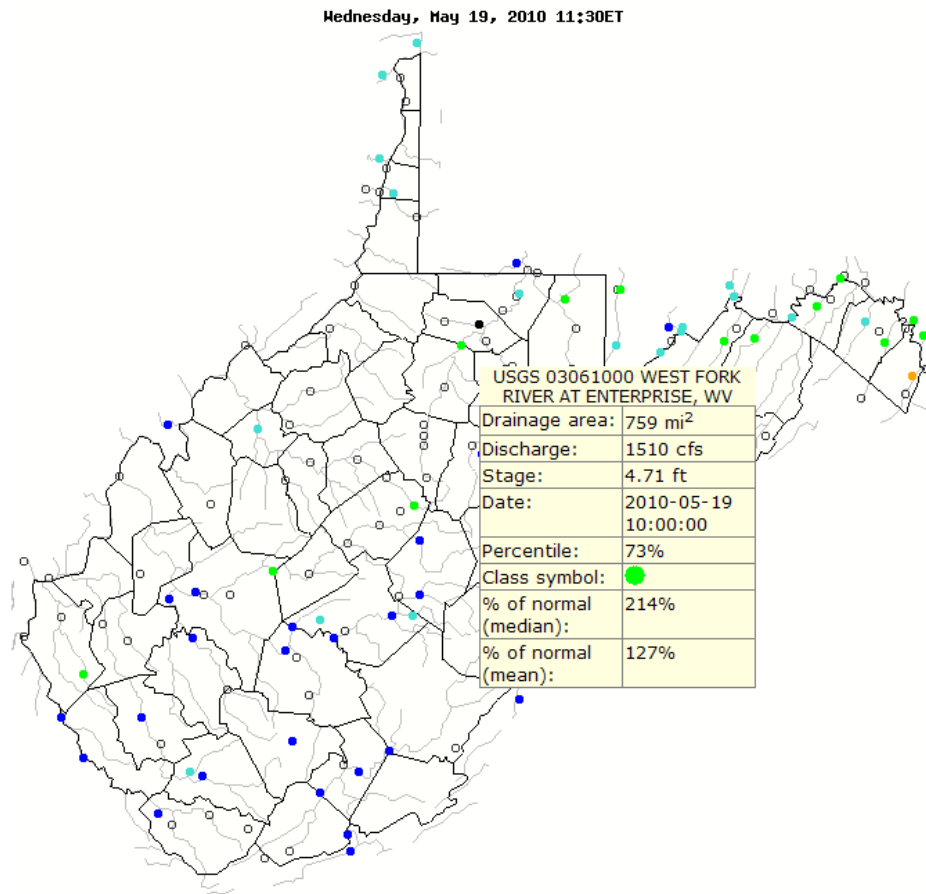
<http://waterdata.usgs.gov/wv/nwis/current?type=dailystagedischarge>

<http://wv.usgs.gov/>

<http://waterwatch.usgs.gov/?m=real&r=wv>

See **Figure 11-2 and Figure 11-3 on the next two pages for examples of USGS website data displays**.

**Map of real-time streamflow compared to historical streamflow for the day of the year (West Virginia)**  
 Google Maps version of this map



**Figure 11-2. Example of USGS stream flow website**  
 (<http://waterwatch.usgs.gov/?m=real&r=ww>)

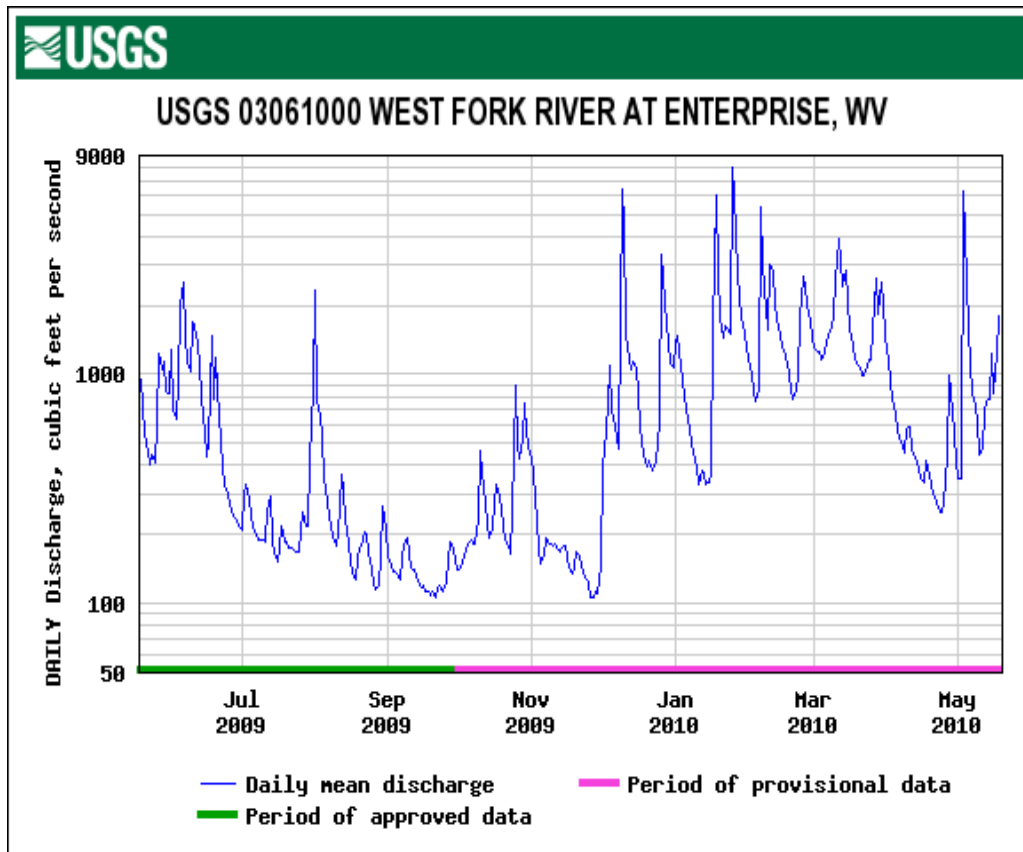


Figure 11-3. Example of USGS Stream Gage Output Graph

Refer to the specific station monitoring information (*see Section C. AMBIENT SAMPLING STATION DESCRIPTIONS starting on the next page 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 2. Section B. APPENDIX #1 - Stream Discharge (Flow) on page 2-110*). 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 11-4. 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

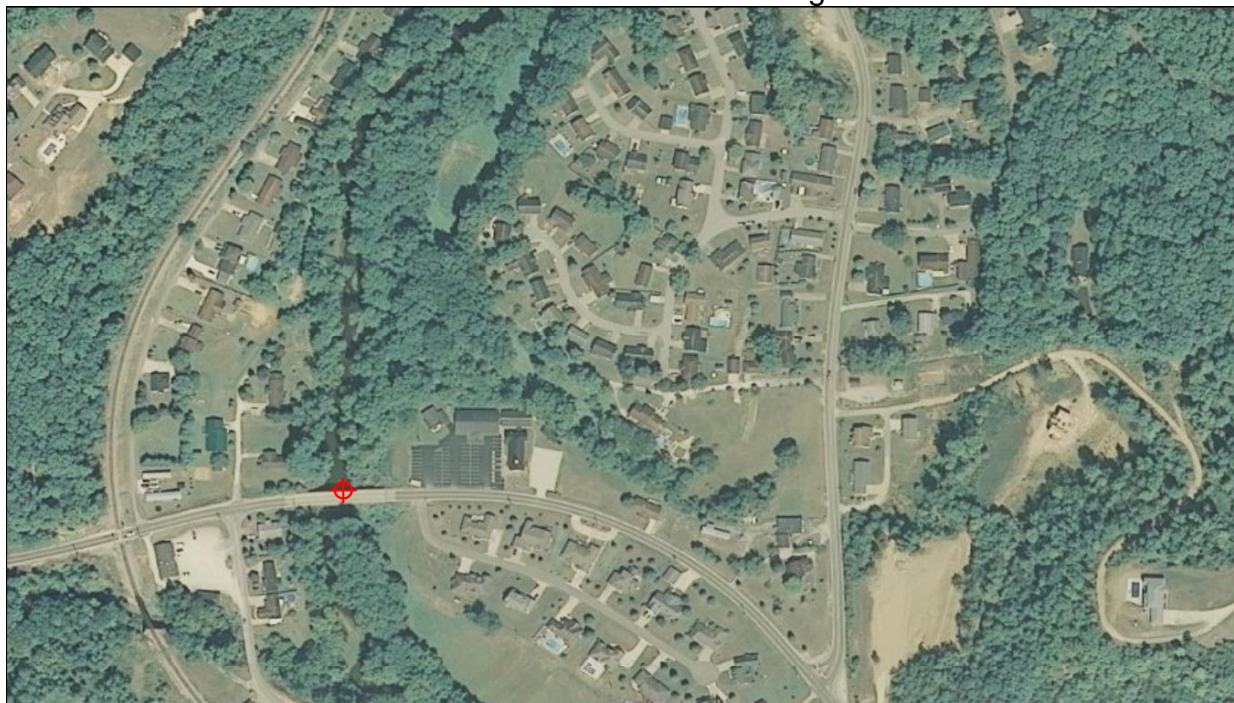


Figure 11-5. 2003 Aerial Photo of the O-2-(8.8) Twelvepole Creek Ambient Sample Site in Wayne Co., WV.

**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 11-6. 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 29<sup>th</sup> 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**

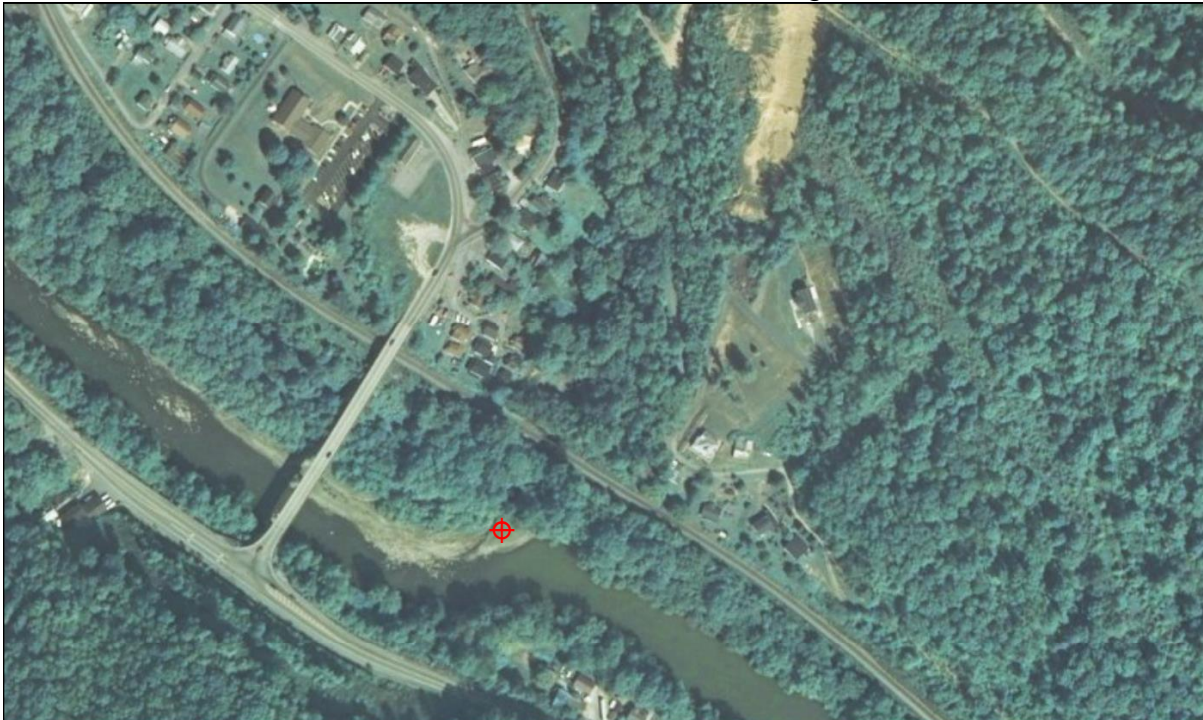
Access USGS website for flow information – Gage site: Branchland, WV / USGS #03204000

**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 11-7. 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 / #03201405

### 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.**

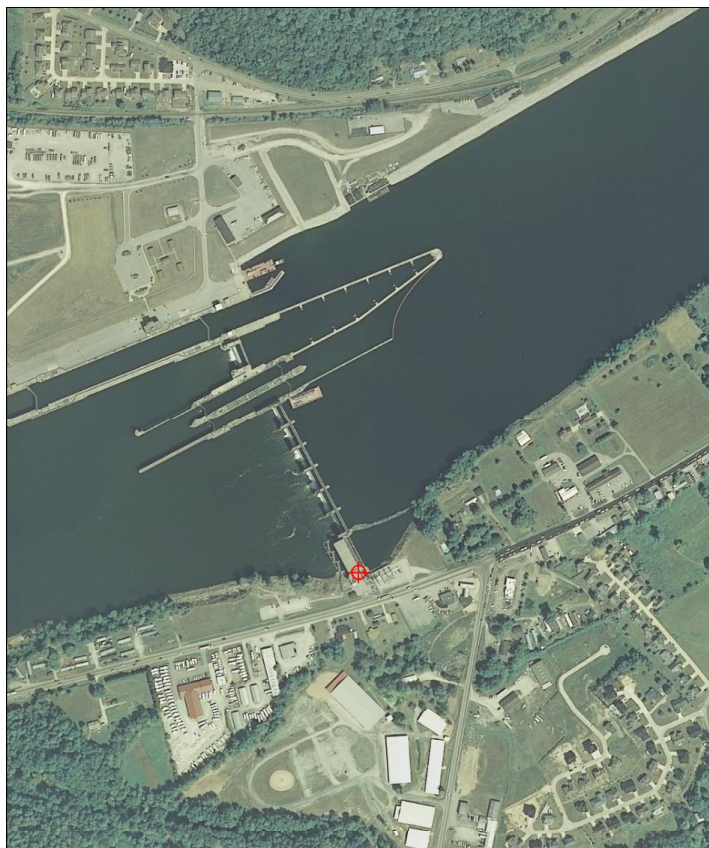


Figure 11-8. 2003 Aerial Photo of the K-(31.7) Kanawha River Ambient Sample Site at Winfield Locks & Dam, WV.



Figure 11-9. Photo of the Winfield Locks & Dam.



Figure 11-10. Photo of the Winfield Locks & Dam Intake Sample Area.

## K-(74.1) 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

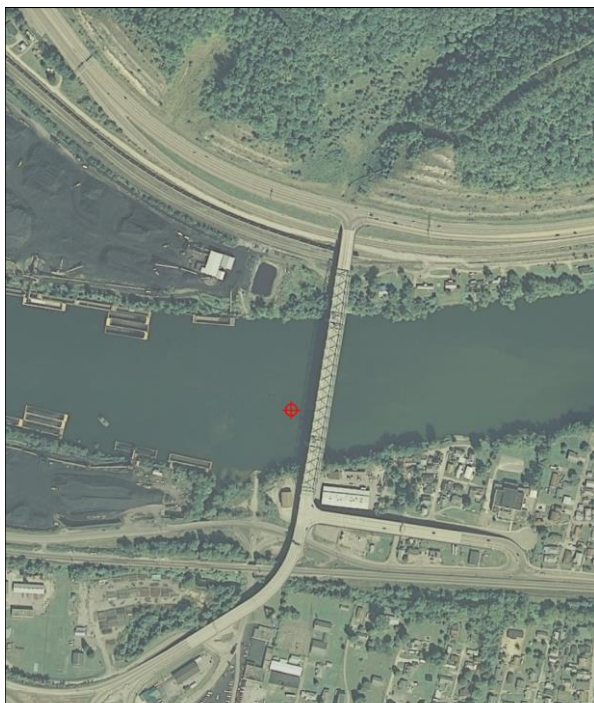


Figure 11-11. 2003 Aerial Photo of the K-(74.1) Kanawha River Ambient Sample Site at Chelyan, WV.



Figure 11-12. 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 11-13. 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.

### 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



Figure 11-14. 2003 Aerial Photo of the KE-(4.3) Elk River Ambient Sample Site at Charleston, WV.

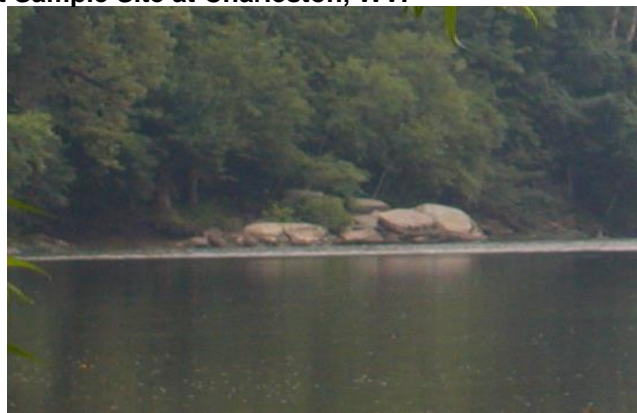


Figure 11-15. Photo of the Elk River Sampling Site from Upstream at Coonskin Park. upstream during summer low flows.

### Flow

Access USGS website for flow information – Gage site: Queen Shoals, WV / #03197000

### Special Instructions

***Wear PFD!!!***



Figure 11-16. Photo of the Elk River Sampling Site Looking Upstream.



**KG-(8.25) 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 11-17. 2003 Aerial Photo of the KG-(8.25) 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**

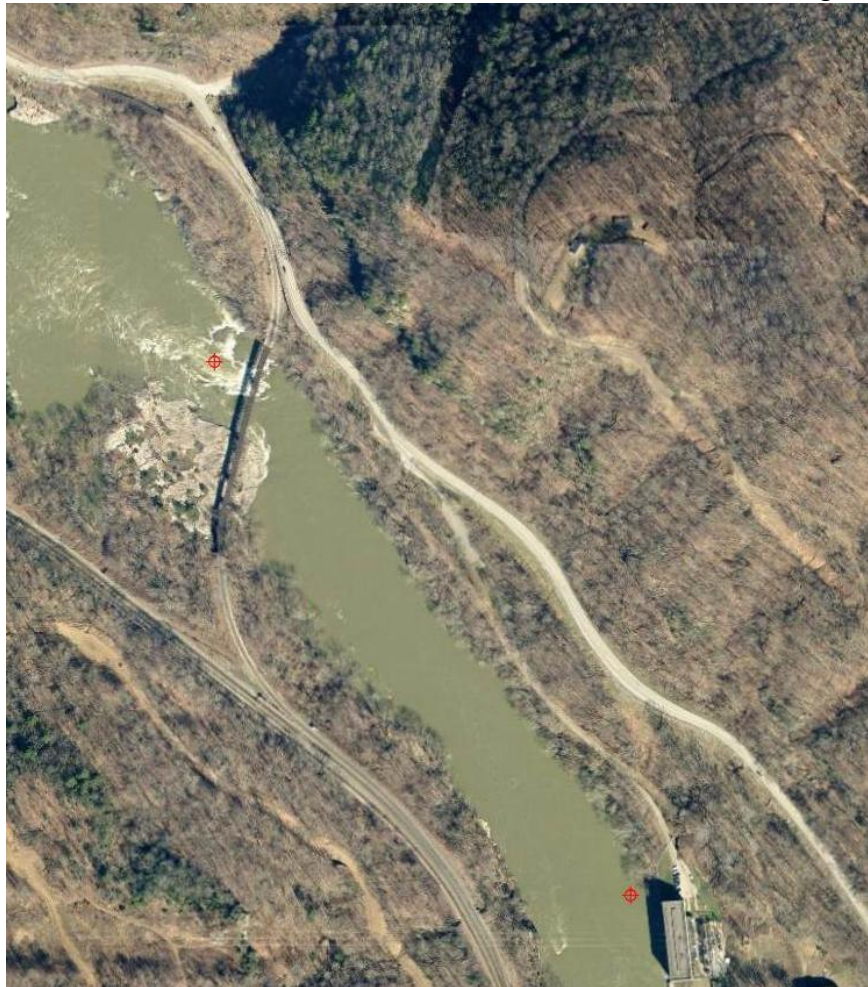
The bridge to access the sampling point experiences a large volume of coal truck traffic.

***Wear orange safety vest and PFD!!!***

**KN-(1.2) New River**

**USGS Quadrangle:** Gauley Bridge, WV  
**Coordinates:** Latitude – 38° 09' 7.4" N

**Basin:** Lower New **County:** Fayette  
 Longitude – 81° 10' 47.7" W



**Figure 11-18. 2010 Aerial Photo of the KN-(1.2) New River Ambient Sample Site near Gauley Bridge, WV. Note the previous location upstream at the Elkem Power Station at 1.55**

**Directions to Sample Site**

Sample site is located just downstream/beneath a railroad trestle downstream of the Elkem Power Station. Parking is located at the Cathedral Falls roadside park, along US Route 60 immediately after the railroad crossing on the left (leaving Gauley Bridge).

**Description of Sampling Point**

Sample is collected from the right descending bank or as far out as one can safely get. Past sampling for this site has occurred mid-stream directly from the Railroad Trestle and on the right descending bank just upstream at the Elkem Power Station at MP 1.55.

**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

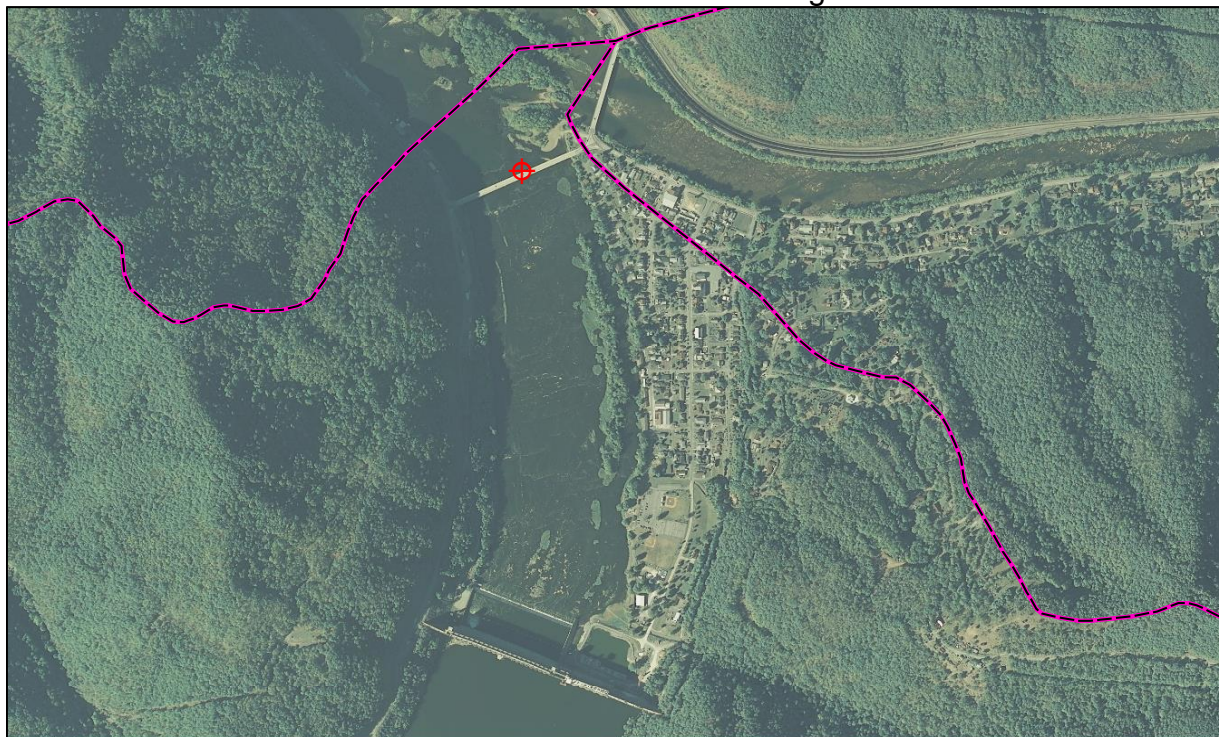


Figure 11-19. 2003 Aerial Photo of the KN-(67.4) New River Ambient Sample Site in Hinton, WV.

**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 11-20. 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

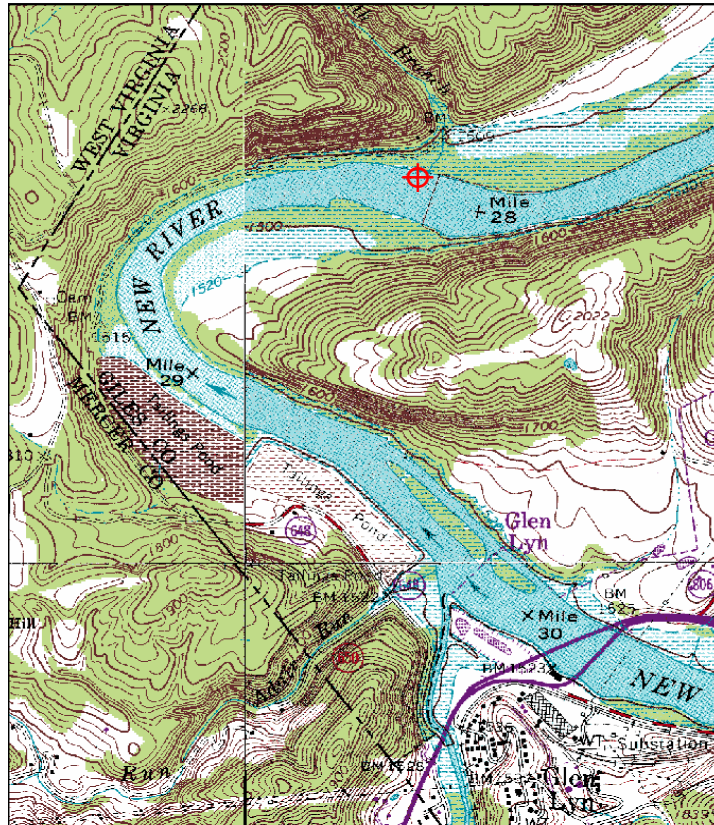
Longitude – 80° 52' 5.8" W

**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



**Figure 11-21. USGS 24k Topographic Map of the KN-(96.2) New River Ambient Sample Site north of Glen Lyn, VA.**

mid-stream as flow levels allow.

**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.

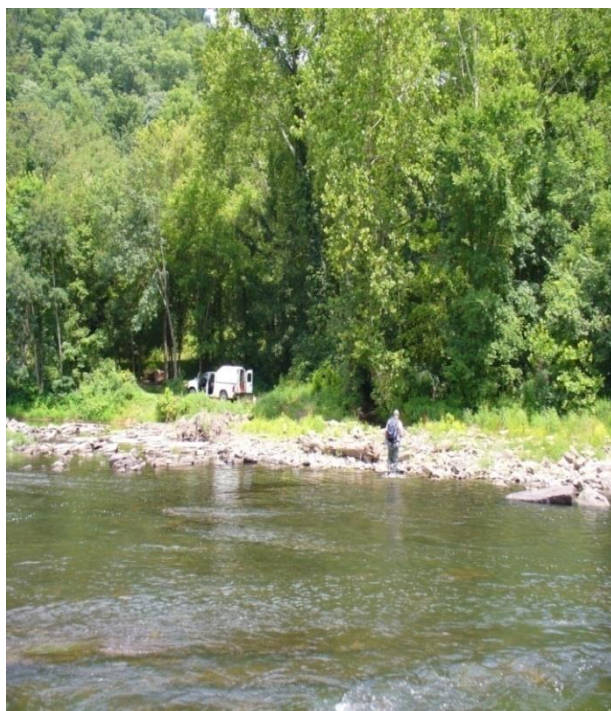
**Flow**

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.**

**Figure 11-22. Photo from X-Site looking toward Left Descending Bank at Mouth of Smith Branch and Parking Area.**





**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 11-23. 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 11-24. 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 11-25. 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 11-26. 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

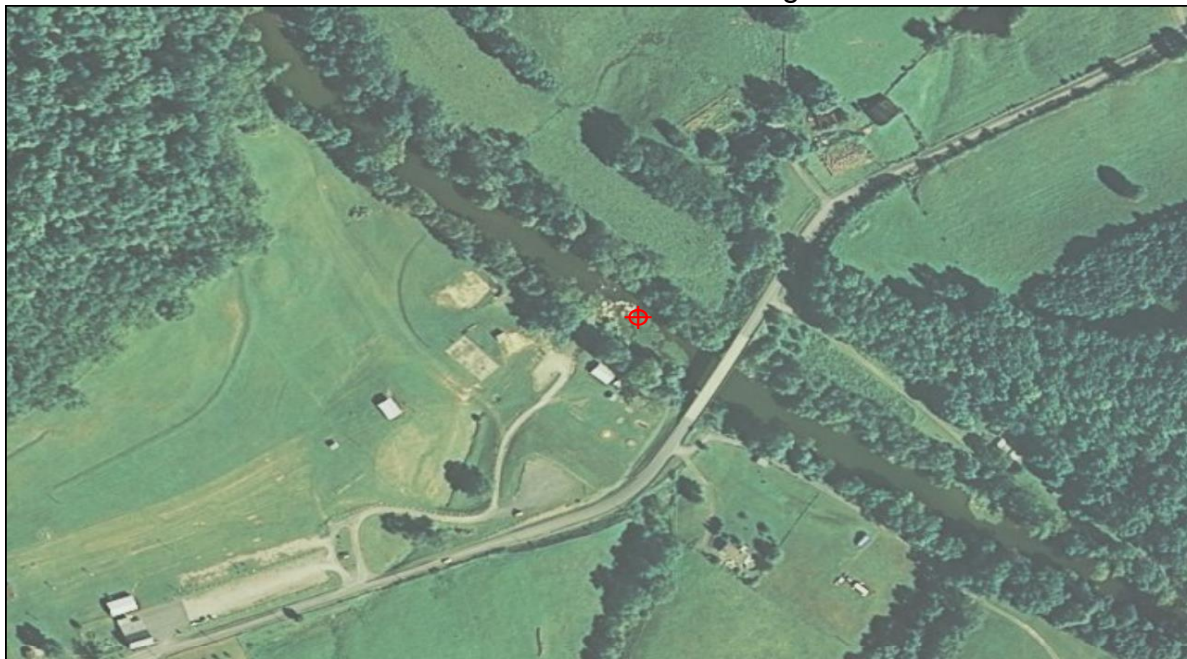


Figure 11-27. 2003 Aerial Photo of the M-1-(20.6) Dunkard Creek Ambient Sample Site at Mason-Dixon Historical Park, WV.

### 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 11-28. 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 11-29. 2003 Aerial Photo of the MW-(12.0) West Fork River Ambient Sample Site at Enterprise, WV.**

**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 11-30. 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 11-31. 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

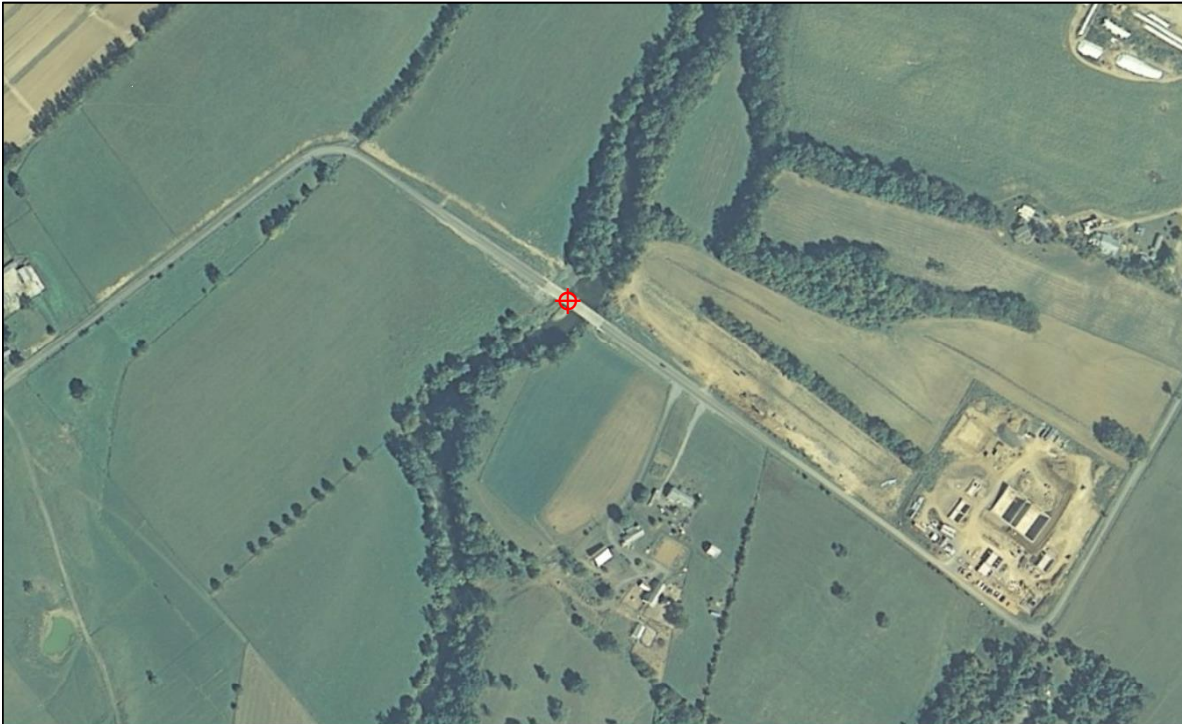


Figure 11-32. 2003 Aerial Photo of the P-4-(3.5) Opequon Creek Ambient Sample Site near Bedington, WV.

### 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

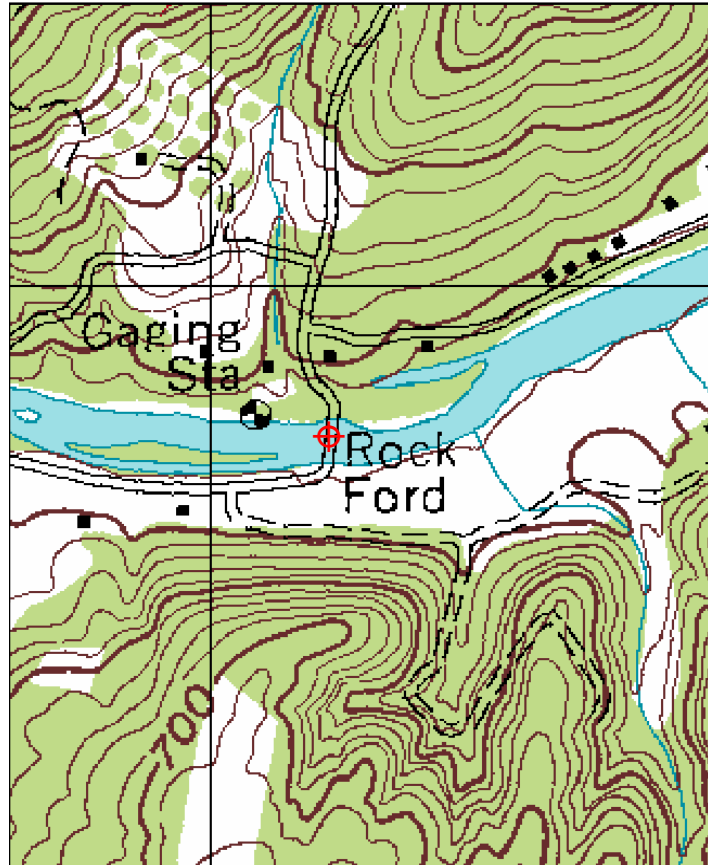


Figure 11-33. USGS 24k Topographic Map of the PC-(6.1) Cacapon River Ambient Sample Site south of Great Cacapon, WV.

VDHS or SSB with rope.

### 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 11-34. 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

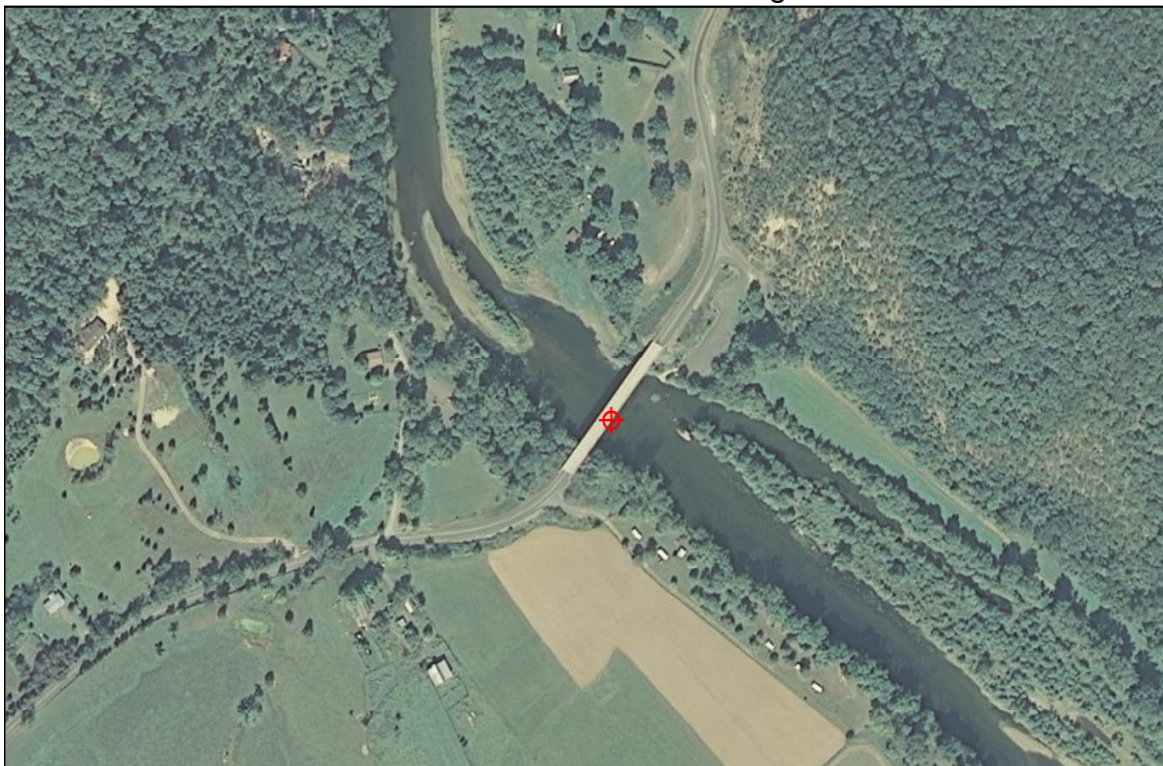


Figure 11-35. 2003 Aerial Photo of the PSB-(13.4) South Branch Potomac River Ambient Sample Site near Springfield, WV.

**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 11-36. 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 in **CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1.**

**This page intentionally left blank.**



## **CHAPTER 12. 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:

**CHAPTER 2 Section C. Guidelines for Completing the Stream Assessment Forms on page 2-28**

**CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1**

**CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1**

### ***Section A. Setting up the Site***

A field crew sampling lakes typically consists of at least two individuals (safety requires at least two people on a boat) 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 second person can operate on the shoreline.

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.

***IMPORTANT: 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 12-7*). Procedures specific to each sample type are discussed below.

## 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.

***WARNING: 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 $\leq$ 20	3
20 < Area $\leq$ 50	4
50 < Area $\leq$ 200	5
200 < Area $\leq$ 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 12-1 on page 12-4 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 12-1 on page 12-4**): 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 12-1. 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		

***IMPORTANT:*** 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:** 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.



***IMPORTANT: 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.

***IMPORTANT: 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 14. Section A. Blanks and Duplicates on page 14-1**), 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 sample containers 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. All duplicate samples and COCs should be labeled as Blind Duplicates for the lab (false Stream Names and AN-Codes) and no indication that it is a Duplicate.

## **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 2. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) on page 2-20.**

Because each lake is visited only 4 times in a season coordinates should be recorded at every lake visit. **Table 2-2 on page 2-21** 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 2. Section B. Part 2. Photographic Documentation on page 2-24.**

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 12-16**).

## **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 2. Section C. Guidelines for Completing the Stream Assessment Forms found on page 2-28.**

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 (e.g., 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.

## Description of Lake Water Quality 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.

### 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 2. Section C. Part 1. Front Side of All Pages on page 2-29 for an example.**

### PAGE 1

#### **IMPORTANT INSTRUCTIONS:**

**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")! Also take photographs of the lake that display the reason why it was not considered target. This is important information and assists in database management. See Figure 12-1 on the next page for an example of PAGE 1 of the Lake Assessment Form.**

For a description of parameters and values not seen below **see CHAPTER 2. Section C. Part 1. PAGE 1-Site Verification on page 2-30 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.



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 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. is 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.

***IMPORTANT: 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.

Single WQ Sample ID: Document the pre-assigned Water Quality Sample IDs used with this visit. This ID is unique and comes pre-printed on labels. It is used whenever a lab water sample is collected. Usually only one (Top) or two (Top & Bottom) water quality samples are taken with lake sampling. If more than two water quality samples are taken during the sampling event (e.g., a waterbody profile), then this information will be documented on **PAGES 5 & 6 (see page 12-16)** of the form with the specific collection information (e.g., depth, distance, transect, etc.).

## **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 2. Section C. Part 1.**

**PAGE 2-Site Activities and Disturbances (Including Roads) on page 2-37 for an example.**

**See Figure 12-2 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**.

Human Activities	<input type="checkbox"/> Yes <input type="checkbox"/> No	Boating Activities	<input type="checkbox"/> Yes <input type="checkbox"/> No
<p>Elaborate on any of the Shore Activities &amp; Disturbances checked above. Which of the above is the greatest detriment to the Lake?</p>			

**Figure 12-2. 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 horsepower 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 in **Figure 12-3 on the next page**, see **CHAPTER 2. Section C. Part 1. PAGE 4 Sediment Characterization on page 2-53 for an example.**

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).

FIELD WATER*****					Reviewers Initials	
ANCode			Date			
WQ Sample Location <input type="checkbox"/> Vertical <input type="checkbox"/> Other:			WQ Type <input type="checkbox"/> Profile <input type="checkbox"/> Other:			
Sonde Method <input type="checkbox"/> Van-Dorn Bottle <input type="checkbox"/> Grab			Lab Water Method <input type="checkbox"/> Van-Dorn Bottle <input type="checkbox"/> 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?		<input type="checkbox"/> Yes <input type="checkbox"/> No
Field Water Notes and Precipitation Comments:						

Figure 12-3. Example of the Field Water Quality Measures (Top of PAGE 3) of the Lake Assessment Form

**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 12-4. 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 disk 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 2. Section C. Part 1. PAGE 12-Photography Log on page 2-103.**

## 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 (*e.g.*, 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.

**IMPORTANT: This is mandatory for each reading.**

Time: The time (military) at which the water parameters were measured at the given depth.

**IMPORTANT: 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 (°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 12-5. 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 (*e.g.*, 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 and then note the depth. Then raise the disk again until it is visible and note the depth. 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.

***IMPORTANT: Remember to account for the extra distance from the end of the measuring tape/rope and the attachment point to the Secchi disk if necessary.***

## **Part 2. Sonde Procedures**

See **CHAPTER 3. Section A. Water Quality Sondes & Sensors: Calibration, Routine Maintenance, & Use** on page 3-1 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 functioning Chlorophyll-a probes are 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.

***IMPORTANT: 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.**

***IMPORTANT: 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 Sensor 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 on page 12-17**).
  - 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.

***IMPORTANT: Do not store the sonde with a full cup of water, as this will lessen the life of the pH Sensor.***

### Part 3. Water Quality Sample Collection and Preservation

#### Lake Water Quality Parameters

- Alkalinity, Fecal coli., TSS, Tot. Phos., TKN, NO<sub>2</sub>-NO<sub>3</sub>-N, Mg (Tot.), Ca (Tot.) & Chlorophyll A

**3 containers (wet iced, HNO<sub>3</sub>, & H<sub>2</sub>SO<sub>4</sub>), 1 container for Chlorophyll Collection, 1 Chlorophyll filter, & fecal**

#### Materials and Reagents

In addition to the Materials and Reagents described in **CHAPTER 3. Section B. Materials and Reagents on page 3-33**, 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 DI (deionized) or distilled 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 3-34*** for information about how to handle water samples and fixatives.

### **Procedures for Collecting Water Quality Samples**

See ***Labeling Sample Containers on page 3-34*** 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 scums. The time of the top and bottom samples should be the same as the time noted on ***PAGE 3-Lake Info (starting on page 12-15)*** of the Lake Assessment 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 DI or distilled 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 containers (e.g., cubitainers). At lake sites, typically 2 are needed for nutrient analysis: 1 unfixed, and 1 sulfuric-fixed.
8. Rinse the container **three times** at least **one-half full** with sample water from the Van Dorn sampler by opening the downward-pointing spigot. During the rinse, secure the lid on the cubitainer, shake for 5 seconds, and then empty.
9. Fill the container with sample water, making sure to limit the amount of organic matter in the sample, 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.

***IMPORTANT: 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 Part 3. Water Quality Sample Collection and Preservation 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 3. Section B. Part 2. Sample Preservation (Filtration, Fixation, & Holding) Filtration on page 3-38** 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 DI or distilled water
7. Ziploc bags
8. Cooler with Ice (Dry Ice preferred)

#### Procedure:

1. Nalgene filtration apparatus should be rinsed with DI or distilled 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 DI or distilled 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.

***IMPORTANT: 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 DI or distilled 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 DI/distilled 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 containers for 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 3. Section B. Part 2. Fixation on page 3-44** for details on how to fix and preserve the other samples collected (*i.e.*, metals, nutrients, unfixed containers, fecal).

### **Holding**

See **CHAPTER 3. Section B. Part 2. Holding on page 3-45** for details on parameter holding times (*i.e.*, metals, nutrients, unfixed containers, fecal).

### **Documentation**

See **CHAPTER 3. Section B. Part 2. Documentation on page 3-46** 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 in **CHAPTER 14. Section A. Blanks and Duplicates on page 14-1.**



## **CHAPTER 13. CONTINUOUS WATER QUALITY MONITORING PROCEDURES**

### ***Overview of Continuous Water Quality Monitoring Program***

The Continuous Water Quality Monitoring Program's purpose is to provide short and long term hourly water quality data in order to understand trends, treatments, changes, and extremes in water chemistry that would be difficult to observe through individual sampling efforts. Deployable meters (continuous monitors or sondes) generally collect the same parameters gathered during a typical WAB discrete water sample (pH, Temperature, Dissolved Oxygen, & Specific Conductance) but records these parameters at a set time interval (e.g., hourly) for the duration of the deployment. Typical study subjects since the program's inception in 2005 include: delineating the efficacy of limestone treatments to acid deposition streams, quantification of daily pH and DO swings in Potomac drainage streams susceptible to periodic unexplained fish kills, recording treatment effects and pH extremes in acid mine drainage streams, studying specific conductance trends in streams affected by resource extraction, and exploratory monitoring of runoff effects. Other WVDEP divisions and State and Federal agencies are frequent cooperators in continuous monitoring efforts and are indispensable partners in collection of these data.

USEPA. 2014. Best Practices for Continuous Monitoring of Temperature and Flow in Wadeable Streams. Global Change Research Program, National Center for Environmental Assessment, Washington, DC. EPA/600/R-13/170. Available online at: [http://www.fs.fed.us/rm/pubs\\_other/rmrs\\_2014\\_stamp\\_i001.pdf](http://www.fs.fed.us/rm/pubs_other/rmrs_2014_stamp_i001.pdf)

### ***Instructions for Establishing/Maintaining the Deployable Site (Including Setting up the Site, Site Documentation, and Guidelines for Completing the Forms)***

The following is a description of how to establish continuous monitoring sites and use the Deployable Monitoring Form to record parameters of interest. Many of the procedures required to conduct this assessment have already been described in other Chapters of the SOP as detailed below:

**CHAPTER 2. INSTRUCTIONS FOR ASSESSING THE STREAM SITE (INCLUDING SETTING UP THE SITE, SITE DOCUMENTATION, AND GUIDELINES FOR COMPLETING THE STREAM ASSESSMENT FORMS) Section C. Guidelines for Completing the Stream Assessment Forms on page 2-28**

**CHAPTER 3. WATER COLLECTION PROTOCOLS starting on page 3-1**

## CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS starting on page 4-1

### *Methods and Procedures*

#### **Section A. Establishing a Site**

Site selection generally begins with a broad study location (e.g., mouth of Sovern Run, or upstream of McClintock Run) or periodically with specific coordinates around which a site must be established. Site selection requires addressing several needs in order to obtain accurate data. A serviceable site should provide enough depth to completely cover the sonde (keeping potential changes in depth in mind), adequate flow to keep the sonde free of sediment and debris, a location for the sonde to blend into the surrounding substrate or be camouflaged into it, and a strong/properly positioned (with respect to depth, flow, and concealment) anchor point. Failure to meet these requirements subjects the data collected to preventable error and the equipment to theft or loss. Upon arrival at the location, the requirements above must be considered when determining the specific point to deploy the continuous monitor.

#### **Part 1. Accessing Site**

Since the Continuous Water Quality Monitoring Program generally has a measure of flexibility in establishing site locations, the majority of the sites should be safely accessible. However, in some cases, due to the remoteness of some sites or access points, travelling to the sample site may require strenuous hikes 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 site. If you feel it is too difficult (e.g., too far to hike) or dangerous (e.g., steep banks) to get to the site, do not attempt it. Discuss it with other personnel who may be willing to try to get the site later.

**WARNING: DO NOT NAVIGATE TO ANY ASSESSMENT SITE THAT PRESENTS A DANGEROUS SITUATION TO YOU OR ANOTHER TEAM MEMBER!**

#### **Part 2. Locating Established X-Site**

When returning to an established site, GPS units should be primarily used to get into the general same stream reach (100 m) previously visited and to confirm the index latitude and longitude that is provided on the list for each index station. If the GPS coordinates and the given index coordinates differ by more than a couple of seconds, re-check your position.

***IMPORTANT:*** *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.*

Once the general stream reach has been located, the exact deployment location should be located by consulting the stream reach drawings from previous installations

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:*** *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.*

***IMPORTANT:*** *Be aware that 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.

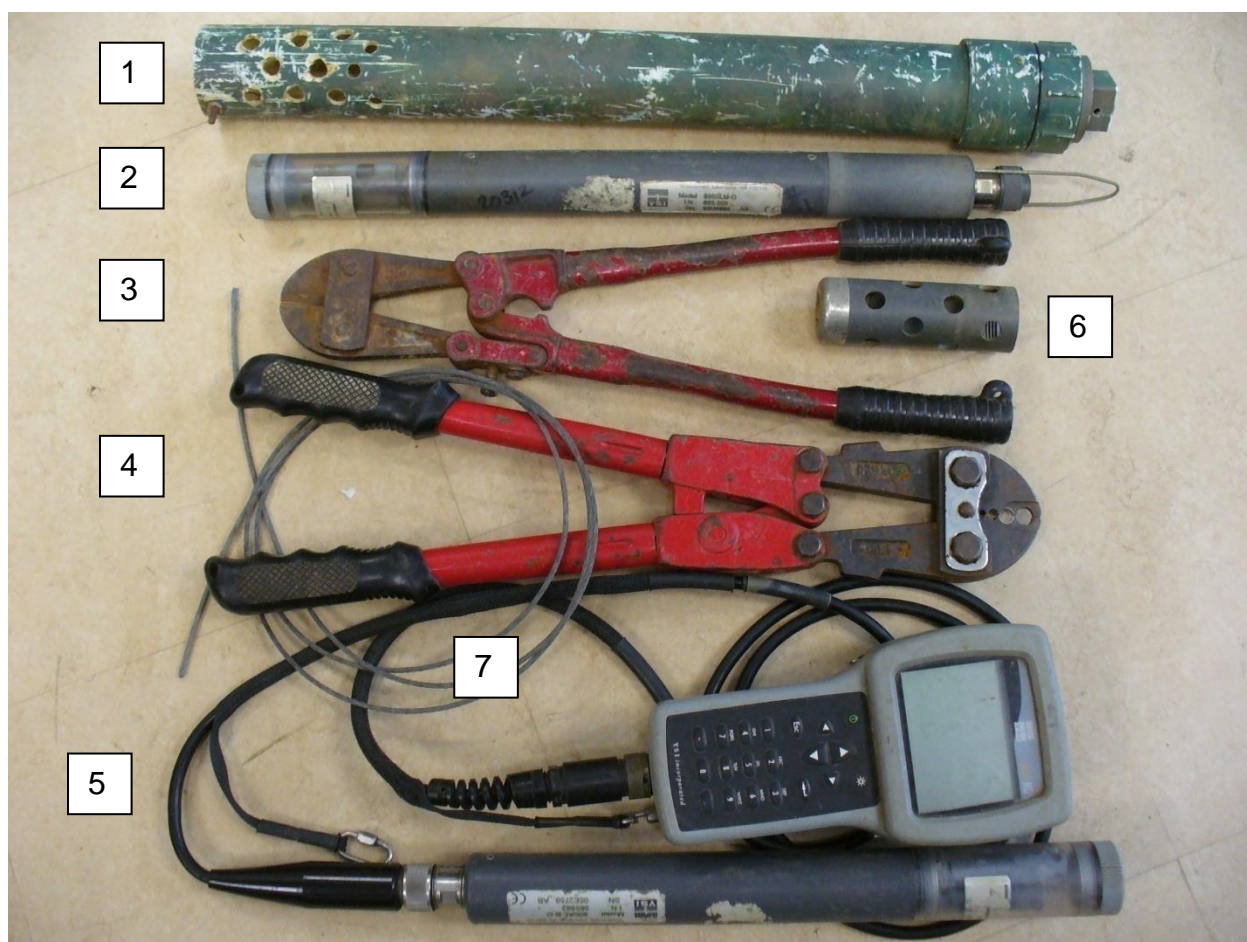
After the X-site has been confirmed (or located via best professional judgment), establish the station.

***IMPORTANT:*** *Collect all physicochemical, water samples, and GPS coordinates at the X site.*

### Part 3. Equipment

The items required for deploying the continuous monitor are pictured in **Figure 13-1 below** and include:

1. Protective Deployable Sonde Case and Cap
2. Deployable Water Quality Meter
3. Cable Cutters
4. Swaging Tool
5. Field Chemistry Multimeter (discrete meter)
6. Deployable Water Quality Meter Guard
7. 1/8" Galvanized Cable
8. 1/8" Ferrules (not pictured)



**Figure 13-1. Items Required for Deploying the Continuous Monitor**

## **Section B. Site Documentation**

### **Part 1. Deployable Coordinates and Global Positioning Systems (GPS)**

Basic guidance on how to use a GPS to document a site can be found in **CHAPTER 2. Section B. Part 1. Coordinates and Global Positioning Systems (GPS) on page 2-20.**

Coordinates must be taken on the initial visit and on any subsequent visits during which the sonde is relocated. **Table 2-2 on page 2-21** outlines some typical frequency of GPS readings for various sample types.

### **Part 2. Deployable Photographic Documentation**

Basic guidance on how to use a camera to document a site can be found in **CHAPTER 2. Section B. Part 2. Photographic Documentation on page 2-24.**

Specifically for continuous monitoring, a minimum of the following are needed from each site to aid in relocating the site if necessary:

- ◆ View upstream from X site
- ◆ View downstream from X site
- ◆ Location of anchor point in wide angle picture with landmark (if available)

In addition, pictures of such items as the following may be useful:

- ◆ Surrounding bridges/roads
- ◆ Close-up of anchor point and sonde location
- ◆ Potential sources of error (sediment, sludge, precipitates) on or around sonde
- ◆ Waterfowl or other wildlife
- ◆ Scenic Views
- ◆ Field crews at work
- ◆ Distinctive views of target streams, buildings, industry or dams along target stream, 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 12-16**).

## **Section C. Form Completion**

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 Deployable Sonde Form have already been addressed in **CHAPTER 2. Section C. Guidelines for Completing the Stream Assessment Forms found on page 2-28.**

The parameters that have already been addressed above will not be described here unless they vary in some way on the Deployable Sonde Form.

What is presented here explains what is found on the Deployable Sonde Form that is unique to Deployables and not found on other forms (e.g., 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.

## Description of Deployable Sonde Form

The Deployable Sonde Form differs only slightly from other general water quality forms. There are two sections of the form that are unique. Instructions for completing those portions are as follows.

### PAGE 1

For a description of parameters and values not seen below, **see CHAPTER 2. Section C. Part 1. PAGE 1-Site Verification on page 2-30 for an example.**

#### Deployable Site Verification

Visit Type: At the top of PAGE 1 (**see Figure 13-2 below**) of the form, there is a check box to indicate the Visit Type that the sampler is performing. One or more boxes will need to be checked to show the purpose of the visit:

Deployable Sonde Form					
Visit Type:	<input type="checkbox"/> Installation	<input type="checkbox"/> Retrieval	<input type="checkbox"/> Re-Deployment	<input type="checkbox"/> Final Retrieval	<input type="checkbox"/> Other:

Figure 13-2. Example of the Deployable Visit Type on PAGE 1 of the Deployable Sonde Form

#### 1. Installation

The Installation box should be marked only when a deployable is being placed in a new location that has not previously been established as a deployable study site. It is not appropriate to check any other boxes during a sonde installation. An installation includes relocating the sonde within the general 100 m reach and should always be accompanied by a new drawing of the specific deployment location and new GPS coordinates.

#### 2. Retrieval

This box refers to any visit during which a previously installed deployable is being recovered for calibration, cleaning, downloading, *etc.* Even if the same sonde is returned to the stream for further data collection, it is still classified as a retrieval visit. Generally, this box is selected with the next one (Re-Deployment) also selected. The only time this would not be the case is when a subsequent trip will be made to deploy a fresh sonde or when the sonde is being temporarily pulled due to seasonal changes (*i.e.*, stream is too low or dry to deploy sonde for the next month) but will be redeployed when desired study conditions reoccur.



**3. Re-Deployment**

The purpose of this box is to indicate any visit in which a fresh sonde is deployed to a previously established site. On many occasions this box will be indicated along with Retrieval box.

**4. Final Retrieval**

Select this box if the visit is to terminate further foreseeable study at that location. No other boxes should be marked when this one is marked. This includes seasonal removals of equipment (e.g., pulling the sonde for the winter months).

**5. Other**

Use this box when none of the above applies. The most common example would be a mid-deployment visit to the station to obtain an additional discrete sample to help later on with data correction (e.g., Discrete Check).

**PAGE 2**

**Discrete Sonde Info**

This unique section of the Deployable Sonde Form is located at the top left of PAGE 2 (see **highlighted area in Figure 13-3 below**). It is similar to the regular Sonde Physicochemical Parameters section on other forms. However, the discrete sonde readings are recorded twice: 1) side-by-side with the deployable sonde at the time of retrieval and 2) side-by-side with the deployable sonde at the time of deployment of a new or serviced sonde. Since this will occur at two separate times, there is additional space to record the exact time of each reading.

Sonde Method <input type="checkbox"/> Grab <input type="checkbox"/> Sample Tube <input type="checkbox"/> Bucket				Lab Water Method <input type="checkbox"/> Grab <input type="checkbox"/> Sample Tube <input type="checkbox"/> Bucket				
Discrete Sonde I.D.				Seasonal Water Level	Water Odors	Surface "Oils"	Turbidity	
Flag	Retrieval Discrete	Physicochemical Parameters	Deploy Discrete	Flag	Below Normal	Normal	None	Clear
		Discrete/QA Time			Normal	Sewage (Not Septic)	Flecks	Slightly Turbid
		Temperature °C		Above Normal	Petroleum	Sheen	Moderately Turbid	
		pH (std. Units)		Flooding	Chemical	Globs	Highly Turbid	
		Dissolved Oxygen (mg/L)		Notes:	Anaerobic (septic)	Slick	Water color:	
		Conductivity (µmhos/cm)			Other:			
		WQ Sample ID			Foam/Suds (Rate 0-4 or NR)			
If any problems occur with the Water Meter or any readings are suspect, record notes in the space to the right.								
ABOVE: Record readings in box for corresponding physicochemical parameter. Insert a 'v' in the box for other categories.								

**Figure 13-3. Example of the Discrete Sonde Info Section on the Top of PAGE 2 of the Deployable Sonde Form**

**Deployment Sonde Info**

The other unique section of the Deployable Sonde Form is located at the bottom of PAGE 2 (*see Figure 13-4 below*). This portion of the form holds positional, programming, and Quality Control data about the deployable sonde.

Deployable Location		<input type="checkbox"/> Mid-Stream <input type="checkbox"/> Bank ( <input type="checkbox"/> Left <input type="checkbox"/> Right) <input type="checkbox"/> Thalweg ( <input type="checkbox"/> Left <input type="checkbox"/> Middle <input type="checkbox"/> Right) <input type="checkbox"/> Left Channel <input type="checkbox"/> Right Channel <input type="checkbox"/> Other:					
Flag	Retrieved Sonde	Physicochemical Parameters		Deployed Sonde	Flag	Target Deployment End Date	
		Deployable Sonde ID				File Name	
		Temperature °C			Deployable Notes & Comments:		
		pH (std. Units)					
		Dissolved Oxygen (mg/L)					
		Conductivity (µmhos/cm)					
		Rate the Following where 0=None, 1=Slight, 2=Moderate, 3=Heavy, 4=Extreme)					
	Sediment on/in Sonde Case	0	1	2	3	4	
	Biofilms on Sonde	0	1	2	3	4	
	Metals/Precipitate on Sonde	0	1	2	3	4	

Figure 13-4. Example of the Deployment Info Section on the Bottom of PAGE 2 of the Deployable

**Deployable Location:** Indicate the cross-sectional location of the deployable sonde: 1) **Mid-Stream**, 2) **Bank** (Left or Right), 3) **Thalweg** (Left, Middle, or Right), 4) **Left Channel**, 5) **Right Channel**, 6) **Other** (please describe).

**Deployable Sonde ID:** Record the retrieved and deployed sonde instrument identification numbers. This is usually marked on the sonde with a black sharpie. 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.

**Target Deployment End Date:** An estimation of the next date the site will be visited for retrieval/redeployment/final retrieval (typically 30 days after deployment).

**File Name:** The name given to the file during programming that will be downloaded upon retrieval of the sonde. Make sure the file name matches exactly the programmed name to ensure future searches and cross checks will be less confusing and less subject to error.

**Retrieved/Deployed Sonde Parameter Check:** This space is for recording the side-by-side values for each of the physicochemical parameters indicated on the form from the retrieved and/or deployed sondes. This requires attaching the deployable sonde to a handheld display or laptop and gathering a discrete measurement from it at the same time as the discrete sonde side-by-side in-stream. This data should be paired with the

discrete sonde readings (*see highlighted area in Figure 13-3 on page 13-7*) at the same exact time.

Sediment in/on Sonde Case: Rate the amount of sediment inside or covering the sonde based on the following scale: **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, or NR-Not Rated**. This is important to evaluate and note before disturbing the sonde during retrieval since sediment can clog the sonde ports and prevent accurate readings.

Biofilms on Sonde: Rate the amount of biofilms (e.g., periphyton, algae, etc.) on the sonde based on the following scale: **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, or NR-Not Rated**. This is important to concentrate on the individual sensors since biofilms can impede accurate measurements and cause drift in the readings.

Metals/Precipitate on Sonde: Rate the amount of metal hydroxides or other precipitates (e.g., Iron Hydroxide, Calcite deposits, etc.) on the sonde based on the following scale: **0-None, 1-Low, 2-Moderate, 3-High, 4-Extreme, or NR-Not Rated**. This is important to concentrate on the individual sensors since these precipitates can impede accurate measurements and cause drift in the readings.

Deployable Notes & Comments: This space is for recording notes that pertain to any conditions observed when retrieving/deploying the meter that may be reflected in the data file such as extreme sediment in sonde case, biofilm on the sonde probes, or sonde washed out of water column upon retrieval. Describe any factors that might affect the accuracy of the data contained on the sonde or any possible explanations for differences in discrete and side by side measures.

## **Section D. Deployable Sonde Operation (Calibration, Programing, and Downloading Protocols)**

### **Part 1. Sonde Calibration Procedures**

The instructions below refer to *Figure 3-3. Example of Sonde Calibration Log Sheet on page 3-5*.

***IMPORTANT:*** *Since all of the readings being calibrated are temperature corrected, it is very important to use room temperature DI or distilled water and calibration solutions when available. The pH solutions can be corrected for using the graphs found on pages 3-19 to 3-21.*

### **YSI Deployables**

YSI sondes may be calibrated using either a compatible YSI Display Unit or the computer via the YSI **Ecowatch software**. Refer to **Section A. Water Quality Sondes & Sensors: Calibration, Routine Maintenance, & Use** starting on *page 3-1* for instructions on the calibration of the YSI deployables using a compatible Display Unit.

Also note that the data ports on the YSI deployable sondes are protected by a gray plastic screw cap which must be removed before connecting the sonde to either a computer or display unit.

Refer to **Part 2. Sonde Programming Procedures-YSI Deployables on page 13-14** for directions on how to program the YSI sonde for deployment.

### **Hydrolab Deployables**

Hydras 3LT software is required to operate Hydrolab brand sondes. If the software is not present, contact the help desk to request installation or use a computer that does have the program. **MAKE SURE THE PROBES ARE CLEAN BEFORE CALIBRATING THE SONDE.** Steps for programming the sonde for deployment are as follows:

1. Remove the rubberized cover over the data port and connect the sonde to the Hydrolab- USB adapter cable and plug into USB port of computer. When plugged in the sonde should emit a 1-2 second monotone beep signaling the unit is powering up. If this does not occur, replace the batteries and perform Step 1 again.
2. Initiate the **Hydras 3LT software** and it will automatically search for any connected sondes.

**NOTE: It is likely that the software will display in German, not English. Press F3 key to switch language to English.**

Upon locating the sonde, the port and sonde name/number will appear in the top box. To the right of this box are 3 gray buttons, select the middle **Operate Sonde** button.

3. Select the **Calibration tab** to open up the calibration interface. Eleven tabs will appear, begin calibration with the **SpCond [µS/cm] tab**. Make certain that calibration is done in µS/cm not mS/cm, as these tabs are adjacent to one another. Open the calibration cup and rinse the cup with DI or distilled water two times, watching the **Current Value** just below the tabs in the left corner ensuring that the values stay near 0, indicating a well rinsed probe. Empty the cup and dry the probes with a paper towel. Type 0 into the white **SpCond [µS/cm]** box above the Calibrate and Reset buttons. Record the **Current Value** as **Initial Sp Cond** on a Calibration Log Sheet and press the **Calibrate** button. Record the **Final Sp Cond** value from **Current Value**. Rinse the calibration cup two times with 1000 µS/cm standard solution and fill the cup above the stirring assembly with the standard. Type 1000 into the white **SpCond [µS/cm]** box above the Calibrate and Reset buttons. Record the **Current Value** as **Initial Sp Cond** on the Calibration Log Sheet and press the **Calibrate** button. Record the final value from **Current Value** on the Calibration Log Sheet as **Final Sp Cond**. Do not pour out standard solution, as it can be used to calibrate the next parameter.

**NOTE:** The Calibration Log Sheet does not have a location for the separate 0 and 1000  $\mu\text{S}/\text{cm}$  initial and final calibration values. Record this on the sheet as 0/1000.

4. Press the **LDO% [Sat] tab**. Rinse the calibration cup 2 times with room temperature DI or Distilled Water. Shake the DI/DW water vigorously for 45 seconds in order to thoroughly aerate the liquid. Fill the calibration cup over the LDO probe and place the lid lightly back on the calibration cup. Let the LDO probe equilibrate for several minutes, or until readings in **Current Value** stabilize. Determine the Barometric Pressure (mmHg) from an YSI unit (which has a built in barometer) or a functioning Lab Barometer and enter the Barometric Pressure in the **LDO\_BP [mmHg]** box. Record the **Current Value** as **Initial % Sat** and the **Atmospheric Pressure (mm Hg)** on the Calibration Log Sheet and press the **Calibrate** button. Record the final value from **Current Value** as **Final % Sat** on the Calibration Log Sheet. Empty the contents of the calibration cup.
5. Click the **pH [Units] tab**. The pH calibration is set up as a 2 point calibration; pH 7 will always be the first calibration standard followed by pH 10 or pH 4 depending on the expected pH range of the stream in which the sonde will be deployed. Use pH 10 buffer if the receiving stream is typically basic or use pH 4 if the stream is typically acidic. Rinse the calibration cup two times with pH 7 standard solution and fill the cup above the stirring assembly with the standard. Let the solution sit for approximately 2 minutes to allow for the temperature to adjust. Type 7 into the white **pH [Units]** box above the Calibrate and Reset buttons. Record the **Current Value** as **Initial pH (7)** on the Calibration Log Sheet and press the Calibrate button. Record the final value from **Current Value** on the Calibration Log Sheet as **Final pH (7)**. Rinse the calibration cup two times with pH 4 or 10 standard solution (select appropriate standard) and fill the cup above the stirring assembly. Type 4 or 10 into the white **pH [Units]** box above the Calibrate and Reset buttons. Record the **Current Value** as **Initial pH (4) or (10)** on the Calibration Log Sheet and press the **Calibrate** button. Record the final value from **Current Value** on the Calibration Log Sheet as **Final pH (4) or (10)**.

The sonde is now calibrated. Refer to **Part 2. Sonde Programming Procedures-Hydrolab Deployables** on **page 13-15** for directions on how to program the Hydrolab sonde for deployment.

### **Eureka Manta Deployables**

Eureka Multiprobe Manager software is required to operate Eureka brand sondes, if the software is not present, contact the help desk to request installation or use a system that does have the program. **MAKE SURE THE PROBES ARE CLEAN BEFORE CALIBRATING THE SONDE.** Steps for calibrating the Manta sonde are as follows:

1. Remove the black plug from the end of the Manta by unscrewing the bolt and pulling the plug out, thus exposing the data port.
2. Connect the sonde to the Manta-USB adapter cable and plug into USB port of computer.
3. Initiate the **Multiprobe Manager software** and it will automatically search for any connected sondes. Upon locating the sonde the program will automatically start downloading the data from the currently running file name. (If there is no new data found on the probe, then a screen showing the current parameters will start cycling).
4. Click on the **Calibration** button, 3<sup>rd</sup> button down in the left column. The window will change to the **Calibration** window. In the middle of the window there will be a scroll down box with Sensor (parameters) list in the left column and Calibrate buttons running down the far right column.
5. Start the calibration by rinsing the probes with clean water. In the **scroll down** box, find the **Sensor DO %Sat** and click on the corresponding **Calibrate** button. The window will change to three line graphs of the real-time values for %Sat DO, Temperature, and Conductivity. Rinse the probes two times with room temperature DI/DW water. Shake the DI/DW water container vigorously for 45 seconds in order to thoroughly aerate the liquid then fill the calibration cup above the tip of the ODO probe. Replace the cap lightly on top of the calibration cup. Click on the **Start** button on the lower-left side of the window. The window will show refreshed line graphs of the above mentioned parameters. Enter the **Barometric Pressure (mmHg)** in the box below the line graphs. This can be determined from an YSI unit (which has a built in barometer) or a functioning lab barometer. Wait for the %Sat DO line graph to stabilize, record the %Sat DO value as **Initial % Sat** and the **Atmospheric Pressure (mm Hg)** on the Calibration Log Sheet. Click on the **Calibrate** button on the lower-left side of the window. The line graphs will refresh. Click the **Finish** button in the lower-left corner to return to the **Calibration** window. No final DO value is provided by program, must be recorded before clicking **Finish**.
6. In the **scroll down** box, find the **Sensor SC (us/cm)** (Specific Conductance) and click on the corresponding **Calibrate** button. This will initiate two line graphs of real-time values for Conductivity and Temperature. Rinse the calibration cup two times with DI or distilled water and fill until DI or distilled water is covering all probes. Specific Conductance should read less than 5 us/cm. Record the Specific Conductance value as the **Low End Check Sp Cond (µmhos/cm)** on the Calibration Log Sheet. If the value is greater than 5 us/cm, clean the probe with a wire brush and repeat the low-end check. If the probe still fails to read less than 5 us/cm, it is likely malfunctioning and will need to be sent to Eureka for maintenance. After completing a successful low end check, proceed with the calibration procedure. Empty the DI or distilled water and dry the probes with a



paper towel. Rinse the probes two times with 1,000 us/cm standard solution. Fill the calibration cup with standard solution ensuring all probes are fully covered. Click on the **Start** button on the lower-left side of the window. The window will show refreshed line graphs of the above mentioned parameters. Wait for the Specific Conductance line graph to stabilize, record the **Initial Specific Conductance** value as **Initial Sp Cond ( $\mu\text{mhos/cm}$ )** on the Calibration Log Sheet. Click on the **Calibrate** button on the lower-left side of the window. The line graphs will refresh, record the **Final Specific Conductance** value as **Final Sp Cond ( $\mu\text{mhos/cm}$ )** on the Calibration Log Sheet. Click the **Finish** button in the lower-left corner to return to the Calibration window.

7. In the **scroll down** box, find the **Sensor pH** and click on the corresponding **Calibrate** button. The two line graphs of the real-time values for pH and Temperature will appear. Rinse the probes twice with pH 7 buffer and fill the calibration cup above all the probes. Click on the **Start** button on the lower-left side of the window. Refreshed line graphs will initiate. Just below the line graphs enter pH 7 into the **pH value** box. Once the pH line graph stabilizes record the **Initial pH value** as **Initial pH (7)** on the Calibration Log Sheet. Click on the **Calibrate** button on the lower-left side of the window. The line graphs will refresh, record the **Final pH value** as **Final pH (7)** on the Calibration Log Sheet. A window will prompt that the next pH solution should be added. Use pH 10 buffer if the receiving stream is typically basic or use pH 4 if the stream is typically acidic. Rinse the probes two times with appropriate pH buffer and fill the calibration cup. Click on the **Start** button on the lower-left side of the window. Refreshed line graphs will appear. Below the line graphs enter the **pH value of the buffer** used. Once the pH line graph stabilizes record the **Initial pH value** as **Initial pH (4) or (10)** on the Calibration Log Sheet. Click on the **Calibrate** button on the lower-left side of the window. The line graphs will refresh, record the **Final pH value** as **Final pH (4) or (10)** on the Calibration Log Sheet. The next window will present three buttons. Click on the **Calibrate** button to end 2 point calibration, **Continue** and repeat the process used for the other two buffers to perform a 3 point calibration (2 point calibration is the preferred method). Record the **Sensor Response Factor** on the Calibration Log Sheet. Click the **Finish** button in the lower-left corner to return to the Calibration window.

The sonde is now calibrated. Replace the plug with the end with the hole toward the circle and replace the screw (this is the logging off position) or continue to **Part 2. Sonde Programming Procedures-Eureka Manta Deployables on page 13-17** for directions on how to program the Eureka sonde for deployment.

### **HOBO - Temperature/Light Intensity & Temperature/Conductivity Loggers**

HOBO (from Onset Comp.) data loggers do not require calibration. Therefore, it is especially important to have a complete set of discrete readings to attempt to document any potential drift. Proceed to **Part 2. Sonde Programming Procedures-HOBO Temperature/Conductivity & Temperature/Light Intensity Loggers on page 13-18**.

## Part 2. Sonde Programming Procedures

### General

All sondes are typically programmed for hourly deployment for a minimum of 30 days. It is advisable to program the meter to run for at least as long as the battery will sustain data collection (around 90 days for YSI and 60 days for Hydrolab). In the event that water level or other priorities prevent timely retrieval of the meter from the site, data will continue to be collected until retrieval is possible. Upon initiating a program, the current date and time need to be verified as correct and the site name must be set up. Site names need to be unique to the site location with the date of the data file within the name. For example, Sovern Run for deployment on May 03, 2010 would be **SOVR0510** for an YSI and **Sovern Run 05032010** for a Hydrolab, HOB0, or Manta. It is important to attempt to maintain the same site name, changing the date only for subsequent deployments. This assists in maintaining neat and orderly collections of data files. Water quality parameters need to be checked to ensure that the file will collect data for all desired parameters using the correct units. Additionally, the programmer must ensure that all previous programs have been terminated before initiating a new file. Some brands will allow multiple files to record concurrently, leading to potential confusion during data analysis.

### YSI Deployables

Ecowatch software is required to operate YSI brand sondes, if the software is not present, contact the help desk to request installation or use a system that has the program installed. Steps for programming the sonde for deployment are as follows:

1. Connect the sonde to the YSI-USB adapter cable and plug into USB port of computer.
2. Open the **Ecowatch software** (if not already open). Single-click the **Sonde Icon** (6 over from the left) on the top bar. A **Select COM Port** window will open, select the **port the USB is connected to** and click the **OK** button. This will open the **Sonde Programming Window**. If this window does not open, no connection is occurring and the issue likely lies with the adapter cable.
3. Within the Sonde Programming Window, a **#** sign or prompt should be present in the top left hand corner. Type **menu** beside the **#** sign to begin programming. This will bring up the **Main Programming Menu**.
4. Select **4** from the Main Menu to bring up the **Status Screen**. Check that the date and time are correct. To edit these, select **2** for **Date** or **3** for **Time** and type in the correct value then **press enter**. Also ensure that the Battery volts are above 5.7 (always deploy a sonde with new batteries) and that Logging is inactive. When all changes have been completed, type **0** or **press escape** to go

backwards to the **Main Menu** (these commands will always take you backwards in any menu screen).

5. Type **1** at the Main Menu to enter the **Run Setup Screen**. Type **2** for **Unattended Sample**. This will bring initiate the **Unattended Setup Screen**. Check that **1-Interval** is set to the correct logging interval, typically **01:00:00 or 1 hour**. The start date and start time default to the day of programming and the beginning of the next hour (for hourly logging). Logging can be postponed by changing these dates/times in the same manner as in the Status Screen (2 for Date; 3 for Time). Postponing the start of logging is advisable if the sondes will not be deployed immediately (within the next 24-48 hours) as it will save some battery life. Check **4 for Duration days** to ensure that the period of deployment is covered plus a window to allow the sonde to continue recording if it is not retrieved when scheduled. Most sondes are set at 90 days.
6. Type **5** and **enter a file name** according to the naming convention described in the section above, then press enter. YSI sondes only allow 8 characters for this field. North Fork of Cranberry River 7/12/10 would need to be recognizable in 4 characters with 4 characters for the date: NFCR0710. Type **6** then **enter the same site designation** and **press enter**. Check that lines 5 and 6 read the same, both in caps.

***IMPORTANT: If you are deploying at the first of the month and anticipate redeploying at the end of the same month, you will need to amend the file name in some manner so that you can distinguish the two files. For example, if you are deploying into the North Fork of Cranberry River on 7/1/11 and 7/31/11 you may use NFCR0711 for the first deployment in July and either NFC0711B (B for the second deployment) or NFCR0811 (indicating August, the next day).***

7. Type **C – Start logging** to initiate the logging program. This will initiate the Start logging screen asking for a confirmation, type **1** to **begin the program**. The **Logging Screen** will open, where all parameters should be read to ensure no errors exist and the program has a start and end time/date.
8. Type **0** two times to return to the **Main Menu**. Type **4** to enter the **Status Screen**, review all entries again paying particular attention to line 7-Logging, it should read “Active.” If it does not, the program has not been initiated correctly. Check that Step 7 was completed correctly, if so, return to Step 4 and redo the entire programming process. When complete, disconnect the sonde and screw the end cap back on the meter and it is ready for deployment.

### **Hydrolab Deployables**

Hydras 3LT software is required to operate Hydrolab brand sondes, if it is not installed on the computer to be used, contact the help desk to request installation or use a

system that does have the program. Steps for programming the sonde for deployment are as follows:

1. Remove the rubberized cover over the data port and connect the sonde to the Hydrolab-USB adapter cable and plug into USB port of computer. When plugged in the sonde should emit a 1-2 second monotone beep signaling the unit is powering up. If this does not occur, replace the batteries and perform Step 1 again.
2. Initiate the **Hydras 3LT software** and it will automatically search for any connected sondes. Upon locating the sonde, the port, and sonde name/number will appear in the top box. To the right of this box are 3 gray buttons, select the middle **Operate Sonde** button.
3. A second, multi-tabbed Hydrolab window will appear with the System tap selected. Check the date and time to ensure they are accurate. If inaccuracies exist, use the **Set clock to PC** button or **Set clock manually** button to remedy the problem(s). Click the **Log Files tab** at the top of the window.
4. Hydrolab sondes only hold 4 log programs at a time. In order to start a new log a check must be performed to determine if any free space exists for a new program. Click the **drop down** box at the top of the screen beside the Log File text and count the number of files listed. If four are there, highlight one (preferably the oldest) of the files then press the **Delete** button at the bottom of the window. If a free spot exists a new log file can be created. **Make sure that the file has definitely already been downloaded before deleting it.**
5. Click the **Create** button at the bottom of the window. An **Enter new file name** box will appear, **fill in the file name** according to the naming convention outlined in the **General** section on **page 13-14** and click the OK button.
6. Enter the **Start Logging** and **Stop Logging** date and times using the boxes in the middle of the window. Be sure to make all of the times on the hour and to set the **deployment period** for at least 60 days so data will continue to be gathered if a sonde is not retrieved in exactly 30 days.
7. Set the **logging interval**, typically 01:00:00 or one hour. Make the sensor and circulator **warm up** 2 minutes and **uncheck the Audio check** box beside circulator warm-up.
8. The bottom portion of the box covers parameter selection. Each project has different parameter needs, but in general, you want to gather every parameter of pertinence that can be accurately measured. This doesn't include unit conversions. The basic set of parameters that should be gathered for a typical Hydrolab deployment are: Temp (C), pH, SpCond ( $\mu\text{s}/\text{cm}$ ), LDO%, LDO (mg/L) and Internal Battery (Volts & %). **Select the parameters** by highlighting them in

the box and clicking the **add** button. Any inadvertent additions are easily removed by highlighting and clicking the **Delete** button. *Be sure to use the SpCond ( $\mu\text{S}/\text{cm}$ ) and not the SpCond ( $\text{mS}/\text{cm}$ ), as the latter will round any values to whole numbers, leaving valuable data unrecorded.* Check the Parameters in Log File box and ensure they are correct.

9. Click the **Save Settings** button at the top right of the window. Read over all logging program information and click the **Enable** button at the bottom of the window to initiate the program. Check the Status line (third line down with a blue count down until start box beside it) and ensure it says "Enabled." When complete, disconnect the sonde and place the rubberized cover over the data port and it is ready for deployment.

### **Eureka Manta Deployables**

Eureka Multiprobe Manager software is required to operate Manta brand sondes, if it is not installed on the computer to be used, contact the help desk to request installation or use a system that does have the program. Steps for programming the sonde for deployment are as follows:

1. Remove the black plug from the end of the Manta by unscrewing the bolt and pulling the plug out, thus exposing the data port.
2. Connect the sonde to the Manta-USB adapter cable and plug into USB port of computer.
3. Initiate the **Multiprobe Manager software** and it will automatically search for any connected sondes. Upon locating the sonde the program will automatically start downloading the data from the currently running file name. If there is no new data found on the probe, then a screen showing the current parameters will start cycling.
4. Once the file has been downloaded, the program will automatically show the **Logged Data page** in the window. This window contains a list of all the downloaded files from that sonde.
5. To set up a new logging profile, click on the **Logging Profile** button, 4<sup>th</sup> from the left at the bottom of the window. This will open the Logging Profile Window. Type the filename of the new data set in the box under the **Description Box** according to the naming convention outlined in the **General** section on **page 13-14**. Next, make sure the **Reading Interval** box is set to 1 hour and the **Warm-up** box is set for 2 minutes. Finally, click the **Set Profile** button to save the changes of the new file name.
6. Now, click on the **Calibration** button, 3<sup>rd</sup> button down in the left column. This will open the **Calibration** window. On the lower-left corner of the window, there will

be an area titled Real-time Clock. Within this area, click the button that reads **Sync w/ PC**. This will change the time on the sonde to that of the computer to which the sonde is connected.

7. Close the program and disconnect the cord from the sonde. Replace the plug with the end with the hole toward the circle and replace the screw (this is the logging off position). In order to **initiate the program**, remove the screw and turn/slide the black plug to the line and away from the circle (logging on position). Check for the **orange and green lights** on the board inside the meter to begin flashing alternately. Once the flashing occurs, **replace the screw** into the plug in its new position and the sonde is ready for deployment.

### **HOBO Temperature/Conductivity & Temperature/Light Intensity Loggers**

HOBOWare Pro software is required to operate HOBO brand loggers, if it is not installed on the computer to be used, contact the help desk to request installation or use a system/setup that does have the program. Steps for programming the sonde for deployment are as follows:

1. Connect the HOBO Optic USB Base Station or Pendant Coupler to the USB port of the computer.
2. Initiate the **HOBOWare Pro software** and click the **Launch Device Icon**, located in the top left of the window under **File**. If a file is currently running it must be stopped and readout or any currently recording file and its corresponding data will be erased.
3. A **Launch Logger** window will appear. Ensure that the battery state is adequate for the deployment. **Type the Site name and deployment date** in the description box according to the naming convention outlined in the **General** section on **page 13-14** (e.g., Coal Fk 031411).
4. Check all appropriate parameters to be collected in the **Channels to Log** box.
  - a. For Temperature/Conductivity loggers choose: Conductivity Low Range, Full Range, and Temperature.
  - b. For Temperature/Light Intensity loggers choose: Temperature and Battery Power Remaining. The WAB does not typically collect light intensity data.
5. Under logging interval, use the boxes to enter the **interval between logging events or data points**. One hour is the default interval to be employed unless another interval is specified for the project.
6. Within the **Launch Options** box, select the start time, ensuring that initiation of the program will occur before the meter is installed into the stream and will not



run for multiple days before deployment if possible. **All files should start on the hour.**

7. Click the **Launch** button in the lower right hand corner to initiate program.

***IMPORTANT: Do not unplug logger until program upload is complete.***

The lower left-hand section of screen will show “Launch Successful” when it is safe to remove from base station. When the HOBOWare PRO window returns to normal, it is safe to unplug the sonde and it is ready for deployment.

### Part 3. Sonde Downloading Procedures

#### General

Downloading is typically a simple process, and will even occur automatically in some deployable brands upon connection to a computer running the appropriate software. Several things must be considered when retrieving data from the sondes. If no file or an attenuated file is on the retrieved meter, this must be noted on the field form with the probable cause of the data loss (water leakage into battery compartment, dead battery, critical sonde failure, etc.). Also, the program must be terminated to prevent further readings to be added to the file, creating potential confusion during data analysis. To prevent loss of data or confusion over file location, upon return from the field, the data file must then be placed on the network as soon at the following location:

Q:\WATER RESOURCES\WAB\DEPLOYABLE DATA\Raw Data

Files should also be left on the sondes until removal is required for new data collection programs (lack of memory space on the sonde). This ensures that for a short period of time any lost files can be retrieved from their sonde of origin.

#### YSI Deployables

Ecowatch software is required to operate YSI brand sondes, if it is not installed on the computer to be used, contact the help desk to request installation or use a system that has the program installed. Steps for downloading data from the sonde are as follows:

1. Perform Steps 1-4 from **Part 2. Sonde Programming Procedures-YSI Deployables** section on **page 13-14**.
2. Type **1** at the **Main Menu** to enter the **Run Setup Screen**. Type **2** for **Unattended Sample**. This will bring initiate the **Logging Screen**. Type **B** – Stop Logging to terminate the program then confirm by typing **1** on the **Stop Logging?** screen. Type **0** two times to return to the **Main Menu**.
3. Type **3** to enter the **File Menu**. Type **2** to enter the **Upload Menu**. Type the number/letter corresponding to the file to be downloaded. The most recent files

are at the bottom of the list. The number to the right of the filename is the number of data points on the file. An hourly file for 30 days should be in the range of 700-750 data points. Any apparent data loss should be documented on the field form.

4. After selecting the file to be downloaded, the **Time Window Screen** shows the start and end date/time of the logged file. Note any errors on the field form, then type **1-Proceed** to bring up the **File Type** screen. Type **1-PC6000** to initiate the download.
5. When the download completes, type **0** to return to the **Main Menu**. When complete, disconnect the sonde and screw the end cap back on the meter and it is ready for calibration and reassignment. Files are automatically downloaded to **C:\Ecowatch\Data** folder on the computer.

### **Hydrolab Deployables**

Hydras 3LT software is required to operate Hydrolab brand sondes, if it is not installed on the computer to be used, contact the help desk to request installation or use a system that does have the program. Steps for downloading data from the sonde are as follows:

1. Connect the sonde to the Hydrolab-USB adapter cable and plug into USB port of computer. When plugged in the sonde should emit a 1-2 second monotone beep signaling the unit is powering up. If this does not occur, replace the batteries and perform Step 1 again.
2. Initiate the **Hydras 3LT software** and it will automatically search for any connected sondes. Upon locating the sonde, the files available for download will appear in the **Log Files** box in the bottom half of the window. Check the files to be downloaded and click the **Download Selected Files** button.
3. When the file completes its download it will be located in the **C:\Program Files\HYDRAS3LT\LogFiles** folder on the computer.

### **Eureka Manta Deployables**

Eureka Multiprobe Manager software is required to operate Manta brand sondes, if it is not installed on the computer to be used, contact the help desk to request installation or use a system that does have the program. Steps for downloading data from the sonde are as follows:

1. Remove the black plug from the end of the Manta by unscrewing the bolt and pulling the plug out, thus exposing the data port.
2. Connect the sonde to the Manta-USB adapter cable and plug into USB port of computer.

3. Initiate the **Multiprobe Manager** software and it will automatically search for any connected sondes. Upon locating the sonde the program will automatically start downloading the data from the currently running file name.
4. Once the file has been downloaded, the program will automatically show the **Logged Data** page in the window. This window contains a list of all the downloaded files from that sonde.
5. Highlight the file names of the data set in which need to be saved (Press Ctrl Key if there is more than one file per data set, usually 2-3). Then, once highlighted, press the **Save Data** button, first button on the left at the bottom of the window. A **Save As** window will appear. Save file under the same name as it was named on the sonde.
6. To save a previously downloaded file (old files) from a sonde, perform Steps 2 and 3 above. The program will automatically start the **Real-time Data** window. Enter the **Logged Data** window by pressing the **Logged Data** button, 2<sup>nd</sup> button down in the left column. Follow steps 4-5 to save the downloaded file.

### **HOBO Conductivity Loggers**

HOBOWare Pro software is required to operate HOBO brand loggers, if it is not installed on the computer to be used, contact the help desk to request installation or use a system that does have the program. Steps for downloading data from the sonde are as follows:

1. Connect the HOBO Optic USB Base Station to the USB port of the computer.
2. Initiate the **HOBOWare Pro** software and click the **Stop Device** Icon, located in the top left of the window under View. Next click the **Readout Device** Icon, two icons to the left of the Stop Device icon.
3. The **Save** window will open, check the **Filename** and make it matches the data file name if it doesn't already. **Click Save**.
4. The **Plot Setup Window** will open. Click **Cancel** to exit and end download or click **Plot** to see a plot of Conductance and Temperature. The location the file will be saved in is **C:\Documents and Settings\{user name/number}\My Documents\HOBOWare** folder on the computer.

### **Section E. Sonde Anchoring and Concealment Procedures**

Anchoring and concealing the sonde is the most critical step in assuring that equipment is not lost due to high flows, theft, or being caught on passing debris. The most stable and concealed location possible should be selected for an anchor point. One must be

keenly aware of high and low flows and the reaction the sonde will have to each and to any moving debris when establishing an anchor point. Will the meter remain covered in flowing water if there is no rain during the 30 day deployment? What will happen to the sonde during a high flow, will it wash up on the bank and stay there or will it roll back into the water? Are there any large pieces of debris that could potentially be washed into the anchor during high flow? Is the anchor point sufficiently positioned to withstand extreme flows?

## Part 1. Anchor Techniques

The deployable should be fastened around a stable anchor point to secure the deployable and provide a stable position for gathering the desired data using a length of 1/8" galvanized cable and a 1/8" ferrule.

Create a loop as pictured in **Figure 13-5 on right** and use the swaging tool to swage the ferrule at least 2 times to attach to the anchor point.



Figure 13-5. Picture of typical anchor loop.



Figure 13-6. Picture of anchor cable looped directly thru the PVC sonde case.

In either case, loop the cable through the holes in the PVC sonde case and, when available, through the loop on the deployable (see **Figure 13-7 on right**).

After the cable attachment to an anchor point has been established, the case and end cap can be secured to the same piece of cable (see **Figure 13-6 on left**) or a loop can be fashioned at the other end for a semi-permanent anchor that can be reused on multiple site visits (see **Figure 13-9 on next page**).



Figure 13-7. Picture of cable looped thru PVC sonde case and sonde.



Figure 13-8. Picture of cable threaded thru PVC sonde case and then looped thru screw cap.



Figure 13-9. Picture of a loop used as a semi-permanent anchor interlocked with a temporary loop attached to the end of the sonde case.



Figure 13-10. Picture of semi-permanent anchor of fixed length in field.

Screw on the end cap (if available) and thread the cable through the hole in the cap as in **Figure 13-8 on left**.

In order to attach to the same cable, loop around the end and swag the ferrule to the anchor cable as in **Figure 13-6 on the previous page**.

In order to attach to a semi-permanent anchor of fixed length (**as in Figure 13-10 below**), attach the cable to the PVC sonde case as stated shown in **Figure 13-8 on left** and swag a ferrule around the two ends and through the anchor as in **Figure 13-9 on left**. The length of the loop of cable should be chosen to allow proper placement of the sonde without unnecessary slack (see **Figure 13-11 on the next page**). On subsequent visits, the loop can be cut just below the ferrule and a new one placed on the newly opened loop with minimal length of cable wasted.





Figure 13-11. Picture of field deployed sonde with adequate anchor cable length to ensure proper placement in stream water column.

### **Rootwads**

Rootwads are the preferred anchoring platform when available. A rootwad provides natural concealment and multiple locations to attach the anchor in a manner that will be very stable under all but the most extreme conditions. The anchor should be looped around the largest accessible piece of root structure that allows the sonde cable to be concealed as well as positioned in the substrate so that it receives proper flow, depth, and concealment. See **Figure 13-12 on the next page** for a typical rootwad used for sonde deployment.





Figure 13-12. Picture of typical rootwad used for anchoring a sonde. Arrow points to actual sonde deployment location.

### **Instream Boulder**

If a rootwad is unavailable or not close enough to the stream, the next best anchor is an instream boulder. In order to anchor to a boulder, it has to be shaped so that the anchor cable cannot slip over the boulder and large enough that the cable will not be pulled out from under it. Ideally the boulder needs to provide protection from debris and high flows while not subjecting the sonde to unacceptable sediment accumulation which would fill the sonde case and inhibit data collection. The cable should be looped around the base of the boulder at a pinch point, where the cable cannot slip over the top of the rock (**see Figure 13-13 above**). The sonde also needs to be located so that it receives proper flow, depth, and concealment.

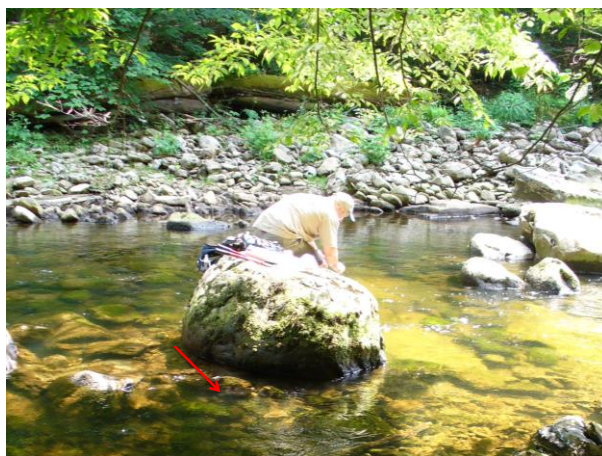


Figure 13-13. Picture of typical instream boulder used for anchoring a sonde. Arrow points to actual sonde deployment location on the downstream side of boulder.



### **Large Stream Debris**

The least preferred anchoring method is to anchor the sonde to a large or seemingly immovable piece of stream debris, such as a downed tree or large trash/debris (e.g., automobile parts, large appliances, etc.; see **Figure 13-14 below**). One must take particular care when attaching sondes to these types of anchor points due to the possibility of them moving downstream during an extreme flow event. Downed trees should be very large (>25 feet long & >4 feet diameter) before even being considered for use. Preference should be given to downed trees that have most or at least some of their mass outside or above (e.g., bridging) the stream channel. Best professional judgment must be used to determine what will work and what will not for these types of anchors.



**Figure 13-14. Pictures of typical large stream debris used for anchoring a sonde. Left picture is a large piece of metal trash/debris (e.g., automobile parts, large appliances, etc.). Right picture is a downed tree that is partially bridging and mostly out of the stream channel.**

### **Anchor Maintenance**

Well-placed and maintained anchors are the primary defense against equipment loss. The best anchor point is useless if the anchor itself is not checked and replaced periodically. Yearly replacements are required. Beyond that, anchors should be checked at each deployment for excessive corrosion, kinks, breakage, or other signs of weakness and replaced immediately if any indications of failure exist.

## Part 2. Concealment

Concealing the sonde serves several purposes. It protects the sonde from being spotted by individuals who might disturb or attempt to steal/sabotage the equipment. Additionally, if done correctly, it provides stability and protection from increases in flow that might batter, dislodge, or move the sonde to dry ground. The best material for camouflage, when available, is in-stream large cobble and boulder-sized substrate. The main method for establishing effective concealment for the deployable is to surround the sonde on all sides with large substrate in a manner that the



**Figure 13-15. Picture of sonde concealed by large substrate.**

probes are protruding from the end of the substrate in a column of moderate to swiftly moving water (**see Figure 13-15 on right**). Care must be taken to place the probe end of the sonde in a manner that will keep it off the bottom and into a flow vector that will minimize sediment accumulation within the sonde case. In locations that do not provide adequate substrate for the construction of this type of structure, creativity will be necessary. Tires, cinder blocks, and pieces of trash or appliances will also serve nicely to provide the necessary materials for concealment. In some cases the anchor point itself can provide adequate concealment, such as when dense rootwads are present. Stream structure can also be used to create effective concealment, try using undercuts, bedrock fractures, or other natural features when other methods are inadequate or unavailable. The following figures (**Figure 13-16 thru Figure 13-21**) show examples of how to properly provide concealment.





**Figure 13-16. Picture of sonde anchor wire before concealment with cobble.**



**Figure 13-17. Picture of sonde anchor wire after concealment with cobble. Note placement of the probe end of the sonde into the channel.**





**Figure 13-18. Picture of sonde anchor wire before concealment with cobble.**



**Figure 13-19. Picture of sonde anchor wire after concealment with cobble.  
Note placement of the probe end of the sonde into the channel.**





**Figure 13-20. Picture of sonde before (left) and after (right close-up) concealment with cobble. Arrow points to sonde placed in stream channel.**



**Figure 13-21. Picture of sonde concealed by cobble in pool created by rootwad.**



## **Section F. Water Quality Sampling Procedures**

If prescribed by the site list or project needs, water samples and flow measurements may also be taken periodically if necessary. Refer to **CHAPTER 3. WATER COLLECTION PROTOCOLS** starting on **page 3-1** and **CHAPTER 4. STREAM FLOW MEASUREMENT PROTOCOLS** starting on **page 4-1** for details on these protocols.

### **Side-By-Side Measurement**

An integral part of validating the data collected on the deployable sonde is gathering discrete readings from the deployable meter, 1<sup>st</sup> during all deployments/redeployments and 2<sup>nd</sup> during any retrievals of the monthly deployment cycle. These measurements allow identification of any calibration shifts, fouling or parameter drift incurred during deployment. Upon retrieval of the deployed sonde, clean the sonde of any biofilms, sediment, *etc.* that may interfere with readings and document any such potential sources of interference on the field form, then connect the meter to the appropriate display for discrete sampling. Place a second “clean” calibrated discrete YSI (or other discrete sampler) directly beside the deployable. Allow both meters to run until all parameters are stable and record all monitored parameters from the deployable in the Retrieved Deployable Sonde Check block at the bottom of the Deployable Sonde form along with the retrieved sonde ID number and location. The same just mentioned steps are followed for all deployments and parameters are recorded in the Deployed Sonde Check block. Make detailed notes about any departures/parameter discrepancies between the two sondes and infer causes when possible why these exist. Also indicate the presence of any significant sediment deposits inside the sonde case that might interfere with the accuracy of the meter.

In some cases, it may be necessary as well as beneficial to complete a discrete check in the middle of the monthly deployment cycle. These mid-deployment checks can help validate data where swings of parameter values occur. All that can be done is to place a discrete meter next to the deployed sonde in the water column and record the readings.

## **Section G. Special Concerns**

### **Part 1. Sediment**

Sediment loads vary widely depending on the system being monitored. A build-up of sediment in the sonde case will eventually fill the cavities in the conductivity/temperature probe and heavily affect the accuracy of the measures of these parameters. Excess sediment will also obscure optical DO probes and surround pH bulbs causing departures from actual and recorded values for these parameters. Care must be taken when anchoring in streams with moderate to high sediment loads to anticipate sediment impacts. Placing the sonde directly on the bottom is inadvisable in any stream and especially so where sedimentation is elevated. Measures to minimize sediment impacts include suspending the sonde from an anchor point in such a manner

that it is hanging without making any contact with the substrate (if depth is sufficient), using a sonde case that is open on the bottom end so that excess sediment can easily escape the case, and periodic mid-deployment revisits to manually remove sediment by agitating the sonde case (a perfect opportunity to also obtain another discrete data point as described above in ***Side-By-Side Measurement on page 13-31***).

## **Part 2. Heavily Populated Sites**

Generally, a deployable sonde should not be anchored in an area that is frequently used for fishing, swimming, boating, partying, or overtly visible to passers-by. The best protection against theft or vandalism of the equipment is keeping their locations as secret as possible. Periodically it is necessary to place a sonde into an area that has increased human traffic, in these instances extreme caution must be exercised. Begin by selecting the most concealed working area possible. The anchor point and the sonde must be completely invisible to onlookers. Use large cobble/small boulders, undercuts, large woody debris, trash, *etc.* to conceal the point of attachment and meter (***see Figure 13-15 to Figure 13-21***). Additionally, it is very important to avoid drawing attention to the work area if anyone is in the area. Return to the site at a later date/time if the sonde cannot be serviced in a clandestine manner.

## **Part 3. Missing Sondes**

In the event a sonde is absent from the anchor location during a retrieval visit, a survey of the surrounding area will lead to discovery of the meter in many instances. Even during very high flow events, the meter will rarely travel beyond the next sizeable pool downstream of the anchor site. If the anchor point is within a pool, many times the meter can be found at the bottom of that particular pool. If the meter is not immediately visible, visualize the stream at bankfull, and project what path is most likely for the highest flow vectors and ascertain where the sonde would likely be moved. Walk the stream and meticulously search the substrate, especially depositional areas and any piles of debris in the area. If a thorough search doesn't result in retrieval of the sonde, report the loss and the sonde identification information on the form and to a supervisor.

## **Part 4. Low Conductivity**

Specific conductance values <100 can cause extended equilibration times in pH values, especially in freshly calibrated meters. In some cases it may require 30 minutes or more to gather an accurate pH values in a stream with a specific conductance value around 20 uS/cm. Extreme caution must be exercised in low conductance streams to ensure side-by-side values are accurate due to this phenomenon. A large pH departure in the side-by-side measurement can provide an easy clue that the discrete meter is not yet ready to be recorded. As a general rule, it is best to calibrate the meter for work in this type of stream before leaving the lab and then place the probe in a small amount of DI or distilled water during travel. Then upon arrival on site, place the discrete meter into the stream immediately before initiating any other assessment activities. The best method for determining the equilibration status of the pH Sensor is to look at the pH mV

value and then recheck this value at 1 minute intervals. If after 5 minutes, the pH mV values are static or bouncing nominally the value is ready to be recorded. If, however, the pH mV values are continuously moving up or continuously down, the pH is not at equilibrium and must continue to run for an accurate discrete value to be obtained. Do not wait in excess of an hour for discreet readings. If several low conductance sites will be visited concurrently, keeping the calibration cup full of water from the site and the meter running during travel will greatly reduce waiting times for equilibration. After completing sampling for the day in low conductance streams, return the sonde to pH 4 buffer so that electrolytes are not leached from the pH Sensor needlessly overnight.

## **Part 5. Large Rivers**

Large rivers present multiple problems to sonde deployment. In some areas, large streams have few rootwads that are near deep enough water to provide an effective anchor point that doesn't place the meter in a position to be inundated with sediment or easily seen. The next anchoring option and in many cases the only option is a downed tree, which is susceptible to being washed downstream during high flows. The other confounding factors are the frequent presence of fishermen, boaters, or onlookers and periodic very high flows. Camouflaging a sonde in these areas is especially important. In some instances stream flow will rise overnight following a hard rain or repeated precipitation events to a level where a sonde cannot be retrieved safely for weeks. Sondes should be visited at the first available instance to check/secure the equipment and prevent a loss should this occur. As a general rule in large rivers, attempt to use a rootwad if at all possible and keep the sonde from resting on the substrate, keeping in mind sediment effects and the necessity of camouflage. Be acutely aware of the need to retrieve the sonde if the water level should rise precipitously without warning and tie high water retrieval anchors if necessary. Use downed trees as anchors if no other option exists. Attempt to use one that is still buried in or rooted to the bank versus one that is only resting on the stream bed. Use only very large trees that were likely moved during the previous spring and likely won't be moved again until a very large rain event occurs. Keep monitoring all available precipitation and hydrograph data to anticipate potential retrieval problems. Preemptively retrieve the sonde before a high flow event may occur if possible. Be aware that equipment is lost more likely in large rivers due to larger flow volumes and difficulty of searching the pools due to depth.

## Section H. *Troubleshooting and Manufacturers*

### Part 1. Troubleshooting by Brand

#### YSI

Problem	Corrective Measures
<p>pH does not calibrate</p> <p>Calibrates but shows large departures from standards, discrete values and/or deployable data files</p>	<p>Check pH millivolts (mV), at pH 7 the values should be <math>\pm 30</math>. Check the date of manufacture on the pH Sensor (imprinted on side of probe). If the millivolts are outside of this range or the manufacture date is &gt;18 months, probe needs to be replaced. Recalibrate.</p> <p>Check for moisture in the contact area. If moisture is present, it will interfere with a proper connection. The moisture must be removed.</p> <p>If probe still fails to function properly the sonde must be sent to Fondriest for diagnostics.</p>
<p>Conductance reads &gt;5 uS/cm in DI or distilled water</p> <p>Conductance/Temperature shows significant departures from discrete values and/or deployable data files</p>	<p>Scrub contacts with cleansing brush, rinse well with DI/distilled water and check zero again.</p> <p>Check the date of manufacture on the Cond/Temp probe (imprinted on side of probe). If &gt;3 years, probe needs to be replaced. Recalibrate.</p> <p>Check for moisture in the contact area. If moisture is present, it will interfere with a proper connection. The moisture must be removed.</p> <p>If probe still fails to function properly the sonde must be sent to Fondriest for diagnostics.</p>
<p>Ecowatch showing lines of data instead of # symbol upon attaching sonde to software</p>	<p>Repeatedly press ESC until # symbol appears</p>
<p>Ecowatch showing random scripts/missing menu options/becoming unresponsive within menus</p>	<p>Unplug sonde, close Ecowatch, restart program and reconnect to sonde</p>

<b>Problem</b>	<b>Corrective Measures</b>
Sonde will not connect to display or Ecowatch program	<p>Make sure the cable is securely fastened to end of sonde.</p> <p>Change the batteries in the sonde.</p> <p>Check for corrosion on the sonde contacts or moisture in the contact area. If either is present, they will interfere with a proper connection. The corrosion and all moisture must be removed.</p> <p>Close the sonde window, click the sonde icon again and change the selected COM port #. Go through COM port numbers and repeat process until # sign appears. If # sign never appears, change USB port and reinitiate software then repeat port selection process.</p> <p>If a connection still fails the sonde must be sent to Fondriest for diagnostics.</p>



**Hydrolab**

<b>Problem</b>	<b>Corrective Measures</b>
<p>pH does not calibrate</p> <p>Calibrates but shows large departures from standards, discrete values and/or deployable data files</p>	<p>Change pH electrolyte solution; ensure that new solution is not expired.</p> <p>Check pH junction cap, if it is more than 18 months old or looks clogged, the cap must be replaced and the meter recalibrated.</p>
<p>LDO (Luminescent Dissolved Oxygen) does not calibrate</p> <p>LDO shows significant departures from discrete values and/or deployable data files but calibrates normally</p>	<p>Twist cap off LDO probe. Check under cap and exposed portion of probe glass for moisture, being very careful not to introduce dirt or moisture. If moisture is present, gently dry off cap/probe. Replace cap and Recalibrate.</p> <p>After performing previous step, check under the probe glass in the blue reflective lens for moisture. If moisture is present under the lens, water has infiltrated the probe seals and must be sent to HACH for diagnostics/repair.</p>
<p>Conductance/Temperature shows standards, significant departures from discrete values and/or deployable data files</p>	<p>Scrub contacts with cleansing brush, rinse well with DI/distilled water and check zero again.</p> <p>Check 0 and 1000 us/cm to determine what departures are. Do a 2 point calibration with these two points and recheck standards.</p> <p>If values continue to significantly depart from standards, must be sent to HACH for diagnostics/repair.</p>
<p>Hydras3LT software will not acknowledge sonde is connected to computer</p>	<p>Change batteries in sonde.</p> <p>Unplug sonde, close software, reinsert plug and restart program.</p>
<p>Hydras3LT software is not in English</p>	<p>Press F3 until language cycles to English</p>

Sonde will not connect to Hydras3LT interface	<p>Make sure the cable is securely fastened to end of sonde.</p> <p>Change the batteries in the sonde. Meter should make an audible beep when batteries are replaced and battery cap is secured. If no beep is heard then sonde is not receiving power.</p> <p>Close down software, unplug and reconnect cable, restart software. This may require multiple attempts.</p> <p>Obtain a different connection cable and attempt operation again.</p> <p>If a connection still fails the sonde must be sent to HACH for diagnostics/repair.</p>
---	---

**Eureka**

<b>Problem</b>	<b>Corrective Measures</b>
<p>pH does not calibrate</p> <p>Calibrates but shows large departures from standards, discrete values and/or deployable data files</p>	<p>Change pH electrolyte solution; ensure that new solution is not expired.</p> <p>Check pH junction cap, if it is more than 18 months old or looks clogged, the cap must be replaced and the meter recalibrated.</p>
<p>Conductance/Temperature shows significant departures from standards, discrete values and/or deployable data files</p>	<p>Scrub contacts with cleansing brush, rinse well with DI/distilled water and check zero again.</p> <p>Check 0 and 1000 us/cm to determine what departures are. Do a 2 point calibration with these two points and recheck standards.</p> <p>If values continue to significantly depart from standards, must be sent to Eureka for diagnostics/repair.</p>
<p>Hydras3LT software freezes while downloading logged files</p>	<p>Unplug sonde, close software, reinsert plug and restart program. Freezing during download can occur several times before download is successfully completed. Repeat the process each time until all files are downloaded.</p>
<p>Eureka Multiprobe Manager software will not acknowledge sonde connected to computer</p>	<p>Unplug sonde, close software, reinsert plug and restart program. Watch electrical board through sonde case for a pair of small lights (one green, one orange) to illuminate within 10 seconds of being plugged into computer. If lights do not come on, sonde must be sent to Eureka for diagnostics/repair. If lights illuminate but sonde is still not recognized by the software, unplug, close, reinsert plug and restart. This may take several attempts before a successful connection is achieved.</p>

## Part 2. Service Contacts

Brand	Company	Contact	Telephone	Address
YSI	Fondriest Environmental	Paul Nieberding	(937) 426-2151	Nexsens Tech Inc. 1415 Research Park Dr. Beavercreek, OH 45432
Hydrolab	HACH Company	Paul Johnson	(800) 949-3766 x 6444	Loveland Service Center 5600 Lindbergh Dr. North Dock Loveland, CO 80538
Eureka	Eureka Environmental	Ric Bertrand	(512) 302-4333 x 111	2113 Wells Br. Pkwy Suite 4400 Austin, TX 78728
HOBO	Onset	Customer Support	(800) 564-4377	470 MacArthur Blvd. Bourne, MA 02532

### Section I. Continuous Monitoring Data Analysis

Upon return to the office, all data files are copied from the field laptops to the network for permanent archive. Within a week, the deployment data file is imported into the AQUARIUS time series software by Aquatic Informatics (see <http://aquaticinformatics.com/aquarius-workstation> for support documentation). The data files are trimmed to match the applicable deployment time period (e.g., readings taken during travel between the office and field location are excised). Discrete information is retrieved from the WABbase or is manually entered from the field forms. Multiple deployments from a particular station are spliced together and then analyzed for potential errors (e.g., calibration drift, sonde out of water, sediment/biofilm interference, and equilibration adjustment period). Corrections to the data are made and noted according to the suspected cause. Corrections can include offset, percent increment, or wholesale deletion. The raw and corrected data are graphed per deployment period and exported to the network for archive. The raw and corrected data are also exported to WABbase for general use.

### Continuous Monitoring 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 Continuous Monitoring is not covered, other sessions (e.g., site documentation and completing the stream assessment forms, sonde calibration, water collection protocols, stream flow measurement, field blanks and duplicates, etc.) are covered. In the field, individuals who are more experienced in Continuous Monitoring 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 maintain 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.

When water quality samples are required, duplicate sampling and field blanks must be performed at a minimum of 2.5% of Continuous Monitoring 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 in **CHAPTER 14. Section A. Blanks and Duplicates starting on page 14-1.**

Continuous monitoring sondes are routinely cross-checked to the results of discrete sonde measurements at the beginning, end of deployment, and in some cases during deployment. Upon recovery, the deployable sondes are used to perform a post-deployment discrete measurement as a “clean”, uncalibrated side-by-side comparison to a recently calibrated discrete meter. This result can help differentiate between probe calibration drift versus other factors that may have altered the readings during deployment (e.g., biofilm, sediment, etc. on or in the probes). The sonde readings (or the data file) from the deployments just prior to and after the deployment of interest are also used as a basis of validation of the data.



## CHAPTER 14. MISCELLANEOUS SAMPLING

### Section A. *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 DI (deionized) or distilled water that are preserved in the field on **weekly basis per team**. 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 DI or distilled 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.
- 4) Document the QA Blank Source Water information on the QA Blank Field Form (**see Figure 14-1 on next page**).

**Field blanks are to be handled and processed 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 Sulfuric Acid Preserved Samples (Total Phosphorous, TKN, NO<sub>2</sub>-NO<sub>3</sub>-N, Unionized NH<sub>3</sub>)

**QA Blank Form**

<b>OFFICE USE ONLY!</b> Actual QA Blank Name will go here		<i>EXAMPLE: Field Blank-Little Kanawha TMDL-Round 3-South Fork List #1</i>					
Select the QA Blank Type below:					Reviewers Initials		
<input type="checkbox"/> <u>Field Blank</u>	A sample of analyte-free water poured into the container <u>in the field</u> , processed, preserved and shipped to the laboratory with field samples. Processing includes the use of the filtration apparatus for dissolved analytes.						
<input type="checkbox"/> <u>Lab Blank</u>	A sample of analyte-free water poured into the container <u>in the lab</u> , processed, preserved and shipped to the laboratory. Processing includes the use of the filtration apparatus for dissolved analytes.						
<input type="checkbox"/> <u>Trip Blank</u>	A clean sample of a matrix that is taken from the laboratory to the sampling site and transported back to the laboratory without having been exposed to the sampling procedure. Note: Typically only for volatile compounds.						
<input type="checkbox"/> <u>Equipment Blank</u>	A sample of analyte-free water poured over or through decontaminated field sampling equipment prior to the collection of environmental samples (i.e., Bucket Sampling from Ambient Sampling & Van Dorn sampler from Lakes Sampling).						
Field Name						See Instructions Below	
Field AN-Code							
Sample Date		Time		Geo		Bio	
Vehicle (Name of person the vehicle is assigned to):							
Field Blank Project Type (Check all that apply):							
<input type="checkbox"/>	Ambient Network	<input type="checkbox"/>	AML Treatment	<input type="checkbox"/>	Acid Rain Deployables	<input type="checkbox"/>	Fecal Construction
<input type="checkbox"/>	Filamentous Algae	<input type="checkbox"/>	Fish	<input type="checkbox"/>	Golden Algae	<input type="checkbox"/>	Lakes Sampling
<input type="checkbox"/>	LTMS	<input type="checkbox"/>	Random	<input type="checkbox"/>	Reference Vulnerability (Climate Change)	<input type="checkbox"/>	South Branch Fish Kill
<input type="checkbox"/>	TDS Deployables	<input type="checkbox"/>	TMDL (Includes associated Deployables)	<input type="checkbox"/>	Trout Survey	<input type="checkbox"/>	USGS Non-Tidal
WAB (Benthic sampled sites that are not part of other projects listed above)							
Other Special Project/Survey:							
QA Blank Source Water	Supplier		Lot #		Date Pulled		
<p><b>INTSTRUCTIONS ON HOW TO CREATE A FIELD NAME AND AN-CODE (if not provided in advance):</b>                  All blanks will be UNTs. Name the UNT after the stream you are currently sampling, and make up a RM (i.e. UNT/Deckers Creek RM 3.6). The AN-Code should be similar, but not identical to the stream you are sampling and have a mile point between 5.0 and 15.0. Using Deckers Creek (M-8-J) as an example, a good Blank AN-Code would be M-8-J-6-{5.2}.</p> <p>Notes about Local Conditions (e.g., dust, exhaust, traffic flow and type, precipitation, general air quality, etc.):</p>							
Notes					WQ Sample ID		

Figure 14-1. Example of QA Blank Form

- 1 full cubitainer for Unfixed Samples (Chlorides, Hot Acidity, Alkalinity, TSS, Sulfates, Lab pH, Lab Cond., Cold Acidity, Total Orthophosphate, *etc.*)
- 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 DI or distilled water is not sterile.**

### **Field Blank Field Procedures**

1. To prepare a field blank, retrieve your pre-filled one-gallon cubitainer with DI or distilled water from storage in the vehicle.
2. Label an appropriate number of one liter sample containers in a manner that it will appear to be an actual water sample to the lab (*i.e.*, a blind sample), but will also be recognizable as a field blank to WAB employees. A fake name and AN-Code is often provided on the Sampling List.
3. Fix and handle the samples as you would do for a stream sample by substituting the DI/distilled 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 (Blind Duplicates)**

All Duplicate samples sent to the Lab should be labeled and documented on the COCs as Blind Duplicates with false Stream Names and AN-Codes and no indication that it is a duplicate.

### **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; e.g., 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.

### **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**

### **Part 1. 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 2. 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 may 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. However, these coordinates were derived using GIS – not GPS field readings. 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.*, Sonde readings), 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 (smash-able 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 3. 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 4. 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.

**This page intentionally left blank.**

## CHAPTER 15. FIELD EQUIPMENT CHECKLIST

<b>Personal Equipment</b>		
	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
<b>Paperwork and Clipboard Stuff</b>		
	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 Network
	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 Internet or the Network
	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.
	Invasive Plant Guide	Keep a copy in clipboard.
<b>Electronic Devices</b>		
	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
	Laptop Computer	Useful for GIS navigation and site confirmation
<b>Daily/Weekly Needs</b>		
	Wet Ice	From Ice Machine in the general garage area or buy at store

<b>Jeep Maintenance</b>		
	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, <i>etc.</i>
	Power Inverter	12 Volt DC to AC used to charge Laptops, Drill, <i>etc.</i>
	Flashlight	<b>MANDATORY!</b> 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.

<b>Safety and Backup Supplies</b>		
	Action Packer	To store seldom used equipment
	First Aid Kit	<b>MANDATORY!</b> Should be checked regularly
	Fire Extinguisher	<b>MANDATORY!</b> Should be checked regularly. Must be ABC type.
	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 Repellent	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	Must be worn if working in water over knee depth or on a boat.
	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 Sensor in field; In Water Lab
	Conductivity Standards	To recalibrate or check Conductivity Sensor 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

<b>Water Quality Sampling (TMDL and WAB)</b>		
	Fecal Coliform Bottles	Stored in Cage #1 in room 1193; provided by Analytical Lab as part of Contract
	Sample Containers	Stored in Cage #1 in room 1193; provided by Analytical Lab as part of Contract
	Lids for Containers	Used if Containers do not come with lids; Stored in Cage #1 in room 1193; provided by Analytical Lab as part of Contract
	HNO <sub>3</sub>	Refills in Water Lab under sinks; provided by Analytical Lab as part of Contract; Be sure to store one extra in Action packer.
	H <sub>2</sub> SO <sub>4</sub>	Refills in Water Lab under sinks; provided by Analytical Lab as part of Contract; Be sure to store 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/distilled Water)	Get from sealed laboratory boxes, put into 1 gallon cubitainer, and mark with a check

<b>Flow Measurement (TMDL and WAB)</b>		
	Measuring Tape	Marked in tenths of a foot; Do not use metric; New ones in Water Lab
	Wading rod w/Vector Ribbon	Marked in tenths of a foot; Do not use metric.
	Flow Meter	Numbered per unit

<b>Secchi Depth &amp; Chlorophyll-a (Lakes)</b>		
	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 <b>container should not be transparent</b> as chlorophyll-a is light-sensitive. This transfer container may be reused from site to site, but should always be rinsed thoroughly with DI (deionized) or distilled 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/distilled Water Bottle; store in large Zip-Lock; Old extra ones in Water Lab; Make sure DI/distilled Water Bottle is not contaminated-Wash regularly
	Aluminum foil	for wrapping chlorophyll filter samples

<b>Benthic Macroinvertebrate Sampling (Mainly Summer WAB)</b>		
	95% EtOH	In Cylinder Room
	Bug Jar Labels	Inner (on waterproof paper) and Outer; on the Network
	Surber-on-a-Stick (Modified Dip Net)	Remember to check for holes; handle marked with depth increments. Mesh Size should be <u>(595-600 <math>\mu</math>m)</u> .
	D-Net	Remember to check for holes; handle marked with depth increments. Mesh Size should be <u>(595-600 <math>\mu</math>m)</u> .
	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?
	# 30 (600 $\mu$ m) Sieve	To sift through benthic material; Extras in Water Lab; Keep an 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



<b>Fish Sampling (Mainly Summer WAB)</b>		
	Fish Jar Labels	Inner (on waterproof paper) and Outer; on the Network
	Backpack Electrofisher	Smith-Root Model 24LR or Model 12 backpack electrofisher; includes the cathode and anode
	Tow barge Electrofisher	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 electrofishers and netters.
	Measuring board and digital scales	
	Hearing protection	Used when using the tow barge/generator.

<b>Periphyton Sampling (Special Sampling Occasions)</b>		
	Periphyton Labels	Same label for inside and out; on the Network
	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.

<b>Golden Algae Sampling (Special Sampling Occasions)</b>		
	1-liter cubitainer	Used as a sample container.
	Filtering Equipment	Filter Flask, Filter Funnel, Vacuum Pump, and Vacuum Tubing
	Filters	<i>Whatman</i> Glass microfiber filters GF/F 47 mm diameter with 0.45 $\mu\text{m}$ pore size
	10% Bleach Solution	Stored in lab- for sterilization
	Distilled drinking water or 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.
	Black or Dark plastic trash bags	To place cubitainers inside of after filtering to keep light from getting to sample

## **CHAPTER 16. QUICK REFERENCE GUIDE**

The Quick Reference Guide on the next few pages is to be used as a quick field guide to this SOP. It can also be found on the network at:

Q:\WATER RESOURCES\WAB\SOP'S\2015.zip

WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015	PAGE 1
<p><b>Filling out the Form:</b> The front page needs to be filled out for every site we visit or make a failed attempt to visit on a team's list, no matter if it was sampled or not. It does not matter what type of stream it was either, non-random, <b>random</b>, reference. This will help us track the streams more efficiently down the road. Location information should still be written down for these sites (e.g. Topo name, coordinates, directions, etc.). <b>If a site is moved any significant distance &gt;0.5, then a form (front page) should be filled out for the original site stating why it was unsuitable &amp; a form should be filled out for the new site making note of the original site.</b></p>	
<p><b>Non-random Sites - Where to Sample?</b> (Also see Reference Sites below): Sample Target sites upstream of bridge or culvert, generally at the first riffle/run. Only sample a stream using MACS or Hand-Pick methodology if you are instructed to do as such on the stream list. If a nasty discharge (AMD stream, point source outfall, etc.) falls within the chosen stream reach, slide the reach upstream of discharge &amp; then take physicochemical samples upstream &amp; downstream of discharge as well as from the discharge itself using a different form for each. If the discharge is a stream &amp; is not on another crew's list, conduct a full Wadeable Benthic assessment on that stream. If you encounter this situation, document the situation thoroughly.</p>	
<p><b>Reference Sites:</b> Some potential reference sites have been identified &amp; are marked as PRS on the crews stream list, these streams have few apparent problems, &amp; should be sampled following reference site protocols (<b>TAKE RANDOM WQ PARAMETERS!</b>). If the site marked on the map is not suitable, try scouting upstream to locate a better site. <b>Every effort should be made to sample the stream as far down in the watershed as allowed by disturbances.</b> Other potential sites have not been identified &amp; are not marked 'PRS' on the crew's list. It is the crew's responsibility to determine if these sites will meet our criteria (see Reference Site section of SOP) for reference sites. Unless otherwise directed to sample them (e.g., via a note on the stream list), do not sample these sites if there are obvious impacts at these sites. If they do look good, then sample them as reference sites. If a potential reference site is encountered that is not listed on the stream list &amp; you are sure no one has it on another list, then sample it if it meets the requirements. <b>Very important:</b> If possible, recon the watershed above the PRS &amp; make notes about the land use quality.</p>	
<p><b>Locating the Random Site:</b> USGS topographic maps with a 1:24,000 scale are marked with an <b>X (highlighted in pink)</b> to designate the sampling station for random sites. <b>This spot is referred to as the X-site &amp; is the downstream end of the 100 m reach that is to be assessed.</b> If you have confidently navigated to the X site, check GPS readings to make sure you are within 10 seconds of the X-site latitude &amp; longitude. If you have good satellite coverage on your GPS (i.e., low EPE), move as close to the provided coordinates as possible. Crew members should collaborate &amp; utilize best professional judgment (BPJ) to decide where the X-site is located (finely tuned map reading skills are important). If the GPS doesn't work, do the best you can using the topo &amp; indicate as such on the field assessment form. <b>If you do not reach the X-site due to landowner denial, etc., you will only fill out the EPA X-site coordinates. The coordinates at farmer Joe's house or a locked gate are not X-site coordinates! Also, you will still need to make an educated guess as to the Target status of the site even if you cannot see it.</b></p>	
<p><b>Determining Random Site Status:</b> Benthic macroinvertebrate data is the primary tool driving the random program. If a benthic macroinvertebrate sample cannot be collected at a site, skip it &amp; move on to the next site. <b>Always complete the first page of the field assessment form regardless of whether the site is sampled or not. All unassessed (as well as assessed) sites will be included in the final analysis of the data. So, it is very important to thoroughly document the reason why a site was not sampled.</b> After establishing the X-site, thoroughly examine the assessment reach &amp; determine if it fits in our target population of random sites. Our target population consists of the following: 1) 1<sup>st</sup> thru 4<sup>th</sup> order streams (check stream list provided by Janice), 2) riffle/run habitat, 3) wadeable, 4) can be sampled using kick (rectangular frame kicknet or D-net) protocols that result in comparable data. Don't confuse riffle/run streams that are heavily embedded (sandy/silty) with true "wetland-like" streams. If stream velocity will effectively carry organisms into the net, then do your best to collect a sample. Look for contours (if present) in the sand/silt &amp; collect sample there. Also, <b>do not</b> avoid sites that are all bedrock or have large substrates (boulder). In the final data analysis, these sites can be classified as less productive habitats &amp; not necessarily impaired. Sample bedrock at areas where there are contours, fissures, &amp;/or rough places (if present), etc. Sites to avoid include the following: 1) too deep to wade safely, 2) too deep to get a comparable benthic sample (water flows over the net) 3) too shallow &amp;/or slow to get a comparable sample using our kicking techniques. Many of these types of sites may be revisited in future years to see if the stream condition was temporary.</p>	
<p><b>Sliding the Random Assessment Reach:</b> In some instances, it may be necessary to slide the reach. <b>Remember: never move the X-site, just slide the reach around it.</b> Do not proceed upstream into a lower order stream when establishing the 100 m reach. If such a confluence is encountered, note the distance &amp; 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. <b>NOTE: the confluence must be within the initial 100m reach for this sliding to apply.</b> Additionally, if the reach contains a lake, reservoir, or pond, mark the water body as the reach end &amp; make up for the loss of the reach length by moving the other end of the reach an equivalent distance from the X-site. However, if the X-site is completely within a lake, pond, or other such impoundment, no sampling can occur &amp; only the front page of the habitat form needs to be filled out describing the situation. Also, don't slide the reach downstream into a larger order stream. This may occur when you are sliding the reach downstream to avoid lakes, ponds, etc. <b>We do not slide the reach to encompass better habitat or to get more riffle/run habitat to sample or to avoid man-made obstacles such as bridges, culverts, rip-rap, or channelization.</b></p>	

WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015									PAGE 2
COC Column	Preservation Code	Water Quality Parameters Suites: <i>Take WQ Sonde readings &amp; Fecal coliform at every site!</i>							
		Parameter	Random/PRS	Acid Rain **	AMD *	Nutrient ***	TDS Ions *****	Oil & Gas ****	Sediment
1	3	Hot Acidity	Yes	Yes	Yes				
1	3	Alkalinity	Yes	Yes	Yes	@	Yes	Yes	
1	3	Sulfate	Yes	Yes	Yes		Yes	Yes	
1	2	Chloride	Yes		Yes		Yes	Yes	
1	6	Fecal coli.	Yes	Yes	Yes	Yes	Yes	Yes	Yes
1	2	Bromide	Yes				Yes		
1	3	Cold Acidity		Yes					
2	3	TDS	Yes		Yes		Yes	Yes	
2	3	TSS	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	4	Total Phosphorus	Yes			Tot. & Dis.			
2	4	TKN	Yes			Yes			
2	4	NO <sub>2</sub> -NO <sub>3</sub> -N	Yes			Yes			
2	4	Ammonia-N (NH <sub>3</sub> )	#		#	##			
2	3/7	Orthophosphate (PO <sub>4</sub> -P)				Tot.			
3	5	Al	Tot. & Diss.	Tot. & Diss.	Tot. & Diss.				
3	5	Fe	Tot. & Diss.	Tot. & Diss.	Tot. & Diss.				Tot. & Diss.
3	5	Mn	Tot.	Tot.	Tot.				
3	5	Se	Tot. (Low Level)		Tot. & Diss.				
3	5	Cu	Diss. (Low Level)						
3	5	Zn	Diss. (Low Level)						
3	5	Mg	Tot.	Tot.	Tot.	@ Tot.	Tot.	Tot.	
3	5	Ca	Tot.	Tot.	Tot.	@ Tot.	Tot.	Tot.	
3	5	K	Tot.				Tot.	Tot.	
3	5	Na	Tot.				Tot.	Tot.	
3	5	Be	Tot.						
3	5	Ba	Tot.					Tot.	
Write-in	5	Sr	Tot.					Tot.	
		# of Containers	4 Cubies & 1 Fecal	3 Cubies & 1 Fecal	3-4 Cubies & 1 Fecal	3-4 Cubies & 1 Fecal	2 Cubies & 1 Fecal	2 Cubie & 1 Fecal	3 Cubies & 1 Fecal

\* AMD: 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.  
 # Ammonia-N (NH<sub>3</sub>) if it is suspected that Ammonia is being used to treat the stream water.  
 \*\* Acid Rain: 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:  
 \*\*\* Nutrient: Take within 24 hours of a significant rain or when animal waste, straight pipes, STP outfalls, etc., may be impacting the stream:  
 @ Take in large rivers that are experiencing exceptionally excessive algal blooms (e.g., Greenbrier River, Tygart River)  
 ## Take Ammonia-N (NH<sub>3</sub>) if cattle or other livestock have direct access to stream or if there is evidence of possible ammonia  
 \*\*\*\* Oil & Gas: Take if oil or gas activities are evident & cond. >200 in absence of other sources like AMD  
 \*\*\*\*\* TDS: Take if sampling in Monongahela Basin (Monongahela, Dunkard, West Fork, Tygart Valley, Cheat, or Youghiogheny)

WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015		PAGE 3												
<p><b>Other Water Quality Notes:</b></p> <ul style="list-style-type: none"> <li>Place fecal bottles in separate zip-lock sandwich size baggies before putting in ice (do not submerge in ice water!).</li> <li>Label each container w/ WVDEP WB, stream name, AN-code, date/time collected, collector (especially if a dup.), &amp; preservative type.</li> <li>Take water samples at lower end of reach for Non-Random targeted sites. <b>Take water samples at lower end of 100 m assessment reach for random sites regardless of the location of the X-site. Rinse containers 3 times!!!!</b></li> <li><b>If Alkalinity is being analyzed, 100% of the air must be expunged from the unfixed cubitainer to avoid contamination.</b></li> <li><b>Collect samples without stirring up sediment or organic debris that can contaminate the sample!</b></li> <li><b>Remember:</b> A net minimum of 200 mL of filtered sample should be turned in for dissolved metal analysis at <b>most labs</b> we deal with.</li> </ul>														
<p><b>Water Chemistry Hold Times</b></p> <table border="1"> <tbody> <tr> <td>Fecal</td> <td>6 or 24 Hours depending on sampling type</td> </tr> <tr> <td>Orthophosphate</td> <td>48 Hours</td> </tr> <tr> <td>TSS</td> <td>7 Days</td> </tr> <tr> <td>Acidity, Alkalinity</td> <td>14 Days</td> </tr> <tr> <td>Chloride, Sulfate, Nutrients</td> <td>28 Days</td> </tr> <tr> <td>Metals</td> <td>6 Months</td> </tr> </tbody> </table>			Fecal	6 or 24 Hours depending on sampling type	Orthophosphate	48 Hours	TSS	7 Days	Acidity, Alkalinity	14 Days	Chloride, Sulfate, Nutrients	28 Days	Metals	6 Months
Fecal	6 or 24 Hours depending on sampling type													
Orthophosphate	48 Hours													
TSS	7 Days													
Acidity, Alkalinity	14 Days													
Chloride, Sulfate, Nutrients	28 Days													
Metals	6 Months													
<p><b>Field Blanks:</b> Each team should prepare field blanks <b>WEEKLY</b> at the sites designated at the time the normal samples are collected. Distilled, deionized water is the blank "sample". Crews should "collect" a clean, well-sealed one gallon cubitainer of DI water from the water lab at the beginning of the week. When the crew arrives at a designated site, they should prepare a set of cubitainers &amp; preserve the blanks as they would normal samples (this includes filtering one blank for dissolved parameters). Use one cubitainer for each type of preserved sample collected (or expected to be collected) that week. <b>Be sure to prepare a field blank for all parameters sampled during the week except Fecal.</b> Blanks are labeled with a false AN-code &amp; stream name (e.g., Surbaugh Branch, KP-100-A-B). A separate "Analysis Request Form" is completed for the field blanks <b>with faked sonde data on the form.</b> Do not indicate on any paperwork given to the lab that it is a field blank until after the sample is submitted to the lab.</p>														
<p><b>Duplicate Sites are not Optional!!</b> Attempt to do the duplicate site at the designated site. 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. Duplicate sites should be large enough to obtain two complete benthic samples (4 kicks + 4 kicks) in comparable habitat (substrate size &amp; velocity in particular), if not, select another stream. Make sure the in stream substrate &amp; velocity of the benthic sampling sites are as similar as possible. Make sure to write the name of the collector &amp; Dup #1 &amp; Dup #2 on the appropriate sample containers. Each team member should complete the habitat forms &amp; collect macroinvertebrate &amp; appropriate water samples as if they are the only person there (<i>i.e.</i>, a one person crew). GPS values can be shared. Hydrolab &amp; flow values should <b>only</b> be recorded on the Dup #1 form. <b>Only filter the sample you collect!</b></p>														



WVDEP WAB IMPORTANT INFORMATION ( <i>QUICK REFERENCE GUIDE</i> ) 4/14/2015	PAGE 4
<p><b>Flow:</b> Locate the best cross section to measure. (Flow parallel to stream bank, minimum of large disturbances, no large eddies, flow not heavily skewed to one side, etc.). Move some substrate to create a spot. <b>ALWAYS USE A TAPE MEASURE!</b></p> <ul style="list-style-type: none"> <li>• Select an optimal section of the reach with a maximum of straight, parallel velocity vectors, stable, level streambed, and a minimum velocity of <b>0.05 ft./s</b> for the transect.</li> <li>• Determine the wetted width &amp; approximate edge of effective flows (EFF) of the stream transect.</li> <li>• <b>Determine the approximate number and average spacing of velocity measurements.</b> <ul style="list-style-type: none"> <li>➢ For streams &lt; 3ft wide, you get as many measurements as possible no closer together than 0.3 ft. using best professional judgment.</li> <li>➢ For streams &gt; 3ft to 10 ft. wide, you should have a minimum of 10 measurements.</li> <li>➢ For streams &gt; 10ft wide, you should have a minimum of 20 measurements.</li> </ul> </li> <li>• Record the distance at one bank (wetted edge) as the 1<sup>st</sup> measurement (vertical). The last measurement is the opposite bank (wetted edge). Make sure to document the bank designation in the location description.</li> <li>• The 2<sup>nd</sup> measurement should be at the EFF. The 2<sup>nd</sup> to last measurement should be at that opposite EFF and the last measurement is the opposite bank (wetted edge). Record the depth and velocity at both EFFs and the EFF description.</li> <li>• Determine the number of velocity readings needed per measurement           <ul style="list-style-type: none"> <li>➢ If the depth is ≤1.5 ft., the velocity is measured only at 4/10ths of depth from the bottom (Middle of the Water Column) at that measurement station=<b>One-Point Method</b>.</li> <li>➢ If the depth is &gt;1.5 ft., take 2 velocity readings at that measurement station: one at 2/10ths of depth from the bottom (Bottom of Water Column) &amp; one at 8/10ths (Top of Water Column) =<b>Two-Point Method</b>.</li> <li>➢ 1) If the depth is &gt;1.5 ft. and               <ul style="list-style-type: none"> <li>○ The <b>Top reading is greater than 2 times the Bottom</b> velocity reading or</li> <li>○ The <b>Bottom reading is greater than the Top</b> reading or</li> <li>○ The <b>Top reading is seriously affected</b> by an obstruction (e.g., ice/ slush cover or overhanging vegetation dipping into the stream channel) or</li> <li>○ The <b>Bottom reading is seriously affected</b> by friction or turbulence produced by the streambed or an obstruction</li> </ul>               Take 3velocity readings at the Top, Middle, and Bottom=<b>Three-Point Method</b>.             </li> </ul> </li> <li>• Stand downstream of the meter facing the bank with the water flowing against the side of one leg and at least 18 inches from the wading rod.</li> <li>• <b>Any negative flow readings should be recorded as that negative value.</b></li> <li>• Always record segment distance &amp; depth in tenths of feet &amp; velocity in ft/second.</li> <li>• Continue taking measurements along the transect by tightening the increments in plumes of high velocity or radically changing depths or by spreading them out in relatively consistent velocity/depth zones until the last measurement is around one half the average segment length from the other edge of E.F. <b>The minimum width increment that can be used is 0.3 ft. or the approximate width of the probe head</b> as any increments smaller than this will be indistinguishable from one another.</li> <li>• If caught behind an unmovable boulder or other obstacle, you need to take the measurement behind the boulder &amp; also on either side of that boulder to get data from higher velocity plumes deflected around the boulder. If the water depth is &gt;1.5, then take a Three-Point reading behind the boulder.</li> <li>• If the wetted edge is associated with a depth or velocity (e.g., at an artificial, near vertical wall) you will need to either:           <ul style="list-style-type: none"> <li>❖ Designate an <b>Edge Factor between 0.9 (smooth wall) to 0.5 (rough wall)</b> if using an <b>OTT MF Pro meter</b>, or</li> <li>❖ Take a measurement at <b>0.2 ft. and 0.5 from the wall</b> if using a <b>Marsh-McBirney Flo-Mate</b>.</li> </ul> </li> <li>• <b>Store the flow meter head separate from the rod to avoid damage &amp; keep the physical structure of the head clear, consistent &amp; smooth.</b></li> </ul> <p><b>Remember: The flow measurement probe is not accurate in depths less than 0.2 ft. as it makes the flow measurement unreliable. Consider this carefully when choosing a location for flow measurement. If a stream simply does not have areas with at least 0.2ft depth, then take what measurement you can &amp; indicate that the flow is not comparable on the form &amp; why. Always not flow comparability &amp; unusual situations.</b></p> <p><b>Note: Extreme conductivity could have an effect adverse on the flow reading; be aware &amp; on the lookout for this when working in such streams.</b></p>	

WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015		PAGE 5
<b>Biology:</b> Check the nets for holes regularly & empty after every kick; always use the small brush on cobble & larger rocks. Put a label on the inside & outside of every sample jar. Sample a variety of riffles (high gradient, intermediate, & low gradient or flat), but sample the most productive or best ones within each group available.		
<b>Comparable Methods:</b>		
- Streams w/ riffles & of adequate size, use the <b>Modified Kick (Surber) net = 4 kicks - 0.5m x 0.5m, 20 seconds each.</b>		
- Streams w/ riffles but too narrow to use Surber, use <b>D-net = 9 kicks - 0.3m (1 foot) x 0.3m, 20 seconds each.</b>		
<b>Non-comparable methods (use only if directed to do so on the stream list):</b>		
- Streams w/ too low of a flow to use other protocols, conduct a <b>Hand pick = 4 areas (0.5m x 0.5m) thoroughly</b> in wetted connections between pools, not in pools. It may not be possible to sample a square 0.5m x 0.5m area (stream too narrow); it is fine to sample non-square areas like 0.25m (~10 inches) x 1.0m. Remember that hand pick samples are not comparable, but this type of sampling can still provide valuable data in certain situations.		
- Streams w/ no flowing water - use <b>Multi-habitat (MACS) = 20 jab/sweeps - 0.5m each.</b> Sample the habitats in proportion to the productive habitat in the reach ( <i>i.e.</i> , if 50 % of the productive habitat is woody snags, sample 10 woody snags). <b>Do MACS sites only if absolutely necessary &amp; if there is plenty of proper habitats to sample.</b> Remember that MACS samples are not comparable, but this type of sampling can still provide valuable data in certain situations.		
<b>Sample Preservation</b> – The samples must be properly preserved!! Each sample must have at least 70% ethanol to adequately preserve organisms. If the sample jar is very full (especially with lots of organic material like filamentous algae or leaves), divide sample into two or more labeled jars. Each sample jar should be no more than half full with the benthic sample. Be sure to clearly indicate '1 of 2' & '2 of 2', etc. on the jars. Also, tape any multiple jar samples together back in the lab. <i>Do not invert the sample jar after putting the organic material (Bugs &amp; Leaf Bits) in on top of the inorganic material (Sand &amp; Silt).</i>		

## WVSCI Thresholds

WVSCI Score	Listing Category	Descriptive Category
78.01-100.00	Unimpaired	Very Good
68.01-78.00	Unimpaired	Good
60.61-68.00	Impaired-Gray Zone	Gray Zone-Impaired
45.01-60.60	Impaired	Slightly Impaired
22.01-45.00	Impaired	Moderately Impaired
0-22.00	Impaired	Severely Impaired

## RBP Thresholds

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

## Relative Bed Stability Thresholds

RBS Score	Category
$\geq 2.0$	Highly Impaired-Too little fine sediment
1.00-1.99	Impaired-Too little fine sediment
0.20-0.99	Good
-1.00-0.19	Impaired-Too much fine sediment
$< -1.00$	Highly Impaired- Too much fine sediment

## Substrate Layer Profile Thresholds

SLP Score	Category
90-100	Not Limiting to Colonization
80-89	Not Likely Limiting to Colonization
70-79	Possibly Limiting to Colonization
60-69	Likely Limiting to Colonization
50-59	Limiting to Colonization
$< 50$	Severely Limiting to Colonization

**WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015 PAGE 6**

**WQ Standards**

	Water Temperature Regime		Water Quality Standards				Units
Water Temperature	Cold or Trout Water		>70				Deg F
	Warm or Warm Water Fishery		>87				
Use Designation	B1 - Warm Water Fishery, B4 - Wetlands		B2 - Trout		C - Contact Recreation	A - Public Water Supply	D - Agr/Wildlife, E - Industrial Water Supply
Parameter	acute	chronic	acute	chronic	C	A	D & E
Bio Impairment	WVSCI <=68	WVSCI <=68	WVSCI <=68	WVSCI <=68			
Fecal Coliform, geo mean- 5 samples min/more than 10% of samples, per 100 ml					200/400	200/400	
Fecal Coliform, per 100 ml, Ohio R. November - April, geo mean 5 samples min					2000		
DO, any time, mg/l minimum	<5	<5	<6, <7 when spawning	<6, <7 when spawning	<5	<5	<5
DO, any time, mg/l minimum (Ohio R; Kan. Zone 1)	<4	<4					
DO, any time, mg/l minimum (Ohio Apr. 15 - June 15)	<5	<5					
DO, daily avg., mg/l minimum (Ohio R)	<5	<5					
pH	<6.0 or >9.0	<6.0 or >9.0	<6.0 or >9.0	<6.0 or >9.0	<6.0 or >9.0	<6.0 or >9.0	<6.0 or >9.0
Aluminum (D), mg/l	0.75	0.75	0.75	0.087			
Iron (T), mg/l		1.5		1		1.5	
Manganese (T), mg/l						1	
Arsenic (T), mg/l					0.05	0.05	0.1
Cyanide (as free), mg/l	0.022	0.005	0.022	0.005	0.005	0.005	
Mercury (T), mg/l	0.0024		0.0024		0.00015	0.00014	
Selenium (T), mg/l	0.02	0.005	0.02	0.005		0.01	
Chromium hexavalent (+6), Diss., mg/l	0.01571	0.01058	0.01571	0.00693			
Ammonia (Un-ionized) mg/l	Need separate spreadsheet						
Chloride, mg/l	860	230	860	230	250	250	
Nitrate (as Nitrate-N), mg/l						10	
Nitrite(as Nitrite-N), mg/l	1		0.06				
Cadmium (D), mg/l	Hardness Dependent				Hardness Dependent		
Lead (D), mg/l	Hardness Dependent						
Nickel (D), mg/l	Hardness Dependent						
Silver (D), mg/l	Hardness Dependent						
Zinc (D), mg/l	Hardness Dependent						

Use Designation	B1- Warm Water Fishery, B4 - Wetlands		B2 - Trout		C - Contact Recreation	A - Public Water Supply	D - Agr/Wildlife, E - Industrial Water Supply
	acute	chronic	acute	chronic	C	A	D & E
Antimony (T), mg/l					4.3	0.014	
Arsenic (D) Trivalent, mg/l	0.34	0.15	0.34	0.15			
Barium (T), mg/l						1	
Beryllium(T), mg/l	0.13		0.13			0.0000077	
Cadmium (D), mg/l (Ohio R)						0.01	
Chromium trivalent (+3), Diss., mg/l	Hardness Dependent						
Copper (T), mg/l						1	
Copper (D), mg/l	Hardness Dependent						
Fluoride (T), mg/l						1.4	2.0 (for D)
Lead (T), mg/l						0.05	
Methylmercury, mg/l		0.000012		0.000012			
Nickel (T), mg/l					4.6	0.51	
Silver (T), mg/l	Hardness Dependent						
Thallium, (T) mg/l					0.0063	0.0017	
Threshold Odor		>8 at 104 deg. F		>8 at 104 deg. F	>8 at 104 deg. F	>8 at 104 deg. F	
Total Residual Chlorine, mg/l	0.019	0.011	No chlorinated discharges				
Turbidity		X		X	X	X	

**Fish Tissue Standard**

Mercury in Fish	0.5	Body Burden value, ug/g
-----------------	-----	-------------------------

**Some Basic Flag Codes for the Field Sheets**

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

**ADB Flag Values**

Conductivity	500	umohs
Total Aluminum	0.75	mg/L
Sulfate	170	mg/L
Hardness	200	20-75 soft
Chloride	230	mg/L
Nitrate plus Nitrite	2	mg/L
Phosphorus	0.1	mg/L
Total Suspended Solids	15	mg/L



WVDEP WAB IMPORTANT INFORMATION (QUICK REFERENCE GUIDE) 4/14/2015				PAGE 8
WV Invasive/Non-Native Species-Flora				Probability of finding specimen in WV
Scientific Name	Common Name	Type	Habitat	
<i>Achillea millefolium</i>	Common Yarrow	Flower	Urban/Roadside	H
<i>Ailanthus altissima</i>	Tree Of Heaven	Tree	Urban	H
<i>Albizia julibrissin</i>	Mimosa, Silk-Tree	Tree	Urban/Ornamental	M
<i>Alliaria petiolata</i>	Garlic Mustard	Ground Cover	Various	H
<i>Berberis thunbergii</i>	Japanese Barberry	Bush	Various	M
<i>Cichorium intybus</i>	Chicory	Flower	Roadside	H
<i>Cleome hassleriana</i>	Spiderflower	Flower		L
<i>Commelina communis</i>	Asiatic Day-Flower	Flower	Bottomland/Riparian	M
<i>Coronilla varia</i>	Crown Vetch	Ground Cover	Roadside/Reclaimed Lands	M
<i>Daucus carota</i>	Queen Anne's Lace, Wild Carrot	Flower	Roadside	H
<i>Elaeagnus umbellata</i>	Autumn Olive	Bush	Roadside/Reclaimed Lands	H
<i>Glechoma hederacea</i>	Ground-Ivy	Vine	Urban	H
<i>Hedera helix</i>	English Ivy	Vine	Urban	M
<i>Hemerocallis fulva</i>	Common Day Lily	Flower	Urban/Riparian	M
<i>Lonicera japonica</i>	Japanese Honeysuckle Vine	Vine	Various	H
<i>Lotus corniculatus</i>	Birdsfoot Trefoil	Flower/Herb	Reclaimed/Disturbed lands	M
<i>Lysimachia nummularia</i>	Moneywort	Vine/Ground Cover	Riparian/Moist Soil	M
<i>Lythrum salicaria</i>	Purple Loosestrife	Tall Herb/Wildflower	Riparian/Wetlands	M
<i>Microstegium vimineum</i>	Japanese Stiltgrass	Grass	Various	H
<i>Myriophyllum aquaticum</i>	Parrot's Feather	Aquatic/Submerged	Ponds/Streams	L
<i>Myriophyllum spicatum</i>	European Water-Milfoil	Aquatic/Submerged	Ponds/Streams	L
<i>Najas minor</i>	Eutrophic Water-Nymph	Aquatic/Submerged	Ponds/Streams	M
<i>Paulownia tomentosa</i>	Princess-Tree	Tree	Roadside/Railroad	M
<i>Phyllostachys aurea</i>	Bamboo	Grass	Urban	M
<i>Polygonum cuspidatum</i>	Japanese Knotweed	Tall Herb	Riparian/Streamside	H
<i>Polygonum perfoliatum</i>	Mile-A-Minute	Vine		L
<i>Potamogeton crispus</i>	Curly Pondweed	Aquatic/Submerged	Ponds/Streams	LM
<i>Pueraria lobata</i>	Kudzu Vine	Vine	Various	H
<i>Rosa multiflora</i>	Multiflora Rose	Bush	Various	H
<i>Rubus phoenicolasius</i>	Wineberry	Bush		M
<i>Trifolium pratense</i>	Red Clover	Herb/Flower	Fields/Pasture/Urban	H
<i>Urtica dioica</i>	Stinging Nettle, Common Nettle	Herb	Riparian	M
<i>Verbascum blattaria</i>	Moth Mullein	Flower		M
<i>Verbascum thapsus</i>	Great Mullein	Flower	Roadside/Disturbed Soil	H
WV Invasive Species-Fauna				Probability of finding specimen in WV
Scientific Name	Common Name	Type	Habitat	
<i>Corbicula fluminea</i>	Asiatic Clam	Mollusca	Stream Substrate (Relic Shells or Live)	H
<i>Dreissena polymorpha</i>	Zebra Mussel	Mollusca	Lake-like habitats/waterways	
<i>Lymantria dispar</i>	Gypsy Moth	Caterpillar	Riparian Forest	H
<i>Agrius planipennis</i>	Emerald Ash Borer	Beetle	Riparian Forest	H
<i>Adelges tsugae</i>	Hemlock woolly adelgid	Aphid-like	Riparian Forest-Hemlock Trees	H

**This page intentionally left blank.**



## **CHAPTER 17. 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 led 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.

For more information about aquatic nuisance species prevention, see <http://www.protectyourwaters.net/>

### **Section A. 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 WVDNR 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.

## **Section B. 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

## **Section C. 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 (USEPA), 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 USEPA 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 USEPA label claims, USEPA 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 USEPA label claims, USEPA accepted protocols must be used in testing specific fungi.

**Bactericide:** kills or inactivates bacteria. For USEPA label claims, USEPA 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 tuberculosis*, an acid fast bacterium which is generally more difficult to kill than most bacteria. Making label claims for tuberculocidal activity requires testing under specific USEPA guidelines.

**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, and 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 non-enveloped 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, and fungicidal. One-step cleaning and disinfectant; hard water or organic soil (5% load) does not affect efficacy of disinfectant.

**Suggested Disinfectant Concentrations**

Table 17-1. 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
<b>Contact Time</b>	10 minutes	30 minutes	10 minutes
		Metals-10 minutes	
<b>Application</b>	Soaking	Soaking/Misting	Cleaning



## CHAPTER 18. FREQUENT TERMINOLOGY

### ***Clean Water Act Basic Terms***

**Clean Water Act (CWA)** – The series of legislative acts that form the foundation for protection of U.S. water resources, including the Water Quality Act of 1965, Federal Water Pollution Control Act of 1972, Clean Water Act of 1977, and Water Quality Act of 1987. CWA Sections 305(b) and 303(d) deal specifically with water quality assessment and TMDL development.

**Water Quality Standard (WQS)** – Forms the legal basis for controlling pollution under the Clean Water Act. A Water Quality Standard includes:

- 1) **Designated Uses** – Including broad categories such as Human Health and Aquatic Life, but are more commonly referred to subcategories such as Public Water Supply, Warm Water Fishery, Trout Waters, Water Contact Recreation, Agriculture and Wildlife, and Industrial Water Supply. Designated Uses are typically assigned by stream segments.
- 2) **Criteria – Numeric** concentrations that limit chemical constituents or **Narrative** requirements that must be adhered to in order to protect the given designated uses. Criteria can be based on Acute and/or Chronic exposure (**see Acute and Chronic**).
- 3) **Antidegradation** – Rules and policies to maintain and protect existing uses and high quality waters. WV's antidegradation rules have tiered system with the highest Tier (Tier 3) having the most stringent requirements for protection.
- 4) **Other General Policies** – Address water quality standard implementation issues (*e.g.*, low flows, variances, mixing zones, *etc.*).

**Acute** – A stimulus severe enough to rapidly induce an effect; in aquatic toxicity tests, an effect observed in 96 hours or less is typically considered acute. When referring to aquatic toxicology or human health, an acute effect is not always measured in terms of lethality.

**Chronic** – A stimulus that lingers or continues for a relatively long period of time, often one-tenth of the life span or more. Chronic should be considered a relative term depending on the life span of an organism. The measurement of a chronic effect can be reduced growth, reduced reproduction, *etc.*, in addition to lethality.

**Point Source** – Any discernible, confined, and discrete conveyance, including but not limited to, any pipe, ditch, channel, tunnel, conduit, well, discrete fissure, container, rolling stock concentrated animal feeding operation (CAFO), landfill leachate collection system, vessel or other floating craft from which pollutants are or may be discharged. This term does not include return flows from irrigated agriculture or agricultural stormwater runoff (**see Nonpoint Source**). Examples include: industrial discharges, wastewater treatment discharges, active (*i.e.*, permitted and not abandoned) mining discharges, and thermal discharges from power plants. Generally, all point sources should be permitted (**see NPDES**) by state and/or federal agencies and should have signage identifying it with permit numbers and names.

**Non-Point Source** – Any source of water pollution that does not meet the legal definition of "point source" in section 502(14) of the Clean Water Act (**see Point Source**). Although the pollutants may have originated from a point source (e.g., a smokestack, tail-pipe) the long-range transport ability and multiple sources over a broad landscape of the pollutants make it a nonpoint source of pollution. Examples include:

- Agricultural runoff (e.g., precipitation runoff with excess fertilizers, herbicides, insecticides);
- Urban runoff (e.g., precipitation runoff from parking lots, roads, and residential areas containing salt, oil, grease & toxic chemicals);
- Sediment from improperly managed construction sites, agricultural and forest lands, and eroding streambanks;
- Atmospheric deposition of sulfuric and nitric acid via precipitation (i.e., acid rain);
- Acid drainage from abandoned mines;
- Bacteria and nutrients from livestock, pet wastes, and faulty septic and sewer systems.

**National Pollutant Discharge Elimination System (NPDES)** – A national program under Section 402 of the Clean Water Act for regulation of discharges of pollutants from point sources to waters of the United States. Discharges are illegal unless authorized by an NPDES permit.

**Discharge Monitoring Report (DMR)** – The form used (including any subsequent additions, revisions, or modifications) to report self-monitoring results by NPDES permittees. DMRs must be used by approved states as well as by EPA.

**Mixing Zone** – An area where an effluent discharge undergoes initial dilution and is extended to cover the secondary mixing in the ambient water body. A mixing zone is an allocated impact zone where water quality criteria can be exceeded as long as acutely toxic conditions are prevented.

**Impaired waterbody** – A waterbody (i.e., stream reaches, lakes, waterbody segments) with chronic or recurring monitored violations of the applicable numeric and/or narrative water quality criteria. All impaired waterbodies must be reported on a 303(d) list (so named after the section of the CWA in which it is described) and have a pollution reduction plan (**see TMDL**) developed to restore the stream to meeting water quality standards.

**Total Maximum Daily Load (TMDL)** – Broadly speaking, a plan of action used to clean up streams that are not meeting water quality standards. Specifically it consists of a calculation of the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards. The calculation consists of wasteload allocations for each existing and future point source of pollution, load allocations for existing and future non-point sources of pollution as well as background sources, and an extra margin of safety. This calculation forms the basis of a pollution budget and helps inform the reduction of pollutants in future NPDES permits and the development of implementation plans that result in the restoration of an impaired stream to meeting water quality standards.

**Best Management Practices (BMPs)** – Physical, structural or managerial practices (*i.e.*, schedules of activities, prohibitions of practices, maintenance procedures) that decreases the potential for facilities to pollute drinking water. They can be used singly or in combination as appropriate in a particular situation. They also include treatment requirements, operating procedures, and practice to control plant site runoff, spillage or leaks, sludge or waste disposal, or drainage from raw material storage. BMPs may be applied to Point Source pollution sources as a part of a NPDES permit or to Nonpoint Source pollution sources.

## ***Oil/Gas Related Terms***

**Natural Gas** – Fossil fuel formed when layers of buried plants, gases, and animals are exposed to intense heat and pressure from being buried deep underground in rock formations over thousands of years. Natural Gas consists primarily of **Methane** (CH<sub>4</sub>), but also can contain other gases such as carbon dioxide, nitrogen, and hydrogen sulfide gases.

**Petroleum (Oil or Crude Oil)** – Fossil fuel formed when large quantities of dead organisms, usually zooplankton and algae, are buried underneath sedimentary rock and subjected to intense heat and pressure. Petroleum (as opposed to Natural Gas) is a liquid at normal atmospheric temperatures and pressures and varies in color (yellow to green to red to dark brown to black) and consistency (thin liquid to thick semi-solid mixed with sand and water also known as **Bitumen** or **Tar Sands**). Petroleum is often found in close proximity to and with natural gas, in subterranean pockets layered with the lighter natural gas acting as a gas cap and the usually heavier saline water sunken beneath the petroleum.

**Biogenic Gas** – Gas, mainly Methane (CH<sub>4</sub>) that is produced by methane-producing organisms (*i.e.*, methanogens such as Archaea) near the earth's surface (*e.g.*, marshes, bogs, landfills, and shallow sediments). Methane that is Biogenic in origin has a unique chemical signature (opposed to **Thermogenic Methane**). This chemical signature can be used in determining the source of methane in contaminated groundwater. Also called **Bacterial Gas**.

**Thermogenic Gas** – Gas that originates from organic material buried in geologic layers (*e.g.*, shale layers) deep in the earth (*i.e.*, **Natural Gas**). Due to the higher temperatures and pressures found at those depths, methane that is produced in these deep geologic layers (**Thermogenic Methane**) often partially transform into larger, longer-chain molecules such as Ethane (C<sub>2</sub>H<sub>6</sub>), Propane (C<sub>3</sub>H<sub>8</sub>), or Butane (C<sub>4</sub>H<sub>10</sub>). Gas containing the longer-chain molecules is often said to be more thermally mature. Opposed to **Biogenic Methane**.

**Dry Gas** – Product from a natural gas well that is mostly methane, which is gaseous at normal atmospheric pressures and temperatures.

**Wet Gas** – Product from a natural gas well that, in addition to methane, contain longer-chain molecules (*e.g.*, Ethane, Propane and Butane) that are liquids at modest pressures. These are also called **Natural Gas Liquids** or **NGLs**. Typically Wet Gas is considered more valuable than Dry Gas since the additional components and long-chain molecules can be converted to other, more useful compounds by processing plants (see **Cracker Plant**). These compounds become the “building

blocks” in the chemical manufacturing chain (e.g., plastics that are made from Ethylene which is, in turn, made from Ethane). Propane and Butane (types of **Liquefied Petroleum Gas** or **LPG**) can also be separated and used directly as fuels.

**Sour Gas** – Euphemism for Natural Gas significantly laced (e.g., 4ppm) with highly toxic hydrogen sulfide. Sour Gas is often **Vented** or **Flared** because of its lack of commercial value.

**Conventional Drilling or Well** – Includes shallow wells and deep vertical drilling. Both processes often involve the use of **Low-Volume Hydraulic Fracturing** (see **Fracturing** below) to help bring the oil or gas to the surface.

**Shallow Wells** are targeting pockets of oil and gas that have migrated toward the surface from much deeper shale layers (e.g., Marcellus, Utica) and have become trapped underneath an impermeable layer of rock.

**Deep Vertical Drilling** goes directly to the originating oil/gas bearing shale formations deep under the surface.

**Unconventional Drilling or Well** – Includes **Horizontal drilling used** in conjunction with **High-Volume Hydraulic Fracturing**.

**Horizontal Drilling** – Drilling process by which at some point the drill bit turns from vertical to horizontal or near horizontal so that the drilled well will run along with the oil/gas bearing deposit. This process increases the surface area of the well in the oil/gas bearing formation, thus increasing the output from one drilled well.

**Fracturing** – Technique by which a substance is injected into the well under pressure to open up fissures in the oil/gas deposit that help the flow of the product up out of the well. Types of fracturing include hydraulic, propane, and nitrogen fracturing and the technique can be used in a relatively small scale in conventional oil and gas drilling as well as the drilling of water and geothermal wells, or in a large scale with unconventional drilling. **Fracking** is a colloquial term used by the media and general public that represents all **Horizontal drilling with high-volume hydraulic fracturing**.

**Shale Plays** – Sedimentary rock formations (e.g., Marcellus and Utica shale) that contain oil and gas. Shale formations are the source rock for oil and gas that gradually (over millions of years) migrate up toward the surface and become trapped in porous rock pockets under a layer of impervious rock underground. These pockets are the gas and oil source of the majority wells drilled worldwide by conventional drilling. Direct extraction of the gas and oil from the source rock occur on a small scale (volume) with Deep Vertical Drilling and a large scale (volume) with Horizontal Drilling. Gas extracted from these shale layers is called **Shale Gas**.

**Fairway** – The main portion of the Marcellus shale formation in Pennsylvania, West Virginia, and parts of New York, Maryland, and Ohio in which the majority of drilling is concentrated. This is due to factors like regional thickness of the shale, natural fractures, and folding of the shale which limit the production efficiency of a well.

**Well pad** – The main location of the head of a well. It is often associated with infrastructure such as well heads, pipes, pumps, storage tanks (for produced water and oil/gas), impoundments, etc. In the case of Unconventional drilling, more than one oil/gas well may be located on a single well pad (also called **Pad Drilling**). A well pad can vary in size (e.g., 1 acre for the relatively smaller conventional wells) to much larger (e.g., 5 acres in unconventional wells).

**Spudded** – A well that is first being drilled.

**Laterals** – The horizontal portion of a well that runs in continual contact with the gas bearing formation for long distances (up to 1-2 miles). Each lateral is a part of one well; several laterals will can radiate out from one well pad. It is a misconception that the laterals from a Marcellus well pad will radiate out in all directions (like a compass or clock). Rather, the laterals will tend to radiate in two directions (usually in a northeastern-southwestern axis) in small angles relative to the well pad (like a bow-tie). This is to take advantage of natural fractures in the Marcellus shale.

**Fracturing Fluid** – The combination of Water (99% of the fluid volume), Chemicals, and Proppant that is injected into the drilled horizontal well under pressure to open up fissures in the shale which facilitates the removal of the natural gas from the shale to the surface. The majority of the fracturing fluid remains in the well and does not return to the surface, but portions of the fracturing fluid can be recovered and reused to some extent.

**Slickwater** – Name for the chemically-laced water used in the hydraulic fracturing process. Chemical components include biocides, friction reducers, oxygen scavengers, acids, chemical cross linkers and scale and corrosion inhibitors.

**Proppant** – A solid material, typically treated sand or man-made ceramic materials, designed to keep an induced hydraulic fracture open, during or following a fracturing treatment.

**Flowback (Backflow Water)** – Wastewater that returns out of the well after initial hydraulic fracturing and contains a portion of the hydraulic fracturing fluid as well as **Produced Water**. Initially the flowback contains mainly the fracturing fluid, but as time goes on (a matter of weeks) the ratio turns to where it consist of mainly produced water, which will return out of the well for years.

**Produced Water** – Naturally occurring water found associated with the oil or gas deposits that returns out of the well in flowback water or with the produced gas or oil. This water is generally very saline in nature (brine) and high in total dissolved solids (barium, calcium, iron magnesium, and sulfur). Additionally low levels of naturally occurring radioactive substances (e.g., Radium) can be found in produced water.

**Venting** – Process by which undesirable gases from a well (including methane in the case of a wet gas well) or processing plant are released directly to the atmosphere.

**Flaring (Gas Flare)** – Process by which undesirable gases from a well (including methane in the case of a wet gas or oil well) or processing plant are combusted as they are being released into the atmosphere. Although the methane is commercially desirable, it may not be recovered either because: a) a lack of pipelines near the well to transport the methane to processing and storage facilities, or b) the process of separating it from the other, more valuable components is not as economically feasible (*i.e.*, it would cost more to extract the methane than could be recovered from its sale on the market).

**Processing Plant** – A centralized location where the gas from multiple gas fields are transported (via pipelines or tankers) to remove impurities (*e.g.*, water, hydrogen sulfide, helium, *etc.*) in order to become pipeline-ready, marketable natural gas. A **Cracker Plant** is a specific type of processing plant where more complex long-chain organic molecules (such as those found in Wet Gas) are broken down (through the use of temperature and catalysts) into simpler, more basic molecules by the breaking (or cracking) of the carbon-carbon bonds in the molecules.

**Pigging** – A pipeline maintenance (cleaning and inspection) device that does not stop the flow of product in the pipeline. The pig (slightly smaller than the diameter of the pipeline) is inserted at a pigging station (launching station) and then flows with the product in the pipeline to the next pigging station (receiving station). The residue that the pig “cleans” from the pipeline can be considered a hazardous waste.

**Compulsory Integration (Forced Pooling)** – Process by which unleased land is forced into oil/gas extraction leases by a company if they own leases on a certain percentage of land (*e.g.*, 60%) inside of a certain amount of space (*e.g.*, one square mile). The percentage and space vary state to state.

### ***Mining Related Terms***

**SMCRA** – Surface Mining Control and Reclamation Act. A 1977 U.S. law that controls surface mining and dictates that all mine lands must be reclaimed after mining has ceased.

**Abandoned Mine Lands** – Mine sites that were either pre-SMCRA law sites or bond forfeited sites where reclamation has not yet occurred.

**Reclamation** – the process by which it is attempted to return mining sites to their pre-mining conditions, or prepared for other higher uses (*e.g.*, land development). Another component of reclamation is to make the site safe for passersby by sealing off mine portals, removing highwalls, or other unnatural hazards. Finally, any water discharges from the reclaimed site must be compliant with water quality standards.

**Subsidence** – The gradual sinking, or sometimes abrupt collapse, of the rock and soil layers into an underground mine. Structures and surface features above the subsidence area can be affected.

**Compensatory Mitigation** – The restoration, creation, enhancement, or in exceptional cases preservation of wetlands and/or other aquatic resources for the purpose of compensating for unavoidable impacts.



## Types of Mining

**Softrock Mining** – Techniques (usually a combination of blasting and tools/machinery; historically hand tools) used to extract geological materials (like coal and salt) from “soft” sedimentary rocks. Opposed to **Hardrock Mining** – Mining of any geological materials (mainly metal containing ores from igneous and metamorphic rocks) that can only be conducted by blasting due to the “hard” nature of the strata.

**Underground mining** – Mining that occurs exclusively underground, usually located several hundred feet below the earth’s surface. **Also called Deep mining.** Includes various subtypes (e.g., **Drift (Horizontal), Slope, and Shaft Mining**) and methods (e.g., **Longwall, Continuous or Room and Pillar, Blast, and Retreat Mining**).

**Punch Mine** – A type of small drift (horizontal) mine used to recover small coal deposits that are otherwise uneconomical to extract (e.g., coal seams in the side of strip-mine highwalls, small exposed seams in a hillside).

**Surface mining** – Any mining that occurs at the surface of the earth by digging down or sideways into a slope.

**Contour mining** – A type of surface mining where a resource (usually coal) is removed from a slope along a level path that roughly follows the contour of the slope. **Also see Highwall.**

**Auger mining** – A type of surface mining where an auger (rotary drill that uses a screw devise) is used to drill laterally into the side of slope to remove the resource (usually coal) while removing a minimal amount of overburden. **Compare to Contour mining.**

**Open-Pit mining** – A type of surface mining where the resource is located close the surface of a relatively flat area and removed by digging it out by creating a large pit or borrow. When used to extract building materials, the open-pit is commonly referred to as a **quarry**. This type of mining is more common in the Western U.S. and is limited to mainly limestone quarries in the Eastern Panhandle near Martinsburg, WV.

**Mountaintop Mining (MTM) or Mountaintop Removal (MTR)** – a type of surface mining where thinner seams of coal that are unobtainable by other means (e.g., Underground or contour mining) are mined by removing all of the overburden above the seam. The overburden (which can include the tops of whole mountains, thus the name) removed can be 10-100s of feet thick and in multiple layers between coal seams. Due to the expansion of rock material during the mining process, excess material is often disposed of in the head of valleys neighboring the mining site (**See Valley Fill**). This type of mining is common in Southern WV due to the steep, mountainous terrain. Often associated with a **Dragline**, which is a large (as big as a house) excavation machine used to remove the overburden.

## Coal Mining Structure and Waste Terms

**Portal** – Typically the surface entrance to an underground mine and used for personnel to enter the mine, drain water, provide ventilation, or remove minerals.

An **Adit** is a blind horizontal or nearly horizontal opening into a mountain, with only one entrance.

A **Tunnel** is a near-horizontal passageway that is open to the surface at both ends and passes completely thru a hill or mountain.

A **Shaft** is a vertical or steeply inclined entrance to a mine.

**Overburden** – The material above or between seams of coal that are removed before or along with the coal and discarded either on-site at the mine (e.g., **see Valley Fill**), saved for later use in reclamation (i.e., restoring the original contour of the topography). It is distinct from Tailings in that it is not processed in any way. **Also called spoil.**

**Gob** – The loose waste in a mine. Can be left inside the mine or it may come out of the mine with the coal and is later separated and discarded into **Spoil Piles**.

**Spoil Pile** – Overburden & discarded material that does not leave the mining site and is often piled up into small to large hummocks. **Also called Gob Piles.** Since the inception of SMCRA, such piles are no longer allowed to be abandoned and must be reclaimed and/or remediated.

**Valley Fill** – When the overburden from a surface mine is disposed of in the head of a small valley; typically associated with Mountaintop Mining, but can also be associated with other large earth removal projects such as road & highway construction.

**Highwall** – The back side of a contour mining job that is distinguished by a large cliff-like drop-off from upslope to downslope. Highwalls are often an indication of either pre-SMCRA law mining or bond forfeited, unreclaimed sites. Highwalls are an obvious safety issue due to the large drop-offs and targeted for abandoned mine reclamation.

## Coal Processing Terms

**Coal Preparation Plant** – A facility that separates coal from the unwanted material (**see Tailings**), crushes and grades it based on size, and often stockpiles it before loading it for transport into the market. The purpose of the plant is to reduce the costs of transportation and increase the market value of the coal by removing the waste material. Typically this material is separated using physical (e.g., size screening), gravity separation and/or washing (**Coal Washing**) processes involving water and high gravity liquid chemicals (typically surfactants). Coal Preparations Plants are typically located in close proximity the mining site or several mines.

**Tailings** – Sometimes called **Slate**. The materials left over after the process of separating the valuable fraction (e.g., coal) from the unwanted portion that was mined. The dry material that separated out is piled up as discard material and typically disposed of at or near a coal processing plant (**see Coal Refuse Pile**). Wet waste material is called **Coal Slurry**.

**Slack Dump** – Slack is small pieces of low-grade bituminous coal often separated out from the more valuable high-grade coal.

**Coal Refuse Pile** – The discard material, or tailings, from the coal preparation process. Due to the inefficiencies of technology at the time, older Coal Refuse Piles often includes minute to large coal particles that could not originally be separated from the tailings. Depending on market prices and coal content, many old Coal Refuse Piles are reclaimed for the content of their coal alone. **Slate Dump** is an older term for a Coal Refuse Pile containing large amounts of slate.

**Red Dog** – Material created from inadvertent slow burning or combustion of small bits of rock and coal in a Spoil or Coal Refuse pile. It is characterized by the red color from minerals containing iron, which is brought out from the burning in a low oxygen environment. Sometimes hard to distinguish from crushed red brick or iron hydroxide coated rocks to a novice.

**Coal Slurry** – A mixture of solid and liquid wastes from the coal preparation washing process; mainly the material that settles to bottom of a basin after the washing of coal and includes minute coal particles (*see Coal Slurry Impoundment*).

**Coal Slurry Impoundment** – Coal Slurry is typically stored and disposed of behind an earthen-fill dam created out of mining overburden and located in the valley of a small stream. Unlike a normal water retention dams, it is raised in succession throughout the life of a mine/coal preparation plant (*i.e.*, as it fills with coal slurry, the dam is raised to accommodate more coal slurry). Since some of the chemicals used in the washing process are toxic, an impervious liner is used to prevent seepage into the groundwater. **Also called a Tailings Dam or Coal Impoundment.**

**Stockpile** – A pile or storage location for bulk materials (*e.g.*, coal). Stockpiles are used in many different areas, such as in a port, refinery, manufacturing facilities, or mine sites. The simple stockpile is created by machinery (*e.g.*, dump truck or conveyor) dumping the material into a pile and/or being pushed into heaps by bulldozers. More controlled stockpiles are created by a stacker and a reclaimer is used to recover the material. Coal is often stored in graded stockpiles for differing designated uses.

## Coal Combustion Waste Terms

**Slag** – Leftover materials from steel mills and coking process; disposed of in piles near the steel mills or coke ovens called **Slag Dumps**. May also refer to a byproduct of burning coal in a boiler (as in power generation) that frequently needs to be removed.

**Ash Pile** – The ash leftover from the burning of coal at a coal fired power plant. Often stored as dry material in a landfill or as wet material behind a head of valley impoundment.

## ***Water Treatment Related Terms***

**Wastewater Treatment Plant (WWTP)** – An industrial structure designed to remove biological or chemical waste products from water, thereby permitting the treated water to be used for other purposes. WWTPs are used to treat domestic wastewater (e.g., sewage - **see Sewage Treatment Plant**), industrial, and agricultural wastewater.

**Sewage Treatment Plant (STP)** – A wastewater treatment plant that is designed primarily to treat sewage. They can vary in size from a large **Publicly Owned Treatment Works (POTW)** to smaller plants that serve a group of or individual homes.

**Publicly Owned Treatment Works (POTW)** – A waste-treatment works owned by a state, unit of local government, or Indian tribe, usually designed to treat municipal wastewaters. These facilities are often large and often designed to treat municipal and industrial wastewater. Privately-owned treatment works, Federally-owned treatment works, and other treatment plants not owned by municipalities are not considered POTWs. Also called **Centralized Wastewater Treatment System**.

**Public Service District (PSD)** – Public corporations established by county commissions with approval of the West Virginia Public Service Commission. These local entities manage the development and maintenance of water, sewage and gas systems covering areas specified by the county commission.

**Combined Sewer** – A wastewater collection system which conveys sanitary wastewaters (domestic, commercial and industrial wastewaters) and stormwater through a single pipe to a publicly owned treatment works for treatment prior to discharge to surface waters. **See combined sewer overflows (CSOs)**.

**Combined Sewer Overflows (CSOs)** – A pre-designed discharge of untreated wastewater from a combined sewer system at a point prior to the headworks (or entry point) of a publicly owned treatment works. CSOs generally occur during wet weather (rainfall or snowmelt). During periods of wet weather, these systems become overloaded, bypass treatment works, and discharge directly to receiving waters. Discharges from CSOs contain not only stormwater but also untreated human and industrial waste, toxic materials, and debris. They are remnants of the country's early infrastructure and so are typically found in older communities and a major water pollution concern.

**Sanitary Sewer** – A pipe or conduit (sewer) intended to carry wastewater or water-borne wastes from homes, businesses, and industries to the point-of-treatment (usually a POTW). Properly designed, operated, and maintained sanitary sewer systems are meant to collect and transport all of the sewage that flows into them to a publicly owned treatment works (POTW). **See Sanitary Sewer Overflows**.

**Sanitary Sewer Overflows (SSOs)** – Unintentional discharges of raw sewage from municipal sanitary sewers occur. They have a variety of causes, including but not limited to blockages, line breaks, sewer defects that allow storm water and groundwater to overload the system, lapses in sewer system operation and maintenance, power failures, inadequate sewer design and vandalism. SSOs contrast with CSOs in that they are not designed to occur.

**Municipal Separate Storm Sewer Systems (MS4s)** – A conveyance or system of conveyances (including roads with drainage systems, municipal streets, catch basins, curbs, gutters, ditches, man-made channels, or storm drains) which are: 1) Designed or used for collecting or conveying stormwater; 2) Not a combined sewer; 3) Owned and operated by a state, city, town, borough, county, parish, district, association, or other public body; and 4) Not part of (*i.e.*, connected to) a publicly owned treatment works (POTW).

**Wastepile** – Non-containerized piles of solid, non-liquid hazardous wastes that are used for temporary storage or treatment. This is opposed to a **Stockpile** which consists of a product (*e.g.*, coal, salt, limestone, etc.).

**Landfill** – Excavated or engineered sites where non-liquid waste is deposited for final disposal and covered.

**Open Dumps** – An illegal, uncovered site used for disposal of waste without environmental controls (as opposed to permitted landfills which use liners, leak detection systems, and water treatment to control leachate). Open dumps tend to be located in out of the way, rural locations (the sides of roads on a hillside) where the dumper can more easily dispose of the garbage without being seen. Open dumps are a breeding ground for pests and vectors which cause disease (*e.g.*, mosquitos breeding in old tires and containers filled with water). They are also a common dumping place for toxic methamphetamine lab waste, thus complicating clean-up.

**Injection Well** – A device that places fluid deep underground into porous rock formations, such as sandstone or limestone, or into or below the shallow soil layer. These fluids may be water, wastewater, brine (salt water), or water mixed with chemicals.

**Underground Storage Tank (UST)** – A tank and any underground piping connected to the tank that has at least 10 percent of its combined volume underground. USTs are typically used to store chemicals, gasoline, or other petroleum products (*e.g.*, gas stations). USTs are a risk to ground and surface water due to the potential for leaks. Compare to **Aboveground Storage Tank (AST)**.

**Animal Feeding Operation (AFO)** – An intensive livestock farming method where a large number of animals are kept in a relatively small area. Such areas are often noted for increased nutrient runoff due to the high density of livestock and increased sediment runoff due to a lack of ground cover (the ground is typically barren soil due to the intense wear by the large number of animals). The animals do not graze on natural vegetation and all food must be supplied externally from the enclosed area. This farming method is often contrasted to more typical and traditional pasture farming where animals are allowed to graze over a wide, fenced area and eat natural vegetation off of the landscape. A lot or facility (other than an aquatic animal production facility) can be classification as an AFO when the following conditions are met: 1) Animals (other than aquatic animals) have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period, and 2) Crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility.

**Concentrated Animal Feeding Operation (CAFO)** – An animal feeding operation that meets and additional size thresholds (*i.e.*, numbers of livestock according to the type of present). The size of a CAFO determines the impact on whether a facility is subject to regulation under the Clean Water Act and can be categorized as Large, Medium or Small.

**Poultry House** – A large building used to house a large number of chickens, turkey, or other domestic fowl for either egg or meat production. Poultry houses are common in the eastern panhandle of WV, especially in the South Branch Potomac River Watershed.

**Pharmaceuticals and Personal Care Products (PPCPs)** – A category of pollutants that are used by individuals for personal health or cosmetic reasons and in agriculture to boost the health of livestock. Examples are such broad categories of chemicals such as over-the-counter and prescription pharmaceuticals, antibiotics, hormones, antimicrobial additives found in soaps. PPCPs are of growing concern because they are not effectively removed by most wastewater treatment methods, their increased use globally, and their detection in the environment in recent decades.



## CHAPTER 19. DOCUMENT REVIEW SIGNATURES

### APPROVED:

\_\_\_\_\_  
Author, Name, Division/Section/Unit Click here to enter a date.  
Date

\_\_\_\_\_  
Peer Reviewer, Name, Division/Section/Unit Click here to enter a date.  
Date

\_\_\_\_\_  
Manager/Supervisor, Name, Division/Section/Unit Click here to enter a date.  
Date

\_\_\_\_\_  
Quality Assurance Reviewer, Name, Division/Section/Unit Click here to enter a date.  
Date



west virginia department of environmental protection



Photo Credits: Front Cover-Brook Trout (*Kevin Seagle*); Otter Creek Wilderness Area near Hendricks, WV, Monongahela National Forest (*Chris Barry*); Perlidae Stonefly (*Mike Whitman*); Back Cover-Mussels (*Mike Whitman*); Shenandoah River near Harpers Ferry, WV (*Mike Whitman*); Pteronarcyidae Stonefly Larvae & Adult (*Kevin Seagle*)

