



United States Environmental Protection Agency
Office of Water
Washington, DC
EPA-841-B-22-006

National Rivers and Streams Assessment 2023/24

Field Operations Manual

Wadeable

Version 1.0



NOTICE

The complete documentation of overall National Rivers and Streams Assessment (NRSA) project management, design, methods, and standards is contained in four companion documents, including:

National Rivers and Streams Assessment 2023-24: Quality Assurance Project Plan EPA-841-B-22-004

National Rivers and Streams Assessment 2023-24: Site Evaluation Guidelines EPA-841-B-22-005

National Rivers and Streams Assessment 2023-24: Wadeable Field Operations Manual EPA-841-B-22-006

National Rivers and Streams Assessment 2023-24: Non-Wadeable Field Operations Manual EPA-841-B-22-007

National Rivers and Streams Assessment 2023-24: Laboratory Operations Manual EPA-841-B-22-008

This document (*Field Operations Manual (FOM)*) contains a brief introduction and procedures to follow at the base location and on-site, including methods for sampling water chemistry (grabs and *in situ* measurements), periphyton, benthic macroinvertebrates, algal toxins, fish assemblage, fish tissue plugs, Enterococci, antimicrobial resistance, and physical habitat. These methods are based on the guidelines developed and followed in the National Rivers and Streams Assessment 2008-2009 (USEPA 2012), Western Environmental Monitoring and Assessment Program (Baker, et al., 1997), the methods outlined in Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers (Flotemersch, et al., 2006), and methods employed by several key states that were involved in the planning phase of this project. Methods described in this document are to be used specifically in work relating to the NRSA 2023/24. All Project Cooperators must follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Details on specific methods for site evaluation and sample processing can be found in the appropriate companion documents. This document and associated QAPP ensure compliance with the EPA policies [Assuring the Competency of EPA Laboratories \(2004\)](#) and [Policy to Assure Competency of Laboratories, Field Sampling, and Other Organizations Generating Environmental Measurement Data under Agency-Funded Acquisitions \(2011\)](#).

The suggested citation for this document is:

USEPA. 2022. National Rivers and Streams Assessment 2023/24: Field Operations Manual – Wadeable. EPA-841-B-22-006. U.S. Environmental Protection Agency, Office of Water Washington, DC.

TABLE OF CONTENTS

NOTICE	II
TABLE OF CONTENTS	IV
LIST OF FIGURES	VII
LIST OF TABLES	VII
ACRONYMS/ABBREVIATIONS	IX
DISTRIBUTION LIST	XI
1 BACKGROUND	1
1.1 SURVEY DESIGN	1
1.2 TARGET POPULATION AND INDEX PERIOD	1
1.3 REPLACING SITES	2
1.4 SELECTION OF NRSA INDICATORS	3
1.5 SUPPLEMENTAL MATERIAL TO THE FIELD OPERATIONS MANUAL	4
1.6 RECORDING DATA AND OTHER INFORMATION	5
2 INTRODUCTION TO WADEABLE SAMPLING	8
2.1 DAILY OPERATIONS	8
2.2 BASE SITE ACTIVITIES.....	10
2.2.1 <i>Pre-departure Activities</i>	10
2.2.2 <i>Post Sampling Activities</i>	12
2.3 SAFETY AND HEALTH.....	15
2.3.1 <i>General Considerations</i>	15
2.3.2 <i>Safety Equipment</i>	16
2.3.3 <i>Safety Guidelines for Field Operations</i>	17
2.4 FORMS (NRSA APP)	18
2.4.1 <i>Field Forms</i>	18
2.4.2 <i>Tracking Form and Packing Slips</i>	19
2.4.3 <i>Equipment and Supplies</i>	20
3 INITIAL SITE PROCEDURES	22
3.1 SITE VERIFICATION ACTIVITIES	22
3.1.1 <i>Locating the X-Site</i>	22
3.1.2 <i>Determining the Sampling Status of a Stream</i>	23
3.1.3 <i>Elevation at Transect A</i>	25
3.1.4 <i>Sampling During or After Rain Events</i>	25
3.1.5 <i>Site Photographs</i>	26
3.2 LAYING OUT THE SAMPLING REACH	26
3.2.1 <i>Sliding the Reach</i>	28
3.3 MODIFYING SAMPLE PROTOCOLS FOR HIGH OR LOW FLOWS.....	29
3.3.1 <i>Streams with Interrupted Flow</i>	29
3.3.2 <i>Braided Rivers and Streams</i>	30
4 IN SITU MEASUREMENTS OF DISSOLVED OXYGEN, PH, TEMPERATURE, AND CONDUCTIVITY	32
4.1 SUMMARY OF METHOD	32
4.2 EQUIPMENT AND SUPPLIES	32
4.2.1 <i>Multi-Probe Sonde</i>	32
4.3 SAMPLING PROCEDURE	33
5 COLLECTION OF ANTIMICROBIAL RESISTANCE SAMPLES	33

5.1	SUMMARY OF METHOD	33
5.2	EQUIPMENT AND SUPPLIES	33
5.3	SAMPLING PROCEDURE	34
5.4	ANTIMICROBIAL RESISTANCE FIELD BLANKS.....	35
6	COLLECTION OF WATER CHEMISTRY AND CHLOROPHYLL-A SAMPLES.....	35
6.1	SUMMARY OF METHOD	35
6.2	EQUIPMENT AND SUPPLIES	36
6.3	WATER CHEMISTRY AND CHLOROPHYLL-A SAMPLING PROCEDURE	36
7	ALGAL TOXINS (MICROCYSTINS AND CYLINDROSPERMOPSIN).....	37
7.1	SUMMARY OF METHOD	38
7.2	EQUIPMENT AND SUPPLIES	38
7.3	SAMPLING PROCEDURE	38
8	BENTHIC MACROINVERTEBRATES	40
8.1	SUMMARY OF METHOD	40
8.2	EQUIPMENT AND SUPPLIES	40
8.3	SAMPLING PROCEDURE	41
8.4	SAMPLE PROCESSING IN FIELD	45
9	PERIPHYTON.....	47
9.1	SUMMARY OF METHOD	47
9.2	EQUIPMENT AND SUPPLIES	47
9.3	SAMPLING PROCEDURE	47
9.4	SAMPLE PROCESSING IN THE FIELD	49
10	PHYSICAL HABITAT CHARACTERIZATION	50
10.1	EQUIPMENT AND SUPPLIES	50
10.2	SUMMARY OF METHODS APPROACH	50
10.3	COMPONENTS OF THE HABITAT CHARACTERIZATION	51
10.4	WORK FLOW FOR THE PHYSICAL HABITAT COMPONENTS.....	52
10.4.1	<i>Channel/Riparian Cross-Sections.....</i>	52
10.4.2	<i>Thalweg Profile and Large Woody Debris Tally.....</i>	52
10.4.3	<i>Channel Constraint and Torrent Evidence.....</i>	53
10.5	HABITAT SAMPLING WITHIN THE REACH	53
10.5.1	<i>Channel and Riparian Measurements at Cross-Section Transects</i>	55
10.5.2	<i>Thalweg Profile and Large Woody Debris Measurements</i>	70
10.6	CROSS-SECTION TRANSECTS ON SIDE CHANNELS	76
10.7	SLOPE AND BEARING	77
10.7.1	<i>Measurement of Slope using Level and Stadia Rod.....</i>	77
10.7.2	<i>Alternate Methods for Obtaining Slope</i>	80
10.7.3	<i>Method for Obtaining Bearing</i>	82
10.8	CHANNEL CONSTRAINT, DEBRIS TORRENTS, AND RECENT FLOODS.....	83
10.8.1	<i>Channel Constraint.....</i>	83
10.8.2	<i>Debris Torrents and Recent Major Floods</i>	85
10.9	ELEVATION AT TRANSECT K.....	86
11	FECAL INDICATOR (<i>ENTEROCOCCI</i>)	87
11.1	SUMMARY OF METHOD	87
11.2	EQUIPMENT AND SUPPLIES	87
11.3	SAMPLING PROCEDURE	87
11.4	SAMPLE PROCESSING IN THE FIELD	88

12	FISH ASSEMBLAGE	89
12.1	SUMMARY OF METHOD	89
12.2	EQUIPMENT AND SUPPLIES	89
12.3	SAMPLING PROCEDURES.....	90
12.3.1	<i>Irruptive Species</i>	91
12.3.2	<i>Small Wadeable Streams</i>	93
12.3.3	<i>Large Wadeable Streams</i>	96
12.4	SEINING	100
12.5	PROCESSING FISH.....	103
12.5.1	<i>Identification and Tallying</i>	103
12.5.2	<i>Unknown Specimens</i>	104
12.5.3	<i>Photovouchering</i>	109
12.5.4	<i>Preparing Preserved Voucher Specimen Samples</i>	109
12.5.5	<i>Preserving Voucher Specimen Samples</i>	110
12.5.6	<i>Processing Unknown/Range Extension (UNK/RNG) Voucher Samples</i>	112
12.5.7	<i>Processing QA Voucher Samples</i>	112
13	FISH TISSUE PLUG SAMPLING METHODS	116
13.1	METHOD SUMMARY.....	116
13.2	EQUIPMENT AND SUPPLIES	116
13.3	SAMPLE COLLECTION PROCEDURES.....	117
14	FINAL SITE ACTIVITIES	121
14.1	OVERVIEW OF FINAL SITE ACTIVITIES	121
14.2	GENERAL SITE ASSESSMENT	122
14.2.1	<i>Elevation at Transect K</i>	122
14.2.2	<i>Watershed Activities and Disturbances Observed</i>	122
14.2.3	<i>Site Characteristics</i>	122
14.2.4	<i>General Assessment</i>	122
14.3	PROCESSING THE FECAL INDICATOR (<i>ENTEROCOCCI</i>), CHLOROPHYLL-A, AND PERIPHYTON SAMPLES	123
14.3.1	<i>Equipment and Supplies (Fecal Indicator Filtering)</i>	123
14.3.2	<i>Procedures for Processing the Fecal Indicator (Enterococci) Sample</i>	123
14.3.3	<i>Equipment and Supplies (Chlorophyll-a from Water Sample Filtering)</i>	125
14.3.4	<i>Procedures for Processing the Chlorophyll-a Water Sample</i>	125
14.3.5	<i>Equipment and Supplies (Periphyton Sample)</i>	126
14.3.6	<i>Procedures for Processing the Periphyton Samples</i>	127
14.4	DATA FORMS AND SAMPLE INSPECTION	131
14.5	LAUNCH SITE CLEANUP	131
15	FIELD QUALITY CONTROL	132
15.1	REVISIT SAMPLING OVERVIEW	132
15.2	REVISIT SAMPLING SITES	132
15.3	FIELD EVALUATION AND ASSISTANCE VISITS	133
15.3.1	<i>Specifications for QC Assurance Field Assistance Visits</i>	134
15.4	REPORTING.....	134
16	REFERENCES	136
APPENDIX A	LIST OF EQUIPMENT AND SUPPLIES	A-1
APPENDIX B	SAMPLE FORMS	B-1
APPENDIX C	SHIPPING AND TRACKING GUIDELINES	C-1
APPENDIX D	COMMON & SCIENTIFIC NAMES OF FISHES OF THE UNITED STATES	D-1

APPENDIX E EXAMPLE ELECTROFISHING SETTINGS E-1

LIST OF FIGURES

Figure 1.1 Example Sample Labels for Sample Tracking and Identification 7
 Figure 2.1 Field Sampling Scenario for Wadeable Sites..... 9
 Figure 2.2 Overview of Base Site Activities 10
 Figure 2.3 Example Sample Packing Slips to Accompany Sample Shipments 19
 Figure 2.4 Electronic Request Form..... 20
 Figure 3.1 Sampling Reach Features for a Wadeable Site 28
 Figure 8.1 Benthic Macroinvertebrate Collection at Wadeable Sites 41
 Figure 8.2 Transect Sample Design for Collecting Benthic Macroinvertebrates at Wadeable Sites 42
 Figure 10.1 Reach Layout for Physical Habitat Measurements for streams 2.5m or greater (plan view).... 54
 Figure 10.2 Substrate Sampling Cross-Section 55
 Figure 10.3 Riparian Zone and Instream Fish Cover Plots for a Stream Cross-Section Transect..... 58
 Figure 10.4 Determining Bank Angle Under Different Types of Bank Conditions 61
 Figure 10.5 Schematic Showing Relationship Between Bankfull Channel and Incision 63
 Figure 10.6 Determining Bankfull and Incision Heights..... 64
 Figure 10.7 Schematic of Modified Convex Spherical Canopy Densiometer..... 65
 Figure 10.8 Proximity Classes for Human Influences in Wadeable Streams 68
 Figure 10.9 Large Woody Debris Influence Zones (modified from Robison and Beschta, 1990). 75
 Figure 10.10 Large Woody Debris Section of Physical Habitat Form in the NRSA App - Wadeable..... 75
 Figure 10.11 Riparian and Instream Fish Cover Plots for a Stream with Minor and Major Side Channels .. 76
 Figure 10.12 Measurements of Bearing Between Transects..... 78
 Figure 10.13 Channel Slope Measurement using a Clinometer 81
 Figure 10.14 Types of Multiple Channel Patterns 85
 Figure 12.1 Reach Layouts for Fish Sampling at Wadeable Sites 92
 Figure 12.2 Unknown/Range Extension Voucher Sample Labels and Tags..... 111
 Figure 12.3 QA Voucher Sample Labels and Tags..... 113
 Figure 14.1 Final Site Activities Summary..... 121
 Figure 15.1 Summary of the Repeat Sampling Design 132

LIST OF TABLES

Table 1.1 Summary Table of Indicators for all NRSA 2023/24 Sites 3
 Table 1.2 Guidelines for Recording Field Measurements and Tracking Information 6
 Table 2.1 Stock Solutions, Uses, and Methods for Preparation 12
 Table 2.2 Post-sampling Equipment Care..... 14
 Table 2.3 General Health and Safety Considerations 16
 Table 2.4 General Safety Guidelines for Field Operations..... 18
 Table 3.1 Equipment and Supplies: for Site Verification 23
 Table 3.2 Procedure: Site Verification 24
 Table 3.3 Guidelines to Determine the Influence of Rain Events..... 26
 Table 3.4 Procedure: Laying Out the Sampling Reach at Wadeable Sites 27
 Table 3.5 Procedure: Sliding the Sampling Reach 29
 Table 3.6 Reach Layout Modifications for Interrupted Streams 30
 Table 3.7 Procedure: Modifications for Braided Rivers and Streams..... 31
 Table 4.1 Equipment and Supplies: DO, pH, Temperature, and Conductivity..... 32
 Table 4.2 Procedure: Temperature, pH, Conductivity and Dissolved Oxygen..... 33
 Table 5.1 Equipment and Supplies: Antimicrobial Resistance Samples 34
 Table 5.2 Procedure: Antimicrobial Resistance Sample Collection (Wadeable Sites) 34

Table 5.3 Procedure: Antimicrobial Resistance Field Blank Collection (Visit 1 to Revisit Sites)	35
Table 6.1 Equipment and Supplies: Water Chemistry Sample Collection and Preservation	36
Table 6.2 Procedure: Water Chemistry and Chlorophyll- <i>a</i> Sample Collection (Wadeable Sites).....	36
Table 7.1 Equipment and Supplies: Microcystin	38
Table 7.2 Procedure: Algal Toxin (Microcystin and Cylindrospermopsin) Collection (Wadeable Sites).....	38
Table 8.1 Equipment and Supplies: Benthic Macroinvertebrate Collection at Wadeable Sites.....	40
Table 8.2 Procedure: Benthic Macroinvertebrates (Wadeable Sites)	43
Table 8.3 Procedure: Compositing Samples for Benthic Macroinvertebrates (Wadeable Sites)	45
Table 9.1 Equipment and Supplies: Periphyton (Wadeable Sites)	47
Table 9.2 Procedure: Collecting Composite Index Samples of Periphyton (Wadeable Sites)	48
Table 10.1 Equipment and Supplies: Physical Habitat.....	50
Table 10.2 Summary of Components of Physical Habitat Characterization at Wadeable Sites	51
Table 10.3 Procedure: Substrate Measurement	56
Table 10.4 Procedure: Estimating Instream Fish Cover.....	59
Table 10.5 Procedure: Measuring Bank Characteristics	59
Table 10.6 Procedure: Canopy Cover Measurements	66
Table 10.7 Procedure: Characterizing Riparian Vegetation Structure.....	67
Table 10.8 Procedure: Estimating Human Influence	69
Table 10.9 Procedure: Thalweg Profile.....	71
Table 10.10 Channel Unit Categories	73
Table 10.11 Procedure: Tallying Large Woody Debris.....	74
Table 10.12 Procedure: Obtaining Slope Data	79
Table 10.13 Modified Procedure: Obtaining Slope Data (without Surveyor’s Level).....	80
Table 10.14 Procedure: Obtaining Bearing Data	82
Table 10.15 Procedure: Assessing Channel Constraint	83
Table 11.1 Equipment and Supplies: Fecal Indicator Sampling (Wadeable Sites).....	87
Table 11.2 Procedure: Fecal Indicator (Enterococci) Sample Collection (Wadeable Sites).....	87
Table 12.1 Equipment and Supplies: Fish Collection (Wadeable Sites).....	89
Table 12.2 Summary of Wadeable Fishing Protocols	91
Table 12.3 Procedure: Electrofishing (Small Wadeable Streams)	93
Table 12.4 Procedure: Electrofishing (Large Wadeable Sites).....	96
Table 12.5 Procedure: Seining (Wadeable Sites).....	100
Table 12.6 Procedure: Processing Fish (Wadeable Sites).....	105
Table 12.7 Procedure: Processing Unknown/Range Extension (UNK/RNG) Voucher Samples.....	114
Table 12.8 Procedure: Processing QA Voucher Samples.....	115
Table 13.1 Equipment and Supplies: Fish Tissue Plug Sample	116
Table 13.2 Recommended Target and Alternate Species for Fish Tissue Plug Collection.....	118
Table 13.3 Procedure: Fish Tissue Plug Samples	119
Table 14.1 Equipment and Supplies: Fecal Indicator (Enterococci) Sample Processing.....	123
Table 14.2 Procedure: Fecal Indicator (Enterococci) Sample Processing.....	123
Table 14.3 Equipment and Supplies: Chlorophyll- <i>a</i> Processing	125
Table 14.4 Procedure: Chlorophyll- <i>a</i> Sample Processing.....	126
Table 14.5 Equipment and Supplies: Periphyton Samples	126
Table 14.6 Procedure: ID/Enumeration Samples of Periphyton	127
Table 14.7 Procedure: Preparing Metagenomic Sample of Periphyton	128
Table 14.8 Procedure: Preparing Chlorophyll Samples of Periphyton	129
Table 14.9 Procedure: Preparing Periphyton Biomass Sample	130
Table 15.1 General Information Noted During Field Evaluation	134

ACRONYMS/ABBREVIATIONS

AFDM	Ash-Free Dry mass
AMR	Antimicrobial resistance
ANC	Acid-neutralizing capacity
Ca	Calcium
Cl	Chloride
CPR	Cardiopulmonary Resuscitation
CWA	Clean Water Act
DELT	Deformities, Eroded Fins, Lesions and Tumors
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DI	Di-ionized Tap Water
EPA	Environmental Protection Agency
FOM	Field Operations Manual
GIS	Geographic Information System
GPP	Generator Powered Pulsator
GPS	Global Positioning Device
IBI	Index of Biotic Integrity
IM	Information Management
K	Potassium
LIMS	Laboratory Information Management System
LOM	Lab Operations Manual
LWD	Large Woody Debris
Mg	Magnesium
MMI	Multimetric Index
MSDS	Methods Safety Data Sheets
Na	Sodium
NAD	North American Datum
NARS	National Aquatic Resources Survey
NHD	National Hydrology Database
NH ₃	Ammonia
NH ₄	Ammonium
NIST	National Institute of Standards and Technology
NO ₂	Nitrite
NO ₃	Nitrate
NRSA	National Rivers and Streams Assessment
O/E	“Observed” over “Expected”
OSHA	Occupational Safety and Health Administration
OW	Office of Water
PESD-AL	Pacific Ecological System Division – Analytical laboratory
PETG	polyethylene terephthalate glycol
PFD	Personal Flotation Device
PP	Polypropylene
PPT	Parts per thousand
Psig	Pounds per square inch - gauge
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QCS	Quality Control Solution
QRG	Quick Reference Guide
SEG	Site Evaluation Guideline
SiO ₂	Silica

SO ₄	Sulfate
SOP	Standard Operating Procedures
Std	Standard
TL	Total Length
TOC	Total Organic Carbon
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UNK/RNG	Unknown Range Extension
USGS	United States Geological Survey

DISTRIBUTION LIST

This FOM and associated manuals or guidelines will be distributed to the following U.S. Environmental Protection Agency (EPA) senior staff participating in the NRSA and to State Water Quality Agencies or cooperators who will perform the field sampling operations. The Quality Assurance (QA) Officers will distribute the Quality Assurance Project Plan (QAPP) and associated documents to participating project staff at their respective facilities and to the project contacts at participating laboratories, as they are determined.

National Monitoring Coordinators		
Richard Mitchell NRSA Project Leader	mitchell.richard@epa.gov 202-564-0064	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Sarah Lehman NRSA Project QA Officer	lehmann.sarah@epa.gov 202-566-1379	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Brian Hasty NRSA Logistics Lead	hasty.brian@epa.gov 202-566-2236	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Lareina Guenzel NRSA ESA Lead	Lareina.guenzel@epa.gov 202-566-0455	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Bernice L. Smith WRAPD QA Coordinator	smith.bernicel@epa.gov 202-566-1244	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Cynthia N. Johnson QA Officer	Johnson.CynthiaN@epa.gov 202-566-1679	U.S. EPA Office of Water 1200 Pennsylvania Ave., NW Washington, DC 20460
Amanda Nahlik, EPA ORD Technical Advisor	Nahlik.Amanda@epa.gov 541-754-4581	U.S. EPA Office of Research and Development 200 S.W. 35th St. Corvallis, OR 97330
Michelle Gover NARS Information Management Coordinator	gover.michelle@epa.gov 541-754-4793 541-754-4799 (fax)	General Dynamics Information Technology 200 S.W. 35 th Street Corvallis, OR 9733
Chris Turner Contract Field Logistics Coordinator	cturner@glec.com 715-829-3737	Great Lakes Environmental Center, Inc. 739 Hastings Street Traverse City, MI 49686
Regional Monitoring Coordinators		
Tom Faber Region 1	Faber.tom@epa.gov 617-918-8672	U.S. EPA – Region I 11 Technology Drive, North Chelmsford, MA 01863-2431
Emily Nering Region 2	nering.emily@epa.gov 732-321-6764	U.S. EPA – Region II 2890 Woodbridge Ave, Edison, NJ 08837-3679
Leah Ettema Region 3	Ettema.leah@epa.gov 215-814-5675	U.S. EPA – Region III 1650 Arch Street, Philadelphia, PA 19103-2029
Elizabeth Belk Region 4	belk.elizabeth@epa.gov 404-562-9377	U.S. EPA - Region IV 61 Forsyth Street S.W., Atlanta, GA 30303-8960
Mari Nord Region 5	nord.mari@epa.gov 312-353-3017	U.S. EPA – Region V 77 West Jackson Blvd, Chicago, IL 60604-3507
Rob Cook Region 6	cook.robert@epa.gov 214-665-7141	U.S. EPA – Region VI 1445 Ross Ave -Ste 1200, Dallas, TX 75202-2733
Laura Webb Region 7	webb.laura@epa.gov 913-551-7435	U.S. EPA – Region VII 300 Minnesota Ave, Kansas City, KS 66101
Liz Rogers/Shera Reems Region 8	rogers.liz@epa.gov /reems.shera@epa.gov 303-312-6974/303-312-6888	U.S. EPA – Region VIII 1595 Wynkoop Street, Denver, CO 80202-1129

Matthew Bolt Region 9	bolt.matthew@epa.gov 415-972-3578	U.S. EPA – Region IX 75 Hawthorne Street, San Francisco, CA 94105
Lillian Herger Region 10	Herger.lillian@epa.gov 206-553-1074	U.S. EPA – Region X, 1200 Sixth Avenue, Seattle, WA 98101

1 BACKGROUND

This manual describes field protocols and daily operations for crews to use in the Wadeable NRSA 2023/24 method. The NRSA is a probability-based survey of our Nation's rivers and streams and is designed to:

- Assess the condition of the Nation's rivers and streams.
- Evaluate changes in condition from NRSA 2008/09, NRSA 2013/14, and NRSA 2018/19.
- Help build State and Tribal capacity for monitoring and assessment and promote collaboration across jurisdictional boundaries.

This is one of a series of water assessments being conducted by states, tribes, the U.S. EPA, and other partners. In addition to rivers and streams, the water assessments will also focus on coastal waters, lakes, and wetlands in a revolving sequence. The purpose of these assessments is to generate statistically valid reports on the condition of our Nation's water resources and identify key stressors to these systems.

1.1 Survey Design

The survey design consists of two separate designs to address the dual objectives of: (1) estimating current status and (2) estimating change in status for all flowing waters:

- Resample design applied to NRSA 2018/19 sites.
- New site design for NRSA 2023/24.

The Resample survey design is a group of sites that were targeted for sampling in NRSA 18/19. These sites all begin with the NRS23_18 prefix. The major objective for this design is change estimation. This results in 790 resample base sites which are part of the 2023/24 design. Allocation of sites to NARS aggregated ecoregions is proportional to the number sampled in the prior surveys. The NRSA 2023/24 survey design includes 1028 new sites which have not been included in previous NRSA surveys. Allocation of a number of sites to states is proportional to stream length and is stratified by state. Unequal probability categories are 27 combinations of NARS nine aggregated ecoregions and three waterbody reach categories (SS – small streams, LS – large streams, and RV – Rivers). A minimum of 20 sites was guaranteed in each state and the maximum number of sites was set at 75 for an individual state. The sample frame was derived from the high-resolution National Hydrography Dataset (NHD), in particular NHDPlus V2. Additional details on the NRSA survey design are found in the National Rivers and Streams Assessment Survey Design: 2023/24 documents.

1.2 Target Population and Index Period

The target population consists of all streams and rivers within the 48 contiguous states that have flowing water during the study index period, including major rivers and small streams. Sites must have > 50% of the reach length with standing water, and sites with water in less than 50% of the reach length must be dropped. All sites must be sampled during base flow conditions.

The target population excludes:

- Tidal rivers and streams up to head of salt (defined as < 0.5 ppt for this study).
- Run-of-the-river ponds and reservoirs with greater than seven-day residence time.

The study index period extends from:

- Beginning of June through end of September for most regions.
- Sites in the select ecoregions or States can be sampled starting in the end of April with approval from the EPA Project Coordinator.

Please refer to the Site Evaluation Guidelines (*EPA- 841-B-22-005* and the NRSA Web site <http://www.epa.gov/national-aquatic-resource-surveys/nrsa>) for more detailed information on the target population and exclusion criteria.

1.3 Replacing Sites

All base sites must be evaluated for sampling. If a stream or river site is determined to be nontarget or otherwise unsampleable, it must be replaced by another site within the same state and same panel. The panels for NRSA 2023/24 are listed below. Note, the FT suffix found in some panels in the site lists refers to “Fish Tissue” sites which may not be applicable to the evaluation process though have been left in should EPA obtain funding for the collection of fish for fish tissue purposes. The evaluation process is the same regardless of the FT suffix and those suffixes have been omitted from the list below.

- NRS23_18RVT2RV: River sites that were selected for sampling in NRSA 2018/19 and have been selected to be sampled twice in 2023/24. This is referred to as a “revisit site” as it will be evaluated and sampled twice during the same year, with at least two weeks in between each visit.
- NRS23_18BaseRV: River sites that were selected for sampling in NRSA 2018/19 and have been selected to be sampled once in 2023/24
- NRS23_18OverRV: River sites to be used as replacements for dropped 18/19 river base sites or as additional sites for state level surveys in some cases.
- NRS23_18RVT2LS: Large stream sites that were selected for sampling in NRSA 2018/19 and have been selected to be sampled twice in 2023/24. This is referred to as a “revisit site” as it will be evaluated and sampled twice during the same year, with at least two weeks in between each visit.
- NRS23_18BaseLS: Large stream sites that were selected for sampling in NRSA 2018/19 and have been selected to be sampled once in 2023/24
- NRS23_18OverLS: Large stream sites to be used as replacements for dropped 18/19 large stream base sites or as additional sites for state level surveys in some cases.
- NRS23_18BaseSS: Small stream sites that were selected for sampling in NRSA 2018/19 and have been selected to be sampled once in 2023/24
- NRS23_18OverSS: Small stream sites to be used as replacements for dropped 18/19 small stream base sites or as additional sites for state level surveys in some cases.
- NRS23_23BaseRV: River sites new to NRSA 2023/24, designated to be sampled once in 2023/24.
- NRS23_23OverRV: River sites to be used as replacements for dropped 23/24 river base sites or as additional sites for state level surveys in some cases. May also be used to replace 2018/19 river base sites if no 18/19 river oversample sites remain.

- NRS23_23BaseLS: Large stream sites new to NRSA 2023/24, designated to be sampled once in 2023/24.
- NRS23_23OverLS: Large stream sites to be used as replacements for dropped 23/24 large stream base sites or as additional sites for state level surveys in some cases. May also be used to replace 2018/19 large stream base sites if no 18/19 large stream oversample sites remain.
- NRS23_23BaseSS: Small stream sites new to NRSA 2023/24, designated to be sampled once in 2023/24.
- NRS23_23OverSS: Small stream sites to be used as replacements for dropped 23/24 small stream base sites or as additional sites for state level surveys in some cases. May also be used to replace 2018/19 small stream base sites if no 18/19 small stream oversample sites remain.

Please refer to the *Site Evaluation Guidelines (EPA- 841-B-22-005)* for more detailed information.

1.4 Selection of NRSA Indicators

As part of the indicator selection process, EPA worked with state, tribal, and other partners through technical conferences and indicator teleconferences. The EPA formed a National Rivers and Streams Assessment Steering Committee with state, tribal, and regional representatives to provide feedback and evaluate core and supplemental indicators to be included in the 2023/24 field season. Key evaluation criteria included indicator applicability on a national scale, the ability of an indicator to reflect various aspects of ecological condition, repeatability, and cost-effectiveness. The core indicators build upon the work done in the NRSA 2008/09, NRSA 2013/14, and NRSA 2018/19. They have been sampled and analyzed on the national scale and have a known applicability to Clean Water Act (CWA) programs. Supplemental indicators were selected based on feedback from the Steering Committee and decisions by EPA management. Supplemental indicators are either in the research phase and their applicability is still being assessed for CWA programs or this is the first time they will be sampled at a national scale. For field sampling purposes, there is no distinction between core and supplemental indicators. Indicators that are included in the NRSA 2023/24 are briefly described in **Table 1.1**.

Table 1.1 Summary Table of Indicators for all NRSA 2023/24 Sites

Indicator	Core or Supplemental Indicator	Specs/Location in Sampling Reach
<i>In Situ</i> measurements (pH, DO, temperature, conductivity)	Core Indicator	Measurements taken at X site at mid-channel; readings are taken at 0.5 m depth, or mid-depth if water depth is less than 1 meter.
Water chemistry (TP, TN, NH ₃ -N, NO ₃ -NO ₂ , NO ₃ , basic anions and cations, silica, alkalinity [Acid-neutralizing capacity (ANC)], DOC, TOC, TSS, conductivity, pH, turbidity, true color)	Core Indicator	Collected at the X site at mid-channel; from a depth of 0.5 m, or mid-depth if water depth is less than 1 meter.

Indicator	Core or Supplemental Indicator	Specs/Location in Sampling Reach
Chlorophyll- <i>a</i>	Core Indicator	Collected as part of water chemistry and periphyton samples
Microcystin and cylindrospermopsin	Supplemental Indicator	Collected at the X site at mid-channel; from a depth of 0.5 m, or mid-depth if water depth is less than 1 meter.
Periphyton composite and periphyton metagenomic	Supplemental Indicator	Collected from 11 locations systematically placed at each site and combined into a single composite sample
Benthic macroinvertebrate assemblage (Littoral)	Core Indicator	Collected from 11 locations systematically placed at each site and combined into a single composite sample
Fish Assemblage	Core Indicator	Sampled throughout the sampling reach at specified locations
Physical habitat assessment	Core Indicator	Measurements collected throughout the sampling reach at specified locations
Fecal indicator (Enterococci)	Supplemental Indicator	Collected at the last transect one meter off the bank at 0.3 m depth
Antimicrobial resistance (genes and bacteria)	Supplemental Indicator	Collected at the X site at mid-channel; from a depth of 0.5 m, or mid-depth if water depth is less than 1 meter.
Fish Tissue Plug	Supplemental Indicator	Tissue sample collected from target species which are collected throughout the sampling reach as part of fish assemblage sampling

1.5 Supplemental Material to the Field Operations Manual

The FOM describes wadeable field protocols and daily operations for crews to use in the NRSA. Following these detailed protocols will ensure consistency across regions and reproducibility for future assessments. Before beginning sampling at a site, crews should prepare a packet for each site containing pertinent information to successfully conduct sampling. This includes a road map and set of directions to the site, topographic maps, landowner access forms, sampling permits (if needed), site evaluation forms, and other information necessary to ensure an efficient and safe sampling day.

Field Crews will collect field data in the NRSA App on an iPad. Within the App, users will find a number of information (*i*) buttons that contain tables and figures summarizing field activities and protocols from the FOM. These informational buttons replace the need for a Quick Reference Guide (QRG) as has been used in previous survey years. In addition to the informational buttons within the App, crews will also have access to this FOM in electronic (Adobe® PDF) format on the iPad. Field Crews are required to know how to access and search the FOM in the field for reference and for possible protocol clarification.

Quality Assurance (QA) is a required element of all EPA-sponsored studies that involve the collection of environmental data (USEPA 2000a, 2000b). Field Crews will be provided a digital copy of the integrated QAPP. The QAPP contains more detailed information regarding quality assurance/quality control (QA/QC) activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, and data reporting activities. For more information on the QA procedures, refer to the *National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA-841-B-22-004)*.

Related NRSA documents include the following: National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA 841-B-22-004), National Rivers and Streams Assessment: Site Evaluation Guidelines (EPA 841-B-22-005), and National Rivers and Streams Assessment: Laboratory Methods Manual (EPA-841-B-22-008). These documents are available at: <http://www.epa.gov/national-aquatic-resource-surveys/nrsa>.

1.6 Recording Data and Other Information

All samples need to be identified and tracked, and associated information for each sample must be recorded. To assist with sample identification and tracking, labels are preprinted with sample ID numbers (**Figure 1.1**).

Field and sample information must be **recorded accurately and consistently**. The cost of a sampling visit coupled with the short index period severely limits the ability to resample a site if the initial information recorded was inaccurate. Guidelines for recording field measurements are presented in **Table 1.2**. At the end of each sampling day, the Field Crew Leader is responsible for reviewing each field form for completeness and accuracy. Field Crews will find a number of data validation routines within each electronic form that will help find missing or possibly incorrect data. These routines can be accessed at any time by tapping the data review button at the top of each form.

Table 1.2 Guidelines for Recording Field Measurements and Tracking Information

ACTIVITY	GUIDELINES
Field Measurements	
Data Recording	<ul style="list-style-type: none"> Record observations and measurement values only using the official NRSA App (provided on EPA owned Apple iPads for all regional, state, and tribal crews). If you make an error when recording data and changes are required, it is best to enter the new value and resubmit that electronic form. Use the correct crew ID assigned during field training. Use the units and formats specified on individual data forms for recording data. For any sample or data where additional explanation is needed, use the provided comment bubble adjacent to the data
Sample Collection	
Sample Labels and Tags	<ul style="list-style-type: none"> Use a writing instrument that leaves clear, dark text to record information (e.g., a No. 2 pencil on paper tags or a water or smear proof fine-point indelible marker on adhesive labels). Use the sample-type appropriate adhesive labels with preprinted Sample ID numbers for each sample. Be sure to fill in any requested information about the sample on the sample label and affix it to the outside of the sample container. Cover completed labels with clear tape. Place a waterproof paper tag inside each benthic macroinvertebrate collection jar and fish voucher jar with the required information written with a No. 2 lead pencil.
Sample Collection Information	<ul style="list-style-type: none"> Record that each sample has been collected on the appropriate data form. Be sure to cross-check the Sample ID number from labels and tags with the Sample IDs populated in the Tracking Form.
QA and Tracking	
Before Leaving Site: Review of Data Forms and Comparison of Sample Labels and Data Forms	<ul style="list-style-type: none"> Review all data forms for accuracy and completeness. Review all sample labels for accuracy, completeness, and legibility. Verify that the information recorded on the sample labels and tags is consistent with all Sample IDs listed on the Tracking Form.
Before Shipping Samples: Review of Sample Labels and Tracking Forms	<ul style="list-style-type: none"> Complete all tracking forms required for all samples being shipped. Review tracking forms for consistency and correctness. Compare labels on samples with the Sample IDs recorded on the Tracking Form for accuracy and completeness before shipping samples.
Review of Data Forms	<ul style="list-style-type: none"> The Field Crew Leader should review the completed forms in the NRSA App as soon as is practicable to ensure they are complete, and all data forms are consistent and correct Confirm that the forms have been reviewed by selecting the reviewed bubble in the App for each electronic data form. If any revisions are made, re-submit the updated form(s) as soon as possible to update the IM Database. After each submission, a data summary email will be sent to the email address which submitted the data. This data summary contains a list of the data forms and their most recent submissions date/time as well as a list of the most critical data points collected at the site. The Field Crew Leader should review this data summary to ensure that the data forms were successfully received and that critical values are present and correct.

Anchor ID: 999000
 Site ID: NRS23_ _____ Visit #: O1 O2

<p>WATER CHEMISTRY (CHEM) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T1 999000</p> <p>PERIPHYTON BIOMASS (PBIO) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Volume Filtered: _____ mL T1 999002</p> <p>WCHL, PBIO, PCHL - OUTER BAG Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 999001, 999002, 999003 T1</p> <p>PERIPHYTON METAGENOMIC (PDNA) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T3 999005</p> <p>BENTHIC MACROINVERTEBRATES (BERW) (Wadeable Sites) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Jar 1 of ____ T4 999007</p> <p>BACTERIA CULTURE (BCUL) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Jar 1 of ____ T2 999009</p> <p>FISH TISSUE PLUG (FPLG) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T3 999011</p>	<p>CHLOROPHYLL-a (WCHL) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Volume Filtered: _____ mL T1 999001</p> <p>PERIPHYTON CHLOROPHYLL (PCHL) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Volume Filtered: _____ mL T1 999003</p> <p>PERIPHYTON ASSEMBLAGE ID (PERI) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Composite Volume: _____ mL T4 999004</p> <p>ALGAL TOXIN (MICX) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T3 999006</p> <p>BENTHIC MACROINVERTEBRATES (BETB) (Boatable Sites) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 Jar 1 of ____ T4 999008</p> <p>BACTERIA DNA (BDNA) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T2 999010</p> <p>QA VOUCHER (VERT) Site ID: NRS23_ _____ Date: ____/____/202__ Visit #: O1 O2 T5 999012</p>
--	---

Figure 1.1 Example Sample Labels for Sample Tracking and Identification.

2 INTRODUCTION TO WADEABLE SAMPLING

2.1 Daily Operations

Field methods for the NRSA are designed to be completed in one field day for most sites. Depending on the time needed for both the sampling and travel for the day, an additional day may be needed to complete sampling or for pre-departure and post-sampling activities (e.g., cleaning equipment, repairing gear, shipping samples, and traveling to the next site). Remote sites with lengthy or difficult approaches may require more time, and Field Crews will need to plan accordingly.

A Field Crew for a wadeable site will typically consist of four people. Any additional crew members may either help collect samples, or may remain on the bank to provide logistical support. Typically, in wadeable sites, two crew members will work on the “habitat” crew, and two or three will work on the “fish” crew.

A daily field sampling scenario showing how the workload may be split between crew members is presented in **Figure 2.1**. The following sections further define the sampling sequence and the protocols for sampling activities.

Field Crews should define roles and responsibilities for each crew member to organize field activities efficiently. While crews may choose to allocate resources as they see fit, the sequence of sampling events presented in **Figure 2.1** cannot be changed and is based on the need to protect some types of samples from potential contamination and to minimize holding times once samples are collected. For example, at sites where fish collections are expected to take longer than physical habitat assessments, crews may choose to task Group A with *in situ* measurements and water collections, but these tasks need to be completed before any tasks near or directly upstream of the water collection are to begin.

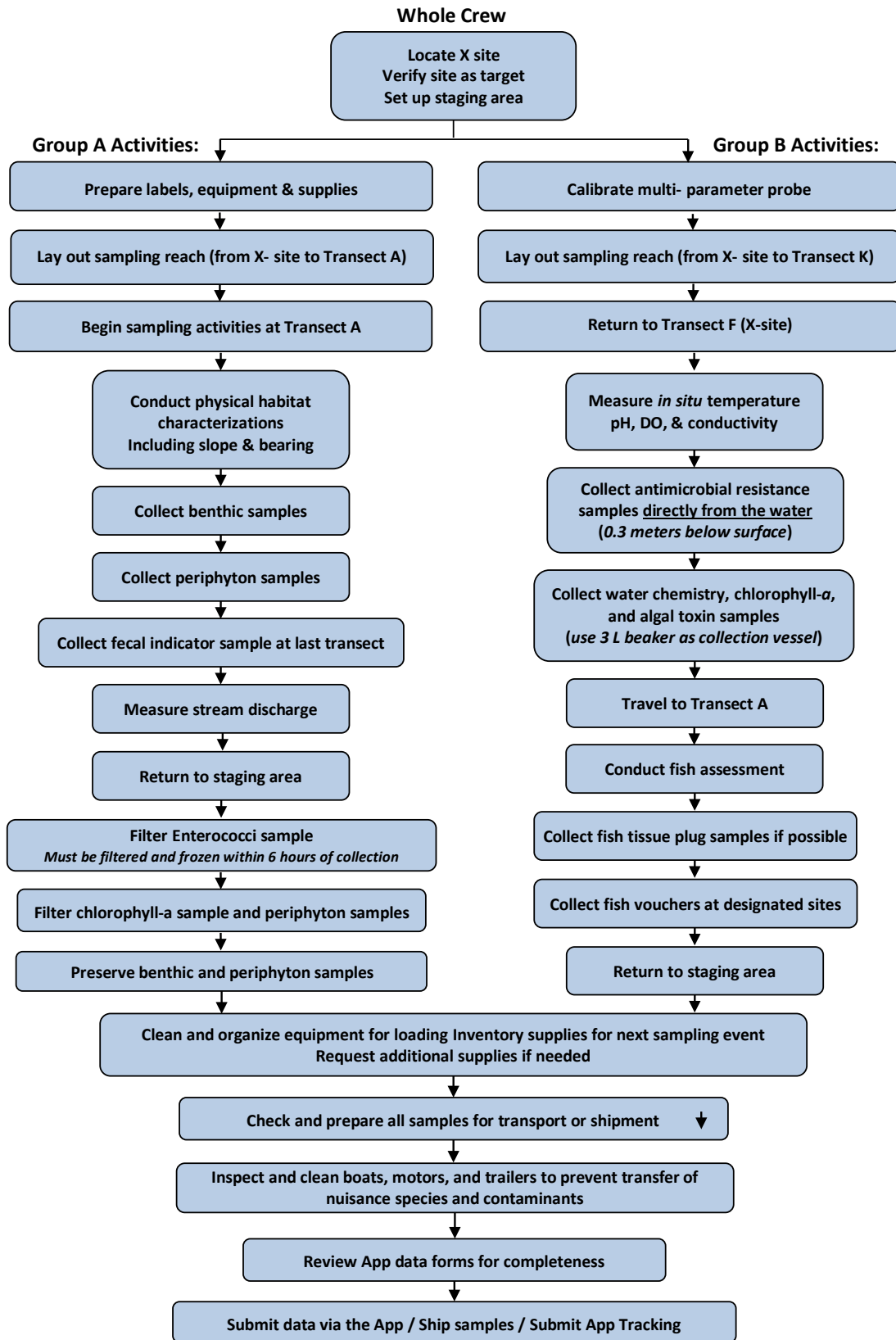


Figure 2.1 Field Sampling Scenario for Wadeable Sites

2.2 Base Site Activities

Field Crews conduct several activities at their base site (i.e., office or laboratory, camping site, or motel). These include tasks that must be completed both before departure to the site and after return from the field (**Figure 2.2**). Close attention to these activities is required to ensure that the Field Crews know: (1) where they are going, (2) that access is permissible and possible, (3) that equipment and supplies are available and in good working order to complete the sampling effort, and (4) that samples are packed and shipped appropriately.

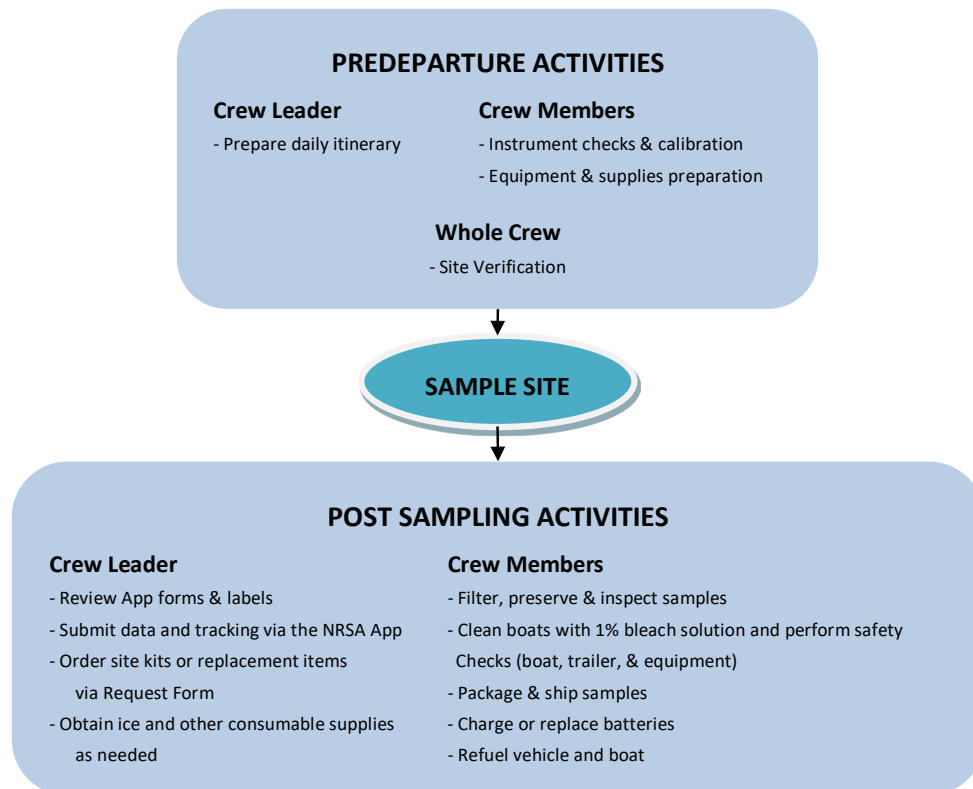


Figure 2.2 Overview of Base Site Activities

2.2.1 Pre-departure Activities

Pre-departure activities include the development of daily itineraries, instrument checks and calibration, and equipment and supply preparation. Procedures for these activities are described in the following sections.

2.2.1.1 Daily Itineraries

The Field Crew Leaders are responsible for developing daily itineraries. This entails compiling maps, contact information, copies of permission letters, and access instructions (a “site packet”). Additional activities include confirming the best access routes, calling the landowners or local contacts, confirming lodging plans, and coordinating rendezvous locations with individuals who must meet with Field Crews prior to accessing a site.

2.2.1.2 Instrument Checks and Calibration

Each Field Crew must test and calibrate instruments prior to sampling. Calibration can be conducted prior to departure for the site or at the site, with the exception of dissolved oxygen (DO) calibration. Because of the potential influence of altitude, **DO calibration is to be performed only at the site.** Field instruments include a global positioning system (GPS) receiver, a multi-probe unit for measuring DO, pH, temperature, and conductivity, and electrofishing equipment. Field Crews should have access to backup instruments if any instruments fail the manufacturer performance tests or calibrations. Prior to departure, Field Crews must:

- Turn on the GPS receiver and check the batteries. Replace batteries immediately if a battery warning is displayed.
- Test and calibrate the multi-probe meter. Each Field Crew should have a copy of the manufacturer's calibration and maintenance procedures. All meters should be calibrated according to manufacturer specifications provided along with the meter.
- Turn on the electrofishing unit and check the batteries. Be sure to have fully charged backup batteries. If using a gas powered electrofishing unit, check the oil and gas supply.

2.2.1.3 Equipment and Supply Preparation

Field Crews must check the inventory of supplies and equipment prior to departure using the equipment and supplies checklists provided in **Appendix A**; use of the lists is mandatory. Specific equipment will be used for wadeable or non-wadeable sites; be sure to bring both sets of equipment if you are unsure what type of site you will be visiting that day. Pack meters, probes, and sampling gear in such a way as to minimize physical shock and vibration during transport. Pack stock solutions as described in **Table 2.1**. Follow the regulations of the Occupational Safety and Health Administration (OSHA) when handling chemicals.

Site kits of consumable supplies for each sampling site will be delivered based on the supply requests each crew submits prior to and during the sampling season. Crews will submit an electronic request form to order site kits, labels, etc. Site kit requests must be submitted at least two weeks before sampling is to take place. If your schedule (and therefore your supply needs) changes, report the change as soon as possible to the Contract Field Logistics Coordinator (Chris Turner, cturner@glec.com, 715-829-3737). The site kit will include sample labels, sample jars, bottles, shipping materials, and other supplies (see complete list in **Appendix A**). The crews must inventory these site kits before departure. Container labels should not be covered with clear tape until all information is completed during sampling at the river/stream. Store at least one extra site kit in the vehicle in the event replacement items are needed immediately.

Table 2.1 Stock Solutions, Uses, and Methods for Preparation

Solution	Use	Preparation										
Bleach (1%)	Clean nets, other gear, and boat.	Add 40 mL bleach to 4L distilled water.										
Bleach (10%)	Clean periphyton sampling equipment.	Add 40 mL bleach to 400 mL distilled water.										
10% Buffered Formalin†	Preservation of periphyton ID sample and fixing Fish Vouchers	<table> <tr> <td>Formaldehyde (37-40%)</td> <td>100 ml</td> </tr> <tr> <td>Distilled water</td> <td>900 ml</td> </tr> <tr> <td>NaH₂PO₄</td> <td>4.0 g</td> </tr> <tr> <td>Na₂HPO₄ (anhydrous)</td> <td>6.5 g</td> </tr> <tr> <td>Mix to dissolve</td> <td></td> </tr> </table>	Formaldehyde (37-40%)	100 ml	Distilled water	900 ml	NaH ₂ PO ₄	4.0 g	Na ₂ HPO ₄ (anhydrous)	6.5 g	Mix to dissolve	
Formaldehyde (37-40%)	100 ml											
Distilled water	900 ml											
NaH ₂ PO ₄	4.0 g											
Na ₂ HPO ₄ (anhydrous)	6.5 g											
Mix to dissolve												
95% Ethanol	Preservative for benthic invertebrate samples and fish vouchers.	No preparation needed (use stock solution as is).										

† 10% Buffered Formalin can also be purchased pre-mixed from various sources

2.2.2 Post Sampling Activities

Upon return to the launching location after sampling, the crew must review all completed data forms and labels for accuracy, completeness, and legibility and make a final inspection of samples. If information is missing from the forms or labels, the Field Crew Leader is to provide the missing information. The Field Crew Leader must review the data summary email received after each data submission. Other post sampling activities include: inspection and cleaning of sampling equipment, supply inventory/reorder, sample and data form shipment, and communications.

2.2.2.1 Review Data Forms and Labels

The Field Crew Leader is ultimately responsible for reviewing all data forms and labels for accuracy and completeness. Ensure that comments use no “shorthand” or abbreviations. The data forms must be thoroughly reviewed by the Field Crew Leader. Upon completing the review, the Field Crew Leader should select the data reviewed button at the top of each form in the App which will run a series of data validation routines to help identify missing or potentially incorrect data. Each sample label must also be checked for accuracy, completeness, and legibility. The Field Crew Leader must cross-check the sample numbers on the labels with those populated on the Tracking Form in the App.

2.2.2.2 Inspect and Prepare Samples

All samples need to be inspected and appropriately preserved and packaged for transport. Check that all samples are labeled, and all labels are filled in completely. Check that each label is covered with clear plastic tape (with the exception of the *Enterococci* labels). Check the integrity of each sample container, and be sure there are no leaks. Make sure that all sample containers are properly sealed. Make sure that all sample containers are properly preserved for storage or immediate shipment.

2.2.2.3 Equipment Cleanup and Check

All equipment and gear must be cleaned and disinfected between sites to reduce the risk of transferring nuisance species and pathogens. Species of primary concern in the U.S. include Eurasian watermilfoil (*Myriophyllum spicatum*), zebra mussels (*Dreissena polymorpha*), New

Zealand mud snails (*Potamopyrgus antipodarum*), *Myxobolus cerebralis* (a sporozoan parasite that causes salmonid whirling disease), and *Batrachochytrium dendrobatidis* (a chytrid fungus that threatens amphibian populations). Field Crews must be aware of regional species of concern, and take appropriate precautions to avoid transfer of these species. There are several online resources regarding invasive species, including information on cleaning and disinfecting gear, such as the Whirling Disease Foundation (www.whirling-disease.org), the USDA Forest Service (*Preventing Accidental Introductions of Freshwater Invasive Species*, available from http://www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf), and the California Dept. of Fish and Game (Hosea and Finlayson 2005). General information about freshwater invasive species is available from the U.S. Geological Survey Nonindigenous Aquatic Species website (<http://nas.er.usgs.gov>), the *Protect Your Waters* website that is co-sponsored by the U.S. Fish and Wildlife Service (<http://www.protectyourwaters.net/hitchhikers>), and the Sea Grant Program (<http://www.sgnis.org>).

Handle and dispose of disinfectant solutions properly, and take care to avoid damage to lawns or other property. **Table 2.2** describes equipment care. Inspect all equipment, including nets, boat trailer, and waders, and clean off any plant and animal material. Inspect, clean, and handpick plant and animal remains from vehicle, boat, motor, trailer and waders. Before moving to the next site, if a commercial car wash facility is available, wash the vehicle, boat, and trailer and rinse thoroughly (hot water pressurized rinse with no soap). Rinse equipment and boat with 1% - 10% bleach solution or other specialized disinfectant to prevent the spread of exotics. Note that many organizations now recommend **against** using felt-soled wading boots in affected areas due to the difficulty in removing myxospores and mudsnails.

2.2.2.4 Supply Inventory

Once a field day is completed, crews should inventory and restock supplies as needed. Ensure that there is a sufficient quantity of site kits to allow sampling at upcoming sites (for at least the next 1-2 weeks). Take note of any supplies that are nearing depletion. Also note any items that may have been lost or damaged during the sampling event. Request additional site kits and/or supplies as needed via the electronic request form. Requests must be made two weeks before needed. Note that not all supplies can be replenished by EPA through the Logistics Contractor, so crews will need to supply some items themselves.

Table 2.2 Post-sampling Equipment Care

Equipment Care after Sampling	
1. Clean for biological contaminants.	
<p>Prior to departing site, drain all water from live wells and buckets used to hold and process fish.</p> <p>Inspect sampling gear, waders, boots, etc. for evidence of mud, snails, plant fragments, algae, animal remains, or debris, and remove using brushes or other tools.</p> <p>At the base location, inspect and rinse seines, dip nets, kick nets, waders, and boots with water and dry. Use one of the procedures below to disinfect gear if necessary.</p> <p>Additional precautions to prevent transfer of Whirling Disease spores, New Zealand mudsnails, and amphibian chytrid fungus is provided below:</p> <p>Before visiting the site, consult the site dossier and determine if it is in an area where whirling disease, New Zealand mud snails, or chytrid fungus are known to exist. Contact the local State fishery biologist to confirm the existence or absence of these organisms.</p> <p>If the stream is listed as “positive” for any of the organisms, or no information is available, <i>avoid using felt-soled wading boots</i>, and, after sampling, disinfect all fish and benthos sampling gear and other equipment that came into contact with water or sediments (i.e., waders, boots, etc.) by one of the following procedures:</p>	
Option A:	
<ol style="list-style-type: none"> 1. Soak gear in a 10% household bleach solution for at least 10 minutes, or wipe or spray on a 50% household bleach solution and let stand for 5 minutes 2. Rinse with clean water (do not use stream water), and remove remaining debris 3. Place gear in a freezer overnight or soak in a 50% solution of Formula 409® antibacterial cleaner for at least 10 minutes or soak gear in 120°F (49°C) water for at least 1 minute. 4. Dry gear in direct sunlight (at least 84 °F) for at least 4 hours. 	
Option B:	
<ol style="list-style-type: none"> 1. Soak gear in a solution of Sparquat® (4-6 oz. per gallon of water) for at least 10 minutes (Sparquat is especially effective at inactivating whirling disease spores). 2. Place gear in a freezer overnight or soak in 120°F (49°C) water for at least 1 minute. 3. Dry gear in direct sunlight (at least 84 °F) for at least 4 hours. 	
2. Clean and dry other equipment prior to storage.	
<p>Rinse coolers with water to clean off any dirt or debris on the outside and inside.</p> <p>Rinse periphyton sampling equipment with tap water at the base location.</p> <p>Make sure conductivity meter probes are rinsed with deionized water and stored moist.</p> <p>Rinse all containers used to collect water chemistry samples three times with deionized water. Place beakers in a 1-gallon sealable plastic bag with a cube container for use at the next stream.</p> <p>Check nets for holes and repair or locate replacements.</p>	
3. Inventory equipment and supply needs and relay orders to the IM Team via the supply request form.	
4. Remove GPS, multi-probe meter, and electrofishing unit from carrying cases and set up for predeparture checks and calibration. Examine the oxygen membranes for cracks, wrinkles, or bubbles. Replace if necessary, allowing sufficient time for equilibration.	
5. Recharge/replace batteries as necessary.	
6. Replenish fuel and oil; if a commercial car wash facility is available, thoroughly clean vehicle and boat (hot water pressurized rinse and no soap).	

2.3 Safety and Health

Collection and analysis of samples can involve significant risks to personal safety and health. This section describes recommended training, communications, and safety considerations, safety equipment and facilities, and safety guidelines for field operations.

2.3.1 General Considerations

Important considerations related to field safety are presented in **Table 2.3**. Please follow your own agency's health and safety protocols, or refer to the *Health and Safety Guidance for Field Sampling: National Rivers and Streams Assessment* (available from the EPA Regional Coordinator) and *Logistics of Ecological Sampling on Large Rivers* (Flotemersch, et al. (editors) 2000). Additional sources of information regarding safety-related training include the American Red Cross (1979), the National Institute for Occupational Safety and Health (1981), U.S. Coast Guard (1987) and Ohio EPA (1990).

Field Crew members should become familiar with the hazards involved with sampling equipment and establish appropriate safety practices prior to using them. They must make sure all equipment is in safe working condition. Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators should meet any state requirements for boat operation and be familiar with U.S. Coast Guard rules and regulations for safe boating contained in a pamphlet, "*Federal Requirements for Recreational Boats*," available from a local U.S. Coast Guard Director or Auxiliary or State Boating Official (U.S. Coast Guard, 1987). **A personal flotation device (PFD) must be worn by crew members at all times on the water.** All boats with motors must have fire extinguishers, boat horns, PFDs or flotation cushions, and flares or communication devices. Boats should stay in visual contact with each other, and should use 2-way radios to communicate.

Primary responsibility for safety while electrofishing rests with the Field Crew Leader. Electrofishing units may deliver a fatal electrical shock, and should only be used by qualified, experienced operators. Field Crew members using electrofishing equipment must be insulated from the water, boat, and electrodes via rubber boots and linesman gloves. All personnel should use chest waders with nonslip soles and linesman gloves. **DO NOT** wear breathable waders while electrofishing. If waders become wet inside, stop fishing until they are thoroughly dry or use a dry pair. Avoid contact with the anode and cathode at all times due to the potential shock hazard. If you perspire heavily, wear polypropylene or some other wicking and insulating clothing instead of cotton. If it is necessary for a crew member to reach into the water to pick up a fish or something that has been dropped, do so only after the electrical current is off and the anode is removed from the water. Do not resume electrofishing until all individuals are clear of the electroshock hazard. Ensure that the backpack electrofishing equipment is equipped with a 45° tilt switch that interrupts the current. Do not make any modifications to the electrofishing unit that would hinder safety. Avoid electrofishing near unprotected people, pets, or livestock. Discontinue activity during thunderstorms or rain. Crew members should keep each other in constant view or communication while electrofishing. For each site, know the location of the nearest emergency care facility. Although the Field Crew Leader has authority, each crew member has the responsibility to question and modify an operation or decline participation if it is unsafe.

Table 2.3 General Health and Safety Considerations

Recommended Training
<ul style="list-style-type: none"> • First aid and cardiopulmonary resuscitation (CPR) • Vehicle safety (e.g., operation of 4-wheel drive vehicles) • Boating and water safety; whitewater safety if applicable • Field safety (weather, personal safety, orienteering, site reconnaissance) • Equipment design, operation, and maintenance • Handling of chemicals and other hazardous materials
Communications
<ul style="list-style-type: none"> ▪ Check-in schedule ▪ Sampling itinerary (vehicle used & description, time of departure & return, travel route) ▪ Contacts for police, ambulance, hospitals, fire departments, search and rescue personnel ▪ Emergency services available near each sampling site and base location ▪ Cell (or satellite) phone and VHF radio if possible
Personal Safety
<ul style="list-style-type: none"> • Field clothing and other protective gear including lifejackets for all crew members • Medical and personal information (allergies, personal health conditions) • Personal contacts (family, telephone numbers, etc.) • Physical exams and immunizations

A communications plan to address safety and emergency situations is essential. All field personnel need to be fully aware of all lines of communication. Field personnel should have a daily check-in procedure for safety. An emergency communications plan should include contacts for police, ambulance, fire departments, hospitals, and search and rescue personnel.

Proper field clothing should be worn to prevent hypothermia, heat exhaustion, sunstroke, drowning, or other dangers. Field personnel must be able to swim, and personal flotation devices must be used. Chest waders made of rubberized or neoprene material must always be worn with a belt to prevent them from filling with water in case of a fall. A PFD and suitable footwear must be worn at all times while on board a boat.

Many hazards lie out of sight in the bottoms of rivers and streams. Broken glass or sharp pieces of metal embedded in the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that can be absorbed through the skin or inhaled may also be present in the water or sediment. Personnel who may be exposed to water known or suspected to contain human or animal wastes that carry causative agents or pathogens must be immunized against tetanus, hepatitis, typhoid fever, and polio. Biological wastes can also be a threat in the form of viruses, bacteria, rickettsia, fungi, or parasites.

2.3.2 Safety Equipment

Appropriate safety apparel such as waders, linesman gloves, safety glasses, etc. must be available and used when necessary. First aid kits, fire extinguishers, and blankets must be readily available in the field. Cellular or satellite telephones and/or portable radios should be provided to Field Crews working in remote areas in case of an emergency. Supplies (e.g., clean water,

antibacterial soap, ethyl alcohol) must be available for cleaning exposed body parts that may have been contaminated by pollutants in the water.

2.3.3 Safety Guidelines for Field Operations

General safety guidelines for field operations are presented in **Table 2.4**. Personnel participating in field activities should be in sound physical condition and have a physical examination annually or in accordance with organizational requirements. All surface waters and sediments should be considered potential health hazards due to potential toxic substances or pathogens. Persons must become familiar with the health hazards associated with using chemical fixing and/or preserving agents. Chemical wastes can be hazardous due to flammability, explosiveness, toxicity, causticity, or chemical reactivity. All chemical wastes must be discarded according to standardized health and hazards procedures (e.g., National Institute for Occupational Safety and Health [1981]; USEPA [1986]).

During the course of field research activities, Field Crews may observe violations of environmental regulations, discover improperly disposed hazardous materials, or observe or be involved with an accidental spill or release of hazardous materials. In such cases it is important that the proper actions be taken and that field personnel do not expose themselves to something harmful. The following guidelines should be applied:

1. First and foremost, protect the health and safety of all personnel. Take necessary steps to avoid injury or exposure to hazardous materials. If you have been trained to take action such as cleaning up a minor fuel spill during fueling of a boat, do it. However, you should always err on the side of personal safety.
2. Field personnel should never disturb or retrieve improperly disposed hazardous materials from the field to bring back to a facility for “disposal”. To do so may worsen the impact, incur personal liability for the crew members and/or their respective organizations, cause personal injury, or cause unbudgeted expenditure of time and money for proper treatment and disposal of the material. Notify the appropriate authorities so they may properly respond to the incident.
3. For most environmental incidents, the following emergency telephone numbers should be provided to all Field Crews: State or Tribal department of environmental quality or protection, U.S. Coast Guard, and the U.S. EPA regional office. In the event of a major environmental incident, the National Response Center may need to be notified at 1-800-424-8802.

Table 2.4 General Safety Guidelines for Field Operations

- Two crew members must be present during all sample collection activities, and no one should be left alone while in the field. Boats should proceed together down the river.
- Use caution when sampling on foot in swift or deep water. Wear a suitable PFD and consider using a safety tether held by an assistant.
- Use extreme care walking on riprap. Rocks can shift unexpectedly and serious falls are possible.
- Field Crew members using electrofishing equipment must be insulated from the water, boat, and electrodes via non-breathable waders and linesman gloves. Use chest waders with nonslip soles.
- Electrofishing units may deliver a fatal electrical shock, and should only be used by qualified, experienced operators.
- Professional quality breathable waders with a belt are recommended for littoral sampling only, and at a safe distance from the electrofishing sampling. Neoprene boots are an alternative, but should have sturdy, puncture resistant soles.
- Exposure to water and sediments should be minimized as much as possible. Use gloves if necessary, and clean exposed body parts as soon as possible after contact.
- All electrical equipment must bear the approval seal of Underwriters Laboratories and must be properly grounded to protect against electric shock.
- Use heavy gloves when hands are used to agitate the substrate during collection of benthic macroinvertebrate samples.
- Use appropriate protective equipment (e.g., gloves, safety glasses) when handling and using hazardous chemicals.
- Crews working in areas with venomous snakes must check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a poisonous snake.
- Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, oak, sumac, etc.) must take proper precautions and have any needed medications handy.
- Field personnel should also protect themselves against deer or wood ticks because of the potential risk of acquiring pathogens that cause Rocky Mountain spotted fever, Lyme disease, and other illnesses.
- Field personnel should be familiar with the symptoms of hypothermia and know what to do in case symptoms occur. Hypothermia can kill a person at temperatures much above freezing (up to 10°C or 50°F) if he or she is exposed to wind or becomes wet.
- Field personnel should be familiar with the symptoms of heat/sun stroke and be prepared to move a suffering individual into cooler surroundings and hydrate immediately.
- Handle and dispose of chemical wastes properly. Do not dispose any chemicals in the field.

2.4 Forms (NRSA App)

Forms are the key to data collection and tracking for the NRSA 2023/24. Field Crews must use electronic forms to submit their field data. If the NRSA App or iPad isn't available, collect information on the provided paper data forms but data must be transferred to the App to be submitted.

2.4.1 Field Forms

Field forms are the primary documents where crews record measures, observations, and collection information during the course of the field day. Additional information regarding specifics of data entry is contained in **Section 1.6**.

- **Electronic Field Forms:** This form of data collection can be collected through an Apple iOS portable electronic device (tablets). The field forms will be optimized for tablet devices. The App will be preloaded on all EPA-owned iPads prior to distribution to Field Crews or can be downloaded from the Apple App Store and installed on the Field Crews'

iPads. It is important for a Field Crew to familiarize themselves with the App prior to field sampling.

2.4.2 Tracking Form and Packing Slips

The Tracking Form in the App describes the status and location of all samples collected during NRSA 2023/24. Field Crew Leaders will submit the pertinent portion(s) of this form electronically through App submissions) whenever samples are shipped. See **APPENDIX C: SHIPPING GUIDELINES** for more information.

Packing slips (**Figure 2.3**) are postcard sized slips pre-populated with sample IDs that match the sample labels. Packing slips are included in the site kit with sample labels. Packing slips are to accompany samples sent to any of the national labs.

T1: NRSA23/24 Daily Water Chemistry Sample Tracking
Site ID: NRS23_ Visit #: 1 2
Date Collected: ___/___/___
Sample ID Lab Comments (For lab staff only)

CHEM	999000	
WCHL	999001	
PBIO	999002	
PCHL	999003	

Rec'd by: _____ Date Rec'd: _____

T2: NRSA23/24 Daily Bacteria Sample Tracking
Site ID: NRS23_ Visit #: 1 2
Date Collected: ___/___/___
Lab Staff: Samples In Box? Sample ID Lab Comments (For lab staff only)

<input type="checkbox"/>	BCUL	999009	
<input type="checkbox"/>	BDNA	999010	
Please cross out sample id(s) not included in shipment.			
<input type="checkbox"/>	AMR Field Blank included in shipment	<input type="checkbox"/>	

T3: NRSA23/24 Frozen Batched Tracking
Site ID: NRS23_ Visit #: 1 2
Date Collected: ___/___/___
Lab Staff: Samples In Box? Sample ID Lab Comments (For lab staff only)

<input type="checkbox"/>	ENTE	999013	
<input type="checkbox"/>	PDNA	999005	
<input type="checkbox"/>	MICX	999006	
<input type="checkbox"/>	FPLG	999011	

Please cross out sample id(s) not included in shipment.

T4: NRSA23/24 Non-Chilled Batched Tracking
Site ID: NRS23_ Visit #: 1 2
Date Collected: ___/___/___
Lab Staff: Samples In Box? Sample ID Lab Comments (For lab staff only)

<input type="checkbox"/>	BERW	999007	
<input type="checkbox"/>	BETB	999008	
<input type="checkbox"/>	PERI	999004	

Please cross out sample id(s) not included in shipment. 9901360974

Figure 2.3 Example Sample Packing Slips to Accompany Sample Shipments

2.4.3 Equipment and Supplies

2.4.3.1 Request Form

Field Crews will submit requests for site kits or other supplies via an electronic request form (Figure 2.4). This form will be submitted to the NARS IM Coordinator who will ensure that the request reaches the appropriate entity. Crews should submit the Request Form at least two weeks prior to their desired sampling date. In addition to typing in specific requests, crews may select one or more of the pre-populated items listed below:

- Site kit: contains all consumable supplies for one site, sample labels, packing slips, FedEx shipping labels to PESD-AL and EPA-Cincinnati, coolers (for sending immediate samples to PESD-AL and EPA-Cincinnati), and cooler liners.
- Frozen batched box: contains box, two-piece dry ice liner, Class 9 hazardous label, FedEx label to GLEC.
- Non-chilled batched cooler: contains cooler, cooler liner, FedEx label to GLEC

NRSA23/24 Request Form		
Request Date: ____ / ____ / ____	Ship to:	
Requester: _____	Name: _____	
State: _____ Crew: _____	Company: _____ <small>OR: HOTEL/FIELD LOCATION</small>	
Phone: _____ - _____ - _____	Address: _____ <small>NO PO BOXES ALLOWED</small>	
Email: _____ <small>REQUESTER EMAIL</small>	City: _____ State: _____	
	Zip Code: _____	
	Email: _____ <small>SHIP TO EMAIL, IF DIFFERENT THAN REQUESTER</small>	
Supplies Needed (Mark all that apply):		
<input type="radio"/> Site Kit (2 coolers with all bottles and consumables for 1 site, including label packet and packing slips, T1 & T2 shipping labels, and 2 cooler liners)		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Frozen Batched Dry Ice box - up to 4 sites max (includes box, 2-piece dry ice liner, T3 shipping label, and Class 9 hazardous label)		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Non-chilled batch cooler (includes 1 cooler, T4/T5 shipping label and cooler liner)		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Replacement Label Packet(s)		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Foil squares (aluminum) - pack of 25		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> 1L Benthic/Fish Voucher Bottles (HDPE, white, wide-mouth)		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Sodium Thiosulfate Tablets - vial of 25		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
<input type="radio"/> Tape strips - packs of 25		
How many? _____	Need by: ____ / ____ / ____	
Comments: _____		
Don't see your item listed above? List items separately below. Refer to equipment lists in FOM for correct terminology		
Item: _____	How many? _____	Need by: ____ / ____ / ____
Item: _____	How many? _____	Need by: ____ / ____ / ____
Item: _____	How many? _____	Need by: ____ / ____ / ____
Item: _____	How many? _____	Need by: ____ / ____ / ____

Figure 2.4 Electronic Request Form

2.4.3.2 Base Kit

The Base Kit is comprised of the subset of durable equipment and supplies needed for NRSA 2023/24 sampling that is provided by EPA through the Contract Field Logistics Coordinator. Typically, one Base Kit is provided to each Field Crew and contains some of the equipment that is used throughout the field season. See **APPENDIX A: EQUIPMENT & SUPPLIES** for a list of the items provided by EPA in the Base Kit.

2.4.3.3 Site Kit

A Site kit contains the subset of consumable supplies (i.e., items used up during sampling or requiring replacement after use) provided by EPA through the Field Logistics Contractor. The site kit will contain all the sample bottles and labels necessary for sampling a single site. A new site kit is provided (upon request) for each site sampled. See **APPENDIX A: EQUIPMENT & SUPPLIES** for the consumable items that will be provided by EPA.

2.4.3.4 Field Crew Supplied Items

The Field Crew will also supply particular items for the field sampling day. These items might include supplies from a previous NRSA, typical field equipment (like a GPS), or a boat. See **APPENDIX A: EQUIPMENT & SUPPLIES** for the items that the Field Crew will need to provide.

3 INITIAL SITE PROCEDURES

When you arrive at a site, you must first confirm that you are at the correct site, and then determine if the site meets the criteria for sampling and data collection activities (See Site Evaluation Guidelines EPA-841-B-22-005). Inspect the selected reach for appropriate access, safety, and general conditions. Decide whether the site is at base flow condition and not unduly influenced by rain events which could affect the representativeness of field data and samples. If you determine that the site can be sampled, lay out a defined reach within which all sampling and measurement activities are conducted.

All field data will be recorded and submitted through the NRSA App. As a Crew begins to sample a site, they access the App and select the correct state, site ID, and visit number (visit 1 for all sampling events except the second visit to a revisit site). The final selection before data entry may begin is the selection of wadeable versus boatable sampling methods (see following section). The choice of wadeable or boatable will allow the user to access the correct set of field forms for the given sampling method.

The first form that is typically completed is the Verification Form. Upon opening this form, enter the sampling date by using the “today” button or by entering the date using the specified format. If a site is sampled over multiple days, use the date the water chemistry sample was collected as the official sampling date. Typically many key site activities will take place on this date, which is why it will be used as the official sampling date. The date entered on the Verification Form will populate to other forms as well, but those dates can be changed manually if needed. Enter or verify the site name (if known) and enter the crew ID that was assigned to the sampling crew prior to the field season.

3.1 Site Verification Activities

3.1.1 Locating the X-Site

River and stream sampling points were chosen using the medium resolution National Hydrography Dataset (NHD), in particular NHD-Plus HR, following a systematic randomized selection process (Stevens and Olsen, 2004). Each point is referred to as the “X-site.” The “X-site” is the mid-point of the sampling reach, and it will determine the location and extent for the rest of the sampling reach. The latitude/longitude of the “X-site” is listed on the site evaluation spreadsheet that was distributed to each Field Crew Leader. **Table 3.1** provides the equipment and supplies needed for site verification.

Note that the coordinates provided on the site evaluation spreadsheet may not be located in the middle of the stream or river; and in some cases, the coordinates may be on dry land next to the stream or river. In these cases, it is important for crews to locate the X-site at a point that is in the middle of the stream or river (e.g., midway between the left and right banks). To do this, simply measure the distance between banks and move the point perpendicular to the nearest bank until it is half-way between the left and right banks. Record these coordinates as the X-site on the Verification Form in the App in the Measured Latitude and Longitude fields. Also record the location of the coordinates (X-site in the case of wadeable sites), the number of satellites your GPS is using and all methods used to verify the site. If the provided coordinates are located on dry land near a stream, move the coordinates to the nearest blue line in NHD-Plus HR during the desktop reconnaissance. Note this movement on the site evaluation spreadsheet and in the comments section of the Verification Form.

Table 3.1 Equipment and Supplies: for Site Verification

<p>For locating and verifying site</p>	<p>Sampling permit and landowner access (if required) Field Operations Manual (PDF Format loaded onto iPad) Site dossier, including access information, site spreadsheet with map coordinates, street and/or topographic maps with “X-site” marked NRSA 2023/24 Community Fact Sheet GPS unit (preferably one capable of recording waypoints) with manual, reference card, extra battery pack Surveyor’s flagging tape (to mark transects) Laser rangefinder 50 m or 100 m measuring tape with reel (if not using rangefinder)</p>
<p>For recording measurements</p>	<p>Verification Form in NRSA App Fine-tipped indelible markers to write on flagging</p>

3.1.2 Determining the Sampling Status of a Stream

After you confirm the location of the X-site, evaluate the stream reach surrounding the X-site and classify the stream into one of three major sampling status categories: sampleable, non-sampleable, or no access (see **Table 3.2**). The primary distinction between “Sampleable” and “Non-Sampleable” streams is based on the presence of a defined stream channel, water content during base flow, and adequate access to the site. By using the information below, the Field Crew will ultimately determine if the site will be sampled or not. In the App, select Yes on No to the question “Did you sample this site”. The selection will open either a list of sampling methods or a list of specific reasons why the site was not sampled.

There must be greater than 50% water throughout the channel reach. If the channel is dry at the X-site, determine if water is present within 20 channel-widths upstream and downstream of the X-site – for small systems, 150 m is the minimum reach length that can be sampled, so the upstream and downstream lengths would be 75 meters each. If there are isolated pools of water within the reach that equal greater than 50% of the reach length, proceed to sample using the modified procedures outlined in **Section 3.1.1**. Do not drop the site if it is dry at the X site, as long as there is greater than 50% water throughout the channel. If less than 50% of the reach has water, classify the site as “Dry-visited” on the Verification Form in the App. NOTE: Do not “slide” the reach (**Section 3.2.1**) for the sole purpose of obtaining more water to sample (e.g., the downstream portion of the reach has water, but the upstream portion does not).

Record the sampling status and pertinent site verification information on the Verification Form in the App. If the site is non-sampleable or inaccessible, no further sampling activities are conducted. Replace the site with the appropriate replacement site (**Section 1.3**).

Table 3.2 Procedure: Site Verification

Site Verification Procedures	
	<ol style="list-style-type: none"> Find the stream/river location in the field corresponding to the X-site coordinates. Record the routes taken and other directions on the Verification Form so that others can visit the same location in the future. If the site is non-wadeable, locate public or private launch sites. Use a GPS receiver to confirm the latitude and longitude at the X-site with the coordinates provided for the site (datum = NAD83). Record these on the Verification Form. Use all available means to ensure you are at the correct stream/river as marked on the map, including 1:24,000 United States Geological Society (USGS) maps, topographic landmarks, road maps, signs, local contacts, etc. Scan the channel upstream and downstream from the X-site, decide if the site is sampleable, and mark the appropriate bubble on the Verification Form. If the channel is dry at the X-site, determine if water is present within 20 channel-widths upstream and downstream of the X-site (150 m is minimum sampling reach length, so in small systems the upstream and downstream lengths would be 75 meters each). Assign one of the following sampling status categories to the stream. Record the category on the Verification Form.
Sampleable Categories	
	<p><u>Wadeable</u>: Continuous water, sampled by wading.</p> <p><u>Boatable</u>: Continuous water, too deep to sample by wading.</p> <p><u>Partial wadeable</u>: Sampled by wading (>50% of reach sampled).</p> <p><u>Partial boatable</u>: Sampled by boat (>50% of reach sampled).</p> <p><u>Wadeable Interrupted</u>: not continuous water along reach, >50% water in reach.</p> <p><u>Boatable Interrupted</u>: not continuous water along reach, >50% water in reach.</p> <p><u>Altered Channel</u>: Stream/river channel present but differs from map.</p>
Non-Sampleable Categories	
	<p style="text-align: center;"><i>PERMANENT</i></p> <p><u>Dry Channel</u>: Less than 50% water within the reach. Record as “Dry-Visited.” If site was determined to be dry (or otherwise non-perennial) from another source and/or field verified before the actual sampling visit, record as “Dry-Not visited” in the site evaluation spreadsheet.</p> <p><u>Wetland</u>: Standing water present, but no definable stream channel. If wetland is surrounding a stream channel, define the site as Target but restrict sampling to the stream channel.</p> <p><u>Map Error</u>: No evidence that a water body or stream channel was ever present at the X-site.</p> <p><u>Impounded stream</u>: Stream is submerged under a lake or pond due to manmade or natural (e.g., beaver dam) impoundments. If the impounded stream is still wadeable, record it as “Altered” and sample.</p> <p><u>Other</u>: Examples would include underground pipelines, or a non-target canal. A sampling site must meet both of the following criteria to be classified as a non-target canal: The channel is constructed where no natural channel has ever existed. The sole purpose/usage of the reach is to transfer water. There are no other uses of the waterbody by humans (e.g., fishing, swimming, and boating).</p> <p style="text-align: center;"><i>TEMPORARY</i></p> <p><u>Not Boatable</u>: need a different crew.</p> <p><u>Not Wadeable</u>: need a different crew.</p>

Other: The site could not be sampled on that particular day, but is still a target site. Examples might include a recent precipitation event that has caused unrepresentative conditions.

No Access to Site Categories

Access Permission Denied: You are denied access to the site by the landowner.

Permanently Inaccessible: Site is unlikely to be sampled by anyone due to physical barriers that prevent access to the site (e.g., cliffs).

Temporarily Inaccessible: Site cannot be reached due to barriers that may not be present at a future date (e.g. forest fire, high water, road temporarily closed, unsafe weather conditions).

Other: explain in the comments section.

6. Do not sample non-target or “Non-sampleable” or “No Access” sites. Select the “NO” bubble for “Did you sample this site?” and mark the appropriate bubble in the “Non-Sampleable” or “No Access” section of the Verification Form; provide a detailed explanation in comments section.
7. If the reason for not sampling the site is a temporary condition, the site should be scheduled for sampling at a later time. The subsequent sampling event, if successful, will use the same visit number and the crew will simply update the previous information recorded on the Verification Form and submit the form with the new data.

3.1.3 Elevation at Transect A

Record the elevation at Transect A using your GPS device. To record this information, record the elevation holding the GPS at approximately 3 feet above the surface of the water. Ensure that the numbers are properly recorded from Transect A on the Verification Form in the App. You will use this same method to record the elevation at Transect K at the end of the sampling day and record that value on the Assessment Form.

3.1.4 Sampling During or After Rain Events

Avoid sampling during high flow rainstorm events. Use your best professional judgement to determine if the stream has risen above baseflow during this recent rain event. It is often unsafe to be in the water during such times. In addition, biological and chemical conditions during such episodes are often quite different from those during baseflow. On the other hand, sampling cannot be restricted to only strict baseflow conditions. It would be next to impossible to define “strict baseflow” with any certainty at an unstudied site. Such a restriction would also greatly shorten the index period when sampling activities can be conducted. Thus, some compromise is necessary regarding whether to sample a given stream because of storm events. To a great extent, this decision is based on the judgment of the Field Crew. Some guidelines to help make this decision are presented in **Table 3.3**. The major indicator of the influence of storm events will be the condition of the stream itself. **If you decide a site is unduly influenced by a storm event, do not sample the site that day.**

Table 3.3 Guidelines to Determine the Influence of Rain Events

- If it is running at bank full discharge or the water seems much more turbid than typical for the class of stream do not sample it that day.
- Do not sample that day if it is unsafe to be in the water.
- Keep an eye on the weather reports and rainfall patterns. Do not sample a stream during periods of prolonged heavy rains.
- If the stream seems to be close to normal summer flows, and does not seem to be unduly influenced by storm events, sample it even if it has recently rained or is raining.

3.1.5 Site Photographs

Taking site photographs is an optional activity, but should be considered if the site has unusual natural or manmade features associated with it. If you do take photographs with a digital camera at a site, date stamp the photograph and include the site ID. Most cell phone cameras also have the ability to attach geographical location data to a particular picture. Alternatively, start the sequence with one photograph of an 8.5 × 11 inch piece of paper with the site ID, waterbody name, and date printed in large, thick letters. After the photo of the site ID information, take at least two photographs at the X-site, one in the upstream direction and one downstream. Take any additional photos you find interesting after these first three pictures. Keep a log of your photographs and briefly describe each one. Photographs can be uploaded to the NARS SharePoint site.

3.2 Laying out the sampling reach

Many of the biological and habitat structure measures require sampling a certain length of a stream to get a representative picture of the ecological community. **A length of 40 times the average wetted width is necessary to characterize the habitat and several biotic assemblages associated with the sampling reach.** Establish the sampling reach about the X-site using the procedures described in **Table 3.4** (wadeable sites).

When you arrive at the site, scout the sampling reach to make sure it is clear of obstacles that would prohibit sampling and data collection activities. Record the channel width used to determine the reach length, and the sampling reach length upstream and downstream of the X-site on the Verification Form in the App. If you did not have to slide the reach, the upstream and downstream lengths will be equal.

Figure 3.1 illustrates the principal features of the established sampling reach for wadeable sites, including the location of 11 cross-section transects used for collecting samples and physical habitat measurements. The figure also shows the specific sampling stations on each transect for collection of periphyton and benthic macroinvertebrate samples.

For each site, crews may prepare an optional map of the reach sampled to help explain specific characteristics or sampling decisions. This can be drawn by hand or produced digitally with any number of mapping programs. In addition to any other interesting features that should be marked on the map, note any landmarks/directions that can be used to find the X-site for future visits. The optional map can be uploaded to the NARS SharePoint site. If a map is uploaded, check the “Crew scanned or submitted a site sketch” box on the Verification Form in the App.

Table 3.4 Procedure: Laying Out the Sampling Reach at Wadeable Sites

1. **Locate the X-site using the coordinates provided for the site.**
2. Use a surveyor's rod, tape measure, or laser range finder to **determine the wetted width of the channel at five places of "typical" width** within approximately five channel widths upstream and downstream from the X-site. **Average the five readings together and round to the nearest 1 m.**
3. **Multiply the average wetted width by 40 to determine the reach length.** If the average width is <4 m, use **150 m as a minimum reach length**. If the average width is >100 m, use **4 km as a maximum reach length**. Record both the average channel width and total intended reach length on the Verification Form in the App.
4. *For channels with "interrupted flow", estimate the width based on the unvegetated width of the channel (again, with a 150 m minimum and 4 km maximum).*
5. **Check the condition of the stream about the X-site** by having one crew member go upstream and one downstream. Each person proceeds until they can see the stream to a distance of 20 times the average channel width (equal to one-half the sampling reach length) determined in Step 3.
6. **Determine if the reach needs to be adjusted** about the X-site due to confluences with higher order streams (downstream), transitions into lower order streams (upstream), impoundments (lakes, reservoirs, ponds), physical barriers (e.g., falls, cliffs), or because of access restrictions to a portion of the initially determined sampling reach. Refer to **Table 3.5**. Record the upstream and downstream reach lengths on the Verification Form in the App.
7. Starting at the X-site (or the new midpoint of the reach if it had to be adjusted as described in Step 6), measure a distance of one-half the reach length down one side of the stream using a GPS unit, laser rangefinder, or tape measure. Be careful not to "cut corners". Enter the channel to make measurements only when necessary to avoid disturbing the stream channel prior to sampling activities. This endpoint is the downstream end of the reach, and is flagged as Transect A.
8. At Transect A, use the seconds display on a digital watch or other random number selection method to select the initial sampling station for standard transect samples: 1-3="Left", 4-6="Center", 7-9=Right. Mark "L", "C", or "R" on the transect flagging; the three potential collection points are roughly equivalent to 25%, 50%, and 75% of the channel width, respectively. Note that left and right sides of the stream are determined while you are facing downstream. It is at these locations that you will collect benthic macroinvertebrate and periphyton samples.
9. Measure 1/10 of the required reach length upstream from Transect A. Flag this spot as Transect B. Assign the sampling station systematically after the first random selection following the repeating pattern L, C, R as you move upstream (**Figure 3.1**).
10. Proceed upstream with the tape measure and flag the positions of nine additional transects (labeled "C" through "K" as you move upstream) at intervals equal to 1/10 of the reach length. Continue to assign the sampling stations systematically.

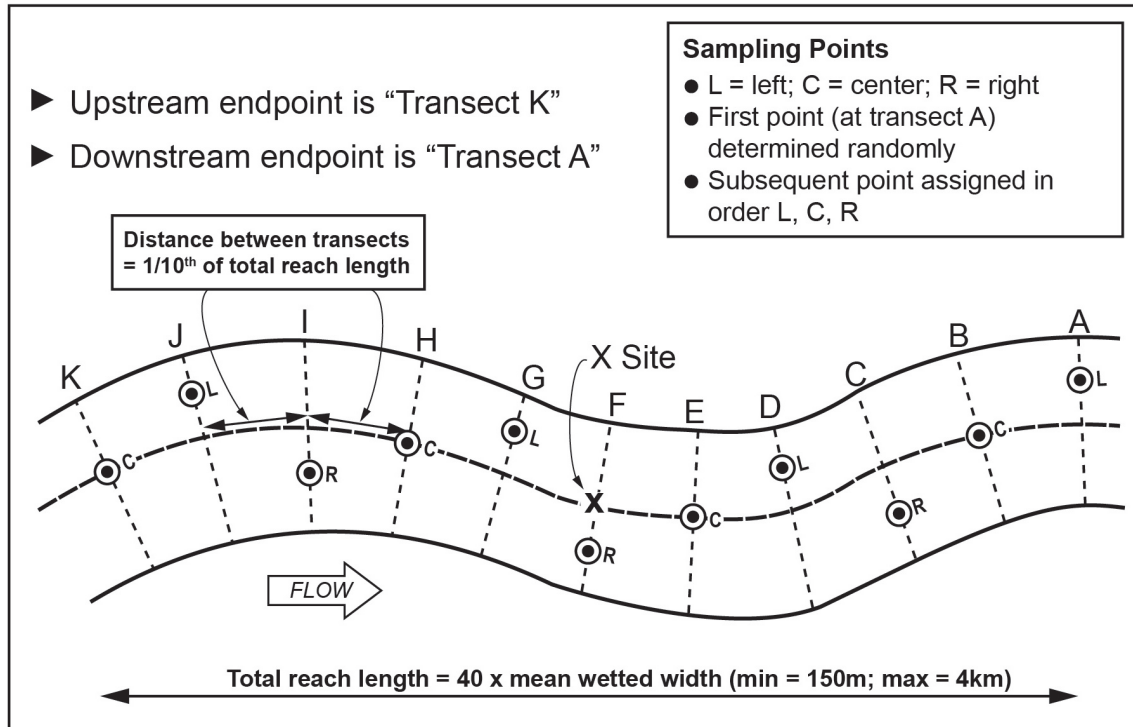


Figure 3.1 Sampling Reach Features for a Wadeable Site

3.2.1 Sliding the Reach

There are some conditions that may require sliding the reach about the X-site (i.e., the X-site is no longer located at the midpoint of the reach) to avoid features we do not wish to or physically cannot sample across. Reasons for sliding the reach include:

- Lack of landowner permission.
- Confluence with higher order waterbody.
- Impoundment.
- Impassable barrier.

Sliding the reach involves noting the distance of the barrier, confluence, or other restriction from the X-site, and flagging the restriction as the endpoint of the reach. Add the distance to the other end of the reach, such that the total reach length remains the same, but it is no longer centered about the X-site. **Table 3.5** describes when you should and should not slide the sampling reach. Record the upstream and downstream reach lengths on the Verification Form in the App.

Table 3.5 Procedure: Sliding the Sampling Reach

<ol style="list-style-type: none">1. Slide the reach if you run into an impoundment (lake, pond, or reservoir), so that the lake/stream confluence is at one end.2. Slide the reach if you run into an impassible barrier (e.g., waterfall, cliff, navigation dam) so that the barrier is at one end.3. Slide the reach if you run into a confluence (another stream meeting the water-body you are sampling) with a higher Strahler Order.4. When you are denied access permission to a portion of the reach, you can slide the reach to make it entirely accessible; use the point of access restriction as the endpoint of the reach.5. Note the distance of the barrier, confluence, or other restriction from the X-site, and flag the restriction as the endpoint of the reach. Add the distance to the other end of the reach, so the total reach length remains the same, but it is no longer centered about the X-site.6. Do not slide the reach so that the X-site falls outside of the reach boundaries.7. Do not proceed upstream into a lower order stream or downstream into a higher order stream when laying out the stream reach (order is based on 1:100,000 scale maps).8. Do not slide a reach to avoid man-made obstacles such as bridges, culverts, rip-rap, or channelization. These represent important features and effects to study.9. Do not slide a reach to gain more water to sample if the flow is interrupted.10. Do not slide a reach to gain better habitat for benthos or fish.

3.3 Modifying Sample Protocols for High or Low Flows

3.3.1 Streams with Interrupted Flow

You cannot collect the full complement of field data and samples from streams that are categorized as “Interrupted” (**Table 3.6**). Note that no data should be collected from streams that are completely “Dry” as defined in **Table 3.6**. Interrupted streams will have some cross-sections amenable to biological sampling and habitat measurements and some that are not. To be considered target, streams must have greater than 50% water in the reach length within the channel (can be isolated pools). Modified procedures for interrupted streams are presented in **Table 3.6**. Samples for water chemistry (**Section 6**) will be collected at the X-site (even if the reach has been adjusted by “sliding” it). If the X-site is dry and there is water elsewhere in the sample reach, collect the sample from a location having water with a surface area >1 m² and a depth >10 cm.

Collect data for the physical habitat indicator along the entire sample reach from interrupted streams, regardless of the amount of water present at each of the transects. Obtain depth measurements along the deepest part of the channel (the “thalweg”) along the entire sampling reach to provide a record of the “water” status of the stream for future comparisons (e.g., the percent of length with intermittent pools or no water). Other measurements associated with characterizing riparian condition, substrate type, etc., are useful to help infer conditions in the stream when water is flowing.

Table 3.6 Reach Layout Modifications for Interrupted Streams

Streams with less than 50% of the reach length containing water (not necessarily continuous) are considered dry and are not sampled.

If more than 50% of the channel has water and if the X-site is dry but there is flowing water or a pool of water having a surface area > 1 m² and a depth > 10 cm somewhere along the defined sampling reach, take the water sample at the pool or flowing water location that is nearest to the X-site. Note that the sample wasn't collected at the X-site and where on the reach the sample was collected on the field data form.

Do not collect a water sample if there is no acceptable location within the sampling reach. Mark the no sample collected box for the water chemistry sample on the Sample Collection Form in the App and use the comment bubble to explain why the sample was not collected.

Physical Habitat, Periphyton and Benthic Macroinvertebrates

Obtain a complete thalweg profile for the entire reach. At points where the channel is dry, record depth as 0 cm and wetted width as 0 m.

At each of the transects (cross-sections), sample the stream depending on flow status:

DRY CHANNEL: No surface water anywhere in cross-section; collect all physical habitat data. Use the unvegetated area of the channel to determine the channel width and the subsequent location of substrate sampling points. Record the wetted width as 0 m. Record substrate data at the sampling points located in the unvegetated, but dry, channel. Do not collect periphyton or benthic macroinvertebrates from this transect.

DAMP CHANNEL: No flowing water at transect, only puddles of water < 10 cm deep; collect all physical habitat data. Do not collect periphyton or benthic macroinvertebrates from this transect.

WATER PRESENT: Transect has flow or pools > 10 cm deep; collect all data and measurements for physical habitat, periphyton, benthic macroinvertebrate, and fish indicators, using standard procedures.

3.3.2 Braided Rivers and Streams

Depending upon the geographic area and/or the time of the sampling visit, you may encounter a stream having “braided” channels, which are characterized by numerous sub-channels that are generally small and short, often with no obvious dominant channel. If you encounter a braided stream, establish the sampling reach using the procedures presented in **Table 3.7**. Figuring the mean width of extensively braided rivers and streams for purposes of setting up the sampling reach length is challenging. For braided channels, measure the mean width and bankfull width as defined in the physical habitat protocols (**Section 10**). For relatively small streams (mean bankfull width ≤15 m) the sampling reach is defined as 40 times the mean bankfull width. For larger streams (>15 m), sum the actual wetted width of all the braids and use that as the width for calculating the 40 x channel width reach length. If there is any question regarding an appropriate reach length for the braided system, it is better to overestimate. Make detailed notes on the Verification Form in the NRSA App about what you did. Also, add additional details to the optional site map and submit it to EPA via the NARS SharePoint site. It is important to remember that the purpose of the 40 x channel width reach length is to sample enough streams to incorporate the variability in habitat types. Generally, the objective is to sample a long enough stretch of a stream to include two to three meander cycles (about six pool riffle habitat sequences). In the case of braided systems, the objective of this protocol modification is to avoid sampling an excessively long stretch of stream. In a braided system where there is a 100 m wide active channel (giving a 4 km reach length based on the standard procedure) and only 10 m

of wetted width (say five, 2 m wide braids), a 400 m long sample reach length is likely to be sufficient, especially if the system has fairly homogenous habitat throughout its length.

Table 3.7 Procedure: Modifications for Braided Rivers and Streams

1. Estimate the mean width as the bankfull channel width as defined in the physical habitat protocol.
 - If the mean width is ≤ 15 m, set up a 40 x channel width sample reach in the normal manner, using the mean bankfull width for your calculations.
 - If > 15 m, sum up the actual wetted width of all the braids and use that as the width for calculating the 40 x channel width reach length. Remember the minimum reach length is always 150 m.
 - If the reach length seems too short for the system in question, set up a longer sample reach, taking into consideration that the objective is to sample a long enough stretch of a stream to include at least two to three meander cycles (about six pool riffle habitat sequences).
2. Make detailed notes on the Verification Form and optional site map about what you did.

4 *In Situ* Measurements of Dissolved Oxygen, pH, Temperature, and Conductivity

4.1 Summary of Method

Measure *in situ* DO, pH, water temperature, and conductivity using a calibrated multi-parameter water quality meter (or sonde). Take the measurements mid-channel at the X-site. Take the readings at 0.5 m depth. Measure the site depth accurately before taking the measurements. If the depth at the X-site is less than 1 meter, take the measurements at mid-depth. Take care to avoid the probe contacting bottom sediments, as the instruments are delicate. Record the measurements on the Field Measurement Form in the App.

4.2 Equipment and Supplies

Table 4.1 provides the equipment and supplies needed to measure dissolved oxygen, pH, temperature, and conductivity.

Table 4.1 Equipment and Supplies: DO, pH, Temperature, and Conductivity

For taking measurements and calibrating the water quality meter	Multi-parameter water quality meter with pH, DO, temperature, and conductivity probes. Extra batteries De-ionized (DI) and tap water Calibration cups and standards Barometer or elevation chart to use for calibration
For recording measurements	Field Measurement Form in NRSA App

4.2.1 Multi-Probe Sonde

Dissolved Oxygen Meter

Calibrate the DO meter prior to each sampling event. It is recommended that the probe be calibrated in the field against an atmospheric standard (e.g., ambient air saturated with water). Note that DO should always be calibrated at the site and should never be calibrated at your base location. Follow your manufacturer’s guidelines for calibration of the DO probe.

pH Meter

Calibrate the pH meter prior to each sampling event. Calibrate the meter in accordance with the manufacturer’s instructions and with the crew agency’s existing Standard Operating Procedures (SOP). Ideally, a quality control solution (QCS) should be used that is similar in ionic strength to the water samples you will be measuring.

Temperature Meter

Check the accuracy of the sensor against a thermometer that is traceable to the National Institute of Standards and Technology (NIST) at least once per sampling season and record the NIST thermometer reading and the sensor reading on the Field Measurement Form. These same values can be recorded each time for the Field Measurement Form unless the accuracy check is conducted again. The entire temperature range encountered in the NRSA should be incorporated in the testing procedure and a record of test results kept on file.

Conductivity Meter

Calibrate the conductivity meter prior to each sampling event. Calibrate the meter in accordance with the manufacturer's instructions. Ideally, a QCS solution should be used that incorporates the entire conductivity range encountered in the NRSA and a record of test results kept on file.

4.3 Sampling Procedure

Table 4.2 presents step by step procedures for measuring dissolved oxygen, pH, temperature, and conductivity.

Table 4.2 Procedure: Temperature, pH, Conductivity and Dissolved Oxygen

1. Check Meter and probes and calibrate according to manufacturer's specifications.
2. Samples are taken mid-channel, at the X site, at a depth of 0.5 meters or at mid-depth if less than 1 meter deep.
3. Lower the sonde in the water and measure DO, pH, temperature, and conductivity at 0.5 m depth (or at mid-depth if less than 1 meter deep).
4. Record the measurements on the Field Measurement Form, noting whether the conductivity value is corrected to 25°C.
5. Provide comments on any measurements that the crew feels needs further explanation or when a measurement cannot be made.

5 Collection of Antimicrobial Resistance Samples

5.1 Summary of Method

Antimicrobial resistance is a naturally occurring phenomenon in the environment. However, human activity can contaminate the environment with antibiotics, antifungals, organisms resistant to these compounds, and genes that confer resistance traits, all of which can alter natural levels of resistance. Rapid spread and emergence of antimicrobial resistance is recognized as a global health problem, but scientists currently do not fully understand the risk of resistance increasing in the environment on human, animal, or environmental health. Studying antimicrobial resistance in surface waters is critical for expanding this knowledge since waterways provide a way for resistance to spread throughout populations.

Collect two antimicrobial resistance samples in pre-sterilized bottles from the X-site at the midpoint of the stream. One sample (Bacteria Culture) will be collected in a 2 L bottle, and the other (Bacteria DNA) will be collected in a 1 L bottle. Both samples will be collected directly from the stream without the use of any additional sampling equipment. Collect the samples by submerging the mouth of each bottle to a depth of 0.3 meters and allowing the bottle to fill completely. If the sampling location is shallow, submerge the mouth of the bottle to a lesser depth and be careful not to contaminate the sample with bottom sediments or detritus. After collection, store both samples on ice in a closed cooler.

5.2 Equipment and Supplies

Table 5.1 provides the equipment and supplies needed to collect antimicrobial resistance samples at the X-site. Record the sample collection data on the Sample Collection Form in the NRSA App.

Table 5.1 Equipment and Supplies: Antimicrobial Resistance Samples

For collecting samples	Nitrile gloves Pre-sterilized 2 L sample bottle (Bacteria Culture) Pre-sterilized 1 L sample bottle (Bacteria DNA) Plastic electrical tape Cooler with ice Field Operations Manual
For collecting field blanks (at Visit 1 to Revisit sites)	Pre-sterilized 500 mL PETG bottle Ultra-pure water (1000 mL bottle) AMR Field Blank Label
For recording measurements	Sample Collection Form in NRSA App Sample label with pre-printed Sample ID Clear tape strips Fine tipped indelible markers for sample labels

5.3 Sampling Procedure

Table 5.2 presents step-by-step procedures for collecting antimicrobial resistance samples at wadeable sites.

Table 5.2 Procedure: Antimicrobial Resistance Sample Collection (Wadeable Sites)

Collection of Antimicrobial Resistance Samples
<ol style="list-style-type: none"> 1. Fill out the pertinent information (Site ID, visit, and date) on the antimicrobial resistance sample labels. 2. Affix the BCUL label to the pre-sterilized 2 L sample bottle and cover with clear tape. 3. Affix the BDNA label to the pre-sterilized 1 L sample bottle and cover with clear tape. 4. Collect the antimicrobial resistance (bacteria culture and bacteria DNA) samples from the X-site in a flowing portion of the stream near the middle of the transect. Use the same location as water chemistry samples collection. 5. Collect the samples directly from the water column without the use of any other sampling devices. Do not rinse the containers. 6. Put on clean, nitrile gloves. 7. Approach the sampling location slowly from downstream or downwind. 8. Remove the lid from the first sample bottle. Do not touch the inside of the sterile container, lip, or lid. When opening sterile sampling containers, work rapidly. Open sterile sampling containers only to admit the sample and close it immediately. 9. Lower the uncapped, inverted sample bottle to a depth of 1 foot (0.3 m) below the water surface, avoiding surface scum, vegetation, and substrates. If the sampling location is shallow, submerge the mouth of the bottle to a lesser depth and be careful not to contaminate the sample with bottom sediments or detritus. 10. Point the mouth of the container away from the body or boat. Right the bottle and raise it through the water column, allowing bottle to fill completely. 11. After removing the container from the water, discard a small portion of the sample to create 1 inch of headspace in the bottle to allow for proper mixing before analysis. 12. Tightly close the lid and seal the cap with electrical tape before shipping. 13. Repeat steps 9-13 for the second sample bottle.

14. Enter the depth of the sample collection (in meters) in the provided fields. *This is the approximate depth from the surface of the water to the mouth of the submerged container, not the depth of the water at the sampling location.*

Storage

Place both bottles in a cooler (on ice or water) and shut the lid. If a cooler is not available, place the bottles in an opaque garbage bag and immerse it in the stream. Once the samples are placed on ice, mark the “Chilled” boxes on the Sample Collection Form in the App.

If sample(s) are not collected, select the “No Sample Collected” box on the form and indicate the reason why targeted sample(s) were not collected in the adjacent comment bubble(s).

5.4 Antimicrobial Resistance Field Blanks

Field blanks for the antimicrobial resistance samples will be collected at all revisit sites during the first visit. The initial order each crew receives will contain the required equipment for the crew’s reported number of revisit sites (**Table 5.1**). Collect the field blank **before** collecting the antimicrobial resistance samples. **Table 5.3** presents step-by-step procedures for collecting antimicrobial resistance field blanks at visit 1 to revisit sites.

Table 5.3 Procedure: Antimicrobial Resistance Field Blank Collection (Visit 1 to Revisit Sites)

Antimicrobial Resistance Field Blanks

1. Fill out the pertinent information (Site ID, visit, and date) on the antimicrobial resistance field blank label.
2. Affix the label to the pre-sterilized 500 mL field blank bottle and cover it with clear tape.
3. Put on clean, nitrile gloves.
4. Open the provided 1000 mL bottle of ultra-pure water and pour 500 mL of water into the sterile 500 mL field blank bottle.
5. Tightly close the lid of the 500 mL sample bottle and seal the cap with electrical tape before shipping.
6. The residual ultra-pure water and the 1000 mL bottle are to be discarded.

Storage

Place the 500 mL field blank bottle in the same cooler with the antimicrobial resistance samples (on ice or water) and shut the lid. The field blank will be shipped to the lab along with the two stream samples.

On the Sample Collection Form in the NRSA App, check the “AMR Field Blank Collected” box to indicate that a field blank was collected.

6 Collection of Water Chemistry and Chlorophyll-*a* Samples

6.1 Summary of Method

The water chemistry samples will be analyzed for total phosphorus (TP), total nitrogen (TN), total ammonium (NH₄), nitrate (NO₃), basic anions, cations, total suspended solids (TSS), turbidity, acid neutralizing capacity (ANC), alkalinity, dissolved organic carbon (DOC), and total organic carbon (TOC). Using a 3 L Nalgene beaker, collect a grab sample into one 4 L cube container (for water chemistry) and one 2 L amber Nalgene bottle (for chlorophyll-*a*) from the X-site at the midpoint of the stream. After collection, store all samples on ice in a closed cooler. After you filter the chlorophyll-*a* sample, the filters must be kept frozen until ready to ship.

6.2 Equipment and Supplies

Table 6.1 provides the equipment and supplies needed to collect water samples at the X-site. Record the water sample collection and preservation data on the Sample Collection Form in the NRSA App.

Table 6.1 Equipment and Supplies: Water Chemistry Sample Collection and Preservation

For collecting samples	Nitrile gloves 4 L cube container 2 L amber Nalgene bottle 3 L Nalgene beaker Cooler with ice Dry Ice Plastic electrical tape DI water (for cleaning beaker and 2 L amber bottle between sites) Field Operations Manual
For recording measurements	Sample Collection Form in NRSA App Water Chemistry sample label with pre-printed Sample ID Clear tape strips Fine tipped indelible markers for sample labels

6.3 Water Chemistry and Chlorophyll-*a* Sampling Procedure

Table 6.2 presents step-by-step procedures for collecting water chemistry samples at wadeable sites.

Table 6.2 Procedure: Water Chemistry and Chlorophyll-*a* Sample Collection (Wadeable Sites)

Sampling Procedure	
Water Chemistry	
1.	Fill out the pertinent information (Site ID, visit number, and date) on the water chemistry label and affix the label to the cube container. Completely cover the label with clear tape.
2.	Collect the water samples from the X-site in a flowing portion near the middle of the stream. Be sure to collect the water samples prior to any disturbance of the stream upstream of the X-site.
3.	Put on nitrile gloves. Make sure not to handle sunscreen or other chemical contaminants until after the sample is collected or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
4.	Rinse the 3 L Nalgene beaker three times with water and discard the rinse downstream.
5.	Remove the cube container lid and expand the cube container by pulling out the sides if needed (the weight of the water alone while filling will often open the container sufficiently). NOTE: DO NOT BLOW into the cube container or place your fingers inside the opening to expand it, because this will cause contamination.
6.	Fill the 3 L beaker with water and slowly pour 30 - 50 mL into the cube container. Cap the cube container and rotate so that the water contacts all the surfaces. Discard the water downstream. Repeat this rinsing procedure two more times.
7.	Fill the beaker with water and pour into the cube container. Repeat as necessary to fill the cube container. Let the weight of the water expand the cube container. Pour the water slowly as the cube container expands. Completely fill the cube container. Rinse the cube container lid with

Sampling Procedure

water. Eliminate any air space from the cube container by squeezing the closed container and opening the cap slightly to allow air to escape, and cap it tightly. Make sure the cap is tightly sealed and not on at an angle.

8. Seal the cap with plastic electrical tape before shipping.

Chlorophyll-*a*

9. Fill the 3 L beaker with water and slowly pour 30 - 50 mL into the 2 L amber Nalgene bottle. Cap the bottle and rotate so that the water contacts all the surfaces. Discard the water downstream. Repeat this rinsing procedure two more times.
10. Fill the beaker with water and pour into the 2 L amber Nalgene bottle, filling the bottle. Cap the bottle tightly. This sample will be filtered later and the bottle will be reused at future sites, therefore it is not necessary to label this bottle.

Storage

Place the cube container and Nalgene bottle in a cooler (on ice or water) and shut the lid. If a cooler is not available, place the cube container in an opaque garbage bag and immerse it in the stream. Once the water chemistry sample is placed on ice, mark the "Chilled" box on the Sample Collection Form in the App.

Use the pertinent comment bubbles to note anything that could influence sample chemistry (heavy rain, potential contaminants, etc.). If the sample was collected at the X-site as planned, select the X-site bubble in the location field. If you had to move to another part of the reach to collect the sample, select the "Other" bubble and place the letter of the nearest transect in the adjacent box. Record more detailed reasons and/or information regarding the sampling location a in the provided comment bubble(s).

If sample(s) are not collected, select the "No Sample Collected" box on the form and indicate the reason why targeted sample(s) were not collected in the adjacent comment bubbles.

Record the Water Chemistry Sample ID on the Tracking Form in the NRSA App. This is also referred to as the *Anchor ID* because it determines the numeric series from which all samples at the site will come. By entering the water chemistry sample ID in the Tracking Form, the rest of the sample IDs will auto-populate in the remainder of the Tracking Form. It is important to keep the labels and packing slips organized so the sample IDs will match when shipping occurs.

7 ALGAL TOXINS (MICROCYSTINS and CYLINDROSPERMOPSIN)

Cyanobacteria naturally occur in surface waters. Under certain conditions, such as in warm water containing an abundance of nutrients, they can rapidly form harmful algal blooms (HABs). HABs can produce toxins known as cyanotoxins, which can be harmful to humans and animals.

Microcystin and cylindrospermopsin are two cyanotoxins known to occur in the surface waters of the United States. Microcystins are the most widespread cyanobacterial toxins and primarily affect the liver but can also affect the kidney and reproductive system.

Cylindrospermopsin is another commonly identified cyanotoxin found in U.S. waters. The primary toxic effects of this toxin are damage to the liver and kidney.

7.1 Summary of Method

The algal toxin (microcystin and cylindrospermopsin) sample is a grab sample taken from the X-site. All Field Crews must collect a grab sample using the 3 L beaker to fill one 500 ml bottle. Collect the sample after the *in situ* measurements and water chemistry samples are collected. Store the sample on wet ice in a closed cooler.

7.2 Equipment and Supplies

Table 7.1 provides the equipment and supplies needed to collect the algal toxin sample at the X-site. Record the water sample collection and preservation data on the Sample Collection Form in the NRSA App.

Table 7.1 Equipment and Supplies: Microcystin

For collecting samples	Nitrile gloves 3 L Nalgene beaker PETG bottle (500 mL, clear, square) – algal toxins (MICX) Plastic electrical tape Cooler with ice Field Operations Manual
For recording measurements	Sample Collection Form in NRSA App Algal toxin sample label with pre-printed Sample ID Clear tape strips Fine tipped indelible markers for labels

7.3 Sampling Procedure

Table 7.2 presents step-by-step procedures for collecting the algal toxin (microcystin and cylindrospermopsin) sample at wadeable sites.

Table 7.2 Procedure: Algal Toxin (Microcystin and Cylindrospermopsin) Collection (Wadeable Sites)

Microcystin Sample Collection	
1.	Fill out the pertinent information (Site ID, visit, and date) on the algal toxin label.
2.	Affix the MICX label to the 500 mL PETG clear square Nalgene bottle. Completely cover the label with clear tape.
3.	Collect the algal toxin sample from the X-site in a flowing portion of the stream near the middle of the transect.
4.	Put on nitrile gloves. Make sure not to handle sunscreen or other chemical contaminants until after the sample is collected or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc.
5.	Rinse the 3 L Nalgene beaker three times with water and discard the rinse downstream.
6.	Rinse the water sample collection container and lid three times with water , discard the rinse downstream.
7.	Fill the beaker with water and pour into the 500 ml Nalgene bottle to the 500 mL mark (or just below the shoulder of the bottle), leaving headspace so that the bottle doesn't burst when frozen.
8.	Seal the cap with plastic electrical tape before shipping.

Storage

Place the 500 ml Nalgene bottle in a cooler (on ice or water) and shut the lid. If a cooler is not available, place the 500 ml bottle in an opaque garbage bag and immerse it in the stream.

Upon returning to your base site (hotel, lab, office, etc.), freeze the sample and keep it frozen until shipping. Mark the “Frozen” box on the form to verify that the sample has been frozen.

If the sample is not collected, select the “No Sample Collected” box on the form and indicate the reason why the targeted sample was not collected in the adjacent comment bubble.

8 BENTHIC MACROINVERTEBRATES

8.1 Summary of Method

Collect a benthic macroinvertebrate composite sample using a D-frame net with 500 µm mesh openings. Take individual samples from the sampling stations at the 11 transects equally distributed along the targeted reach (**Figure 8.1**). Multiple habitats will be encountered and sampled using this approach. Habitats will include various types of bottom substrate as well as woody debris, macrophytes, and leaf packs. Composite all sample material from all 11 sampling locations and field preserve with ~95% ethanol.

8.2 Equipment and Supplies

Table 8.1 shows the checklist of equipment and supplies required to complete the collection of benthic macroinvertebrates. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the site. Record collection data on the Benthic Collection Form in the NRSA App.

Table 8.1 Equipment and Supplies: Benthic Macroinvertebrate Collection at Wadeable Sites

<p>For collecting samples</p>	<p>Modified kick net (D-frame with 500 µm mesh) and 52" handle Watch with timer or stopwatch Sieve bucket with 500 µm mesh openings (U.S. std No. 35) 5 gallon bucket Watchmakers' forceps Wash bottle, 1 L capacity labeled "STREAM WATER" Funnel, with large bore spout</p>	<p>Small spatula, spoon, or scoop to transfer sample Sample jars, 1 L HDPE plastic suitable for use with ethanol 95% ethanol, in a proper container Cooler (with absorbent material) for transporting ethanol & samples Plastic electrical tape Scissors Field Operations Manual</p>
<p>For recording measurements</p>	<p>Composite benthic sample labels with & without preprinted ID Sample ID numbers Blank labels on waterproof paper for inside of jars</p>	<p>Soft (#2) lead pencils Fine-tip indelible markers Clear tape strips Benthic Collection Form in the App</p>

8.3 Sampling Procedure

Figure 8.1 summarizes how samples will be collected from wadeable sites. The transect sample design for collecting benthic macroinvertebrates is shown in **Figure 8.2**. Collect a sample from **1 m downstream** of each of the 11 cross-section transects at the assigned sampling station. The process for selecting the sample stations is described in the Initial Site Procedures (**Section 3**). At transects assigned a “Center” sampling point where the stream width is between one and two net widths wide, pick either the “Left” or “Right” sampling point instead. If the stream is only one net wide at a transect, place the net across the entire stream width and consider the sampling point to be “Center”. If a sampling point is located in water that is too deep or unsafe to wade, select an alternate sampling point on the transect at random.

The procedure for collecting samples at each transect is described in **Table 8.2**. At each sampling point, determine if the habitat is a “riffle/run” or a “pool/glide” (any area where there is not sufficient current to extend the net is operationally defined as a pool/glide habitat). Record the dominant substrate type (fine/sand, gravel, coarse substrate (coarse gravel or larger) or other (e.g., bedrock, hardpan, wood, aquatic vegetation, etc.) and the habitat type (pool, glide, riffle, or rapid) for each sample collected on the Benthic Collection Form in the NRSA App. As you proceed upstream from transect to transect, combine all samples into a bucket.

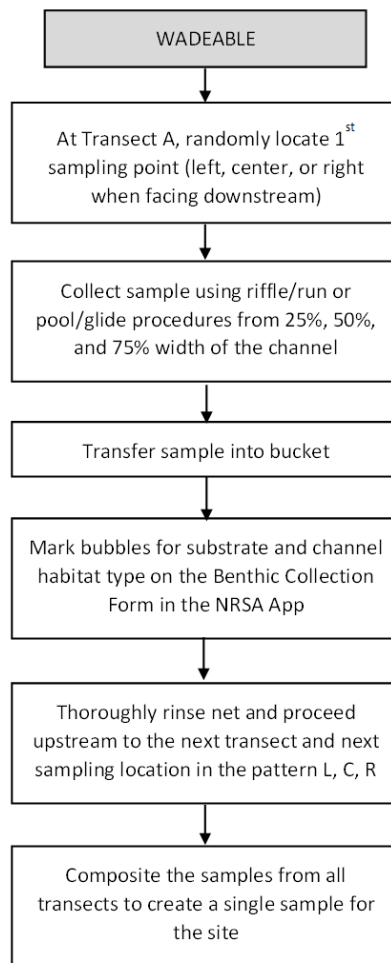


Figure 8.1 Benthic Macroinvertebrate Collection at Wadeable Sites

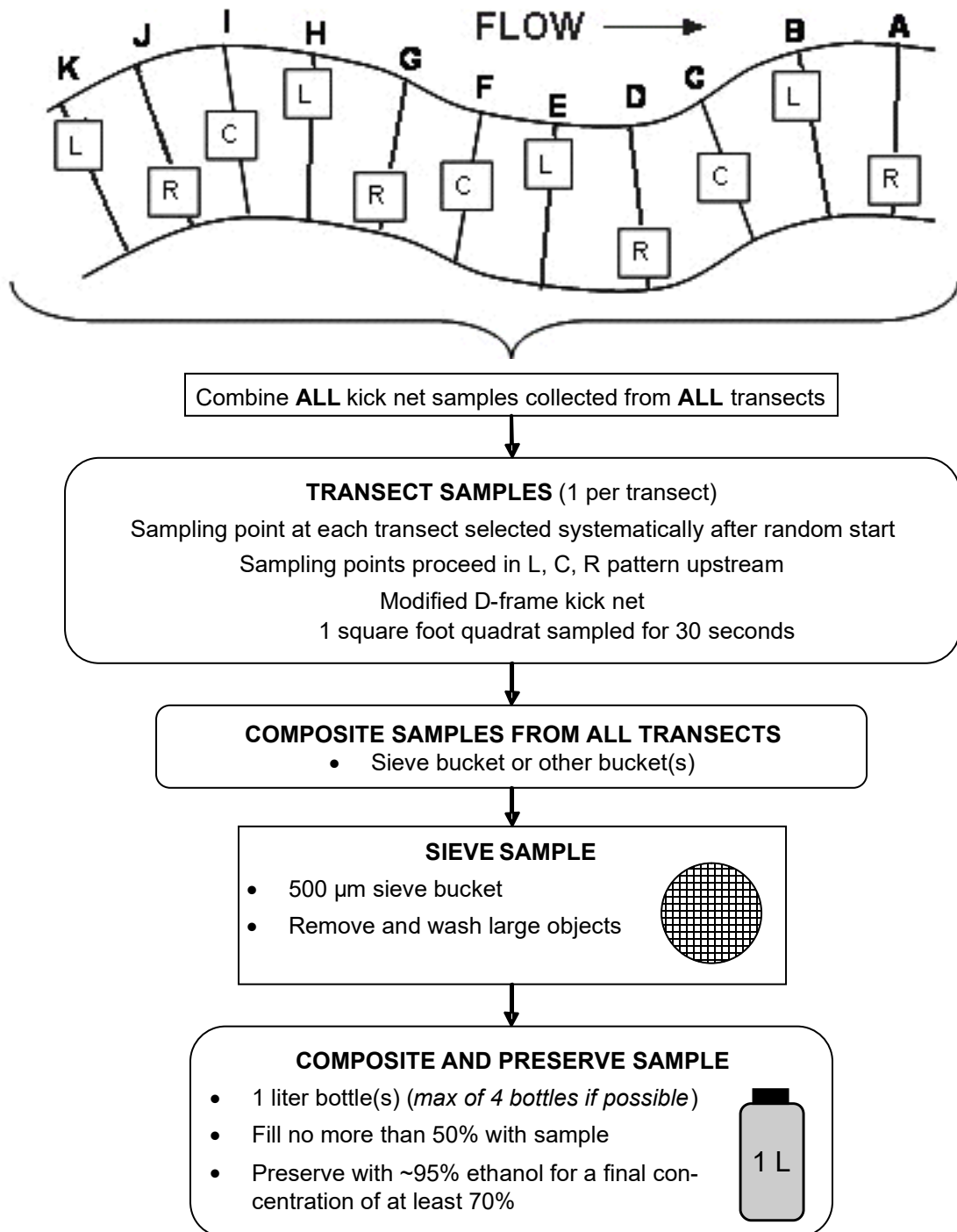


Figure 8.2 Transect Sample Design for Collecting Benthic Macroinvertebrates at Wadeable Sites

Table 8.2 Procedure: Benthic Macroinvertebrates (Wadeable Sites)

Collecting Benthic Macroinvertebrate Sample	
<ol style="list-style-type: none">1. At 1 m downstream of each transect, beginning with Transect "A", randomly locate the first sampling station (Left, Center, or Right as you face downstream) as 25%, 50%, and 75% of the wetted width, respectively. If you cannot collect a sample at the designated point because of deep water or unsafe conditions, move to another point on the same transect as close to the planned location as possible.2. Determine if there is sufficient current in the area at the sampling station to fully extend the net. If so, classify the habitat as "riffle/run" and proceed to Step 3. If not, use the sampling procedure described for "pool/glide" habitats starting at Step 9. <p><i>NOTE: If the net cannot be used, hand-pick a sample for 30 seconds from about 1 ft² of substrate at the sampling point. For vegetation choked sampling points, sweep the net through the vegetation within a 1 ft² quadrat for 30 seconds. Place this hand-picked sample directly into the sample container. Provide a comment for the sample and indicate which transect(s) required the modified collection procedure. Go to Step 13.</i></p>	
Riffle/Run Habitats:	
<ol style="list-style-type: none">3. With the net opening facing upstream, quickly position the net securely on the stream bottom to eliminate gaps under the frame. Avoid large rocks that prevent the net from seating properly on the stream bottom. <p><i>NOTE: If there is too little water to collect the sample with the D-net, randomly pick up 10 rocks from the riffle and pick and wash the organisms off them into a bucket which is half full of water.</i></p> <ol style="list-style-type: none">4. Holding the net in position on the substrate, visually define a quadrat that is one net width wide and long upstream of the net opening. The area within this quadrat is 1 ft².5. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball sized or larger and which are at least halfway into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.6. Hold the D-net securely in position. Starting at the upstream end of the quadrat, vigorously kick the remaining finer substrate within the quadrat for 30 seconds (use a stopwatch). <p><i>NOTE: For samples located within dense beds of long, filamentous aquatic vegetation (e.g., algae or moss), kicking within the quadrat may not be sufficient to dislodge organisms in the vegetation. Usually these types of vegetation are lying flat against the substrate due to current. Use a knife or scissors to remove only the vegetation that lies within the quadrat (i.e., not entire strands that are rooted within the quadrat) and place it into the net.</i></p> <ol style="list-style-type: none">7. Pull the net up out of the water. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net. Avoid having any water or material enter the mouth of the net during this operation.8. Go to Step 13.	
Pool/Glide Habitats:	
<ol style="list-style-type: none">9. Visually define a quadrat that is one net width wide and long at the sampling point. The area within this quadrat is 1 ft².10. Check the quadrat for heavy organisms, such as mussels and snails. Remove these organisms by hand and place them into the net. Pick up loose rocks or other larger substrate particles in the quadrat. Use your hands to dislodge organisms and wash them into the net. Scrub all rocks that are golf ball sized or larger and which are at least halfway into the quadrat. After scrubbing, place the substrate particles outside of the quadrat.	

Collecting Benthic Macroinvertebrate Sample

11. Vigorously kick the remaining finer substrate within the quadrat with your feet while dragging the net repeatedly through the disturbed area just above the bottom. Keep moving the net all the time so that the organisms trapped in the net will not escape. Continue kicking the substrate and moving the net for 30 seconds.

NOTE: If there is too little water to use the kick net, stir up the substrate with your gloved hands and use a sieve with 500 μ m mesh size to collect the organisms from the water in the same way the net is used in larger pools.

12. After 30 seconds, remove the net from the water with a quick upstream motion to wash the organisms to the bottom of the net.

All samples:

13. Invert the net into a sieve bucket and transfer the sample. Remove as much gravel as possible so that the organisms do not get damaged. Inspect the net for any residual organisms clinging to the net and deposit them into the bucket. Use forceps if necessary to remove organisms from the net. Carefully inspect any large objects (such as rocks, sticks, and leaves) in the bucket and wash any organisms found off of the objects and into the bucket before discarding the object. Remove as much detritus as possible without losing organisms.

NOTE: It is recommended that crews carry a sample bottle containing a small amount of ethanol with them to enable them to immediately preserve larger predaceous invertebrates such as hellgrammites and water beetles. Doing so will help reduce the chance that other specimens will be consumed or damaged prior to the end of the field day.

14. Determine the **predominant** substrate size/type you sampled within the sampling quadrat. Mark the sampled substrate type on the Benthic Collection Form under the pertinent heading and transect row. The substrate types are:
 - **Fine/sand (F)**: not gritty (silt/clay/muck <0.06 mm diam.) to gritty, up to ladybug sized (2 mm)
 - **Gravel (G)**: fine to coarse gravel (ladybug to tennis ball sized; 2 mm to 64 mm)
 - **Coarse (C)**: cobble to boulder (tennis ball to car sized; 64 mm to 4000 mm)
 - **Other (OT)**: bedrock (larger than car sized; > 4000 mm), hardpan (firm, consolidated fine substrate), wood of any size, aquatic vegetation, etc.). Note type of "other" substrate in comment bubbles on field form.
15. Identify the channel habitat type where the sampling quadrat was located. Indicate the channel habitat type on the Benthic Collection Form under the pertinent heading and transect row. The channel habitat types are:
 - **Pool (P)**: Still water; low velocity; smooth, glassy surface; usually deep compared to other parts of the channel
 - **Glide (GL)**: Water moving slowly, with smooth, unbroken surface; low turbulence
 - **Riffle (RI)**: Water moving, with small ripples, waves, and eddies; waves not breaking, and surface tension is not broken; "babbling" or "gurgling" sound.
 - **Rapid (RA)**: Water movement is rapid and turbulent; surface with intermittent "white water" with breaking waves; continuous rushing sound.
16. If any transects could not be sampled for benthos, leave the substrate and channel bubbles empty for the transect and use the adjacent comment bubble to explain why the sample could not be collected at the transect.

Thoroughly rinse the net before proceeding to the next sampling station. Proceed upstream to the next transect (through Transect K, the upstream end of the reach) and repeat steps 1 - 15. Assign the sampling station systematically after the first random selection following the repeating pattern L, C, R as you move upstream. Combine all kick net samples from riffle/run and pool/glide habitats into the bucket.

Collecting Benthic Macroinvertebrate Sample

- Record the number of transects that were sampled throughout the reach on the Benthic Collection Form in the App.

8.4 Sample Processing in Field

Use a 500 µm mesh sieve bucket placed inside a larger bucket full of site water while sampling to carry the composite sample as you travel around the site. Once the composite sample from all stations is sieved and reduced in volume, store in a 1 L jar and preserve with 95% ethanol. Do not fill jars more than ½ full of material. Multiple jars may be required if detritus is heavy (**Table 8.3**). If there is a large amount of organic material in the sample, or there are adverse field conditions (i.e., hot, humid weather), place sample in a 1 L jar with ethanol after each station.

Try to use no more than four jars per site. If more than one jar is used for a composite sample, use the “extra jar” label provided; record the SAME sample ID number on this “extra jar” label. **DO NOT use two different sample numbers on two jars containing one single sample.** Cover the labels with clear tape. The sample ID number (as well as other pertinent sample information) is recorded with a No. 2 lead pencil on a waterproof label that is placed inside each jar. Be sure the inside label and outside label describe the same sample.

Record information for each composite sample on the Benthic Collection Form in the NRSA App. Place the samples in a cooler or other secure container for transporting and/or shipping to the laboratory (see **Appendix C**).

Table 8.3 Procedure: Compositing Samples for Benthic Macroinvertebrates (Wadeable Sites)

Compositing Benthic Macroinvertebrate Sample					
<ol style="list-style-type: none"> Pour the entire contents of the bucket into a sieve bucket with 500 µm mesh size. Remove any large objects and wash off any clinging organisms back into the sieve before discarding. Remove any large inorganic material, such as cobble or rocks. Using a wash bottle filled with river water, rinse all the organisms from the bucket into the sieve. This is the composite sample for the reach. Estimate the total volume of the sample in the sieve and determine how many 1 L jars will be required. Try to use no more than four jars per site. Fill in a sample label with the Site ID, date of collection, visit, and jar number (i.e., Jar 1 of 2). Attach the completed label to the jar and cover it with a strip of clear tape. Verify the sample ID number for the composite sample matches the ID on the Tracking Form in the App. Wash the contents of the sieve to one side by gently agitating the sieve in the water. Wash the sample into a jar using as little water from the wash bottle as possible. Use a large bore funnel if necessary. If the jar is too full pour off some water through the sieve until the jar <u>is not more than ½ full</u>, or use a second jar if necessary. Carefully examine the sieve for any remaining organisms and use watchmakers’ forceps to place them into the sample jar. <ul style="list-style-type: none"> <i>If a second jar is needed, fill in a sample label that does not have a pre-printed ID number on it. Record the ID number from the pre-printed label prepared in Step 4 in the “SAMPLE ID” field of the label. Attach the label to the second jar and cover it with a strip of clear tape. Record the number of jars required for the sample on the Benthic Collection Form. Make sure the number you record matches the actual number of jars used. Write “Jar N of X” on each sample label using a waterproof marker (“N” is the individual jar number, and “X” is the total number of jars for the sample).</i> Place a waterproof label inside each jar with the following information written with a number 2 lead pencil: <table border="0" style="width: 100%; margin-top: 10px;"> <tr> <td style="width: 50%;">Site ID</td> <td style="width: 50%;">Collectors initials</td> </tr> <tr> <td>Type of sampler</td> <td>Number of stations sampled</td> </tr> </table> 	Site ID	Collectors initials	Type of sampler	Number of stations sampled	
Site ID	Collectors initials				
Type of sampler	Number of stations sampled				

BENTHIC MACROINVERTEBRATES

Compositing Benthic Macroinvertebrate Sample

Name of site

Date of collection

Jar "N" of "X"

7. Completely fill the jar with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved. Existing water in the jar should not dilute the concentration of ethanol below 70%. Once preserved, mark the "Preserved" bubble on the data form in the App.

NOTE: Composite samples can be transported back to the vehicle before adding ethanol if necessary. In this case, fill the jar with stream water, which is then drained using the net (or sieve) across the opening to prevent loss of organisms, and replace with ethanol.

8. Replace the cap on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic tape.
9. Store labeled composite samples in a container with absorbent material that is suitable for use with 70% ethanol until transport or shipment to the laboratory.
10. If no benthic sample were collected, select the "No Sample Collected" box on the data form and indicate the reason why targeted samples were not collected in the adjacent comment bubble.

9 PERIPHYTON

9.1 Summary of Method

Collect periphyton from the 11 cross-section transects (“A” through “K”) established within the sampling reach. Collect periphyton samples at the same transect location (L, C, or R) as the benthic macroinvertebrate samples (**Section 8**) directly after collecting the benthic macroinvertebrate sample. Prepare one composite sample of periphyton for each reach. At the completion of the day's sampling activities, but before leaving the site, **prepare four types of laboratory samples** (an ID/enumeration sample to determine taxonomic composition and relative abundances, a metagenomic sample, a chlorophyll-*a* sample, and a biomass sample (for ash-free dry mass [AFDM])) from the composite periphyton sample.

9.2 Equipment and Supplies

Table 9.1 is a checklist of equipment and supplies required to conduct periphyton sample collection and processing activities. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the site.

Table 9.1 Equipment and Supplies: Periphyton (Wadeable Sites)

For collecting samples	Large Funnel (15-20 cm diameter) 12 cm ² area delimitter (3.8 cm diameter pipe, 3 cm tall) Stiff-bristle toothbrush with handle bent at 90° angle 1 L wash bottle for DI water 500 mL graduated plastic bottle for the composite sample 60 mL plastic syringe with tip removed, and length of tubing (20 mL) Timer or stopwatch Cooler (small soft-sided preferred) Wet ice Field Operations Manual
For recording measurements	Sample Collection Form in NRSA App Fine-tipped indelible markers for filling out sample labels Sample labels (4 per site) with pre-printed Sample ID numbers Clear tape strips for covering labels
For cleaning equipment	10% Bleach solution

9.3 Sampling Procedure

At each of the 11 transects, collect samples from the sampling station assigned during the layout of the reach (L, C, or R). Collect the substrate selected for sampling from a depth no deeper than 0.5 m. If a sample cannot be collected because the location is too deep, pick another point along the transect as close to the planned location as possible. The procedure for collecting samples and preparing a composite sample is presented in **Table 9.2**. Collect one sample from each of the transects and composite into one bottle to produce one composite sample for each site. Record number of transects sampled and the total the volume of the composite sample on the Sample Collection Form in the NRSA App.

Table 9.2 Procedure: Collecting Composite Index Samples of Periphyton (Wadeable Sites)

Periphyton Composite Sample
<p>1. Starting with Transect “A”, collect a single sample from the assigned sampling station using the procedure below.</p> <p>a) If coarse substrate (cobbles, woody materials, etc.) are present that can be removed from the water:</p> <ul style="list-style-type: none">i) Collect a sample of substrate (rock or wood) that is small enough (< 15 cm diameter) and can be easily removed from the water. Place the substrate in or over a plastic funnel which drains into a 500 mL plastic bottle with volume graduations marked on it.ii) Use the area delimiter to define a 12 cm² area on the upper surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter into the funnel by brushing with a stiff-bristled toothbrush for 30 seconds. Take care to ensure that the upper surface of the substrate is the surface that is being scrubbed, and that the entire surface within the delimiter is scrubbed.iii) Fill a wash bottle with DI water. Using water from this bottle, wash the dislodged periphyton from the funnel into the 500 mL bottle. Use an amount of water (~45 mL) that brings the composite volume up to the next graduation mark on the bottle.iv) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite). <p>b) If large coarse substrate is present that is too large to remove from the water (bedrock, large woody materials, boulders, etc.):</p> <ul style="list-style-type: none">v) Use the area delimiter to define a 12 cm² area on the upper surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter using the clear tube attached to the tip of the syringe in a scraping motion.vi) While dislodging periphyton with the tube, simultaneously pull back to 25 mL on the syringe plunger to draw the dislodged periphyton into the syringe. The 25 mL in the syringe combined with the 20 mL in the tube equals the target volume of 45 mL.vii) Empty the syringe and tube into the same 500 mL plastic bottle as above. If the volume of the vacuumed sediment is not enough to raise the composite volume to the next graduation on the bottle (~45 mL), add additional DI water to the bottle to raise the level to the next graduation.viii) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite.) <p>c) If no coarse sediments (cobbles or larger) are present:</p> <ul style="list-style-type: none">ix) Use the area delimiter to confine a 12 cm² area of soft sediments.x) Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60 mL syringe with attached clear tube up to the 25 mL line of the syringe.xi) Empty the syringe into the same 500 mL plastic bottle as above. If the volume of the vacuumed sediment is not enough to raise the composite volume to the next graduation on the bottle (~45 mL), add additional DI water to the bottle to raise the level to the next graduation.xii) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)

2. Repeat Step 1 for transects “B” through “K”. Place the sample collected at each sampling station into the single 500 mL bottle to produce the composite index sample.

Storage

3. After samples have been collected from all 11 transects (or as many transects as possible), thoroughly mix the 500 mL bottle regardless of substrate type. Record the total volume of the composite sample in the periphyton section of the Sample Collection Form. The graduations on the collection bottle are placed at 45 mL increments, so the total volume of the composite sample should be the number of transects sampled times 45.
4. Record the number of transects sampled. If all 11 transects were not sampled, record the reason(s) for any missed transects in the comment bubble adjacent to the “Number of Transects” field.
5. If sample(s) are not collected at all, select the “No Sample Collected” box on the data form in the NRSA App and indicate the reason why targeted sample(s) were not collected in the adjacent comment bubble(s).

Clean up

6. After preparing the four types of laboratory samples (see **Section 14.3.6**), thoroughly clean each of the pieces of periphyton equipment (delimiter, brush, funnel, syringe, and composite bottle) with a 10% Bleach solution and rinse with tap or DI water.

9.4 Sample Processing in the Field

You will prepare four different types of laboratory samples from the composite sample: an **ID/enumeration sample** (to determine taxonomic composition and relative abundances), a **metagenomic sample**, a **chlorophyll-*a* sample**, and a **biomass sample** (for AFDM). All of the methods for processing the four samples are found in the Final Site Activities (**Section 14**) portion of the manual. All the sample containers required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination (e.g., dust, dirt, or mud) that are present at site shorelines.

10 PHYSICAL HABITAT CHARACTERIZATION

Field measurements for physical habitat are made at two scales of resolution along the mid-channel length of the reach, and the results are later aggregated and expressed for the entire reach. The protocol defines the length of each sampling reach proportional to stream channel wetted width and then systematically places measurements to statistically represent the entire reach.

10.1 Equipment and Supplies

Table 10.1 lists the equipment and supplies required to conduct all the activities described for characterizing physical habitat. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the stream. Use this checklist to ensure that equipment and supplies are organized and available at the river site in order to conduct the activities efficiently.

Table 10.1 Equipment and Supplies: Physical Habitat

<p>For making measurements</p>	<p>Convex spherical canopy densiometer (Lemmon Model B), modified with taped “V”</p> <p>GPS</p> <p>1 roll each colored surveyor’s flagging tape (2 colors)</p> <p>2 pair chest waders</p> <p>1 or 2 fisherman’s vest with lots of pockets and snap fittings.</p> <p>Digital camera with extra memory card & battery (optional)</p> <p>50 m or 100 m measuring tape with reel</p> <p>Meter stick for bank angle measurements</p> <p>Laser rangefinder (400 ft. distance range) and clear waterproof bag</p> <p>Clinometer</p> <p>Binoculars (optional)</p> <p>Bearing compass</p> <p>Surveyor’s telescoping leveling rod</p> <p>Sounding rod or wading staff</p> <p>Level tripod</p> <p>CST Berger SAL 20 Automatic Level</p> <p>Field Operations Manual</p>
<p>For recording data</p>	<p>In the NRSA App:</p> <p>Physical Habitat Form</p> <p>Slope Form</p> <p>Channel Constraint Form</p> <p>Torrent Evidence Form</p> <p>Site Assessment Form</p>

10.2 Summary of Methods Approach

Physical habitat in streams includes all those physical attributes that influence or provide sustenance to organisms within the stream. The physical habitat of a stream varies naturally, thus expectations differ even in the absence of anthropogenic disturbance. The procedures are employed on a **reach length 40 times its mean wetted width** at the time of sampling.

Measurement points are systematically placed to statistically represent the entire reach. Stream depth and wetted width are measured at very tightly spaced intervals, whereas channel cross-section profiles, substrate, bank characteristics and riparian vegetation structure are measured at larger intervals. Woody debris is tallied along the full length of the sampling reach. The tightly spaced depth and width measures allow calculation of indices of channel structural complexity, objective classification of channel units such as pools, and quantification of residual pool depth, pool volume, and total stream volume.

10.3 Components of the Habitat Characterization

There are six components of the physical habitat characterization (**Table 10.2**). Physical habitat measurements are recorded using the NARS App. Similarly, slope and bearing measurements, the degree of channel constraint, and evidence of debris torrents or recent major flooding are recorded in the NRSA App.

Table 10.2 Summary of Components of Physical Habitat Characterization at Wadeable Sites

Component	Description
Channel and Riparian Characterization	<ul style="list-style-type: none"> At 11 transects placed at equal intervals along reach: <ul style="list-style-type: none"> Measure channel cross-section dimensions, bank height, bank undercut distance, bank angle, slope and compass bearing (backsight), and riparian canopy density (with densiometer). Visually estimate^a: substrate size class, embeddedness and water depth at five equidistant points on cross-section; areal cover class and type (e.g., woody trees) of riparian vegetation in canopy, understory, and ground cover; areal cover class of fish concealment features, aquatic macrophytes and filamentous algae. Observe and record^a: presence and proximity of human disturbances. At 10 cross-sections that are midway between the 11 transects above: Visually estimate^a substrate size class at 5 equidistant points on each cross-section
Wetted Width / Bar Width	<ul style="list-style-type: none"> Measure wetted width and bar width (if present) and evaluate substrate particle size classes at 11 cross-section transects and midway between them (21 width measurements and substrate notations along entire reach).
Thalweg Profile	<ul style="list-style-type: none"> Measure maximum depth, classify habitat and check presence of backwaters, side channels and loose, soft deposits of sediment particles at 10-15 equally spaced intervals between each of 11 transects (100 or 150 individual measurements along entire reach). The number of thalweg measurements is specified by the stream's mean wetted width.
Woody Debris Tally	<ul style="list-style-type: none"> Between each of the channel cross-sections, tally large woody debris numbers within and above the bankfull channel according to specified length and diameter classes (10 separate tallies).
Assessment of Channel Constraint, Debris Torrents, and Major Floods	<ul style="list-style-type: none"> After completing thalweg and transect measurements and observations, identify features causing channel constraint, estimate the percentage of the channel margin that is constrained for the whole reach, and estimate the bankfull and valley widths. Check for evidence of recent major floods and debris torrent scour or deposition.

^a Substrate size class is estimated for a total of 105 particles taken at 5 equally spaced points along each of 21 cross-sections. Depth is measured and embeddedness estimated for the 55 particles located along the 11 regular transects A through K. Cross-sections are defined by laying the surveyor's rod or tape to

span the wetted channel. Woody debris is tallied over the distance between each cross-section and the next cross-section upstream. Riparian vegetation and human disturbances are observed 5m upstream and 5m downstream from the cross-section transect. They extend shoreward 10m from left and right banks. Fish cover types, aquatic macrophytes, and algae are observed within the channel 5m upstream and 5m downstream from the cross-section stations. These boundaries for visual observations are estimated by eye.

10.4 Work Flow for the Physical Habitat Components

The six components (**Table 10.2**) of the habitat characterization are organized into four grouped activities described below in the following sections.

10.4.1 Channel/Riparian Cross-Sections

At each of the 11 transects, one person proceeds with the channel cross-section dimension measurements and substrate observations as described below; and also makes measurements of bank angle (using rod and clinometer) and canopy cover (using densiometer). The second person records those measurements in the specific sections of the Physical Habitat Form in the NRSA App while making visual estimates of riparian vegetation structure, instream fish cover, and human disturbance specified on that form.

Slope is measured by measuring the difference in elevation between each transect and bearing is determined by backsighting to the previous transect. Supplementary points may need to be located and flagged (using a different color) if the stream is extremely brushy, sinuous, or steep to the point that you cannot sight for slope and bearing measures between two adjacent transects.

The work flow for the thalweg profile and channel cross-section described above can be modified by delaying the measurements for slope and bearing and/or the woody debris tally until after reaching the upstream end of the reach. Backsighting and/or wood tallies can be done on the way back downstream (note that in this case, the slope and bearing data form would have to be completed in reverse order). Crews may also elect to return to Transect A and record slope and bearing measurements on a second trip upstream through the reach.

10.4.2 Thalweg Profile and Large Woody Debris Tally

“*Thalweg*” refers to the flow path of the deepest water in a river channel. The *thalweg profile* is a longitudinal survey of maximum depth and several other selected characteristics. Thalweg spacing is calculated such that either 10 or 15 evenly spaced measurements are made between each transect (see **Section 10.5.2**). Two people proceed upstream from the downstream end of the sampling reach making observations and measurements at the calculated increment spacing. One person is in the channel making width and depth measurements and determining whether soft/small sediment deposits are present under his/her wading staff. The other person records these measurements, classifies the channel habitat, records presence/absence of side channels and off-channel habitats (e.g., backwater pools, sloughs, alcoves), and tallies large woody debris. Each time the crew reaches a flag marking a new cross-section transect, they start filling out a new portion of the Physical Habitat Form in the NRSA App by selecting the appropriate Transect bubble at the top of the form. They interrupt the thalweg profile and woody debris tallying activities to complete data collection at each cross-section transect as they come to it. When the crew member in the water makes a width measurement at channel locations midway between regular transects (i.e. at the fifth or seventh thalweg measurement in each sub-reach), she or he also locates and estimates the size class of the substrate particles on the left channel margin (0%) and at positions 25%, 50%, 75%, and 100% of the distance across

the wetted channel. Procedures for this substrate tally are the same as for those at regular cross-sections, but data are recorded in a different portion- of the field form.

10.4.3 Channel Constraint and Torrent Evidence

After completing observations and measurements along the thalweg and at all 11 transects, the Field Crew completes the overall reach assessments of channel constraint and evidence of debris torrents and major floods.

10.5 Habitat Sampling within the Reach

Measurements are made at two scales along the length of the reach; the results are later aggregated for the entire reach using procedures described by Kaufmann et al. (1999). **Figure 10.1** illustrates the locations within the reach where data for the different components of the physical habitat characterization are collected. Most channel and riparian features are characterized on 11 cross-sections and pairs of riparian plots spaced at 4 channel width intervals (i.e., transect spacing = $1/10^{\text{th}}$ the total reach length). Thalweg profile measurements will be spaced evenly over the entire reach. In addition, they must be sufficiently close together that they do not miss deep areas and major habitat units.

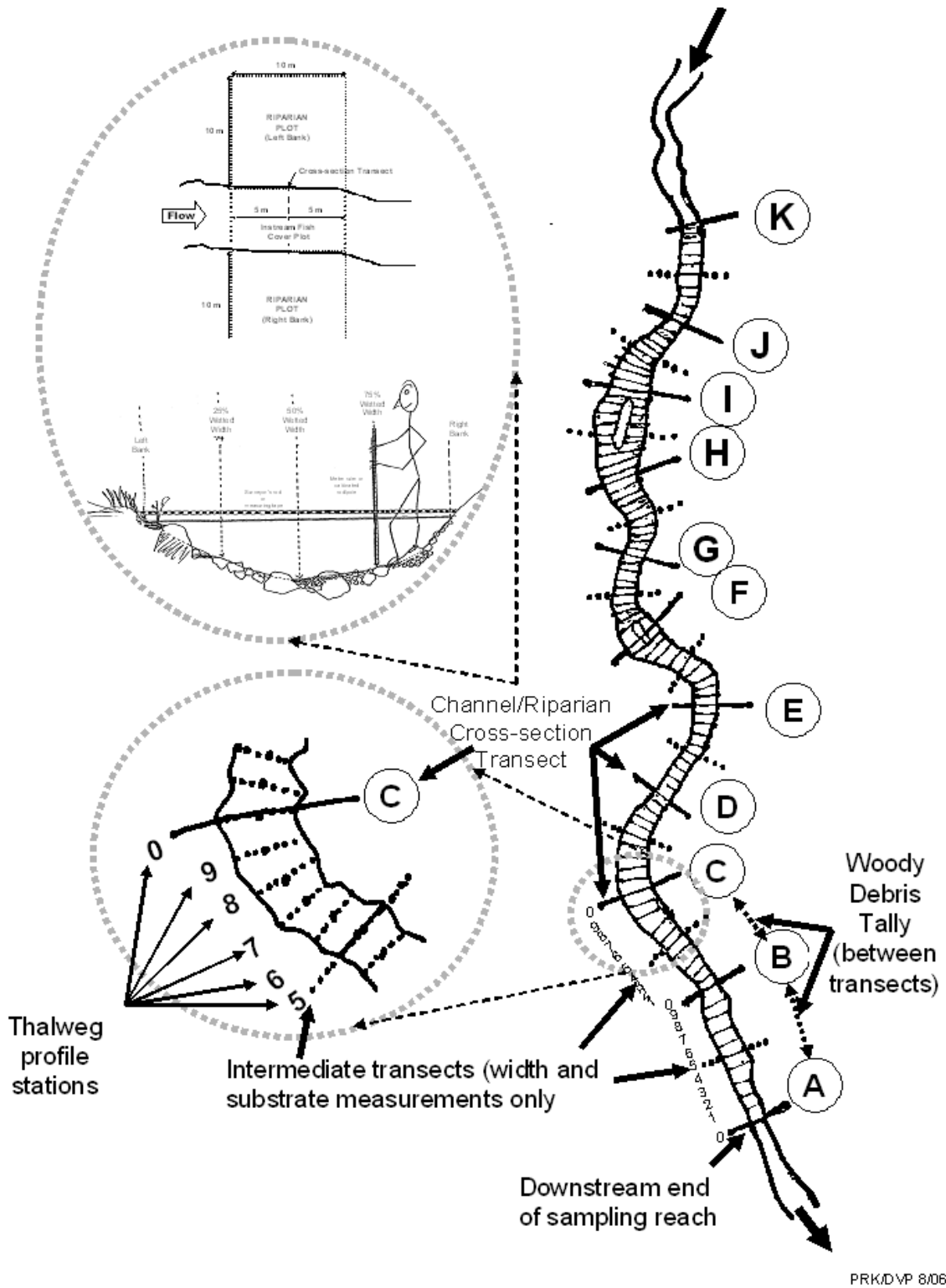
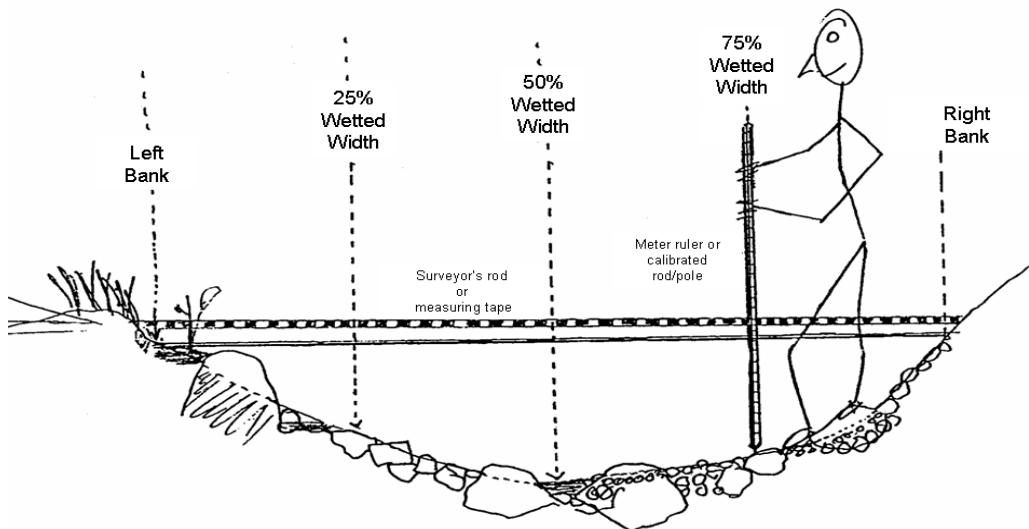


Figure 10.1 Reach Layout for Physical Habitat Measurements for streams 2.5m or greater (plan view)

10.5.1 Channel and Riparian Measurements at Cross-Section Transects

10.5.1.1 Substrate Size and Channel Dimensions

Substrate size and embeddedness are evaluated at 5 points at each of the 11 transects (refer to **Figure 10.2**). Substrate size (but not embeddedness) is also evaluated at 10 additional cross-sections located midway between each of the 11 regular transects (A-K) during the thalweg profile. In the process of measuring substrate particle sizes at each transect, the water depth at each substrate sample point is measured (at the 10 midway cross-sections, depth to the substrate point is not recorded). If the wetted channel is split by a mid-channel bar (**Section 10.5.2**), the five substrate points are centered between the wetted width boundaries regardless of the mid-channel bar in between. Consequently, substrate particles selected in some cross-sections may be “high and dry.” For cross-sections that are entirely dry, make measurements across the **unvegetated portion** of the channel.



PRK/DVP 8/06

Figure 10.2 Substrate Sampling Cross-Section

The substrate sampling points along the cross-section are located at 0, 25, 50, 75, and 100 percent of the measured wetted width, with the first and last points located at the water's edge just within the left and right banks. The procedure for obtaining substrate measurements is described in **Table 10.3** (including all particle size classifications). Record these measurements in the Substrate Cross-sectional Information section of the Physical Habitat Form in the NRSA App,

To minimize bias in selecting a substrate particle for size classification, it is important to concentrate on correct placement of the measuring stick along the cross-section, and to select the particle right at the bottom of the stick (not, for example, a more noticeable large particle that is just to the side of the stick). Classify the particle into one of the size classes listed on the data form based on the middle dimension of its length, width, and depth. This *median dimension* determines the sieve size through which the particle can pass. When you record the

size class as *Other*, describe the substrate type in the adjacent comment bubble on the field form.

At substrate sampling locations on the 11 regular transects (A-K), examine particles larger than sand for surface stains, markings, and algal coatings to estimate embeddedness. Embeddedness is the fraction of a particle’s volume that is surrounded by (embedded in) sand or finer sediments on the stream bottom. For particles 16mm diameter and larger, (e.g., coarse gravel [GC] and above), estimate the embeddedness of the volume of the entire individual particle at the point of the wading rod. For fine gravel (GF), which are particles between 2 and 16mm diameter, estimate the average embeddedness of all particles within a 10 cm diameter circle around the substrate sampling point. By definition, the embeddedness of sand and fines (silt, clay, and muck) is *100 percent*, and the embeddedness of hardpan and bedrock is *zero percent*. When these size classes are selected in the App, the correct embeddedness value will automatically be entered.

Table 10.3 Procedure: Substrate Measurement

<p>1. Select the appropriate cross-section transect at the top of the Physical Habitat Form in the NRSA App. At the transect, extend the surveyor’s rod or metric tape across the channel perpendicular to the flow, with the “zero” end at the left bank (facing downstream).</p> <p><i>NOTE: If a side channel is present, and contains 16 - 49% of the total flow, establish a secondary cross-section transect. Mark the “IS THIS A SIDE CHANNEL” checkbox near the top of the form to record data for the side channel. Unchecking the side channel box will display the primary transect data once again. Collect all channel and riparian cross-section measurements from the side channel as well as the primary channel. While collecting thalweg profile data (including mid-station substrate size class) and large woody debris tallies, record the cumulative data on the primary transect only (e.g., with the side channel check box unchecked).</i></p> <p>2. Enter 0 as the left bank starting distance on the top row data form and record the distance to the right bank (e.g., the wetted width at the transect) to the nearest 0.1 meters on the fifth row. Doing so will prompt the App to divide the wetted channel width by 4 to locate substrate measurement points on the cross-section. The App will enter these values in the three remaining fields of the form. These calculated distances correspond 25% (<i>LEFT CENTER</i>), 50% (<i>CENTER</i>), 75% (<i>RIGHT CENTER</i>).</p> <p>3. Place your sharp ended meter stick or calibrated pole at the <i>LEFT</i> location (0 m). Measure the depth and record it on the field data form.</p> <ul style="list-style-type: none"> • Depth entries at the left and right banks may be 0 (zero) if the banks are gradual. • If the bank is nearly vertical, let the base of the measuring stick fall to the bottom (i.e., the depth at the bank will be > 0 cm), rather than holding it suspended at the water surface. <p>4. Pick up the substrate particle that is at the base of the meter stick (unless it is bedrock or boulder), and visually estimate its particle size, according to the following table. Classify the particle according to its median diameter (the middle dimension of its length, width, and depth). Record the size class code on the data form by tapping the blue data field and selecting the correct size class.</p>																												
<table border="1"> <thead> <tr> <th>Code</th> <th>Size Class</th> <th>Size Range (mm)</th> <th>Description</th> </tr> </thead> <tbody> <tr> <td>RS</td> <td>Bedrock (Smooth)</td> <td>>4000</td> <td>Smooth surface rock bigger than a car</td> </tr> <tr> <td>RR</td> <td>Bedrock (Rough)</td> <td>>4000</td> <td>Rough surface rock bigger than a car</td> </tr> <tr> <td>RC</td> <td>Concrete/Asphalt</td> <td>Regardless of Size</td> <td>Artificial materials</td> </tr> <tr> <td>XB</td> <td>Large Boulders</td> <td>>1000 to 4000</td> <td>Yard/meter stick to car size</td> </tr> <tr> <td>SB</td> <td>Small Boulders</td> <td>>250 to 1000</td> <td>Basketball to yard/meter stick size</td> </tr> <tr> <td>CB</td> <td>Cobbles</td> <td>>64 to 250</td> <td>Tennis ball to basketball size</td> </tr> </tbody> </table>	Code	Size Class	Size Range (mm)	Description	RS	Bedrock (Smooth)	>4000	Smooth surface rock bigger than a car	RR	Bedrock (Rough)	>4000	Rough surface rock bigger than a car	RC	Concrete/Asphalt	Regardless of Size	Artificial materials	XB	Large Boulders	>1000 to 4000	Yard/meter stick to car size	SB	Small Boulders	>250 to 1000	Basketball to yard/meter stick size	CB	Cobbles	>64 to 250	Tennis ball to basketball size
Code	Size Class	Size Range (mm)	Description																									
RS	Bedrock (Smooth)	>4000	Smooth surface rock bigger than a car																									
RR	Bedrock (Rough)	>4000	Rough surface rock bigger than a car																									
RC	Concrete/Asphalt	Regardless of Size	Artificial materials																									
XB	Large Boulders	>1000 to 4000	Yard/meter stick to car size																									
SB	Small Boulders	>250 to 1000	Basketball to yard/meter stick size																									
CB	Cobbles	>64 to 250	Tennis ball to basketball size																									

GC	Gravel (Coarse)	>16 to 64	Marble to tennis ball size
GF	Gravel (Fine)	> 2 to 16	Ladybug to marble size
SA	Sand	>0.06 to 2	Smaller than ladybug; gritty between fingers
FN	Fines	<0.06	Silt Clay Muck (not gritty between fingers)
HP	Hardpan	>4000	Firm, consolidated fine substrate
WD	Wood	Regardless of Size	Wood & other organic particles
OT	Other	Regardless of Size	Concrete, metal, tires, car bodies, etc. (describe in comment bubble)

5. Evaluate substrate embeddedness as follows at each of the five points. For particles larger than sand, examine the surface for stains, markings, and algae. For particles 16mm diameter and larger, (e.g., coarse gravel [GC] and above), estimate the embeddedness of the volume of the entire individual particle at the point of the wading rod. For fine gravel (GF), which are particles between 2 and 16mm diameter, estimate the average embeddedness of all particles within a 10 cm diameter circle around the substrate sampling point. By definition, sand and fines are embedded 100%; bedrock and hardpan are embedded 0%. These values will automatically populate the form. For sand and smaller particles, you will not be able to pick up an individual particle, but a “pinch” of fine particles between your fingers. Determine and record the dominant size of particles in the “pinch.” If a fine flocculent coating coats the particle at the end of the wading rod, we are interested in the diameter of the particle, not the flocculent coating of fine material ---- unless the coating is so thick that it obscures the larger particles. For example, if the tip of the wading rod falls on a cobble, and there is a thin layer of fine sediment that can be scoured away by waving your hand over the particle or by picking it up, note the presence of these fine coatings in comments, but record the diameter and embeddedness of the larger particle.
6. Move to the next location on the transect, and repeat Steps 3 - 5 at each location. Repeat Steps 1 - 5 at each transect, including any additional side channel transects established if side channels are present.

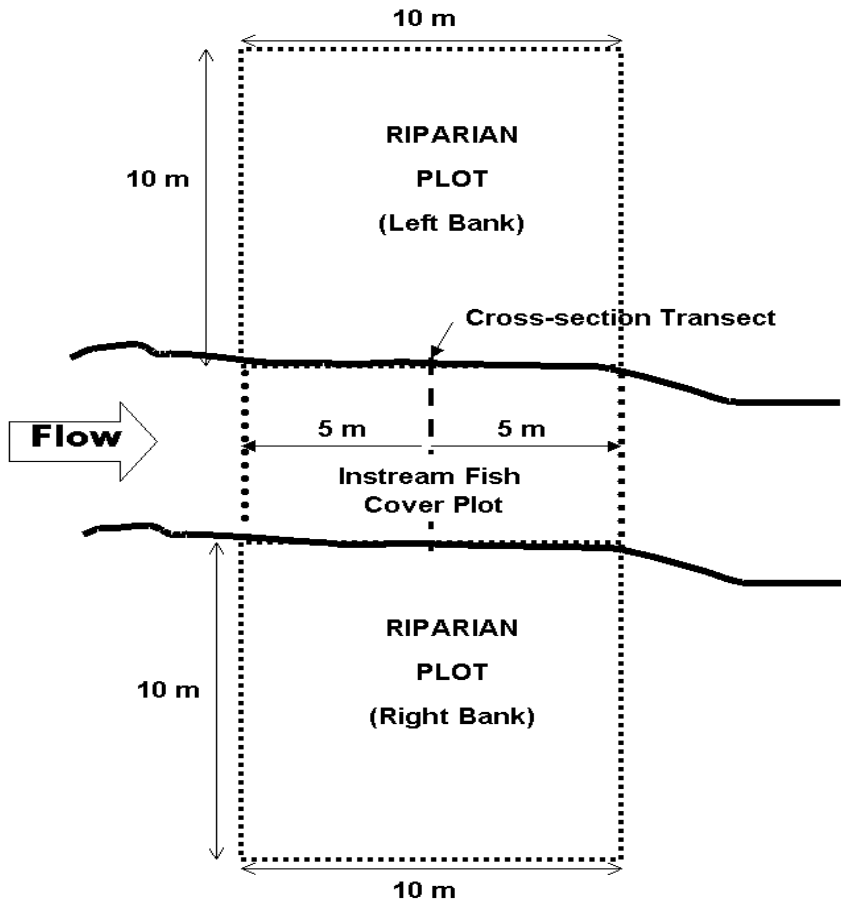
10.5.1.2 Instream Fish Cover, Algae, and Aquatic Macrophytes

Over a defined area upstream and downstream of the sampling transects (**Figure 10.3**), crews shall estimate by eye and/or by sounding the proportional cover of fish cover features and trophic level indicators including large woody debris, rootwads and snags, brush, live trees in the wetted channel, undercut banks, overhanging vegetation, rock ledges, aquatic macrophytes, filamentous algae, and artificial structures.

The procedure to estimate the types and amounts of instream fish cover is outlined in **Table 10.4**. Data are recorded in the Fish Cover section of the Physical Habitat Form in the NRSA App. Estimate the areal cover of all of the fish cover and other listed features that are in the water and on the banks 5 m upstream and downstream of the cross-section (**Figure 10.3**). The areal cover classes of fish concealment and other features are the same as those described for riparian vegetation (**Section 10.5.1.5**).

The entry *FILAMENTOUS ALGAE* refers to long streaming algae that often occur in slow moving waters. *MACROPHYTES* are water loving plants, including mosses, in the stream that could provide cover for fish or macroinvertebrates. If the stream channel contains live wetland grasses, include these as aquatic macrophytes. *WOODY DEBRIS* are the larger pieces of wood that can provide cover and influence stream morphology (i.e., those pieces that would be included in the large woody debris tally [**Section 10.5.2**]). *BRUSH/WOODY DEBRIS* refers to smaller wood pieces that primarily affect cover but not morphology. *LIVE TREES OR ROOTS* are living trees that are

within the channel – estimate the areal cover provided by the parts of these trees or roots that are inundated. *OVERHANGING VEGETATION* includes tree branches, brush, twigs, or other small debris that is not in the water but is close to the stream (within 1 m of the surface) and provides potential cover. For ephemeral channels, estimate the proportional cover of these trees that is inundated during bankfull flows. *BOULDERS* are typically basketball to car-sized particles. *ARTIFICIAL STRUCTURES* include those designed for fish habitat enhancement, as well as in-channel structures that have been discarded (e.g., concrete, asphalt, cars, or tires) or deliberately placed for diversion, impoundment, channel stabilization, or other purposes.



PRK/DVP 8/06

Figure 10.3 Riparian Zone and Instream Fish Cover Plots for a Stream Cross-Section Transect

Table 10.4 Procedure: Estimating Instream Fish Cover

Instream Fish Cover Measurements	
1.	Standing at a point along the transect where riparian observations can be made effectively, estimate a 5 m distance upstream and downstream (10 m total width).
2.	Examine the water and both banks within the 10 m segment of stream for the following features and types of fish cover: <i>filamentous algae, macrophytes, large woody debris, brush and small woody debris, in-channel live trees or roots, overhanging vegetation, undercut banks, boulders, and artificial structures.</i>
3.	For each cover type, estimate the areal cover. Record the appropriate cover class in the <i>FISH COVER</i> section of the Physical Habitat Form in the App: <i>0=absent: zero cover,</i> <i>1=sparse: <10%,</i> <i>2=moderate: 10-40%,</i> <i>3=heavy: >40-75%, or</i> <i>4=very heavy: >75%.</i>
4.	Repeat Steps 1 through 3 at each cross-section transect (including any additional side channel transects established when islands are present).

10.5.1.3 Bank Characteristics

The procedure for obtaining bank and channel dimension measurements is presented in **Table 10.5**. Data are recorded in the *BANK MEASUREMENTS* section of the Physical Habitat Form in the NRSA App. Bank angle and bank undercut distances are determined on the left and right banks at each cross-section transect. **Figure 10.4** illustrates how bank angle is determined for several different situations. Measure bank angle on wadeable streams at the scale of approximately 0.5 m using a short (approx. 1 m long) pole. When measuring the angle, try to ensure that at least half (0.5 m) of the pole length is in contact with the bank. Other features include the wetted width of the channel, the width of exposed mid-channel bars of gravel or sand, estimated incision height, and the estimated height and width of the channel at bankfull stage as described in **Figure 10.5**. *Bankfull height* and *incised height* are both measured relative to the present water surface (i.e., the level of the wetted edge of the stream). This is done by placing the base of the small measuring rod at the bankfull elevation and sighting back to the survey rod placed at the water's edge using the clinometer as a level (i.e., positioned so the slope reading is 0%). The height of the clinometer above the base of the smaller rod is subtracted from the elevation sighted on the surveyor's rod. Bankfull flows are large enough to erode the stream bottom and banks, but frequent enough (every one to two years) to prevent substantial growth of upland terrestrial vegetation. Consequently, in many regions, it is these flows that have determined the width and depth of the channel.

Table 10.5 Procedure: Measuring Bank Characteristics

1.	To measure <i>bank angle</i> , lay a meter ruler or a short (approx. 1 m long) rod down against the left bank (determined as you face downstream), with one end at the water's edge. At least 0.5 m of the ruler or rod should be <i>resting comfortably</i> on the ground to determine bank angle. If the ground adjacent to the water's edge is not indicative of the predominant angle of the 1 meter shoreline, it may be necessary to move the end of the rod away from the water's edge to correctly measure the predominant angle of the 1 meter shoreline. Lay the clinometer on the rod, and read the bank angle in degrees from the external scale on the clinometer. Record the angle in the field for the left bank in the <i>BANK MEASUREMENTS</i> section of the Physical Habitat Form in the App.
----	--

- A *vertical bank* is 90°, *overhanging banks* have angles >90° approaching 180°, and more gradually sloped banks have angles <90°. To measure bank angles >90°, turn the clinometer (which only reads 0 to 90°) over and subtract the angle reading from 180°.
 - If there is a large boulder or log present at the transect, measure bank angle at a nearby point where conditions are more representative.
2. If the bank is *undercut*, measure the horizontal distance of the undercutting to the nearest 0.01 m. The undercut distance is the distance from the deepest point of the undercut out to the point where a vertical plumb line from the bank would hit the water's surface. Record the distance on the field data form. Measure submerged undercuts by thrusting the rod into the undercut and reading the length of the rod that is hidden by the undercutting. If the bank is not undercut, record 0 in the in the Undercut Distance field.
 3. Repeat Steps 1 and 2 on the right bank.
 4. Verify the *wetted width* value determined when locating substrate sampling points. The *WETTED WIDTH* field in the bank measurements section of the form will be populated based on the wetted width entered earlier and is not editable here. Also determine the *bankfull channel width* and the *width of exposed midchannel bars* (if present). Record these values in the *BANK MEASUREMENTS* section of the form. If no bars exist at the transect, enter 0 in the bar width field.
 5. While still holding the surveyor's rod as a guide, and sighting with the clinometer as a level, examine both banks to measure and record the *height of bankfull flow above the present water level*. Look for evidence on one or both banks such as:
 - An obvious slope break that differentiates the channel from a relatively flat floodplain terrace higher than the channel.
 - A transition from exposed stream sediments to terrestrial vegetation.
 - Moss growth on rocks along the banks.
 - Presence of drift material caught on overhanging vegetation.
 - A transition from flood and scour tolerant vegetation to that which is relatively intolerant of these conditions.
 6. Hold the surveyor's rod vertical, with its base planted at the water's edge. Examine both banks, then determine the channel *incision* as the *height up from the water surface to elevation of the first terrace of the valley floodplain* (Note, this is at or above the bankfull channel height). Whenever possible, use the clinometer as a level (positioned so it reads 0% slope) to measure this height by transferring (backsighting) it onto the surveyor's rod. Record this value in the *INCISED HEIGHT* field of the bank measurement sections on the form.
 7. Repeat Steps 1 through 6 at each cross-section transect, (including any additional side channel transects established when islands are present).

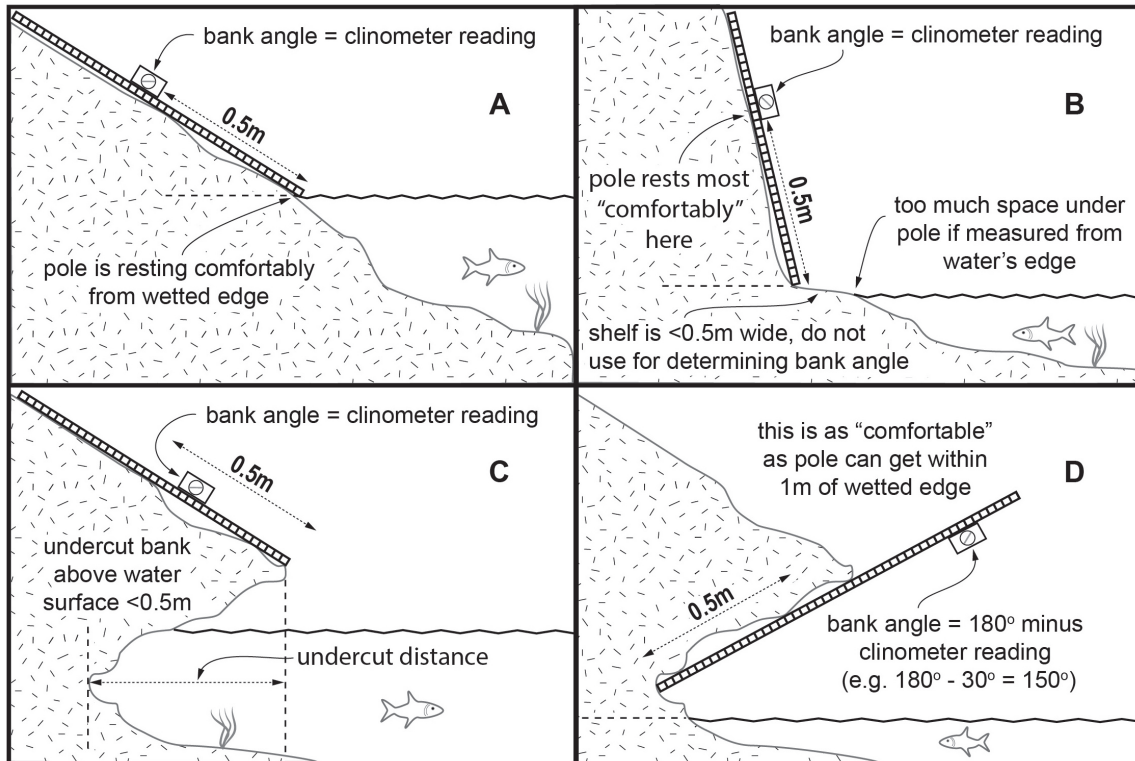


Figure 10.4 Determining Bank Angle Under Different Types of Bank Conditions

(A) typical, (B) incised channel, (C) undercut bank (less than 0.5 m), and (D) overhanging bank (greater than 0.5 m).

Unfortunately, we have to depend upon evidence visible during the low flow sampling season. If available, consult published rating curves relating expected bankfull channel dimensions to stream drainage area within the region of interest. Graphs of these rating curves can help you get a rough idea of where to look for field evidence to determine the level of bankfull flows. Curves such as these are available from the USGS for streams in most regions of the U.S. (e.g., Dunne and Leopold 1978; Harrelson et al. 1994, Leopold 1994). To use them, you need to know the contributing drainage area to your sample site. Interpret the expected bankfull levels from these curves as a height above the streambed in a riffle, but remember that your field measurement will be a height above the present water surface of the stream. Useful resources to aid your determination of bankfull flow levels in streams in the United States are video presentations produced by the USDA Forest Service for western streams (USDA Forest Service 1995) and eastern streams (USDA Forest Service 2002).

After consulting rating curves that show where to expect bankfull levels in a given size of stream, estimate the bankfull flow level by looking at the following indicators:

First look at the stream and its valley to determine the active floodplain. This is a depositional surface that frequently is flooded and experiences sediment deposition under the current climate and hydrological regime.

Then look specifically for:

- An obvious break in the slope of the banks.
- A change from water loving and scour tolerant vegetation to more drought tolerant vegetation.

- A change from well sorted stream sediments to unsorted soil materials.

In the absence of clear bankfull indications, consider the previous season's flooding as the best evidence available (note: you could be wrong if very large floods or prolonged droughts have occurred in recent years.). Look for:

- Drift debris (“sticky wickets” left by the previous seasons flooding).
- The level where deciduous leaf fall is absent on the ground (carried away by previous winter flooding).
- Unvegetated sand, gravel or mud deposits from previous years flooding.

In years that have experienced large floods, drift material and other recent high flow markers may be much higher than other bankfull indicators. In such cases, base your determination on less transient indicators such as channel form, perennial vegetation, and depositional features. In these cases, provide a comment associated with the height of drift material in the provided comment bubble on the form.

We use the vertical distance (height) from the observed water surface up to the level of the first major valley depositional surface (**Figure 10.5**) as a measure of the degree of *incision* or *downcutting* of the stream below the general level of its valley. This value is recorded in the *INCISED HEIGHT* field. It may not be evident at the time of sampling whether the channel is downcutting, stable, or aggrading (raising its bed by depositing sediment). However, by recording incision heights measured in this way and monitoring them over time, we will be able to tell if streams are incising or aggrading.

If the channel is not greatly incised, bankfull channel height and incision height will be the same (i.e., the first valley depositional surface is the active floodplain). However, if the channel is incised greatly, the bankfull level will be below the level of the first terrace of the valley floodplain, making bankfull channel height less than incision height (**Figure 10.6**). *Bankfull height is never greater than incision height*. You may need to look for evidence of recent flows (within about one year) to distinguish bankfull and incision heights. 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 at the edge of the valley is not necessarily an indication of recent incision. In such a case, the opposite bank may be lower, with a more obvious terrace above bankfull height; choose that bank for your measurement of incised height. Examine both banks to more accurately determine incision height and bankfull height. Remember that incision height is measured as *the vertical distance to the first major depositional surface above bankfull* (whether or not it is an active floodplain or a terrace. If terrace heights differ on left and right banks (both are above bankfull), choose the lower of the two terraces. In many cases your sample reach may be in a “V” shaped valley or gorge formed over eons, and the slope of the channel banks simply extends uphill indefinitely, not reaching a terrace before reaching the top of a ridge. In such cases, record incision height values equal to bankfull values and make appropriate comment that no terrace is evident. Similarly, when the stream has extremely incised into an ancient terrace, (e.g., the Colorado River in the Grand Canyon), you may crudely estimate the terrace height if it is the first one above bankfull level. If you cannot estimate the terrace height, make appropriate comments describing the situation.

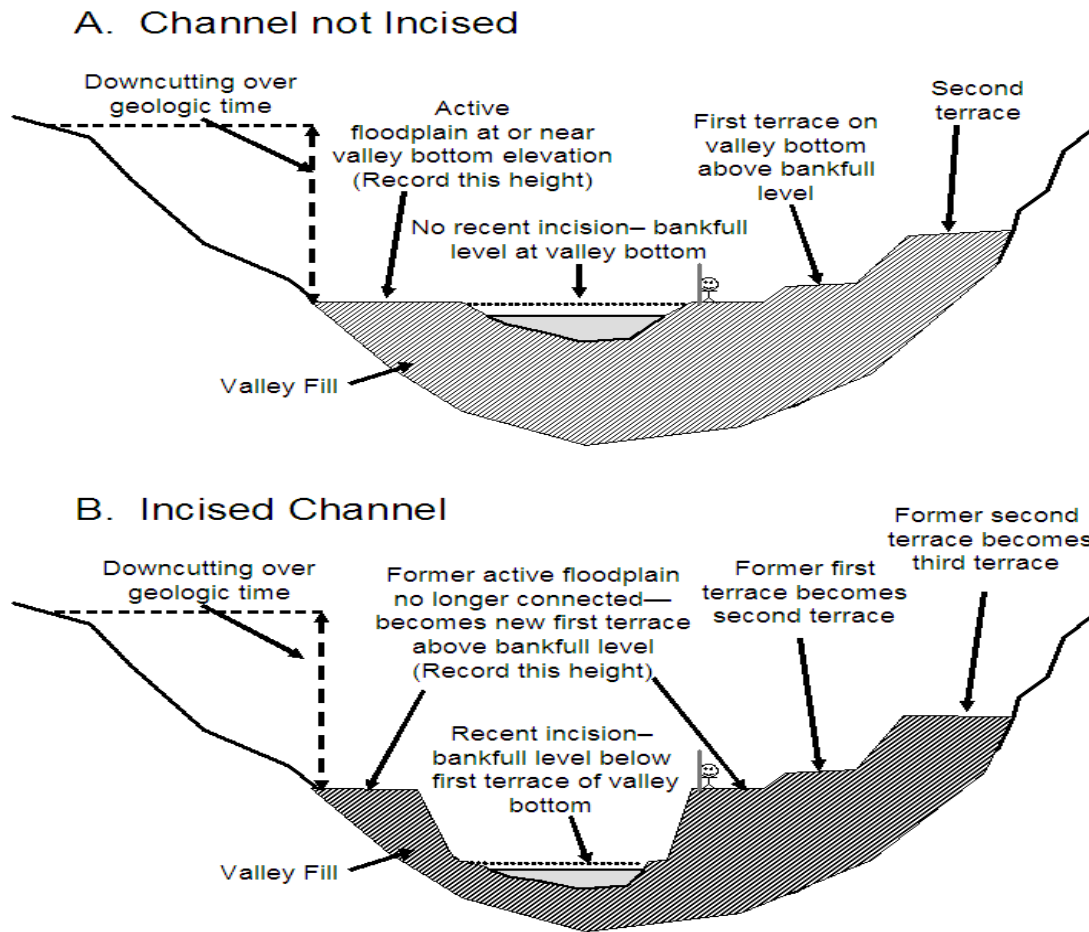
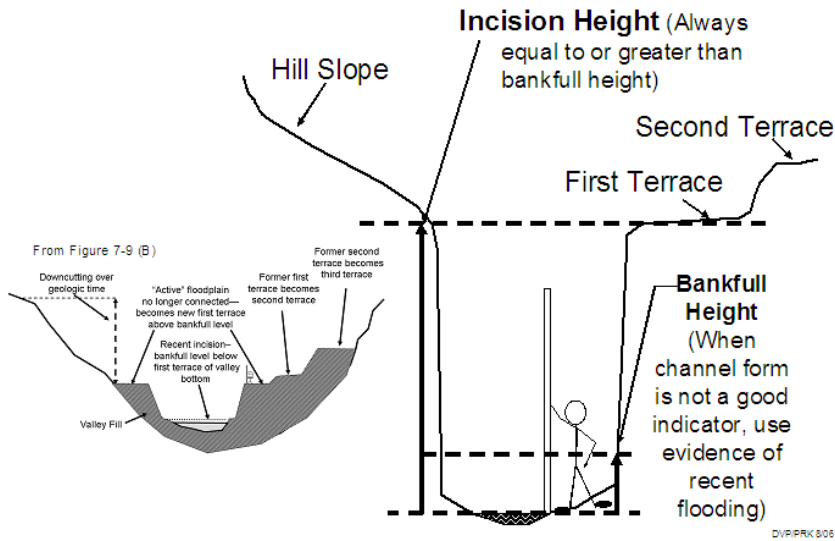


Figure 10.5 Schematic Showing Relationship Between Bankfull Channel and Incision
(A) Not recently incised, and (B) recently incised into valley bottom. Note level of bankfull stage relative to elevation of first terrace (abandoned floodplain) on valley bottom. (Stick figure included for scale).

A) Deeply Incised Channel



B) Small stream constrained in V-shaped valley

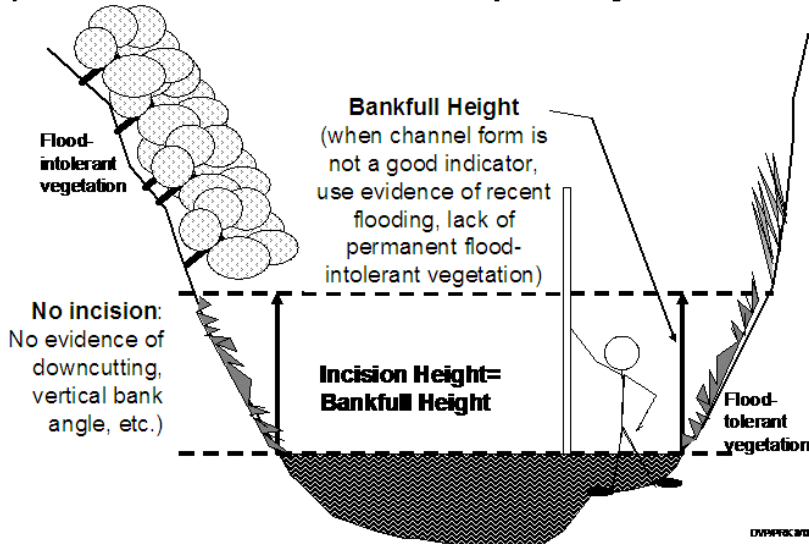


Figure 10.6 Determining Bankfull and Incision Heights

(A) Deeply Incised Channels, and (B) Streams in Deep V Shaped Valleys (Stick figure included for scale).

10.5.1.4 Canopy Cover Measurements

Canopy cover over the stream is determined at each of the 11 cross-section transects. A spherical densiometer (model A **convex type**) is used (Lemmon 1957) and is provided in the base kit to each crew. Mark the densiometer with a permanent marker or tape exactly as shown in **Figure 10.7** to limit the number of square grid intersections read to 17. Densiometer readings can range from 0 (no canopy cover) to 17 (maximum canopy cover). Six measurements are obtained at each cross-section transect (four measurements in each of four directions at mid-channel and one at each bank).

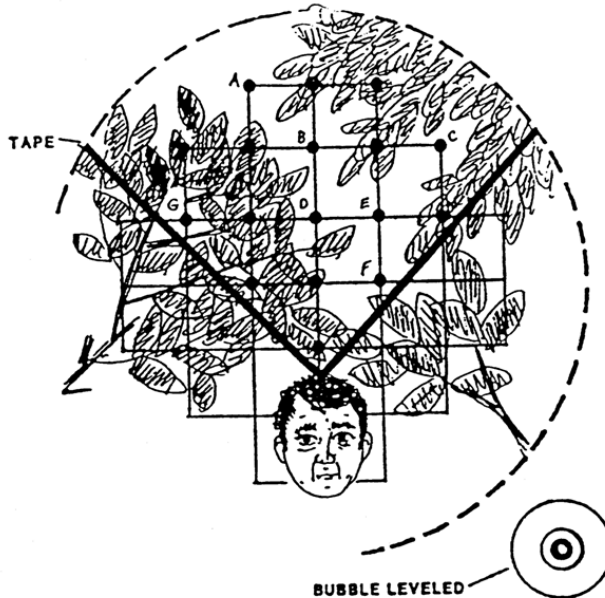


Figure 10.7 Schematic of Modified Convex Spherical Canopy Densiometer.

From Mulvey et al. (1992). In this example, 10 of the 17 intersections show canopy cover, giving a densiometer reading of 10. Note proper positioning with the bubble leveled and face reflected at the apex of the "V".

The procedure for obtaining canopy cover data is presented in **Table 10.6**. Hold the densiometer level (using the bubble level) 0.3 m above the water surface with your face reflected just below the apex of the taped "V", as shown in **Figure 10.7**. Concentrate on the 17 points of grid intersection on the densiometer that lie within the taped "V". If the reflection of a tree or high branch or leaf overlies any of the intersection points, that particular intersection is counted as having cover. For each of the six measurement points, record the number of intersection points (maximum=17) that have vegetation or other overhead objects covering them in the *CANOPY COVER MEASUREMENTS* section of the Physical Habitat Form in the NRSA App.

Table 10.6 Procedure: Canopy Cover Measurements

Canopy Cover Measurements with Densiometer	
1.	At each cross-section transect, stand in the stream at mid-channel 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 either a tree, a leaf, or a high branch. Record the value (0 to 17) in the <i>CENTER UP</i> field of the canopy cover measurements section of the Physical Habitat Form in the App.
4.	Face toward the left bank (left as you face downstream). Repeat Steps 2 and 3, recording the value in the <i>CENTER LEFT</i> field of the form.
5.	Repeat Steps 2 and 3 facing downstream, and again while facing the right bank (right as you look downstream). Record the values in the <i>CENTER DOWN</i> and <i>CENTER RIGHT</i> fields of the form.
6.	Move to the water’s edge (either the left or right bank). Repeat Steps 2 and 3 again, this time facing the bank. Record the value in the <i>LEFT</i> or <i>RIGHT</i> fields of the form. Move to the opposite bank and repeat.
7.	Repeat Steps 1 through 6 at each cross-section transect (including any additional side channel transects established when islands are present).

10.5.1.5 Visual Riparian Estimates

10.5.1.5.1 Riparian Vegetation Structure

The previous section described methods for quantifying the cover of canopy over the stream channel. The following visual estimation procedures supplement those measurements with a semi-quantitative evaluation of the type and amount of various types of riparian vegetation.

Riparian vegetation observations apply to the riparian area upstream five meters and downstream five meters from each of the 11 cross-section transects. They include the visible area from the stream back a distance of 10 m (~30 ft.) shoreward from both the left and right banks, creating a 10 m x 10 m riparian plot on each side of the stream (**Figure 10.3**). The riparian plot dimensions are estimated, not measured. On steeply sloping channel margins, the 10 m x 10 m plot boundaries are defined as if they were projected down from an aerial view. **Table 10.7** presents the procedure for characterizing riparian vegetation structure and composition.

Riparian estimates are recorded in the Visual Riparian Estimates section of the Physical Habitat Form. Conceptually divide the riparian vegetation into 3 layers: the *Canopy* layer (> 5 m high), the *Understory* layer (0.5 to 5 m high), and the *Ground cover* layer (< 0.5 m high). Note that several vegetation types (e.g., grasses or woody shrubs) can potentially occur in more than one layer. Similarly note that some things other than vegetation are possible entries for the *Ground cover* layer (e.g., barren ground).

Before estimating the areal coverage of the vegetation layers, record the type of *woody* vegetation (*broadleaf Deciduous*, *Coniferous*, *broadleaf Evergreen*, *Mixed*, or *None*) in each of the two taller layers (Canopy and Understory). Consider the layer *Mixed* if more than 10% of the areal coverage is made up of the alternate vegetation type. If there is no woody vegetation in the canopy or understory layer, record the type as *None*.

Estimate the areal cover separately in each of the three vegetation layers. Note that the areal cover can be thought of as the amount of shadow cast by a particular layer alone when the sun

is directly overhead. *The maximum cover in each layer is 100%, so the sum of the areal covers for the combined three layers could add up to 300%.* The four areal cover classes are *Absent (0%), Sparse (<10%), Moderate (10 to 40%), Heavy (40 to 75%), and Very Heavy (>75%)*. These cover classes and their corresponding codes are shown on the form for reference. When rating vegetation cover types for a single vegetation layer, mixtures of two or more subdominant classes might all be given *Sparse (1), Moderate (2), or Heavy (3)* ratings. One *Very Heavy* cover class with no clear subdominant class might be rated *4* with all the remaining classes rated as either *Moderate (2), Sparse (1) or Absent (0)*. Note that within a given vegetation layer, two cover types with 40-75% cover can both be rated *3*, but no more than one cover type could receive a rating of *4*.

Table 10.7 Procedure: Characterizing Riparian Vegetation Structure

Riparian Vegetative Structure Measurements	
1.	Standing at a point along the transect where riparian observations can be made effectively, estimate a 5 m distance upstream and downstream (10 m total length).
2.	Facing the left bank (left as you face downstream), estimate a distance of 10 m back into the riparian vegetation. On steeply sloping channel margins, estimate the distance into the riparian zone as if it were projected down from an aerial view.
3.	Within this 10 m x 10 m area, conceptually divide the riparian vegetation into 3 layers: a <i>Canopy Layer</i> (> 5 m high), an <i>Understory</i> (0.5 to 5 m high), and a <i>Ground Cover</i> layer (<0.5 m high).
4.	Within this 10 m x 10 m area, determine the dominant woody vegetation type for the <i>CANOPY LAYER</i> (vegetation >5 m high) as either <u>D</u> eciduous, <u>C</u> oniferous, broadleaf <u>E</u> vergreen, <u>M</u> ixed, or <u>N</u> one. Consider the layer <i>Mixed</i> if more than 10% of the areal coverage is made up of the alternate vegetation type. If the canopy layer contains no vegetation or the dominant vegetation type in the canopy layer is not woody, record the vegetation type as " <u>N</u> one". Indicate the appropriate vegetation type in the <i>VISUAL RIPARIAN ESTIMATES</i> section of the Physical Habitat Form.
5.	Determine separately the areal cover class of large trees (≥0.3 m [1 ft] diameter at breast height [dbh]) and small trees (<0.3 m dbh) within the canopy layer. Estimate areal cover as the amount of shadow that would be cast by a particular layer alone if the sun were directly overhead. Record the appropriate cover class on the form (<i>0=absent: zero cover, 1=sparse: <10%, 2=moderate: 10-40%, 3=heavy: 40-75%, or 4=very heavy: >75%</i>).
6.	Look at the <i>UNDERSTORY</i> layer (vegetation between 0.5 and 5 m high). Determine the dominant woody vegetation type for the understory layer as described in Step 4 for the canopy layer. If the understory layer contains no vegetation or the dominant vegetation type in the understory is not woody (e.g., herbaceous), record the vegetation type as " <u>N</u> one".
7.	Determine the areal cover class for woody shrubs and saplings (as well as trunks and branches of trees) separately from non-woody vegetation within the understory, as described in Step 5 for the canopy layer.
8.	Look at the <i>GROUND COVER</i> layer (vegetation <0.5 m high). Determine the areal cover class for woody shrubs and seedlings (including trunks and branches of trees), non-woody vegetation, and the amount of bare ground or duff (dead organic material present as described in Step 5 for the canopy layer). Include all non-vegetative materials such as buildings, pavement, standing water, etc. In the "barren, bare dirt, or duff" category.
9.	Repeat Steps 1 through 8 for the right bank.
10.	Repeat Steps 1 through 9 for all cross-section transects (including any additional side channel transects established when islands are present).

10.5.1.5.2 Human Influence

For the left and right banks at each of the 11 detailed Channel and Riparian Cross-sections, evaluate the presence/absence and the proximity of 11 categories of human influences with the procedure outlined in **Table 10.8**. Record data in the Human Influence section of the Physical Habitat Form in the App. Relate your observations and proximity evaluations to the stream and riparian area within 5 m upstream and 5 m downstream from the station (**Figure 10.8**). Four proximity classes are used: In the stream or on the bank within 5 m upstream or downstream of the cross-section transect (B), contained within the 10 m x 10 m riparian plot but not in the stream or on the bank (C), present outside of the riparian plot (P), and absent (0). If a disturbance is within more than one proximity class, record the one that is closest to the stream (e.g., present in riparian plot “C” takes precedence over outside of riparian plot “P”).

You may mark “P” more than once for the same human influence observed outside of more than one riparian observation plot (e.g., at both Transects D and E). **The rule is that you count human disturbance items as often as you see them, BUT NOT IF you have to site through another transect or its 10x10 meter riparian plot.**

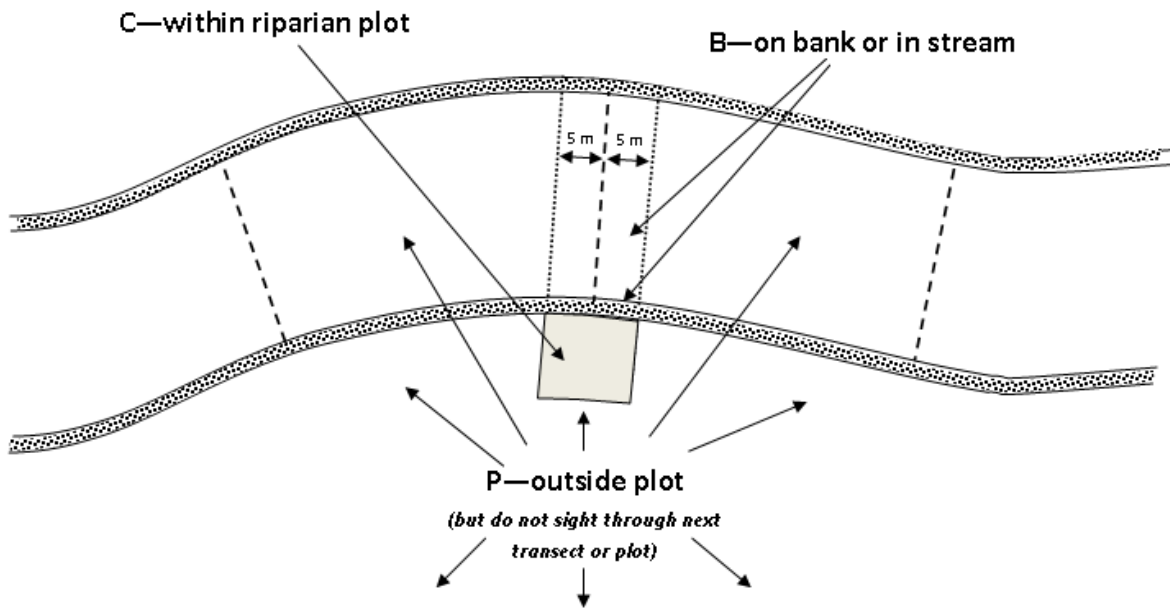


Figure 10.8 Proximity Classes for Human Influences in Wadeable Streams

Table 10.8 Procedure: Estimating Human Influence

Estimate Human Influence									
1.	Standing at a point along the transect where riparian observations can be made effectively, look toward the left bank (left when facing downstream), and estimate a 5 m distance upstream and downstream (10 m total width). Also, estimate a distance of 10 m back into the riparian zone to define a riparian plot area.								
2.	Examine the channel, bank and riparian plot area adjacent to the defined stream segment for the following human influences: (1) <i>walls, dikes, revetments, riprap, and dams</i> ; (2) <i>buildings</i> ; (3) <i>pavement/cleared lots</i> (e.g., paved, graveled, dirt parking lot, foundation); (4) <i>roads or railroads</i> , (5) <i>inlet or outlet pipes</i> ; (6) <i>landfills or trash</i> (e.g., cans, bottles, trash heaps); (7) <i>parks or maintained lawns</i> ; (8) <i>row crops</i> ; (9) <i>pastures, rangeland, hay fields, or evidence of livestock</i> ; (10) <i>logging</i> ; and (11) <i>mining</i> (including gravel mining). If human influences are observed that do not match one of the listed categories, choose the category that best describes the influence and add further explanation in the adjacent comment bubble.								
3.	For each type of influence, determine if it is present and what its proximity is to the stream and riparian plot area. Consider human disturbance items as present if you can see them from the cross-section transect. Do not include them if you have to sight through another transect or its 10 m x 10 m riparian plot.								
4.	For each type of influence, record the appropriate proximity class in the <i>HUMAN INFLUENCE</i> part of the <i>VISUAL RIPARIAN ESTIMATES</i> section of the Physical Habitat Form in the App. If a disturbance is within more than one proximity class, record the one that is closest to the stream. Proximity classes (listed in order of priority) are: <table border="0" data-bbox="357 945 1429 1165"><tr><td><i>B (Bank)</i></td><td>Present within the defined 10 m stream segment and located in the stream or on the stream bank.</td></tr><tr><td><i>C (Contained)</i></td><td>Present within the 10 x 10 m riparian plot area.</td></tr><tr><td><i>P (Present)</i></td><td>Present, but outside the riparian plot area.</td></tr><tr><td><i>O (Absent)</i></td><td>Not present within or adjacent to the 10 m stream segment or the riparian plot area at the transect</td></tr></table>	<i>B (Bank)</i>	Present within the defined 10 m stream segment and located in the stream or on the stream bank.	<i>C (Contained)</i>	Present within the 10 x 10 m riparian plot area.	<i>P (Present)</i>	Present, but outside the riparian plot area.	<i>O (Absent)</i>	Not present within or adjacent to the 10 m stream segment or the riparian plot area at the transect
<i>B (Bank)</i>	Present within the defined 10 m stream segment and located in the stream or on the stream bank.								
<i>C (Contained)</i>	Present within the 10 x 10 m riparian plot area.								
<i>P (Present)</i>	Present, but outside the riparian plot area.								
<i>O (Absent)</i>	Not present within or adjacent to the 10 m stream segment or the riparian plot area at the transect								
5.	Repeat Steps 1 through 4 for the right bank.								
6.	Repeat Steps 1 through 5 for each cross-section transect, (including any additional side channel transects established when islands are present).								

10.5.2 Thalweg Profile and Large Woody Debris Measurements

10.5.2.1 Thalweg Profile

“Thalweg” refers to the flow path of the deepest water in a stream channel. The *thalweg profile* is a longitudinal survey of maximum flow path depth. Data from the thalweg profile allows calculation of indices of residual pool volume, stream size, channel complexity, and the relative proportions of habitat types such as riffles and pools. Thalweg profile increments are spaced evenly throughout the sampling reach to accurately represent the maximum stream depths throughout the entire reach and must be spaced close enough to provide data on both major and minor habitat units.

Follow these guidelines for choosing the increment between thalweg profile measurements:

Channel Width < 2.5 m:

- Minimum reach length of 150 m is used
- Thalweg increment = 1.0 m (resulting in 15 thalweg measurements per subreach)
- A total of 150 evenly spaced thalweg profile measurements will be made, 15 between each channel cross-section
- Mid-subreach measurements are made at the 7th thalweg location

Channel Width 2.5 to 3.5 m:

- Minimum reach length of 150 m is used
- Thalweg increment = 1.5 m (resulting in 10 thalweg, measurements per subreach)
- A total of 100 evenly spaced thalweg profile measurements will be made, 10 between each channel cross-section
- Mid-subreach measurements are made at the 5th thalweg location

Channel Width > 3.5 m:

- Reach length is 40 times channel width (maximum of 4 km)
- Thalweg increment = $0.01 \times$ reach length (resulting in 10 thalweg measurements per subreach)
- A total of 100 evenly spaced thalweg profile measurements will be made, 10 between each channel cross-section
- Mid-subreach measurements are made at the 5th thalweg location

The procedure for obtaining thalweg profile measurements is presented in **Table 10.9**. Record data in the Thalweg Profile and Large Woody Debris sections of the Physical Habitat Form in the NRSA App Use the surveyor's rod and a metric ruler or calibrated rod or pole to make the required depth and width measurements at each station, and to measure off the distance between stations as you proceed upstream. You may need to make minor adjustments to align each 10th measurement to be one increment short of the next transect. In streams with average widths less than 2.5 m, make thalweg measurements at 1 meter increments. Because the minimum reach length is set at 150 meters, there will be 15 measurements on a field data form: Station 0 at the transect plus 14 additional stations between it and the next transect upstream.

Use the five extra lines on the thalweg profile portion of the data form to record measurements 10-14.

Measure thalweg depths at all stations. Provide a comment for any missing measurements in the comment bubbles on the data form. At points where a direct depth measurement cannot be made, make your best estimate of the depth, record it on the form, and comment that it is an estimated value. Where the thalweg points are too deep for wading, measure the depth by extending the surveyor's rod or weighted line at an angle to reach the thalweg point. On the field form enter a comment associated with the thalweg station (to indicate a nonstandard technique), and calculate the thalweg depth based on the water level on the rod or line, and the rod/line angle, as determined using the external scale on the clinometer (vertical = 90).

Table 10.9 Procedure: Thalweg Profile

1. Determine the increment distance between measurement stations based on the wetted width used to determine the length of the reach. Using a laser rangefinder or surveyor's rod:
 - For widths < 2.5 m, establish stations every 1 m (150 total).
 - For widths ≥ 2.5 and ≤ 3.5 m, establish stations every 1.5 m (100 total).
 - For widths > 3.5 m, establish stations at increments equal to 0.01 times the reach length (100 total).
2. With Transect A selected on the Physical Habitat Form in the App, complete the Thalweg Profile header information. Record the increment distance determined in Step 1 in the *INCREMENT* field and the total reach length (e.g., the total distance from Transect A to Transect K) in the *TOTAL REACH LENGTH* field. It is not necessary to repeat these entries on the remainder of the Physical Habitat transect forms, the App will populate the remainder of the fields based on the entries in Transect A.
3. Begin at the downstream end (*station 0*) of the first transect (Transect A).
4. Verify the wetted width at station 0. *The Wetted Width field for station 0 will be populated based on the wetted width entered earlier and is not editable here.* You will also measure and record the wetted width at either station 5 (if the stream width defining the reach length is ≥ 2.5 m), or station 7 (if the stream width defining the reach length is < 2.5 m). Wetted width is measured across and over mid-channel bars and boulders. Record the width on the field data form to the nearest 0.1 m. For streams with interrupted flow, where no water is in the channel at the station or transect, record zeros for wetted width.

NOTE: If a mid-channel bar is present at a station where wetted width is measured, measure the wetted width across and including the bar, but also measure the bar width and record it on the field data form.

5. At station 5 or 7 (see above) classify the size of the bed surface particle at the tip of your depth measuring rod at the left wetted margin (0%) and at positions 25%, 50%, 75%, and 100% of the distance across the wetted width of the stream. This procedure is identical to the substrate size evaluation procedure described for regular channel cross-sections (Transects A - K), except that for these midway supplemental cross-sections, substrate size is entered in the Thalweg Profile section of the Physical Habitat Form in the App.
6. At each thalweg profile station, use a calibrated pole or rod to locate the deepest point within the deepest flow path (*the thalweg*), which may not always be found at mid-channel (and may not always be the absolute deepest point in every channel cross-section). Measure the thalweg depth to the nearest cm from the substrate surface to the water surface, and record it on the form. Read the depth on the **side** of the rod to avoid inaccuracies due to the wave formed by the rod in moving water.

NOTE: For streams with interrupted flow if there is no water at a transect, record zeros for depth.

NOTE: Obtain thalweg depths at all stations. If the thalweg is too deep to measure directly, stand in shallower water and extend the surveyor's rod or pole at an angle to reach the thalweg. Determine the angle by resting the clinometer on the upper surface of the rod and reading the angle on the external scale

of the clinometer. Calculate the thalweg depth based on your measurements and record a comment in the provided comment bubble to indicate a non-standard procedure was used. For deeper depths, use the same procedure with a taut string as the measuring device. Tie a weight to one end of a length of string or fishing line, and toss the weight into the deepest channel location. Draw the string up tight and measure the length of the line that is under water. Measure the string angle with the clinometer exactly as done for the surveyor's rod. If a direct measurement cannot be obtained, make the **best estimate** you can of the thalweg depth, and use a comment to identify it as an estimated measurement.

7. At the point where the thalweg depth is determined, observe if unconsolidated, loose (*soft*) deposits of small diameter ($\leq 16\text{mm}$) sediments are present directly beneath your ruler, rod, or pole. *Soft/small sediments* are defined here as fine gravel, sand, silt, clay or muck readily apparent by "feeling" the bottom with the rod. Record presence or absence using the checkboxes in the *SOFT/SMALL SEDIMENT* column on the form (an unchecked box indicates absence). *Note: A thin coating of fine sediment or silty algae coating the surface of cobbles should not be considered soft/small sediment. However, fine sediment coatings should be identified in the comments section of the field form when determining substrate size and type.*
8. Determine the channel unit code for the station. Record this on the form by tapping the blue data field and selecting from the codes provided. For dry and intermittent streams, where no water is in the channel, record habitat type as dry channel (*DR*).
9. If the station cross-section intersects a mid-channel bar, indicate the presence of the bar by using the checkboxes in the *BAR PRESENT* column on the form (an unchecked box indicates absence). However, a measurement of the bar width is only taken if the bar intersects a station at either the endpoint or midpoint of a subreach (e.g., station 0 and station 5 or 7). If this is the case, measure and record the bar width to the nearest 0.1 meter in the provided data field.
10. Record the presence or absence of a side channel at the station's cross-section using the checkboxes in the *SIDE CHANNEL* column on the form (an unchecked box indicates absence).
11. Record the presence or absence of quiescent off-channel aquatic habitats, including sloughs, alcoves and backwater pools using the checkboxes in the *BACKWATER* column of the (an unchecked box indicates absence).
12. Proceed upstream the specified increment to the next station, and repeat Steps 4 through 11.
13. Repeat Steps 4 through 12 until you reach the next transect (the last station will be one increment prior to the next transect). At this point, select the next transect bubble at the top of the Physical Habitat Form and complete Channel/Riparian measurements at the new transect (**Section 10.4.1**). Then record Thalweg Profile and Woody Debris data and repeat Steps 4 through 12 for each of the reach segments, until you reach the upstream end of the sampling reach (Transect *K*). At Transect *K*, you will have completed 10 separate Thalweg Profiles, one for each subreach (*A* to *B*, *B* to *C*, etc.).

At every thalweg increment, determine by sight or feel whether deposits of *soft/small sediments* are present on the channel bottom. These particles are defined as substrate equal to or smaller than fine gravel ($\leq 16\text{ mm}$ diameter). These soft/small sediments are **different** from *Fines* described when determining the substrate particle sizes at the cross-section transects (**Section 10.5.1.1**). If the channel bottom is not visible, determine if soft/small sediment deposits are readily obvious by feeling the bottom with your boot, the surveyor's rod, or a calibrated rod or pole.

Measure wetted width at each transect (station *0*), and midway between transects (station 5 for larger streams having 100 measurement points, or station 7 for smaller streams having 150 measurement points). The wetted width boundary is the point at which substrate particles are no longer surrounded by free water. Estimate substrate size for five locations evenly spaced

across each midway cross-section at regular cross-sections (**Figure 10.1**), but at the supplemental cross-sections, only the size class (not distance and depth) data are recorded.

While recording the width and depth measurements and the presence of soft/small sediments, the second person evaluates and records the habitat class (**Table 10.10**) applicable to each of the 100 (or 150) measurement points along the length of the reach. Make channel unit scale habitat classifications at the thalweg of the cross-section. The habitat unit itself must meet a minimum size criteria in addition to the qualitative criteria listed in **Table 10.10**. Before being considered large enough to be identified as a channel unit scale habitat feature, the unit should be at least as long as the channel is wide. For instance, if there is a small deep (pool like) area at the thalweg within a large riffle area, do not record it as a pool unless it occupies an area about as wide or long as the channel is wide. If a backwater pool **dominates the channel**, record *PO* (*Pool*) as the dominant habitat unit class. If the backwater is a pool that **does not dominate** the main channel, or if it is an **off channel** alcove or slough (large enough to offer refuge to small fishes), select the checkbox to indicate presence of a backwater in the *BACKWATER* column of the form, but classify the main channel habitat unit type according to characteristics of the main channel. *Sloughs* are backwater areas having marsh like characteristics such as vegetation, and *alcoves* (or *side pools*) are deeper areas off the main channel that are typically wide and shallow (Helm 1985, Bain and Stevenson 1999).

Table 10.10 Channel Unit Categories

Channel Unit Habitat Classes ¹	
Class (Code)	Description
Pool (PO)	Still water, low velocity, a smooth, glassy surface, usually deep compared to other parts of the channel
Glide (GL)	Water moving slowly, with a <i>smooth, unbroken surface</i> . Low turbulence.
Riffle (RI)	Water moving, with <i>small ripples, waves and eddies</i> waves not breaking, <i>surface tension not broken</i> . Sound: babbling, gurgling.
Rapid (RA)	Water movement rapid and turbulent, surface with <i>intermittent whitewater</i> with breaking waves. Sound: continuous rushing, but not as loud as cascade.
Cascade (CA)	Water movement rapid and very turbulent over steep channel bottom. Much of the water surface is broken in <i>short, irregular plunges, mostly whitewater</i> . Sound: roaring.
Falls (FA)	<i>Free falling water</i> over a vertical or near vertical drop into plunge, water turbulent and white over high falls. Sound: from splash to roar.
Dry Channel (DR)	No water in the channel, or flow is submerged under the substrate (<i>hyporheic flow</i>).

¹ Note that in order for a channel habitat unit to be distinguished, it must be at least as wide or long as the channel is wide (except for off channel backwater pools, which are noted as present regardless of size).

10.5.2.2 Large Woody Debris Tally

Large Woody Debris (LWD) is defined here as woody material with a small end diameter of at least 10 cm (4 in.) **and** a length of at least 1.5 m (5 ft.). This includes any portion of woody material that meets those minimum size categories (e.g., include any LWD piece with at least 1.5 meters of its length having a diameter of 10 cm or greater). The procedure for tallying LWD is presented in **Table 10.11**. The tally includes all pieces of LWD that are at least partially in the baseflow channel (Zone 1), in the *bankfull channel* (Zone 2, flood channel up to bankfull stage), or spanning above the bankfull channel (Zone 3), as shown in **Figure 10.9**. LWD in zones 1 and 2 will be tallied together (considered all or in-part in the bankfull channel). The *bankfull channel* is defined as the channel that is filled by moderate sized flood events that typically recur every one to two years. LWD in or above the bankfull channel is tallied over the entire length of the reach, including the area between the channel cross-section transects. Pieces of LWD that are not at least partially within Zones 1, 2, or 3 are not tallied.

For each tally (Pieces All/Part in Bankfull Channel and Pieces Bridge Above Bankfull Channel), the field form (**Figure 10.10**) provides 12 entry boxes for tallying debris pieces visually estimated within three length and four diameter class combinations. Tally each LWD piece in only one box.

For each LWD piece, first visually estimate its length and its large and small end diameters and place it in one of the diameter and length categories. The diameter classes on the field form (**Figure 10.10**) refer to the large end diameter. Sometimes LWD is not cylindrical, so it has no clear “diameter”. In these cases, visually estimate what the diameter would be for a piece of wood with circular cross-section that would have the same volume. When evaluating length, include only the part of the LWD piece that has a diameter >10 cm. Count each of the LWD pieces as one tally entry and include the whole piece when assessing dimensions. If you encounter massive, complex debris jams, estimate their length, width, and height. Estimate the diameter and length of large “key” pieces and judge the average diameter and length of the other pieces making up the jam. Record this information in the comments section of the form.

Table 10.11 Procedure: Tallying Large Woody Debris

Large Woody Debris Tally Form
<p>Note: Tally pieces of large woody debris (LWD) within each segment of stream while the thalweg profile is being determined. Include all pieces in the tally whose large end is found within the subreach.</p> <ol style="list-style-type: none"> Scan the stream segment between the two cross-section transects where thalweg profile measurements are being made. Tally all LWD pieces within the segment that are at least partially within the bankfull channel. Determine if a piece is LWD (<i>any portion with a small end diameter ≥ 10 cm [4 in.], and a length ≥ 1.5 m [5 ft.]</i>). For each piece of LWD, determine the class based on the <i>diameter of the large end</i> (0.1 m to <0.3 m, 0.3 m to <0.6 m, 0.6 m to <0.8 m, or >0.8 m), and the class based on the <i>length of the piece</i> (1.5m to <5.0m, 5m to <15m, or >15m). If the piece is not cylindrical, visually estimate what the diameter would be for a piece of wood with circular cross-section that would have the same volume. When estimating length, include only the part of the LWD piece that has a diameter >10 cm (4 in). Tally each piece of LWD in the appropriate diameter and length class tally box in the <i>PIECES ALL/PART IN BANKFULL CHANNEL</i> columns in the Large Woody Debris section of the Physical Habitat Form in the App. Tally all LWD pieces within the segment that are not actually within the bankfull channel, but are at least partially spanning (bridging) the bankfull channel. For each piece, determine the class based on

- the diameter of the large end (0.1 m to <0.3 m, 0.3 m to <0.6 m, 0.6 m to <0.8 m, or >0.8 m), and the class based on the length of the piece (1.5 m to <5.0 m, 5 m to <15 m, or >15 m).
- Tally each piece of LWD the appropriate diameter and length class tally box in the *PIECES BRIDGE ABOVE BANKFULL CHANNEL* columns in the Large Woody Debris section of the Physical Habitat Form in the App
- Note that when entering data via the NRSA App, numbers can be typed directly into tally boxes, or the “+” and “-” buttons can be used to incrementally change the number.
- If no LWD is present in the subreach for a given diameter/length class, enter 0 in the tally box. The NRSA App contains a “Populate blank boxes with zeros” button which will automatically enter zeros into all the blank LWD fields. This can be done after all LWD is tallied to quickly enter zeroes in the remaining fields.
- Steps 1 through 5 will be repeated at each subreach.

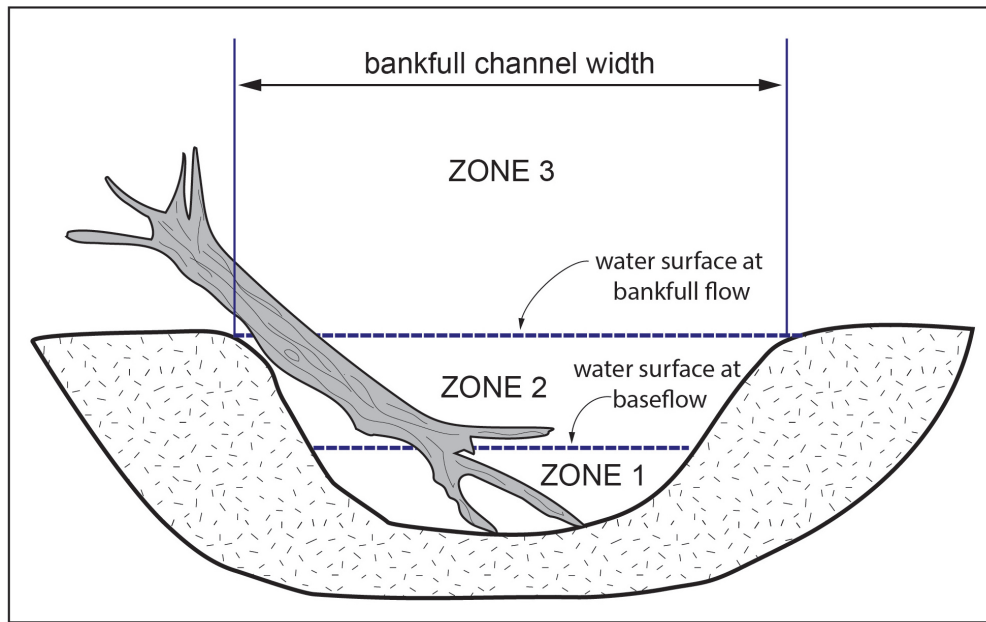


Figure 10.9 Large Woody Debris Influence Zones (modified from Robison and Beschta, 1990).

LARGE WOODY DEBRIS (10cm small end diameter; 1.5m length) ⓘ

Populate blank boxes with zeros

DIAMET...	PIECES ALL/PART IN BANKFULL CHANNEL			PIECES BRIDGE ABOVE...		
	1.5-5m	5-15m	>15m	1.5-5m	5-15m	>15m
0.1 - <0.3m	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +
0.3 - 0.6m	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +
0.6 - 0.8m	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +
> 0.8m	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +	- <input type="text"/> +

Figure 10.10 Large Woody Debris Section of Physical Habitat Form in the NRSA App - Wadeable

10.6 Cross-section Transects on Side Channels

If the wetted channel at a transect is split by an island, and the estimated flow in the side channel is less than 15% of the total flow, the bank and riparian measurements are made at each side of the main channel only (the minor side channel is ignored other than to note its presence on the Thalweg Profile section of the Physical Habitat Form), so one riparian plot is established on the island as shown in **Figure 10.11** (side A). If an island is present at a transect that creates a major side channel containing **15% or more** of the total flow, an additional cross-section transect is established for the side channel as shown in **Figure 10.11** (side B). Separate substrate, bank and riparian measurements are made for side channel transects. Data from the additional side channel transect are recorded by checking the “IS THIS A SIDE CHANNEL” checkbox near the top of the Physical Habitat Form. Unchecking the side channel box will display the primary transect data once again. Riparian plots established on the island for each transect may overlap (and be < 10 m shoreward) if the island is less than 10 m wide at the transect. Islands are distinguished from mid-channel bars by their relationship to bankfull flow: Islands are not inundated at bankfull stage; bars are part of the main channel and are inundated at bankfull flow. Collect all channel and riparian cross-section measurements from the side channel as well as the primary channel. While collecting thalweg profile data (including mid-station substrate size class) and large woody debris tallies, record the cumulative data on the primary transect only (e.g., with the side channel check box unchecked).

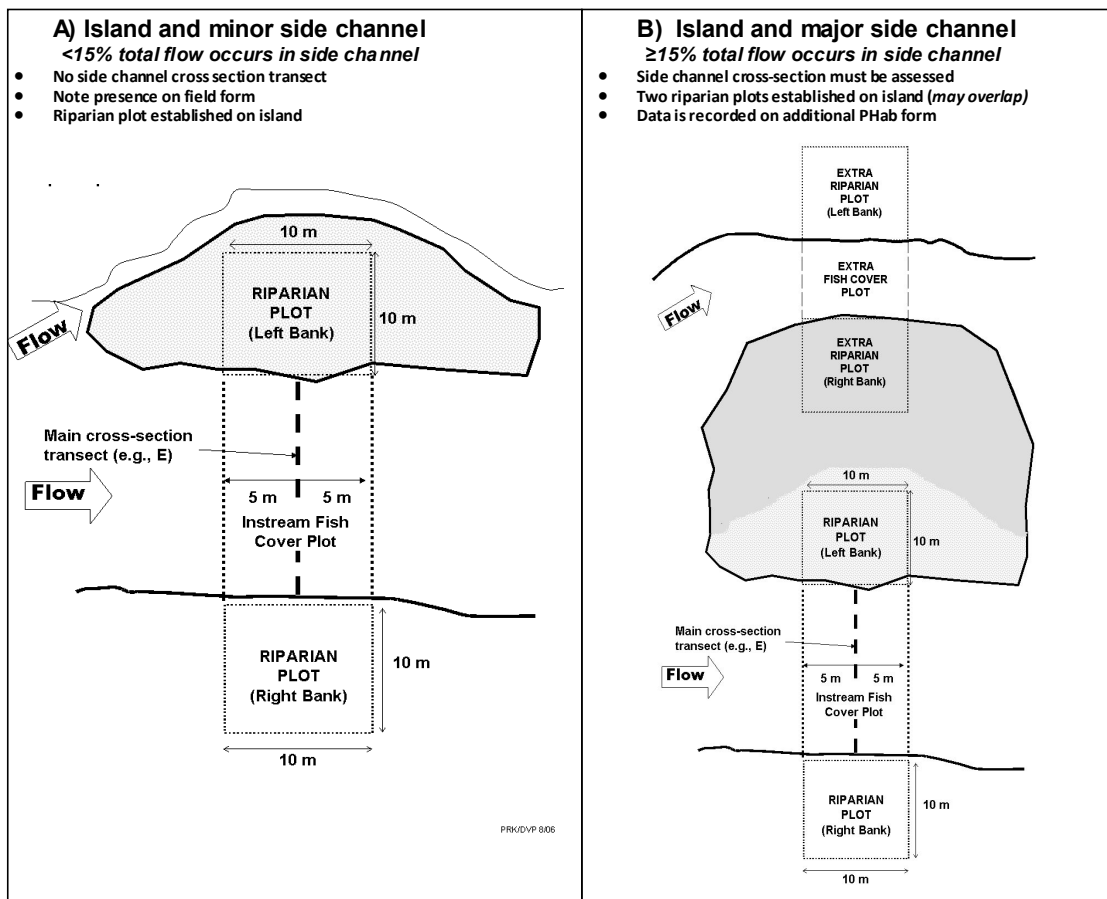


Figure 10.11 Riparian and Instream Fish Cover Plots for a Stream with Minor and Major Side Channels

10.7 Slope and Bearing

Measure slope and bearing by *backsighting* between transects (e.g., transect *B* and *A*, *C* and *B*, etc.). To measure the slope between adjacent transects, follow the procedure presented in **Table 10.12** or the alternate procedures presented in **Table 10.13**. Measure bearing following the procedure presented in **Table 10.14**. Record slope and bearing data on the Slope and Bearing Form in the NRSA App.

10.7.1 Measurement of Slope using Level and Stadia Rod

Slope is typically measured by two people, one holding a surveyor's rod and the second sighting through the surveyor's level. Be sure that the person is holding the marked pole or rod at the surface of the water. The intent is to get a measure of the *water surface* slope, which may not necessarily be the same as the bottom slope. The surveyor's level is leveled according to the manufacturer's recommendations, which is generally to adjust the three leveling feet until the bubble is centered. Level is checked in all planes to be measured. If the level does not "self level" in all measured planes the user should check the instruction manual for suggested options. Relative elevation readings are made at each transect and the difference between each elevation reading is calculated and recorded as the change in elevation. **NOTE: Multiple transect elevations can often be made for each setup of the level, but every time the tripod and level are moved, a second measurement of the last elevation from the last setup is required. You cannot use elevations from previous setups because the relative height of the level has changed.**

To calculate sinuosity from bearing measurements, it does not matter whether or not you adjust your compass bearings for magnetic declination, but it is important that you are **consistent** in the use of magnetic or true bearings throughout all the measurements you make on a given reach. Provide a comment on the Slope and Bearing Form about which type of bearings you are taking, so the measurements can be used to describe reach aspect. Also, guard against recording *reciprocal bearings* (erroneous bearings 180 degrees from what they should be). The best way to do this is to know where the primary (cardinal) directions are in the field: (north [0 degrees], east [90 degrees], south [180 degrees], and west [270 degrees]), and ensure that your bearings "make sense."

As stated earlier, it may be necessary to set up intermediate (supplemental) bearing points between a pair of cross-section transects if you do not have direct line-of-sight along (and within) the channel between stations (**Figure 10.12**). This can happen if brush is too heavy, or if there are sharp slope breaks or tight meander bends. *If you would have to sight across land to measure bearing between two transects, then you need to make one or more supplemental measurements* (i.e., do not "short-circuit" a meander bend). Mark these supplemental locations with a different color of plastic flagging than used for the cross-section transects to avoid confusion. Record these supplemental bearing measurements, along with the proportion of the stream segment between transects included in each supplemental measurement, in the appropriate sections of the Slope and Bearing Form in the NRSA App. Note that the main bearing observations are always downstream of supplemental observations (i.e., **to** the downstream transect). Similarly, first supplemental observations are always downstream of second supplemental observations.

Because measurements of slope are a calculation of the elevation difference between transects, you may sight over land for the purposes of slope only (**Figure 10.12**). You may need to use supplemental points in your measurement of slope if visibility is severely limited, but

supplemental points are not required for slope as they are for measurements of bearing. As a result, there may be times when you record bearing data for a supplemental point (or two) on the Slope and Bearing Form; but only record the total elevation change between the two transects in the primary slope field.

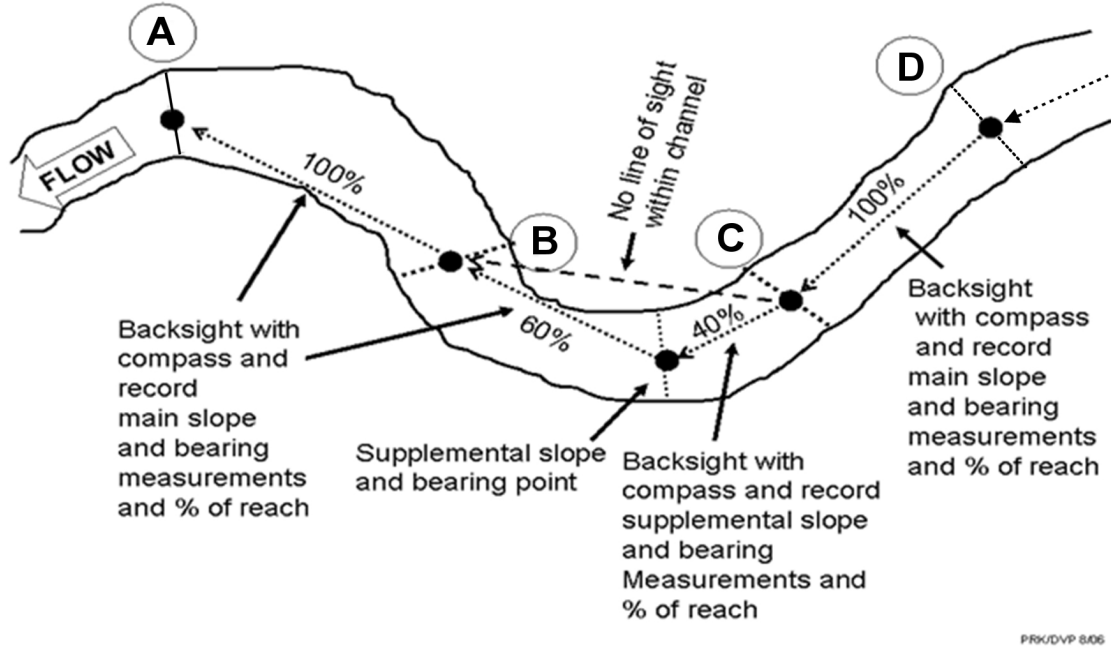


Figure 10.12 Measurements of Bearing Between Transects

Table 10.12 Procedure: Obtaining Slope Data

Slope Method with Surveyors Level
<p><u>Instrument Setup:</u></p> <ol style="list-style-type: none">1. Set up the instrument upstream of Transect A where a clear line of sight is possible to Transect A. If visibility allows, the instrument may be placed as far upstream as possible, as long as Transect A is still visible. <p><i>Note: In small streams with a clear line of site it may be possible to set the instrument up once and make readings to several transects from a single set up. Simply record the readings for each transect and do not skip transects.</i></p> <ol style="list-style-type: none">2. Extend the tripod legs to approximately eye level and set the legs firmly into the ground; adjust the legs so that they form a regular triangle and are firmly set with no wobble. Adjust the legs so that the base plate is approximately level.3. Hold the instrument on the tripod and start the centering screw. Ensure the adjustable feet are roughly evenly adjusted. While the centering screw is still loose, slide the instrument on the base plate until the bubble is approximately centered in the circular level. Tighten the centering screw.4. Adjust the leveling foot screws until the bubble is exactly level in the center circle.5. Self-Leveling instruments can now be swiveled gently on the base plate and should maintain level as long as the tripod remains steady. Check to ensure the bubble indicates the instrument is level across all planes at which measurements are to be made.6. Adjust focus, brightness and parallax according to manufacturer's specifications. The instrument is ready to make measurements. <p><u>Taking Measurements:</u></p> <ol style="list-style-type: none">1. Determine a location along Transect A to hold a surveyor's rod that will be visible from the location of the instrument:<ol style="list-style-type: none">a. Position the staff at Transect A, holding the bottom of the staff at the water level and the staff as vertical as possible and the numbers facing the instrument.b. Site the staff through the instrument and record the reading to the nearest 0.5 centimeter in a field notebook or other workspace (this value is not entered on the field form).c. Move the staff to Transect B and gently swivel the instrument to face the next reading. Hold the staff as before, vertically, with the bottom at the water level and the numbers facing the instrument.d. Site the staff and record the reading to the nearest 0.5 centimeter (again in a field notebook or elsewhere).e. Subtract the elevation reading at Transect A from the reading at Transect B.f. The difference in the readings is the elevation difference that is recorded on the Slope and Bearing Form. Also be sure to select the "cm" bubble to indicate the units on the value entered.g. Repeat measurements between each transect.2. Proceed to the next cross-section transect (or supplementary point), and repeat Steps a - f above. For each transect pair where the above method is used, select "TR" (for surveyors level/transit) in the Method field. If the above method cannot be used for one or more transect pair(s), see Section 10.7.2 for alternative methods^a. <p>NOTE: If you are sighting to a supplemental point (required for bearing measurements in some streams), you may record the elevation difference in the appropriate <i>SUPPLEMENTAL</i> section(s) of the Slope and Bearing Form. You may also calculate the <u>total</u> elevation change from one transect to the next and entered only that value in the form. In this case, values would be entered for bearing in the supplemental field(s), but not for slope.</p>

^a Method codes are: *CL*=clinometer, *TR*=surveyor's level / transit, *HL*=hand level, *WT*=Water tube, *LA*=laser level, *OTHER*=method not listed (describe in comments section of form).

10.7.2 Alternate Methods for Obtaining Slope

Because of ease of use, portability, and cost, hand-held clinometers were previously used to determine slope. In NRSA, the Field Crews will have access to more sophisticated instrumentation (e.g., surveyor's level), and have field personnel who are experienced in the use of these instruments. Clinometers should only be used if the slope is greater than 2.75% or if the surveyors level malfunctions. However, note that when properly used, a roofer's level (hydrostatic level) can yield slope measurements more precise than even a laser level. The Slope and Bearing Form in the NRSA App is designed to allow for different methods and/or different units of measuring elevations or direct measurements of slope. Select the appropriate method (instead of *TR*; method codes are identified in **Table 10.12**) and mark the % *BUBBLE* (instead of the *CM* bubble) if the method or instrument measures the percent slope rather than the difference in elevation (**Table 10.13**).

Table 10.13 Modified Procedure: Obtaining Slope Data (without Surveyor's Level)

Modified Slope Method - Only Use if Surveyor Level is NOT Possible

Use this procedure (**Figure 10.13**) if you are starting at the upstream Transect (*K*), after completing the thalweg profile and other cross-section measurements at Transects *A* through *K*. **It should only be used if you cannot use the surveyor's level.**

1. Stand in the channel at the upstream cross-section transect. Determine if you can see the next transect downstream. If not, you may have to take supplementary slope measurement(s). While sighting over land is not prohibited during any slope measurement, the clinometer method is more difficult to use over larger distances, so supplementary measurements are more likely to be needed.
 - a. If the next transect downstream still cannot be seen, you will have more than one supplemental measurements (e.g., you will measure the second supplemental, then the first supplemental, and finally the main).
2. Mark a surveyor's rod and a calibrated rod (or meter ruler) at the same height. If a shorter pole or ruler is used, measure the height from the ground to the opening of the clinometer when it is resting on top.
3. Have one person take the marked surveyor's rod to the downstream transect. Hold the rod vertical with the bottom at the same level as the water surface. If no suitable location is available at the stream margin, position the rod in the water and note the depth.
4. Place the base of the calibrated rod at the same relative height as the surveyor's rod (either at the water surface or at the same depth in the water).
5. Place the clinometer on the calibrated rod at the height determined in Step 2. With the clinometer, sight downstream to the flagged height on the surveyor's rod at the downstream transect (or at the supplementary point).

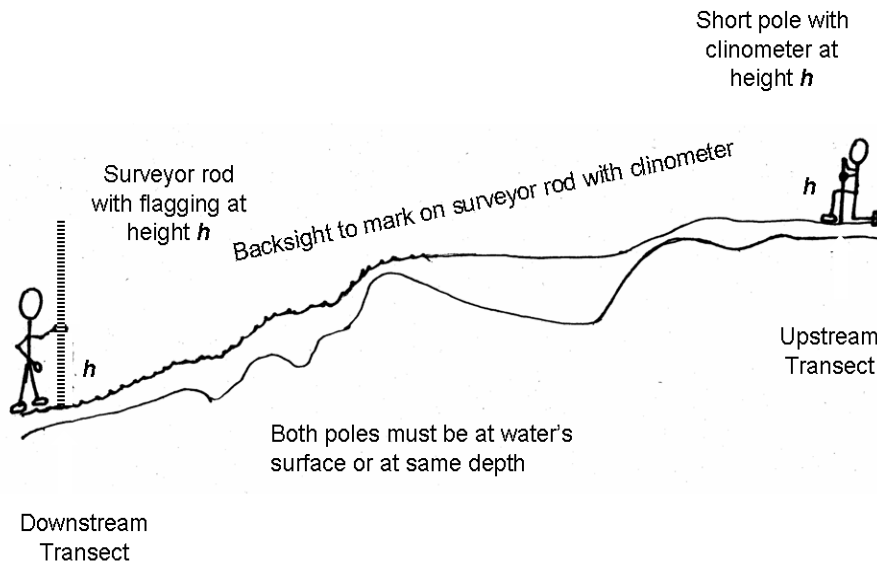
If you have determined in Step 1 that supplemental measurements are required for this segment, walk downstream to the furthest point where you can stand in the center of the channel and still see the center of the channel at the upstream transect. Mark this location with a different color flagging than that marking the transects.

If you are sighting to the next downstream transect, read and record the percent slope in the *MAIN* field on the Slope and Bearing Form for the downstream transect (e.g., *J < K*), which is at the bottom of the form (i.e., you are completing the form in reverse order). Record the *PROPORTION* as 100%.

If you are sighting from a supplementary point, record the slope (%) and proportion (%) of the stream segment that is included in the measurement in the appropriate *SUPPLEMENTAL* field of the Slope and Bearing Form. The last sighting to a downstream transect (from either the upstream transect or the nearest upstream supplementary point) is always recorded as

the *MAIN* reading. This method requires the form to be filled out from the bottom up when supplemental points are needed. If it was determined in Step 1 that two supplemental points are needed, you will fill out the third row first.

6. Stand in the middle of the channel at the upstream transect (or supplemental point), and backsight with your compass to the middle of the channel at the downstream transect (or supplemental point). Record the bearing (degrees) in the same section of the Slope and Bearing Form (Supplemental or Main) as you recorded the slope in Step 5 (see **Table 10.14** for details).
7. Proceed to the next cross-section transect (or to a supplementary point) and repeat Steps 3 through 6 above.



PRK/DVP 6/06

Figure 10.13 Channel Slope Measurement using a Clinometer

10.7.3 Method for Obtaining Bearing

Table 10.14 presents the steps necessary to obtain bearing data with a compass in wadeable streams.

Table 10.14 Procedure: Obtaining Bearing Data

Obtaining Bearing Data

Use this procedure to backsight from one transect to another using a bearing sighting compass.

Readings (in degrees) are taken from the center of one transect to the center of the next transect downstream (e.g., when using standard workflow and working upstream, you are sighting back to the transect you just left).

It does not matter whether you use true or magnetic bearings as long as you are consistent for each reading.

Take care not to accidentally take a reciprocal reading (one that is 180 degrees off).

1. Stand in the center of the channel at the upstream transect. If you cannot see the next transect downstream without sighting across land, you will have to take supplementary bearing measurements (i.e., do not “short-circuit” a meander bend).
2. Hold the compass in line with your body and sight down the “lubber line” or through the sight window to the center of the next transect downstream (or supplemental point). It will help to have another person at the center of the transect to which you are sighting. Remember that your line of sight cannot “cross land.”
3. While pointing the compass toward the middle of the transect or supplemental point, read the bearing (in degrees)
 - For many navigational compasses, you will rotate the bezel until the index marks are centered over magnetic north needle. The base of the lubber line or index mark on the compass now points to the compass heading on the bezel.
 - For some direct reading compasses, the bearing will be displayed already.
4. Record the reading on the Slope and Bearing Form.
5. If you are sighting to the next downstream transect, read and record the bearing in the *MAIN* section on the Slope and Bearing Form. Record the *PROPORTION* as 100%.
6. If you are sighting from a supplemental point, record the bearing and proportion (%) of the stream segment that is included in the measurement in the appropriate *SUPPLEMENTAL* field of the Slope and Bearing Form. The sighting to a downstream transect (from either the upstream transect or the nearest upstream supplemental point) is always recorded as the *MAIN* reading.
 - a. The first measurement taken is from the supplemental point to the downstream transect. This is recorded in the *MAIN* row.
 - b. The second measurement taken is from either the upstream transect to the first supplemental, or from the second supplemental to the first supplemental. This is recorded in the first supplemental row.
 - c. If two supplementals are needed, then the third measurement taken is from the upstream transect to the second supplemental. This is recorded in the second supplemental row.
 - d. It will be extremely rare to need more than two supplemental points, but if this were to happen, record additional supplemental measurements in the comment bubble provided.
7. Proceed to the next transect (or supplemental point), and repeat steps 1 through 6 above.

10.8 Channel Constraint, Debris Torrents, and Recent Floods

10.8.1 Channel Constraint

After completing the thalweg profile and riparian/channel cross-section measurements and observations, envision the stream at bankfull flow and evaluate the degree, extent and type of channel constraint, using the procedures presented in **Table 10.15**. Record data on the Channel Constraint Form in the NRSA App. First, classify the stream reach channel pattern as predominantly a *single channel*, an *anastomosing channel*, or a *braided channel* (**Figure 10.14**):

1. Single channels may have occasional in-channel bars or islands with side channels, but feature a predominant single channel, or a dominant main channel with a subordinate side channel.
2. Anastomosing channels have relatively long major and minor channels (but no predominant channel) in a complex network, diverging and converging around many vegetated islands. Complex channel pattern remains even during major floods.
3. Braided channels also have multiple branching and rejoining channels, (but no predominant channel) separated by unvegetated bars. Channels are generally smaller, shorter, and more numerous, often with no obvious dominant channel. During major floods, a single continuous channel may develop

After classifying the channel pattern, determine whether the channel is constrained within a narrow valley, constrained by local features within a broad valley, unconstrained and free to move about within a broad floodplain, or free to move about, but within a relatively narrow valley floor. Then examine the channel to ascertain the bank and valley features that constrain the stream. Entry choices for the type of constraining features are bedrock, hillslopes, terraces/alluvial fans, and human land use (e.g., a road, a dike, landfill, rip-rap, etc.). Estimate the percent of the channel margin in contact with constraining features (for unconstrained channels, this is 0%). To aid in this estimate, you may wish to refer to the individual transect assessments of incision and constraint. Finally, estimate the “typical” bankfull channel width and estimate the average width of the valley floor either with a topographic map or visually. If you cannot directly estimate the valley width (e.g., it is further than you can see, or if your view is blocked by vegetation), record the distance you can see and mark the appropriate bubble on the field form.

Table 10.15 Procedure: Assessing Channel Constraint

Channel Constraint
<p>NOTE: These activities are conducted after completing the thalweg profile and littoral-riparian measurements and observations, and represent an evaluation of the entire stream reach.</p> <p><i>CHANNEL CONSTRAINT:</i> Determine the degree, extent, and type of channel constraint based on envisioning the stream at bankfull flow.</p> <ol style="list-style-type: none">1. Classify the stream reach channel pattern as predominantly a single channel, an anastomosing channel, or a braided channel.<ul style="list-style-type: none">• <i>Single channels may have occasional in-channel bars or islands with side channels</i>, but feature a predominant single channel, or a dominant main channel with a subordinate side channel.• <i>Anastomosing channels have relatively long major and minor channels</i> branching and rejoining in a complex network separated by vegetated islands, with no obvious dominant channel.

Channel Constraint

- *Braided channels also have multiple branching and rejoining channels, separated by unvegetated bars. Subchannels are generally small, short, and numerous, often with no obvious dominant channel.*
2. After classifying the channel pattern, determine whether the channel is constrained within a narrow valley, constrained by local features within a broad valley, unconstrained and free to move about within a broad floodplain, or free to move about, but within a relatively narrow valley floor.
 3. Then examine the channel to ascertain the bank and valley features that constrain the stream. Entry choices for the type of constraining features are bedrock, hillslopes, terraces/alluvial fans, and human land use (e.g., a road, a dike, landfill, rip-rap, etc.).
 4. Based on your determinations from Steps 1 through 3, select and record one of the constraint classes shown on the Channel Constraint Form.
 5. Estimate the percent of the channel margin in contact with constraining features (for unconstrained channels, this is 0%). Record this value on the Channel Constraint Form.
 6. Finally, estimate the “typical” bankfull channel width, and visually estimate the average width of the valley floor. Record these values on the Channel Constraint Form.

NOTE: To aid in this estimate, you may wish to refer to the individual transect assessments of incision and constraint that were recorded on the Physical Habitat Form.

NOTE: If the valley is wider than you can directly estimate, record the distance you can see and mark the box on the form that indicates this fact.

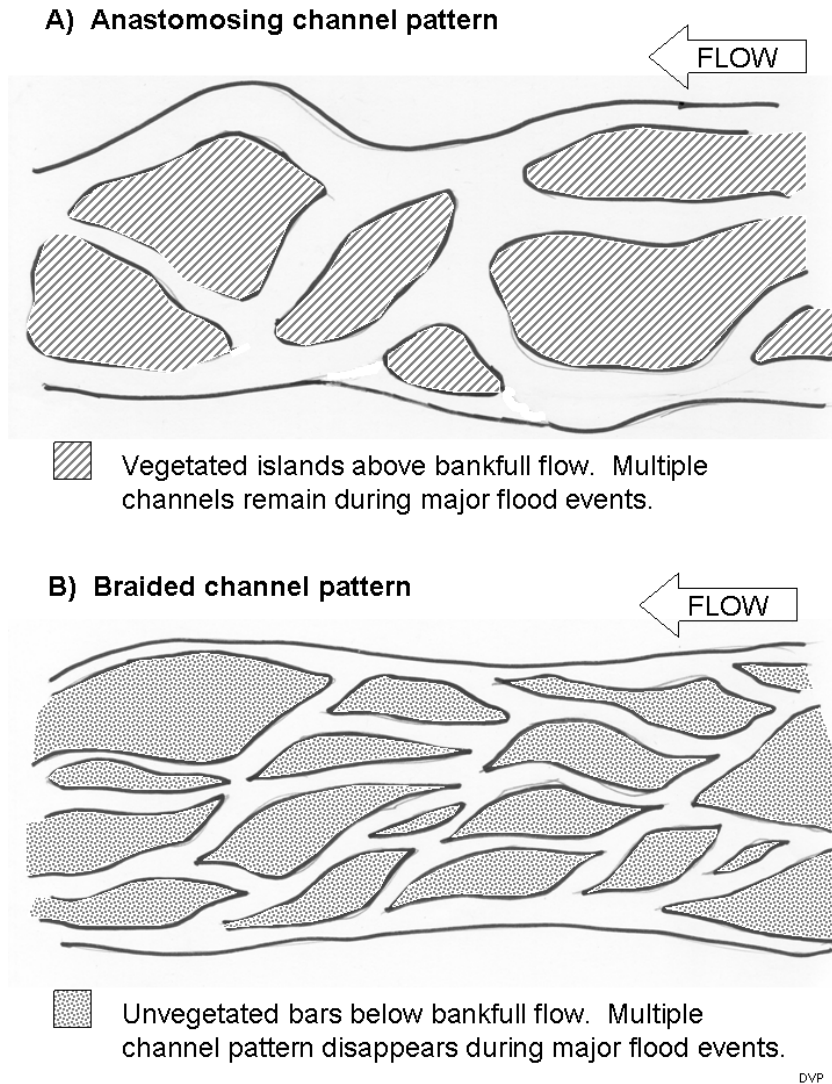


Figure 10.14 Types of Multiple Channel Patterns

10.8.2 Debris Torrents and Recent Major Floods

Debris torrents, or *lahars*, differ from conventional floods in that they are flood waves of higher magnitude and shorter duration, and their flow consists of a dense mixture of water and debris. Their high flows of dense material exert tremendous scouring forces on streambeds. For example, in the Pacific Northwest, flood waves from debris torrents can exceed 5 meters deep in small streams normally 3 m wide and 15 cm deep. These torrents move boulders in excess of 1 m diameter and logs >1 m diameter and >10 m long. In temperate regions, debris torrents occur primarily in steep drainages and are relatively infrequent, occurring typically less than once in several centuries.

Because they may alter habitat and biota substantially, infrequent major floods and torrents can confuse the interpretation of measurements of stream biota and habitat in regional surveys and monitoring programs. Therefore, it is important to determine if a debris torrent or major flood has occurred within the recent past. After completing the thalweg profile and channel/riparian measurements and observations, examine the stream channel along the entire sample reach,

including its substrate, banks, and riparian corridor, checking the presence of features described on the Torrent Evidence Form in the NRSA App. It may be advantageous to look at the channel upstream and downstream of the actual sample reach to look for areas of torrent scour and massive deposition to answer some of the questions on the field form. For example, you may more clearly recognize the sample reach as a torrent deposition area if you find extensive channel scouring upstream. Conversely, you may more clearly recognize the sample reach as a torrent scour reach if you see massive deposits of sediment, logs, and other debris downstream.

10.9 Elevation at Transect K

Record elevation at Transect K using your GPS device. To record this information, record the elevation holding the GPS at approximately 3 feet above the surface of the water. Ensure that the numbers are properly recorded for Transect K on the Assessment Form in the NRSA App.

11 FECAL INDICATOR (*ENTEROCOCCI*)

11.1 Summary of Method

Collect a fecal indicator sample at the last transect (Transect K) after all other sampling is completed. **Filters must be frozen within six hours of collection.** Use a pre-sterilized, 250 ml bottle and collect the sample approximately 1 m off the bank at about 0.3 meter (12 inches) below the water. Following collection, place the sample in a cooler and maintain on ice prior to filtration of two 50 mL volumes. Again, samples must be filtered and frozen on dry ice within six hours of collection. In addition to collecting the sample, look for signs of disturbance throughout the reach that would contribute to the presence of fecal contamination to the waterbody. Record these disturbances on the Site Assessment Form.

11.2 Equipment and Supplies

Table 11.1 provides the equipment and supplies needed to collect the fecal indicator sample. Record the sample data on the Sample Collection Form in the NRSA App.

Table 11.1 Equipment and Supplies: Fecal Indicator Sampling (Wadeable Sites)

For collecting samples	nitrile gloves pre-sterilized, 250 ml sample bottle sodium thiosulfate tablet Wet ice cooler
For recording measurements	Sample Collection Form in NRSA App

11.3 Sampling Procedure

The procedure for collecting the fecal indicator sample is presented in **Table 11.2**.

Table 11.2 Procedure: Fecal Indicator (Enterococci) Sample Collection (Wadeable Sites)

Enterococci Sample
<ol style="list-style-type: none"> 1. Put on clean nitrile gloves. 2. Select a sampling location at Transect K that is approximately 1 m from the bank. Approach the sampling location slowly from downstream or downwind. 3. Lower the uncapped, inverted 250 ml sample bottle to a depth of 1 foot (0.3 m) below the water surface, avoiding surface scum, vegetation, and substrates. If the water depth 1 meter from the shore is less than 0.3 meters, submerge the bottle to a lesser depth and be careful not to contaminate the sample with bottom sediments or detritus. 4. Point the mouth of the container away from the body or boat. Right the bottle and raise it through the water column, allowing bottle to fill completely. 5. If the depth along the transect at 1 m from the bank is too shallow to allow for the sample to be collected without contamination from bottom sediments, take the sample at an alternate location along the transect and provide a comment on the form describing the sampling location. If needed, adjust the sampling depth to avoid contaminating the sample with bottom sediments or detritus. 6. After removing the container from the water, discard a small portion of the sample to allow for proper mixing before filtering (down to the 250 mL mark on the bottle). 7. Add the sodium thiosulfate tablet, cap, and shake bottle 25 times. 8. On the Sample Collection Form in NRSA App, record the time of collection for the samples by using the "Now" button or by entering the time in 24-hr format.

9. Enter the depth of the sample collection (in meters) in the provided fields. *This is the approximate depth from the surface of the water to the mouth of the submerged container, not the depth of the water at the sampling location.*

Storage

10. Store the sample in a cooler on ice to chill (do not freeze immediately). Chill for at least 15 minutes before filtering.
11. Sample must be filtered and all filters frozen within six hours of collection

11.4 Sample Processing in the Field

You will need to process two separate filters for the Enterococci sample. All the filters required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination. Please refer to **Section 14.3** for information regarding processing the Enterococci samples.

12 FISH ASSEMBLAGE

12.1 Summary of Method

The fish sampling method is designed to provide a representative sample of the fish community, collecting all but the rarest fish taxa inhabiting the site. It is intended to accurately represent species richness, species guilds, relative abundance, size, and presence of anomalies. The intended uses of the fish assemblage data are to calculate predictive models of multimetric indicators (MMIs; similar to an Index of Biotic Integrity (IBI); Pont et al. 2008, USEPA 2013a) and possibly Observed/Expected (O/E) taxa richness. In addition, the fish assemblage data provides a starting point for developing potential indicators of ecosystem services related to fish.

For wadeable streams, collect fish using a bank or towed (e.g., a towed barge, small watercraft, or float tube) electrofishing unit (2,500- to 5,000-V; 2.5-5.0 Generator Powered Pulsator [GPP] unit or equivalent) or backpack electrofisher. Use a backpack electrofishing unit in smaller streams, when conductivity is appropriate, or in larger streams that are inaccessible with a towed unit. As a last option, use seining as an alternate method when electrofishing is precluded by high or low conductivity or extreme levels of turbidity.

There are different protocols for collecting fish from wadeable streams of different sizes (see **Section 12.3**). For all wadeable sites, conduct sampling in an upstream direction (i.e., from the downstream end of the reach), allocating effort (button time) within *subreaches* (areas between the cross-section transects). At smaller streams (mean channel width [CW] rounded to the nearest meter and recorded on the Verification Form is less than or equal to 12 m), sample all available habitats over the entire sampling reach (40 CW; see **Section 3.2**). At large wadeable streams (mean channel width is greater than or equal to 13 m), the initial length of the fish sampling reach is less than the entire sampling reach, and effort is focused on habitats along the stream margins. At large wadeable streams, the minimum sample reach is 20 CW (five subreaches). If you have not collected 500 individuals at the end of the 20 CW, sample additional subreaches in their entirety until you obtain at least 500 individuals, or until you have sampled the entire sampling reach (40 CW; 10 subreaches). Record information related to sampling effort on the Fish Gear Form in the NRSA App. Record species identification and enumeration data on the Fish Collection Form in the NRSA App.

12.2 Equipment and Supplies

Table 12.1 shows the checklist of equipment and supplies required to complete the fish assessment. This checklist is similar to the one presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the site.

Table 12.1 Equipment and Supplies: Fish Collection (Wadeable Sites)

<p>For collecting samples</p>	<p>Bank or towed electrofishing equipment (e.g., towed barge or mounted on a small watercraft or float tube), including variable voltage pulsator unit, wiring cables, generator, and electrodes</p> <p>Backpack electrofishing unit with anode and cathode</p> <p>Linesman gloves, boots, and other necessary safety equipment</p>	<p>1 Scalpel for slitting open large fish before preservation.</p> <p>1 container of 10% buffered formalin</p> <p>Small mesh bags or several Leak proof HDPE jars (various sizes from 250 mL - 4L) for fish voucher specimens</p> <p>2 non-conducting dip nets with ¼ inch mesh</p>
--------------------------------------	---	---

	<p>Dip nets Extra electrofishing unit batteries Scientific collection permit(s) Digital camera with extra memory card & battery 1 Laser rangefinder (optional) Polarized sunglasses and hats 10 ft x 6 ft Minnow or Bag Seine with ¼ inch mesh (additional 4' depth seine may also be used)</p>	<p>1 Minnow net for dipping small fish from live well 2 measuring boards (3 cm size classes) 1 set Fish ID keys Field Operations Manual 20 ft x 6 ft Minnow or Bag Seine with ¼ inch mesh (additional 4 ft depth seine may also be used) Buckets</p>
For recording measurements	<p>Sheet of sample labels and voucher specimen tags (for unknown/range extension voucher samples) Sheet of sample labels and voucher specimen tags (for QA voucher samples) Fish Gear Form in NRSA App Fish Collection Form in NRSA App</p>	<p>Clear tape strips Soft (#2) lead pencils for writing on voucher inner labels Fine-tip indelible markers</p>

12.3 Sampling Procedures

The reach length sampled for fish varies based on the mean width of the stream used to establish the length of the sampling reach (which has been rounded to the nearest meter as per the directions in **Section 3.2**) and on the number of individuals collected (**Figure 12.1**). For small wadeable streams (mean CW from the Verification Form ≤ 12 meters), follow the protocol presented in **Section 12.3.2**. Sample the entire reach (150 m to 40 CW, 10 subreaches) and move the electrofishing unit (towed barge or backpack) within each subreach to sample both shorelines as well as the mid-channel.

For large wadeable streams (mean CW from the Verification Form ≥ 13 m), follow the protocols described in **Section 12.3.3**. For large wadeable streams the minimum length for fish sampling is 20 CW (5 of the 10 subreaches). If a minimum of 500 fish are not collected after sampling this minimum fishing reach, sample additional subreaches in their entirety until at least 500 fish are collected, or all 10 subreaches have been sampled. Stop sampling when you reach Transect K (the end of the sampling reach), regardless of the number of individuals collected.

Table 12.2 summarizes the fishing protocols for small and large wadeable streams. More detailed steps for fishing small and large wadeable streams are found in the following sections.

If conditions prohibit any type of electrofishing, collect fish by seining as described in **Table 12.5**. The objective of seining is to collect species and relative abundance data that is comparable to what would have been obtained by electrofishing at the site. If seining is used, record all fish collected with seining protocols on separate lines of the Fish Collection Form.

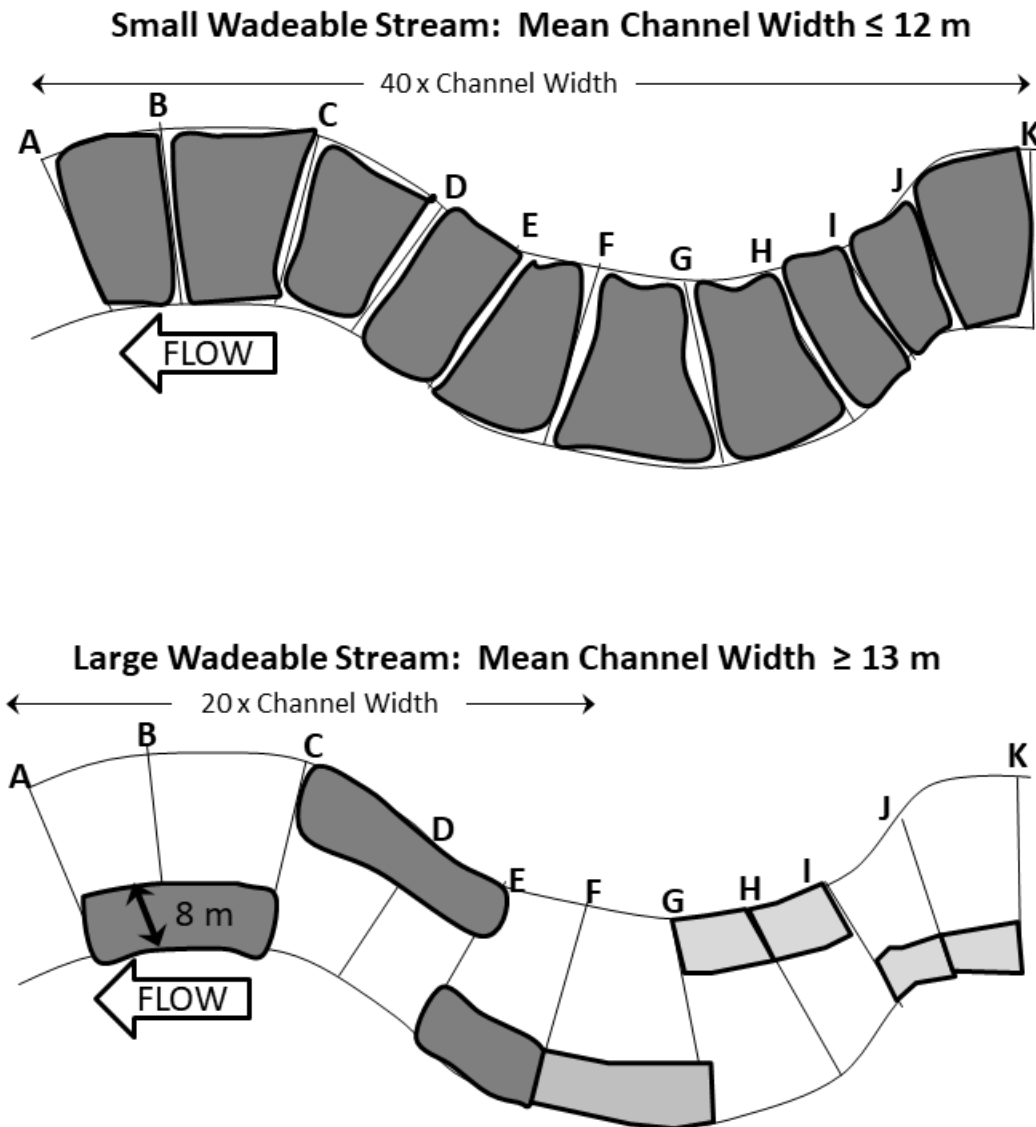
It is important that you record the total reach length that was sampled for fish, as this is used along with the number of fish collected to determine sampling sufficiency. Data from streams that were not sufficiently sampled for fish cannot be used to assess stream condition based on the fish assemblage.

12.3.1 Irruptive Species

For the purposes of NRSA, the term *irruptive species* will be used to describe fish species which are found in locally abundant “patches” in one or two small places within the sampling reach. These are distinct from dominant species which are in abundance throughout most of the reach. As such, irruptive species may artificially skew necessary effort to reach 500 individuals; and, if included the overall assemblage counts, may artificially skew the calculations of relative abundance of fish species in the reach. To avoid the impact of irruptive species, move quickly through large isolated schools of a single species (e.g., shad, certain shiners, etc.). Also, when tallying total fish at the end of the designated fish sampling reach, calculate the percentage of irruptive species to total individuals captured. If any single irruptive species comprises greater than or equal to 50% of the total sample, continue fishing one or more additional subreaches until the percentage of the irruptive species decreases to less than 50%.

Table 12.2 Summary of Wadeable Fishing Protocols

<p><u>Small Wadeable (mean channel width [CW] from Verification Form \leq 12 meters)</u></p> <ul style="list-style-type: none">• Fish sampling reach length will be between 150 m (CW < 4 m) and 40 CW.• Subreaches will be between 15 meters and 4 channel widths long<ul style="list-style-type: none">✓ Sample all 10 subreaches in their entirety from bank to bank starting at Transect A✓ Total button time will range from 500-700 seconds per subreach<ul style="list-style-type: none">• You do not have to expend equal button time among the 10 subreaches—you can devote more button time to subreaches with more complex habitat.✓ No minimum fish number
<p><u>Large Wadeable (mean channel width [CW] from Verification Form \geq 13 meters)</u></p> <ul style="list-style-type: none">• Initial minimum fish sampling reach will be 20 CW (5 subreaches).• Subreaches will be 1/10 of the sampling reach in length<ul style="list-style-type: none">✓ Fish each subreach in a swath 8 meters from bank in pairs of subreaches starting at a random bank at Transect A✓ Button time is roughly 700 seconds per subreach<ul style="list-style-type: none">• Depending upon the habitat complexity, you can vary the distance actively fished to allocate the available button time throughout the subreach. Put another way, sample for 700 seconds and if you have not reached the next transect, stop fishing and move to that transect.✓ Minimum fish number is 500 or until 10 subreaches have been fished.<ul style="list-style-type: none">➤ After fishing 5 subreaches, if 500 fish have not been collected, add subreaches one at a time (but fish them in their entirety) until 500 fish are collected or all 10 subreaches have been fished.



If < 500 individuals have been collected after fishing 20 CW (5 subreaches), continue fishing next subreach (alternating bank after every two subreaches) until either 500 individuals are collected, or Transect K is reached (10 subreaches [40 CW] have been sampled)

Figure 12.1 Reach Layouts for Fish Sampling at Wadeable Sites

Dark shaded areas indicate the minimum length of the fish sampling reach. Light shaded areas are sampled as needed to meet the required 500 individuals.

12.3.2 Small Wadeable Streams

Table 12.3 describes the procedure for collecting fish in small wadeable streams. The sampling crew should consist of one electrofisher operator, one dip-netter (1/4" mesh dip net), and an optional bucket carrier (who may also have a net to aid in transferring fish to the livewell). An anode with a net cannot substitute for a netter. For safety, all crew members are required to wear non-breathable waders and insulated gloves. To aid vision, wear polarized sunglasses and a hat or visor. See **Appendix E** for example starting settings for electrofishing using backpack, towed barge, and boat (Temple 2018). These are only suggestions; the final determination of settings is decided by the lead fish taxonomist.

Begin sampling at the downstream end of the sampling reach defined for the site (**Figure 12.1**) and proceed upstream. Sample the entire reach, which will be between 150 m and 40 channel widths (10 subreaches). Total button time will vary between 500 and 700 seconds per subreach. Conduct sampling by subreach (area between transects), but you do not have to allocate effort equally among all 10 subreaches. Whenever possible, process fish at the end of each subreach to minimize mortality and stress to fish.

Table 12.3 Procedure: Electrofishing (Small Wadeable Streams)

Electrofishing Procedures in Small Wadeable Streams	
1)	Verify or enter the fish sampling date in the header section of the Fish Gear Form in the NRSA App. The date in this field will be automatically entered based on the date recorded on the Verification Form. If you have not filled out the Verification Form or if the date of the fish collection is different, you can edit the date here. Note however that changing the value on this form will not change the value on the Verification Form.
2)	Decide if you will be able to sample the site for fish. <ul style="list-style-type: none"> a) Review all collecting permits to determine if any sampling restrictions are in effect for the site. In some cases, you may have to cease sampling if you encounter certain State or Federally listed species. If you cannot sample at all because of permit restrictions, select <i>Not Fished – No Permit</i>. b) If site conditions prevent barge or backpack electrofishing (e.g., no access, safety concerns, ambient conductivity is too high or too low to use a barge or backpack electrofishing unit), determine if you can sample by seining. <ul style="list-style-type: none"> i) If yes, follow the procedures presented in Table 12.5. ii) If not, select <i>Not Fished – Site Conditions Prohibit Sampling</i>. Provide a comment describing why site conditions prohibit sampling. c) If you can determine that > 50% of the required fish sampling reach (75 m or 20 CW; 5 subreaches) cannot be sampled, select <i>Not Fished/Fishing Suspended – Can't sample ≥50% of required reach</i>. d) If you cannot sample because of equipment problems, select <i>Not Fished – Equipment Failure</i>. e) At a very small and very shallow stream, if you cannot attempt to sample, but are very confident that no fish are present (i.e., you do not observe any at any point along the sampling reach), then select <i>Not Fished – No Fish Observed</i>. f) If you cannot sample for any other reason, select <i>Not Fished – Other</i> and provide a comment indicating why sampling cannot occur.
3)	If you can begin to sample, select <i>Small Wadeable</i> in the <i>Fish Sampling Protocol</i> section. <ul style="list-style-type: none"> a) Proceed to the downstream end of the sampling reach (Transect A). b) For safety, everyone must wear personal floatation devices, non-breathable waders, foot protection, and insulated linesman's gloves. c) To aid vision while netting fish, wear polarized sunglasses and a hat or visor.

<p>4) Select the appropriate <i>Water Visibility</i> conditions on the form. <i>Poor</i> implies that your ability to electrofish effectively is compromised because of poor visibility. Record the water temperature and conductivity (note whether the conductivity value is corrected to 25 °C).</p>
<p>5) Mark either <i>Backpack, Bank or Towed Barge</i> in the <i>Primary Electrofishing Gear</i> section of the Fish Gear Form. Mark Towed Barge for any electrofishing unit that is towed (e.g., canoe, kayak or float tube).</p> <p>a) Do not use any secondary electrofishing gear in a small wadeable stream.</p>
<p>6) Operation of Bank or Towed Electrofisher</p> <p>a) Set unit to pulsed DC and mark it in the <i>Wave Form</i> section of the Fish Gear Form.</p> <p>b) Select the initial voltage setting based on the conductivity of the stream.</p> <p>i) See Tables in Appendix E.</p> <p>ii) If the electrofishing system only lets you select High and Low voltage (rather than a specific voltage), record the setting used on the Fish Gear Form.</p> <p>iii) If your conductivity meter cannot measure ambient conductivity, you can “uncorrect” specific conductance at 25 °C to ambient conductivity using the following equation: (1) $\text{Ambient conductivity} = \text{Specific conductance} \times (1 + ([\text{water temp} - 25 \text{ °C}] \times 0.02))$.</p> <p>c) Select the initial pulse rate and width.</p> <p>i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.</p> <p>ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz.</p> <p>d) If the electrofishing system only lets you adjust the percent of power, record the value on the Fish Gear Form.</p> <p>e) Turn the electrofisher on, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width.</p> <p>f) Once you have determined the appropriate settings, record them on the Fish Gear Form. Start cleared clocks and resume fishing.</p> <p>g) Note: Some electrofishers do not meter all the requested settings; provide what you can.</p> <p>h) If button time is not metered, estimate it with a stop watch and provide a comment in the NRSA App.</p>
<p>7) Operation of Backpack Electrofisher</p> <p>a) Set unit to pulsed DC and mark it in the <i>Wave Form</i> section of the Fish Gear Form.</p> <p>b) Select the initial voltage setting based on the conductivity of the stream.</p> <p>i) See Tables in Appendix E.</p> <p>ii) If your conductivity meter cannot measure ambient conductivity, you can “uncorrect” specific conductance at 25 °C to ambient conductivity using the following equation: (1) $\text{Ambient conductivity} = \text{Specific conductance} \times (1 + ([\text{water temp} - 25 \text{ °C}] \times 0.02))$.</p> <p>c) Select the initial pulse rate and width.</p> <p>i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.</p> <p>ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz.</p> <p>d) Turn the electrofisher on, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width.</p>

- e) Once you have determined the appropriate settings, record them on the Fish Gear Form. Start cleared clocks and resume fishing.
 - f) Note: some electrofishers do not meter all the requested settings; provide what you can.
 - g) If button time is not metered, estimate it with a stop watch and provide a comment in the NRSA App.
- 8) Once the settings on the electrofisher are adjusted properly to sample effectively and minimize injury and mortality, begin sampling at the downstream end of the reach (Transect A) and fish in an upstream direction.
- a) The minimum reach length is 150 m. The maximum reach length for this protocol is 40 CW.
 - i) Search for fish even if the stream is extremely small, and it appears that sampling may produce no specimens.
 - ii) Button time should range from 500 to 700 sec per subreach. Actual time will depend upon the water conditions, the diversity and complexity of the available habitat, and on the number of fish present in the reach.
 - (1) If the electrofisher is highly effective and the fish are staying stunned longer and the water is clear and flowing slowly then button time will be much lower than it will be in a system where it is more turbid, flowing faster, and the fish are not being stunned as well.
 - (2) You do not have to expend equal button time among the 10 subreaches—you can devote more button time to subreaches with more complex habitat and less time to subreaches with simple habitat.
 - b) Depress the switch and slowly sweep the electrode from side to side.
 - c) Sample all habitat types (deep, shallow, fast, slow, complex, and simple). **Avoid the temptation to focus sampling only in the richest habitat types.**
 - i) For available cut-bank and snag habitats, move the anode wand into cover with the current off, turn the anode on when in the cover, and then remove the wand quickly to draw fish out.
 - ii) In fast, shallow water, sweep the anode and fish downstream into a net.
 - iii) In stretches with deep pools, fish the margins of the pool as much as possible, being extremely careful not to step or slide into deep water.
 - d) Keep the cathode near the anode if fish catch is low.
- 9) The netter, holds the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket.
- a) **Irruptive species:** If you encounter a large school of a **single species** (e.g., shad, certain shiners, etc.), quickly move through it to ensure you can sample the entire subreach within the allotted button time.
- 10) Continue upstream until you reach the next transect (end of subreach).
- a) Process fish and/or change water after each subreach to reduce mortality and stress.
 - i) Although not required, you may note amphibians and reptiles captured on the Fish Collection Form.
 - b) Release fish in a location that eliminates the likelihood of recapture.
- 11) Repeat Steps 8-10 until all 10 subreaches are sampled (i.e., you reach transect K).
- 12) After sampling all 10 subreaches, record the final length of the fish sampling reach in the *Primary Electrofishing Gear* section of the Fish Gear Form.
- a) If you suspend sampling before completing all 10 subreaches, record the actual length that was sampled, and mark the reason for the suspension in the *Fish sampling – Not Routine or Suspended* section of the Fish Gear Form.

b) If you did not collect any fish, mark <i>Fished – None Collected</i> in the <i>Fish sampling – Not Routine or Suspended</i> section of the Fish Gear Form.
13) In the <i>Primary Electrofishing Gear</i> section of the Fish Gear Form, record the total button time expended for electrofishing, the total time spent sampling, and the length of the total fish sampling reach (recorded in Step 12) sampled by electrofishing.
14) Indicate whether conditions allowed for sufficient sampling on the Fish Gear Form (<i>Yes, No, Not Sure</i>). If you marked <i>No</i> or <i>Not Sure</i> , provide an explanatory comment.
15) Note the general response of fish to your final electrofishing settings as either: <ul style="list-style-type: none"> a) <i>Immobilized</i> (no swimming motions due to electrical field). Includes narcosis (slack muscles) and tetany (rigid muscles). b) <i>Inhibited Swimming</i> (unbalanced swimming induced by the electrical field). Includes taxis (movement, usually towards the anode), pseudo-taxis (movement, but fish are unconscious and belly-up), and oscillotaxis (movement without orientation). c) <i>Escape</i> (upright avoidance swimming).
16) Record the total length of the stream that was sampled for fish on the Fish Gear Form.

12.3.3 Large Wadeable Streams

Table 12.4 describes the procedure for collecting fish in large wadeable streams. The electrofishing crew should consist of one electrofishing operator, and one dip netter and an optional bucket carrier (who may also have a net to aid in transferring fish to the live well). An anode with a net cannot substitute for a netter. For safety, all crew members are required to wear non-breathable waders and insulated gloves. Polarized sunglasses and caps to aid vision are also required. See **Appendix E** for example starting settings for electrofishing using backpack, towed barge, and boat (Temple 2018). These are only suggestions; the final determination of settings is decided by the lead fish taxonomist.

For large wadeable streams with a mean channel width (from the Verification Form) ≥ 13 m, the minimum fish sampling reach is 20 channel widths (5 subreaches). As shown in **Figure 12.1**, begin sampling at Transect A on a randomly determined bank and fish a section of the subreach that extends approximately 8 m from the bank in an upstream direction. Within each subreach, fish the near bank habitat as well as midstream habitat within the 8 meter sampling area for a button time of ~700 seconds. When 700 seconds are reached, stop electrofishing unless you are “pushing” a large school of fish, in which case continue fishing until you capture them at a break. To reduce stress and mortality, net immobilized fish immediately and deposit into a bucket or live-well for processing. Whenever possible, process fish at the end of each subreach to minimize mortality and stress to fish. At the end of the minimum fish sampling reach (20 CW or 5 subreaches), determine if you have collected at least 500 individuals. If so, stop sampling. If not, sample additional subreaches (one at a time) until at least 500 individuals are captured. If irruptive species make up $\geq 50\%$ of the sample, sample one or more additional subreaches to bring the proportion of the irruptive species below 50%. Stop sampling when you reach Transect K (the end of the entire 40 CW sampling reach), regardless of the number of individuals collected. Once the decision is made to fish an additional subreach, it should be completely fished as described above (do not stop sampling partway through a subreach).

Table 12.4 Procedure: Electrofishing (Large Wadeable Sites)

Electrofishing Procedures in Large Wadeable Streams
1) Verify or enter the fish sampling date in the header section of the Fish Gear Form in the NRSA App. The date in this field will be automatically entered based on the date recorded on the Verification

Electrofishing Procedures in Large Wadeable Streams	
	Form. If you have not filled out the Verification Form or if the date of the fish collection is different, you can edit the date here. Note however that changing the value on this form will not change the value on the Verification Form.
2)	Decide if you will be able to sample the site for fish. <ul style="list-style-type: none">a) Review all collecting permits to determine if any sampling restrictions are in effect for the site. In some cases, you may have to cease sampling if you encounter certain State or Federally listed species. If you cannot sample at all because of permit restrictions, select <i>Not Fished – No Permit</i>.b) If site conditions prevent towed or backpack electrofishing (e.g., no access, safety concerns, conductivity is too high or too low to use a barge or backpack electrofishing unit), determine if you can sample by seining.<ul style="list-style-type: none">i) If yes, follow the procedures presented in Table 12.5.c) If not, select <i>Not Fished – Site Conditions Prohibit Sampling</i>. Provide a comment indicating why site conditions prohibit sampling.d) If you can determine that > 50% of the required fish sampling reach cannot be sampled (20 CW; 5 subreaches), select <i>Not Fished/Fishing Suspended – Can't sample >=50% of required reach</i>.e) If you cannot sample because of equipment problems, select <i>Not Fished – Equipment Failure</i>.e) If you cannot sample for any other reason, select <i>Not Fished – Other</i> and provide a comment about why sampling cannot be completed.
3)	If you can begin to sample, select <i>Large Wadeable</i> in the <i>Fish Sampling Protocol</i> section. <ul style="list-style-type: none">a) Proceed to the downstream end of the reach (Transect A).b) For safety, everyone must wear personal floatation devices, non-breathable waders, foot protection, and insulated linesman's gloves.c) To aid vision while netting fish, wear polarized sunglasses and a hat or visor.
4)	Select the appropriate <i>Water Visibility</i> conditions on the form. <i>Poor</i> implies that your ability to electrofish effectively is compromised because of poor visibility. Record the water temperature and conductivity.
5)	Select either <i>Backpack</i> or <i>Bank or Towed Unit</i> in the <i>Primary Electrofishing Gear</i> section of the Fish Gear Form. <ul style="list-style-type: none">a) Do not use any secondary electrofishing gear in a large wadeable stream.
6)	Operation of Bank or Towed Electrofisher <ul style="list-style-type: none">a) Set unit to pulsed DC and mark it in the Wave Form section of the Fish Gear Form.b) Select the initial voltage setting based on the ambient conductivity of the river (i.e., not corrected to 25 °C).<ul style="list-style-type: none">i) See Tables in Appendix E.ii) If the electrofishing system only lets you select High and Low voltage (rather than a specific voltage), record the setting used on the Fish Gear Form.c) Select the initial pulse rate and width.<ul style="list-style-type: none">i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz.d) If the electrofishing system only lets you adjust the percent of power, record the value on the Fish Gear Form.e) Turn the electrofisher on, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to

Electrofishing Procedures in Large Wadeable Streams

minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width.

- f) Once you have determined the appropriate settings, record them on the Fish Gear Form. Start cleared clocks and resume fishing.
- g) Note: some electrofishers do not meter all the requested settings; provide what you can.
- h) If button time is not metered, estimate it with a stop watch and provide a comment in the NRSA App.

7) Operation of Backpack Electrofisher

- a) Set unit to pulsed DC and mark it in the Wave Form section of the Fish Gear Form.
- b) Select the initial voltage setting based on the **ambient** conductivity of the river.
 - i) See Tables in **Appendix E**.
- c) Select the initial pulse rate and width.
 - i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.
 - ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz.
- d) Turn the electrofisher on, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width.
- e) Once you have determined the appropriate settings, record them on the Fish Gear Form. Start cleared clocks and resume fishing.
- f) Note: some electrofishers do not meter all the requested settings; provide what you can.
- g) If button time is not metered, estimate it with a stop watch and provide a comment in the NRSA App.

8) Once the settings on the electrofisher are adjusted properly to sample effectively and minimize injury and mortality, begin sampling at the downstream end of the reach (Transect A). Randomly choose a bank on which to start and fish in an upstream direction within 8 m of the shoreline.

For the large stream protocol, the minimum initial fish sampling reach length is 5 subreaches

- 9) When using a towed electrofishing unit, the minimum crew size for electrofishing is three.
- a) The operator must remain actively at the control box and navigate the towed electrofishing unit.
 - b) The probe operator will use one probe.

10) When using a backpack electrofishing unit, the minimum crew size is two (one operator with the probe and one netter). An anode outfitted with a net cannot substitute for a netter.

11) Depress the switch and slowly sweep the electrode from side to side.

- a) Search for fish even if it appears that sampling may produce no specimens.
- b) Sample all habitat types (deep, shallow, fast, slow, complex, and simple). Avoid the temptation to focus sampling only in the richest habitat types.
 - i) In slack water area (e.g., available cut bank and snag habitats), move the anode wand into cover with the current off, turn the anode on when in the cover, and then remove the wand quickly to draw fish out.
 - ii) In fast, shallow water, sweep the anode and fish downstream into a net.
 - iii) In stretches with deep pools, fish the margins of the pool as much as possible, being extremely careful not to step or slide into deep water.
- c) Keep the cathode near the anode if fish catch is low.

Electrofishing Procedures in Large Wadeable Streams

- 12) The netter, holds the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket.
 - a) **Use a 6 mm (1/4 inch) mesh dip net** to collect stunned fish. Actively capture stunned fish from the electric field and **immediately place them into the live well. Devote special attention to net small and benthic fishes** as well as fishes that may respond differently to the electric current.
 - b) **Irruptive species:** If you encounter a large school of a **single species** (e.g., shad, certain shiners, etc.), quickly move through it to ensure you can sample the entire subreach within the allotted button time.
- 13) Continue upstream until you reach the next transect (end of subreach).
 - a) The total button time within each subreach should be ~700 sec. depending upon the habitat complexity, you can vary the distance actively fished to allocate the available button time throughout the subreach.
 - b) Process fish after each subreach to reduce mortality and stress. Release fish in a location that eliminates the likelihood of recapture.
 - i) Although not required, you may note amphibians and reptiles captured on the Fish Collection Form.
- 14) Move to the opposite bank when necessary (see **Figure 12.1**). Repeat Steps 11-14 until you have sampled the required length of stream.
 - a) If you have to suspend sampling before completing the required fish sampling reach, record the actual length that was sampled, and mark the reason for the suspension in the *Fish sampling – Not Routine or Suspended* section of the Fish Gear Form.
- 15) If the minimum reach length has been met (20 X mean stream width) determine the total number of individuals collected.
 - a) If the total is < 500, sample one or more additional subreaches in their entirety until at least 500 individuals have been collected and processed, or you sample all 10 subreaches. Go to Step 16.
 - b) If you collect ≥ 500 individuals, determine if a single irruptive species comprises > 50% of the total number of individuals.
 - i) If an irruptive species makes up ≥ 50% of the sample, sample one or more additional subreaches to bring the proportion of the irruptive species below 50%. Go to Step 16.
 - ii) If not, go to Step 16.
 - c) If you have sampled all 10 subreaches (i.e., you have reached Transect K), go to Step 16.
- 16) After sampling, record the final length of the fish sampling reach in the *Primary Electrofishing Gear* section of the Fish Gear Form.
 - a) If you did not collect any fish, select *Fished – None Collected* in the *Fish sampling – Not Routine or Suspended* section of the Fish Gear Form.
- 17) In the *Primary Electrofishing Gear* section of the Fish Gear Form, record the total button time expended for electrofishing (should be ~700 sec per subreach sampled), the total time spent sampling, and the length of the total fish sampling reach (recorded in Step 15 or 16) sampled by electrofishing.
- 18) Indicate whether conditions allowed for a sufficient sampling effort on the Fish Gear Form (*Yes, No, Not Sure*). If you marked either *No* or *Not Sure*, provide an explanatory comment.
- 19) Note the general response behavior of fish to your final electrofishing settings on the Fish Gear Form as either:
 - a) *Immobilized* (no swimming motions due to electrical field). Includes narcosis (slack muscles) and tetany (rigid muscles).

Electrofishing Procedures in Large Wadeable Streams
<ul style="list-style-type: none"> b) <i>Inhibited Swimming</i> (unbalanced swimming induced by the electrical field). Includes taxis (movement, usually towards the anode), pseudo-taxis (movement, but fish are unconscious and belly-up), and oscillotaxis (movement without orientation). c) <i>Escape</i> (upright avoidance swimming).
20) Record the total length of the stream that was sampled for fish on the Fish Gear Form. This total length should coincide with the end of a subreach unless fishing was suspended prematurely.

12.4 Seining

In small or large wadeable streams where conditions prohibit electrofishing, use seining **only as the last option for collecting fish**. Seining is not to be used in concert with electrofishing. If conditions are such that seining is the only method used, provide a justification in the comment bubble provided in the *Sampling Protocol* section at the top of the Fish Gear Form. **Table 12.5** presents the procedure for seining wadeable streams. The intent of the seining effort is to provide comparable data to electrofishing.

Although wadeable electrofishing techniques typically work best in an upstream direction, seining may work best moving downstream. Allocate seine hauls so that the snag, edge, and mid-channel habitats are fished thoroughly. In general, edge and snag habitats will be sampled using narrower seines over shorter distances, while mid-channel habitats will be sampled using longer seines over longer distances. Generalized habitat seining procedures are presented in **Table 12.5**. Depending upon habitat types and complexity, use 2 to 3 crew members. Two crew members move the seine; an optional third person creates and maintains a bag in the seine in area with higher velocities, or agitates rocks in riffles or snags.

To avoid mortality, process fish after each seine haul. Record all fish collected with seining protocols on separate lines on the Fish Collection Form from those lines used for fish collected by electrofishing.

If you seine, record information for the seine hauls on the Seine Gear sections of the Fish Gear Form to track effort.

Table 12.5 Procedure: Seining (Wadeable Sites)

Procedures for Seining at All Wadeable Sites
1) Use seining as a last option only (e.g., when electrofishing is ineffective due to high conductivity or extremely high turbidity). Do not use seining as a supplementary method to electrofishing. Fish sampling reach must be safely wadeable. <ul style="list-style-type: none"> a) If site conditions are such that only seining is used, provide an explanation in the comment bubble provided in the Sampling Protocol Comments section at the top of the Fish Gear Form. b) At the end of each seine haul, immediately place all fish in one or more live wells to minimize injury and mortality, and so that most fish can be returned to the river alive.
2) Verify or enter the fish sampling date in the header section of the Fish Gear Form in the NRSA App. The date in this field will be automatically entered based on the date recorded on the Verification Form. If you have not filled out the Verification Form or if the date of the fish collection is different, you can edit the date here. Note however that changing the value on this form will not change the value on the Verification Form.
3) Select the pertinent protocol and size class in the <i>Fish Sampling Protocol</i> section. <ul style="list-style-type: none"> a) Proceed to the downstream end of the reach (Transect A).

<ul style="list-style-type: none"> i) At some sites, seining may be more effective while working downstream (from Transect K) instead of upstream. <ul style="list-style-type: none"> (1) If working downstream in a large wadeable stream, reverse the transects in Figure 12.1 and move to the opposite bank where indicated. b) For safety, everyone must wear personal floatation devices and foot protection. c) To aid vision while seining, wear polarized sunglasses and a hat or visor.
<p>4) Select the appropriate <i>Water Visibility</i> conditions on the form. <i>Poor</i> implies that your ability to seine effectively is compromised because of poor visibility. Record the water temperature and conductivity. Note whether the conductivity value is corrected to 25 °C.</p>
<p>5) Select the type of seine being used (<i>Bag Seine</i> or <i>Minnow Seine</i>) in the <i>Primary Seine Net</i> section of the Fish Gear Form. This is the seine that will be used for sampling the majority of the fish sampling reach.</p> <ul style="list-style-type: none"> a) Record the number of crew members (2-3), and the net dimensions (height, total length, and mesh size) on the Fish Gear Form. b) If you have to use a second type of seine for parts of the sampling reach, select the type and record the dimensions in the <i>Secondary Seine Net</i> section of the Fish Gear Form.
<p>6) Determine the length of the fish sampling reach and the number of subreaches that should be sampled (refer to Table 12.2).</p>
<p>7) To maximize capture efficiency, please do the following:</p> <ul style="list-style-type: none"> a) Always use 10 and 20 ft. seines. When necessary, reduce the width by rolling seine poles and floats into the net. b) When narrowing seines, always keep lead line outside of the pole. c) When working edge habitats, only roll the inner side of the seine, while keeping the near bank pole extended. d) As a default, use seines that are 2 meters in depth. A 1.25 meter seine may be used in shallow habitats. e) Keep the float line above the surface (avoid dragging it below the surface while pulling). f) Maintain the lead line along the river bottom. g) Either tie the seine to the poles tightly, or roll the seine into the poles. h) Always maintain the bag behind the poles.
<p>8) Seining habitats include large riffles or gravel bars, pools (which include backwater areas), glides or runs, edges, and snags. Seine width and haul length is dependent upon the water velocity, depth, and/or complexity of the habitat.</p> <ul style="list-style-type: none"> a) The objective of the seining effort is to acquire a comparable collection of fish (in terms of species richness and relative abundance, and allocation of effort throughout the fish sampling reach) to that obtained if the site was electrofished. <ul style="list-style-type: none"> i) Avoid extended seine hauls that collect hundreds of individuals. ii) Seine as many available habitat types as possible within each subreach (one haul each). iii) Total time spent seining a site should be comparable to what would have been spent electrofishing.
<p>b) Riffle Habitats</p> <ul style="list-style-type: none"> i) Use two crew members, each tending a seine pole. Place the seine perpendicular to the current across the downstream end of the riffle. Ensure that the lead line is on the bottom. Tilt the net slightly downstream to form a bag to trap aquatic vertebrates. ii) Starting no more than 3 m upstream, a third crew member kicks the substrate and overturns rocks, proceeding quickly downstream toward the net.

<p>iii) When the area is thoroughly kicked, quickly raise and bag the net.</p>
<p>c) Pool, Backwater, and Bar Habitats (Slack water)</p> <p>i) Use two crew members, each tending a seine pole. Pull the seine across the pool using shallow riffles or banks as barriers. A third crew member creates and maintains a bag in the seine.</p> <p>ii) In areas with current, pull the net downstream and then sweep toward the bank with one or both poles, or post one pole on the bank and sweep the other end in a wide arc from midstream to the same bank.</p> <p>iii) You can work pools in short to long hauls and use seines of varying width depending on the complexity and depth of the pool. Keep the seine depth constant at 2 meters.</p> <p>iv) Pull the bag completely to shore at a predesignated point.</p>
<p>d) Glide or Run Habitats (noticeable current)</p> <p>i) Use two crew members, each tending a seine pole. Pull the seine diagonally across the glide towards the bank. If necessary, a third crew member creates and maintains a bag in the seine.</p> <p>ii) Pull the net quickly downstream along the glide moving diagonally toward the bank. When you reach the bank with the outer edge of the seine, post the pole and sweep the other end in a wide arc from midstream to the same bank.</p> <p>iii) Because of decreased complexity and shallower depths, seine hauls in glides or runs are typically longer and use wider nets. You can use a 1.25 m deep seine in shallow glides.</p> <p>iv) Pull the bag completely to shore at a predesignated point.</p>
<p>e) Edge Habitats</p> <p>i) Edge habitats may be shallow too deep with complex to uniform habitat, and may include undercut banks.</p> <p>ii) Use two crew members, each tending a seine pole. Seine along the nearshore area.</p> <p>iii) The near bank crew member moves along the shore while jabbing along any undercut or small structure. The other crew member stays ahead of the shoreline pole to maintain a “J” in the seine bag. At a predesignated point, post the near shore pole and sweep the seine towards and up on the bank.</p> <p>iv) Depending on edge complexity and depth, seine width and haul length may vary. Use wider seines and longer hauls in shallower, less complex habitats. As complexity, depths, and flow increase, shorten the seine width and haul length accordingly. Seine depth may vary depending on depth.</p>
<p>f) Snag Habitats</p> <p>i) Snag habitats often require creativity in terms of seine length and approach. You can use a 1.25 meter deep seine to avoid snagging the net on structure, but use a 2 m deep seine in deeper areas. Narrow seine widths and short hauls are preferred.</p> <p>ii) Use two crew members, each tending a seine pole. Jab seining is often the most effective method. Quickly jab a shortened seine (< 2 m wide) under the cover and near the river bottom, then quickly lift the seine to the water surface. You can use a third crew member to agitate the snag to move fish out toward the seine.</p> <p>iii) For small snags along the bank, seining along the edge may work best. The near snag crew member moves along the snag, while jabbing along its length. The other crew member stays ahead of the shoreline pole to maintain a “J” in the seine bag. At a predesignated point, quickly pull the seine to the surface.</p>
<p>9) To minimize mortality, process fish (i.e., identify, count, and prepare preserved voucher specimens or photovoucher images) after each seine haul (rather than at the end of a subreach).</p>

a) Record identifications, tallies, and voucher information on the Fish Collection Form. Record all fish collected with seining protocols on separate lines on the field Fish Collection Form from those lines used for fish collected by electrofishing.
10) At the end of all sampling, determine the total number of hauls, the average haul length, the total time spent seining, and the total fish sampling reach length sampled for each type of seine. Record the totals in the <i>Primary</i> and <i>Secondary Seine Net</i> sections of the Fish Gear Form.
11) Indicate whether conditions allowed for sufficient sampling on the Fish Gear Form (<i>Yes, No, Not Sure</i>). If you marked <i>No</i> or <i>Not Sure</i> , provide an explanatory comment.
12) Record the total length of the river that was sampled for fish on the Fish Gear Form. This total length should coincide with the end of a subreach unless fishing was suspended prematurely.

12.5 Processing Fish

Process the fish at the end of each subreach or pairs of subreaches, as described in **Table 12.6**. However, if fish show signs of stress (e.g., loss of righting response, gaping, gulping air, excessive mucus) in the middle of a subreach, change the water in the live well or stop fishing and initiate processing. Always process and release individuals of State or Federally listed threatened or endangered species or large game fish immediately after collection. After processing fish, release them in a location that prevents the likelihood of their recapture.

If you use a seine to collect fish, please record all fish collected with seining protocols on separate lines on the field Fish Collection Form from those lines used for fish collected by electrofishing.

12.5.1 Identification and Tallying

Record species identifications, tallies, and other information for individuals collected on the Fish Collection Form in the NRSA App. While recording data on the Fish Collection Form in the App, select the species from the drop-down list and use the tally boxes to enter the number of each fish species in each size category. As you collect additional fish, edit the tallies in the existing lines of data or add new lines of data for new species. If more than 14 lines of fish data are needed, tap the *Add New Fish Collection Line* button to add a new data line to the form.

When entering data in the tally boxes, numbers can be typed directly into tally boxes, or the “+” and “-” buttons can be used to incrementally change the number. The same species can be entered on multiple rows if necessary (e.g., you did not realize the species had already been collected or an unknown fish is later determined to be a species already collected). As fish are tallied, a running total of fish collected is automatically calculated at the top of the form.

Do not process individuals with total length < 25 mm (1 inch), as these are likely young of year individuals that cannot be identified confidently to species. Only crew members designated as “taxonomic specialists” by EPA regional coordinators can identify fish species. Tally fish by species and major size class (15 cm [6 inch] intervals), and examine them for the presence of DELT (Deformities, Eroded Fins, Lesions and Tumors) anomalies. Use common names of species established by the American Fisheries Society *Common and Scientific Names of Fishes from the United States, Canada and Mexico* (Nelson, et al. 2004, Page et al. 2013). **Appendix D** provides a list of species names to be used, based on the current cumulative taxa list developed for NRSA.

If you believe a specimen is nonindigenous to the site, mark it as *Introduced* on the Fish Collection Form. If you suspect it represents a potential range extension for the species, prepare

one or more specimens (preserved if possible but photographs if not). Physical specimens are required in order to publish reports of range extensions. Include specimens to document suspected range extensions are included as part of the preserved *Unknown/Range Extension* voucher sample (UNK/RNG; **Section 12.5.6**).

12.5.2 Unknown Specimens

If you cannot positively identify individuals to species in the field, record taxonomic information of the collection form using scientific names rather than common names. If you can identify a specimen only to family, record the scientific rather than the common family name (e.g., *UNKNOWN PERCID A*, not *UNKNOWN PERCH A*) on the Fish Collection Form by typing directly into the *Common Name* field. If you can identify a specimen to genus, record the scientific name rather than the common name (e.g., *UNKNOWN PERCINA A*, not *UNKNOWN DARTER*) on the Fish Collection Form. Using scientific rather than common names for unknowns reduces ambiguity, since some common names may in fact refer to multiple genera (e.g., “darter”, “shiner”, “sucker”, “sunfish”, etc.). If you identify an unknown species to Genus, retain a small number (up to 20 individuals per putative species) as part of the preserved UNK/RNG voucher sample (see **Section 12.5.6**) or take good digital photographs (**Section 12.5.3**) for laboratory identification. If you are only able to identify an unknown to Family, retain as many of the individuals as possible for later identification. Use the UNK/RNG Voucher label(s) to label your jar(s) of unknown to track from which sites the unknowns originated.

Table 12.6 Procedure: Processing Fish (Wadeable Sites)

Fish Processing	
1)	Ensure that the date of fish collection is accurately recorded on the top of the Fish Gear Form. This date will be attributed to all fishing activities including the collection of vouchers and fish plug tissue samples. If fishing occurs over multiple days, use the first day of fishing as the date entered on the Fish Gear Form and include a comment in the <i>Sampling Protocol</i> section of the Fish Gear Form that provides all dates of fish collection.
2)	Process individuals collected as often as necessary to avoid mortality. You may record a single species on multiple lines of the collection form (e.g., use separate lines for individuals collected in multiple subreaches or collected with a secondary gear type) or simply amend the tallies of fish on data rows previously used. Fish do not need to be separated by subreach. <ul style="list-style-type: none"> a) Process species listed as threatened and endangered first as described in Step 4. <ul style="list-style-type: none"> i) Photograph specimens for voucher purposes if conditions permit and stress to individuals will be minimal. Mark as <i>Photo</i> in the <i>Voucher</i> section of the collection form. ii) If individuals die due to sampling, prepare them as part of the local voucher sample and preserve them in the field. Comply with the conditions of your collection permit in regards to mortality of listed species. iii) Return individuals to the stream immediately after processing. Release fish in an area where recapture is unlikely.
3)	Only identify and process individuals > 25 mm (1 inch) in total length (TL). Ideally handle specimens only once. Although not required, you may note amphibians and reptiles captured on the Fish Collection Form.
4)	Identify each individual to the lowest possible taxonomic level: <ul style="list-style-type: none"> a) If you can confidently identify the individual to species, record the common name on the first blank line in the <i>Common Name</i> field of the Fish Collection Form. <ul style="list-style-type: none"> i) Common names should follow those recognized by the American Fisheries Society. Use of alternative names is discouraged. Use names presented in Appendix D, which are based on those used in previous NRSA surveys. <ul style="list-style-type: none"> (1) Record the complete common name. Avoid using shortened names (e.g., <i>stoneroller</i>, <i>carp</i>, <i>bass</i>, etc.). Select the common name from the provided drop-down list to ensure correct spelling, etc. The search bar in the drop-down selection window can be used to search for any part of a fish name to speed data entry. Once you see the common name you are searching for, tap on it to enter it into the data form. (2) If you use a non-standard name, you must type the name directly into the <i>Common Name</i> field and include a comment which provides the taxonomic reference for the name. b) If you cannot positively identify an individual to the species level: <ul style="list-style-type: none"> i) Identify it to the lowest taxonomic level (i.e., family or genus). Record the putative name as UNKNOWN plus the scientific name of the family or genus (e.g., <i>UNKNOWN CATOSTOMID A</i>, <i>UNKNOWN MOXOSTOMA A</i>) by typing in the <i>Common Name</i> field. ii) If you are permitted to retain the specimen, assign it the next available sequential tag number (starting with <i>01</i>) in the <i>Voucher Tag Number</i> field and see Step 9. c) If you believe the individual is a hybrid: <ul style="list-style-type: none"> i) Mark as <i>Hybrid?</i> on the collection form. ii) If the hybrid has an accepted standard common name (e.g., <i>Tiger muskellunge</i>, <i>Saugeye</i>, <i>Wiper</i>, etc.), record that name by selecting it from the drop-down list. For other hybrids record the common name of both species (e.g., <i>Green sunfish x Bluegill</i>, <i>Cutthroat trout x</i>

	<p><i>Rainbow trout</i>) which may also be included in the drop-down list. Avoid using non-specific terms such as <i>Hybrid sunfish</i>.</p> <p>iii) If you are unsure of the identification and are permitted to retain the specimen, assign it the next available sequential tag number (starting with 01) in the <i>Voucher Tag Number</i> field and see Step 9.</p>
5)	<p>If you know the species is not native to this location, mark as <i>Introduced?</i> If you cannot evaluate the native/introduced status of fish collected, select the checkbox at the top of the collection form. This will confirm that blank values for the <i>Introduced?</i> field are missing, rather than being presumed as native.</p>
6)	<p>Visually estimate the total length of each individual (a measuring board is not necessary but can be used to calibrate one's eye to the size groupings or can be used to verify a fish length). Keep a running tally in the appropriate <i>Tally and Counts</i> section (< 6 in., 6-12 in., 12-18 in., or > 18 in.) of the Fish Collection Form. When entering data in the tally boxes, numbers can be typed directly into tally boxes, or the "+" and "-" buttons can be used to incrementally change the number.</p> <p>a) If all individuals of a species appear to be the same size, provide a comment for the line if you believe the population is stunted.</p>
7)	<p>Examine each individual for external anomalies. Readily identify external anomalies including missing organs (eye, fin), skeletal deformities, shortened operculum, eroded fins, irregular fin rays or scales, tumors, lesions, ulcerous sores, blisters, cysts, blackening, white spots, bleeding or reddening, excessive mucus, and fungus. After you process all of the individuals of a species, record the total number of individuals observed with one or more anomaly in the <i>Anom Count</i> field of the Fish Collection Form.</p> <p>a) NOTE: Do not include injuries from collecting, handling, or processing fish, or from parasites in the external anomaly tally.</p>
8)	<p>If an individual has died due to electrofishing or handling, include it in the running tally for the species as normal. After you process all of the individuals of a species, record the total mortality for the species in the <i>Mortality/Count</i> field of the Fish Collection Form.</p>
9)	<p>If you are retaining individuals of the species as part of the preserved Unknown/Range Extension Unk/Rng voucher sample:</p> <p>a) Select the <i>Voucher Unk/Rng</i> checkbox on the form.</p> <p>b) Assign the species the next available voucher specimen tag, and record the number in the <i>Voucher Tag #</i> field.</p> <p>i) If you take one or more photographs of the species instead of or in addition to preserving specimens, select the <i>Voucher Photo</i> box. Include the specimen tag in all photos of the species. Ideally, take photos of all species collected at a site that are not being preserved.</p> <p>c) Record the number of individuals retained for the preserved voucher sample in the <i>Vouchers Retained</i> field of the Fish Collection Form.</p> <p>i) NOTE: Do not keep separate tallies of voucher and non-voucher specimens. Record all individuals in the appropriate area of the <i>Tally and Counts</i> section. The retained voucher specimens represent a subsample of the total count.</p> <p>ii) Place the specimens in a jar which has been labeled with the site ID. You can have multiple individuals of the same species in the jar, but each species will have a separate voucher tag number (i.e. one tag number per line on the collection form). Line numbers and tag numbers do not need to be the same because the same species may occur on multiple lines, but any given species should only be assigned a single tag number.</p>
10)	<p>If you are retaining specimens as part of a preserved QA voucher sample for the site:</p> <p>a) Select the <i>Voucher QA</i> checkbox on the form.</p>

- i) NOTE: This should be marked at least once for all species collected at the site (including unknowns) to create a complete voucher collection for the site.
 - b) Use the sheet of labels and tags for the QA voucher sample (the primary jar label has a preprinted sample ID number and is found on the same label sheet as other sample labels provided in the site kit and extra jar labels are provided on a separate sheet). Assign the species the next available voucher specimen tag number (start with Tag 01 and assign tag number sequentially). Record the specimen tag number in the *Voucher Tag #* field of the Fish Collection Form.
 - c) If you take one or more photographs of the species instead of or in addition to preserving specimens, select the Voucher Photo box. Include the specimen tag in all photos of the species.
 - d) Record the number of individuals retained for the preserved voucher sample in the *Vouchers Retained* field of the Fish Collection Form.
 - i) NOTE: Do not keep separate tallies of voucher and non-voucher specimens. Record all individuals in the appropriate area of the *Tally and Counts* section. The retained voucher specimens represent a subsample of the total count.
 - ii) Place the specimens in a fine mesh bag (or separate jar) along with the voucher specimen tag that matches the number recorded on the collection form. You can have multiple bags (or jars) of the same species, but each bag (or jar) will have a separate voucher tag number (i.e., one tag per line on the collection form). Line numbers and tag numbers do not need to be the same because the same species may occur on multiple lines, but any given species should only be assigned a single tag number.
- 11) Repeat Steps 2 through 10 for each subreach sampled. Add to the tally counts by editing the numbers in the tally field or by using the “+” and “-” buttons. The same species can be entered on multiple rows if necessary (e.g., you did not realize the species had already been collected or an unknown fish is later determined to be a species already collected). As fish are tallied, a running total of fish collected is automatically calculated at the top of the form.
- 12) Record the fish collected with seining methods on a separate lines from those lines used for fish collected by electrofishing.
- 13) At the end of sampling, follow the appropriate procedure to prepare the preserved voucher samples (UNK/RNG and/or QA Vouchers) and/or select specimens for tissue (fish plug) samples.
- a) For all voucher samples, use a sufficient volume of 10% buffered formalin (the volume of formalin solution used must exceed the volume of specimens). Use additional jars if necessary. Slit large individuals (TL > 200 mm [~8 in.]) along the right side in the lower abdominal cavity to allow penetration of the formalin solution.
- 14) Complete a sample jar label for the UNK/RNG voucher sample. Attach it to the sample jar and cover it with clear tape. Note that sample IDs are not assigned to UNK/RNG Voucher samples.
- 15) Complete an inner label by filling in the site ID, date and visit number with a pencil. For QA Vouchers, also write the sample ID in the space provided.
- 16) If you did not prepare a QA voucher sample, mark *No Voucher Preserved* on the bottom of the Fish Collection Form (this is akin to the ‘no sample collected’ checkbox associated with other sample types).
- a) Otherwise complete a sample jar label for the QA Voucher sample. Attach it to the sample jar and cover it with clear tape. Extra jar labels are provided that will be used to label additional jars belonging to the same voucher sample by writing the Voucher Sample ID on the label and indicating the number of the jar on the label (e.g., Jar 1 of 2).
 - b) Record the total number of jars in the “# of Jars” field. Also select the “Preserved” checkbox to confirm that the voucher sample has been properly preserved.

- 17) Record the file names of any photovouchers taken on the Fish Collection Form in the NRSA App by completing the Following steps.
 - a) Enter the tag number assigned to the species in the *Voucher Tag #* field. Use 2-digit format (e.g., 01, 02, etc.).
 - b) Select the *Voucher Photo* checkbox. Doing so will open a Photo File Name field that is automatically populated with the base portion of the photo naming convention (Site ID + Visit number + tag number).
 - c) Each photo taken of the same individual or species will need to be assigned a letter (starting with “a”). Indicate the beginning and the end of the sequence in the *Sequence* field. For example, a series of four photos of an individual would be assigned a sequence of “a-d”.
 - d) Prior to uploading the images to the NARS SharePoint site, name each image file as: Site ID + Visit number + tag number + sequence (e.g., NRS238_WY-10001_V1_tag01a).
- 18) If you did not collect any fish from the entire fish sampling reach, select *Fished – None Collected* in the *Fish Sampling – Not Routine or Suspended* section of the Fish Gear Form.

12.5.3 Photovouchering

Use digital imagery for fish species that cannot be preserved as voucher specimens (e.g., rare, threatened, and endangered species; very large bodied). Ideally, take photos of all species collected a site (that are not preserved) to provide a minimal level of documentation of occurrence. Take photographs of entire specimens and additional specific morphological features that are appropriate and necessary for an independent taxonomist to accurately identify the specimen. Additional detail for these guidelines is provided in Stauffer et al. (2001), which is provided to all Field Crews in electronic format.

The recommended specifications for digital images to be used for photovouchering include: 16 bit color at a minimum resolution of 1024x768 pixels; macro lens capability allowing for images to be recorded at a distance of less than 4 cm; and built-in or external flash for use in low light conditions. Specimens (or morphological features) should occupy as much of the field of view as possible. Use a fish measuring board, ruler, or some other calibrated device to provide a reference to scale. Provide an adequate background color for photographs (e.g., fish measuring board). Include a card with site ID number, site name, and date in each photograph so that photos can be identified if file names become corrupted. In addition, if the specimen is part of either the unknown/range extension or QA voucher collection, include the voucher specimen tag that you assign to the species to provide a link to the line on the Fish Collection Form. For each photovoucher specimen, include at least a full body photo (preferably of the left side of the fish), and other macro images of important morphological features (e.g., lateral line, ocular/oral orientation, fin rays, gill arches, mouth structures, etc.). It may also be necessary to photograph males, females, or juveniles to depict key identifying features.

Save images in medium to high quality jpeg format. It is important that time and date stamps are accurate, as this information can also be useful in tracking the origin of photographs. Transfer images stored in the camera to a personal computer or external storage device (e.g., thumb drive or flash memory card) at the first available opportunity. At this time, rename the original files to include the site ID, visit number, voucher specimen tag number, and photo sequence (e.g., NRS23_WY-10001_V1_tag01a.jpg). Record the file names on the Fish Collection Form. You should review your photos to confirm that they provide sufficient details to allow someone else to confidently confirm your identification using only your image files.

Maintain a complete set of your photovoucher files in a safe location (e.g., an office computer that is backed up regularly) for the duration of the sampling season. At this time, you will post all images to the NRSA SharePoint site.

12.5.4 Preparing Preserved Voucher Specimen Samples

There are two different types of samples for preserved voucher specimens. The *UNK/RNG* voucher samples are used to identify specimens that cannot be confidently identified in the field, and to provide physical specimens of suspected range extensions. After submitting the initial fish collection data via the NRSA App (which should be done immediately after leaving the site), the data can easily be amended by editing the Fish Collection Form and resubmitting that form to update the database.

In addition to a *UNK/RNG* voucher sample (if needed), you will prepare an additional *QA voucher sample* (**Section 12.5.7**) at some sites. A QA voucher sample will be performed at a pre-designated set of sites and includes preserved specimens (and/or photographs) of **all** species collected at a site (including the unknowns). Use the voucher specimen tags and sample labels

designated for QA voucher samples. QA voucher samples are eventually sent to an independent taxonomist as a check on the accuracy of each fish taxonomist.

12.5.5 Preserving Voucher Specimen Samples

Preserve UNK/RNG and QA voucher specimens in the field with a 10% buffered formalin solution. The volume of formalin must be equal to or greater than the total volume of specimens. Use additional jars if necessary to ensure proper preservation. For individuals having a total length larger than 200 mm (~8 in.), make a slit along the right side of the fish in the lower abdominal cavity to allow penetration of the preservative solution. Follow all the precautions for handling formalin outlined in the SDS. **Formalin is a potential carcinogen. Handle with extreme caution, as vapors and solution are highly caustic and may cause severe irritation on contact with skin, eyes, or mucus membranes. Wear vinyl or nitrile gloves and safety glasses, and always work in a well-ventilated area.**

Once you have completed preserving all jars of voucher specimens, complete the appropriate inner and outer jar labels (**Figure 12.2** for UNK/RNG samples, and **Figure 12.3** for QA voucher samples). Attach the completed label to the jar and cover with clear shipping tape. Extra jar labels are provided for each type of voucher collection. If you have multiple jars of either type of sample, use the extra jar labels provided to prepare a label for each additional jar. For the QA voucher sample, write the unique sample ID number on the extra jar label (this is found on the pre-printed QA voucher label). On each jar label, use the spaces provided to record "Jar N of X", where "N" is the individual jar number, and "X" is the total number of jars for the sample.

UNK/RNG VOUCHER Site ID: NRS23_ _____ ____ / ____ / 202__		UNK/RNG VOUCHER Site ID: NRS23_ _____ ____ / ____ / 202__	
UNK/RNG VOUCHER BAG TAG: 41	UNK/RNG VOUCHER BAG TAG: 42	UNK/RNG VOUCHER BAG TAG: 43	UNK/RNG VOUCHER BAG TAG: 44
UNK/RNG VOUCHER BAG TAG: 37	UNK/RNG VOUCHER BAG TAG: 38	UNK/RNG VOUCHER BAG TAG: 39	UNK/RNG VOUCHER BAG TAG: 40
UNK/RNG VOUCHER BAG TAG: 33	UNK/RNG VOUCHER BAG TAG: 34	UNK/RNG VOUCHER BAG TAG: 35	UNK/RNG VOUCHER BAG TAG: 36
UNK/RNG VOUCHER BAG TAG: 29	UNK/RNG VOUCHER BAG TAG: 30	UNK/RNG VOUCHER BAG TAG: 31	UNK/RNG VOUCHER BAG TAG: 32
UNK/RNG VOUCHER BAG TAG: 25	UNK/RNG VOUCHER BAG TAG: 26	UNK/RNG VOUCHER BAG TAG: 27	UNK/RNG VOUCHER BAG TAG: 28
UNK/RNG VOUCHER BAG TAG: 21	UNK/RNG VOUCHER BAG TAG: 22	UNK/RNG VOUCHER BAG TAG: 23	UNK/RNG VOUCHER BAG TAG: 24
UNK/RNG VOUCHER BAG TAG: 17	UNK/RNG VOUCHER BAG TAG: 18	UNK/RNG VOUCHER BAG TAG: 19	UNK/RNG VOUCHER BAG TAG: 20
UNK/RNG VOUCHER BAG TAG: 13	UNK/RNG VOUCHER BAG TAG: 14	UNK/RNG VOUCHER BAG TAG: 15	UNK/RNG VOUCHER BAG TAG: 16
UNK/RNG VOUCHER BAG TAG: 09	UNK/RNG VOUCHER BAG TAG: 10	UNK/RNG VOUCHER BAG TAG: 11	UNK/RNG VOUCHER BAG TAG: 12
UNK/RNG VOUCHER BAG TAG: 05	UNK/RNG VOUCHER BAG TAG: 06	UNK/RNG VOUCHER BAG TAG: 07	UNK/RNG VOUCHER BAG TAG: 08
UNK/RNG VOUCHER BAG TAG: 01	UNK/RNG VOUCHER BAG TAG: 02	UNK/RNG VOUCHER BAG TAG: 03	UNK/RNG VOUCHER BAG TAG: 04

Figure 12.2 Unknown/Range Extension Voucher Sample Labels and Tags

12.5.6 Processing Unknown/Range Extension (UNK/RNG) Voucher Samples

Table 12.7 outlines the procedure for determining the identification of unknown specimens from each UNK/RNG sample. A more detailed procedure for conducting the laboratory identifications is presented in the NRSA 2023/24 laboratory operations manual. Identify unknown specimens using whatever resources are necessary (magnification, literature, reference collections/specimens, including dissected anatomical features or in-house colleagues).

Following positive laboratory identification, update the Fish Collection Form in the NRSA App to reconcile the unknown records to reflect revisions to the actual species identifications, counts, and any other information recorded on the Fish Collection Form. Resubmit the form to update the database with the revised data.

If all specimens for an unknown record are a single species, simply record the final identification (as common name from the standard list [**Appendix D**]) in the *Common Name* field, and enter any changes to the original counts in the appropriate *Tally/Counts* fields. If you determine that a single unknown record is actually >1 species, update the original UNKNOWN record with the information for the most abundant species. Record the information for additional species from this original unknown in a new data line but retain the voucher specimen tag number of the original unknown record. For example, if a sample of 20 specimens of *UNKNOWN COTTUS A* is later identified as 15 individuals of one species and 5 individuals of another, record the common name for the first (most abundant) species on the same line as the original unknown record, and assign 75% of the original total count to it. Record the common name of the second species on the first available blank line, and assign 25% of the original total count to this second species.

If you use a non-standard name (i.e., one that is not listed in Appendix D), use the adjacent comment bubble to provide the taxonomic reference for the name. Submit the revised form via the NRSA App as soon as possible after completing the laboratory identifications. Retain the preserved UNK/RNG voucher samples from each site. Contact your regional EPA coordinator if you cannot store the samples at your facility.

If your attempts at identification do not yield a positive identification for 100% of the fish you retained, contact the Field Logistics Coordinator for further guidance (Chris Turner, cturner@glec.com, 715-829-3737). There are provisions under which fish can be identified by a contracted lab and the results returned to you.

12.5.7 Processing QA Voucher Samples

Prepare the QA voucher sample as outlined in **Table 12.8**. Prepare the QA voucher sample separately from the UNK/RNG voucher sample. Processing involves ensuring that the sample jar(s) and photovoucher files include representative specimens of ALL species (including unknowns and common species) collected from the site. Each unique species (including unknowns) should have a unique QA voucher specimen tag number assigned (**Figure 12.3**). Record information about the preserved QA voucher sample on the bottom of the Fish Collection Form in the App.

Retain all of your QA voucher samples (including digital image files) until given direction by EPA regarding where to send them. When you are ready to ship the samples, complete a sample Tracking Form as described in **Appendix C**. QA voucher samples may require shipping as “dangerous goods,” and packing and documentation requirements will differ depending on

whether the samples contain formalin or ethanol, the size of individual bottles, and on the particular shipping service used.

QA VOUCHER (VERT) Site ID: NRS23_ _____ ___ / ___ / 202__ 999012		QA VOUCHER (VERT) Site ID: NRS23_ _____ ___ / ___ / 202__ 999012	
QA VOUCHER – BAG TAG: 41	QA VOUCHER – BAG TAG: 42	QA VOUCHER – BAG TAG: 43	QA VOUCHER – BAG TAG: 44
QA VOUCHER – BAG TAG: 37	QA VOUCHER – BAG TAG: 38	QA VOUCHER – BAG TAG: 39	QA VOUCHER – BAG TAG: 40
QA VOUCHER – BAG TAG: 33	QA VOUCHER – BAG TAG: 34	QA VOUCHER – BAG TAG: 35	QA VOUCHER – BAG TAG: 36
QA VOUCHER – BAG TAG: 29	QA VOUCHER – BAG TAG: 30	QA VOUCHER – BAG TAG: 31	QA VOUCHER – BAG TAG: 32
QA VOUCHER – BAG TAG: 25	QA VOUCHER – BAG TAG: 26	QA VOUCHER – BAG TAG: 27	QA VOUCHER – BAG TAG: 28
QA VOUCHER – BAG TAG: 21	QA VOUCHER – BAG TAG: 22	QA VOUCHER – BAG TAG: 23	QA VOUCHER – BAG TAG: 24
QA VOUCHER – BAG TAG: 17	QA VOUCHER – BAG TAG: 18	QA VOUCHER – BAG TAG: 19	QA VOUCHER – BAG TAG: 20
QA VOUCHER – BAG TAG: 13	QA VOUCHER – BAG TAG: 14	QA VOUCHER – BAG TAG: 15	QA VOUCHER – BAG TAG: 16
QA VOUCHER – BAG TAG: 09	QA VOUCHER – BAG TAG: 10	QA VOUCHER – BAG TAG: 11	QA VOUCHER – BAG TAG: 12
QA VOUCHER – BAG TAG: 05	QA VOUCHER – BAG TAG: 06	QA VOUCHER – BAG TAG: 07	QA VOUCHER – BAG TAG: 08
QA VOUCHER – BAG TAG: 01	QA VOUCHER – BAG TAG: 02	QA VOUCHER – BAG TAG: 03	QA VOUCHER – BAG TAG: 04

Figure 12.3 QA Voucher Sample Labels and Tags

Table 12.7 Procedure: Processing Unknown/Range Extension (UNK/RNG) Voucher Samples

Processing UNK/RNG Voucher Samples	
1)	Following fixation for 5 to 7 days, decant and properly discard the formalin solution. Formalin is a potential carcinogen and should be used with extreme caution, as vapors and solution are highly caustic and may cause severe irritation on contact with skin, eyes, or mucus membranes. Wear vinyl or nitrile gloves and safety glasses, and always work in a well-ventilated area. a) Formalin must be disposed of properly. Contact your regional EPA coordinator if your laboratory does not have the capability of handling waste formalin.
2)	Replace the formalin with tap water and soak specimens over a 4-5 day period. Soaking may require periodic water changes and should continue until the odor of formalin is barely detectable.
3)	Decant the tap water. Use 45%-50% isopropyl alcohol or 70% ethanol as a final preservative for specimens.
4)	Process unknowns by tag number— do not combine multiple bags (or jars) of the same unknown before determining the final identifications . Corrections and updates need to be linked back to the original voucher specimen tag number you recorded on the Fish Collection Form.
5)	Make any corrections to the original collection data by revising names and/or counts in the Fish Collection Form in the App. a) Use common names from the standard list (Appendix D) as revised names. b) If you must use a non-standard name, provide the taxonomic reference in the adjacent comment bubble.
6)	If an unknown turns out to include > 1 species, correct the final counts based on the proportion of each species found in the original unknown bag. a) Record the revised name and count for one species (the most abundant) on the line of the original unknown. b) Record the revised name and count for the second species as a new line of data on the Fish Collection Form. i) Record the specimen tag number from the original unknown record in the new line of data.
7)	After reconciling all of the unknowns, and correcting any other information on the Fish Collection Form, submit the revised form via the NRSA App to update the database with the new data. Retain the preserved UNK/RNG voucher samples. Contact your regional EPA Regional Coordinator if you cannot store the samples at your facility.

Table 12.8 Procedure: Processing QA Voucher Samples

1) Ensure that all species collected at a site are represented by either preserved voucher specimens or photovouchers. There should be a unique QA voucher specimen tag number assigned to every species recorded on the Fish Collection Form.
2) Before submitting the QA Voucher sample, ensure that all specimens have been positively identified. If your attempts at identification do not yield a positive identification for 100% of the fish you retained, contact the Contract Field Logistics Coordinator for further guidance (Chris Turner, cturner@glec.com , 715-829-3737).
3) After preparing the preserved QA voucher sample, check that the sample ID number recorded on the Tracking Form matches the preprinted label attached to each sample jar, and that the number of jars recorded on the Fish Collection Form is correct. Also ensure that each extra jar is labelled with the same Sample ID as the primary jar.
4) Complete an inner label by filling in the site ID, date, visit number and sample ID with a pencil. Place a completed inner label inside each jar of specimens.
5) Retain the QA voucher samples in appropriate storage space for formalin until you receive information regarding where to send them from the NRSA staff at EPA Office of Water or EPA Regional Coordinator.
6) If you are storing the preserved QA voucher samples for an extended period, you may need to replace the formalin fixative with ethanol. a) Following fixation for 5 to 7 days, decant and properly discard the formalin solution. Formalin is a potential carcinogen handle with extreme caution, as vapors and solution are highly caustic and may cause severe irritation on contact with skin, eyes, or mucus membranes. Wear vinyl or nitrile gloves and safety glasses, and always work in a well-ventilated area. b) Formalin must be disposed of properly. Contact your regional EPA Regional Coordinator if your laboratory does not have the capability of handling waste formalin.
7) Replace the formalin with tap water and soak specimens over a 4-5 day period. Soaking may require periodic water changes and should continue until the odor of formalin is barely detectable.
8) Decant the tap water. Use 45%-50% isopropyl alcohol or 70% ethanol as a final preservative for specimens.
9) When ready to ship all of the QA voucher samples, complete a sample Tracking Form as described in Appendix C .
10) Package the preserved samples properly for either formalin or ethanol and prepare all required documentation and safety measures for the shipment.
11) Post all photovoucher files for each QA voucher sample to SharePoint. Use the file names that are recorded on the Fish Collection Form.

13 FISH TISSUE PLUG SAMPLING METHODS

13.1 Method Summary

Because many fish spend their entire life in a particular water body they can be important indicators of water quality, especially for toxic pollutants (e.g., pesticides and trace elements). Toxic pollutants, which may be present in the water column or sediments at concentrations below our analytical detection limits, can be found in fish tissue due to bioaccumulation.

Typical fish tissue collection methods require the fish to be sacrificed, whether it be a whole fish or a skin-on fillet tissue sample. This can be problematic when there is a need to collect large trophy-sized fish for contaminant analysis or when a large sample size is necessary for statistical analysis. The following describes an alternative method for the collection of fish tissue samples for a single contaminant of concern (mercury), which uses a tissue plug instead of a skin-on fillet. A plug sample consisting of two fish tissue plugs for mercury analysis will be collected from two fish of the same species (one plug per fish) from the target list (below) at all sites where suitable fish species and lengths are available. Specimens must be at least 190 mm to qualify for tissue plug sampling. DO NOT collect tissue plugs from any fish less than 190 mm in total length. These fish are often collected during the fish assemblage sample collection effort (**Section 12**) but additional effort can be used to collect specimens for tissue samples if desired. A plug tissue sample is collected by inserting a biopsy punch into a de-scaled thicker area of dorsal muscle section of a live fish. After collection, antibiotic salve is placed over the wound and the fish is released. If only one qualifying specimen is collected, collect both tissue plugs from the same individual.

13.2 Equipment and Supplies

Table 13.1 lists the equipment and supplies necessary for Field Crews to collect fish tissue plug samples. This list is comparable to the checklist presented in **Appendix A**, which provides information to ensure that Field Crews bring all of the required equipment to the site. Record the fish tissue plug sampling data on the Fish Collection Form in the NRSA App.

Table 13.1 Equipment and Supplies: Fish Tissue Plug Sample

<p>For fish tissue plug samples</p>	<ul style="list-style-type: none"> Fish measuring board Fish weigh scale Plastic bags Sterile 20 mL glass scintillation vial Coolers with ice Cooler with dry ice Nitrile gloves 8 millimeter disposable biopsy punch Sterile disposable scalpel Sterile forceps Laboratory pipette bulb. Antibiotic salve. Fish collection gear (electrofisher, nets, livewell, etc.) Dip net Field Operations Manual
<p>For recording measurements</p>	<ul style="list-style-type: none"> Fish tissue plug sample labels Fish Collection Form in NRSA App

	Fine-tipped indelible markers for filling out sample labels Clear tape strips for covering labels
--	--

13.3 Sample Collection Procedures

Collection of individual fish specimens for the fish tissue plug indicator occurs in the sample reach during the fish assemblage sampling effort, using the same gear used to collect the fish assemblage samples. Fish tissue plug samples should be taken from the species listed in the target list found in **Table 13.2**. If the target species are unavailable, the fisheries biologist will select an alternative species (i.e., a species that is commonly consumed in the study area, with specimens of harvestable or consumable size) to obtain a plug sample. Recommended and alternate target species are given in **Table 13.2**. The procedures for collecting and processing fish plug samples are presented in **Table 13.3**.

If the fish assemblage sample collection effort does not yield specimens that are appropriate for the collection of the tissue plugs, the crew should expend a reasonable amount of additional effort to collect specimens for the tissue plug samples. This additional effort will not be included as part of the fish assemblage effort and specimens collected during this additional effort should not be recorded as part of the assemblage collection. Crews may target areas of habitat that were not sampled as part of the fish assemblage effort or concentrate additional effort on habitat likely to hold target fish.

Table 13.2 Recommended Target and Alternate Species for Fish Tissue Plug Collection

	FAMILY NAME	COMMON NAME	SCIENTIFIC NAME	MINIMUM LENGTH GUIDELINE
Target Species	Centrarchidae	Spotted bass	<i>Micropterus punctulatus</i>	The minimum length guideline is ≥190 mm for ALL species.
		Largemouth bass	<i>Micropterus salmoides</i>	
		Smallmouth bass	<i>Micropterus dolomieu</i>	
		Black crappie	<i>Pomoxis nigromaculatus</i>	
		White crappie	<i>Pomoxis annularis</i>	
	Ictaluridae	Channel catfish	<i>Ictalurus punctatus</i>	
		Blue catfish	<i>Ictalurus furcatus</i>	
		Flathead catfish	<i>Pylodictis olivaris</i>	
	Percidae	Sauger	<i>Sander canadensis</i>	
		Walleye	<i>Sander vitreus</i>	
		Yellow perch	<i>Perca flavescens</i>	
	Moronidae	White bass	<i>Morone chrysops</i>	
	Esocidae	Northern pike	<i>Esox lucius</i>	
		Chain pickerel	<i>Esox niger</i>	
		Muskellunge	<i>Esox masquinongy</i>	
	Salmonidae	Brown trout	<i>Salmo trutta</i>	
Cutthroat trout		<i>Oncorhynchus clarkii</i>		
Rainbow trout		<i>Oncorhynchus mykiss</i>		
Brook trout		<i>Salvelinus fontinalis</i>		
Alternates ^B	Catostomidae ^A	Largescale sucker	<i>Catostomus macrocheilus</i>	
		Quillback	<i>Carpionides cyprinus</i>	
		River carpsucker	<i>Carpionides carpio</i>	
		River redhorse	<i>Moxostoma carinatum</i>	
		Smallmouth buffalo	<i>Ictiobus bubalus</i>	
		White sucker	<i>Catostomus commersonii</i>	
	Centrarchidae	Bluegill	<i>Lepomis macrochirus</i>	
		Rock bass	<i>Ambloplites rupestris</i>	
		Redbreast sunfish	<i>Lepomis auritus</i>	
		Redear sunfish	<i>Lepomis microlophus</i>	
	Cyprinidae	Northern pikeminnow	<i>Ptychocheilus oregonensis</i>	
		Sacramento pikeminnow	<i>Ptychocheilus grandis</i>	
		Common carp	<i>Cyprinus carpio</i>	
	Ictaluridae	Black bullhead	<i>Ameiurus melas</i>	
		Brown bullhead	<i>Ameiurus nebulosus</i>	
		White catfish	<i>Ameiurus catus</i>	
		Yellow bullhead	<i>Ameiurus natalis</i>	
	Lepisosteidae	Longnose gar	<i>Lepisosteus osseus</i>	
	Moronidae	Striped bass	<i>Morone saxatilis</i>	
		White perch	<i>Morone americana</i>	
	Percidae	Saugeye	<i>Sander vitreus x Sander canadensis</i>	
	Salmonidae	Mountain whitefish	<i>Prosopium williamsoni</i>	
Sciaenidae	Freshwater drum	<i>Aplodinotus grunniens</i>		

^A These are only a few examples of the many sucker species that would be acceptable alternative species.

^B Only send if preferred species are not available.

Table 13.3 Procedure: Fish Tissue Plug Samples

Fish Tissue Plug Methods

1. Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellent until after the plug sample has been collected.
2. Rinse potential target species/individuals in ambient water to remove any foreign material from the external surface and place in clean holding containers (e.g., livewells, buckets). Return non-target fishes or small specimens to the river or stream.
3. Retain two individuals of the same target species from each site. The fish should be of adequate size to sample (minimum size for all species is 190 mm). Select fish based on the following criteria:
 - is on the target list
 - both the same species
 - both satisfy legal requirements of harvestable size for the sampled river, or at least be of consumable size if no legal harvest requirements are in effect
 - are of similar size, so that the smaller individual is no less than 75% of the total length of the larger individual.
4. Remove one fish selected for plug sampling from the clean holding container(s) (e.g., livewell) using clean nitrile gloves.
5. Measure the fish to determine total body length. Measure total length of the specimen in millimeters, from the anterior-most part of the fish to the tip of the longest caudal fin ray (when the lobes of the caudal fin are depressed dorsoventrally).
6. Weigh the fish in grams using the fish weigh scale.
7. Note any anomalies (e.g., lesions, cuts, sores, tumors, fin erosion) observed on the fish.
8. Record the collection method, species, and specimen length and weight in the Fish Tissue Plug section of the Fish Collection Form in the NRSA App. If the specimen is one of the fish species listed in the assemblage portion of the form, it will appear in the drop-down list in the fish tissue plug common name field. If the species was not collected as part of the assemblage effort, type the common name directly into the box, taking care to spell it correctly.
9. Prepare a Sample Label for the sample with the Site ID, date and visit number. Affix the label to a sterile 20 milliliter scintillation vial and cover with clear tape.
10. On a meaty portion of the left side dorsal area of the fish between the dorsal fin and the lateral line, clear a small area of scales with a sterile disposable scalpel.
11. Wearing clean nitrile gloves, insert the 8 millimeter biopsy punch into the dorsal muscle of the fish through the scale-free area. The punch is inserted with a slight twisting motion cutting the skin and muscle tissue. Once full depth of the punch is achieved a slight bending or tilting of the punch is needed to break off the end of the sample. Remove biopsy punch taking care to ensure sample remains in the punch. **Note: The full depth of the punch should be filled with muscle tissue, which should result in collecting a minimum of 0.25 to 0.35 grams of fish tissue for mercury analysis.**
12. Apply a generous amount of antibiotic salve to the plug area and gently return the fish to the water.
13. Using a laboratory pipette bulb placed on the end of the biopsy punch, give a quick squeeze, blowing the tissue sample into a sterile 20 milliliter scintillation vial.
14. Repeat steps 2-13 for the second fish, collecting a second fish plug sample. Place the second plug in the same scintillation vial as the first. The two plugs should provide at least 0.5 grams of tissue.

If only one qualifying specimen is collected, collect both tissue plugs from the same individual (one plug from each side of the fish). Leave the second set of length and weight fields blank and include a comment

Fish Tissue Plug Methods

in the adjacent comment bubble to verify that both plugs were collected from the single fish listed and the blank fields were left blank on purpose.

15. Place the sample immediately on dry ice for shipment.
16. Dispose of gloves, scalpel and biopsy punch.
17. Keep the samples frozen on dry ice or in a freezer at $\leq -20^{\circ}\text{C}$ until shipment.
18. Frozen samples will subsequently be packed on dry ice and shipped to the batched sample laboratory via priority overnight delivery service within 1 week of collection.

14 FINAL SITE ACTIVITIES

14.1 Overview of Final Site Activities

Prior to leaving the site, make a general visual assessment of the site and its surrounding catchment. The objective of the site assessment is to record observations of catchment and site characteristics that are useful for future data interpretation, ecological value assessment, development of associations, and verification of stressor data. Your observations and impressions are extremely valuable.

You will filter and process the fecal indicator, chlorophyll-a, and periphyton samples, as well as conduct a final check of the data forms, labels, and samples. The purpose of the second check of data forms, labels, and samples is to assure completeness of all sampling activities. Finally, clean and pack all equipment and supplies, and clean the launch site and staging areas. After you leave the site, submit any and all data collected and ship or store the samples. Making an initial data submission as soon as possible is important so that the data is stored in the EPA database, preventing data loss due to a lost or broken iPad. Data can easily be updated in the NRSA App and resubmitted at any time. Activities described in this section are summarized in **Table 14.1**.

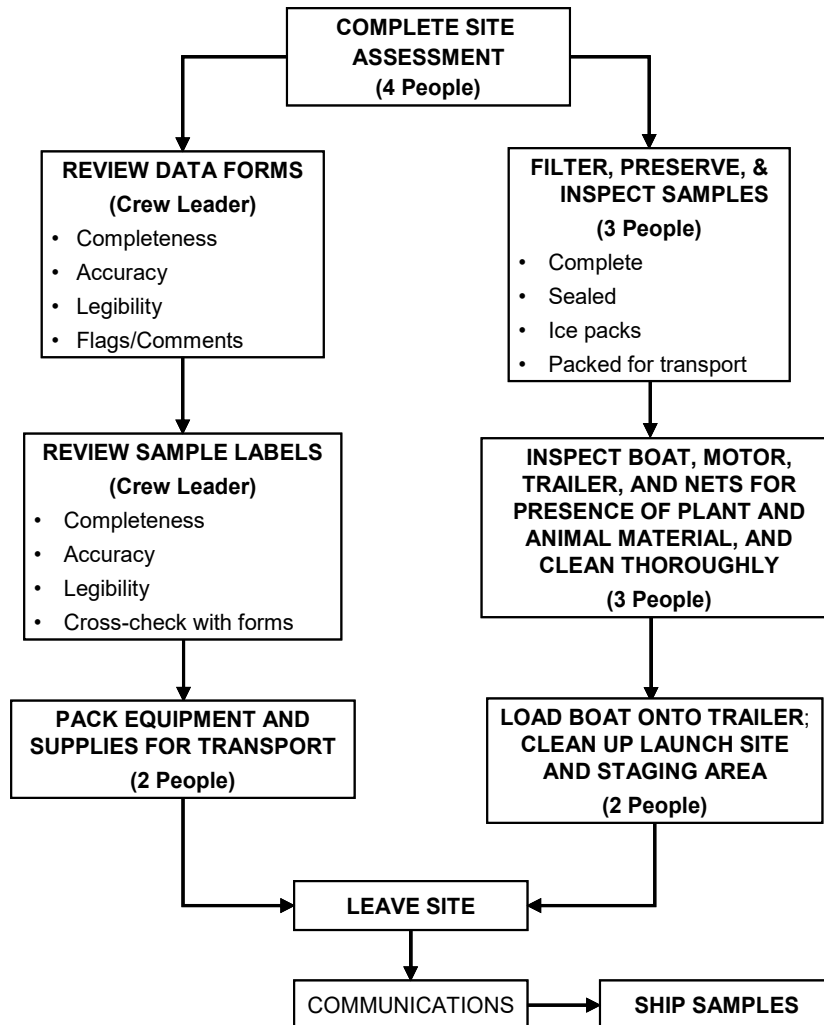


Figure 14.1 Final Site Activities Summary

14.2 General Site Assessment

Complete the Site Assessment Form in the NRSA App at the end of the sampling day, recording all observations from the site that were noted during the course of the visit. This Site Assessment Form is designed as a template for recording pertinent field observations. It is by no means comprehensive, and any additional observations should be recorded in the General Assessment section.

14.2.1 Elevation at Transect K

Ensure that the elevation at Transect K has been taken with your GPS and is recorded on the Assessment Form. To record this information, record the elevation holding the GPS at approximately 3 feet above the surface of the water.

14.2.2 Watershed Activities and Disturbances Observed

Record any of the sources of potential stressors listed in the “Watershed Activities and Disturbances Observed” section on the Site Assessment Form. Include those that were observed while on the site, while driving or walking through the site catchment, or while flying over the site and catchment. For activities and stressors that you observe, rate their abundance or influence as low (L), moderate (M), or heavy (H) on the line next to the listed disturbance. Leave the line blank for any disturbance not observed and be sure to verify that blank field indicate absence by filling in the bubble at the top of the section. The distinction between low, moderate, and heavy will be subjective. For example, if there are two to three houses on a site, select “L” for low next to “Residences.” If the site is lined with houses, rate it as heavy (H). Similarly, a small patch of clear-cut logging on a hill overlooking the site would rate a low ranking. Logging activity right on the site shore, however, would get a heavy disturbance ranking. This section includes residential, recreational, agricultural, industrial, and stream management categories.

To confirm that lines left blank were done so to indicate absence of the activity or disturbance, select the “*ALL activities were evaluated*” bubble at the top of this section of the Site Assessment Form in the App.

14.2.3 Site Characteristics

Record observations regarding the general characteristics of the site on the Site Assessment Form. When assessing these characteristics, look at a 200 m riparian distance on both banks. Rank the site between “pristine” and “highly disturbed”, and between “appealing” and “unappealing.” Document any signs of beaver activity and flow modifications. Record the dominant land use and forest age class. Document the weather conditions on the day of sampling and any extreme weather conditions in the days prior to sampling.

14.2.4 General Assessment

Record any additional information and observations in this narrative section. Information to include could be observations on biotic integrity, vegetation diversity, presence of wildlife, local anecdotal information, or any other pertinent information about the site or its catchment. Record any observations that may be useful for future data interpretation.

14.3 Processing the Fecal Indicator (*Enterococci*), Chlorophyll-*a*, and Periphyton Samples

14.3.1 Equipment and Supplies (Fecal Indicator Filtering)

Table 14.1 provides the equipment and supplies needed for Field Crews to collect the fecal indicator sample.

Table 14.1 Equipment and Supplies: Fecal Indicator (*Enterococci*) Sample Processing

<p>For processing samples</p>	<p>Nitrile gloves sterile screw-cap 50 mL PP tube Filtration apparatus with collection flask Sterile filter holder, Nalgene 145/147 Vacuum pump (electric pump may be used if available) Sterile phosphate buffered saline (PBS) Millipore 47 mm polycarbonate sterile filters Sterile disposable forceps Petri dishes (60 x 15, disposable) 2 sterile microcentrifuge tubes containing sterile glass beads 1 additional sterile microcentrifuge tube if collecting filter blank Bubble bag Zip-top bag Dry ice Cooler Field Operations Manual</p>
<p>For recording measurements</p>	<p>Sample Collection Form in NRSA App Fine-tipped indelible markers for filling out sample labels Fecal Indicator sample labels (2 vial labels and 1 bag label) Filter blank label if collecting filter blank</p>

14.3.2 Procedures for Processing the Fecal Indicator (*Enterococci*) Sample

The fecal indicator sample **must** be filtered **before** the chlorophyll-*a* and periphyton samples, since the filtering apparatus needs to be sterile for this sample. The procedures for processing the fecal indicator sample are presented in **Table 14.2**. The sample must be filtered and frozen within six hours of collection.

Table 14.2 Procedure: Fecal Indicator (*Enterococci*) Sample Processing

<p>Filtering for the fecal indicator (<i>Enterococci</i>) sample</p>
<ol style="list-style-type: none"> Put on nitrile gloves. Set up sample filtration apparatus on flat surface and attach vacuum pump. Set out 50 mL sterile PP tube, sterile 60 mm Petri dish, two bottles of chilled phosphate buffered saline (PBS), Millipore 47 mm polycarbonate sterile filter box, and two filter forceps. Chill Filter Extraction tubes with beads on dry ice. Aseptically transfer two polycarbonate filters from filter box to base of opened Petri dish. Close filter box and set aside (this step is to prevent wind from disturbing the remaining filters in the box but is optional in calm/controlled environments).

5. Remove the pre-loaded cellulose nitrate (CN) filter (the filter with grid design on it) from funnel and discard. Be sure to leave the support pad in the filter funnel.
6. Load filtration funnel with sterile polycarbonate filter on support pad (shiny side up).
7. Gently shake sample bottle 25 times to mix well.
8. Measure 25 mL of the mixed water sample in the sterile graduated sterile PP tube and pour into filter funnel.
9. Replace cover on filter funnel and pump to generate a vacuum (do not generate more than 7 inches of Hg of vacuum [3.44 psig]). Keep pumping until all liquid is in filtrate collection flask.
10. If the first 25 mL volume passes readily through the filter, add another 25 mL and continue filtration. If it was very difficult to filter the first 25 mL, proceed to step 11. If the filter clogs before completely filtering the first or second 25 mL volume, discard the filter and repeat the filtration using a lesser volume.
11. Pour approx. 10 mL of the chilled phosphate buffered saline (PBS) into the graduated PP tube used for the sample. Cap the tube and shake 5 times. Remove the cap and pour rinsate into filter funnel to rinse filter.
12. Filter the rinsate and repeat with another 10 mL of phosphate buffered saline (PBS).
13. Remove filter funnel from base without disturbing filter. Using sterile disposable forceps remove the filter (touching only the filter edges) and fold it in half, in quarters, in eighths, and then in sixteenths (filter will be folded four times).
14. Insert filter into chilled filter extraction tube (with beads). Filter should be inserted open end down (pointed side up) into the tube. Replace and tighten the screw cap.
15. Record the volume of sample filtered through the filter on the small yellow label and apply the label to the extraction tube (DO NOT cover with clear tape).
16. Record the volume of sample filtered through the filter on the outer bag label and apply the label to the bubble bag (DO NOT cover with clear tape).
17. Insert tube into bubble bag and zip-top bag on dry ice for preservation during transport and shipping.
18. Record the volume of water sample filtered through each filter on the Sample Collection Form in the App. Record the filtration start time and finish time for the sample as well as the time the filters were frozen.
19. Repeat steps 6 to 15 for the remaining 50 mL sub-sample volume to be filtered. Make every effort to filter the same volume of sample through each of the two filters.

Processing Procedure—fecal indicator (Enterococci) filter blank

Enterococci filter blanks will be prepared at all revisit sites during the first visit. Prepare the filter blanks **before** filtering the stream sample.

1. Set up sample filtration apparatus using same procedure as used for the river sample. Chill Filter Extraction tubes with beads on dry ice.
2. Aseptically transfer 1 polycarbonate filter from filter box to base of opened Petri dish. Close filter box and set aside (this step is to prevent wind from disturbing the remaining filters in the box but is optional in calm/controlled environments).
3. Remove the pre-loaded cellulose nitrate (CN) filter (the filter with grid design on it) from funnel and discard. Be sure to leave the support pad in the filter funnel.
4. Load filtration funnel with sterile polycarbonate filter on support pad (shiny side up).
5. Measure 10 mL of the chilled phosphate buffered saline (PBS) in the sterile graduated PP tube and pour into filter funnel.

6. Replace cover on filter funnel and pump to generate a vacuum (do not generate more than 7 inches of Hg of vacuum [3.44 psig]). Keep pumping until all liquid is in filtrate collection flask.
7. Remove filter funnel from base without disturbing filter. Using sterile disposable forceps remove the filter (touching only the filter edges) and fold it in half, in quarters, in eighths, and then in sixteenths (filter will be folded 4 times).
8. Insert filter into chilled filter extraction tube (with beads). Filter should be inserted open end down (pointed side up) into the tube. Replace and tighten the screw cap.
9. Record the volume of PBS filtered through the filter on the small yellow label and apply the label to the extraction tube (DO NOT cover with clear tape). Note that there is a specific label for the blank sample. At sites where a blank is not collected, this label will be discarded.
10. Insert tube into bubble bag and zip-top bag on dry ice for preservation during transport and shipping.
11. Package and submit this sample to the lab with the standard samples.
12. Indicate that you have collected a filter blank by selecting the “Blank Collected” checkbox on the Sample Collection Form.

14.3.3 Equipment and Supplies (Chlorophyll-*a* from Water Sample Filtering)

Table 14.3 provides the equipment and supplies needed to process the chlorophyll-*a* water sample.

Table 14.3 Equipment and Supplies: Chlorophyll-*a* Processing

For filtering chlorophyll-<i>a</i> sample	Whatman GF/F 0.7 μm glass fiber filter Filtration apparatus with graduated filter holder and collection flask Vacuum pump (electric pump may be used if available) 50 mL screw-top centrifuge tube Aluminum foil square 250 mL graduated cylinder DI water Nitrile gloves Forceps Dry ice Zip-top bag Plastic electrical tape
For recording measurements	Sample Collection Form in NRSA App Sample labels Fine-tipped indelible markers Clear tape strips

14.3.4 Procedures for Processing the Chlorophyll-*a* Water Sample

The procedures for processing chlorophyll-*a* water samples are presented in **Table 14.4**. Whenever possible, sample processing should be done in subdued light, out of direct sunlight.

Table 14.4 Procedure: Chlorophyll-a Sample Processing

Filtering for the chlorophyll a water sample	
1.	Put on nitrile gloves.
2.	Use clean forceps to place a Whatman GF/F 0.7 µm glass fiber filter in the graduated filter holder apparatus with the gridded side of the filter facing down.
3.	Retrieve the 2 liter chlorophyll sample bottle from the cooler and gently shake the bottle to homogenize the sample. While filtering sample, keep the bottle in the cooler on ice.
4.	Measure 250 mL of water with a graduated cylinder and pour into the filter holder, replace the cap, and use the vacuum pump to draw the sample through the filter (do not exceed 7 inches of Hg [3.44 psig]). If 250 mL of site water will not pass through the filter, change the filter, rinse the apparatus with DI water, and repeat the procedures using 100 mL of site water. <ul style="list-style-type: none"> • <i>NOTE: IF the water is green or turbid, use a smaller volume to start.</i>
5.	Observe the filter for visible color. If there is readily visible color, proceed; if not, repeat steps 3 & 4 until color is visible on the filter or until a maximum of 2,000 mL have been filtered. Record the actual sample volume filtered on the Sample Collection Form in the App.
6.	Rinse the upper portion of the filtration apparatus and graduated cylinder thoroughly with DI water to include any remaining cells adhering to the sides and pump through the filter. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump. Remove the bottom portion of the apparatus and pour off the water from the bottom as often as needed.
7.	Remove filter funnel from base without disturbing filter.
8.	Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored side folded in on itself.
9.	Place the folded filter into a 50 mL screw-top centrifuge tube and cap. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met. Failure to tighten the lid completely could allow water to infiltrate into the sample and may compromise its integrity.
10.	Record the sample volume filtered on a chlorophyll label and attach it to the centrifuge tube. Ensure that all written information is complete and legible. Cover with a strip of clear tape.
11.	Wrap the tube in aluminum foil and place in the provided leak-proof zip-top plastic bag labelled with the completed chlorophyll outer bag label. Cover the outer label with clear tape. Place this bag immediately on dry ice to freeze.

14.3.5 Equipment and Supplies (Periphyton Sample)

Table 14.5 lists the equipment and supplies needed to process the periphyton sample.

Table 14.5 Equipment and Supplies: Periphyton Samples

For preparing periphyton samples	Whatman 47 mm 0.7 micron GF/F glass fiber filter Whatman 47 mm 1.2 micron GF/C glass fiber filter Filtration apparatus with collection flask and graduated filter holder Vacuum pump (electric pump may be used) 25 or 50 mL graduated cylinder Pipette and pipette bulb (2 mL) 3 50 mL screw-top centrifuge tubes 125 mL sterile PETG bottle 60 mL syringe with tip removed
---	--

	Aluminum foil squares Forceps Nitrile gloves deionized water in wash bottle plastic electrical tape dry ice wet ice coolers formalin
For data recording	Sample Collection Form Sample labels Fine-tipped indelible markers Clear tape strips
For cleaning equipment	10% Bleach solution

14.3.6 Procedures for Processing the Periphyton Samples

Four different types of laboratory samples are prepared from the composite periphyton sample: an ID/enumeration sample (to determine taxonomic composition and relative abundances), periphyton metagenomics sample, chlorophyll *a* sample, and a biomass sample (for ash-free dry mass [AFDM]). All the sample containers required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination (e.g., dust, dirt, or mud) that are present at site shorelines.

14.3.6.1 ID/Enumeration Sample

Prepare the ID/Enumeration sample as a 50 mL aliquot from the composite periphyton sample, following the procedure presented in **Table 14.6**. Preserve each sample with 2 mL of formalin. Record the sample ID number from the container label and the total volume of the periphyton composite sample in the appropriate fields on the Sample Collection Form in the NRSA App. Store the preserved samples upright in a container containing absorbent material.

Table 14.6 Procedure: ID/Enumeration Samples of Periphyton

Periphyton ID Sample Processing Procedure	
1.	Prepare a sample label (with pre-printed sample ID number sample type "PERI"). Record the site ID, date, and visit number as well as the total volume of the composite sample on the label. Attach completed label to a 50 mL centrifuge tube. Cover the label completely with a clear tape strip.
2.	Record the volume of the subsample (typically 50 mL) and the total volume of the composite sample on the Sample Collection Form.
3.	Thoroughly mix the bottle containing the composite sample.
4.	Immediately after mixing, pour 50 mL of sample into pre-labeled 50 mL centrifuge tube.
5.	Use a syringe or bulb pipette to add 2 ml of 10% formalin to the tube. Cap the tube tightly and seal with plastic electrical tape. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met.
6.	Shake gently to distribute preservative.

14.3.6.2 *Periphyton Metagenomic Sample*

Prepare the periphyton metagenomic sample as a 100 mL aliquot from the composite index sample, following the procedure presented in **Table 14.7**.

Table 14.7 Procedure: Preparing Metagenomic Sample of Periphyton

Periphyton metagenomic Sample Processing Procedure

1. Prepare a sample label (with pre-printed sample ID number sample type “PDNA”). Record the site ID, date, and visit number on the label. Attach completed label to the sterile 125 mL PETG bottle. Cover the label completely with a clear tape strip.
2. Record the total volume of the composite sample on the Sample Collection Form.
3. Put on nitrile gloves.
4. Remove the cap from the bottle.
5. Do not rinse the bottle and avoid touching the inside of the bottle or the inside of the cap.
6. Thoroughly mix the bottle containing the composite sample and immediately pour 100 mL of the mixed sample into the labeled 125 mL PETG bottle. Use the graduations on the bottle to gauge the volume of sample poured.
7. Carefully replace the cap on the sample bottle. Seal the cap with plastic electrical tape.
8. Immediately after sample is collected, place in a cooler with ice to minimize exposure to light and begin chilling the sample. The sample should be frozen as soon as is practicable and should remain frozen until and during shipping.

14.3.6.3 *Periphyton Chlorophyll a Sample*

Prepare the periphyton chlorophyll-*a* sample by filtering a 25 mL aliquot of the composite sample through a 47 mm 0.7 micron GF/F glass fiber filter. The procedure for preparing the periphyton chlorophyll-*a* sample is presented in **Table 14.8**. Chlorophyll-*a* can degrade rapidly when exposed to bright light. If possible, prepare the samples in subdued light (or shade), filtering as quickly as possible after collection to minimize degradation. If using the same filtration chamber that was used for *Enterococci* and index site chlorophyll-*a* samples, rinse it with deionized water prior to filtering the periphyton chlorophyll-*a* sample. If you are reusing a filtration chamber from a previous site, you should rinse it with DI water each day before use at the base site and then seal in a plastic bag until use at the stream (be sure to use a new chamber at each site for the *Enterococci* sample as it needs to be filtered in a sterile chamber). Keep the glass fiber filters in a dispenser inside a sealed plastic bag until use.

It is important to measure the volume of the sample being filtered accurately (± 1 mL) with a graduated cylinder. During filtration, do not exceed 7 inches of Hg (3.44 psig) to avoid rupturing cells. If the vacuum pressure exceeds 7 inches of Hg, prepare a new sample. If the filter clogs completely before all the sample in the chamber has been filtered, discard the sample and filter, and prepare a new sample using a smaller volume of sample.

Table 14.8 Procedure: Preparing Chlorophyll Samples of Periphyton

Periphyton Chlorophyll *a* Sample Processing Procedure

1. Rinse the sides of the filter funnel and the filter with a small volume of deionized water to prevent contamination from the previously filtered samples.
2. Using clean forceps, place a Whatman GF/F 0.7 μm glass fiber filter on the filter holder gridded side down. If needed, use a small amount of deionized water from a wash bottle to help settle the filter properly. Attach the filter funnel to the filter holder and filter chamber, and then attach the vacuum pump to the filter flask.
3. Rinse a 25 mL or 50 mL graduated cylinder three times with small volumes of deionized water and discard.
4. Mix the composite sample bottle thoroughly.
5. Measure 25 mL (± 1 mL) of sample into the graduated cylinder.
NOTE: For a composite sample containing fine sediment, allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.
6. Pour the 25 mL aliquot into the filter funnel, replace the cap, and pull the sample through the filter using the vacuum pump. Vacuum pressure from the pump should not exceed 7 inches of Hg (3.44 psig) to avoid rupture of fragile algal cells.
NOTE: If 25 mL of sample will not pass through the filter, discard the filter and rinse the chamber thoroughly with deionized water. Collect a new sample using a smaller volume of sample, measured to ± 1 mL. Be sure to record the actual volume sampled on the sample label and the Sample Collection Form.
7. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump. Remove the bottom portion of the apparatus and pour off the water from the bottom as often as needed.
8. Rinse the upper portion of the filtration apparatus and graduated cylinder thoroughly with DI water to include any remaining cells adhering to the sides and pump through the filter.
9. Remove the filter chamber from the filter holder being careful not to disturb the filter. Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored sample (filtrate) side folded in on itself. Place the folded filter in a 50 mL centrifuge tube.
10. Tighten the cap as tightly as possible. The cap will seal tightly after an additional $\frac{1}{4}$ turn past the point at which initial resistance is met. Seal the cap with plastic electrical tape.
11. Prepare a sample label (with pre-printed sample ID number sample type "PCHL") including the site ID, date, visit number, and volume filtered, and attach it to the centrifuge tube. Cover the label completely with a strip of clear tape.
12. Place the centrifuge tube into the provided leak-proof zip-top plastic bag with the water column chlorophyll sample.
13. Record the total volume of the composite sample on the Sample Collection Form. Record the volume filtered in the "Periphyton Chlorophyll" field on the Sample Collection Form. Double check that the volume recorded on the collection form matches the total volume recorded on the sample label.
14. Place the centrifuge tube containing the filter on dry ice.

14.3.6.4 Periphyton Biomass Sample

Prepare the periphyton biomass sample by filtering a 25 mL aliquot of the composite index sample through a 47 mm 1.2 micron GF/C glass fiber filter. The procedure for preparing the biomass sample is presented in **Table 14.9**. Using the same filtration chamber that was used for *Enterococci* and chlorophyll-*a* samples, rinse it with deionized water prior to filtering the

periphyton biomass sample. If you are reusing a filtration chamber from a previous site, you should rinse it with DI water each day before use at the base site and then seal in a plastic bag until use at the stream (be sure to use a new chamber at each site for the *Enterococci* sample as it needs to be filtered in a sterile chamber). Keep the glass fiber filters in a dispenser inside a sealed plastic bag until use.

It is important to measure the volume of the sample being filtered accurately (± 1 mL) with a graduated cylinder. During filtration, do not exceed 7 inches of Hg (3.44 psig) to avoid rupturing cells. If the vacuum pressure exceeds 7 inches of Hg prepare a new sample. If the filter clogs completely before all the sample in the chamber has been filtered, discard the sample and filter, and prepare a new sample using a smaller volume of sample.

Table 14.9 Procedure: Preparing Periphyton Biomass Sample

Periphyton AFDM Sample Processing Procedures	
1.	Rinse the sides of the filter funnel and the filter with a small volume of deionized water to prevent contamination from the previously filtered sample.
2.	Using clean forceps, place a Whatman 47 mm 1.2 micron GF/C glass fiber filters on the filter holder gridded side down. If needed, use a small amount of deionized water from a wash bottle to help settle the filter properly. Attach the filter funnel to the filter holder and filter chamber, then attach the hand vacuum pump to the filter flask.
3.	Rinse a 25 mL or 50 mL graduated cylinder three times with small volumes of deionized water and discard.
4.	Mix the composite sample bottle thoroughly.
5.	Measure 25 mL (± 1 mL) of sample into the graduated cylinder. NOTE: For a composite sample containing fine sediment, allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.
6.	Pour the 25 mL aliquot into the filter funnel, replace the cap, and pull the sample through the filter using the vacuum pump. Vacuum pressure from the pump should not exceed 7 inches of Hg (3.44 psig) to avoid rupture of fragile algal cells. NOTE: If 25 mL of sample will not pass through the filter, discard the filter and rinse the chamber thoroughly with deionized water. Collect a new sample using a smaller volume of sample, measured to ± 1 mL. Be sure to record the actual volume sampled on the sample label and the Sample Collection Form.
7.	Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump. Remove the bottom portion of the apparatus and pour off the water from the bottom as often as needed.
8.	Rinse the upper portion of the filtration apparatus and graduated cylinder thoroughly with DI water to include any remaining cells adhering to the sides and pump through the filter.
9.	Remove the filter chamber from the filter holder being careful not to disturb the filter. Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored sample (filtrate) side folded in on itself. Place the folded filter in a 50 mL centrifuge tube.
10.	Tighten the cap as tightly as possible. The cap will seal tightly after an additional $\frac{1}{4}$ turn past the point at which initial resistance is met. Seal the cap with plastic electrical tape.
11.	Prepare a sample label (with pre-printed sample ID number sample type "PBIO"), including the site ID, date, visit number, and volume filtered, and attach it to the centrifuge tube. Cover the label completely with a strip of clear tape. Place the centrifuge tube into the provided leak-proof zip-top plastic bag with the water column and periphyton chlorophyll samples.

12. Record the total volume of the composite sample on the Sample Collection Form. Record the volume filtered in the “Periphyton Biomass” field on the Sample Collection Form. Double check that the volume recorded on the collection form matches the total volume recorded on the sample label.
13. Place the centrifuge tube containing the filter on dry ice.

14.3.6.5 Cleaning of Periphyton Equipment

Once all four laboratory samples have been prepared, discard any remaining sample and thoroughly clean all periphyton sampling equipment (including brush, delimitter, composite bottle, funnel, and syringe) with a 10% bleach solution to disinfect the equipment and limit the possible spread of periphyton DNA to future samples. After cleaning, thoroughly rinse all the equipment with tap or DI water. Store the equipment in a clean plastic bag.

14.4 Data Forms and Sample Inspection

After the Site Assessment Form is completed, the Field Crew Leader reviews all of the data forms and sample labels for accuracy, completeness, and legibility. The other crew members inspect all sample containers and package them in preparation for transport, storage, or shipment. Refer to **Appendix C** for details on preparing samples for shipping.

Ensure that all required data forms for the site have been completed. Confirm that the Site ID, the visit number, and date of visit are correct. On each form, verify that all information has been recorded accurately and that any data needing additional explanation has a comment associated with it.

In each data form you will find a number of data validation routines that will help find missing or possibly incorrect data. These routines can be accessed at any time by tapping the data review button at the top of each form.

Ensure that all samples are labeled, all labels are completely filled in, and each label is covered with clear plastic tape (with the exception of *Enterococci* labels). Compare sample label information with the information recorded on the corresponding field data forms (e.g., the Sample Collection Form) to ensure accuracy. Make sure that all sample containers are properly sealed. Ensure that the water chemistry (CHEM) sample ID is entered into the top of the Tracking Form to allow the App to populate the other IDs for samples that were collected.

14.5 Launch Site Cleanup

Inspect all nets for pieces of macrophyte or other organisms and remove as much as possible before packing the nets for transport. Pack all equipment and supplies in the vehicle and trailer for transport. Keep equipment and supplies organized so they can be inventoried using the equipment and supply checklists presented in **Appendix A**. Lastly, be sure to clean up all waste material at the launch site and dispose of or transport it out of the site if a trash can is not available.

15 FIELD QUALITY CONTROL

Standardized training and data forms provide the foundation to help assure that data quality standards for field sampling are met. This section for field sampling and data collection are the primary guidelines for all cooperators and Field Crews. In addition, repeat sampling and field evaluation and assistance visits will address specific aspects of the data quality standards for the NRSA.

15.1 Revisit Sampling Overview

Revisit sampling will provide data to make variance estimates (for measurement variation and index period variation) that can be used to evaluate the NRSA design for its potential to estimate status and detect trends in the target population of sites. A summary of the repeat sampling design is provided in **Figure 15.1**.

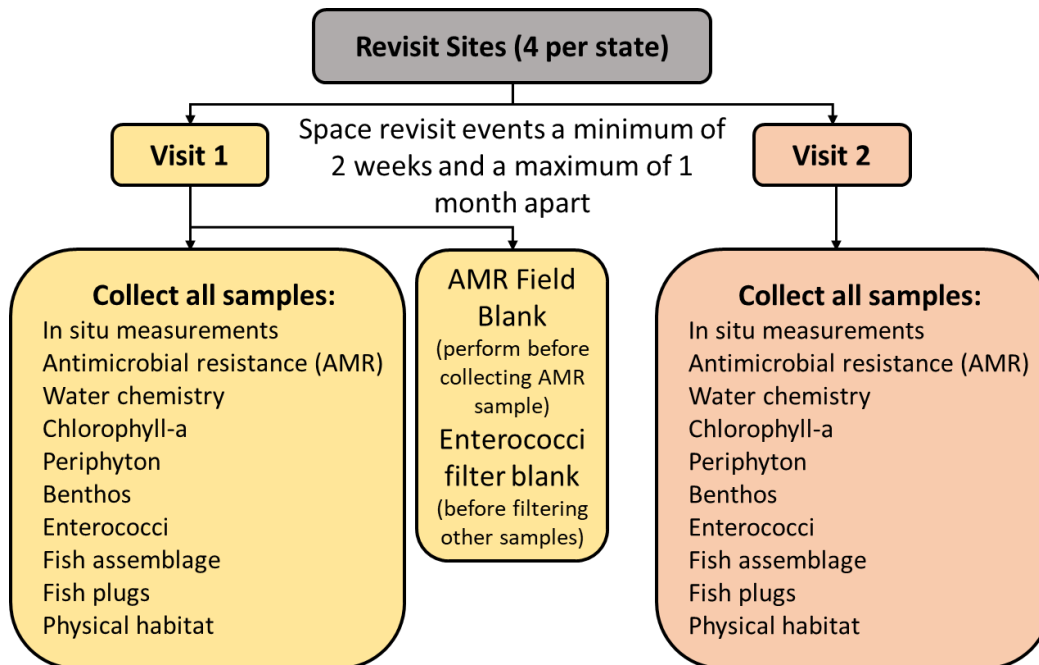


Figure 15.1 Summary of the Repeat Sampling Design

15.2 Revisit Sampling Sites

A total of 192 (approximately 10%) of the target sites visited will be revisited during the same sample year by the same Field Crew that initially sampled the site. Revisit samples and measurements are taken from the same reach as the first visit. **Each state has four revisit sites;** two likely wadeable and two likely non-wadeable sites. For each state these sites are:

Wadeable Revisit sites:

- The two likely wadeable revisit sites are resample sites from the NRSA 2018/19; one from the Large Stream (LS) panel and one from the Small Stream (SS) panel. The revisit sites are labeled as NRS23_18RVT2LS and NRS23_18RVT2SS, respectively, and are

located in the Large and Small Streams tabs within the state specific site evaluation spreadsheet.

Non-Wadeable Revisit sites:

- The two likely non-wadeable revisit sites are resample sites from the NRSA 2018/19 from the River (RV) panel. The revisit sites are labeled as NRS23_18RVT2RV_FT and are located in the Rivers tab within the state specific site evaluation spreadsheet.

If a site selected for revisit sampling is dropped, then the next available base site in the same state and panel should be revisited and the next available oversample site in the state and panel is used to replace the re-designated base site. If there are no base sites remaining in the panel, replace the revisit site with the first available oversample site in the panel and consider it the new revisit site. The primary purpose of this “revisit” set of sites is to collect temporal replicate samples to provide variance estimates for both measurement variation and index period variation. The revisit will include the full set of indicators and associated parameters. The time period between the initial and repeat visit to a site is not less than 2 weeks (**Figure 15.1**). Label the data and samples Visit 2 to indicate that they are from the second sampling event at a revisit site.

At each revisit site, a field blank will be collected for the antimicrobial resistance sample during the first sampling visit (Visit 1). The crews will pour a provided bottle of ultra-pure water into a 500mL PETG pre-sterilized bottle. Detailed description of the AMR field blank is found in **Section 5.4**.

Additionally at each revisit site, a filter blank will be collected for *Enterococci* during the first sampling visit (Visit 1). The crews will filter a small amount (10 mL) of sterile buffer through 1 filter, label it with the “filter blank” label and use the provided checkbox on the Sample Collection Form to indicate the blank was collected. The filter blank should be run before the stream sample is filtered and the filter blank will be sent to the lab along with the stream samples. Detailed description of the filter blank is found in **Table 14.2**.

15.3 Field Evaluation and Assistance Visits

A rigorous program of field and laboratory evaluation and assistance visits has been developed to support the National Rivers and Streams Assessment Program. These evaluation and assistance visits are explained in detail in the QAPP for the NRSA. The following sections will focus only on the field evaluation and assistance visits.

These visits provide a QA/QC check for the uniform evaluation of the data collection methods, and an opportunity to conduct procedural reviews as required minimizing data loss due to improper technique or interpretation of field procedures and guidance. Through uniform training of Field Crews and review cycles conducted early in the data collection process, sampling variability associated with specific implementation or interpretation of the protocols will be significantly reduced. The field evaluations will be based on the Field Evaluation Plan and Checklists. This evaluation will be conducted for each unique crew collecting and contributing data under this program (EPA will make a concerted effort to evaluate every crew, but will rely on the data review and validation process to identify unacceptable data that will not be included in the final database).

15.3.1 Specifications for QC Assurance Field Assistance Visits

Field evaluation and assistance personnel are trained in the specific data collection methods detailed in this FOM. A plan and checklist for field evaluation and assistance visits have been developed to detail the methods and procedures. The plan and checklist are included in the QAPP. **Table 15.1** summarizes the plan, the checklist, and corrective action procedures.

It is anticipated that evaluation and assistance visits will be conducted with each Field Crew early in the sampling and data collection process, and that corrective actions will be conducted in real time. If the Field Crew misses or incorrectly performs a procedure, the Evaluator will note this on the checklist and immediately point this out so the mistake can be corrected on the spot. The role of the Evaluator is to provide additional training and guidance so that the procedures are being performed consistent with the FOM, all data are recorded correctly, and paperwork is properly completed at the site.

Table 15.1 General Information Noted During Field Evaluation

<p>Field Evaluation Plan</p>	<ul style="list-style-type: none"> • EPA Logistics Coordinator will arrange the field evaluation visit with each Field Crew, ideally within the first two weeks of sampling. • The Evaluator will observe the performance of a crew through one complete set of sampling activities. • If the Crew misses or incorrectly performs a procedure, the Evaluator will note it on the checklist and immediately point it out so the mistake can be corrected on the spot. • The Evaluator will review the results of the evaluation with the Field Crew before leaving the site, noting positive practices and problems.
<p>Field Evaluation Checklist</p>	<ul style="list-style-type: none"> • The Evaluator observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and NRSA protocols are followed. • The Evaluator checks the sample containers to verify that they are the correct type and size, and checks the labels to be sure they are correctly and completely filled out. • The Evaluator confirms that the Field Crew has followed NRSA protocols for locating the site. • The Evaluator observes the complete set of sampling activities, confirming that all protocols are followed. • The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Check List.
<p>Corrective Action Procedures</p>	<ul style="list-style-type: none"> • If the Evaluator's findings indicate that the Field Crew is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Crew until certain of the Crew's ability to conduct the sampling properly so that data quality is not adversely affected. • If the Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact a NRSA QA Project Coordinator.

15.4 Reporting

When the sampling operation has been completed, the Evaluator will review the results of the evaluation with the Field Crew before leaving the site (if practicable), noting positive practices and problems (i.e., weaknesses [might affect data quality] or deficiencies [would adversely affect data quality]). The Evaluator will ensure that the Field Crew understands the findings and will be able to perform the procedures properly in the future. The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Check List. After the

Evaluator completes the Field Evaluation and Assistance Check List, including a brief summary of findings, all Field Crew members must read and sign off on the evaluation.

If the Evaluator's findings indicate that the Field Crew is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Crew until certain of the Crew's ability to conduct the sampling properly so that data quality is not adversely affected. If the Evaluator finds major deficiencies in the Field Crew operations (e.g., major misinterpretation of protocols, equipment or performance problems) the Evaluator must contact the following QA official:

Sarah Lehmann, EPA National Rivers and Streams Assessment Project QA Officer

The QA Officer will contact the Project Manager to determine the appropriate course of action. Data records from sampling sites previously visited by this Field Crew will be checked to determine whether any sampling sites must be redone.

16 REFERENCES

- American Red Cross. 1979. Standard First Aid and Personal Safety. American National Red Cross. 269 pp.
- Bain, M.B., and N.J. Stevenson (editors). 1999. Aquatic habitat assessment: common methods. American Fisheries Society, Bethesda, Maryland.
- Baker, J.R., D.V. Peck, and D.W. Sulton (editors). 1997. *Environmental Monitoring and Assessment Program Surface Waters Field Operations Manual for Lakes*. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC.
- Dunne, T., and L.B. Leopold. 1978. Water in environmental planning. W.H. Freeman, New York. 818 p.
- Flotemersch, J.E.1, B.C. Autrey2, and S.M. Cormier1 (editors). 2000. Logistics of Ecological Sampling on Large Rivers. U.S. Environmental Protection Agency, Cincinnati OH.
- Flotemersch, J.E., J.B.Stribling, and M.J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. EPA 600-R-06-127. U.S. Environmental Protection Agency, Cincinnati, OH.
- Harrelson, C.C., C.L. Rawlins, and J.P. Potyondy. 1994. Stream channel reference sites: an illustrated guide to field technique. USDA Forest Service, General Technical Report RM-245, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 61p.
- Helm, W.T. 1985. Aquatic habitat inventory: standard methods and glossary. American Fisheries Society, Western Division, Bethesda, Maryland.
- Hosea, R. C., and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails on wading gear. California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005-02, Sacramento.
- Kaufmann, P.R., P. Levine, E.G. Robison, C. Seeliger, and D.V. Peck. 1999. Quantifying Physical Habitat in Wadeable Streams. EPA 620/R-99/003. Environmental Monitoring and Assessment Program, U.S. Environmental Protection Agency, Corvallis, OR.
- Lemmon, P.E. 1957. A new instrument for measuring forest overstory density. *Journal of Forestry* 55:667-669.
- Leopold, L.B. 1994. A view of the river. Harvard University Press, Cambridge, Massachusetts. 298 p.
- Linsley, R.K., M.A. Kohler, and J.L.H. Paulhus. 1982. Hydrology for engineers. McGraw-Hill Book Co. New York, NY. 508 p.
- Mulvey, M., L. Cato, and R. Hafele. 1992. Oregon Nonpoint Source Monitoring Protocols Stream Bioassessment Field Manual: For Macroinvertebrates and Habitat Assessment. Oregon Department of Environmental Quality Laboratory Biomonitoring Section. Portland, Oregon. 40pp.
- National Institute for Occupational Safety and Health. 1981. Occupational Health Guidelines for Chemical Hazards (Two Volumes). NIOSH/OSHA Publication No. 81-123.

- Nelson, J.S., E.J. Crossman, H. Espinosa-Perez, L.T. Findley, C.R. Gilbert, R.N. Lea, and J.D. Williams. 2004. Common and Scientific Names of Fishes from the United States, Canada, and Mexico. American Fisheries Society, Special Publication 29, Bethesda, Maryland.
- Ohio EPA. 1990. Ohio EPA Fish Evaluation Group Safety Manual. Ohio Environmental Protection Agency, Ecological Assessment Section, Division of Water Quality Planning and Assessment, Columbus, Ohio.
- Pont, D., R.M. Hughes, T.R. Whittier, and S. Schmutz. 2009. A predictive index of biotic integrity model for aquatic-vertebrate assemblages of western U.S. streams. *Transactions of the American Fisheries Society* 138:292-305.
- Rantz, S. E., et al., 1982. Measurement and Computation of Streamflow, Vols. 1 and 2. U.S. Geological Survey Water-Supply Paper 2175.
- Robison, E.G. and R.L. Beschta. 1990. Characteristics of coarse woody debris for several coastal streams of southeast Alaska, USA. *47(9):1684-1693*.
- Stauffer, Dr. Jay R., J. Karish and T.D. Stecko. 2001. Guidelines for Using Digital Photos as Fish Vouchers for Pennsylvania Fishes. The Pennsylvania State University and National Park Service.
- Stevens, D. L., Jr., and A. R. Olsen. 2004. Spatially-balanced sampling of natural resources in the presence of frame imperfections. *Journal of American Statistical Association:99:262-278*.
- U.S. Coast Guard. 1987. Federal Requirements for Recreational Boats. U.S. Department of Transportation, United States Coast Guard, Washington, D.C.
- USDA Forest Service, 1995. A guide to field identification of bankfull stage in the western United States. Rocky Mountain Forest and Range Experiment Station, Stream Systems Technology Center, Fort Collins, Colorado (31 minute video, closed captioned).
- USDA Forest Service, 2002. Identifying bankfull stage in forested streams in the eastern United States. Rocky Mountain Forest and Range Experiment Station, Stream Systems Technology Center, Fort Collins, Colorado (46 minute video, closed captioned).
- USEPA. 1986. Occupational Health and Safety Manual. Office of Planning and Management U.S. Environmental Protection Agency, Washington, D.C. USEPA. 2009. National Rivers and Streams Assessment: Field Operations Manual. EPA 841-B-07-009. U.S. Environmental Protection Agency, Office of Water and Office of Research and Development, Washington, D.C.
- USEPA. 2000a. EPA Quality Manual for Environmental Programs 5360A1. May 2000. <http://www.epa.gov/quality/qs-docs/5360.pdf>
- USEPA. 2000b. EPA Order 5360.1 A2 CHG2, Policy and Program Requirements for Mandatory Agency-wide Quality System, May 5, 2000. <http://www.epa.gov/quality/qs-docs/5360-1.pdf>

Web Pages:

- US EPA Aquatic Monitoring Research: <http://www.epa.gov/nheerl/arm>
- NHD Plus: <http://www.horizon-systems.com/nhdplus>