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National Rivers and Streams Assessment 2013/14

Field Operations Manual

Wadeable

Version 1.0



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NOTICE

The complete documentation of overall NRSA project management, design, methods, and standards is contained in four companion documents, including:

National Rivers and Streams Assessment: Quality Assurance Project Plan EPA-841-B-12-007

National Rivers and Streams Assessment: Site Evaluation Guidelines EPA-841-B-12-008

National Rivers and Streams Assessment: Field Operations Manual EPA-841-B-12-009 a and b

National Rivers and Streams Assessment: Laboratory Methods Manual EPA 841-B-12-010

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, microcystins, fish assemblage, fish tissue plugs, whole fish tissue, Enterococci, and physical habitat. These methods are based on the guidelines developed and followed in the National Rivers and Streams Assessment 2008-2009 (EPA 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 National Rivers and Streams Assessment 2013/14. 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 document.

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ACRONYMS/ABBREVIATIONS

AFDM	Ash-Free Dry mass
ANC	Alkalinity
Ca	Calcium
CAS	Chemical Abstract Service
Cl	Chloride
CPR	Cardiopulmonary Resuscitation
CSDGM	Content Standards for Digital Geospatial Metadata
CWA	Clean Water Act
DELT	Deformities, Eroded Fins, Lesions and Tumors
DO	Dissolved Oxygen
DOC	Dissolved Organic Compound
DI	Di-ionized Tap Water
DQO	Data Quality Objective
EMAP	Environmental Monitoring and Assessment Program
EPA	Environmental Protection Agency
FGDC	Federal Geographic Data Committee
FOIA	Freedom of Information Act
FOM	Field Operations Manual
GIS	Geographic Information System
GPS	Global Positioning Device
HQ	Head Quarters
IBI	Index of Biotic Integrity
IQG	Information Quality Guideline
IM	Information Management
ITIS	Integrated Taxonomic Information System
K	Potassium
LIMS	Laboratory Information Management System
LOM	Lab Operations Manual
LRL	Laboratory Recording Levels
LWD	Large Woody Debris
ISO	International Organization for Standardization
MDL	Method Detection Levels (limit)
Mg	Magnesium
MMI	Multimetric Indicators
MQO	Measurement Quality Objective
MSDS	Methods Safety Data Sheets
Na	Sodium
NABS	North American Benthic Society
NAD	North American Datum
NARS	National Aquatic Resources Survey
NAWQA	National Water-Quality Assessment Program
NCCA	National Coastal Condition Assessment
NELAP	National Environmental Laboratory Accreditation Program
NERL	New England Regional Laboratory
NHD	National Hydrology Database
NH ₃	Ammonia
NH ₄	Ammonium
NIST	National Institute of Standards
NLA	National Lakes Assessment
NLCD	National Land Cover Dataset
NO ₂	Nitrite

NO ₃	Nitrate
NRC	National Research Council
NRSA	National Rivers and Streams Assessment
NWCA	National Wetland Condition Assessment
O/E	“Observed” over “Expected”
OSHA	Occupational Safety and Health Administration
OW	Office of Water
PC	Personal Computer
PD	Percent Difference
PDE	Percent Difference in Enumeration
PDF	Personal Flotation Device
PP	Polypropylene
PPT	Parts per thousand
PRISM	Parameter-Elevation Regressions on Independent Slopes Model
PTD	Percent Taxonomic Disagreement
QA	Quality Assurance
QAPP	Quality Assurance Protection Plan
QA/QC	Quality Assurance/Quality Control
QCS	Quality Check Solution
QRG	Quick Reference Guide
RL	Reporting Limit
RTE	Rare, Threatened and Endangered
RVT	Revisit
SAS	Statistical Analysis System
SDTD	Spatial Data Transfer Standard
SEG	Site Evaluation Guideline
SiO ₂	Silica
SO ₄	Sulfate
SOP	Standard Operating Procedures
SQL	Standard Query Language
Std	Standard
STORET	Storage and Retrieval Data Warehouse
TL	Total Length
TOC	Total Organic Carbon
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UNK/RNG	Unknown Range Extension
USGAO	United States General Accounting Office
USGS	United States Geological Survey
WED	Western Ecology Division
WSA	Wadeable Streams Assessment
WQX	Water Quality Exchange

DISTRIBUTION LIST

This Field Operation Manual (FOM) and associated manuals or guidelines will be distributed to the following 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.

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1 BACKGROUND

This manual describes field protocols and daily operations for crews to use in the National Rivers and Streams Assessment (NRSA) 2013/14. 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 the 2008/09 NRSA; and
- 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. Environmental Protection Agency (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

EPA selected sampling locations using a probability based survey design. The design is an unequal probability design that selects 900 sites classified as Strahler order 1-4 and 900 sites classified as Strahler order 5th and above. To evaluate change from the 2008/09 NRSA, 420 of the 900 1-4th order sites are resampled from the 2008/09 NRSA and 390, 5th orders and above sites are revisits from the 2008-2009 NRSA. Approximately 10%, or 200, of the total NRSA sites are scheduled for repeated sampling (revisit sites) in the same field season. The sample frame was derived from the National Hydrography Dataset (NHD), NHD-Plus, from 1:100,000 scale maps. Additional details on the NRSA survey design are found in the National Rivers and Streams Assessment Survey Design: 2013-2014 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. This includes major rivers, and small streams. Sites must have > 50% of the reach length with standing water. 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 < .05ppt for this study).
- Run-of-the-river ponds and reservoirs with greater than 7 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-012-008*) and the NRSA Web site (<http://www.epa.gov/owow/riverssurvey/index.html>) 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 unsampleable, it must be replaced by another site within the state in the same category. The six general categories for each state are:

- NRSA 2008-2009 resample 1 – 4th Strahler order sites.
- NRSA 2008-2009 resample 5th and above Strahler order sites.
- Small Stream- new 1-3 Strahler order sites.
- Large Streams- new 3-5 Strahler order sites.
- Major Rivers- new 5 and above order sites. Rivers identified as major rivers or additional rivers in the book: Rivers of North America.
- Other Rivers- new 5 and above order sites that are not considered Major Rivers.

Please refer to the *Site Evaluation Guidelines (EPA-841-B-12-008)* for more detailed information.

1.4 Selection of NRSA Indicators

As part of the indicator selection process, EPA worked with state and tribal partners 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 2013/14 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. 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 2013/14 are briefly described in **Table 1.1**.

Table 1.1 Summary Table of Indicators for all NRSA 2013/14 Sites

Indicator	Core or Supplemental Indicator	Specs/Location in Sampling Reach
In Situ measurements (pH, DO, temperature, conductivity)	Core Indicator	Measurements taken at X site at midchannel; readings are taken at 0.5 m depth, or mid-depth if water depth is less than 1 meter.
Water chemistry (TP, TN [NH ₄ , NO ₃], basic anions and cations, alkalinity [ANC], DOC, TOC, TSS, conductivity)	Core Indicator	Measurements taken at X site at midchannel; Collected from a depth of 0.5 m, or mid-depth if water depth is less than 1 meter.
Chlorophyll-a	Core Indicator	Collected as part of water chemistry and periphyton samples

Microcystin	Supplemental Indicator	Collected from index site
Periphyton	Core 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 (<i>Enterococci</i>)	Supplemental Indicator	Collected at the last transect one meter off the bank at .3 m depth
Fish Tissue Plug	Supplemental Indicator	Target species collected throughout the sampling reach as part of fish assemblage sampling
Whole Fish Tissue	Supplemental Indicator at select sites	Target species collected throughout the sampling reach as part of fish assemblage sampling

1.5 Supplemental Material to the Field Operations Manual

The Field Operations Manual describes 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.

Site maps have been provided to assist in the site evaluation process. Three maps are available: an aerial image, topographic map, and road map, see **Figure 1.1**. These maps provide an overlay of the NHD waterbody layer with the coordinate and label for the x-site and/or waterbody, if available. Each site is symbolized by the panel the site is considered within (see Section 1.3). Other important information that may assist in site evaluation is included on the map including: state, EPA region, latitude, longitude, ownership, and stream order. These maps will be helpful in the planning and preparation for visiting and sampling a particular NRSA 2013/14 site. These maps will become part of your site packet.

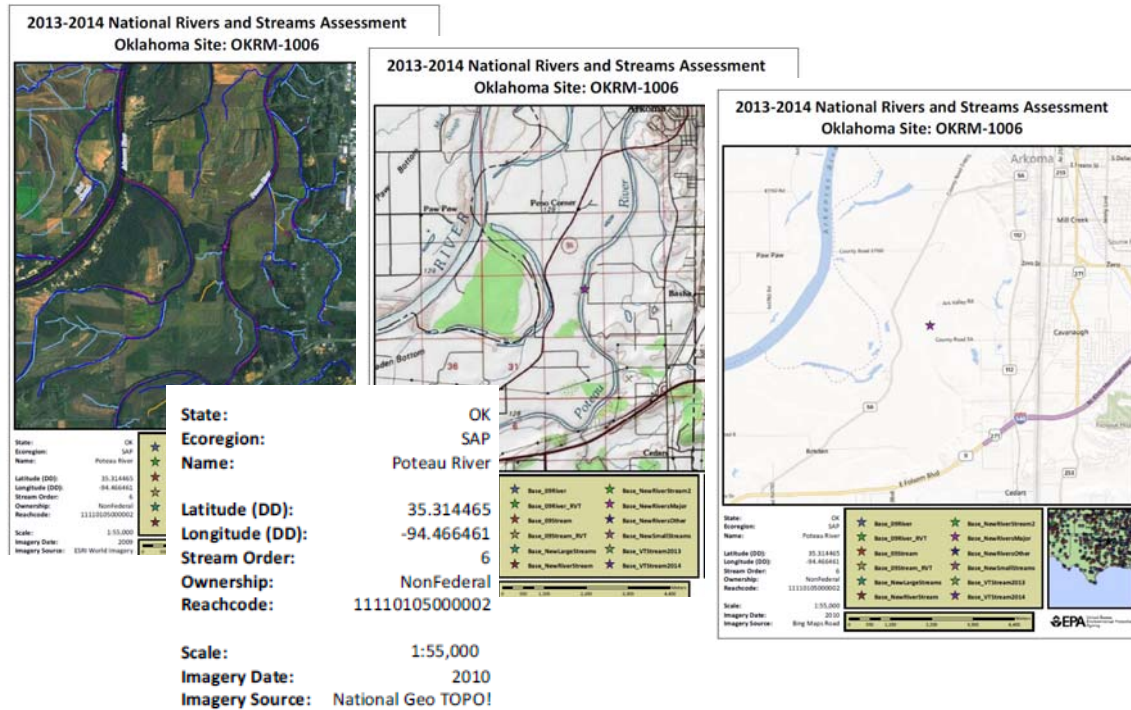


Figure 1.1 Example NRSA Site Maps

Field crews will also receive a Quick Reference Guide (QRG) that contains tables and figures summarizing field activities and protocols from the Field Operations Manual (FOM). This waterproof handbook will be the primary field reference used by field crews after reading the FOM and completing the required field training session. The field crews are also required to keep the field operations manual available in the field for reference and for possible protocol clarification.

Quality assurance 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 copy of the integrated Quality Assurance and Project Plan (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 Quality Assurance procedures, refer to the *National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA 841-B-012-007)*.

Related NRSA documents include the following: National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA 841-B-12-007), National Rivers and Streams Assessment: Site Evaluation Guidelines (EPA 841-B-012-008), and National Rivers and Streams Assessment: Laboratory Methods Manual (EPA 841-B-012-010). These documents are available at: <http://www.epa.gov/owow/riverssurvey/index.html>.

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 1.2**).

It is imperative that field and sample information be **recorded accurately, consistently, and legibly**. 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 or illegible. Guidelines for recording field measurements are presented in **Table 1.2**.

Table 1.2 Guidelines for Recording Field Measurements and Tracking Information

Activity	Guidelines
Field Measurements	
Data Recording	<ul style="list-style-type: none"> • Record measurement values and observations on data forms preprinted on water-resistant paper. • Use No. 2 pencil only (fine-point indelible markers can be used if necessary) to record information on forms. • Record data and information using correct format as provided on data forms. • Be sure to accurately record site IDs and sample numbers. For all primary sampling visits indicate the event as Visit 1. For revisit sites use Visit 2 to indicate the second sampling event during the same season. • Print legibly (and as large as possible). Clearly distinguish letters from numbers (e.g., 0 versus O, 2 versus Z, 7 versus T or F, etc.), but do not use slashes. • In cases where information is recorded repeatedly on a series of lines (e.g., physical habitat characteristics), do not use “ditto marks” (“) or a straight vertical line. Record the information that is repeated on the first and last lines, and then connect these using a wavy vertical line. • When recording comments, print or write legibly. Make notations in comments field only; avoid marginal notes. Be concise, but avoid using abbreviations or “shorthand” notations. If you run out of space, attach a sheet of paper with the additional information, rather than trying to squeeze everything into the space provided on the form.
Data Qualifiers (Flags)	<p>Use only defined flag codes and record on data form in appropriate field.</p> <p style="margin-left: 20px;">K = Measurement not attempted or not recorded.</p> <p style="margin-left: 20px;">Q = Failed quality control check; remeasurement not possible.</p> <p style="margin-left: 20px;">U = Suspect measurement; remeasurement not possible.</p> <p style="margin-left: 20px;">Fn = Miscellaneous flags (<i>n</i> = 1, 2, etc.) assigned by a field crew during a particular sampling visit (also used for qualifying samples).</p> <p>Explain reason for using each flag in comments section on data form.</p>

Activity	Guidelines
Sample Labels	<ul style="list-style-type: none"> • Use adhesive labels with preprinted ID numbers and follow the standard recording format for each type of sample. • Use a pencil to record information on label. Cover the completed label with clear tape. • Record sample ID number from label and associated collection information on sample collection form preprinted on water-resistant paper. • Sample IDs from a single label sheet are in sequential order. Do not mix labels from different sheets.
Sample Collection and Tracking	
Sample Qualifiers (Flags)	<p>Use only defined flag codes and record on sample collection form in appropriate field.</p> <p>K = Sample not collected or lost before shipment; resampling not possible.</p> <p>U = Suspect sample (e.g., possible contamination, does not meet minimum acceptability requirements, or collected by non-standard procedure).</p> <p>Fn = Miscellaneous flags (n=1, 2, etc.) assigned by a field crew during a particular sampling visit (also used for field measurements).</p> <p>Explain reason for using flags in “Comments” on sample collection form.</p>
Review of Labels and Data Collection Forms	<ul style="list-style-type: none"> • Compare information recorded on labels and sample collection form for accuracy before leaving site. • Review labels and data collection forms for accuracy, completeness, and legibility before leaving site. • The Field Crew Leader must review all labels and data collection forms for consistency, correctness, and legibility before transfer to the Information Management Center.

<p>CHEMISTRY (CHEM) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 990000</p>	<p>WATER COLUMN CHLOROPHYLL (CHLA) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Volume Filtered: ____mL 990001</p>
<p>PERIPHYTON ASSEMBLAGE ID (PERI) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Composite Volume: ____mL 990002</p>	<p>PERIPHYTON CHLOROPHYLL (PCHL) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Volume Filtered: ____mL 990003</p>
<p>PERIPHYTON BIOMASS (PBIO) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Volume Filtered: ____mL 990004</p>	<p>ALGAL TOXIN (MICX) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Volume Collected: ____mL 990005</p>
<p>BENTHIC MACROINVERTEBRATES (BERW) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Jar 1 of ____ 990006</p>	<p>BENTHIC MACROINVERTEBRATES (BETB) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Jar 1 of ____ 990007</p>
<p>FISH TISSUE PLUG (FPLG) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 990008</p>	<p>QA VOUCHER (VERT) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 990009</p>
<p>UNK/RNG VOUCHER (VERT) Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2</p>	<p>Sample Type: _____ Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Sample ID: _____</p>
<p>Sample Type: _____ Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Sample ID: _____</p>	<p>Sample Type: _____ Site ID: _____ Date: ____/____/201__ Visit #: ○1 ○2 Sample ID: _____</p>

Figure 1.2 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 wadeable sites 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 work load 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 the **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.

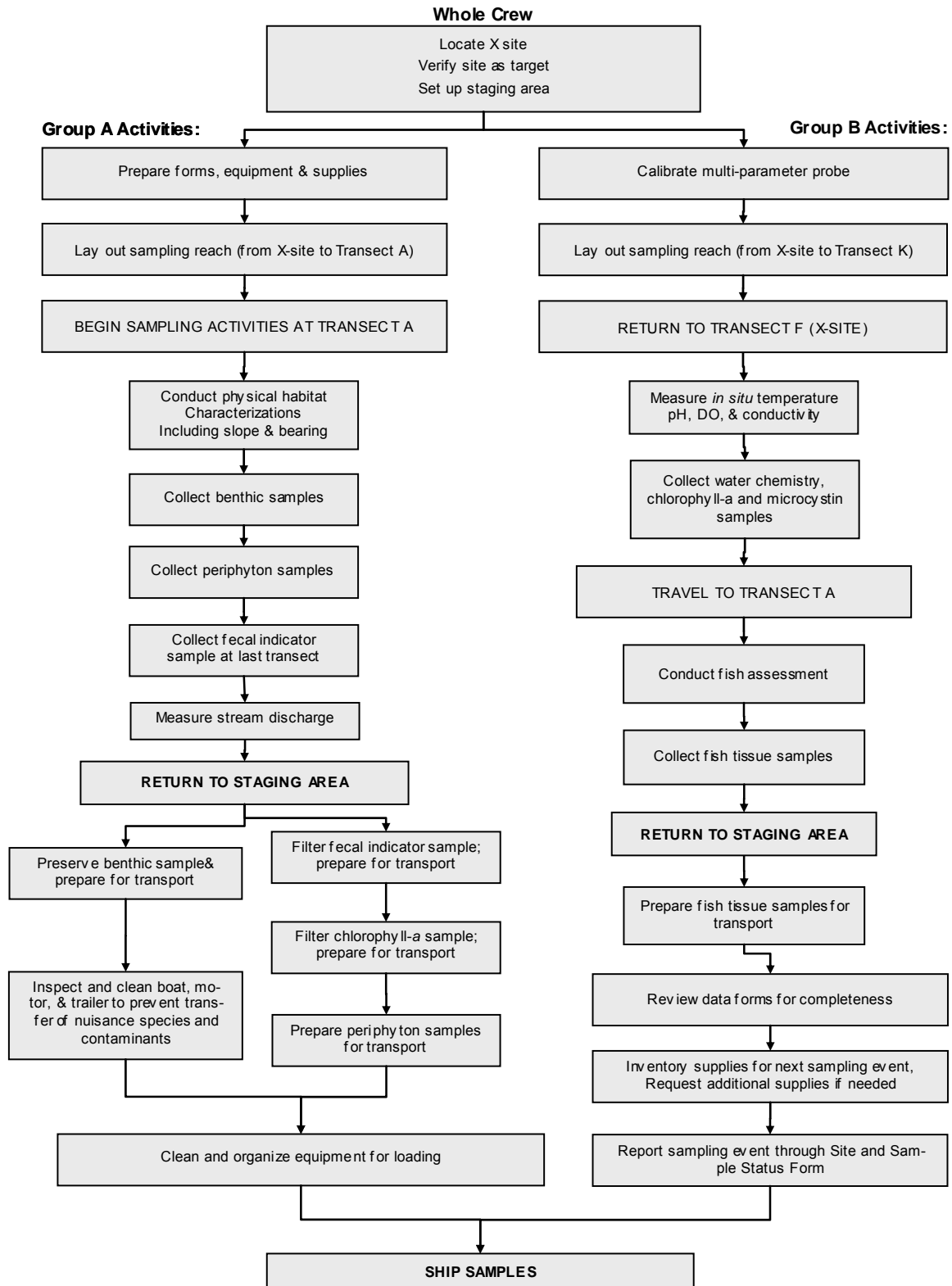


Figure 2.1 Field Sampling Scenario for Wadeable Sites

2.2 Base Site Activities

Field crews conduct a number of 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.

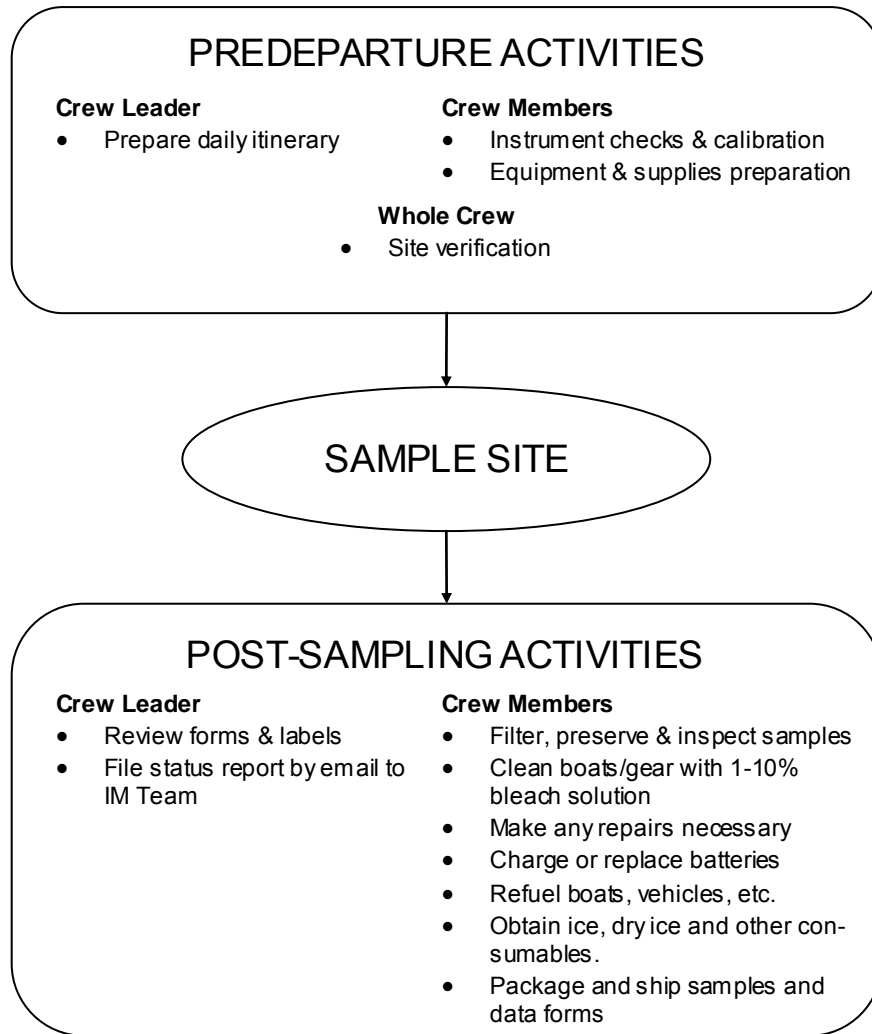


Figure 2.2 Overview of Base Site Activities

2.2.1 Pre-departure Activities

Pre-departure activities include the development of a 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. Changes in the itinerary during the week, such as canceling a sampling day, must be relayed by the crew leader to the Field Logistics Coordinator as soon as possible.

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. Once a week, crews should check their multi-probe against a Quality Check Solution (QCS).
- 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).

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, forms, labels, etc. Site kit requests must be submitted at least three weeks before sampling is to take place. **Field crew leaders MUST provide a tentative schedule in order to receive the site kits. Crews should include in this schedule the primary fish taxonomist at each site.** If your schedule changes, report the change as soon as possible to the Field Logistics Coordinator (Chris Turner, cturner@glec.com, (715)829-3737). The site kit will include data forms, labels, sample jars, bottles, 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 4 L distilled water.
QCS Solution*	Weekly check of meter calibration A 1:100 dilution of the standard solution (RIGHT) produces a solution with the following theoretical values: pH 6.98 Conductivity 75.3 μ S/cm @ 25oC	STANDARD SOLUTION: KH ₂ PO ₄ 3.4022 g Na ₂ HPO ₄ 3.5490 g Deionized water 1000 ml Mix to dissolve
10% Buffered Formalin†	Preservation of periphyton ID sample and fixing Fish Vouchers	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).

* QCS or “confidence” solutions can also be purchased pre-mixed from various sources.

† 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 is to initial all data forms after review. If obtainable samples are missing, the site should be rescheduled for complete sampling. 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, completeness, and legibility. Ensure that written comments use no “shorthand” or abbreviations. The data forms must be thoroughly reviewed. Upon completing the review, the field crew leader must initial the field forms to indicate that they are ready to be sent to the Information Management Center (a similar review process is used for electronic forms). 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 recorded on the data forms.

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 completely filled in. Check that each label is covered with clear plastic tape. 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, and trailer, and clean off any plant and animal material. Prior to leaving a site, drain all bilge water and live wells in the boat. Inspect, clean, and handpick plant and animal remains from vehicle, boat, motor, and trailer. Before moving to the next site, if a commercial car wash facility is available, wash vehicle, boat, and trailer and rinse thoroughly (hot water pressurized rinse no soap). Rinse equipment and boat with 1% - 10% bleach solution 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 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 three 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
<p>1. Clean for biological contaminants.</p> <p>Prior to departing site, drain all water from live wells and buckets used to hold and process fish, and drain all bilge water from the boat.</p> <p>Inspect motor, boat, trailer, 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</p>

Equipment Care after Sampling

amphibian chytrid fungus.

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.

If the stream is listed as “positive” for any of the organisms, or no information is available, *avoid using felt-soled wading boots*, 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:

Option A:

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:

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 min.
3. Dry gear in direct sunlight (at least 84 °F) for at least 4 hours.

2. Clean and dry other equipment prior to storage.

Rinse coolers with water to clean off any dirt or debris on the outside and inside.

Rinse periphyton sampling equipment with tap water at the base location.

Make sure conductivity meter probes are rinsed with deionized water and stored moist.

Rinse carboy and all beakers 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.

Check nets for holes and repair or locate replacements.

3. Inventory equipment and supply needs and relay orders to the Field Logistics Coordinator.

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, 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). **Life jackets must be worn by crew members at all times on the water.** All boats with motors must have fire extinguishers, boat horns, life jackets 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 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 this safety switch. 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 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

- 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 personal flotation device (PDF) 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]; U.S. EPA [1986]).

During the course of field research activities, field crews may observe violations of environmental regulations, may discover improperly disposed hazardous materials, or may 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.
- Do not attempt to collect samples from vertical or near vertical banks.
- 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 poisonous 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 and Lyme disease.
- 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 10oC or 50oF) 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 (Paper or Electronic)

Forms are the key to data collection and tracking for the NRSA 2013/14. Electronic forms have been developed as well as paper forms. These electronic forms should streamline data collection. Field crews will have the option of using paper or electronic forms.

2.4.1 Field Forms

Field forms are the primary documents where we 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.

- Paper Field Forms: A paper field form packet (wadeable or non-wadeable) for each site will be provided by the NARS IM Coordinator if you have elected to use paper field data collection. You will need to add these forms to the site packet prior to going in the field. After a site is sampled, the completed NRSA 2013/14 paper field forms are checked for completeness and organized sequentially into a Data Packet. The Data Packets from several sites are batched together and sent every 1-2 weeks to the NARS IM Center and accompanied by a Tracking form to track which data packets have been shipped. Extra paper field forms will be provided to field crews to serve as backup copies in case of lost forms or problems with electronic field forms.
- Electronic Field Forms: This form of data collection can be collected through 3 platforms: an iOS, Android or a Windows portable electronic device (tablets, phones). This will require a field crew to download or install the developed Application (or “App”) onto the device. The field forms will be optimized for tablet devices. Once downloaded and the App launched, the field forms will be split into sections or “form-lets” for easier data entry. It is important for a field crew to familiarize themselves with the App prior to field sampling.

2.4.2 Tracking Forms

Tracking forms describe the status and location of all samples collected during NRSA 2013/14. Field crew leaders will typically transmit these forms electronically (by emailing a fillable PDF form) to the NARS IM Center at specified times and you will pack hard copies in shipping containers with the samples. See APPENDIX C: SHIPPING GUIDELINES for more information.

- Site and Sample Status/Water Chemistry Lab Tracking: Transmitted within 24 hours of sampling or visiting a site to report on the status of the site (e.g. sampleable or not), to record the Sample ID numbers, and to indicate the status of all samples collected at the

site (immediate shipment and batch shipments). This also serves as the tracking form for sample shipped to the WRS lab.

- Tracking – Packets: Accompany packets that are batched together from multiple sites and shipped every 1 or 2 weeks. These are sent to the NARS IM Center.
- Tracking –Samples: Accompany samples that are batched together from multiple sites and shipped every 1 or 2 weeks. Whenever batched samples are shipped to their designated lab for analysis, the appropriate tracking form, which lists the Sample ID numbers for all samples packed in a shipping container, is included in the shipping package and is also transmitted electronically to the NARS IM Center. Separate forms exist for the tracking of frozen batched samples, non-chilled batched samples and whole fish samples

2.4.3 Equipment and Supplies

2.4.3.1 Request Form

Field Crews will submit requests for field forms, labels and site kits via an electronic Request Form. This form will be submitted to the NARS Information Management (IM) Coordinator who will ensure that the request reaches the appropriate entity. Crews must submit sampling schedules at or before the time of submitting request forms. Crews should submit the Request Form at least 2 weeks prior to their desired sampling date.

2.4.3.2 Base Kit

The Base Kit is comprised of the subset of durable equipment and supplies needed for NRSA 2013/14 sampling that is provided by USEPA 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 USEPA in the Base Kit. We anticipate that this equipment will be available for use in future NRSA efforts.

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 USEPA through the Contract Field Logistics Coordinator. The site kit will contain all the sample bottles necessary for sampling a single site. A new Site Kit is provided for each site sampled. See APPENDIX A: EQUIPMENT & SUPPLIES for the consumable items that will be provided by USEPA.

2.4.3.4 Field Crew Supplied Items

The field crew will also supply particular items for the field sampling day. These might include supplies from the previous NRSA, typical field equipment (like a GPS), or boat equipment. 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 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-12-008). 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.

3.1 Site Verification Activities

3.1.1 Locating the X-Site

River and stream sampling points were chosen using the National Hydrography Dataset (NHD), in particular NHD-Plus, 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 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 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. If the site falls on dry land near a stream, move the X site to the nearest blue line. Record the coordinates as the x-site on 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 and laminated Quick Reference Guide Site dossier, including access information, site spreadsheet with map coordinates, street and/or topographic maps with “X-site” marked NRSA 2013-14 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>Clipboard #2 pencils Verification Form 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 (**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.

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 75 m upstream and downstream of the X-site. 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. 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 (**Figure 3.1**). If the site is non-sampleable or inaccessible, no further sampling activities are conducted. Replace the site with the first oversample site on the state list within the same category based on Strahler order and whether it is a 2008/09 resample site (Section 1.3).

NRSA 2013/14 VERIFICATION (Front)					Reviewed by (initial): _____
Site ID: _____		Visit: <input type="radio"/> 1 <input type="radio"/> 2		Date: ____/____/____	
Site Name: _____			State of Site Location: _____		Field Crew: _____
STREAM/RIVER VERIFICATION INFORMATION					
Stream/River verified by (Mark all that apply): <input type="radio"/> GPS <input type="radio"/> Local Contact <input type="radio"/> Signs <input type="radio"/> Roads <input type="radio"/> Topo. Map					
<input type="radio"/> Other (Describe Here): _____					
Coordinates	Latitude	Longitude	Type of GPS Fix	Elevation at transect A	
GPS Decimal Degrees NAD 83	_____	- _____	<input type="radio"/> 2D <input type="radio"/> 3D	_____	
Location: <input type="radio"/> X-Site (wadeable) <input type="radio"/> Transect A (non-wadeable)				<input type="radio"/> ft <input type="radio"/> m	
DID YOU SAMPLE THIS SITE?					
<input type="radio"/> YES If Yes, check one below			<input type="radio"/> NO If No, check one below		
SAMPLEABLE (Choose method used) <input type="radio"/> Wadeable - Continuous water, greater than 50% wadeable <input type="radio"/> Boatable <input type="radio"/> Partial - Sampled by wading (>50% of reach sampled). Explain below. <input type="radio"/> Partial - Sampled by boat (>50% of reach sampled). Explain below. <input type="radio"/> Wadeable Interrupted - Not continuous water along reach <input type="radio"/> Boatable Interrupted - Not continuous water along reach <input type="radio"/> Altered - Stream/River Channel Present but differs from Map			NON-SAMPLEABLE-PERMANENT <input type="radio"/> Dry - Visited <input type="radio"/> Dry - Not visited <input type="radio"/> Wetland (No Definable Channel) <input type="radio"/> Map Error (No evidence channel/waterbody ever present) <input type="radio"/> Impounded (> 7 day residence time) <input type="radio"/> Tidal (exceeds salinity threshold) NON-SAMPLEABLE-TEMPORARY <input type="radio"/> Other (explain in comments) <input type="radio"/> Not boatable - Need a different crew - Reschedule for this year <input type="radio"/> Not wadeable - Need a different crew - Reschedule for this year NO ACCESS <input type="radio"/> Other (Explain in comments) <input type="radio"/> Access Permission Denied <input type="radio"/> Permanently Inaccessible (Unable/Unsafe to Reach Site) <input type="radio"/> Temporarily Inaccessible-Fire, etc. - Reschedule for next year		
ADDITIONAL SITE CHARACTERISTICS <input type="radio"/> Tidally Influenced <input type="radio"/> Blackwater <input type="radio"/> Not Applicable					
GENERAL COMMENTS					
DIRECTIONS TO SITE					
04/08/2013 2013 Verification				5409334985	

Figure 3.1 Verification Form (front)

Table 3.2 Procedure: Site Verification

1. **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.
2. **Use a GPS receiver to confirm the latitude and longitude at the X-site** with the coordinates provided for the site (datum = NAD 83). Record these on the Verification Form.
3. **Use all available means to insure 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.
4. **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.**
5. If the channel is dry at the X-site, determine if water is present within 75 m upstream and downstream of the X-site. Assign one of the following sampling status categories to the stream. Record the category on the Verification Form.

Sampleable Categories

Wadeable: Continuous water, sampled by wading.

Boatable: Continuous water, too deep to sample by wading.

Partial wadeable: Sampled by wading (>50% of reach sampled).

Partial boatable: Sampled by boat (>50% of reach sampled).

Wadeable Interrupted: not continuous water along reach, >50% water in reach.

Boatable Interrupted: not continuous water along reach, >50% water in reach.

Altered Channel: Stream/river channel present but differs from map.

Non-Sampleable Categories

PERMANENT

Dry Channel: 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".

Wetland: 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.

Map Error: No evidence that a water body or stream channel was ever present at the X-site.

Impounded stream: 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.

Other: 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).

TEMPORARY

Not Boatable: need a different crew.

Not Wadeable: need a different crew.

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).

6. Do not sample non-target or “Non-sampleable” or “No Access” sites. Fill in 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.

3.1.3 Elevation at Transect A

Record 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 Site Verification 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. 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 Sharefile 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 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 (back) as shown in **Figure 3.2**. **Figure 3.3** 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 figures also show the specific sampling stations on each transect for collection of periphyton, and benthic macroinvertebrate samples.

Before leaving the stream, complete a rough sketch map of the stream reach you sampled on page 2 of the Verification Form (**Figure 3.2**). 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.

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 5 places of "typical" width within approximately 5 channel widths upstream and downstream from the X-site. Average the 5 readings together and round to the nearest 1 m. 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 this width on page 2 of the Verification Form.
3. *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).*
4. 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 2.
5. 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 Section 3.2.1.
6. Starting at the X-site (or the new midpoint of the reach if it had to be adjusted as described in Step 4), measure a distance of 20 channel widths 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".
7. At Transect A, use the seconds display on a digital watch 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 3 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.

8. 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 3.3).
9. Proceed upstream with the tape measure and flag the positions of 9 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.

NRSA 2013/14 VERIFICATION (Back)				
Site ID: <input style="width: 80%;" type="text"/>	Visit: <input type="radio"/> 1 <input type="radio"/> 2	Date: <input style="width: 20%;" type="text"/> / <input style="width: 20%;" type="text"/> / <input style="width: 20%;" type="text"/>	Reviewed by (initial): <input style="width: 50%;" type="text"/>	
STREAM/RIVER REACH DETERMINATION				
Channel Width Used to Define Reach (m)	DISTANCE (m) FROM X-SITE		Total Reach Length Intended (m):	Comment:
	Upstream Length:	Downstream Length:		
<input style="width: 100%;" type="text"/>	<input style="width: 100%;" type="text"/>	<input style="width: 100%;" type="text"/>	<input style="width: 100%;" type="text"/>	<input style="width: 100%;" type="text"/>
SKETCH MAP				
Arrow Indicates North; Mark site L=Launch X=Index T= Take Out NOTE: If an outline map is attached here, use a continuous strip of clear tape across the top edge. You can also attach a separate sheet with the outline map on it. For boatable sites you can attach topo map with reach, X-site and transect locations marked.				
PERSONNEL				
Crew Leader: <input style="width: 90%;" type="text"/>	Name: <input style="width: 90%;" type="text"/>			
Fish Taxonomist: <input style="width: 90%;" type="text"/>	Name: <input style="width: 90%;" type="text"/>			
Name: <input style="width: 90%;" type="text"/>	Name: <input style="width: 90%;" type="text"/>			
Name: <input style="width: 90%;" type="text"/>	Name: <input style="width: 90%;" type="text"/>			
04/08/2013 2013 Verification			4426334983	

Figure 3.2 Verification Form (back)

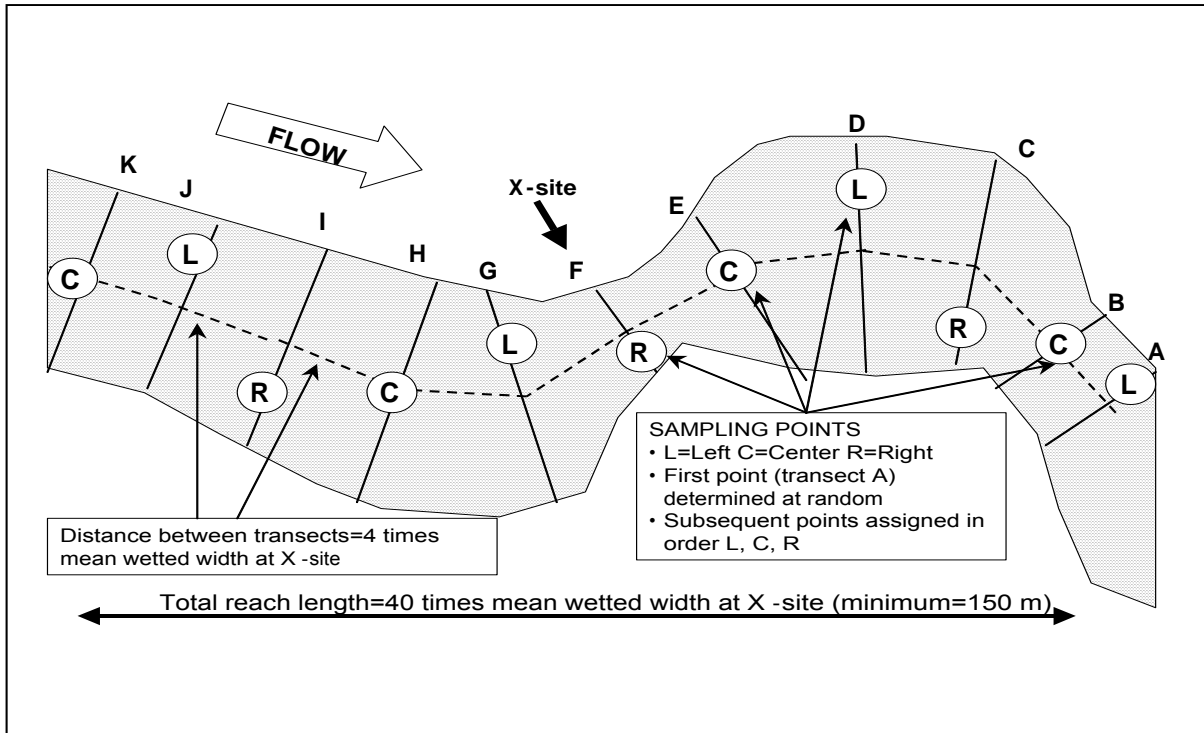


Figure 3.3 Sampling Reach Features for a Wadeable Site

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:

- Landowner access;
- 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.

Table 3.5 Procedure: Sliding the Sampling Reach

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 **impassable 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 0) 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 $>1 \text{ m}^2$ and a depth $>10 \text{ cm}$.

Collect data for the physical habitat indicator along the entire sample reach from interrupted streams, regardless of the amount of water present at 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 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 $> 1 \text{ m}^2$ and a depth $> 10 \text{ cm}$ 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. Record a "K" flag for the water chemistry sample on the sample collection form and explain why the sample was not collected in the comments section of the form.

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 $< 10 \text{ cm}$ deep; collect all physical habitat data. Do not collect periphyton or benthic macroinvertebrates from this transect.

WATER PRESENT: Transect has flow or pools $> 10 \text{ cm}$ 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 **Error! Reference source not found.**). For relatively small streams (mean bankfull width $\leq 15 \text{ m}$) the sampling reach is defined as 40 times the mean bankfull width. For larger streams ($> 15 \text{ m}$), sum the actual wetted width of all the braids and use that as the width for calculating the 40 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 and sketches on the Verification Form (**Figure 3.1** and **Figure 3.2**) about what you did. It is important to remember that the purpose of the 40 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 2 to 3 meander cycles (about 6 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 2 to 3 meander cycles (about 6 pool riffle habitat sequences).
2. Make detailed notes and sketches on the Verification Form about what you did.

4 WATER CHEMISTRY / CHLOROPHYLL-a SAMPLE COLLECTION AND PRESERVATION

4.1 In Situ Measurements of Dissolved Oxygen, pH, Temperature, and Conductivity

4.1.1 Summary of Method

Measure dissolved oxygen (DO), pH, 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, as seen in **Figure 4.1**.

4.1.2 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 Pencils (for data forms)

4.1.2.1 Multi-Probe Sonde

Dissolved Oxygen Meter

Calibrate the DO meter prior to each sampling event. We recommend that the probe be calibrated in the field against an atmospheric standard (ambient air saturated with water) prior to launching the boat. 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).

Temperature Meter

You must check the accuracy of the sensor against a thermometer that is traceable to the National Institute of Standards (NIST) at least once per sampling season. 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. The entire conductivity range encountered in the NRSA should be incorporated in the testing procedure and a record of test results kept on file.

4.1.3 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. Check the calibration against the QCS solution for pH and conductivity and record the results on the field sheet as the QCS Measured value. This should be done at least once a week.
3. Record the true value of the QCS solution from the stock solution container on the field sheet as QCS True.
4. Samples are taken mid-channel, at the X site, at a depth of 0.5 meters or at a mid-depth if less than 1 meter deep.
5. 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).
6. Record the measurements on the Field Measurement Form
7. Flag any measurements that the crew feels needs further comment or when a measurement cannot be made.

4.2 Water Chemistry Samples

4.2.1 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). All field crews must collect a grab sample in one 4-L cube container and in one 2-L amber Nalgene bottle from the X site at the midpoint of the reach. Store all samples on ice in a closed cooler.

4.2.2 Equipment and Supplies

Table 4.3 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, as seen in **Figure 4.2**.

Table 4.3 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 DI water (for cleaning beaker between sites) Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Sample Collection Form Water Chemistry sample label with pre-printed Sample ID Clear tape strips Field Measurement Form Pencils (for data forms) Fine tipped indelible markers

4.2.3 Water Chemistry and Chlorophyll-*a* Sampling Procedure

Table 4.4 presents step-by-step procedures for collecting water chemistry samples at wadeable sites.

Table 4.4 Procedure: Water Chemistry and Chlorophyll-*a* Sample Collection (Wadeable Sites)

Sampling Procedure	
Water Chemistry	
<ol style="list-style-type: none"> 1. Fill out the pertinent information (Site ID 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. 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. NOTE: DO NOT BLOW into the cube container or place your fingers inside the opening to expand it, this will cause contamination. 6. Fill the 3-liter 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 2 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. Fill the cube container to at least three-fourths of its maximum volume. Rinse the cube container lid with water. Eliminate any air space from the cube container, and cap it tightly. Make sure the cap is tightly sealed and not on at an angle. 	
Chlorophyll-<i>a</i>	
<ol style="list-style-type: none"> 8. Fill the 3-liter 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 2 more times. 9. 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	
<p>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.</p> <p>Record the Sample ID on the Sample Collection Form along with the pertinent stream information (stream name, ID, date, etc.). Note anything that could influence sample chemistry (heavy rain, potential contaminants) in the Comments section. If the sample was collected at the X-site, darken the X-site bubble in the "STATION COLLECTED" field. If you had to move to another part of the reach to collect the sample, place the letter of the nearest transect in the "STATION COLLECTED" field. Record more detailed reasons and/or information in the Comments section.</p>	

NRSA 2013/14 SAMPLE COLLECTION (Front)										Reviewed by (initial): _____	
Site ID: _____			Date: ____ / ____ / ____								
CHEMISTRY (CHEM) STATION COLLECTED:										No Sample Collected <input type="radio"/>	
(Target Volume = 4L) <input type="radio"/> X-Site (wadeable) <input type="radio"/> Transect A (non-wadeable) <input type="radio"/> Other Transect: _____											
Sample ID		Chilled		Comments							
_ _ _ _ _ _ _ _		<input type="radio"/>									
WATER COLUMN CHLOROPHYLL (CHLA)										No Sample Collected <input type="radio"/>	
(Target Volume = 1000mL; max vol = 2000 mL)											
Sample ID		Volume Filtered (ml)		Frozen		Comments					
_ _ _ _ _ _ _ _				<input type="radio"/>							
COMPOSITE PERIPHYTON										No Sample Collected <input type="radio"/>	
Composite Volume		No. of Transects		Comments							
_ _ _ _ _ _ _ _		_ _ _ _ _ _ _ _									
Assemblage ID (PERI) (50-mL tube)			Chlorophyll (PCHL) (GF/F Filter)				Biomass (PBIO) (GF/C Filter)				
Sample ID			Sample ID				Sample ID				
Volume (ml)	Preserved	Flag	Volume (ml)	Frozen	Flag	Volume (ml)	Frozen	Flag			
_ _ _ _ _ _ _ _	<input type="radio"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	<input type="radio"/>	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	<input type="radio"/>	_ _ _ _ _ _ _ _			
Flag	Comments										
_ _ _ _ _ _ _ _											
ALGAL TOXIN (Microcystin) (MICX)										No Sample Collected <input type="radio"/>	
(Target Volume = 500 mL)											
Sample ID		Frozen		Comments							
_ _ _ _ _ _ _ _		<input type="radio"/>									
ENTEROCOCCI (ENTE)										No Sample Collected <input type="radio"/>	
(Target Volume = 250 mL)										Blank Collected <input type="radio"/>	
Sample ID	Time Collected (hhmm)	Depth Collected (m)	Sample Volume (mL)	Filt. Start Time (hhmm)	Volume Filtered (Target = 50 mL)		Filt. End Time (hhmm)	Time Frozen (hhmm)			
_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _	Filt. 1 Filt. 2		_ _ _ _ _ _ _ _	_ _ _ _ _ _ _ _			
Comments											
Filter blank is collected during visit 1 at all revisit sites.											
04/08/2013 2013 Sample Collection							3054051198				

Figure 4.2 Sample Collection Form (front)

5 MICROCYSTINS

Microcystis is a microscopic organism that is found naturally at low concentrations in freshwater systems. Under optimal conditions (such as high light and calm weather, usually in summer), *Microcystis* occasionally forms a bloom, or dense aggregation of cells, that floats on the surface of the water forming a thick layer or “mat.” At higher concentrations, *Microcystis* blooms are so dense that they resemble bright green paint that has been spilled in the water. These blooms potentially affect water quality as well as human health (*Microcystis* produces microcystin, a potent liver toxin) and natural resources. Decomposition of large blooms can lower the concentration of dissolved oxygen in the water, resulting in hypoxia (low oxygen) or anoxia (no oxygen). Sometimes, this results in fish kills. The blooms can also be unsightly, often floating at the surface in a layer of decaying, odiferous, gelatinous scum.

Although the likelihood of people being affected by a *Microcystis* bloom is low, minor skin irritation can occur with contact, and gastrointestinal discomfort can also occur if water from a bloom is ingested. People recreationally exposed (e.g., swimmers or personal watercraft operators) to microcystins have also reported minor skin irritation. Health problems may occur in animals if they are chronically exposed to fresh water with *Microcystis* present. Just as livestock and domestic animals can be poisoned by drinking contaminated water, fish and bird mortalities have been reported in waterbodies with persistent *Microcystis* blooms.

5.1 Summary of Method

The microcystin (algal toxin) 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 a 500ml bottle. Collect this sample after the in situ measurements and water chemistry samples are collected. Store all samples on ice in a closed cooler.

5.2 Equipment and Supplies

Table 5.1 provides the equipment and supplies needed to collect microcystin sample at the index site. Record the Water Sample Collection and Preservation data on the Sample Collection Form, as seen in **Error! Reference source not found.**

Table 5.1 Equipment and Supplies: Microcystin

For collecting samples	Nitrile gloves 3-L Nalgene beaker 500 ml Nalgene bottle Cooler with ice Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Sample Collection Form Microcystin sample label with pre-printed Sample ID Clear tape strips Field Measurement Form Pencils (for data forms) and Fine tipped indelible markers for labels

5.3 Sampling Procedure

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

Table 5.2 Procedure: Microcystin Sample Collection (Wadeable Sites)

Microcystin Sample Collection

1. Fill out the pertinent information (Site ID and date) on the microcystin label and affix the label to the 500 mL Nalgene bottle. Completely cover the label with clear tape.
2. **Collect the microcystin samples from the X-site** in a flowing portion near the middle of the site.
3. **Put on nitrile gloves.** Make sure not to handle sunscreen or other chemical contaminants until after the sample is collected.
4. **Rinse the 3-L Nalgene beaker three times with water,** and discard the rinse downstream.
5. **Fill the 3 liter beaker with water and slowly pour 30 - 50 mL into the 500 ml Nalgene bottle.** Cap the bottle and rotate so that the water contacts all the surfaces. Discard the water downstream. Repeat this rinsing procedure 2 more times.
6. **Fill the beaker with water and pour into the 500 ml Nalgene bottle.** Leave one inch of head space in the bottle to allow for expansion when frozen.

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 cube container in an opaque garbage bag and immerse it in the stream.

Record the Sample ID on the Sample Collection Form along with the pertinent site information (site name, ID, date, etc.).

Upon returning to your base site (hotel, lab, office, etc.), freeze the entire sample and keep frozen until shipping.

6 BENTHIC MACROINVERTEBRATES

6.1 Summary of Method

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

6.2 Equipment and Supplies

Table 6.1 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 back of Sample Collection Form (**Figure 6.1**).

Table 6.1 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 4-ft handle Watch with timer or stopwatch Buckets, plastic, 8 to 10 qt (optional) Sieve bucket with 500 µm mesh openings (U.S. std No. 35) 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 or laminated Quick Reference Guide</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 Sample Collection Form</p>

NRSA 2013/14 SAMPLE COLLECTION (Back)																							
Site ID: _____												Date: ___ / ___ / ___											
BENTHIC MACROINVERTEBRATES (BERW) - WADEABLE																							No Sample Collected <input type="radio"/>
Sample ID	Number of jars					Preserved (ETOH)			No. of Transects			Comments											
						○																	
REACH-WIDE BENTHOS - WADEABLE																							
TRANSECT		A		B		C		D		E		F		G		H		I		J		K	
SUBSTRATE	CHAN.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.
Fine/Sand	Pool	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P	○ F	○ P
Gravel	Glide	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL	○ G	○ GL
Coarse	Riffle	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI	○ C	○ RI
Other:	Rapid	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA	○ OT	○ RA
If other, flag and explain in comments		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag	
BENTHIC MACROINVERTEBRATES (BETB) - BOATABLE																							No Sample Collected <input type="radio"/>
Sample ID	Number of jars					Preserved (ETOH)			No. of Transects			Comments											
						○																	
TRANSECT BENTHOS - BOATABLE																							
Habitat: C = Coarse Substrate / LWD L = Leaf Pack F = Organic Fine Muds / Sand M = Macrophyte beds OT = Other (Flag and explain in comment section below) Substrate: F = Fine / Sand G = Gravel C = Coarse substrate OT = Other (Flag and explain in comment section below) Channel: P = Pool GL = Glide RI = Riffle RA = Rapid OT = Other (Flag and explain in comment section below)																							
TRANSECT	A		B		C		D		E		F		G		H		I		J		K		
Location (L/R):	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	○ L	○ R	
Dominant Habitat: (ONE PER TRANSECT)	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	○ C	○ L	
Secondary Habitat: (ONE PER TRANSECT)	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	○ F	○ M	
Substrate: (ONE PER TRANSECT)	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	○ F	○ C	
Channel: (ONE PER TRANSECT)	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	○ P	○ RA	
		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag		Flag	
Flag	Comments																						
04/08/2013 2013 Sample Collection																				0629051193			

Figure 6.1 Sample Collection Form (back)

6.3 Sampling Procedure

Figure 6.2 summarizes how samples will be collected from wadeable sites. The transect sample design for collecting benthic macroinvertebrates is shown in **Figure 6.3**. 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 0). 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 6.2**. 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 Sample Collection Form as shown in **Figure 6.1**. As you proceed upstream from transect to transect, combine all samples into a bucket.

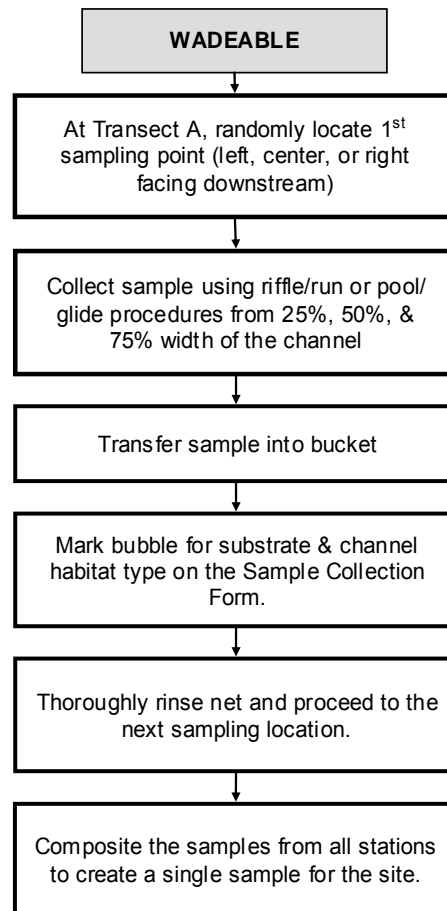


Figure 6.2 Benthic Macroinvertebrate Collection at Wadeable Sites

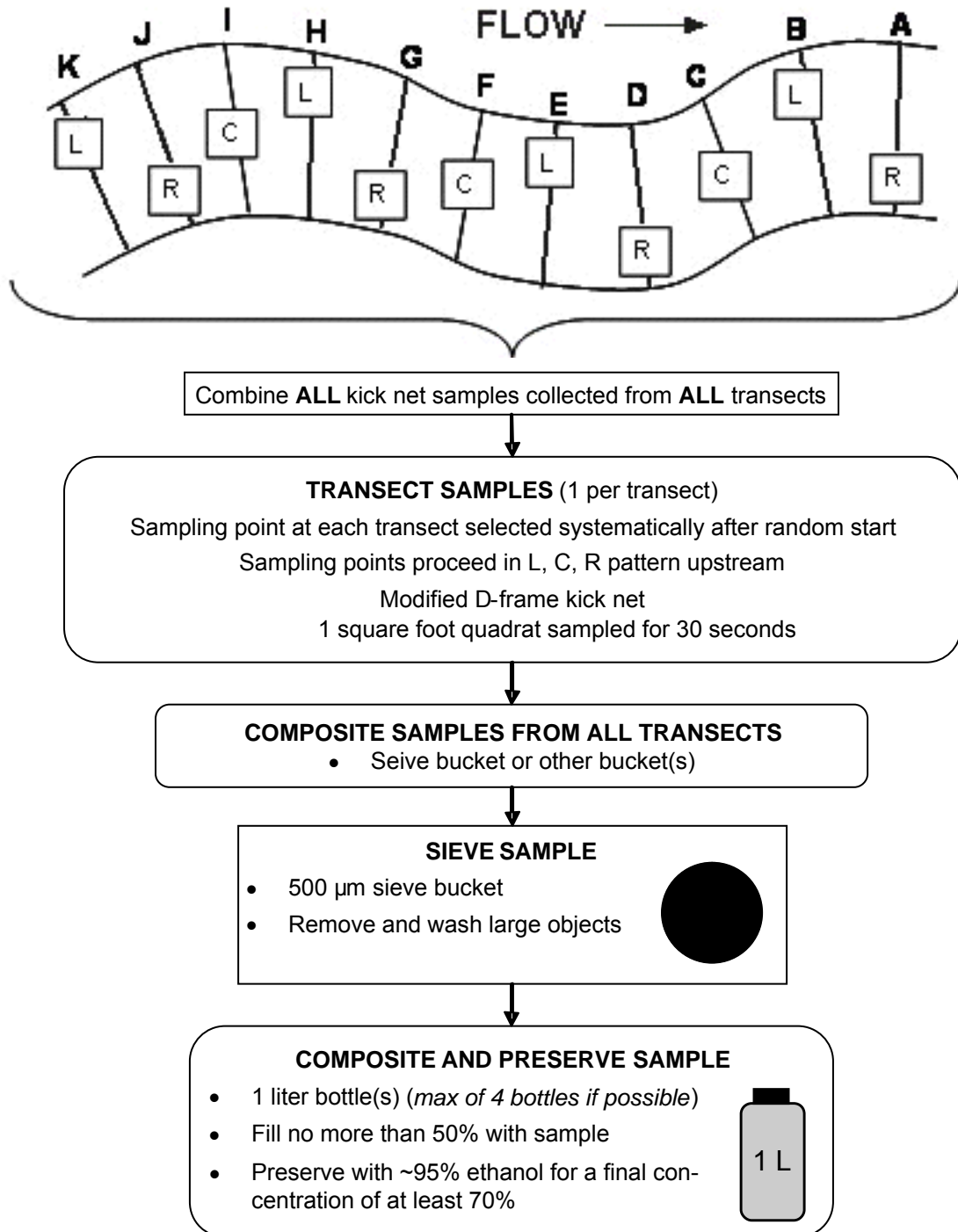


Figure 6.3 Transect Sample Design for Collecting Benthic Macroinvertebrates at Wadeable Sites

Table 6.2 Procedure: Benthic Macroinvertebrates (Wadeable Sites)

Collecting Benthic Macroinvertebrate Sample

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, relocate to another random point on the same transect.
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.

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. Assign a "U" flag (non-standard sample) to the sample and indicate which transect(s) required the modified collection procedure in the comments section. Go to Step 13.

Riffle/Run Habitats:

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.

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.

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 or a scrub brush 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).

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.

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:

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 or a scrub brush 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.
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 µm 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 appropriate substrate type for the transect on the Sample Collection Form. 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 comments on field form.

NOTE: If there are co-dominant substrate types, you may indicate more than one substrate type; note the co-dominants in the comments section of the form.

15. Identify the channel habitat type where the sampling quadrat was located. Indicate the appropriate channel habitat type for the transect on the Sample Collection Form. 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.
 - **Other (OT)**: Note type of “other” channel habitat in comments on field form.
16. 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. Combine all kick net samples from riffle/run and pool/glide habitats into the bucket.
17. Record the number of transects that were sampled throughout the reach.

6.4 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-liter 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 6.3). Try to use no more than 4 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 is also 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 Sample Collection Form as shown in **Figure 6.1**. Place the samples in a cooler or other secure container for transporting and/or shipping to the laboratory (see Appendix C). If the sample contains primarily organic material, or if adverse weather conditions exist (i.e. hot humid weather) process the sample at each station by placing it in a 1-L Nalgene jar with ethanol.

Table 6.3 Procedure: Compositing Samples for Benthic Macroinvertebrates (Wadeable Sites)

Compositing Benthic Macroinvertebrate Sample									
1.	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.								
2.	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.								
3.	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 4 jars per site.								
4.	Fill in a sample label with the Sample ID and date of collection. Attach the completed label to the jar and cover it with a strip of clear tape. Record the sample ID number for the composite sample on the Sample Collection Form. For each composite sample, make sure the number on the form matches the number on the label.								
5.	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 Sample 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> 								
6.	Place a waterproof label inside each jar with the following information written with a number 2 lead pencil:								
	<table border="0" style="width: 100%;"> <tr> <td style="width: 50%;">Site ID</td> <td>Collectors initials</td> </tr> <tr> <td>Type of sampler and mesh size used</td> <td>Number of stations sampled</td> </tr> <tr> <td>Name of site</td> <td></td> </tr> <tr> <td>Date of collection</td> <td>Jar “N” of “X”</td> </tr> </table>	Site ID	Collectors initials	Type of sampler and mesh size used	Number of stations sampled	Name of site		Date of collection	Jar “N” of “X”
Site ID	Collectors initials								
Type of sampler and mesh size used	Number of stations sampled								
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%.								
	<i>NOTE: Composite samples can be transported back to the vehicle before adding ethanol if necessary. In this</i>								

Compositing Benthic Macroinvertebrate Sample

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.

7 PERIPHYTON

7.1 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 6) directly after collecting the benthic macroinvertebrate sample. Prepare one composite “index” sample of periphyton for each site. At the completion of the day's sampling activities, but before leaving the site, **prepare three types of laboratory samples** (an ID/enumeration sample to determine taxonomic composition and relative abundances, a chlorophyll sample, and a biomass sample (for ash-free dry mass [AFDM]) from the composite periphyton sample.

7.2 Equipment and Supplies

Table 7.1 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 7.1 Equipment and Supplies: Periphyton (Wadeable Sites)

For collecting samples	Large Funnel (15-20 cm diameter) 12-cm ² area delimiter (3.8 cm diameter pipe, 3 cm tall) Stiff-bristle toothbrush with handle bent at 90° angle 1-L wash bottle for stream water 500-mL graduated plastic bottle for the composite sample 60-mL plastic syringe with tip removed, and length of tubing Field Operations Manual and a laminated Quick Reference Guide
For recording measurements	Sample Collection Form Soft (#2) lead pencils for recording data on field forms Fine-tipped indelible markers for filling out sample labels Sample labels (4 per set) with the same Sample ID Number Clear tape strips for covering labels

7.3 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, skip the transect. The procedure for collecting samples and preparing a composite sample is presented in **Table 7.2**. Collect one sample from each of the transects and composite in one bottle to produce one composite sample for each site. Record the volume of the composite sample on the Sample Collection Form as shown in **Figure 4.2**.

Table 7.2 Procedure: Collecting Composite Index Samples of Periphyton (Wadeable Sites)

Periphyton Composite Sample

1. Starting with Transect "A", collect a single sample from the assigned sampling station using the procedure below.
 - If coarse substrate (cobbles, woody materials, etc.) are present that can be removed from the water:**
 - (a) 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 a plastic funnel which drains into a 500-mL plastic bottle with volume graduations marked on it.
 - (b) 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.
 - (c) Fill a wash bottle with river 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.
 - (d) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The samples need to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)
 - If large coarse substrate is present that is too large to remove from the water (bedrock, large woody materials, boulders, etc.):**
 - (a) 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 tip of the syringe in a scraping motion.
 - (b) While dislodging periphyton with the syringe tip, simultaneously pull back on the syringe plunger to draw the dislodged periphyton into the syringe.
 - (c) 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 stream water to the bottle to raise the level to the next graduation.
 - (d) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The samples need to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)
 - If no coarse sediment (cobbles or larger) are present:**
 - (a) Use the area delimiter to confine a 12-cm² area of soft sediments.
 - (b) Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60-mL syringe.
 - (c) 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 stream water to the bottle to raise the level to the next graduation.
 - (d) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The samples need 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 site into the single 500-mL bottle to produce the composite index sample.

Storage

3. After samples have been collected from all 11 transects, 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.
4. **If all 11 samples are not collected, record the number of transects collected and reason for any missed collection on the field forms.**

7.4 Sample Processing in the Field

You will prepare three different types of laboratory samples from the composite index samples: an **ID/enumeration sample** (to determine taxonomic composition and relative abundances), a **chlorophyll sample**, and a **biomass sample** (for ash-free dry mass [AFDM]). All of the methods for processing the three samples are found in the Final Site Activities section 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.

8 PHYSICAL HABITAT CHARACTERIZATION

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

8.1 Equipment and Supplies

Table 8.1 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 river. 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 8.1 Equipment and Supplies: Physical Habitat

<p>For making measurements</p>	<p>Convex spherical canopy densiometer (Lemmon Model B), modified with taped "V" GPS 1 roll each colored surveyor's flagging tape (2 colors) 2 pair chest waders 1 or 2 fisherman's vest with lots of pockets and snap fittings. Digital camera with extra memory card & battery 50 m or 100 m measuring tape with reel Meter stick for bank angle measurements Laser rangefinder (400 ft. distance range) and clear waterproof bag Clinometer Binoculars Bearing compass (Backpacking type) Current velocity meter, probe, and operating manual Top-set wading rod for use with current velocity meter Surveyor's telescoping leveling rod Sounding rod or wading staff Level tripod CST Berger SAL 20 Automatic Level Field Operations Manual and/or laminated Quick Reference Guide</p>
<p>For recording data</p>	<p>2 covered clipboards (lightweight, with strap or lanyard) Soft (#2) lead pencils 11 plus extras Channel/Riparian Transect forms 11 plus extras Thalweg Profile forms 1+ extras field Form: Stream Verification Form 1+ extras field Form: Field Measurement Form 1+ extras field Form: Sample Collection Form 1+ extras field Form: Channel Constraint Form 1+ extras field Form: Visual Assessment Form</p>

8.2 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 baseflow wetted width**. 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, and discharge is measured at one location. 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.

8.3 Components of the Habitat Characterization

There are six components of the physical habitat characterization (**Table 8.2**). Measurements are recorded on 11 copies of a two-sided field form, and separate forms for recording slope and bearing measurements, assessing the degree of channel constraint, recording evidence of debris torrents or recent major flooding, and for stream discharge measurements.

Table 8.2 Summary of Components of Physical Habitat Characterization at Wadeable Sites

Component	Description
Thalweg Profile	<ul style="list-style-type: none"> Measure maximum depth, classify habitat and pool forming features, 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).
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).
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).
Channel and Riparian Characterization	<ul style="list-style-type: none"> At 11 transects placed at equal intervals along reach: Measure channel cross-section dimensions, bank height, bank undercut distance, bank angle, slope and compass bearing (back sight), and riparian canopy density (with densiometer). Visually Estimate^a: substrate size class and embeddedness; areal cover class and type (e.g., woody trees) of riparian vegetation in Canopy, Mid-Layer and Ground Cover; areal cover class of fish concealment features, aquatic macrophytes and filamentous algae. Observe & Record^a: Presence and proximity of human disturbances.
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 ratio of bankfull/valley width. Check evidence of recent major floods and debris torrent scour or deposition.
Discharge	<ul style="list-style-type: none"> Measure water depth and velocity at 0.6 depths at 15 to 20 equally spaced intervals across one carefully chosen channel cross-section.

- In very small streams, measure discharge by timing the passage of a neutrally buoyant object through a segment whose cross-sectional area has been estimated or by timing the filling of a bucket.

^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.

8.4 Work Flow for the Physical Habitat Components

The six components (**Table 8.2**) of the habitat characterization are organized into four grouped activities described below in the following sections.

8.4.1 Thalweg Profile and Large Woody Debris Tally

Thalweg spacing is calculated such that either 10 or 15 evenly spaced measurements are made between each transect (see section 8.5). 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 this crew reaches a flag marking a new cross-section transect, they start filling out a new copy of the Thalweg Profile and Woody Debris 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 on the thalweg profile side of the field form.

8.4.2 Channel/Riparian Cross-Sections

At each of the 11 transects, one person proceeds with the channel cross-section dimension and substrate notations as described above; and also makes measurements of bank angle (using rod and clinometer) and canopy cover (using densitometer). The second person records those measurements on the Channel/ Riparian Cross-section Form 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 the woody debris tally until

after reaching the upstream end of the reach. Backsighting and 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).

8.4.3 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.

8.4.4 Stream Discharge

Discharge measurements can be performed during or at the end of the field sampling, but need to be made after collecting the water chemistry sample so as not to disturb the water and compromise the sample. The measurements are taken at a chosen optimal cross-section (but not necessarily at a sampling transect) near the X-site. However, do not use the electromagnetic current meter close to where electrofishing is taking place. Furthermore, if a lot of channel disruption is necessary and sediment must be stirred up, wait on this activity until all chemical and biological sampling has been completed.

8.5 Habitat Sampling Locations within the Reach

Measurements are made at two scales along the length of the reach; the results are later aggregated for the entire reach. Figure 8.1 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 = 1/10th the total reach length). The thalweg profile measurements must 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.

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

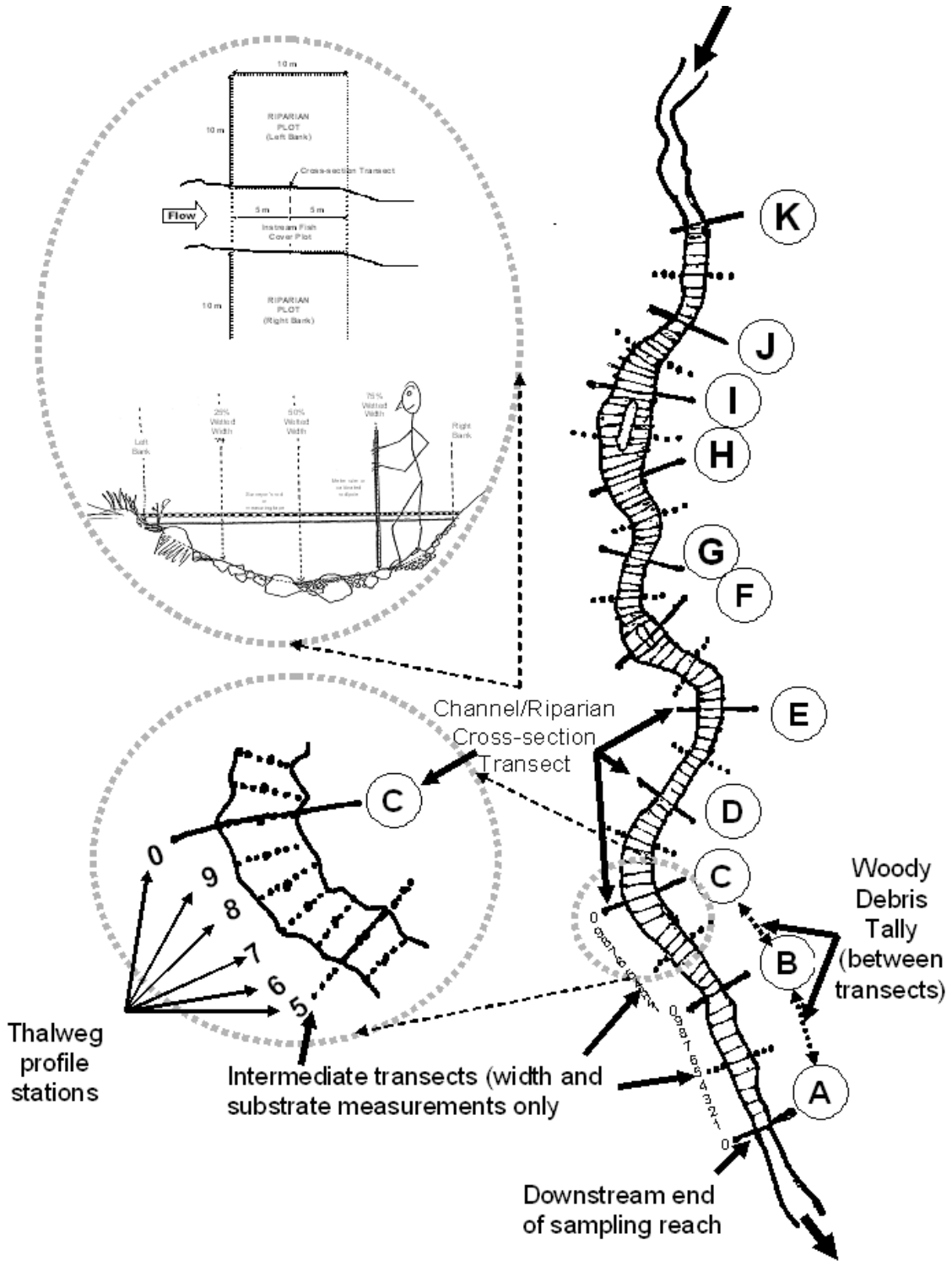
Channel Width 2.5 to 3.5 m:

- 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

- 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



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Figure 8.1 Reach Layout for Physical Habitat Measurements (plan view)

8.5.1 Thalweg Profile and Large Woody Debris Measurements

8.5.1.1 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.

The procedure for obtaining thalweg profile measurements is presented in **Table 8.3**. Record data on the Thalweg Profile and Woody Debris Data Form as shown in **Figure 8.2**. 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 (**Figure 8.2**) to record measurements 10-14.

Measure thalweg depths at all stations. Flag any missing measurements using a K code and explain the reason in the comments section of the field data form. At points where a direct depth measurement cannot be made, make your best estimate of the depth, record it on the field form, and flag the value using a U code (nonstandard measurement), explaining that it is an estimated value in the comments section of the field data form. 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 U code in the thalweg depth column (to indicate a nonstandard technique), and record the water level on the rod or line, and the rod/line angle, as determined using the external scale on the clinometer (vertical = 90) in the comment field (i.e. "depth of xx cm taken at an angle of xx degrees"). If a direct measurement of the thalweg depth is not possible, make the best estimate you can of the depth, record it, and use a U flag and a comment to note it is an estimated value.

Table 8.3 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. Complete the header information on the Thalweg Profile and Woody Debris Form, noting the transect pair (downstream to upstream). Record the increment distance determined in Step 1 in the *INCREMENT* field on the field data form used for subreach A-B. Also record the total reach length on this form. It is not necessary to repeat these calculations on the remainder of the Thalweg Profile and Woody Debris forms.
3. Begin at the downstream end (*station 0*) of the first transect (transect A).
4. Measure the wetted width at station 0, and 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 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 on the thalweg profile side of the field form.
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 thalweg profile 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. Record a U flag in the thalweg depth column to indicate a non-standard procedure was used. Record the water level on the rod and the rod angle in the comments section of the field data form. 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 U flag 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 (≤ 16 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 in the *SOFT/SMALL SEDIMENT* field on

the field data form. *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 field data form using the standard 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 filling in the bubble in the *BAR WIDTH* field on the field data form. However, a measurement of the bar width is only taken if the bar intersects a transect at either the endpoint or midpoint of a subreach.
10. Record the presence or absence of a side channel at the station's cross-section in the *SIDE CHANNEL* field on the field data form.
11. Record the presence or absence of quiescent off channel aquatic habitats, including sloughs, alcoves and backwater pools in the *BACKWATER* column of the field form.
12. Proceed upstream to the next station, and repeat Steps 2 through 11.
13. Repeat Steps 2 through 12 until you reach the next transect. At this point complete Channel/ Riparian measurements at the new transect (Section 8.4.2). Then prepare a new Thalweg Profile and Woody Debris Form and repeat Steps 2 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 copies of the Thalweg Profile and Woody Debris Form, one for each segment (*A to B*, *B to C*, etc.).

NRSA 2013/14 PHAB: THALWEG PROFILE & WOODY DEBRIS - WADEABLE ONLY Reviewed by (initial): _____

Site ID: _____ Date: _____ / _____ / _____

TRANSECT: OA-B OB-C OC-D OD-E OE-F
OF-G OH-H OI-J OJ-K

Total Reach Length (m): _____

For Transect A-B ONLY: Increment (m): X.X

THALWEG PROFILE		THALWEG PROFILE COMMENTS					
STA-TION	THALWEG DEPTH (cm) (XXX)	WETTED WIDTH (m) (XXX.X)	BAR WIDTH 1 Present	SOFT /SMALL SEDIMENT	CHANNEL UNIT CODE	SIDE CHANNEL	BACK WATER
	XX.X	XX.X	XX.X	XX.X	XX.X	XX.X	XX.X
0							
1							
2							
3							
4							
*5							
6							
*7							
8							
9							
10							
11							
12							
13							
14							

CHANNEL UNIT CODES: PO = Pool GL = Glide RI = Riffle RA = Rapid CA = Cascade FA = Falls DR = Dry

SUBSTRATE	Station (5 or 7)				LARGE WOODY DEBRIS (10 cm small and diameter; 1.5 m length)	FLAG
	LFT	LCR	CTR	RGT		
RS = BEDROCK (SMOOTH) - (LARGER THAN A CAR) RR = BEDROCK (ROUGH) - (LARGER THAN A CAR) RC = CONCRETE ASPHALT RB = LG BOULDER (1000 TO 4000 mm) - (HETEROSICK TO GAR) RS = LG BOULDER (400 TO 1000 mm) - (HETEROSICK TO GAR) CS = COBBLE (64 TO 250 mm) - (TENNIS BALL TO BASKETBALL) GC = COARSE GRAVEL (16 TO 64 mm) - (MARBLE TO TENNIS BALL) S = SAND (0.06 TO 2 mm) - (GRITTY - UP TO LADYBUG SIZE) GF = FINE GRAVEL (2 TO 16 mm) - (LADYBUG TO MARBLE) FN = SILT / CLAY / MUCK - (NOT GRITTY) FP = HARDPAN / PERL CONSOLIDATED FINE SUBSTRATE) MP = MUD / PUTTY / MUCK - (NOT GRITTY) OT = OTHER (FLAG AND EXPLAIN IN COMMENTS)					DIAMETER LARGE END 0.1-<0.3 m 0.3-0.6 m 0.6-0.8 m >0.8 m	PIECES ALL PART IN BANKFULL CHANNEL Length 1.5-5m >15m 5-15m >15m
FLAG COMMENTS (for SUBSTRATE and LWD)						

1 = Measure Bar Width at Station 0 and Mid-Station (5 or 7).

Flag Codes: K = no measurement made; U = suspect measurement; F1, F2, etc. = flags assigned by each field crew. Explain all flags in comments.
04/08/2013 2013 Phab Thalweg Profile - Wadeable

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Figure 8.2 Thalweg Profile and Woody Debris Form

At every thalweg station, 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 (≤ 16 mm diameter). These soft/small sediments are **different** from *Fines* described when determining the substrate particle sizes at the cross-section transects (Section 8.7). 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 using procedures described for substrate at regular cross-sections (**Figure 8.1**), 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 8.4**) 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 8.4**. 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 *PB* 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), fill in the *Y* bubble to indicate presence of a backwater in the *BACKWATER* column of the field 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). When trying to identify the pool forming element for a particular pool, remember that most pools are formed at high flows, so you may need to look for elements that are dry at baseflow, but still within the bankfull channel (e.g., boulders or large woody debris).

Table 8.4 Channel Unit Categories

Channel Unit Habitat Classes ¹	
Class (Code)	Description
Pools: Still water, low velocity, a smooth, glassy surface, usually deep compared to other parts of the channel	
Plunge Pool (PP)	Pool at base of plunging cascade or falls.
Trench Pool (PT)	Pool like trench in the center of the stream
Lateral Scour Pool (PL)	Pool scoured along a bank.
Backwater Pool (PB)	Pool separated from main flow off the side of the channel (large enough to offer refuge to small fishes). Includes sloughs (backwater with marsh characteristics such as vegetation), and alcoves (a deeper area off a wide and shallow main channel)
Impoundment Pool (PD)	Pool formed by impoundment above dam or constriction.
Pool (P)	Pool (unspecified type).
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>).

8.5.1.2 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.). The procedure for tallying LWD is presented in Table 8.5. 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 8.3**. LWD in zones 1 and 2 will be tallied together (considered all or in-part within 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.

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).

Table 8.5 Procedure: Tallying Large Woody Debris

Large Woody Debris Tally Form	
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.	
1.	Scan the stream segment between the two cross-section transects where thalweg profile measurements are being made.
2.	Tally all LWD pieces within the segment that are at least partially within the bankfull channel. Determine if a piece is LWD (<i>small end diameter ≥ 10 cm [4 in.], and length ≥ 1.5 m [5 ft.]</i>)
3.	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> (1.5 m to < 5.0 m, 5 m to < 15 m, or > 15 m). 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)
4.	Place a tally mark in the appropriate diameter \times length class tally box in the <i>PIECES ALL/PART IN BANKFULL CHANNEL</i> section of the Thalweg Profile and Woody Debris Form.
5.	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).
6.	Place a tally mark for each piece in the appropriate diameter \times length class tally box in the <i>PIECES BRIDGE ABOVE BANKFULL CHANNEL</i> section of the Thalweg Profile and Woody Debris Form.
7.	After all pieces within the segment have been tallied, write the total number of pieces for each diameter \times length class in the small box at the lower right-hand corner of each tally box.
8.	Repeat Steps 1 through 7 for the next subreach, using a new Thalweg Profile and Woody Debris Form.

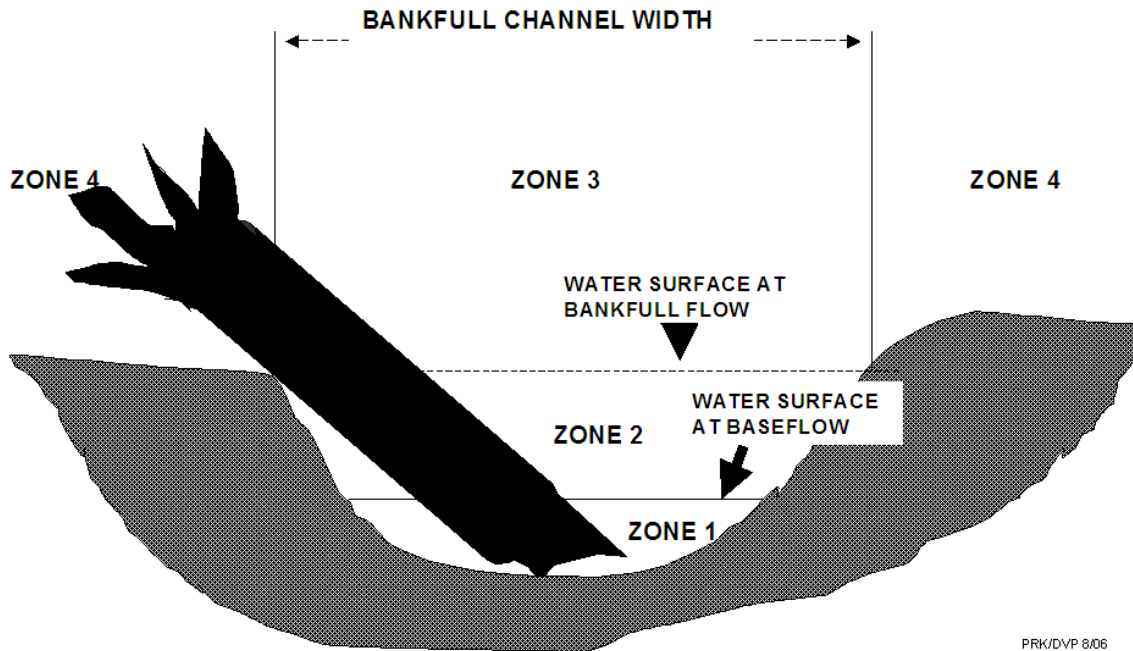


Figure 8.3 Large Woody Debris Influence Zones (modified from Robison and Beschta, 1990).

8.6 Channel and Riparian Measurements at Cross-Section Transects

8.6.1 Slope and Bearing

Measure slope and bearing by *sighting* 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 8.6**. Measure bearing following the procedure presented in **Table 8.8**. Record slope and bearing data on the Slope and Bearing Form as shown in **Figure 8.4**.

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. Elevation readings are made at each transect and the difference between each elevation reading is 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 transect 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. Note in the comments section of the Slope and Bearing Form 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 insure 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 8.5**. 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 (**Figure 8.4**). 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 8.5**). You may need to use supplemental points in your measurement of slope if visibility is severely limited, but supplemental points are not required 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 in the MAIN column.

Table 8.6 Procedure: Obtaining Slope Data

Slope Method with Surveyors Level
<p><u>Instrument Setup:</u></p> <ol style="list-style-type: none">1. 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.2. 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.3. Adjust the leveling foot screws until the bubble is exactly level in the center circle.4. 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.5. Adjust focus, brightness and parallax according to manufactures specifications. The instrument is ready to make measurements. <p><u>Taking Measurements:</u></p> <ol style="list-style-type: none">1. Determine a location at transect K to hold a surveyor’s rod that will be visible from a point between transect J and transect K:<ol style="list-style-type: none">a. Set up the instrument at a point approximately halfway between transects J and K and where a clear line of sight is possible. If visibility allows, the instrument may be placed farther upstream, as long as transect K is still visible.b. Position the staff at transect K, holding the bottom of the staff at the water level and the staff as vertical as possible and the numbers facing the instrument.c. Site the staff through the instrument and record the reading to the nearest centimeter in a field

notebook or other workspace (this value is **not** entered on the field form).

- d. Move the staff to transect J 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.
- e. Site the staff and record the reading to the nearest centimeter (again in a field notebook or elsewhere).
- f. Subtract the elevation reading at transect J from the reading at transect K.
- g. The difference in the readings is the elevation difference that is recorded on the Slope and Bearing Form.
- h. Repeat measurements between each transect.

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.

- If you are back sighting to a supplemental point, you may record the elevation difference in the appropriate *SUPPLEMENTAL* section of the Slope and Bearing Form.

2. Proceed to the next cross-section transect (or supplementary point), and repeat Steps a - h above.

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

Site ID: _____ NRSA 2013/14 PHAB: SLOPE AND BEARING - WADEABLE ONLY Date: ____/____/____ Reviewed by (initials): _____		FIRST SUPPLEMENTAL			SECOND SUPPLEMENTAL			FLAG
MAIN (always used)		BEARING 0 - 359	PROPOR- TION %	Slope(%) or Elev. Diff. (cm)	BEARING 0 - 359	PROPOR- TION %	Slope(%) or Elev. Diff. (cm)	PROPOR- TION %
TRANSECT & METHOD	Slope(%) or Elev. Diff. (cm)							
<small>Mark method for every Transsect. Mark Units for every Transsect.</small>								
A < B	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
B < C	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
C < D	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
D < E	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
E < F	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
F < G	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
G < H	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
H < I	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
I < J	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
J < K	<input type="radio"/> CL <input type="radio"/> TR <input type="radio"/> HL <input type="radio"/> WT <input type="radio"/> LA <input type="radio"/> Other	<input type="radio"/> % <input type="radio"/> cm						
Flag	Comments							

Figure 8.4 Slope and Bearing Form

Flag codes: U = Suspect sample; F1, F2, M = Method - used for method comment only) = flag assigned by field crew. Explain all flags in comment sections.
 CL=Chinometer; HL=Hand Level; LA=Laser rangefinder with electronic clinometer; TR=Transit; surveyors level or total station; WT=Water Tubing.
 04/08/2013, 2013 Slope and Bearing

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Because of ease of use, portability, and cost, hand-held clinometers were previously used to determine slope. In this instance, 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. The Slope and Bearing Form (Figure 8.4) is designed to allow for different methods and/or different units of measuring slope. Mark the appropriate method bubble (instead of *CL*; method codes are identified in **Table 8.6**) and mark the % *BUBBLE* (instead of the *CM* bubble) if the method or instrument measures the percent slope rather than the change in elevation (**Table 8.7**).

Table 8.7 Modified Procedure: Obtaining Slope Data (without Surveyor's Level)

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

Use this procedure 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 cross-section transect downstream.. If not, you may have to take supplementary slope measurements. 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.
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.

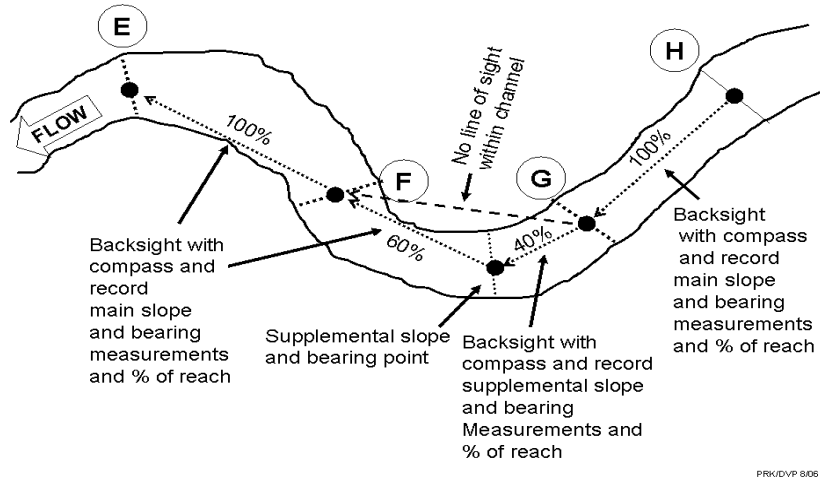
If you have determined in Step 1 that supplemental measurements are required for this segment, walk downstream to the furthest point where you can stand in the center of the channel and still see the center of the channel at the upstream cross-section transect.. Mark this location with a different color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

4. Place the base of the calibrated rod at the level 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 are sighting to the next downstream transect, read and record the percent slope in the *MAIN* section 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%.

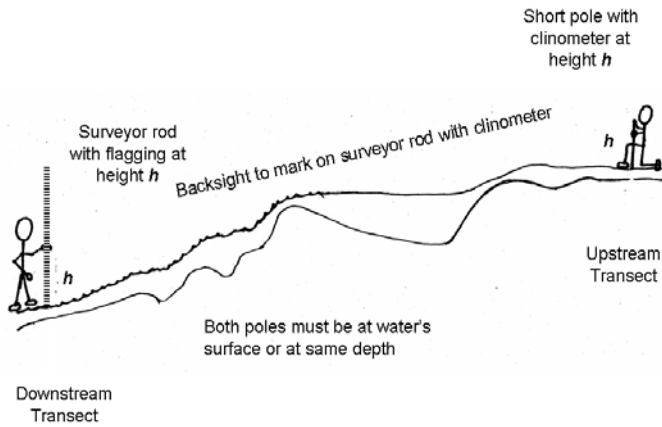
If you are backsighting from a supplemental point, record the slope (%) and proportion (%) of the stream segment that is included in the measurement in the appropriate *SUPPLEMENTAL* section of the Slope and Bearing Form. The last sighting to a downstream transect (from either the upstream transect or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

6. Stand in the middle of the channel at upstream transect (or at a supplemental point), and sight with your compass to the middle of the channel at the downstream transect (or at a 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 6.
7. Proceed to the next cross-section transect (or to a supplementary point), and repeat Steps 3 through 7 above.



PRR/DVP 806

Figure 8.5 Measurements of Bearing Between Transects



PRR/DVP 606

Figure 8.6 Channel Slope Measurement using a Clinometer

Table 8.8 Procedure: Obtaining Bearing Data

Obtaining Bearing Data

Use this procedure to backsight from one transect to another with the use of a sighting compass.

Readings (in degrees) are taken from the center one of transect to the center of the next transect downstream.

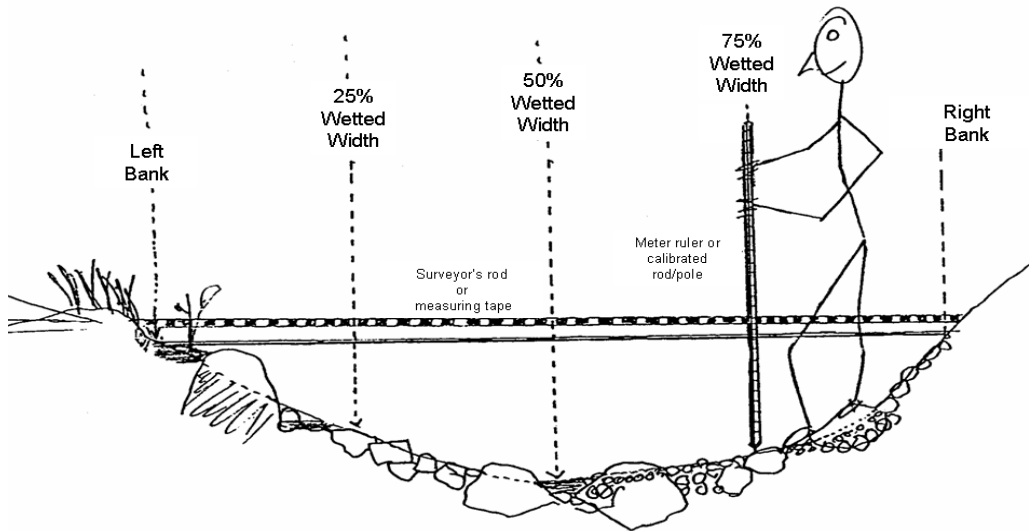
It does not matter whether you use true or magnetic bearings as long as you are consistent for each reading.

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 cross-section transect. Determine if you can see the next cross-section transect downstream without sighting across land (i.e., do not “short-circuit” a meander bend). If not, you will have to take supplementary bearing measurements
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 sight 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 backsighting from a supplemental point, record the bearing and proportion (%) of the stream segment that is included in the measurement in the appropriate *SUPPLEMENTAL* section of the Slope and Bearing Form. The last sighting to a downstream transect (from either the upstream transect or the nearest upstream supplemental point) is always recorded as the *MAIN* reading.
7. Proceed to the next transect (or supplemental point), and repeat steps 1 through 6 above.

8.7 Substrate Size and Channel Dimensions

Substrate size and embeddedness are evaluated at 5 points at each of the 11 transects (refer to **Figure 8.7**). Substrate size is also evaluated at 10 additional cross-sections located midway between each of the 11 regular transects (A-K). In the process of measuring substrate particle sizes at each channel cross-section, the wetted width of the channel and the water depth at each substrate sample point are measured (at the 10 midway cross-sections, only substrate size and wetted width are recorded). If the wetted channel is split by a mid-channel bar (Section 8.4.1), 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 8.7 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 8.9 (including all particle size classifications). Record these measurements on the Channel/Riparian Cross-section side of the field form, as shown in **Figure 8.8**.

For the supplemental cross-sections midway between regular transects, record substrate size and wetted width data on the thalweg profile side of the field form. 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 field data form (**Figure 8.8**) 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*, assign an *Fn* flag on the field data form and describe the substrate type in the comments section of the field form, as shown in **Figure 8.8**.

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 of all particles in the 10 cm diameter circle around the substrate sampling point. Embeddedness is the fraction of a particle's volume that is surrounded by (embedded in) sand or finer sediments on the stream bottom. By definition, record the embeddedness of sand and fines (silt, clay, and muck) as *100 percent*, and record the embeddedness of hardpan and bedrock as *0 percent*.

Table 8.9 Procedure: Substrate Measurement

1. Fill in the header information on page 1 of a Channel/Riparian Cross-section Form. Indicate the cross-section transect. 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).
NOTE: If a side channel is present, and contains 16 - 49% of the total flow, establish a secondary cross-section transect. Use a separate field data form to record data for the side channel, designating it as a secondary transect by marking both the EXTRA SIDE CHANNEL bubble and the associated primary transect letter (e.g., A, B, etc.). Collect all channel and riparian cross-section measurements from the side channel.
2. Divide the wetted channel width channel by 4 to locate substrate measurement points on the cross-section. In the *DISTLB* fields of the form, record the distances corresponding to 0% (*LEFT*), 25% (*LCTR*), 50% (*CTR*), 75% (*RCTR*), and 100% (*RIGHT*) of the measured wetted width. Record these distances at Transects A-K, but just the wetted width at midway cross-sections.
3. Place your sharp ended meter stick or calibrated pole at the *LEFT* location (0 m). Measure the depth and record it on the field data form. (Cross-section depths are measured only at regular transects A-K, not at the 10 midway cross-sections).
 - 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.
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 field data form. (Cross-section side of form for transects A-K; special entry boxes on Thalweg Profile side of form for midway cross-sections.)

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
HP	Hardpan	>4000	Firm, consolidated fine substrate
LB	Boulders (large)	>1000 to 4000	Yard/meter stick to car size
SB	Boulders (small)	>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 size; gritty between fingers
FN	Fines	≤0.06	Silt Clay Muck (not gritty between fingers)
WD	Wood	Regardless of Size	Wood & other organic particles
RC	Concrete	Regardless of size	Record size class in comment field
OT	Other	Regardless of Size	Metal, tires, car bodies etc. (describe in comments)

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. Estimate the average % embeddedness of particles in the 10 cm circle around the measuring rod. Record this value on the field data 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.” By definition, sand and fines are embedded 100%; bedrock and hardpan are embedded 0%.

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.

8.7.1 Bank Characteristics

The procedure for obtaining bank and channel dimension measurements is presented in **Figure 8.10**. Data are recorded in the *BANK MEASUREMENTS* section of the Channel/Riparian Cross-section Form as shown in **Figure 8.8**. Bank angle and bank undercut distances are determined on the left and right banks at each cross-section transect. **Figure 8.9** illustrates how bank angle is determined for several different situations. The scale at which bank angle is characterized is approximately 0.5 m. A short (approx. 1 m long) pole is used to determine bank angle. The angle is determined based on the pole resting on the ground for about 0.5 m. Other features include the wetted width of the channel (as determined in Section 8.4.1), 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 8.10. *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 1 to 2 years) to not allow substantial growth of upland terrestrial vegetation. Consequently, in many regions, it is these flows that have determined the width and depth of the channel.

NRSA 2013/14 PHAB: CHANNEL/RIPARIAN CROSS-SECTION - WADEABLE ONLY Reviewed by (initial): _____

Site ID: _____ Date: _____ / _____ / _____ TRANSECT: **OA OB OC OD OE OF** **OG OH OI OJ OK OL** Extra Side Channel

SUBSTRATE CROSS-SECTIONAL INFORMATION		FISH COVER/OTHER		VISUAL RIPARIAN ESTIMATES									
Dist LB XX.XX m	Depth XXX cm	Size Class Code	Embed. (%) 0-100%	Flag	Cover in Channel								
Left													
LClr													
Clr													
RClr													
Right													
SUBSTRATE SIZE CLASS CODES RS = Bedrock (Smooth) - (Larger than a cat) RL = Bedrock (Rough) - (Larger than a cat) XE = Concrete/Asphalt (to 4000 mm) - (Meterstick to cat) XB = Single Boulder (250 to 1000 mm) - (Ball to Meterstick) XS = Single Boulder (250 to 1000 mm) - (Ball to Meterstick) SB = Single Boulder (250 to 1000 mm) - (Ball to Meterstick) CB = Cobble (64 to 250 mm) - (Tennis ball to Basketball) CG = Coarse Gravel (16 to 64 mm) - (Marble to Tennis ball) GF = Fine Gravel (2 to 16 mm) - (Ladybug to marble) SA = Sand (0.06 to 2 mm) - (Gritty - up to Ladybug size) SI = Silt / Clay / Muck - (Not Gritty) HP = Hardpan - (Firm, Consolidated Fine Substrate) WD = Wood - (Any Size) OT = Other (Write comment below)													
		Embed. (%)		Flag									
		0											
		0											
RIPARIAN VEGETATION COVER Canopy (>5 m high) Left Bank Right Bank Flag:													
Woody Vegetation Type													
BIG Trees (Trunk >0.3 m DBH)													
SMALL Trees (Trunk <0.3 m DBH)													
Understory (0.3 to 5 m high)													
Woody Vegetation Type													
Woody Shrubs & Saplings													
Non-Woody Herbs, Grasses, & Forbs													
Ground Cover (<0.5 m high)													
Woody Shrubs & Saplings													
Non-Woody Herbs, Grasses, & Forbs													
Barren, Bare Dirt or Duff													
HUMAN INFLUENCE 0 = Not Present P = >10 m C = Within 10 m plot B = On Bank													
Wall/Dike/Revetment/Rap/Dam													
Buildings													
Pavement/Cleared Lot													
Road/Railroad													
Pipes (Inlet/Outlet)													
Landfill/Trash													
Park/Lawn													
Row Crops													
Pasture/Range/Hay Field													
Logging Operations													
Mining Activity													
CANOPY COVER MEASUREMENTS DENSITOMETER (0-17/Max) Flag <table border="1" style="margin-left: auto; margin-right: auto;"> <tr> <td>CentUp</td> <td>CentR</td> <td>CentL</td> <td>CentDwn</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> </tr> </table>						CentUp	CentR	CentL	CentDwn				
CentUp	CentR	CentL	CentDwn										
BANK MEASUREMENTS Bank Angle 0 - 360 Undercut Dist. (m) Flag													
Left													
Right													
Wetted Width XXX.X m													
Bar Width XX.X m													
Bankfull Width XXX.X m													
Bankfull Height XX.X m													
Incised Height XX.X m													
Flag Comments													

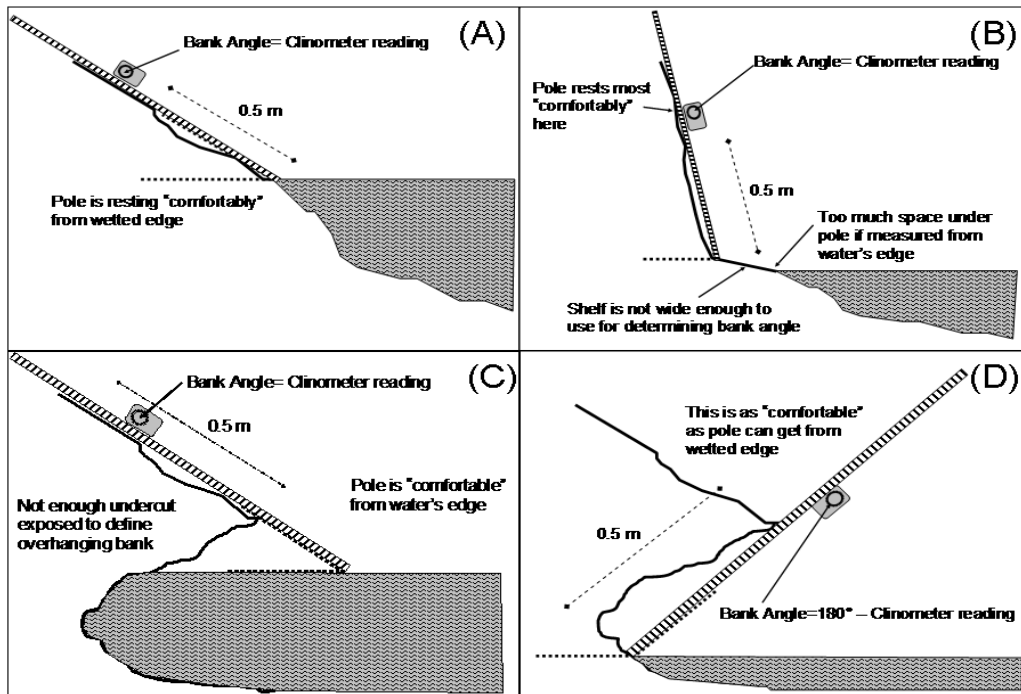
04/08/2013 2013 Phab Channel Riparian - Wadeable 998 9625187

Flag codes: K = Sample not collected; U = Suspect sample; F1, F2, etc. = flag assigned by field crew. Explain all flags in comment sections.

Figure 8.8 Channel Riparian Cross-Section Form

Table 8.10 Procedure: Measuring Bank Characteristics

1. To measure *bank angle*, 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 *resting comfortably* on the ground to determine bank angle. 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 *BANK MEASUREMENT* section of the Channel/Riparian Cross-section Form.
 - A *vertical bank* is 90° , *overhanging banks* have angles $>90^\circ$ approaching 180° , and more gradually sloped banks have angles $<90^\circ$. To measure bank angles $>90^\circ$, 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 water's edge 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.
3. Repeat Steps 1 and 2 on the right bank.
4. 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 (back sighting) it onto the surveyor's rod. Record this value in the *INCISED HEIGHT* field of the bank measurement section on the field data form.
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. Record the *wetted width* value determined when locating substrate sampling points in the *WETTED WIDTH* field in the bank measurement section of the field data form. Also determine the *bankfull channel width* and the *width of exposed mid-channel bars* (if present). Record these values in the *BANK MEASUREMENT* section of the field data form.
7. Repeat Steps 1 through 6 at each cross-section transect, (including any additional side channel transects established when islands are present). Record data for each transect on a separate field data form.



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Figure 8.9 Determining Bank Angle Under Different Types of Bank Conditions (A) typical, (B) incised channel, (C) undercut bank, and (D) overhanging bank.

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, flag your data entry and also record the height of drift material in the comments section of the field data form.

We use the vertical distance (height) from the observed water surface up to the level of the first major valley depositional surface (**Figure 8.10**) 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 8.11**). *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.

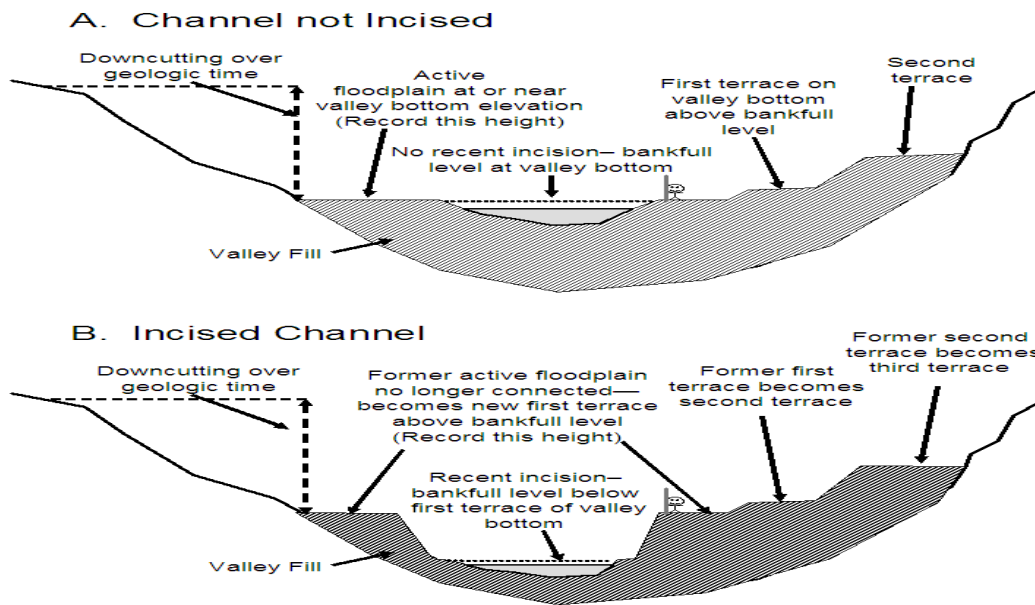
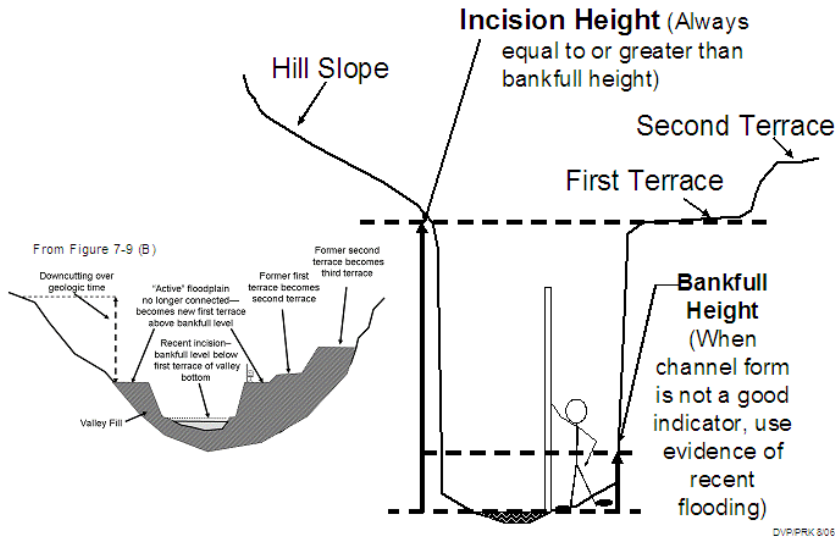


Figure 8.10 Schematic Showing Relationship Between Bankfull Channel and Incision

(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

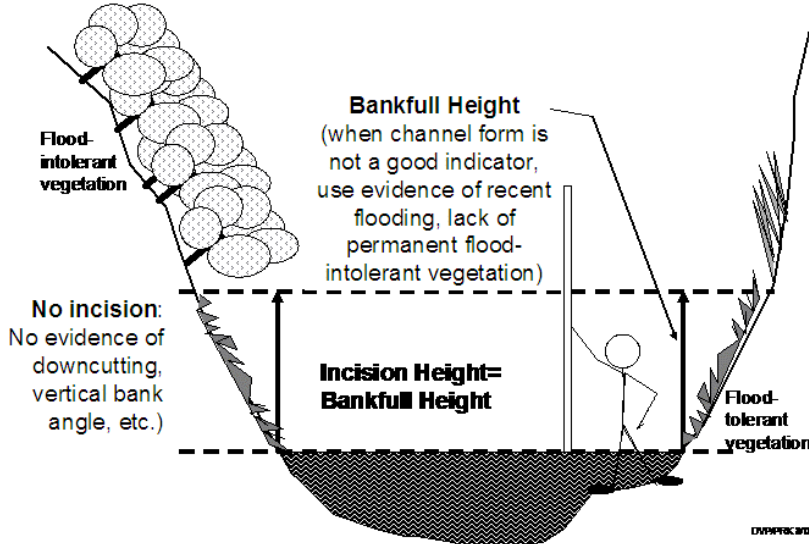


Figure 8.11 Determining Bankfull and Incision Heights for (A) Deeply Incised Channels, and (B) Streams in Deep V Shaped Valleys (Stick figure included for scale).

8.8 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). Mark the densiometer with a permanent marker or tape exactly as shown in **Figure 8.12** 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).

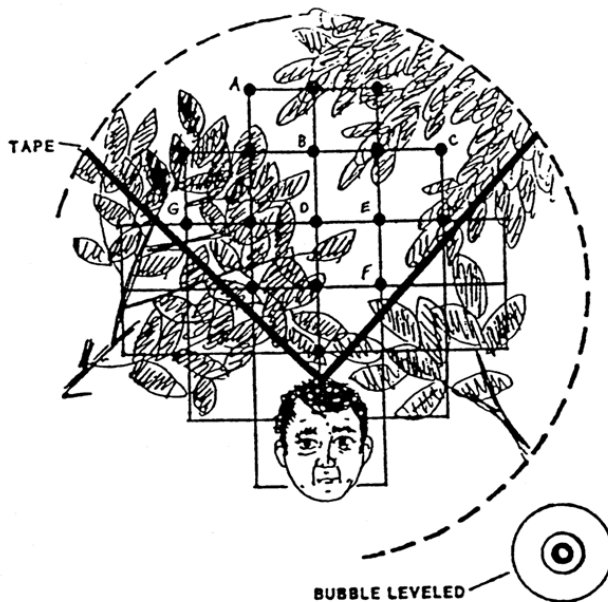


Figure 8.12 Schematic of Modified Convex Spherical Canopy Densiometer.

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

The procedure for obtaining canopy cover data is presented in **Table 8.11**. 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 8.12. 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 covering them in the *CANOPY COVER MEASUREMENT* section of the Channel/Riparian Cross-section Form as shown in **Figure 8.8**.

Table 8.11 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>CENUP</i> field of the canopy cover measurement

Canopy Cover Measurements with Densiometer

section of the Channel/Riparian Cross-section and Thalweg Profile Form.

4. Face toward the left bank (left as you face downstream). Repeat Steps 2 and 3, recording the value in the *CENL* field of the field data 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 *CENDWN* and *CENR* fields of the field data 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 *LEFT* or *RIGHT* fields of the field data 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). Record data for each transect on a separate field data form.

8.9 Visual Riparian Estimates

8.9.1 Riparian Vegetation Structure

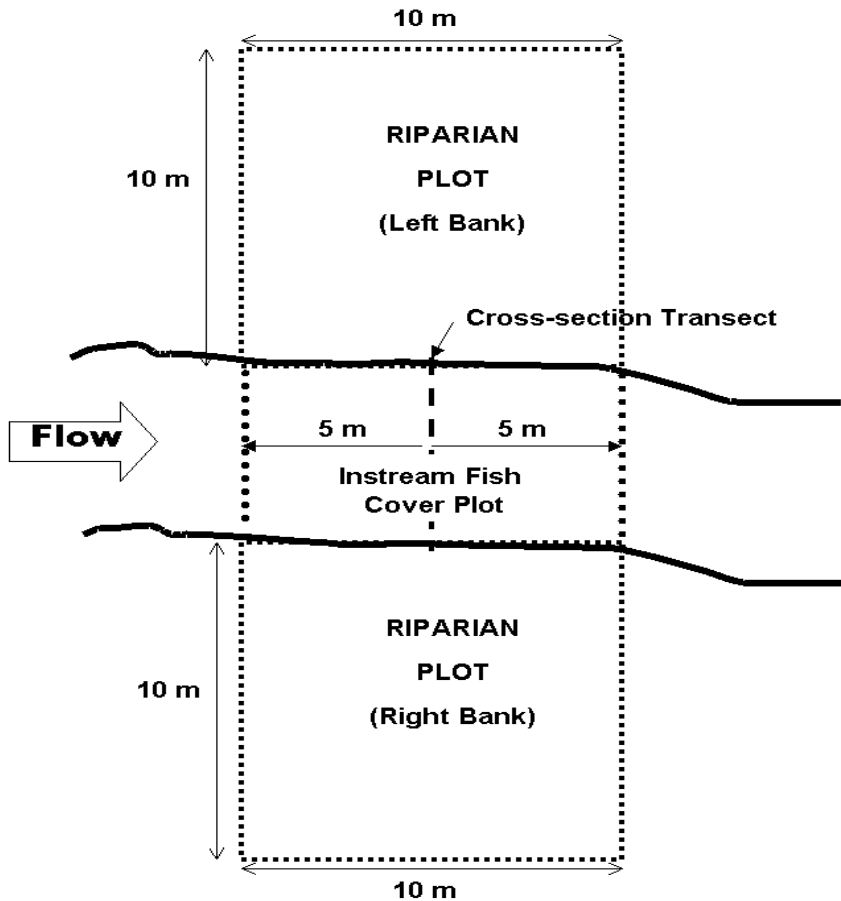
The previous section described methods for quantifying the cover of canopy over the stream channel. The following visual estimation procedures supplement those measurements with a semi-quantitative evaluation of the type and amount of various types of riparian vegetation.

Riparian vegetation observations apply to the riparian area upstream 5 meters and downstream 5 meters from each of the 11 cross-section transects. They include the visible area from the stream back a distance of 10m (~30 ft.) shoreward from both the left and right banks, creating a 10 m × 10 m riparian plot on each side of the stream (Figure 8.13). The riparian plot dimensions are estimated, not measured. On steeply sloping channel margins, the 10 m × 10 m plot boundaries are defined as if they were projected down from an aerial view. Table 8.12 presents the procedure for characterizing riparian vegetation structure and composition. Figure 8.8 illustrates how measurement data are recorded on the Channel/Riparian Cross-section 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 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*, *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 field data form (Figure 6.2-7). 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.



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Figure 8.13 Riparian Zone and Instream Fish Cover Plots for a Stream Cross-Section Transect

Table 8.12 Procedure: Characterizing Riparian Vegetation Structure

Riparian Vegetative Structure Measurements	
1.	Standing in mid-channel at a cross-section transect, 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 × 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 × 10 m area, determine the dominant vegetation type for the <i>CANOPY LAYER</i> (vegetation >5 m high) as; Deciduous, <i>Coniferous</i> , <i>broadleaf Evergreen</i> , <i>Mixed</i> , or <i>None</i> . Consider the layer <i>Mixed</i> if more than 10% of the areal coverage is made up of the alternate vegetation type. Indicate the appropriate vegetation type in the <i>VISUAL RIPARIAN ESTIMATES</i> section of the

Channel/Riparian Cross-section 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 field data form (*0=absent*: zero cover, *1=sparse*: $< 10\%$, *2=moderate*: 10-40%, *3=heavy*: 40-75%, or *4=very heavy*: $> 75\%$).
6. Look at the *UNDERSTORY* 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 there is no woody vegetation in the understory layer, record the type as *None*.
7. Determine the areal cover class for woody shrubs and saplings separately from non-woody vegetation within the understory, as described in Step 5 for the canopy layer.
8. Look at the *GROUND COVER* layer (vegetation < 0.5 m high). Determine the areal cover class for woody shrubs and seedlings, non-woody vegetation, and the amount of bare ground present as described in Step 5 for large canopy trees.
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). Uses a separate field data form at each transect.

8.10 Instream Fish Cover, Algae, and Aquatic Macrophytes

Over a defined area upstream and downstream of the sampling transects (**Figure 8.13**), crews shall estimate by eye and 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 8.13**. Data are recorded on the Channel/Riparian Cross-section Form as shown in **Figure 8.8**. 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 8.13**). The areal cover classes of fish concealment and other features are the same as those described for riparian vegetation (Section 8.9.1).

The entry *FILAMENTOUS ALGAE* refers to long streaming algae that often occur in slow moving waters. *AQUATIC 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 influence cover and stream morphology (i.e., those pieces that would be included in the large woody debris tally [Section 8.5.1]). *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. *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.

Table 8.13 Procedure: Estimating Instream Fish Cover

Instream Fish Cover Measurements	
1.	Standing mid-channel at a cross-section transect, estimate a 5m distance upstream and downstream (10 m total length).
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, aquatic 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/OTHER</i> section of the Channel/Riparian Cross-section Form: <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). Record data from each transect on a separate field data form.

8.11 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 8.14**. Relate your observations and proximity evaluations to the stream and riparian area within 5 m upstream and 5 m downstream from the station (**Figure 8.14**). Four proximity classes are used: In the stream or on the bank within 5 m upstream or downstream of the cross-section transect, present within the 10 m × 10 m riparian plot but not in the stream or on the bank, present outside of the riparian plot, and absent. Record data on the Channel/Riparian Cross-section Form (**Figure 8.8**). If a disturbance is within more than one proximity class, record the one that is closest to the stream (e.g., C takes precedence over 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 Transect 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 a previously counted transect or its 10x10 meter riparian plot.**

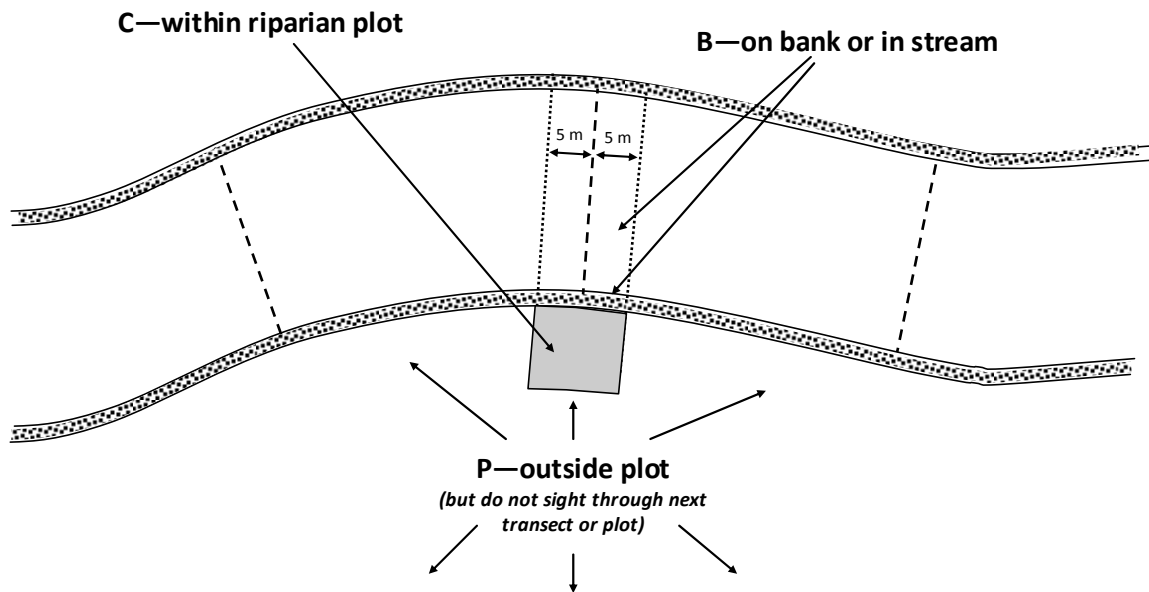


Figure 8.14 Proximity Classes for Human Influences in Wadeable Streams

Table 8.14 Procedure: Estimating Human Influence

Estimate Human Influence	
1.	Standing mid-channel at a cross-section transect, look toward the left bank (left when facing downstream), and estimate a 5 m distance upstream and downstream (10 m total length). 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, gravelled, 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).
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 Channel/Riparian Cross-section Form. Proximity classes are: <ul style="list-style-type: none"> <i>B (Bank)</i> Present within the defined 10 m stream segment and located in the stream or on the stream bank. <i>C (Close)</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). Record data for each transect on a separate field form.

8.12 Cross-section Transects on Side Channels

If the wetted channel is split by an island, and the estimated flow in the side channel is less than or equal to 15% of the total flow, the bank and riparian measurements are made at each side of the main channel (the minor side channel is ignored other than to note its presence on the Thalweg Profile Form), so one riparian plot is established on the island as shown in **Figure 8.15** (side A). If an island is present that creates a major side channel containing **more than 15%** of the total flow, an additional cross-section transect is established for the side channel as shown in **Figure 8.15** (side B). Separate substrate, bank and riparian measurements are made for side channel transects. Data from the additional side channel transect are recorded on a separate Channel/Riparian Cross-section Form as shown in **Figure 8.16**. 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.

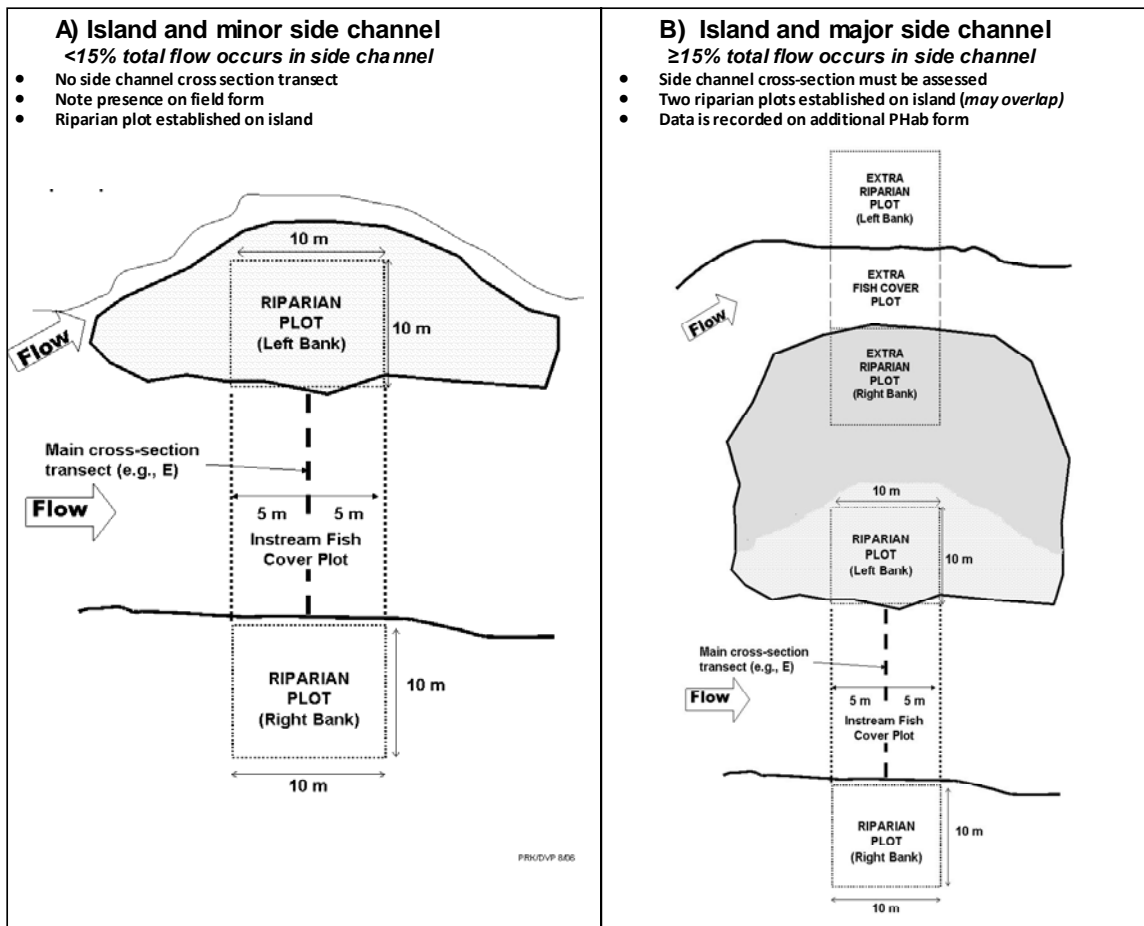


Figure 8.15 Riparian and Instream Fish Cover Plots for a Stream with Minor and Major Side Channels

Reviewed by (initials):

NRSA 2013/14 PHAB: CHANNEL/RIPARIAN CROSS-SECTION - WADEABLE ONLY

Site ID: MS9-0928 Date: 06/06/2013 TRANSECT: 0A 0B 0C 0D 0E 0F 0G 0H 0I 0J 0K 0L Extra Side Channel

SUBSTRATE CROSS-SECTIONAL INFORMATION		FISH COVER/OTHER			
Dist LB XX.XX m	Depth XXX cm	Size Class Code	Embed. (%) 0-100%	Flag	Flag
Left	0.00	0	FN	100	
Lctr	0.50	2.0	SA	100	
Ctr	1.00	2.5	SA	100	
Rctr	1.50	1.5	FN	100	
Right	2.00	0	FN	100	

SUBSTRATE SIZE CLASS CODES
 RS = Bedrock (Smooth) - (Larger than a car)
 RB = Bedrock (Rough) - (Larger than a car)
 RC = Cobble (1000 to 4000 mm) - (Meterstick to car)
 RB = Large Boulder (1000 to 1000 mm) - (Basketball to meterstick)
 SB = Small Boulder (250 to 1000 mm) - (Basketball to meterstick)
 CB = Cobble (64 to 250 mm) - (Tennis ball to Basketball)
 GC = Coarse Gravel (16 to 64 mm) - (Tennis ball to Basketball)
 GF = Fine Gravel (2 to 16 mm) - (Ladybug to marble)
 SA = Sand (0.06 to 2 mm) - (Gritty - up to Ladybug size)
 FS = Silt / Clay / Muck - (Not Gritty)
 HP = Hardpan - (Firm, Consolidated Fine Substrate)
 WD = Wood - (Any Size)
 OT = Other - (Write comment below)

BANK MEASUREMENTS		CANOPY COVER MEASUREMENT	
Bank Angle 0 - 360	Undercut Dist. (m)	Flag	Flag
Left	80	0	
Right	45	0	
Wetted Width	XXX.X m	2.0	
Bar Width	XX.X m	0.0	
Bankfull Width	XXX.X m	3.5	
Bankfull Height	XX.X m	0.3	
Incised Height	XX.X m	0.9	

DENSITOMETER (0-17Max)		Flag	
CentUp	CentL	CentR	Flag
12	16	6	
13	16	16	
		6	

Visual Riparian Estimates
 0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%)
 4 = Very Heavy (>75%)
 D = Deciduous C = Coniferous E = Broadleaf Evergreen M = Mixed N = None

RIPARIAN VEGETATION COVER		Flag	
Canopy (>5 m high)	Left Bank	Right Bank	Flag
Woody Vegetation Type			
BIG Trees (Trunk >0.3 m DBH)			
SMALL Trees (Trunk <0.3 m DBH)			
Undercut (>0.3 m high)			
Woody Shrubs & Saplings			
Non-Woody Herbs, Grasses, & Forbs			
Ground Cover (<0.5 m high)			
Woody Shrubs & Saplings			
Non-Woody Herbs, Grasses and Forbs			
Barren, Bare Dirt or Duff			

HUMAN INFLUENCE 0 = Not Present P = >10 m C = Within 10 m plot B = On Bank

Wall/Dike/Retement /Riprap/Dam	Buildings	Pavement/Cleared Lot	Road/Railroad	Pipes (Inlet/Outlet)	Landfill/Trash	Park/Lawn	Row Crops	Pasture/Range/Hay Field	Logging Operations	Mining Activity

Comments
Pasture

Flag codes: K = Sample not collected; U = Suspect sample; F1, F2, etc. = flag assigned by field crew. Explain all flags in comment sections.

03/05/2013 2013 Phab Channel Riparian - Wadeable 9989625187

Figure 8.16 Channel/Riparian Cross-Section Form for an Additional Major Side Channel Transect.

8.13 Channel Constraint, Debris Torrents, Recent Floods, and Discharge

8.13.1 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 8.15**. Record data on the Channel Constraint Assessment Form (**Figure 8.17**). First, classify the stream reach channel pattern as predominantly a *single channel*, an *anastomosing channel*, or a *braided channel* (**Figure 8.18**):

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 8.15 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>CHANNEL CONSTRAINT: 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.• <i>Braided channels also have multiple branching and rejoining channels</i>, separated by unvegetated bars. Subchannels are generally small, short, and numerous, often with no obvious

Channel Constraint

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 Channel/Riparian Cross-Section Forms.

NOTE: If the valley is wider than you can directly estimate, record the distance you can see and mark the bubble on the field form.

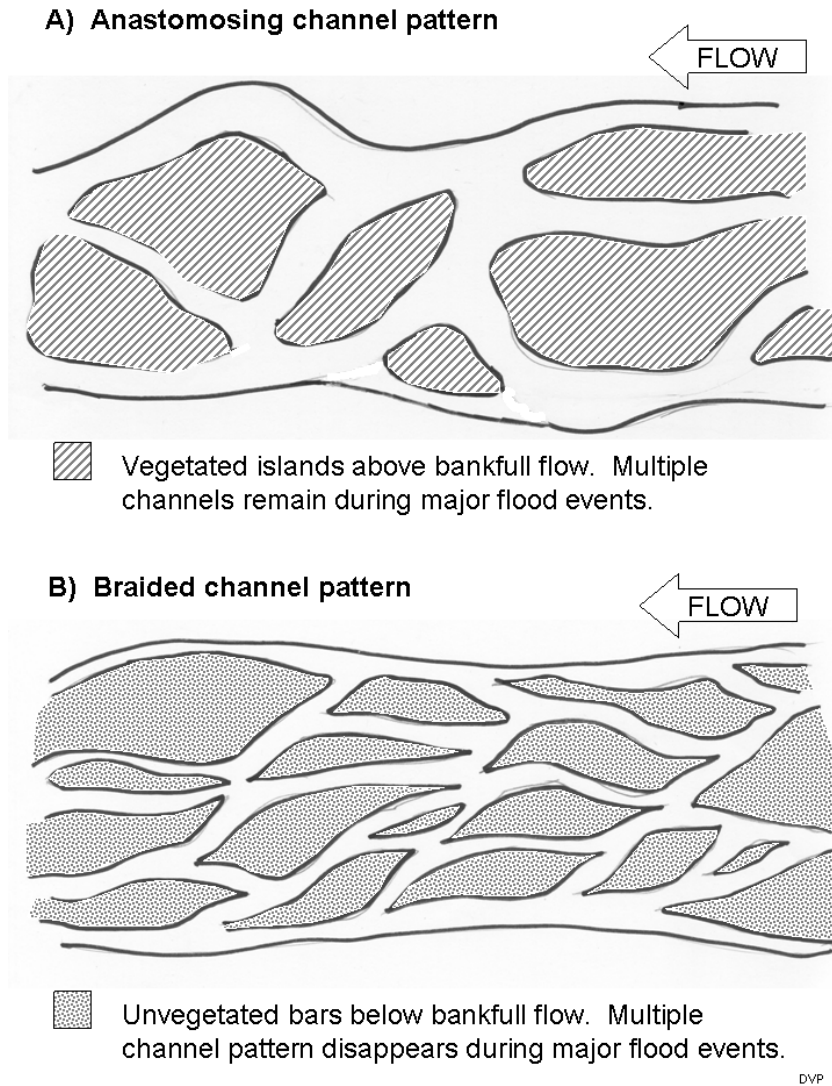


Figure 8.18 Types of Multiple Channel Patterns

8.13.2 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 Assessment Form (**Figure 8.19**). 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.

8.14 Stream Discharge

No single method for measuring discharge is applicable to all types of stream channels. The preferred procedure for obtaining discharge data is based on “velocity-area” methods (e.g., Rantz and others, 1982; Linsley et al., 1982). For streams that are too small or too shallow to use the equipment required for the velocity-area procedure, two alternative procedures are presented. One procedure is based on timing the filling of a volume of water in a calibrated bucket. The second procedure is based on timing the movement of a neutrally buoyant object (e.g., an orange or a small rubber ball) through a measured length of the channel, after measuring one or more cross-sectional depth profiles within that length.

8.14.1 Velocity-Area Procedure

Because velocity and depth typically vary greatly across a stream, accuracy in field measurements is achieved by measuring the mean velocity and cross-sectional area of many increments across a channel (**Figure 8.20**). Each increment gives a subtotal of the stream discharge, and the whole is calculated as the sum of these parts. Discharge measurements are made **at only one carefully chosen channel cross-section within the sampling reach**. It is important to choose a channel cross-section that is as much like a canal as possible. A glide area with a “U” shaped channel cross-section that is free of obstructions provides the best conditions for measuring discharge by the velocity-area method. You may remove rocks and other obstructions to improve the cross-section before any measurements are made. However, because removing obstacles from one part of a cross-section affects adjacent water velocities, you must not change the cross-section once you commence collecting the set of velocity and depth measurements.

The procedure for obtaining depth and velocity measurements is outlined in

Table 8.16. Record the data from each measurement on the Stream Discharge Form as shown in **Figure 8.21**. In the field, data will be recorded using only one of the available procedures.

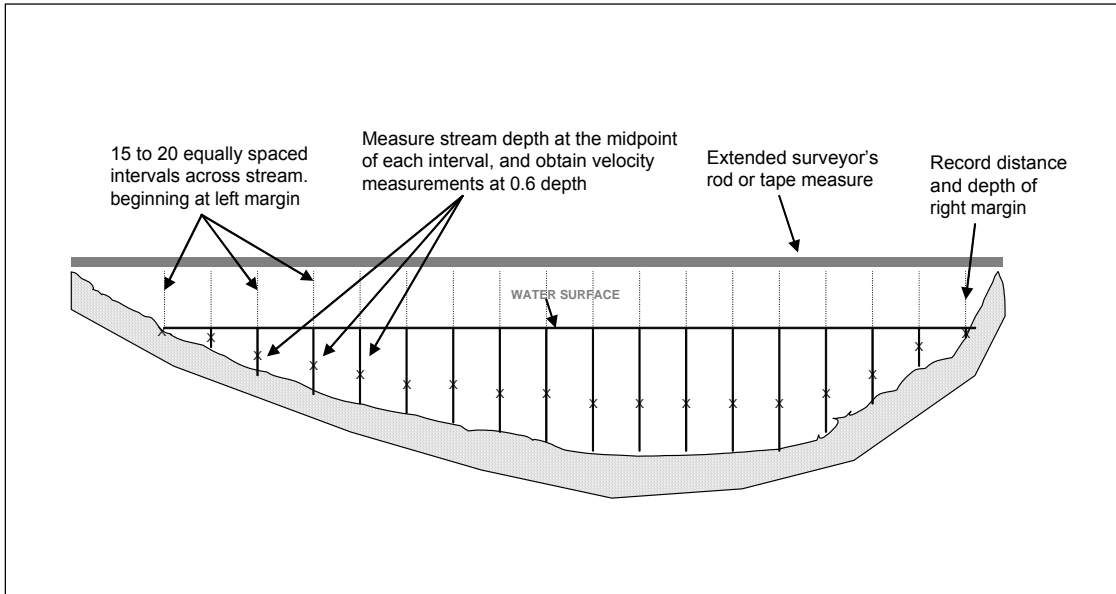


Figure 8.20 Layout of Channel Cross-Section for Obtaining Discharge Data by the Velocity-Area Procedure

Table 8.16 Procedure: Determining Stream Discharge – Velocity-Area

Stream Discharge Velocity- Area Option	
1.	<p>Locate a cross-section of the stream channel for discharge determination that has most of the following qualities (based on Rantz and others, 1982):</p> <ul style="list-style-type: none">• Segment of stream above and below cross-section is straight• Depths mostly greater than 15 centimeters, and velocities mostly greater than 0.15 meters/second. Do not measure discharge in a pool.• “U” shaped, with a uniform streambed free of large boulders, woody debris or brush, and dense aquatic vegetation.• Flow is relatively uniform, with no eddies, backwaters, or excessive turbulence.
2.	<p>Lay the surveyor’s rod (or stretch a measuring tape) across the stream perpendicular to its flow, with the “zero” end of the rod or tape on the left bank, as viewed when looking downstream. Leave the tape tightly suspended across the stream, approximately one foot above water level.</p>
3.	<p>Attach the velocity meter probe to the calibrated wading rod. Check to ensure the meter is functioning properly and the correct calibration value is displayed. Calibrate (or check the calibration) the velocity meter and probe as directed in the meter’s operating manual. Darken in the “VELOCITY AREA” bubble on the Stream Discharge Form.</p>
4.	<p>Divide the total wetted stream width into 15 to 20 equal sized intervals. To determine interval width, divide the width by 20 and round up to a convenient number. Intervals should not be less than 10 cm wide, even if this results in less than 15 intervals. The first interval is located at the left margin of the stream (left when looking downstream), and the last interval is located at the right margin of the stream (right when looking downstream).</p>
5.	<p>Stand downstream of the rod or tape and to the side of the first interval point (closest to the left bank if looking downstream).</p>
6.	<p>Place the wading rod in the stream at the interval point and adjust the probe or propeller so that it is at the water surface. Darken in the appropriate bubbles for “Distance Units” and “Depth Units” on the Stream Discharge Form. Record the distance from the left bank and the depth indicated on the wading rod on the Stream Discharge Form.</p> <p><i>Note:</i> For the first interval, distance equals 0 cm, and in many cases depth may also equal 0 cm. For the last interval, distance will equal the wetted width (in cm) and depth may again equal 0 cm.</p>
7.	<p>Stand downstream of the probe or propeller to avoid disrupting the stream flow. Adjust the position of the probe on the wading rod so it is at 0.6 of the measured depth below the surface of the water. Face the probe upstream at a right angle to the cross-section, even if local flow eddies hit at oblique angles to the cross-section.</p>
8.	<p>Wait 20 seconds to allow the meter to equilibrate, then measure the velocity. Darken the appropriate “Velocity Units” bubble on the Stream Discharge Form. Record the value on the Stream Discharge Form. Note for the first interval, velocity may equal 0 because depth will equal 0.</p> <ul style="list-style-type: none">• For the electromagnetic current meter (e.g., Marsh-McBirney), use the lowest time constant scale setting on the meter that provides stable readings.• For the impeller type meter (e.g., Swoffer 2100), set the control knob at the mid-position of “DISPLAY AVERAGING”. Press “RESET” then “START” and proceed with the measurements.
9.	<p>Move to the next interval point and repeat Steps 6 through 8. Continue until depth and velocity measurements have been recorded for all intervals. Note for the last interval (right margin), depth and velocity values may equal 0.</p> <p>At the last interval (right margin), record a “Z” flag on the field form to denote the last interval sampled.</p> <p>If using a meter that computes discharge directly, darken the “Q” bubble on the discharge form, and record calculated discharge value. In this case, you do not have to record the depth and velocity data for each interval.</p>

NRSA 2013/14 DISCHARGE - WADEABLE ONLY					Reviewed by (Initial): _____			
Site ID: _____					Date: ____ / ____ / ____			
<input type="radio"/> Velocity Area					<input type="radio"/> Timed Filling			
Distance Units <input type="radio"/> ft <input type="radio"/> cm		Depth Units <input type="radio"/> ft <input type="radio"/> cm		Velocity Units <input type="radio"/> ft/s XX.X <input type="radio"/> m/s X.XX				
	Dist. from Bank	Depth	Velocity	Flag	Repeat	Volume (L)	Time (s)	Flag
1	0				1			
2					2			
3					3			
4					4			
5					5			
6								
7								
8								
9								
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
<input type="radio"/> Neutral Buoyant Object								
					Float 1	Float 2	Float 3	
	Float Dist.	<input type="radio"/> ft <input type="radio"/> m						
	Float Time (s)							
	Flag							
Cross Sections on Float Reach								
					Upper Section	Middle Section	Lower Section	
	Width	<input type="radio"/> ft <input type="radio"/> m						
	Depth 1	<input type="radio"/> ft <input type="radio"/> cm						
	Depth 2							
	Depth 3							
	Depth 4							
	Depth 5							
<input type="radio"/> Q Value					If discharge is determined directly in field, record value here: Q = _____			
					<input type="radio"/> cfs	<input type="radio"/> m ³ /s	FLAG	<input type="text"/>
Flag	Comments							
04/08/2013 2013 Discharge					8103048936			

Figure 8.21 Discharge Form, showing data recorded for All Discharge Measurement Procedures

8.14.2 Timed Filling Procedure

In channels too “small” for the velocity-area method, discharge can sometimes be measured by filling a container of known volume and timing the duration to fill the container. “Small” is defined as a channel so shallow that the current velocity probe cannot be placed in a cross-section of the stream that contains one or more natural spillways or plunges that collectively include the entire stream flow, or where the channel is broken up and irregular due to rocks and debris, and a suitable cross-section for using the velocity area procedure is not available. . This can be an extremely precise and accurate method, but requires a natural or constructed spillway of freefalling water. A temporary spillway can also be constructed using a portable V-notch weir, plastic sheeting, or other materials that are available onsite. If obtaining data by this procedure will result in a lot of channel disturbance or stir up a lot of sediment, wait until after all biological and chemical measurements and sampling activities have been completed.

Choose a location within the sampling reach that is narrow and easy to block when using a portable weir. Position the weir in the channel so that the entire flow of the stream is completely rerouted through its notch (**Figure 8.22**). Impound the flow with the weir, making sure that water is not flowing beneath or around the side of the weir. Use mud or stones and plastic sheeting to get a good waterproof seal. The notch must be high enough to create a small spillway as water flows over its sharp crest.

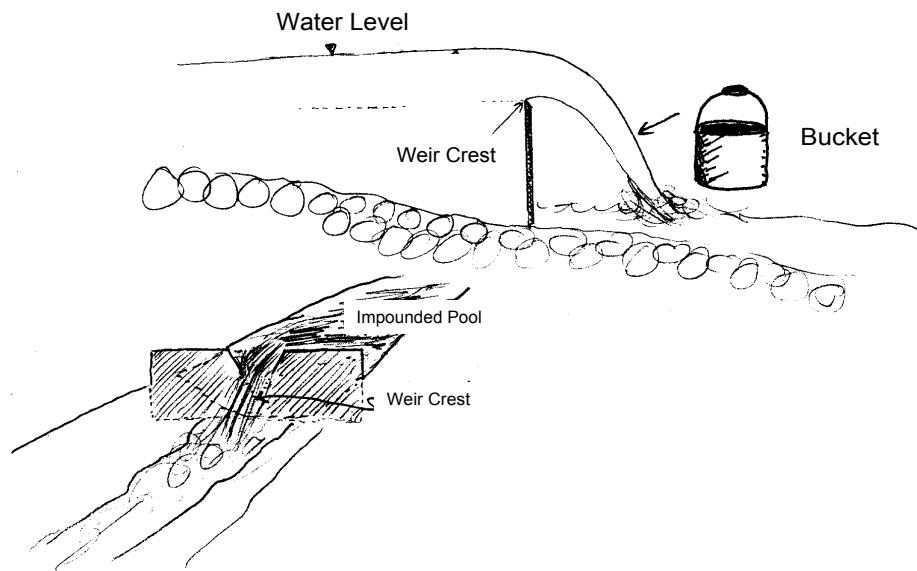


Figure 8.22 Use of a Portable Weir in Conjunction with a Calibrated Bucket to Obtain an Estimate of Stream Discharge

The timed filling procedure is presented in **Table 8.17**. Make sure that the entire flow of the spillway is going into the bucket. Record the time it takes to fill a measured volume on the Discharge Measurement Form as shown in **Figure 8.21**. Repeat the procedure 5 times. If the cross-section contains multiple spillways, you will need to do separate determinations for each spillway. If so, clearly indicate which time and volume data replicates should be averaged together for each spillway; use additional Stream Discharge Form if necessary.

Table 8.17 Procedure: Determining Stream Discharge – Timed Filling

Stream Discharge Timed filled Option
<p>NOTE: If measuring discharge by this procedure will result in significant channel disturbance or will stir up sediment, delay determining discharge until all biological and chemical measurement and sampling activities have been completed.</p> <ol style="list-style-type: none"> 1. Choose a cross-section that contains one or more natural spillways or plunges, or construct a temporary one using on-site materials, or install a portable weir using a plastic sheet and on-site materials. 2. Darken the “TIMED FILLING” bubble in the stream discharge section of the Stream Discharge Form. 3. Position a calibrated bucket or other container beneath the spillway to capture the entire flow. Use a stopwatch to determine the time required to collect a known volume of water. Record the volume collected (in liters) and the time required (in seconds) on the Stream Discharge Form. 4. Repeat Step 3 a total of 5 times for each spillway that occurs in the cross-section. If there is more than one spillway in a cross-section, you must use the timed filling approach on all of them. Additional spillways may require additional data forms

8.14.3 Neutrally Buoyant Object Procedure

In very small, shallow streams with no waterfalls, where the standard velocity-area or timed filling methods cannot be applied; the neutrally buoyant object method may be the only way to obtain an estimate of discharge. The required pieces of information are the mean flow velocity in the channel and the cross-sectional area of the flow. The mean velocity is estimated by measuring the time it takes for a neutrally buoyant object to flow through a measured length of the channel. The channel cross-sectional area is determined from a series of depth measurements along one or more channel cross-sections. Since the discharge is the product of mean velocity and channel cross-sectional area, this method is conceptually very similar to the standard velocity-area method.

The neutrally buoyant object procedure is described in **Table 8.18**. Examples of suitable objects include plastic golf balls (with holes), small sponge rubber balls, or small sticks. The object must float, but very low in the water. It should also be small enough that it does not “run aground” or drag bottom. Choose a stream segment that is roughly uniform in cross-section, and that is long enough to require 10 to 30 seconds for an object to float through it. Select one to three cross-sections to represent the channel dimensions within the segment, depending on the variability of width and/or depth. Determine the stream depth at 5 equally spaced points at each cross-section. Three separate times, measure the time required for the object to pass through the segment that includes all of the selected cross-sections. Record data on the Stream Discharge Form as shown in **Figure 8.21**.

Table 8.18 Procedure: Determining Stream Discharge - Neutrally Buoyant Object

Stream Discharge Neutrally Buoyant Object Option
<ol style="list-style-type: none"> 1. Darken the “NEUTRALLY BUOYANT OBJECT” bubble on the Stream Discharge Form. 2. Select a segment of the sampling reach that is deep enough to float the object freely and long enough that it will take between 10 and 30 seconds for the object to travel. Mark the units used and record the length of the segment in the “FLOAT DIST.” field of the Stream Discharge Form. 3. If the channel width and/or depth change substantially within the segment, measure widths and depths at three cross-sections, one near the upstream end of the segment, a second near the middle of the segment, and a third near the downstream end of the segment. If there is little change in channel width and/or depth, obtain depths from a single “typical” cross-

section within the segment.

4. At each cross-section, measure the wetted width using a surveyor's rod or tape measure, and record both the units and the measured width on the Stream Discharge Form. Measure the stream depth using a wading rod or meter stick at points approximately equal to the following proportions of the total width: 0.1, 0.3, 0.5, 0.7, and 0.9. Record the units and the depth values (not the distances) on the Stream Discharge Form.
5. Repeat Step 4 for the remaining cross-sections.
6. Use a stopwatch to determine the time required for the object to travel through the segment. Record the time in the "FLOAT TIME" field of the Stream Discharge Form.
7. Repeat Step 6 two more times. The float time may differ somewhat for the three trials.

9 FECAL INDICATOR (ENTEROCOCCI)

9.1 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 6 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 6 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 (**Error! Reference source not found.**).

9.2 Equipment and Supplies

Table 9.1 provides the equipment and supplies needed to collect the fecal indicator sample. Record the sample data on the Sample Collection Form (**Error! Reference source not found.**).

Table 9.1 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 Fecal Indicator sample labels (2 vial labels and 1 bag label) Pencils (for data forms) Fine tipped indelible markers (for labels) Clear tape strips

9.3 Sampling Procedure

The procedure for collecting the fecal indicator sample is presented in **Table 9.2**.

Table 9.2 Procedure: Fecal Indicator (Enterococci) Sample Collection (Wadeable Sites)

Enterococci Sample
<ol style="list-style-type: none"> 1. Put on sterile, nitrile gloves. 2. Select a sampling location at transect K that is approximately 1 m from the bank and approximately 0.3m deep. Approach the sampling location slowly from downstream or downwind. 3. Lower the uncapped, inverted 250 ml sample bottle to a depth of 1 foot below the water surface, avoiding surface scum, vegetation, and substrates. 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 does not reach 0.3m along transect at 1 m from the bank, take the sample and flag it on the field form. 6. After removing the container from the water, discard a small portion of the sample to allow for proper mixing before filtering. 7. Add the sodium thiosulfate tablet, cap, and shake bottle 25 times.

Storage

8. Store the sample in a cooler on ice to chill (do not freeze immediately). Chill for at least 15 minutes.
9. Sample must be filtered and all filters frozen within 6 hours of collection

9.4 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 13.3 for information regarding processing the Enterococci samples.

10 FISH ASSEMBLAGE

10.1 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 barge electrofishing unit (2,500- to 5,000-V; 2.5-5.0 GPP unit or equivalent). Use a backpack electrofishing unit in smaller streams, when conductivity is appropriate. As a last option, use seining as an alternate method when electrofishing is precluded by high conductivity or extreme levels of turbidity.

There are different protocols for collecting fish from wadeable streams of different sizes (see Section 10.3). At smaller streams (<12.5 m wide), sample all available habitats over the entire support reach (40 channel widths [CW]). At medium and large wadeable streams (≥ 12.5 m wide), 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. 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 medium and large wadeable streams, if you have not collected 500 individuals at the end of the defined fish sampling reach, sample additional subreaches until you obtain at least 500 individuals or until you have sampled the entire reach. Record information related to sampling effort on the front of the Fish Gear and Sample Information Form (**Figure 10.1**). Record species identification and enumeration data on one or more pages of the Fish Collection Form (**Figure 10.2**). There are several revisions and clarifications to the wadeable sampling protocols from those used in the NRSA 2008/09.

The layout of the fish sampling reach and how to allocate effort across the reach for smaller and larger wadeable streams is clarified. The Fish Gear Form is modified to include the specific protocol used to sample (wadeable or large wadeable), to clarify reasons for not sampling. The collection form is modified to include information about introduced species and hybrids and to break down total counts by major size classes (to begin to look at ecosystem service-related indicators). Measuring minimum and maximum lengths for each species collected is not required. An additional form to record seining effort information is used to evaluate the sufficiency of the seining results and the ability to combine them with electrofishing data. The procedure for preparing voucher specimens is clarified to distinguish *unknown/range extension vouchers* (those taken back to a local facility for identification) and quality assurance (QA) *vouchers* (a sample of all species from a site sent to an external facility for confirmation of all field identifications). Guidance for recording unknown taxa is clarified to minimize ambiguity. The procedure to update species identifications based on the results from unknown/range extension voucher specimens is clarified and uses an additional data form.

Site ID: <input type="text"/>	Reviewed by (initials): <input type="text"/>	NRSA 2013/14 FISH GEAR AND SAMPLING INFORMATION (Front)		Date: <input type="text"/> / <input type="text"/> / <input type="text"/>	
FISH SAMPLING PROTOCOL (select one): <input type="radio"/> BOATABLE (20 to 40 Channel Widths (CW)) <input type="radio"/> L.G. WADEABLE - (>=12.5 m wide) (500 m to 40 CW) <input type="radio"/> WADEABLE - (<12.5 m wide) (40 CW) Final Length of Fishing Reach (m): <input type="text"/>					
FISH SAMPLING - NOT CONDUCTED OR SUSPENDED (select one): <input type="radio"/> Fished - None Collected <input type="radio"/> Not Fished/Fishing suspended - Can't sample >= 50% of required reach: - Boatable (10 CW) - Lg. Wadeable (250m or 10 CW, whichever is longer) - Wadeable (20 CW) <input type="radio"/> Not Fished - No Permit <input type="radio"/> Not Fished - Site Conditions Prohibit Sampling (Describe in comments) <input type="radio"/> Fishing Suspended - Permit Restriction (Listed species encountered) <input type="radio"/> Not Fished - Equipment Failure <input type="radio"/> Not Fished - Other Explain: _____					
Sampling Protocol Comments: _____					
FISH GEAR INFORMATION					
Water Visibility: <input type="radio"/> Good <input type="radio"/> Poor <input type="radio"/> Cond (uS/cm): <input type="text"/>					
Primary Electrofishing Gear # of Netters (1): <input type="text"/> Wave Form: <input type="radio"/> AC <input type="radio"/> DC <input type="radio"/> Pulsed DC Anodes: <input type="text"/> Volts: (50-1000) <input type="text"/> Number: <input type="text"/> Watts: likely 400 (bp), 2500 or 5000 (boatraft) <input type="text"/> Diameters: <input type="text"/> In. <input type="text"/> cm <input type="text"/> Pulsed Rate: <input type="text"/> pps or Hz <input type="radio"/> BOAT (Motor) <input type="radio"/> RAFT (No motor) <input type="radio"/> BACKPACK <input type="radio"/> BANK OR TOWED BARGE Amps: (may not be provided for bp) <input type="text"/>					
Secondary Electrofishing Gear # of Netters (1): <input type="text"/> Wave Form: <input type="radio"/> AC <input type="radio"/> DC <input type="radio"/> Pulsed DC Anodes: <input type="text"/> Volts: (50-1000) <input type="text"/> Number: <input type="text"/> Watts: likely 400 (bp), 2500 or 5000 (boatraft) <input type="text"/> Diameters: <input type="text"/> In. <input type="text"/> cm <input type="text"/> Pulsed Rate: <input type="text"/> pps or Hz <input type="radio"/> BOAT (Motor) <input type="radio"/> RAFT (No motor) <input type="radio"/> BACKPACK <input type="radio"/> BANK OR TOWED BARGE Amps: (may not be provided for bp) <input type="text"/>					
Primary Seine Net: <input type="radio"/> BAG SEINE <input type="radio"/> MINNOW SEINE No. of crew members: <input type="text"/> Height (m): <input type="text"/> Length (m): <input type="text"/> Mesh (mm): <input type="text"/> Avg. Haul Length (m): <input type="text"/> No. of Hauls: <input type="text"/> Total Seining Time (min): <input type="text"/> % of Fishing Reach Sampled: <input type="text"/>					
Secondary Seine Net: <input type="radio"/> BAG SEINE <input type="radio"/> MINNOW SEINE No. of crew members: <input type="text"/> Height (m): <input type="text"/> Length (m): <input type="text"/> Mesh (mm): <input type="text"/> Avg. Haul Length (m): <input type="text"/> No. of Hauls: <input type="text"/> Total Seining Time (min): <input type="text"/> % of Fishing Reach Sampled: <input type="text"/>					
GEAR INFORMATION COMMENTS					
04/11/2013 2013 Fish Gear 8573529111					

Figure 10.1 Fish Gear and Sampling Information Form (front)

10.2 Equipment and Supplies

Table 10.1 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 10.1 Equipment and Supplies: Fish Collection (Wadeable Sites)

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

10.3 Sampling Procedures

The reach length sampled for fish varies based on the width of the stream used to establish the length of the support reach and on the number of individuals collected (**Figure 10.3**). For small wadeable streams (with a mean channel width [CW] < 12.5 meters), follow the protocol presented in Section 0. Sample the entire support reach (150 to 500 m) and move the electrofishing unit (towed barge or backpack) within each subreach to sample both shorelines as well as the mid-channel.

For medium and large wadeable streams (mean CW ≥ 12.5 m), follow the protocols described in Section 10.3.3. In medium wadeable streams (mean CW between 12.5 and 25 meters) the minimum length for fish sampling is 500 m, which will include between 5 and 10 subreaches. If you reach the required distance within a subreach, extend the length of the fish sampling reach to the end of the subreach so you end fishing efforts at a transect. For large wadeable stream (mean CW > 25 meters), the minimum length for fish sampling is 5 of the 10 subreaches. In both

medium and large wadeable streams, if a minimum of 500 fish are not collected after sampling the minimum fishing reach, additional subreaches will be sampled until 500 fish are collected or all 10 subreaches have been sampled. **Table 10.2** summarizes the fishing protocols for each of three sizes of wadeable streams.

If conditions prohibit any type of electrofishing, collect fish by seining as described in **Table 10.3**. 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.

10.3.1 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 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 10.2 Summary of Wadeable Fishing Protocols

<p><u>Small Wadeable (less than 12.5 meters wide)</u></p> <ul style="list-style-type: none"> • Sampling reach will be between 150 and 500 meters • Subreaches will be between 15 and 50 meters each <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>Medium Wadeable (12.5 to 25 meters wide)</u></p> <ul style="list-style-type: none"> • Sampling reach will be between 500 and 1000 meters • Subreaches will be between 50 and 100 meters each • Minimum fishing length = 500 meters which will be between 5 and 10 subreaches • If needed, extend fishing length to end at a transect <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. ✓ Minimum fish number is 500 unless all 10 subreaches have been fished. <ul style="list-style-type: none"> ➤ After fishing at least 500 meters, 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.
<p><u>Large Wadeable (25 + meters wide)</u></p> <ul style="list-style-type: none"> • Sampling reach will be between 1000 and 4000 meters

- Subreaches will be between 100 and 400 meters each
- Minimum fishing length = 5 subreaches (which will equal between 500 and 2000 meters)
 - ✓ 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
 - Depending upon the habitat complexity, you can vary the distance actively fished to allocate the available button time throughout the subreach.
 - ✓ Minimum fish number is 500 unless all 10 subreaches have been fished.
 - 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.

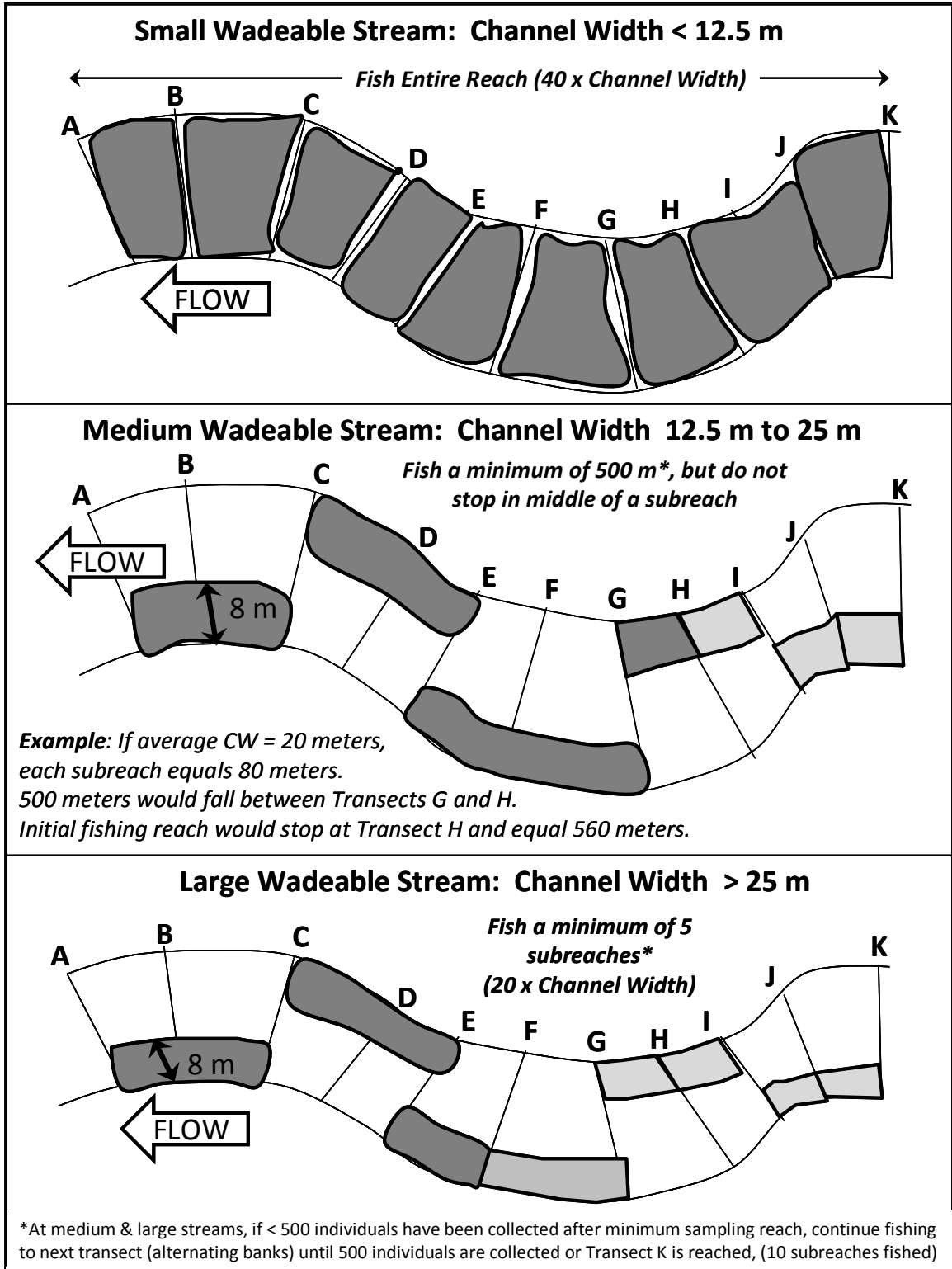


Figure 10.3 Reach Layouts for Fish Sampling at Wadeable Sites

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.

10.3.2 Small Wadeable Streams

Table 10.3 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). 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. Begin sampling at the downstream stream end of the support reach defined for the site (**Figure 10.3**) and proceed upstream. Sample the entire support reach, which will be between 150 and 500 m long. 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. You can use multiple lines per species on the Fish Collection Form if necessary (e.g., you collect a large number of individuals and need additional space for tallying, or collect the same species at non-adjacent subreaches [e.g., A-B and G-H]).

Table 10.3 Procedure: Electrofishing (Small Wadeable Streams)

Electrofishing Procedures in Small Wadeable Streams
1) Complete the header section of the fish gear form (Site ID and date).
2) Decide if you will be able to sample the site. <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, mark <i>Not Fished – No Permit</i>. b) If site conditions prevent barge 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 10.5. ii) If not, mark <i>Not Fished – Site Conditions Prohibit Sampling</i>. Note the conditions in the <i>Sampling Protocol Comments</i>. c) If you can determine that > 50% of the required fish sampling reach cannot be sampled, mark <i>Not Fished/Fishing Suspended – Can't sample >=50% of required reach</i>. d) If you cannot sample because of equipment problems, mark <i>Not Fished – Equipment Failure</i>. e) If you cannot sample for any other reason, mark <i>Not Fished – Other</i> and note the reason in the <i>Explain</i> field
3) If you can begin to sample, mark <i>Wadeable</i> in the <i>Fish Sampling Protocol</i> section. <ul style="list-style-type: none"> a) Proceed to the downstream end of the support 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) Mark 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) Mark either <i>Backpack</i> , <i>Bank</i> or <i>Towed Barge</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 small wadeable stream.
6) Operation of Bank or Towed Barge Electrofisher—

- a) Set unit to pulsed DC and mark it in the *Wave Form* section of the fish gear form.
- b) Test settings outside of the sampling area. Start the electrofisher, set the timer, and depress the switch to begin fishing. Typical settings are:
 - i) 500-1000 V DC
 - ii) 8-20 A
 - iii) 120 Hz.
- c) 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.
- d) **If no fish can be collected or electrofishing is ineffective due to high conductivity or extreme turbidity, refer to seining protocol in Table 10.5.**

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 conductivity of the river.
 - i) 150-400 V for high conductivity (>300 $\mu\text{S/cm}$).
 - ii) 500-800 V for medium conductivity (100 to 300 $\mu\text{S/cm}$).
 - iii) 900-1100 V for low conductivity (< 100 $\mu\text{S/cm}$).
- c) Select the initial pulse rate and width.
 - i) In waters with strong swimming fish (length >200 mm [8 in]), 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 60-70 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) **If no fish can be collected or electrofishing is ineffective due to high conductivity or extreme turbidity, refer to seining protocol in Table 10.5.**
- f) Once you have determined the appropriate settings, record them on the fish gear form. Start cleared clocks and resume fishing.
 - i) 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 flag the data.

- 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 500 m.
 - 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 support 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.

<p>(3) Consider that the sampling effort should provide a sample of ~500 individuals, if these numbers exist within the support reach.</p> <p>b) Depress the switch and slowly sweep the electrode from side to side.</p> <p>c) Sample all habitat types (deep, shallow, fast, slow, complex, and simple). Avoid the temptation to focus sampling only in the richest habitat types.</p> <p>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.</p> <p>ii) In fast, shallow water, sweep the anode and fish downstream into a net.</p> <p>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.</p> <p>d) Keep the cathode near the anode if fish catch is low.</p>
<p>9) The netter, with the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket.</p>
<p>10) Continue upstream until you reach the next transect (end of subreach).</p> <p>a) Process fish and/or change water after each subreach to reduce mortality and stress.</p> <p>i) Although not required, you may note amphibians and reptiles captured on the fish collection form.</p> <p>b) Release fish in a location that eliminates the likelihood of recapture.</p>
<p>11) Repeat Steps 8-10 until all 10 subreaches are sampled (i.e., you reach transect K).</p>
<p>12) After sampling all 10 subreaches, record the final length of the fish sampling reach in the <i>Fish Sampling Protocol</i> section of the fish gear form.</p> <p>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 <i>Fish sampling – Not Conducted or Suspended</i> section of the fish gear form.</p> <p>b) If you did not collect any fish, mark <i>Fished – None Collected</i> in the <i>Fish sampling – Not Conducted or Suspended</i> section of the fish gear form.</p>
<p>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 percentage of the total fish sampling reach (recorded in Step 12) sampled by electrofishing (should be 100%).</p>

10.3.3 Medium and Large Wadeable Streams

Table 10.4 describes the procedure for collecting fish in medium and 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 livewell). 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.

For medium wadeable streams with a mean channel width between 12.5 and 25 m, the minimum fish sampling reach is ~500 m (Table 10.2). If 500 m falls within a subreach, complete sampling the subreach (so you will sample between 5 and 10 subreaches). For large wadeable streams with a mean channel width >25 m, the minimum fish sampling reach is 20 channel widths (5 subreaches).

As shown in Figure 10.3, 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. For streams <16 m wide, or wider streams with simple habitat, fish the subreach in its entirety (from bank to bank). Within each subreach, fish the near bank habitat as well as midstream

habitat within the 8-m 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. You can use multiple lines per species on the fish collection form if necessary (e.g., you collect a large number of individuals and need additional space for tallying, or collect the same species at non-adjacent subreaches [e.g., A-B and G-H]).

At the end of the designated fish sampling reach, 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. Stop sampling when you reach Transect K (the end of the entire support 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 10.4 Procedure: Electrofishing (Medium and Large Wadeable Sites)

Electrofishing Procedures in Medium and Large Wadeable Streams	
1)	Complete the header section of the fish gear form (Site ID and date).
2)	Decide if you will be able to sample the site. <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, mark <i>Not Fished – No Permit</i>. b) If site conditions prevent barge 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 10.5. ii) If not, mark <i>Not Fished – Site Conditions Prohibit Sampling</i>. Note the conditions in the <i>Sampling Protocol Comments</i>. c) If you can determine that > 50% of the required fish sampling reach cannot be sampled, mark <i>Not Fished/Fishing Suspended – Can’t sample >=50% of required reach</i>. d) If you cannot sample because of equipment problems, mark <i>Not Fished – Equipment Failure</i>. e) If you cannot sample for any other reason, mark <i>Not Fished – Other</i> and note the reason in the <i>Explain</i> field
3)	If you can begin to sample, mark <i>Wadeable</i> in the <i>Fish Sampling Protocol</i> section. <ul style="list-style-type: none"> a) Proceed to the downstream end of the support 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)	Mark 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)	Mark either <i>Backpack</i> or <i>Bank</i> or <i>Towed Barge</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 Barge Electrofisher—

Electrofishing Procedures in Medium and Large Wadeable Streams	
<ul style="list-style-type: none"> a) Set unit to pulsed DC and mark it in the <i>Wave Form</i> section of the fish gear form. b) Test settings outside of the sampling area. Start the electrofisher, set the timer, and depress the switch to begin fishing. Typical settings are: <ul style="list-style-type: none"> i) 500-1000 V DC ii) 8-20 A iii) 120 Hz. c) 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>7) Operation of Backpack Electrofisher</p> <ul style="list-style-type: none"> a) Set unit to pulsed DC and mark it in the <i>Wave Form</i> section of the fish gear form. b) Select the initial voltage setting based on the conductivity of the stream. <ul style="list-style-type: none"> i) 150-400 V for high conductivity (>300 $\mu\text{S/cm}$). ii) 500-800 V for medium conductivity (100 to 300 $\mu\text{S/cm}$). iii) 900-1100 V for low conductivity (< 100 $\mu\text{S/cm}$). c) Select the initial pulse rate and width. <ul style="list-style-type: none"> i) In waters with strong swimming fish (length >200 mm [8 in]), 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 60-70 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) If no fish can be collected or electrofishing is ineffective due to high conductivity or extreme turbidity, refer to seining protocol in Table 10.5. f) Once you have determined the appropriate settings, record them on the fish gear form. Start cleared clocks and resume fishing. <ul style="list-style-type: none"> i) 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 flag the data. 	
<p>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.</p> <ul style="list-style-type: none"> a) For the medium stream protocol, the minimum initial fish sampling reach length is 500 m. If 500 m or 2000 m falls within a subreach, finish sampling the subreach. b) For the large stream protocol, the minimum initial fish sampling reach length is 5 subreaches <ul style="list-style-type: none"> i) For streams <16 m wide, or wider streams with simple habitat, fish the subreach in its entirety (from bank to bank). 	
<p>9) When using a towed electrofishing unit (barge or pram), the minimum crew size for electrofishing is three.</p> <ul style="list-style-type: none"> a) The barge operator must remain actively at the control box and navigate the barge. b) The probe operator will use one probe. 	
<p>10) When using a backpack electrofishing unit, the minimum crew size is two (one operator with the probe and one netter).</p>	
<p>11) Depress the switch and slowly sweep the electrode from side to side.</p>	

Electrofishing Procedures in Medium and Large Wadeable Streams

- 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) 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.
 - c) Keep the cathode near the anode if fish catch is low.
- 12) The netter, with the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket.
- 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 10.3). Repeat Steps 12-14 until you have sampled the required length of stream (Step 8).
 - 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 Conducted or Suspended* section of the fish gear form.
- 15) If the required length did not include all 10 subreaches, determine the total number of individuals collected.
 - a) If the total is < 500, sample one or more additional subreaches until at least 500 individuals have been collected and processed, or you sample all 10 subreaches. Go to Step 17.
 - 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 make up > 50% of the sample, sample one or more additional subreaches to bring the proportion of the irruptive species below 50%. Go to Step 17.
 - ii) If not, go to Step 17.
 - c) If you have sampled all 10 subreaches (i.e., you have reached Transect K), go to Step 17.
- 16) After sampling, record the final length of the fish sampling reach in the *Fish Sampling Protocol* section of the fish gear form.
 - a) If you did not collect any fish, mark *Fished – None Collected* in the *Fish sampling – Not Conducted 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 percentage of the total fish sampling reach (recorded in Step 15 or 17) sampled by electrofishing (should be 100%).

10.4 Seining

In small or large wadeable streams where conditions prohibit electrofishing, use seining **only as the last option for collecting fish**. If conditions are such that seining is the only method used, provide a justification in the *Sampling Protocol Comments* section of the fish gear form (Figure 10.1). **Table 10.5** presents the procedure for seining wadeable streams. The intent of the seining effort is to provide comparable data to electrofishing.

Although electrofishing typically works 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 10.5**. Depending upon habitat types and complexity, use 2 to 3 crew members. Two crewmembers 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. If necessary, you can use additional lines on the fish collection form (**Figure 10.2**) to record species collected with different nets or in different hauls.

If you seine, record information for each seine haul on the Seining Information Form to track effort (**Figure 10.4**). Denote the bank as right or left as you face downstream. Restrict each haul to a single habitat type. After fish sampling is completed for the site, use the information from the seining information form to complete the information in the *Primary Seine Net* section of the fish gear form (**Figure 10.1**). Include the seining information form with the packet of completed field forms you submit to the NRSA information management staff.

Table 10.5 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, note the reason in the <i>Sampling Protocol Comments</i> section. 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)	Complete the header section of the fish gear form (Site ID and date).
3)	Mark 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 support 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 10.3 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.
4)	Mark 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.
5)	Mark 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>a) Record the number of crewmembers (2-3), and the net dimensions (height, total length, and mesh size) on the fish gear form.</p> <p>b) If you have to use a second type of seine for parts of the sampling reach, Mark the type and record the dimensions in the <i>Secondary Seine Net</i> section of the fish gear form.</p>
<p>6) Determine the length of the fish sampling reach and the number of subreaches that should be sampled (refer to Table 10.2).</p>
<p>7) To maximize capture efficiency, please do the following:</p> <p>a) Always use 10 and 20 ft. seines. When necessary, reduce the width by rolling seine poles and floats into the net.</p> <p>b) When narrowing seines, always keep lead line outside of the pole.</p> <p>c) When working edge habitats, only roll the inner side of the seine, while keeping the near bank pole extended.</p> <p>d) As a default, use seines that are 2 meters in depth. A 1.25 meter seine may be used in shallow habitats.</p> <p>e) Keep the float line above the surface (avoid dragging it below the surface while pulling).</p> <p>f) Maintain the lead line along the river bottom.</p> <p>g) Either tie the seine to the poles tightly, or roll the seine into the poles.</p> <p>h) Always maintain the bag behind the poles.</p>
<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> <p>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.</p> <p>i) Avoid extended seine hauls that collect hundreds of individuals.</p> <p>ii) Seine as many available habitat types as possible within each subreach (one haul each).</p> <p>iii) Total time spent seining a site should be comparable to what would have been spent electrofishing.</p>
<p>b) Riffle Habitats</p> <p>i) Use two crewmembers, 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.</p> <p>ii) Starting no more than 3 m upstream, a third crewmember kicks the substrate and overturns rocks, proceeding quickly downstream toward the net.</p> <p>iii) When the area is thoroughly kicked, quickly raise and bag the net. . Process fish (i.e., enumerate, identify and voucher fish) and record tally information on the fish collection form (Figure 10.2). You may use separate lines on the fish collection form to record species information from seine hauls.</p> <p>iv) Record seine characteristics and haul length, habitat, and time on the seining information form (Figure 10.4).</p>
<p>c) Pool, Backwater, and Bar Habitats (Slack water)</p> <p>i) Use two crewmembers, each tending a seine pole. Pull the seine across the pool using shallow riffles or banks as barriers. A third crewmember 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</p>

<p>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 crewmembers, each tending a seine pole. Pull the seine diagonally across the glide towards the bank. If necessary, a third crewmember 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 crewmembers, each tending a seine pole. Seine along the nearshore area.</p> <p>iii) The near bank crewmember moves along the shore while jabbing along any undercut or small structure. The other crewmember 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 crewmembers, 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 crewmember 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 crewmember moves along the snag, while jabbing along its length. The other crewmember 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> <p>a) Record identifications, tallies, and voucher information on the fish collection form (Figure 10.2). You may use separate lines on the fish collection form to record species information from separate seine hauls.</p> <p>b) Tally seine characteristics and haul length, habitat, and time on the seining information form (Figure 10.4).</p>
<p>10) At the end of all sampling, use the seining information form to determine the total number of hauls, the average haul length, the total time spent seining, and the proportion of the total fish sampling reach sampled (recorded in the <i>Fish Sampling Protocol</i> section of the fish gear form) for each type of</p>

seine. Record the totals in the *Primary* and *Secondary Seine Net* sections of the fish gear form.

NRSA 2013/14 SEINING INFORMATION

Reviewed by (initials):

Site ID: Date: / /

Subreach (e.g., A-B)	Bank	Habitat Sampled (choose one)	If other, describe:	Seine Width (m)	Seine Depth (m)	Approx Haul Distance (m)	Approx Haul Time (sec)	Fish Collected?	Additional Person	Flag
	<input type="radio"/> L <input type="radio"/> R <input type="radio"/> CH	<input type="radio"/> PL <input type="radio"/> GL <input type="radio"/> RF <input type="radio"/> BW <input type="radio"/> ED <input type="radio"/> SN <input type="radio"/> Other:						<input type="radio"/>	<input type="radio"/>	
	<input type="radio"/> L <input type="radio"/> R <input type="radio"/> CH	<input type="radio"/> PL <input type="radio"/> GL <input type="radio"/> RF <input type="radio"/> BW <input type="radio"/> ED <input type="radio"/> SN <input type="radio"/> Other:						<input type="radio"/>	<input type="radio"/>	
	<input type="radio"/> L <input type="radio"/> R <input type="radio"/> CH	<input type="radio"/> PL <input type="radio"/> GL <input type="radio"/> RF <input type="radio"/> BW <input type="radio"/> ED <input type="radio"/> SN <input type="radio"/> Other:						<input type="radio"/>	<input type="radio"/>	
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BANK: L = Left Bank (facing downstream) R = Right Bank (facing downstream) CH = In Channel
 HABITAT SAMPLED: PL = Pool, GL = GlideRun, RF = Riffle, ED = Edge, SN = Snag, BW = Backwater or side channel, OT = Other (describe)
 FLAG CODES: K = No measurement made, U = Suspect measurement, F1,F2, etc. = flags assigned by each field crew. Explain all flags in comments.

Flag _____

Comments

02/27/2013 2013 Seining Information 9533043973

Figure 10.4 Seining Information Form

10.5 Processing Fish

Process fish at the end of each subreach, as described in **Table 10.6**. 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 the information on a separate line on the field form.

10.5.1 Identification and Tallying

Record species identifications, tallies, and other information for individuals collected on the Fish Collection Form (**Figure 10.2**). Use multiple pages of the form as needed to record all species collected. It is important to record page numbers correctly because page number is one of the variables used to uniquely identify a species record. You can record separate collections of the same species on multiple lines of the collection form (e.g., when you encounter a species in non-adjacent subreaches, or collect a species with a secondary gear type). 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 (6 inch [15 cm] 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). Appendix D provides a list of species names reported from the NRSA 2008/09.

If you believe a specimen is nonindigenous to the site, mark it as *Introduced* on the 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 10.5.2).

10.5.2 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. 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 0) or take good digital photographs (Section 10.5.3) 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 on the label sheet to label your jar of unknown to track from which sites the unknowns originated.

Table 10.6 Procedure: Processing Fish (Wadeable Sites)

Fish Processing	
1)	Complete all header information accurately and completely on the fish collection form. It is important to paginate the collection forms correctly (e.g., start with page 1, do not duplicate page numbers, etc.), as page number is part of the unique record identifier for the fish count data.
2)	<p>Process individuals collected at the end of each subreach. You can record a single species on multiple lines of the collection form (e.g., use separate lines for individuals collected in nonadjacent subreaches, collect with a secondary gear type, or if you need additional space to record tally marks, etc.).</p> <p>a) Process species listed as threatened and endangered first as described in Step 4.</p> <p>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.</p> <p>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.</p> <p>iii) Return individuals to the river immediately after processing.</p>
3)	Only identify and process individuals > 25mm (1 inch) in total length (TL). Ideally handle specimens only once.
4)	<p>Identify each individual to the lowest possible taxonomic level:</p> <p>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.</p> <p>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 the NRSA 2008/09.</p> <p>(1) Record the complete common name. Avoid using shortened names (e.g., <i>stoneroller</i>, <i>carp</i>, <i>bass</i>, etc.).</p> <p>(2) If you use a non-standard name, you must assign a flag to the line and provide the taxonomic reference for the name in the <i>Comments</i> section of the collection form.</p> <p>b) If you cannot positively identify an individual to the species level:</p> <p>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>) in the <i>Common Name</i> column of the collection form.</p> <p>ii) If you are permitted to retain the specimen, assign it the next available sequential tag number (starting with 01) in the <i>Voucher Tag Number</i> column and see Step 9.</p> <p>c) If you believe the individual is a hybrid:</p> <p>i) Mark as <i>Hybrid?</i> on the collection form.</p> <p>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. For other hybrids record the common name of both species (e.g., <i>Green sunfish x Bluegill</i>, <i>Cutthroat trout x Rainbow trout</i>). 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> column and see Step 9.</p>

5)	If you know the species is not native to this location, mark as <i>Introduced?</i>
6)	Visually estimate the total length of each individual (a measuring board is not necessary). 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. a) If all individuals of a species appear to be the same size, provide a flag and comment for the line if you believe the population is stunted.
7)	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 observed in the <i>Anom Count</i> column of the collection form.
8)	If an individual has died due to electrofishing or handling, include it in the running tally for the species. After you process all of the individuals of a species, record the total number observed in the <i>Mortality/Count</i> column of the collection form.
9)	If you are retaining individuals of the species as part of the preserved Unknown/Range Extension Unk/Rng voucher sample: a) Mark as <i>Unk/Rng</i> in the <i>Voucher</i> section of the collection form. b) Assign the species the next available voucher specimen tag, and record the number in the <i>Voucher Tag #</i> column of the collection form. i) If you take one or more photographs of the species instead of preserving specimens, assign the next available voucher specimen tag number in the <i>Voucher Tag #</i> column of the collection form. Include the specimen tag in all photos of the species. Mark <i>Photo</i> in the <i>Voucher</i> section of the collection form. (1) Ideally, take photos of all species collected at a site that are not being preserved. c) Record the number of individuals retained for the preserved voucher sample in the <i>Vouchers Retained</i> column of the collection form. 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. 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).
10)	If you are retaining specimens as part of a preserved QA voucher sample for the site: a) Mark as <i>QA</i> in the <i>Voucher</i> section of the fish collection form. i) NOTE: This should be marked at least once for all species collected at the site (including unknowns). b) Use the sheet of labels and tags for the QA voucher sample (the jar label has a preprinted sample ID number). Assign the species the next available voucher specimen tag number. Record the specimen tag number in the <i>Voucher Tag #</i> column of the collection form. i) If you take one or more photographs of the species instead of preserving specimens, assign the next available voucher specimen tag number, and record the number in the <i>Voucher Tag #</i> column of the collection form. Include the specimen tag in all photos of the species. Mark <i>Photo</i> in the <i>Voucher</i> section of the collection form. c) Record the number of individuals retained for the preserved voucher sample in the <i>Vouchers Retained</i> column of the collection form. 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.
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).
11)	Repeat Steps 2 through 10 for each subreach sampled. Use additional fish collection form sheets as needed, being careful to paginate each sheet correctly.
12)	Record the fish collected with seining methods on a separate line on the field form.
13)	At the end of sampling, follow the appropriate procedure to prepare the preserved voucher samples (UNK/RNG and/or QA) and/or select specimens for tissue 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.
15)	If you did not prepare a QA voucher sample, mark <i>No Voucher Preserved</i> on the back of the fish gear form. a) Otherwise complete a sample jar label for the QA voucher sample. Attach it to the sample jar and cover it with clear tape. b) Record QA voucher sample label information on the back of the fish gear form.
16)	Record the file names of any photovouchers taken on the back of the fish gear form. a) Use only one line per voucher tag, even if you took multiple photos (record the beginning and end of the sequence in the <i>Sequence</i> column). Make sure the page and line numbers you record match those on the collection form. b) Name image files as: Site ID + Visit number + tag number + sequence (e.g., CTLS1001_V1_tag01a).
17)	If you did not collect any fish from the entire fish sampling reach, mark <i>Fished – None Collected</i> in the <i>Fish Sampling – Not Conducted or Suspended</i> section of the fish gear form.

10.5.3 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 as a handout.

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, 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.

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 (PC) 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., CTLS1001_V1_tag01a.jpg). Record the file names on the back of the fish gear form (**Figure 10.6**).

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 transfer all images to the NRSA information management (IM) staff. You will also send copies of your image files as part of your QA voucher samples (Section 10.5.3).

10.5.4 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 fish collection form to the NRSA IM staff, you will receive an update form that lists only the records for unknown species recorded on the fish collection form (including photovouchers) that were marked as being part of the UNK/RNG voucher sample.

In addition to a UNK/RNG voucher sample (if needed), you will prepare an additional *QA voucher sample* (Section 10.5.6). A QA voucher sample will be performed at a pre-designated set of sites and includes preserved specimens (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.

10.5.5 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 MSDS. **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 jar label (**Figure 10.5** for UNK/RNG samples, and **Figure 10.6** for QA voucher samples). Attach the completed label to the jar and cover with clear shipping tape. If you have > 2 jars of either type of sample, prepare a hand-made label for each additional jar (this is more important for the QA voucher sample, which has a unique sample ID number).

FISH VOUCHER – UNK/RNG Site ID: _____ ___ / ___ / 201__		FISH VOUCHER – UNK/RNG Site ID: _____ ___ / ___ / 201__	
FISH – BAG TAG: 41	FISH – BAG TAG: 42	FISH – BAG TAG: 43	FISH – BAG TAG: 44
FISH – BAG TAG: 37	FISH – BAG TAG: 38	FISH – BAG TAG: 39	FISH – BAG TAG: 40
FISH – BAG TAG: 33	FISH – BAG TAG: 34	FISH – BAG TAG: 35	FISH – BAG TAG: 36
FISH – BAG TAG: 29	FISH – BAG TAG: 30	FISH – BAG TAG: 31	FISH – BAG TAG: 32
FISH – BAG TAG: 25	FISH – BAG TAG: 26	FISH – BAG TAG: 27	FISH – BAG TAG: 28
FISH – BAG TAG: 21	FISH – BAG TAG: 22	FISH – BAG TAG: 23	FISH – BAG TAG: 24
FISH – BAG TAG: 17	FISH – BAG TAG: 18	FISH – BAG TAG: 19	FISH – BAG TAG: 20
FISH – BAG TAG: 13	FISH – BAG TAG: 14	FISH – BAG TAG: 15	FISH – BAG TAG: 16
FISH – BAG TAG: 09	FISH – BAG TAG: 10	FISH – BAG TAG: 11	FISH – BAG TAG: 12
FISH – BAG TAG: 05	FISH – BAG TAG: 06	FISH – BAG TAG: 07	FISH – BAG TAG: 08
FISH – BAG TAG: 01	FISH – BAG TAG: 02	FISH – BAG TAG: 03	FISH – BAG TAG: 04

Figure 10.5 Unknown/Range Extension Voucher Sample Labels and Voucher Specimen Tags

NRSA 2013/14 FISH GEAR AND SAMPLING INFORMATION (Back)

Site ID: _____ Reviewed by (initial): _____

Date: _____ / _____ / _____

NO VOUCHERS PRESERVED

QA VOUCHER SAMPLE INFORMATION (VERT)

Sample ID	# of Jars	Preserved	Comments
	<input type="radio"/>	<input type="radio"/>	

PHOTO VOUCHER FILE INFORMATION

Page	Line	Photo File Name (SiteID_V(Visit#)_Tag#) (e.g.: SSCT001_V1_Tag01)	Sequence (e.g. a-c)	Comments	Page	Line	Photo File Name (SiteID_V(Visit#)_Tag#) (e.g.: SSCT001_V1_Tag01)	Sequence (e.g. a-c)	Comments

FISH TISSUE PLUG SAMPLES (FPLG)

Collection Method: ELECTROFISHING HOOK & LINE SEINING OTHER:

Sample ID	Common Name	Length(mm)	Weight(g)	Comments

NO SAMPLE COLLECTED

04/11/2013, 2013 Fish Gear
5036529112

Figure 10.6 Fish Gear and Sampling Information Form (back)

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

Table 10.7 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 laboratory operations manual (USEPA 2013b). 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, use the update form for the sample (**Figure 8.10**) to reconcile the unknown records to reflect the actual species identifications and numbers. **It is important to update counts and identifications by voucher tag—do not combine multiple samples of the same unknown before updating.**

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 *Revised Name* column, and enter the original count in the *Revised Count* column. If you determine that a single unknown record is actually >1 species, you will record the new species on blank lines. 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, correct the total count for the unknown assigning 75% of the original total count to the first species and 25% of the original total count to the second species. Record the information (revised common name and revised count) for the first species on the same line as the original unknown record. Record the information for additional species from this original unknown as new data records, but retain the page, line number, and voucher specimen tag number of the original unknown record. Record the name and count in the *Revised Name* and *Revised Count* columns.

If you use a non-standard name, enter the page, line number, tag number, and taxonomic reference for the name in the *Comments* section of the update form. Submit your completed update forms to the NRSA IM staff 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.

10.5.6 Processing QA Voucher Samples

Prepare the QA voucher sample as outlined in **Table 10.8**. Prepare the QA voucher sample separately from the UNK/RNG voucher sample. Processing involves ensuring that the sample jar(s) and photovoucher files include 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 assigned (**Figure 10.7**). Record information about the preserved QA voucher sample on the back side of the fish gear form (**Figure 10.6**).

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, and on the particular shipping service used.

QA FISH VOUCHER (VERT) Site ID: _____ ___ / ___ / 201__ 990011		QA FISH VOUCHER (VERT) Site ID: _____ ___ / ___ / 201__ 990011	
FISH - BAG TAG: 41 990011	FISH - BAG TAG: 42 990011	FISH - BAG TAG: 43 990011	FISH - BAG TAG: 44 990011
FISH - BAG TAG: 37 990011	FISH - BAG TAG: 38 990011	FISH - BAG TAG: 39 990011	FISH - BAG TAG: 40 990011
FISH - BAG TAG: 33 990011	FISH - BAG TAG: 34 990011	FISH - BAG TAG: 35 990011	FISH - BAG TAG: 36 990011
FISH - BAG TAG: 29 990011	FISH - BAG TAG: 30 990011	FISH - BAG TAG: 31 990011	FISH - BAG TAG: 32 990011
FISH - BAG TAG: 25 990011	FISH - BAG TAG: 26 990011	FISH - BAG TAG: 27 990011	FISH - BAG TAG: 28 990011
FISH - BAG TAG: 21 990011	FISH - BAG TAG: 22 990011	FISH - BAG TAG: 23 990011	FISH - BAG TAG: 24 990011
FISH - BAG TAG: 17 990011	FISH - BAG TAG: 18 990011	FISH - BAG TAG: 19 990011	FISH - BAG TAG: 20 990011
FISH - BAG TAG: 13 990011	FISH - BAG TAG: 14 990011	FISH - BAG TAG: 15 990011	FISH - BAG TAG: 16 990011
FISH - BAG TAG: 09 990011	FISH - BAG TAG: 10 990011	FISH - BAG TAG: 11 990011	FISH - BAG TAG: 12 990011
FISH - BAG TAG: 05 990011	FISH - BAG TAG: 06 990011	FISH - BAG TAG: 07 990011	FISH - BAG TAG: 08 990011
FISH - BAG TAG: 01 990011	FISH - BAG TAG: 02 990011	FISH - BAG TAG: 03 990011	FISH - BAG TAG: 04 990011

Figure 10.7 QA Voucher Sample Labels and Voucher Specimen Tags

Table 10.7 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. <ul style="list-style-type: none"> 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)	You will receive a Fish Update Identification and Count Form for each UNK/RNG sample from the NARS IM staff. This form lists all records from the original collection form that were marked as being part of the UNK/RNG voucher sample. Identify unknown fish to species in the laboratory, using the procedure described in the NRSA laboratory operations manual, which is briefly described below. <ul style="list-style-type: none"> a) 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 page and line number, and voucher specimen tag you recorded on the collection form.
5)	Record the final identification(s) and count for each unknown record in the <i>Revised Name</i> and <i>Revised Count</i> columns of the update form. <ul style="list-style-type: none"> a) Use common names from the standard list (Appendix D) as revised names. b) If you must use a non-standard name, provide the page, line number, specimen tag number, and the taxonomic reference in the <i>Comments</i> section of the update form.
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. <ul style="list-style-type: none"> a) Record the revised name and count for one species on the line of the original unknown. b) Record the revised name and count for the second species as a new record on the update form. <ul style="list-style-type: none"> i) Record the page, line number, and specimen tag number from the original unknown record on the next available blank line of the update form. ii) Mark <i>New Record</i> Leave the <i>Original Name</i> and <i>Original Count</i> columns blank. iii) Record the revised name and count in the <i>Revised Name</i> and <i>Revised Count</i> columns.
7)	After reconciling all of the unknowns, submit the completed update forms to the NARS IM staff in Corvallis. Retain the preserved UNK/RNG voucher samples. Contact your regional EPA coordinator if you cannot store the samples at your facility.

Table 10.8 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 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 Field Logistics Coordinator for further guidance (Chris Turner, cturmer@glec.com, 715-829-3737).
3) After preparing the preserved QA voucher sample, check that the sample ID number recorded on the fish gear form matches the preprinted label attached to each sample jar, and that the number of jars recorded on the fish gear form is correct.
4) 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.
5) 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 coordinator if your laboratory does not have the capability of handling waste formalin.
6) 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.
7) Decant the tap water. Use 45%-50% isopropyl alcohol or 70% ethanol as a final preservative for specimens.
8) When ready to ship all of the QA voucher samples, complete a sample tracking form as described in Appendix C.
9) Package the preserved samples properly for either formalin or ethanol and prepare all required documentation and safety measures for the shipment.
10) Include a CD with the photovoucher files for each QA voucher sample in the shipment. Use the file names that are recorded on the fish gear form.

11 FISH TISSUE PLUG SAMPLING METHODS

11.1 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 which uses a tissue plug instead of a skin-on fillet. 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 every site. These fish are collected during the fish assemblage sample collection effort (Section 10). 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.

11.2 Equipment and Supplies

Table 11.1 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 Gear and Voucher/Tissue Sample Information Form (**Figure 10.6**).

Table 11.1 Equipment and Supplies: Fish Tissue Plug Sample

For fish tissue plug samples	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 (Acuderm brand Acu-Punch or equivalent) Sterile disposable scalpel Sterile forceps Laboratory pipette bulb. Antibiotic salve. Fish collection gear (electrofisher, nets, livewell, etc.) Dip net Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Fish tissue plug sample labels Fish Gear and Sampling Form Soft (#2) lead pencils for recording data on field forms Fine-tipped indelible markers for filling out sample labels Clear tape strips for covering labels

11.3 Sample Collection Procedures

The fish tissue plug indicator samples will be collected using the same gear and procedures used to collect the fish assemblage. Collection of individual specimens for fish tissue occurs in the sample reach during the fish assemblage sampling. Samples should be taken from the species listed in the target list found in **Table 11.2**. 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 sample from the species that are available. Recommended and alternate target species are given in **Table 11.2**. The procedures for collecting and processing fish plug samples are presented in **Table 11.3**.

Table 11.2 Recommended Target and Alternate Species for Fish Tissue Plug Collection

	Family name	Common name	Scientific name	Length Guideline (Estimated Minimum)
Target Species	Centrarchidae	Spotted bass	<i>Micropterus punctulatus</i>	~280 mm
		Largemouth bass	<i>Micropterus salmoides</i>	~280 mm
		Smallmouth bass	<i>Micropterus dolomieu</i>	~300 mm
		Black crappie	<i>Pomoxis nigromaculatus</i>	~330 mm
		White crappie	<i>Pomoxis annularis</i>	~330 mm
	Ictaluridae	Channel catfish	<i>Ictalurus punctatus</i>	~300 mm
		Blue catfish	<i>Ictalurus furcatus</i>	~300 mm
		Flathead catfish	<i>Pylodictis olivaris</i>	~300 mm
	Percidae	Sauger	<i>Sander canadensis</i>	~380 mm
		Walleye	<i>Sander vitreus</i>	~380 mm
		Yellow perch	<i>Perca flavescens</i>	~330 mm
	Moronidae	White bass	<i>Morone chrysops</i>	~330 mm
	Esocidae	Northern pike	<i>Esox lucius</i>	~430 mm
		Chain pickerel	<i>Esox niger</i>	~430 mm
	Salmonidae	Brown trout	<i>Salmo trutta</i>	~300 mm
		Cutthroat trout	<i>Oncorhynchus clarkii</i>	~300 mm
Rainbow trout		<i>Oncorhynchus mykiss</i>	~300 mm	
Brook trout		<i>Salvelinus fontinalis</i>	~330 mm	
Alternates	Cyprinidae	Northern pikeminnow	<i>Ptychocheilus oregonensis</i>	~300 mm
	Centrarchidae	Bluegill	<i>Lepomis macrochirus</i>	~200 mm
		Rock bass	<i>Ambloplites rupestris</i>	~200 mm
		Redbreast sunfish	<i>Lepomis auritus</i>	~200 mm

Table 11.3 Procedure: Fish Tissue Plug Samples

Fish Tissue Plug Methods
<ol style="list-style-type: none">1. Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellent until after the composite sample has been collected, measured, and wrapped.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 (refer to Table 11.2 for minimum species length guidelines). Select fish based on the following criteria:<ul style="list-style-type: none">• is on the target list,• both the same species• both satisfy legal requirements of harvestable size (or weight) for the sampled river, or at least be of consumable size if no legal harvest requirements are in effect, and• 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 retained for analysis 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 site ID, date, sample ID, species, and specimen length and weight on the back of the Fish Gear and Sampling Form in the Fish Tissue Plug section (Figure 10.6). Make sure the sample ID numbers and specimen numbers/lengths that are recorded on the collection form match those on the sample tracking form and labels where applicable.9. Prepare a Sample Identification Label for the sample, ensuring that the label information matches the information recorded on the Fish Tissue Plug section of the Fish Gear and Sampling Form. Affix 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 scalpel11. 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.15. Place the sample immediately on dry ice for shipment.16. Dispose of gloves, scalpel and biopsy punch.

Fish Tissue Plug Methods

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.

12 WHOLE FISH SAMPLING METHOD

12.1 Method Summary

Fish are important integrators of toxic contaminants that are bioavailable in the water column and in sediment. EPA monitors the occurrence of toxic chemicals in fish fillet samples to assess the potential health impacts to people who consume fish. Results from the 2008/09 NRSA provided the first statistically representative national data for fish contamination in U.S. rivers. Collecting whole fish tissue samples and submitting them to the laboratory for filleting and homogenization during the 2013/14 NRSA allows consistency with fish tissue methods of the previous NRSA, and provides tissue amounts that are sufficient to allow the analysis of multiple chemical contaminants of concern. It will also provide the first opportunity for temporal analysis of probability-based national fish contamination trends in U.S. rivers. Additionally, collecting fish at locations sampled during the previous NRSA will reduce the variability in data for trends analysis.

Whole fish tissue sampling procedures are described in detail in **Table 2.3**. The objective is to collect one whole fish tissue sample from each of the 453 designated target sites. The focus is on fish species that commonly occur throughout the region of interest and that are sufficiently abundant within a sampling reach. Each whole fish tissue sample will consist of five adult fish of the same species that are similar in size (the smallest individual in the sample is no less than 75% of the total length of the largest individual). Collection occurs in the sampling reach. Whole fish samples are shipped to the laboratory designated for fish sample preparation. The laboratory fillets the fish and homogenizes the fillet tissue for analysis of mercury and other contaminants (e.g., perfluorinated compounds).

12.2 Equipment and Supplies

Table 11.4 lists the equipment and supplies necessary for field crews to collect whole fish tissue 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 sampling data on the Whole Fish Tissue Collection Form (**Figure 11.1**).

Table 11.4 Equipment and Supplies: Whole Fish Tissue Sample Collection

<p>For collecting whole fish tissue sample</p>	<p>Electrofishing equipment (including variable voltage pulsator unit, wiring cables, generator, electrodes, dip nets, protective gloves, boots, and necessary safety equipment) Scientific collection permit Sampling vessel (including boat, motor, trailer, oars, gas, and all required safety equipment)</p>	<p>Coast Guard approved personal floatation devices Maps of target sites & access routes Global Positioning System (GPS) unit Livewell and/or buckets Measuring board (millimeter scale) Clean nitrile gloves</p>
<p>For storing and preserving whole fish tissue sample</p>	<p>Aluminum foil (solvent rinsed and baked) Heavy-duty food grade polyethylene tubing Large plastic (composite) bags</p>	<p>Knife or scissors Dry Ice Plastic cable ties Coolers</p>
<p>For documenting the whole fish tissue sample</p>	<p>Whole Fish Tissue Collection Form Clipboard Clear tape strips</p>	<p>Sample Identification Labels #2 pencils Fine tipped indelible markers</p>
<p>For shipping the whole fish tissue samples</p>	<p>Preaddressed FedEx airbill Coolers</p>	<p>Tracking Form Chain-of-custody labels Packing/strapping tape</p>

12.3 Sampling Procedures

The whole fish tissue samples will be collected using the same gear and procedures used to collect the fish assemblage samples. Collection of individual specimens for whole fish samples occurs in the sample reach during the fish assemblage sampling. Ideally, each fish sample will contain 5 fish of the same species that are similar in size. Depending on the size of the fish, fewer than 5 fish may be acceptable or more than 5 fish will be necessary to meet the 500-gram tissue requirement for chemical analysis and archived tissue (refer to Frequently Asked Questions in the sampling kits). If sufficient fish are not collected during the fish assemblage sampling, sample for up to one additional hour. If no fish can be collected, call the Field Logistics Coordinator at the end of the day and record “no sample collected” on the whole fish tissue collection form, along with the reason in the comments section of the form. 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, and in sufficient numbers to yield a fish sample with adequate tissue for analysis) to obtain a whole fish sample from the species that are available. Recommended target species are given in **Table 11.5**. The procedures for collecting and processing whole fish tissue samples are presented in

Table 11.6.

Table 11.5 Recommended Target Species for Whole Fish Tissue Collection

Target Species	Family name	Common name	Scientific name	Length Guideline (Estimated Minimum)
	Target Species	Centrarchidae	Spotted bass	<i>Micropterus punctulatus</i>
Largemouth bass			<i>Micropterus salmoides</i>	~280 mm
Smallmouth bass			<i>Micropterus dolomieu</i>	~300 mm
Black crappie			<i>Pomoxis nigromaculatus</i>	~330 mm
White crappie			<i>Pomoxis annularis</i>	~330 mm
Ictaluridae		Channel catfish	<i>Ictalurus punctatus</i>	~300 mm
		Blue catfish	<i>Ictalurus furcatus</i>	~300 mm
		Flathead catfish	<i>Pylodictis olivaris</i>	~300 mm
Percidae		Sauger	<i>Sander canadensis</i>	~380 mm
		Walleye	<i>Sander vitreus</i>	~380 mm
		Yellow perch	<i>Perca flavescens</i>	~330 mm
Moronidae		White bass	<i>Morone chrysops</i>	~330 mm
Esocidae		Northern pike	<i>Esox lucius</i>	~430 mm
		Chain pickerel	<i>Esox niger</i>	~430 mm
Salmonidae		Brown trout	<i>Salmo trutta</i>	~300 mm
		Cutthroat trout	<i>Oncorhynchus clarkii</i>	~300 mm
	Rainbow trout	<i>Oncorhynchus mykiss</i>	~300 mm	
	Brook trout	<i>Salvelinus fontinalis</i>	~330 mm	

Table 11.6 Sampling Procedures for Whole Fish Tissue Samples

Whole Fish Tissue Method	
1.	Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellent until after the whole fish sample has been collected, measured, and wrapped.
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.	Collect one target species sample from each designated site. The sample should consist of 5 fish of adequate size to provide a total of 500 grams of edible tissue for analysis (refer to Table 11.5 for minimum species length guidelines). Select fish for each sample based on the following criteria: <ul style="list-style-type: none">• all are of the same species,• all satisfy legal requirements of harvestable size (or weight) for the sampled river, or at least be of consumable size if no legal harvest requirements are in effect,• all are of similar size, so that the smallest individual in a composite is no less than 75% of the total length of the largest individual, and• all are collected at the same time, i.e., collected as close to the same time as possible, but no more than one week apart (Note: Individual fish may have to be frozen until all fish to be included in the sample are available for delivery to the designated laboratory). <p>Accurate taxonomic identification is essential in assuring and defining the organisms that have been collected and submitted for analysis. Do not mix species in a single whole fish sample.</p>
4.	Measure each individual fish to determine total body length. Measure total length of each 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).
5.	Record site ID, date, sample ID, species (common name), and specimen length on the Whole Fish Tissue Collection Form (Figure 11.1) in black ink. Fill in site type (“Wadeable” or “Boatable”) at the top of the form. Address the two sample criteria in the space above the fish specimen data to confirm compliance. All samples must meet these two criteria (i.e., fish are all the same species and fish lengths are all within 75% of the largest specimen length). Make sure the sample ID numbers and specimen numbers/lengths that are recorded on the collection form match the corresponding information on each individual specimen label.
6.	Remove each fish selected for analysis from the clean holding container(s) (e.g., livewell) using clean nitrile gloves. Dispatch each fish using a clean wooden bat (or equivalent wooden device).
7.	Wrap each fish in extra heavy-duty aluminum foil with the dull side in (foil provided by EPA as solvent-rinsed, oven-baked sheets).
8.	Prepare a Sample Identification Label for each sample, ensuring that the label information matches the information recorded on the Whole Fish Tissue Collection Form.
9.	Cut a length of food grade tubing (provided by EPA) that is long enough to contain each individual fish and to allow extra length on each end to secure with cable ties. Place each foil wrapped specimen into the appropriate length of tubing. Seal each end of the tubing with a plastic cable tie. Attach the fish sample label to the outside of the food grade tubing with clear tape and secure the label by taping around the entire fish (so that tape sticks to tape).
10.	Place all the wrapped fish in the sample from each site in a large plastic bag and seal with another cable tie.
11.	After each sample is packaged, place it immediately on dry ice for shipment. If samples will be carried back to a laboratory or other facility to be frozen before shipment, wet ice can be used to transport wrapped and bagged fish samples in the coolers to a laboratory or other interim facility.
12.	If possible, keep all (five) specimens designated for a particular sample in the same shipping

Whole Fish Tissue Method

container (ice chest) for transport.

13. Samples may be stored temporarily on dry ice (replenishing the dry ice daily). You have the option, depending on site logistics, of:
 - shipping the samples packed on dry ice in sufficient quantities to keep samples frozen for up to 48 hours (50 pounds are recommended), via priority overnight delivery service (e.g., Federal Express), so that they arrive at the sample preparation laboratory within less than 24 hours from the time of sample collection, or
 - freezing the samples within 24 hours of collection at $\leq -20^{\circ}\text{C}$, and storing the frozen samples until shipment within 2 weeks of sample collection (frozen samples will subsequently be packed on dry ice and shipped to the sample preparation laboratory via priority overnight delivery service).
14. Ship fish tissue samples to the designated laboratory for fish sample preparation on Monday through Thursday (no Saturday delivery to the laboratory).

NRSA 2013/14 WHOLE FISH TISSUE COLLECTION				
		<input type="radio"/> WADEABLE <input type="radio"/> BOATABLE		Reviewed by (initial): _____
Site ID: _____	Date: ____ / ____ / ____		PAGE: ____ OF ____	
WHOLE FISH TISSUE FILLET SAMPLE (FTIS)			SAMPLE ID: _____	NO SAMPLE COLLECTED <input type="radio"/>
<input type="radio"/> FISH ARE ALL THE SAME SPECIES		<input type="radio"/> FISH ALL WITHIN 75% OF LARGEST SPECIMEN		
	Common Name	Total Length (mm)	Frozen	Comments
.01			<input type="radio"/>	
.02		<input type="radio"/>		
.03		<input type="radio"/>		
.04		<input type="radio"/>		
.05		<input type="radio"/>		
.06*		<input type="radio"/>		
.07*		<input type="radio"/>		
.08*		<input type="radio"/>		
.09*		<input type="radio"/>		
.10*		<input type="radio"/>		
*Additional specimens for smaller fish species to ensure sufficient tissue is available for chemical analysis of fillet tissue.				
04/08/2013 2013 Fish Tissue Collection		2227506532		

Figure 11.1 Whole Fish Tissue Collection Form

13 FINAL SITE ACTIVITIES

13.1 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, report the sampling event to the Information Management Coordinator, and ship or store the samples. Activities described in this section are summarized in **Figure 13.1**.

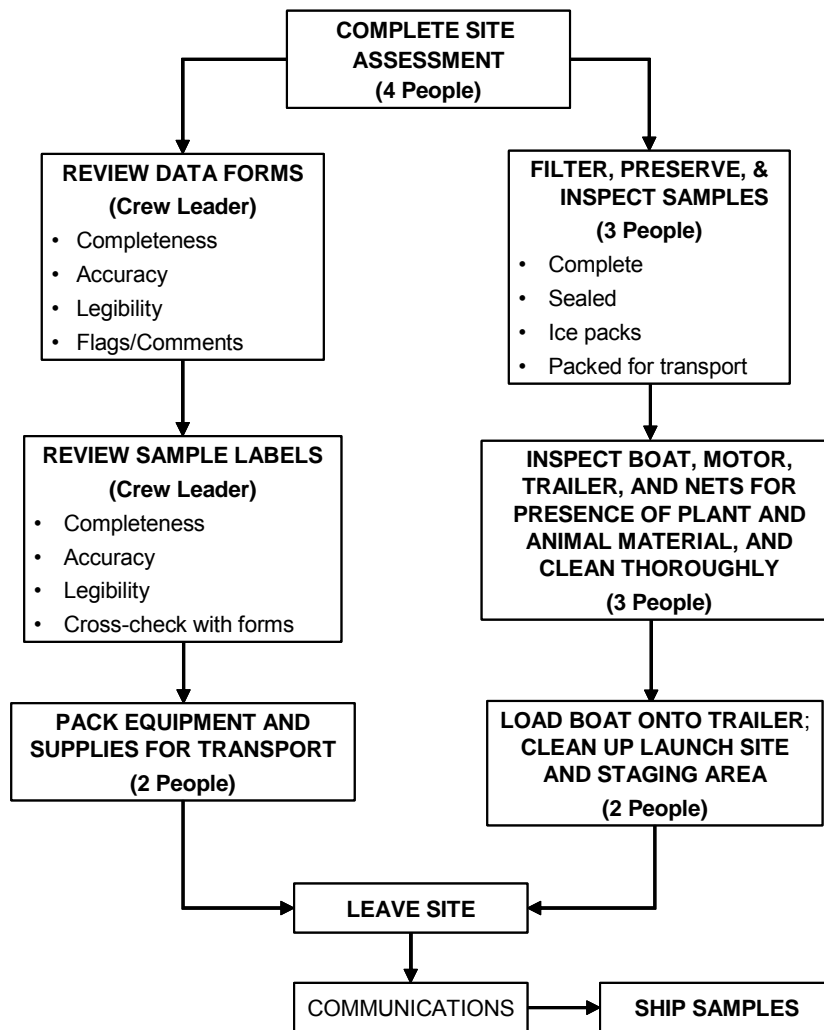


Figure 13.1 Final Site Activities Summary

13.2 General Site Assessment

Complete the Site Assessment Form (**Figure 13.2**) after sampling, 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.

13.2.1 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.

13.2.2 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 (**Figure 13.2**). 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. The distinction between low, moderate, and heavy will be subjective. For example, if there are two to three houses on a site, circle “L” for low next to “Houses.” If the site is ringed 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.

13.2.3 Site Characteristics

Record observations regarding the general characteristics of the site on the Site Assessment Form (**Figure 13.2 & Figure 13.3**). 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 just in the days prior to sampling.

13.2.4 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.

NRSA 2013/14 ASSESSMENT (Front)					Reviewed by (initial): _____
Site ID: _____		Date: ____ / ____ / ____			
Elevation at transect K: _____ <input type="radio"/> ft <input type="radio"/> m					
WATERSHED ACTIVITIES AND DISTURBANCES OBSERVED					BLANK FIELD INDICATES ABSENCE: <input type="radio"/>
(Intensity: Blank=Not observed, L=Low, M=Moderate, H=Heavy)					
Residential	Recreational	Agricultural	Industrial	Stream Management	
<input type="radio"/> <input type="radio"/> <input type="radio"/> Residences <input type="radio"/> <input type="radio"/> <input type="radio"/> Maintained Lawns <input type="radio"/> <input type="radio"/> <input type="radio"/> Construction <input type="radio"/> <input type="radio"/> <input type="radio"/> Pipes, Drains <input type="radio"/> <input type="radio"/> <input type="radio"/> Dumping <input type="radio"/> <input type="radio"/> <input type="radio"/> Roads <input type="radio"/> <input type="radio"/> <input type="radio"/> Bridges/Causeway <input type="radio"/> <input type="radio"/> <input type="radio"/> Sewage Treatment	<input type="radio"/> <input type="radio"/> <input type="radio"/> Hiking Trails <input type="radio"/> <input type="radio"/> <input type="radio"/> Parks, Campgrounds <input type="radio"/> <input type="radio"/> <input type="radio"/> Primitive Parks, Camping <input type="radio"/> <input type="radio"/> <input type="radio"/> Trash/Litter <input type="radio"/> <input type="radio"/> <input type="radio"/> Surface Films, Scums, or Slicks	<input type="radio"/> <input type="radio"/> <input type="radio"/> Cropland <input type="radio"/> <input type="radio"/> <input type="radio"/> Pasture <input type="radio"/> <input type="radio"/> <input type="radio"/> Livestock Use <input type="radio"/> <input type="radio"/> <input type="radio"/> Orchards <input type="radio"/> <input type="radio"/> <input type="radio"/> Poultry <input type="radio"/> <input type="radio"/> <input type="radio"/> Feedlot <input type="radio"/> <input type="radio"/> <input type="radio"/> Water Withdrawal	<input type="radio"/> <input type="radio"/> <input type="radio"/> Industrial Plants <input type="radio"/> <input type="radio"/> <input type="radio"/> Mines/Quarries <input type="radio"/> <input type="radio"/> <input type="radio"/> Oil/Gas Wells <input type="radio"/> <input type="radio"/> <input type="radio"/> Power Plants <input type="radio"/> <input type="radio"/> <input type="radio"/> Logging <input type="radio"/> <input type="radio"/> <input type="radio"/> Evidence of Fire <input type="radio"/> <input type="radio"/> <input type="radio"/> Odors <input type="radio"/> <input type="radio"/> <input type="radio"/> Commercial	<input type="radio"/> <input type="radio"/> <input type="radio"/> Liming <input type="radio"/> <input type="radio"/> <input type="radio"/> Chemical Treatment <input type="radio"/> <input type="radio"/> <input type="radio"/> Angling Pressure <input type="radio"/> <input type="radio"/> <input type="radio"/> Dredgong <input type="radio"/> <input type="radio"/> <input type="radio"/> Channelization <input type="radio"/> <input type="radio"/> <input type="radio"/> Water Level Fluctuations <input type="radio"/> <input type="radio"/> <input type="radio"/> Fish Stocking <input type="radio"/> <input type="radio"/> <input type="radio"/> Dams	
SITE CHARACTERISTICS (200m radius)					
WATERBODY CHARACTER					
PRISTINE: <input type="radio"/> 5 <input type="radio"/> 4 <input type="radio"/> 3 <input type="radio"/> 2 <input type="radio"/> 1 Highly Disturbed					
APPEALING: <input type="radio"/> 5 <input type="radio"/> 4 <input type="radio"/> 3 <input type="radio"/> 2 <input type="radio"/> 1 Unappealing					
BEAVER					
Beaver Signs: <input type="radio"/> Absent <input type="radio"/> Rare <input type="radio"/> Common					
Beaver Flow Modifications: <input type="radio"/> None <input type="radio"/> Minor <input type="radio"/> Major					
DOMINANT LAND USE					
Dominant Land Use Around 'X' <input type="radio"/> Forest <input type="radio"/> Agriculture <input type="radio"/> Range <input type="radio"/> Urban <input type="radio"/> Suburban/Town					
If Forest, Dominant Age Class <input type="radio"/> 0 - 25 yrs. <input type="radio"/> 25 - 75 yrs. <input type="radio"/> > 75 yrs.					
WEATHER					
CONDITIONS AND LOCAL CONTACTS					
OBSERVATIONS (e.g. accessibility, boating, fishing, swimming, health concerns):					
04/08/2013 2013 Assessment		0351596807			

Figure 13.2 Site Assessment Form (front)

NRSA 2013/14 ASSESSMENT (Back)			Reviewed by (Initial): _____
Site ID: _____	Date: _____ / _____ / _____		
GENERAL ASSESSMENT AND COMMENTS			
INVASIVE OR NUISANCE SPECIES OF LOCAL INTEREST			
<small>Record species of plants and animals that were observed but are not on the invasive plant form. Examples would be Zebra Mussel or New Zealand Mud Snail, or invasive plants or animals of concern to a particular state. Indicate your level of confidence in your identification, and provide some idea of how prevalent it is in the sampling reach or adjacent riparian area.</small>			
Species (Common Name)	Confidence	Prevalence	Comments
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
	<input type="radio"/> LOW <input type="radio"/> HIGH	<input type="radio"/> DOMINANT <input type="radio"/> SPARSE <input type="radio"/> COMMON	
04/08/2013 2013 Assessment		0986596801	

Figure 13.3 Site Assessment Form (back)

13.3 Processing the Fecal Indicator, Chlorophyll-*a*, and Periphyton Samples

13.3.1 Equipment and Supplies (Fecal Indicator Filtering)

Table 13.1 provides the equipment and supplies needed for field crews to collect the fecal indicator sample.

Table 13.1 Equipment and Supplies: Fecal Indicator Sample Processing

For processing samples	<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) Osmotics 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 Dry ice Cooler Field Operations Manual and laminated Quick Reference Guide</p>
For recording measurements	<p>Sample Collection Form Soft (#2) lead pencils for recording data on field forms 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 Clear tape strips for covering labels</p>

13.3.2 Procedures for Processing the Fecal Indicator 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 13.2**. The sample must be filtered and frozen within 6 hours of collection.

Table 13.2 Procedure: Fecal Indicator Sample Processing

Filtering for the Enterococci (fecal indicator) sample
<ol style="list-style-type: none"> 1. Put on nitrile gloves. 2. Set up sample filtration apparatus on flat surface and attach vacuum pump. Set-out 50-mL sterile PP tube, sterile 60-mm Petri dish, 2 bottles of chilled phosphate buffered saline (PBS), Osmotics 47 mm polycarbonate sterile filter box, and 2 filter forceps. 3. Chill Filter Extraction tubes with beads on dry ice. 4. Aseptically transfer 2 polycarbonate filters from filter box to base of opened Petri dish. Close filter box and set aside. 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. Shake sample bottle(s) 25 times to mix well.
 8. Measure 25-mL of the mixed water sample in the sterile graduated 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 pressure). 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 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 4 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, insert tube(s) into ziplock bag on dry ice for preservation during transport and shipping.
 15. Record the volume of water sample filtered through each filter and the volume of buffer rinsate each filter was rinsed with on the Sample Collection Form, Side 2. Record the filtration start time and finish time for each sample.

Repeat steps 6 to 15 for the remaining 50-mL sub-sample volume to be filtered.

Processing Procedure—fecal indicator filter blank

Enterococci filter blanks will be prepared at all revisit sites during the first visit. Prepare the filter blanks **before** filtering the river 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.
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 pressure). 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, insert tube(s) into ziplock bag on dry ice for preservation during transport and shipping.
9. Label the samples as “blank” on the label and field form, and package and submit this sample to the lab with the standard samples.
10. Indicate that you have collected a filter blank by filling in the “Blank Collected” button on the

Sample Collection Form.

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

Table 13.3 provides the equipment and supplies needed to process the chlorophyll-*a* water sample.

Table 13.3 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
For recording measurements	Sample Collection Form Sample labels #2 pencils Fine-tipped indelible markers Clear tape strips

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

The procedures for processing chlorophyll-*a* water samples are presented in **Table 13.4**. Whenever possible, sample processing should be done in subdued light, out of direct sunlight.

Table 13.4 Procedure: Chlorophyll-*a* Sample Processing

Filtering for the chlorophyll <i>a</i> water sample
<ol style="list-style-type: none"> 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 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). 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 with.</i> 5. Observe the filter for visible color. If there is 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. 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 (do not cover the volume markings on the 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 a self-sealing plastic bag. Place this bag immediately on dry ice to freeze.

13.3.5 Equipment and Supplies (Periphyton Sample)

Table 13.5 lists the equipment and supplies needed to process the periphyton sample.

Table 13.5 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 60-mL syringe with tip removed Aluminum foil squares Forceps deionized water in wash bottle plastic electrical tape dry ice wet ice coolers formalin
For data recording	Sample Collection Form Sample labels Pencils Fine-tipped indelible markers Clear tape strips

13.3.6 Procedures for Processing the Periphyton Samples

Three different types of laboratory samples are prepared from the composite index samples: an ID/enumeration sample (to determine taxonomic composition and relative abundances), 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.

13.3.6.1 ID/Enumeration Sample

Prepare the ID/Enumeration sample as a 50-mL aliquot from the composite index sample, following the procedure presented in Table 13.6. Preserve each sample with formalin. Record the sample ID number from the container label and the total volume of the periphyton sample in the appropriate fields on the Sample Collection Form as shown in **Error! Reference source not found.** Store the preserved samples upright in a container containing absorbent material.

Table 13.6 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 volume of the subsample (typically 50 mL) and the volume of the composite index sample on the label. Attach completed label to a 50-mL centrifuge tube; avoid covering the volume graduations and markings. Cover the label completely with a clear tape strip.
2. Record the sample ID number of the label and the total volume of the composite index sample on the Sample Collection Form.
3. Rinse a 60-mL syringe with deionized water.
4. Thoroughly mix the bottle containing the composite sample.
5. Immediately after mixing, withdraw 50 mL of the mixed sample into the syringe and place the contents of syringe into the labeled 50-mL centrifuge tube.
6. Use a syringe or bulb pipette to add 2ml 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.
7. Shake gently to distribute preservative.
8. Record the volume of the sample in the centrifuge tube (excluding the volume of preservative) in "Assemblage ID Subsample Vol." field of the Sample Collection Form.

13.3.6.2 Periphyton Chlorophyll *a* Sample

Prepare the periphyton chlorophyll *a* sample by filtering a 25-mL aliquot of the composite index sample through a 47 mm 0.7 micron GF/F glass fiber filter. The procedure for preparing periphyton chlorophyll *a* samples is presented in Table 13.7. 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 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 13.7 Procedure: Preparing Chlorophyll Samples of Periphyton

Periphyton Chlorophyll *a* Sample Processing Procedure

1. 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.
2. Rinse the sides of the filter funnel and the filter with a small volume of deionized water to prevent cross-contamination from the previously filtered sample.
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 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. 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.
9. 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.
10. Prepare a sample label (with pre-printed sample ID number sample type "PCHL") including the volume filtered, and attach it to the centrifuge tube. Cover the label completely with a strip of clear tape.
11. Place the centrifuge tube into a self-sealing plastic bag.
12. Record the sample ID number of the label and the total volume of the composite index 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.
13. Place the centrifuge tube containing the filter on dry ice.

13.3.6.3 Periphyton Biomass Sample (AFDM)

Prepare the ash-free dry mass (AFDM) 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 AFDM samples is presented in **Table 13.8**. If 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 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 13.8 Procedure: Preparing Ash-Free Dry Mass (AFDM) Samples of Periphyton

Periphyton AFDM Sample Processing Procedures

1. 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.
2. 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.
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 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. 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.
9. 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.
10. Prepare a sample label (with pre-printed sample ID number sample type "PBIO"), including the volume filtered, and attach it to the centrifuge tube. Cover the label completely with a strip of clear tape. Place the centrifuge tube into a self-sealing plastic bag.
11. Record the sample ID number of the label and the total volume of the composite index 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.
12. Place the centrifuge tube containing the filter on dry ice.

13.4 Data Forms and Sample Inspection

After the Site Assessment Form is completed, the Field Team Crew Leader reviews all of the data forms and sample labels for accuracy, completeness, and legibility. The other team 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 all forms. On each form, verify that all information has been recorded accurately, the recorded information is legible, and any flags are explained in the comments section. Ensure that written comments are legible, with no “shorthand” or abbreviations. Make sure there is no marking s in the scan code boxes. Make sure the header information is completed on all pages of each form. After reviewing each form initial the upper right corner of each page of the form.

Ensure that all samples are labeled, all labels are completely filled in, and each label is covered with clear plastic tape. 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.

13.5 Launch Site Cleanup

Load the boat on the trailer and inspect the boat, motor, and trailer for evidence of weeds and other macrophytes. Clean the boat, motor, and trailer as completely as possible before leaving the launch site. 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.

14 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 National Rivers and Streams Assessment.

14.1 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 14.1**.

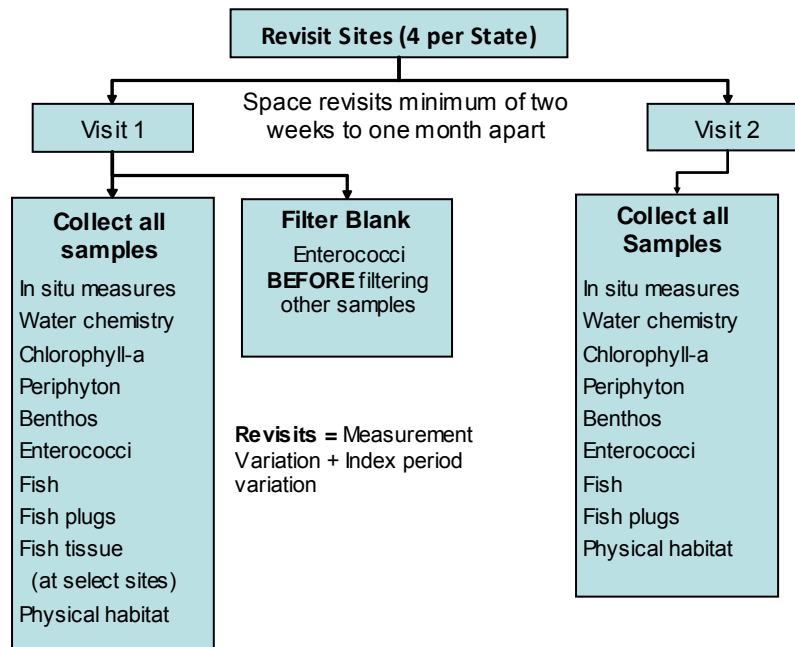


Figure 14.1 Summary of the Repeat Sampling Design

14.2 Revisit Sampling Sites

A total of 200 (approximately 10%) of the target sites visited will be revisited during the same field season 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 wadeable and two non-wadeable sites. For each state these sites are:

Wadeable Revisit sites:

- The two wadeable revisit sites are re samples from the NRSA 2008/09 (1-4th order). They are in the base sites in NRSA09, and have _RVT in the panel column. For most States, these are the first two sites in that category, but this is not always the case.

Non-Wadeable Revisit sites:

- The two non-wadeable revisit sites are resamples from the NRSA 2008/09 (5th and above order). They are in the base sites in NRSA09, and have _RVT in the panel column. For most States, these are the first two sites in that category, but this is not always the case.

If a site selected for revisit sampling is dropped, then the alternate assigned to replace it should be revisited. The alternate site is the next site on the sampling list, in the same category (i.e. Repeat NRSA 1-4th order). If a non-wadeable site is sampled with wadeable methods, the next non-wadeable site should be selected as the 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 and not more than one month. Label the samples Visit 2 to indicate that they are samples from revisit sites. We will not be collecting replicate data on whole fish tissue. Whole fish tissue will only be collected on the first visit.

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 them and write “blank” on the label and field form, and package and submit these samples to the lab. The filter blanks should be run before the sample is filtered. The filter blanks should be collected on the first field visit (Visit 1) (**Figure 14.1**). Detailed description of the filter blanks is found in **Table 13.2**.

14.3 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 Quality Assurance Project Plan (QAPP) for the NRSA. The following sections will focus only on the field evaluation and assistance visits.

These visits provide a quality assurance/quality control (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).

14.3.1 Specifications for QC Assurance Field Assistance Visits

Field evaluation and assistance personnel are trained in the specific data collection methods detailed in this Field Operations Manual. 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 14.1** 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 Field Operations Manual, all data are recorded correctly, and paperwork is properly completed at the site.

Table 14.1 General Information Noted During Field Evaluation

<p>Field Evaluation Plan</p>	<p>Field Logistics Coordinator will arrange the field evaluation visit with each Field Crew, ideally within the first two weeks of sampling.</p> <p>The Evaluator will observe the performance of a crew through one complete set of sampling activities.</p> <p>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.</p> <p>The Evaluator will review the results of the evaluation with the Field Crew before leaving the site, noting positive practices and problems.</p>
<p>Field Evaluation Checklist</p>	<p>The Evaluator observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and NRSA protocols are followed.</p> <p>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.</p> <p>The Evaluator confirms that the Field Crew has followed NRSA protocols for locating the site.</p> <p>The Evaluator observes the complete set of sampling activities, confirming that all protocols are followed.</p> <p>The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Check List.</p>
<p>Corrective Action Procedures</p>	<p>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.</p> <p>If the Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact a NRSA QA Project Coordinator.</p>

14.4 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 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.

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