Non Wadeable- Cliff notes (Make sure to read summary on pg 70-76)

1. Measure three places of typical wetted width on google earth to determine reach length with a maximum reach length of 4 km.

2. Record mid-channel GPS coordinates for each transect from google earth and determine random bank (Note: banks switch every two transects and boatable transects are reversed with A being the most upstream transect.(pg 44).

3. Water chemistry is taken at transect A which is now the most upstream transect.

4. Bugs- Do a 1 m sweep of dominant habitat in a plot 10 m along shore X 15 m streamward or to 0.5 to 1 m depth section if the former is too deep.(pg 60-61). We are not recording dominant or secondary substrate types.

5. Littoral/Riparian Cross-Sections (pg 80-83):
   - Wetted width and Mid-channel bar width (laser range finder).
   - Bankfull width (laser)
   - Bankfull and incision height (stadia rod and hand level).
   - Bank angle from wetted width 5 m into the bankfull margin (estimate) on chosen bank
   - Riparian canopy cover (densiometer) in four directions from chosen bank.
   - Shoreline Substrate in the first 1 m above waterline (estimated dominant and subdominant size class).

20m along shore X 10m streamward from chosen bank:

- At 5 locations systematically-spaced within plot record littoral depth, dominant, and subdominant substrate (depth rod). Note: we are not measuring substrate.
- Tally large woody debris in littoral plot and in bankfull channel by size and length class (pg 82). Note our size classes will be finer and are the same ones we are using for the wadeable protocol.
- Fish cover (same as wadeable)

20m along shore X 10m up **from bankfull** on BOTH banks:

- Riparian cover (same as wadeable)
- Human influence (same as wadeable but we have added some categories that the EPA field forms do not have)

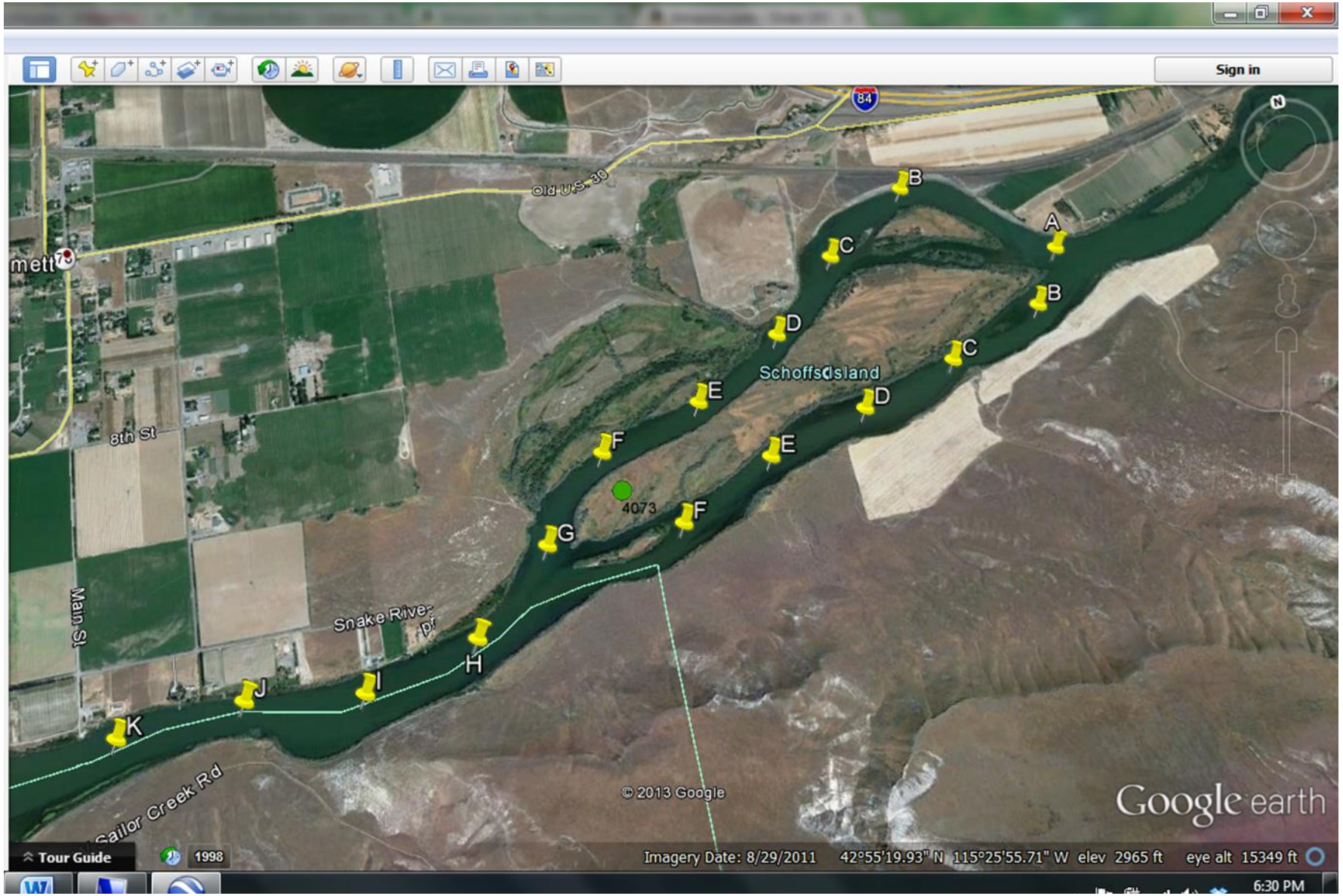
6. Thalweg Profile:
   - Measure thalweg every 40m (if the reach is 4 km) using Sonar or stadia rod
   - Note: we are NOT classifying habitat type, record presence of backwater and off-channel habitats.
   - Stadia Rod Drag (near impossible to do)
     - Determine dominant substrate
     - Record the presence of mid-channel snags

7. Channel constraint and assessment forms

Example reach layout and maps that need to be made for each site

Example mid-river coordinates for each transect with random chosen bank:

A -115.4220,42.9369 ---Right Bank
LB -115.4220,42.9369---Right Bank
RB-115.4267,42.9423---Right Bank
LC-115.4267,42.9355---Right Bank
RC-115.4309,42.9401---Right Bank
LD-115.4313,42.9343---Left Bank
RD-115.4344,42.9375---Left Bank
LE-115.4360,42.9333---Left Bank
RE-115.4386,42.9356---Left Bank
LF-115.4404,42.9316---Right Bank
RF-115.4433,42.9345---Right Bank
G-115.4464,42.9317---Right Bank
H-115.4500,42.9292---Left Bank
I-115.4548,42.9282---Left Bank
J-115.4598,42.9287---Right Bank
K-115.4650,42.9283---Right Bank


Sign in
84
Old U.S. 30
mett
8th St
Main St
Schoffsdsland
4073
Snake River
Gailor Creek Rd
A
B
C
D
E
F
G
H
I
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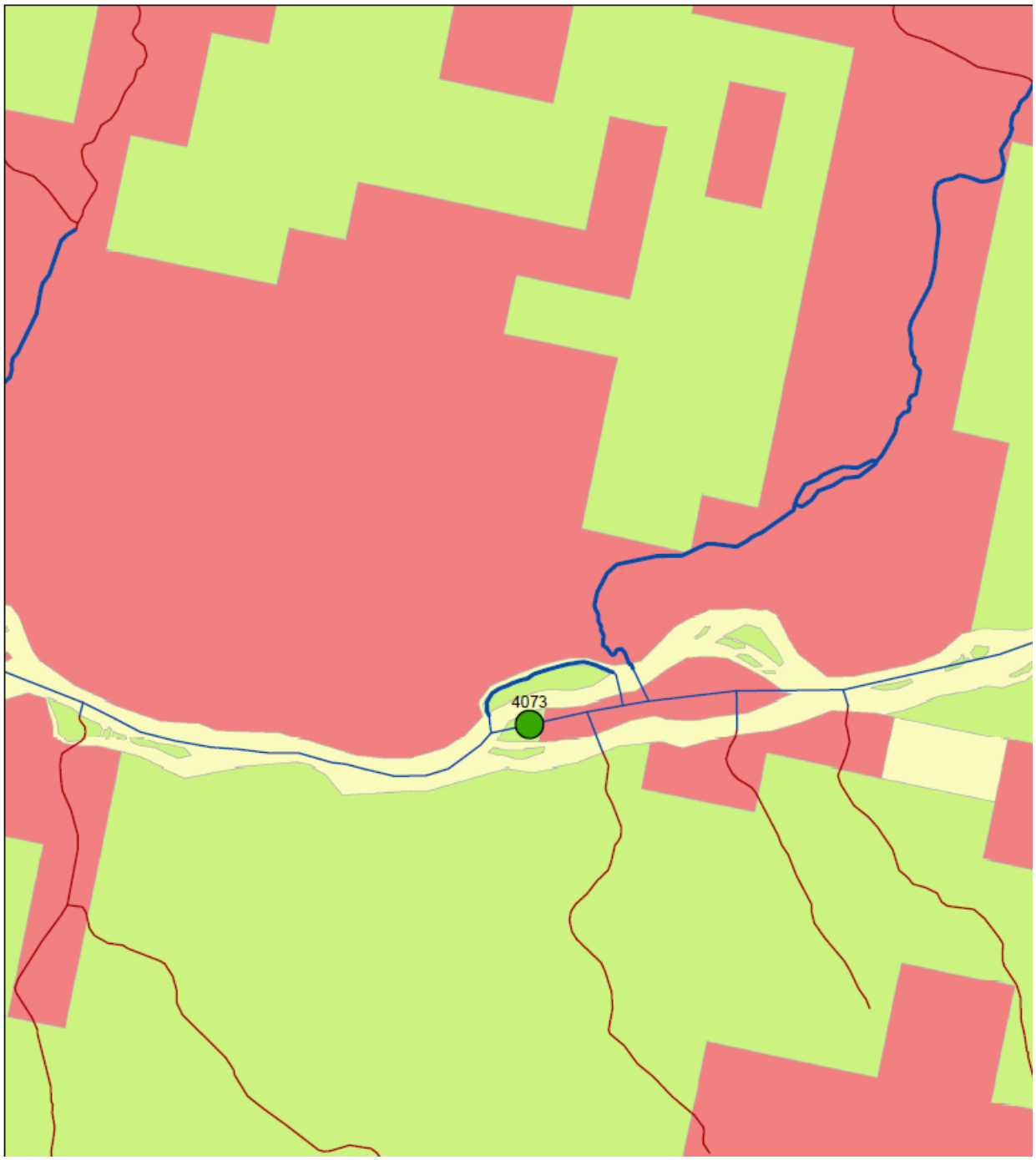
K
© 2013 Google
Google earth
Tour Guide
1998
Imagery Date: 8/29/2011 42°55'19.93" N 115°25'55.71" W elev 2965 ft eye alt 15349 ft
6:30 PM



Pink is private- green is BLM

United States Environmental Protection Agency
Office of Water
Office of Environmental Information
Washington, DC
EPA-841-B-12-009

National Rivers and Streams Assessment 2013/14

Field Operations Manual

Non-Wadeable

Draft Version 1

March 2013

DRAFT

DRAFT

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

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.

The suggested citation for this document is:

USEPA. 2013 (draft). National Rivers and Streams Assessment: Integrated Quality Assurance Project Plan. EPA-841-B-12-007. U.S. Environmental Protection Agency, Office of Water Washington, DC.

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ACRONYMS/ABBREVIATIONS

AFDM	Ash-Free Dry mass
ANC	Alkialinity
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
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
Na	Sodium
NABS	North American Benthic Society
NAD	North American Datum
NARS	National Aquatic Resources Survey
NAWQA	National Water-Quality Assessment Program
NCCA	National Costal Condition Assessment
NELAP	National Environmental Laboratory Accreditation Program
NERL	New England Regional Laboratory
NHD	National Hydrology Database
NH_3	Ammonia
NH_4	Ammonia-Nitrogen
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
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
RBS	Relative Bed Stability
RL	Reporting Limit
RTE	Rare, Threatened and Endangered
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
WT	Water Tube
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, 5^{th} 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 un-sampleable, 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 0-2 Strahler order sites.
- Large Streams- new 3-4 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 Transect A at mid-channel; readings are taken at 0.5 m depth, or mid-depth if water depth is less than 1 meter.
Water chemistry (TP, TN [NH_4, NO_3), basic anions and cations, alkalinity [ANC], DOC, TOC, TSS, conductivity	Core Indicator	Measurements taken at Transect A at mid-channel; 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 (*Enterococci*)	Supplemental Indicator	Collected at the last transect one meter off the bank
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.

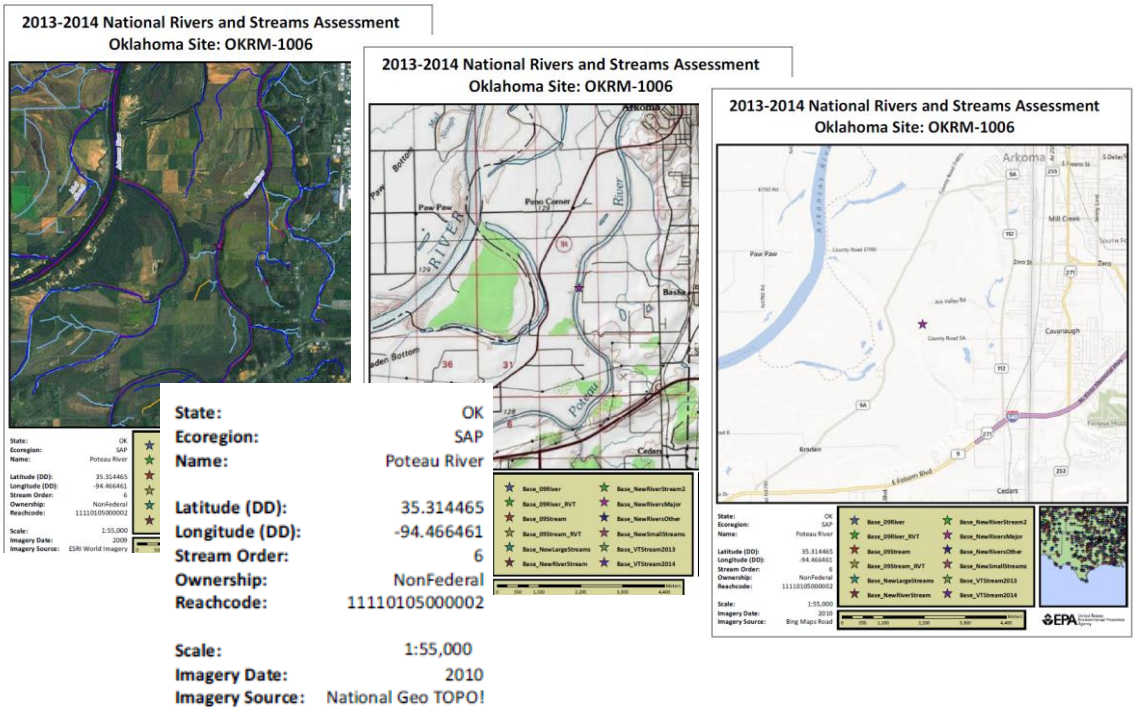


Figure 1.1 Example NRSA Site Maps

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).

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	• 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)	Use only defined flag codes and record on data form in appropriate field. K = Measurement not attempted or not recorded. Q = Failed quality control check; remeasurement not possible. U = Suspect measurement; remeasurement not possible. F*n* = Miscellaneous flags (*n* = 1, 2, etc.) assigned by a field crew during a particular sampling visit (also used for qualifying samples). Explain reason for using each flag in comments section on data form.
Sample Labels	• 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.

Activity	Guidelines
Sample Collection and Tracking	
Sample Qualifiers (Flags)	Use only defined flag codes and record on sample collection form in appropriate field. K = Sample not collected or lost before shipment; resampling not possible. U = Suspect sample (e.g., possible contamination, does not meet minimum acceptability requirements, or collected by non-standard procedure). Fn = Miscellaneous flags (n=1, 2, etc.) assigned by a field crew during a particular sampling visit (also used for field measurements). Explain reason for using flags in "Comments" on sample collection form.
Review of Labels and Data Collection Forms	• 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.

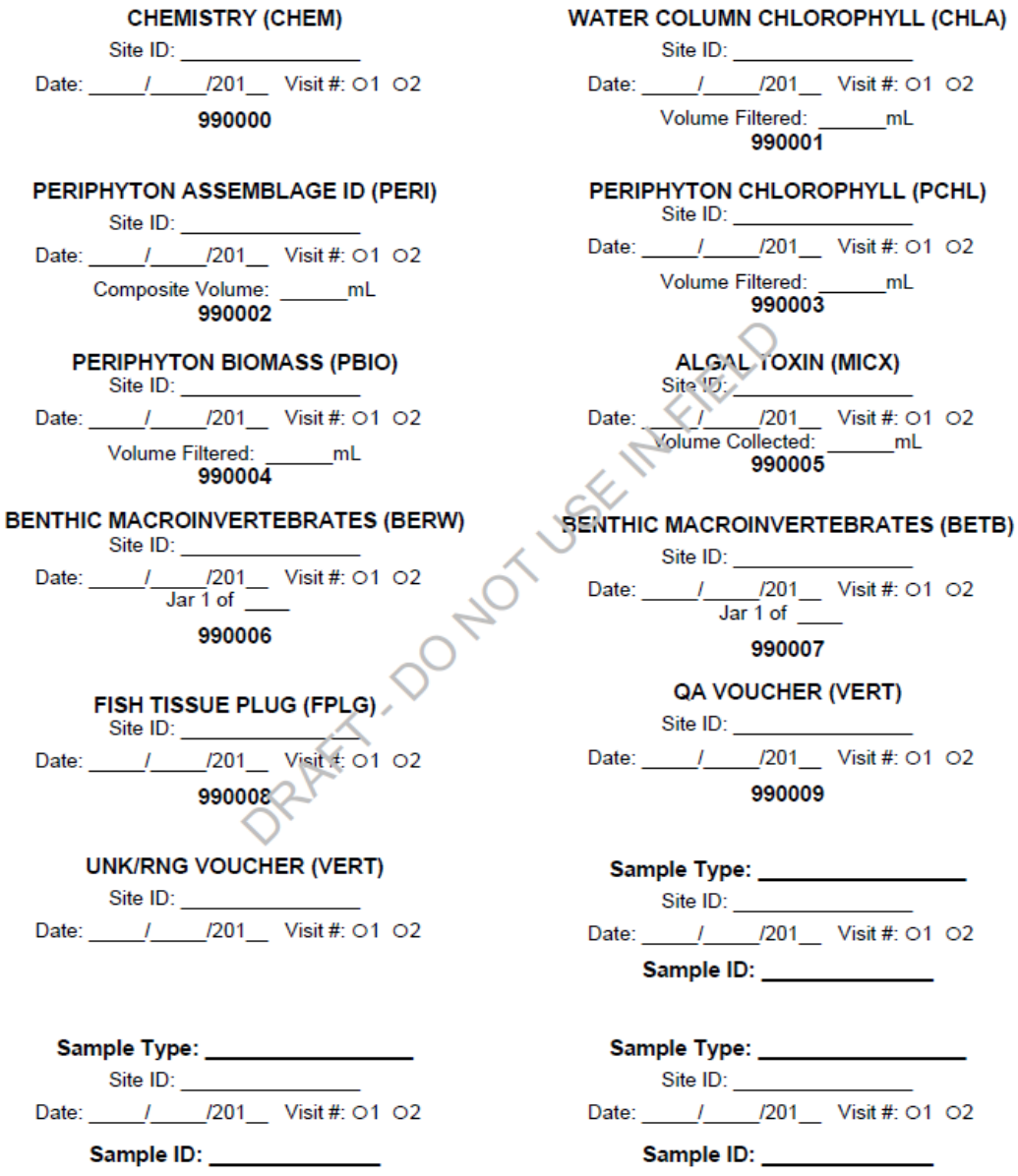


Figure 1.2 Example Sample Labels for Sample Tracking and Identification

2 INTRODUCTION TO NON-WADEABLE SAMPLING

2.1 Daily Operations

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

A field crew for a non-wadeable field crew typically will consist of four or five people in 2 boats. Additional crew members may either help collect samples, or may remain on the bank to provide logistical support. A minimum of two people are **always required** in a boat together to execute the sampling activities and to ensure safety.

Typically, in non-wadeable sites, two crew members will work in the "habitat" boat, and two or three will work in the "fish" boat. One crew member on each boat is primarily responsible for boat operation and navigation.

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.

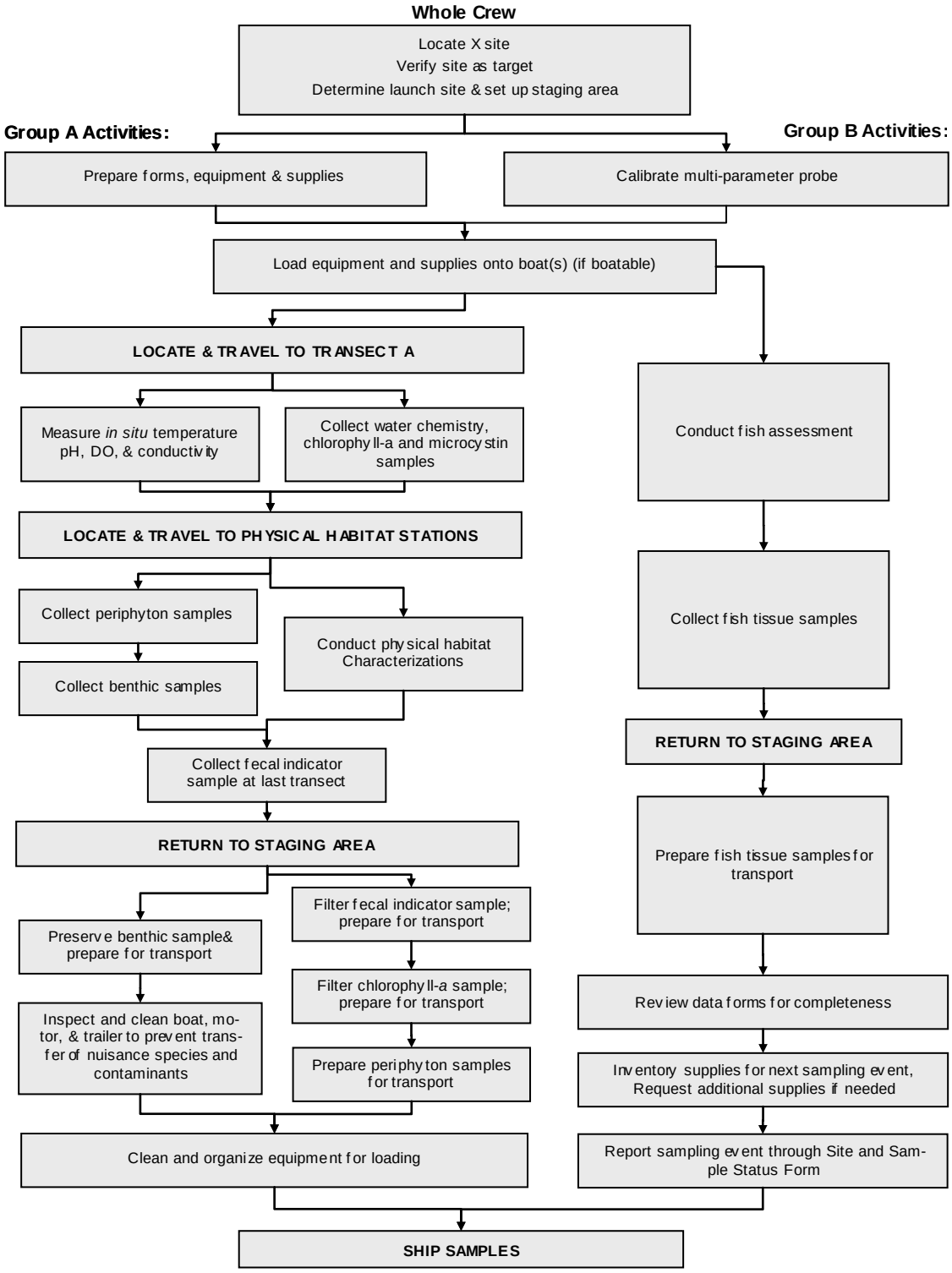


Figure 2.1 Field Sampling Scenario for Non-Wadeable Sites

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.

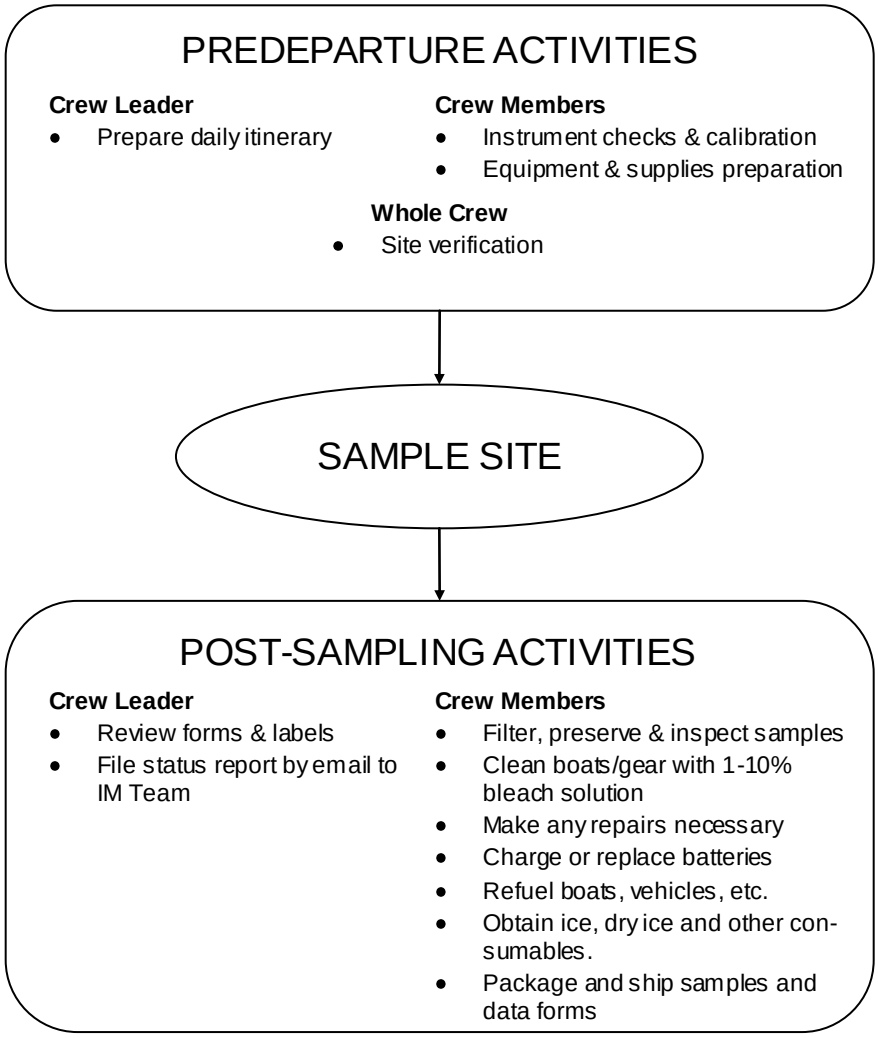


Figure 2.2 Overview of Base Site Activities

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. **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 (1-10%)	Clean nets, gear, and inside of boat	Add 10 -100 mL bleach to 1 L distilled water.
Calibration QCS	QCS for pH and conductivity calibration	Per manufacturer's guidelines or purchase already prepared)
95% Ethanol	Preserve benthic samples	None
10% Buffered Formalin	Preserve fish voucher samples and preserve periphyton ID sample	None

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 1-2 weeks). Take note of any supplies that are nearing depletion. Also note any items that may have been lost or damaged during the sampling event. Request additional site kits and/or supplies as needed via the electronic request form. Note that not all supplies can be replenished by EPA through the Logistics Contractor, so crews will need to supply some items themselves.

Table 2.2 Post-sampling Equipment Care

Equipment Care after Sampling
1. Clean for biological contaminants.
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. 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. 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. Additional precautions to prevent transfer of Whirling Disease spores, New Zealand mudsnails, and 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:

Equipment Care after Sampling
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
• 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
• 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 Guidleines 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 coordinates are located on dry land near a stream, move the coordinates to the nearest blue line during the desktop reconnaissance. Note this movement on the site recon tracking form and in the comments section of the Site Verification form

Table 3.1 Equipment and Supplies List for Site Verification

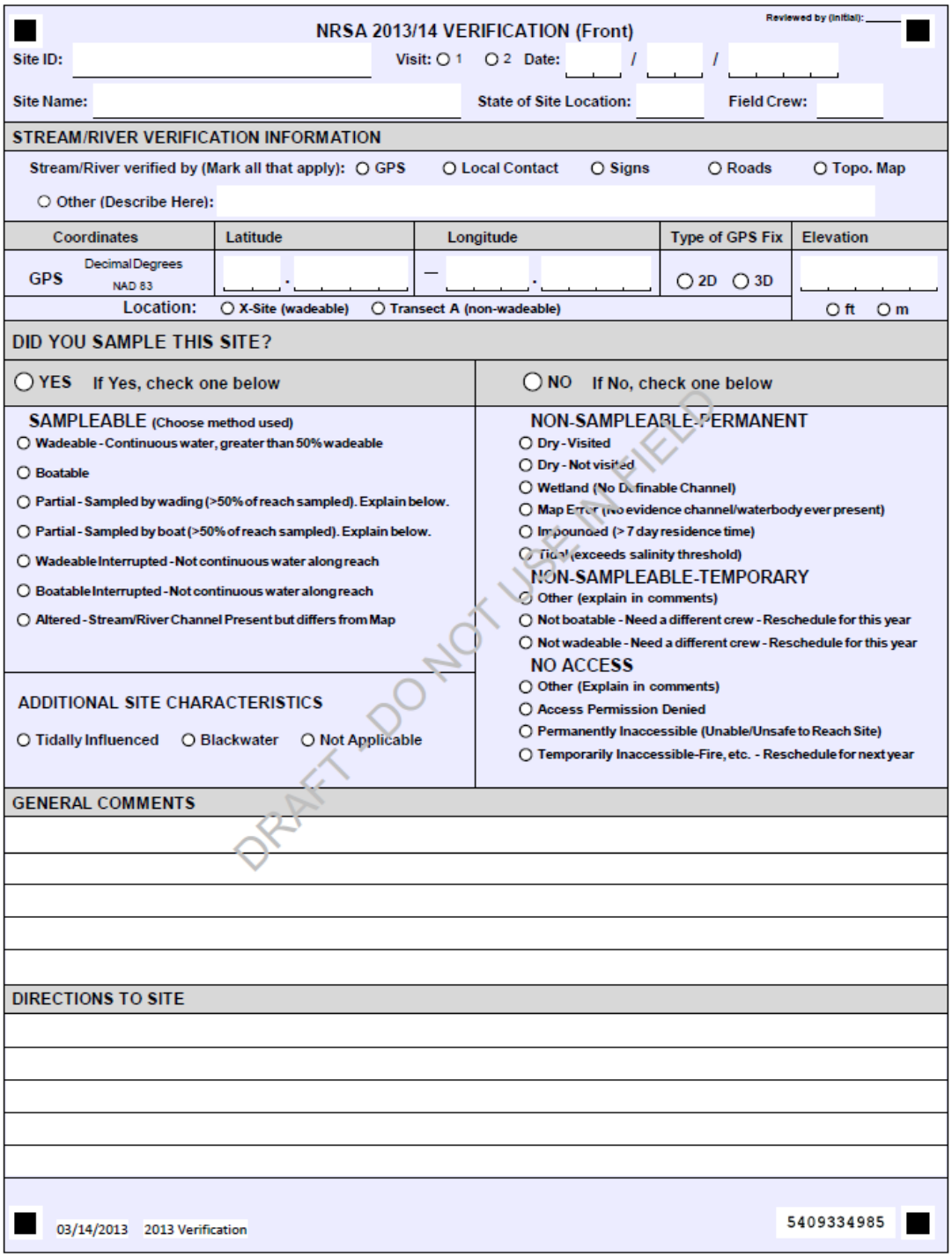
For locating and verifying site	Sampling permit and landowner access (if required). Field Operations Manual and laminated Quick Reference Guide. Site dossier, including access information, site spreadsheet with map coordinates, street and/or topographic maps with "X-site" marked. NRSA Community Fact Sheets. 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).
For recording measurements	Clipboard #2 pencils Site Verification Form Fine-tipped indelible markers to write on flagging

3.1.2 Determining the Sampling Status of a Stream

After you confirm the location of the X-site, evaluate the stream reach surrounding the X-site and classify the stream into one of three major sampling status categories: sampleable, non-sampleable, or no access (see Table 3.2). The primary distinction between "Sampleable" and "Non-Sampleable" streams is based on the presence of a defined stream channel, water content during base flow, and adequate an 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: ○ 1 ○ 2 Date: ___ / ___ / _____

Site Name: _____ State of Site Location: _____ Field Crew: _____

STREAM/RIVER VERIFICATION INFORMATION

Stream/River verified by (Mark all that apply): ○ GPS ○ Local Contact ○ Signs ○ Roads ○ Topo. Map

○ Other (Describe Here): _____

Coordinates	Latitude	Longitude	Type of GPS Fix	Elevation
GPS Decimal Degrees NAD 83	___ . _____	— ___ . _____	○ 2D ○ 3D	_____
Location: ○ X-Site (wadeable) ○ Transect A (non-wadeable)				○ ft ○ m

DID YOU SAMPLE THIS SITE?

○ YES If Yes, check one below

SAMPLEABLE (Choose method used)

- ○ Wadeable - Continuous water, greater than 50% wadeable
- ○ Boatable
- ○ Partial - Sampled by wading (>50% of reach sampled). Explain below.
- ○ Partial - Sampled by boat (>50% of reach sampled). Explain below.
- ○ Wadeable Interrupted - Not continuous water along reach
- ○ Boatable Interrupted - Not continuous water along reach
- ○ Altered - Stream/River Channel Present but differs from Map

ADDITIONAL SITE CHARACTERISTICS

○ Tidally Influenced ○ Blackwater ○ Not Applicable

○ NO If No, check one below

NON-SAMPLEABLE-PERMANENT

- ○ Dry - Visited
- ○ Dry - Not visited
- ○ Wetland (No Definable Channel)
- ○ Map Error (no evidence channel/waterbody ever present)
- ○ Impounded (> 7 day residence time)
- ○ Tidal (exceeds salinity threshold)

NON-SAMPLEABLE-TEMPORARY

- ○ Other (explain in comments)
- ○ Not boatable - Need a different crew - Reschedule for this year
- ○ Not wadeable - Need a different crew - Reschedule for this year

NO ACCESS

- ○ Other (Explain in comments)
- ○ Access Permission Denied
- ○ Permanently Inaccessible (Unable/Unsafe to Reach Site)
- ○ Temporarily Inaccessible-Fire, etc. - Reschedule for next year

GENERAL COMMENTS

DIRECTIONS TO SITE

03/14/2013 2013 Verification

5409334985

DRAFT - DO NOT USE IN FIELD

Figure 3.1 Verification Form (page 1)

Table 3.2 Site Verification Procedures

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 Sampling During or After Rain Events

Avoid sampling during high flow rainstorm events. 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.

3.1.4 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.

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 channel 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 (non-wadeable sites). It is **highly recommended** that you lay out the sampling reach for large, non-wadeable sites before you go in the field using maps, aerial photos, and/or GIS software. This will save time on the field day.

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 page 2 of the Verification Form as shown in Figure 3.2 Verification Form (page 2)

Figure 3.3 illustrates the principal features of the established sampling reach for non-wadeable sites, including the location of the 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 Laying Out the Sampling Reach at Non-Wadeable Sites

Laying out the sampling reach at the base site (recommended at boatable sites)

1. Using GIS, an aerial photo or a 1:100:000 topographic map; **locate the X-site using the coordinates provided for the site**.
2. **Determine the average wetted width of the channel at the X-site** using GIS or if not available maps and/or aerial photographs. To get an average, 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.**
3. **Multiply the average wetted width by 40 to determine the reach length**. If the average width is <4 m, use 150 m as a **minimum** reach length. If the average width is >100 m, use 4 km as a **maximum** reach length.
4. **From the X-site, measure a distance of 20 channel widths downstream using GIS**. Be careful to measure all of the bends of the river/stream; do not artificially straighten out the line of measurement. The **downstream endpoint is marked as Transect** K. Measure 20 channel widths upstream from the X-site; **the upstream end of the reach is marked as**

Transect A.

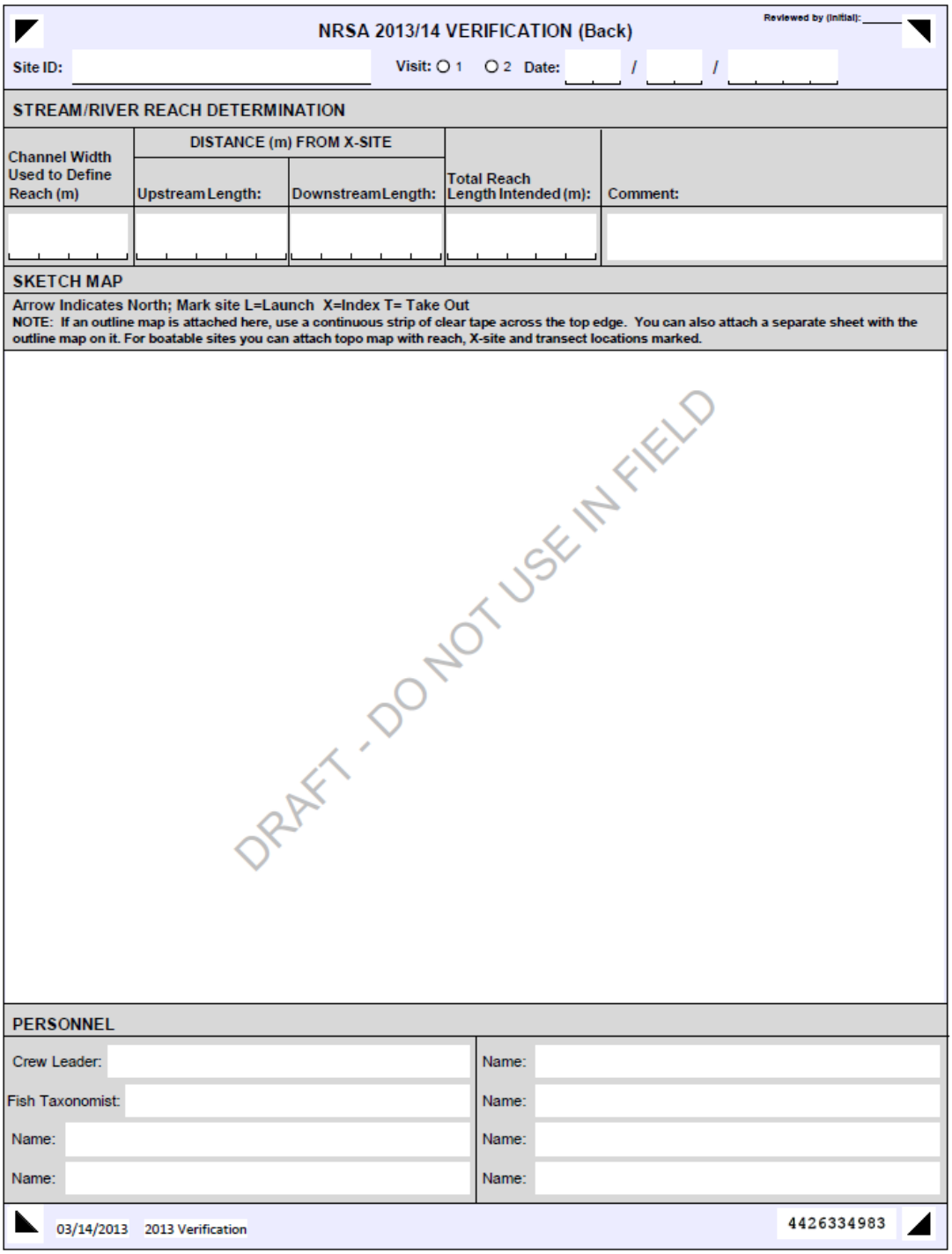
5. **Measure 1/10 of the reach length downstream from Transect A, and mark this spot as Transect B**. **Continue marking the 11 transects A - K in increments of 1/10 of the reach length.** Enter the waypoints for transects into a GPS unit so transects are easy to find on the sampling day.
6. **Assign the sampling station at Transect A randomly** (e.g., use the seconds display on a digital watch to select the initial sampling station: 1 - 5 = Left Bank, 6 – 9 = Right Bank). From here, three stations will be on the first (randomly selected) side of the river, then 2 on the other, then 2 on the first side, and so on through Transect K (as shown in Figure 3.3). 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.
7. When you are at the site, "ground truth" the wetted width measurements and proceed to Steps 9 & 10 to see if the layout needs to be adjusted.

Laying out the sampling reach in the field

1. Locate the X-site using the coordinates provided for the site.
2. Use a 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 Site 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 Table 3.5 for specific instructions.
6. Starting at the X-site (or the new midpoint of the reach if it had to be adjusted as described in Step 8), measure a distance of 20 channel widths downstream using a GPS unit, laser rangefinder, or tape measure. Be careful to measure all of the bends of the river/stream; do not artificially straighten out the line of measurement. Enter the channel to make measurements only when necessary to avoid disturbing the stream channel prior to sampling activities. The downstream endpoint is flagged as Transect K. The upstream end of the reach is flagged as Transect A.
7. At Transect A, use the seconds display on a digital watch to select the initial sampling station for transect samples: 1 - 5 = Left Bank, 6 – 9 = Right Bank. Mark "L" or "R" on the transect flagging. 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 reach length downstream from Transect A. Flag this spot as

Transect B. Assign the sampling station systematically after the first random selection as shown in Figure 3.3. Three stations will be on the first side of the river, then 2 on the other, then 2 on the first side, and so on through Transect K.

9. Proceed downstream with a GPS unit, laser rangefinder, or tape measure and flag the positions of 9 additional transects (labeled "C" through "K" as you move downstream) at intervals equal to 1/10 of the reach length. Continue to assign the sampling stations systematically.



NRSA 2013/14 VERIFICATION (Back)

Reviewed by (initial): _____

Site ID: _____ Visit: ○ 1 ○ 2 Date: ____ / ____ / ____

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:		

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.

DRAFT - DO NOT USE IN FIELD

PERSONNEL

Crew Leader:		Name:	
Fish Taxonomist:		Name:	
Name:		Name:	
Name:		Name:	

03/14/2013 2013 Verification 4426334983

Figure 3.2 Verification Form (page 2)

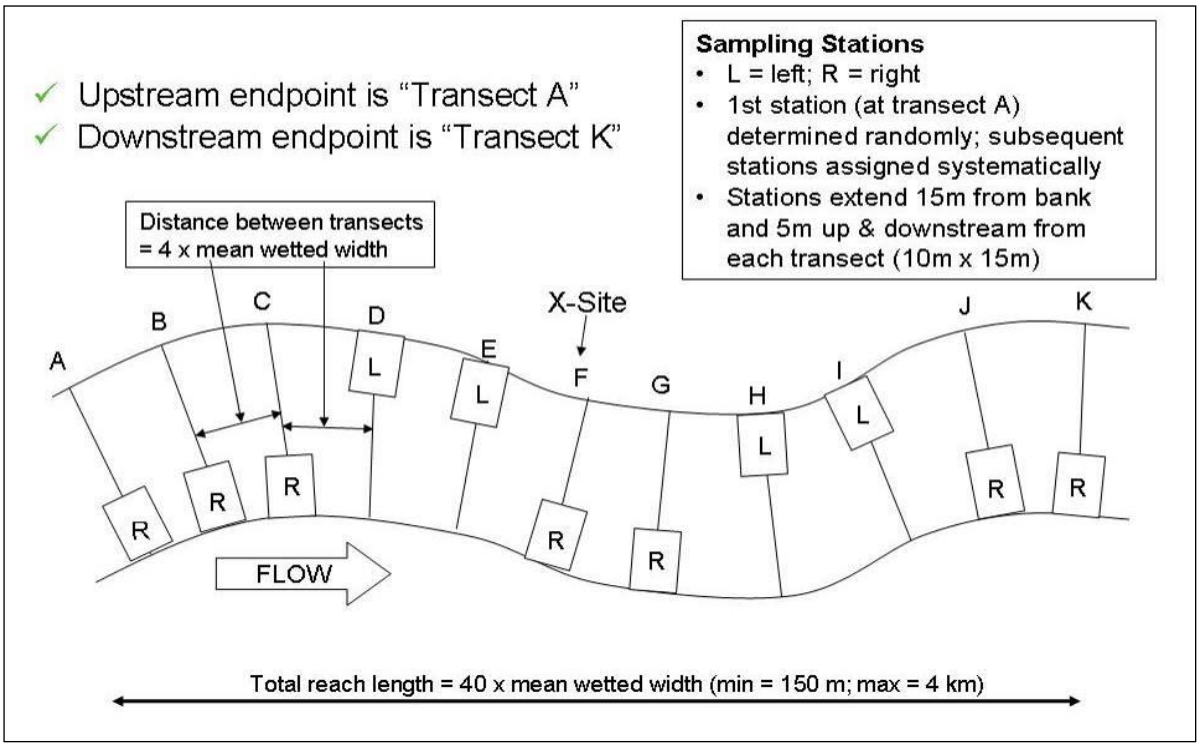


Figure 3.3 Sampling Reach Features for a Non-Wadeable Site

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 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 **impassible barrier** (e.g., waterfall, cliff, navigation dam) so that the barrier is at one end. 3. Slide the reach if you run into a **confluence** (another stream meeting the water-body you are sampling) with a higher Strahler Order.

4. When you are d**enied 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 4) will be collected at Transect A (even if the reach has been adjusted by “sliding” it). If Transect A is dry and there is water elsewhere in the sample reach, collect the sample from a location having water with a surface area >1 m^2 and a depth >10 cm.

.

Collect data for the physical habitat indicator along the entire sample reach from interrupted streams, regardless of the amount of water present at 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 m^2 and a depth > 10 cm somewhere along the defined sampling reach, take the water sample at the pool or flowing water location that is nearest to the X-site. Note that the sample wasn't collected at the X-site and where on the reach the sample was collected on the field data form. Do not collect a water sample if there is no acceptable location within the sampling reach. 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 cm deep; collect all physical habitat data. Do not collect periphyton or benthic macroinvertebrates from this transect. WATER PRESENT: Transect has flow or pools > 10 cm deep; collect all data and measurements for physical habitat, periphyton, benthic macroinvertebrate, and fish indicators, using standard procedures.

3.3.2 Braided Rivers and Streams

Depending upon the geographic area and/or the time of the sampling visit, you may encounter a stream having "braided" channels, which are characterized by numerous sub-channels that are generally small and short, often with no obvious dominant channel. If you encounter a braided stream, establish the sampling reach using the procedures presented in Table 3.7**.** Figuring the mean width of extensively braided rivers and streams for purposes of setting up the sampling reach length is challenging. For braided channels, measure the mean width and bankfull width as defined in the physical habitat protocols (Section 8). For relatively small streams (mean bankfull width ≤15 m) the sampling reach is defined as 40 times the mean bankfull width. For larger streams (>15 m), sum the actual wetted width of all the braids and use that as the width for calculating the 40 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 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. It is recommended 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 SOP.

Temperature Meter

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

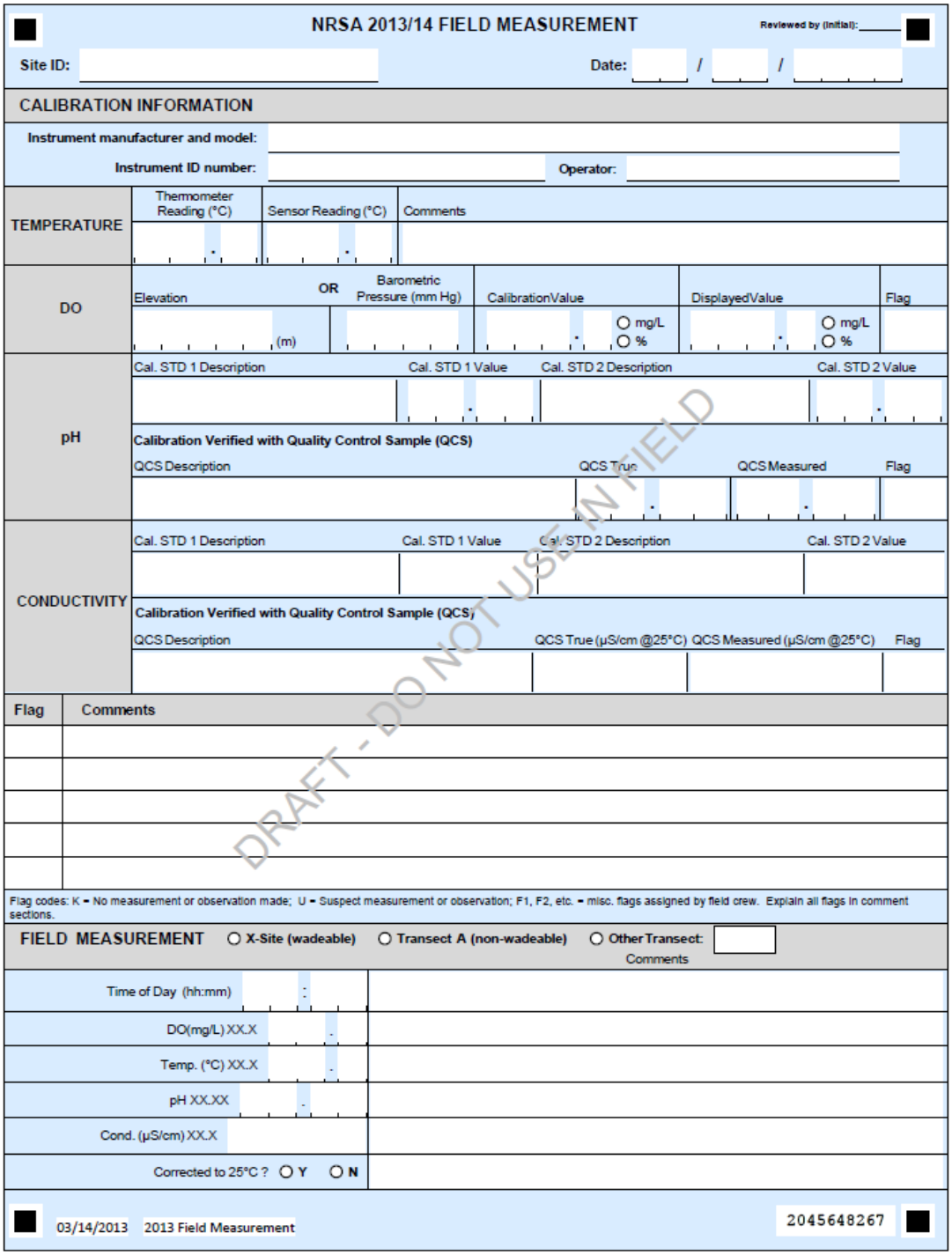
Calibrate the conductivity meter prior to each sampling event. Calibrate the meter in accordance with the manufacturer's instructions. The entire conductivity range encountered in the NRSA should be incorporated in the testing procedure and a record of test results kept on file.

4.1.3 Sampling Procedure

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

Table 4.2 Sampling 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.



NRSA 2013/14 FIELD MEASUREMENT

Reviewed by (Initial): _____

Site ID: _____ Date: ___ / ___ / _____

CALIBRATION INFORMATION

Instrument manufacturer and model: _____

Instrument ID number: _____ Operator: _____

TEMPERATURE

Thermometer Reading (°C)	Sensor Reading (°C)	Comments
.	.	

DO

Elevation (m)	OR Barometric Pressure (mm Hg)	CalibrationValue		DisplayedValue		Flag
		.	○ mg/L ○ %	.	○ mg/L ○ %	

pH

Cal. STD 1 Description	Cal. STD 1 Value	Cal. STD 2 Description	Cal. STD 2 Value
	.		.

Calibration Verified with Quality Control Sample (QCS)

QCS Description	QCS True	QCS Measured	Flag
	.	.	

CONDUCTIVITY

Cal. STD 1 Description	Cal. STD 1 Value	Cal. STD 2 Description	Cal. STD 2 Value

Calibration Verified with Quality Control Sample (QCS)

QCS Description	QCS True (µS/cm @25°C)	QCS Measured (µS/cm @25°C)	Flag

Flag	Comments

Flag codes: K = No measurement or observation made; U = Suspect measurement or observation; F1, F2, etc. = misc. flags assigned by field crew. Explain all flags in comment sections.

FIELD MEASUREMENT ○ X-Site (wadeable) ○ Transect A (non-wadeable) ○ Other Transect: ____ Comments

Time of Day (hh:mm) :	
DO(mg/L) XX.X .	
Temp. (°C) XX.X .	
pH XX.XX .	
Cond. (µS/cm) XX.X	
Corrected to 25°C ? ○ Y ○ N	

03/14/2013 2013 Field Measurement 2045648267

DRAFT - DO NOT USE IN FIELD

Figure 4.1 Field Measurement Form

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 ammonia nitrogen (NH_4), nitrate (NO_3), 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. After Collection, store all samples in darkness on ice in a closed cooler. After you filter the chlorophyll-*a* sample, the filters must be kept frozen until ready to ship.

4.2.2 Equipment and Supplies

Table 4.3 provides the equipment and supplies needed to collect water samples at Transect A. 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

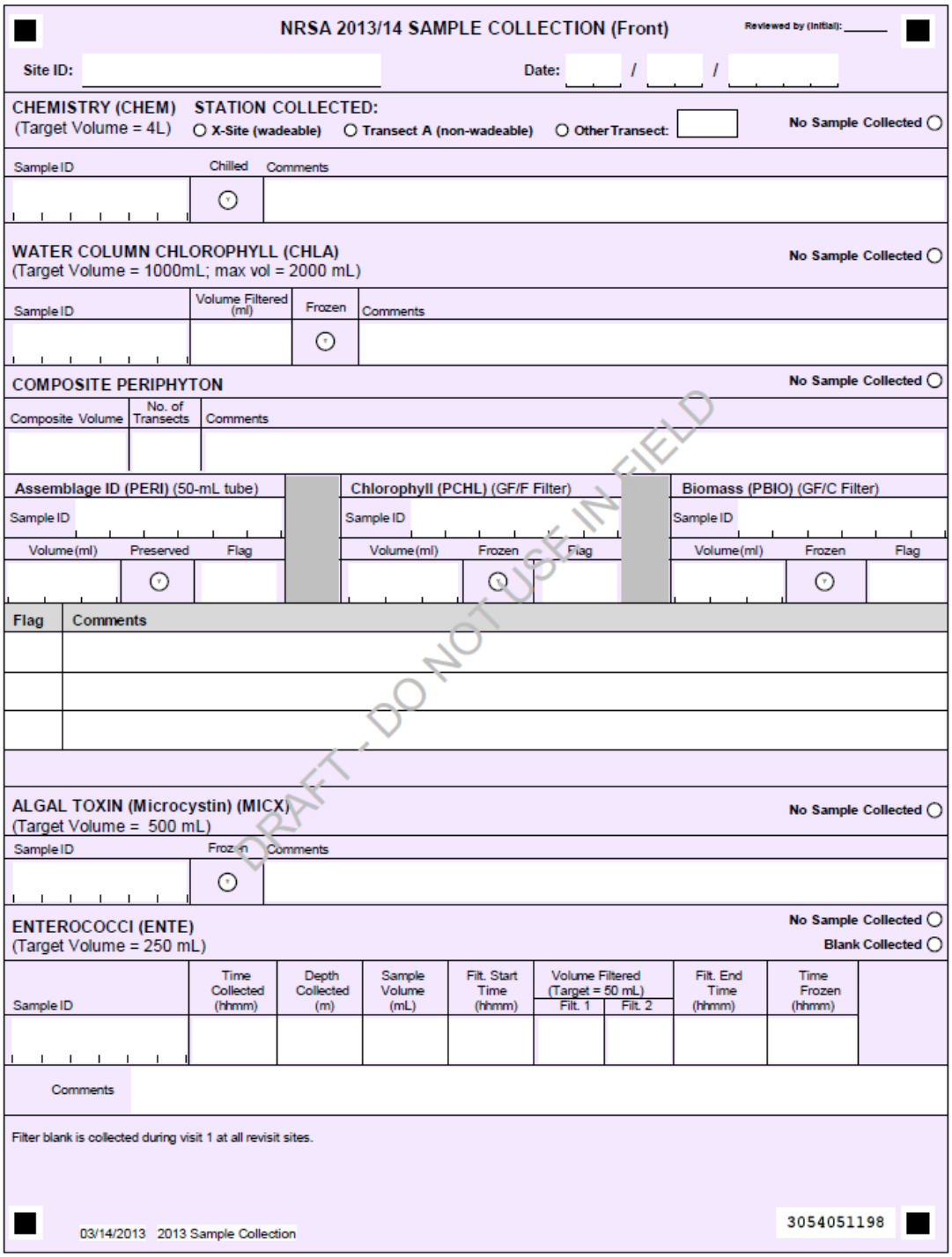
For collecting samples	Nitrile gloves 4-L cube container 2-L amber Nalgene bottle 3-L Nalgene beaker Cooler with ice DI water (for cleaning beaker between sites) Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Sample Collection Form Water Chemistry sample label 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 describes the sampling procedures for collecting water chemistry samples in non-wadeable streams and rivers.

Table 4.4 Sampling Procedure for Non-Wadeable Sites - Water Chemistry and Chlorophyll-a Sample Collection

Sampling Procedure
Water Chemistry
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 Transect A 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. 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-*a*
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. Cap the bottle tightly. This sample will be filtered later and the bottle will be reused at future sites, therefore it is not necessary to label this bottle.
Storage
Place the cube container and Nalgene bottle in a cooler (on ice or water) and shut the lid. If a cooler is not available, place the cube container in an opaque garbage bag and immerse it in the stream. **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 Transect A, darken the Transect A 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.



NRSA 2013/14 SAMPLE COLLECTION (Front)

Reviewed by (initial): _____

Site ID: _____ Date: ___ / ___ / _____

CHEMISTRY (CHEM) (Target Volume = 4L) **STATION COLLECTED:** ○ X-Site (wadeable) ○ Transect A (non-wadeable) ○ Other Transect: ___ No Sample Collected ○

Sample ID	Chilled	Comments
	○	

WATER COLUMN CHLOROPHYLL (CHLA) (Target Volume = 1000mL; max vol = 2000 mL) No Sample Collected ○

Sample ID	Volume Filtered (ml)	Frozen	Comments
		○	

COMPOSITE PERIPHYTON No Sample Collected ○

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
	○			○			○	

Flag	Comments

ALGAL TOXIN (Microcystin) (MICX) (Target Volume = 500 mL) No Sample Collected ○

Sample ID	Frozen	Comments
	○	

ENTEROCOCCI (ENTE) (Target Volume = 250 mL) No Sample Collected ○ Blank Collected ○

Sample ID	Time Collected (hhmm)	Depth Collected (m)	Sample Volume (mL)	Filt. Start Time (hhmm)	Volume Filtered (Target = 50 mL) Filt. 1	Volume Filtered (Target = 50 mL) Filt. 2	Filt. End Time (hhmm)	Time Frozen (hhmm)

Comments

Filter blank is collected during visit 1 at all revisit sites.

03/14/2013 2013 Sample Collection 3054051198

DRAFT - DO NOT USE IN FIELD

Figure 4.2 Sample Collection Form, Side 1

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 Transect A. 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 sample 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 Figure 4.2.

Table 5.1 Equipment and Supplies - Microcystin Sample

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 non-wadeable sites.

Table 5.2 Sampling Procedure for Non-Wadeable Sites - Microcystin Sample Collection

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 Transect A** 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.). As soon as you return 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

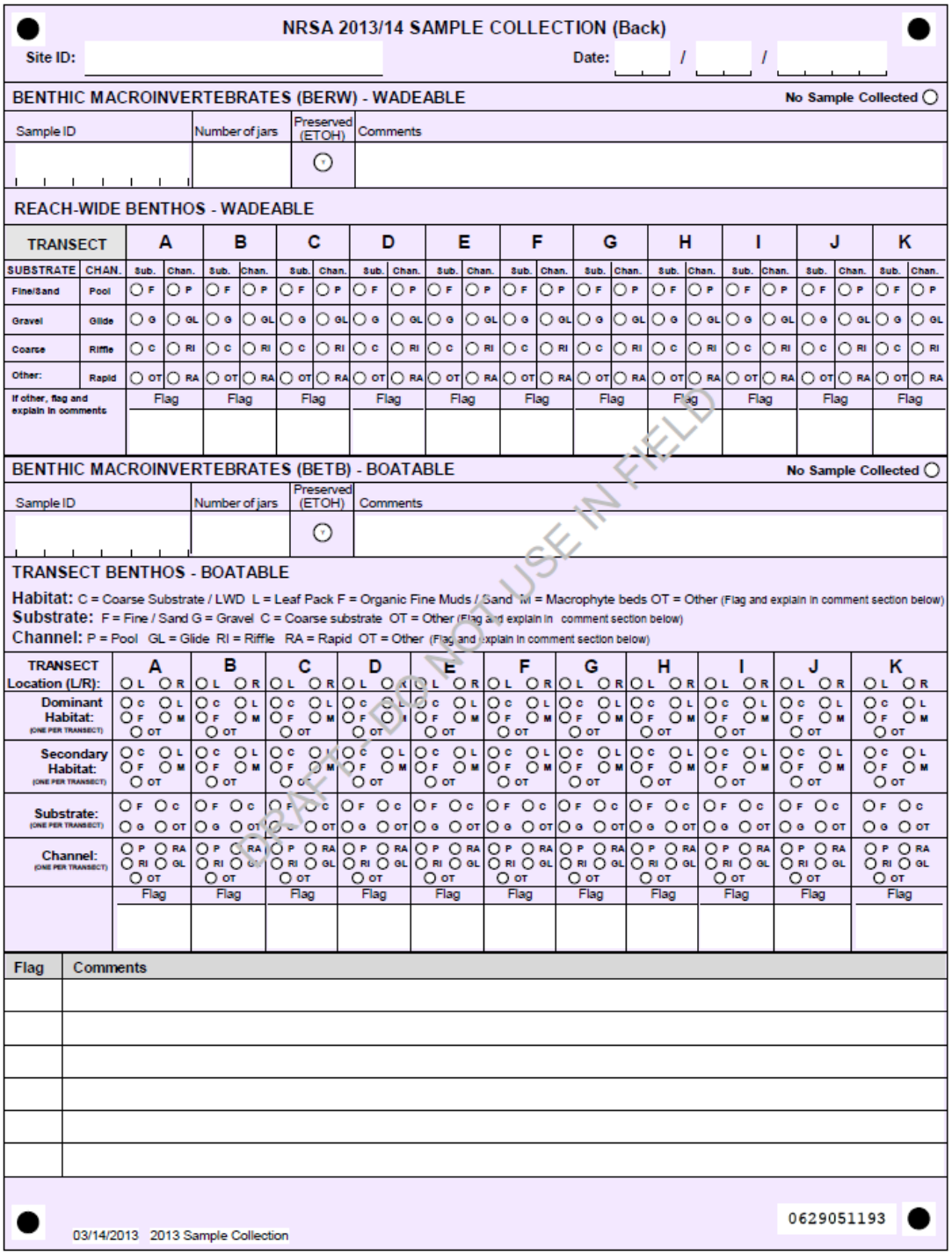
Collect benthic macroinvertebrate composite samples using a D-frame net with 500 µm mesh openings. Take the samples from the sampling stations at the 11 transects equally distributed along the targeted reach. Multiple habitats will be encountered and sampled using this approach. They include bottom substrate 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 List for Benthic Macroinvertebrate Collection at Wadeable Sites

For collecting samples	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	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
For recording measurements	Composite benthic sample labels with & without preprinted ID Sample ID numbers Blank labels on waterproof paper for inside of jars	Soft (#2) lead pencils Fine-tip indelible markers Clear tape strips Sample Collection Form



NRSA 2013/14 SAMPLE COLLECTION (Back)

Site ID: Date: / /

BENTHIC MACROINVERTEBRATES (BERW) - WADEABLE — No Sample Collected ○

Sample ID	Number of jars	Preserved (ETOH)	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 ○

Sample ID	Number of jars	Preserved (ETOH)	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 Location (L/R):	A	B	C	D	E	F	G	H	I	J	K
	○ 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 ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT
Secondary Habitat: (ONE PER TRANSECT)	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT	○ C ○ L ○ F ○ M ○ OT
Substrate: (ONE PER TRANSECT)	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT	○ F ○ C ○ G ○ OT
Channel: (ONE PER TRANSECT)	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT	○ P ○ RA ○ RI ○ GL ○ OT
	Flag	Flag	Flag	Flag	Flag	Flag	Flag	Flag	Flag	Flag	Flag

Flag	Comments

03/14/2013 2013 Sample Collection

0629051193

DRAFT - DO NOT USE IN FIELD

Figure 6.1 Sample Collection Form (back)

6.3 Sampling Procedure

Figure 6.2 summarizes how samples will be collected from non-wadeable sites. Collect benthic macroinvertebrate samples at the 11 transects and within the sampling stations for non-wadeable streams (Figure 6.3). The process for selecting the sample stations is described in the Initial Site Procedures (Section 3). Collect all benthic samples at non-wadeable sites from the dominant habitat type within the 10 m x 15 m randomly selected sampling station at each transect.

The procedure for collecting samples at each transect is described in Table 6.2. Take 1 linear meter sweep at the dominant habitat type. Record the benthic macroinvertebrate collection data on the Sample Collection Form as seen in Figure 6.1. As you proceed from transect to transect, combine all samples into a bucket.

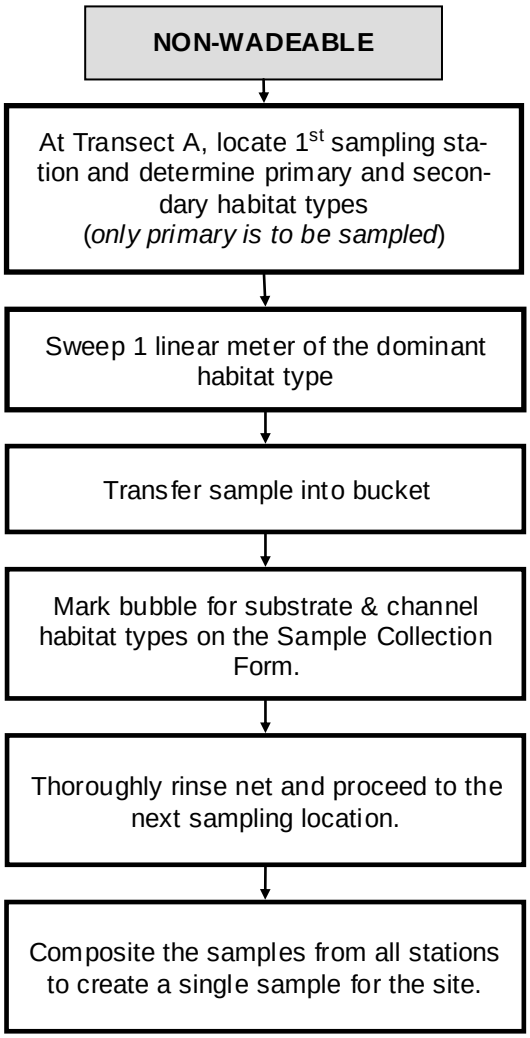


Figure 6.2 Benthic Macroinvertebrate Collection at Non-Wadeable Sites

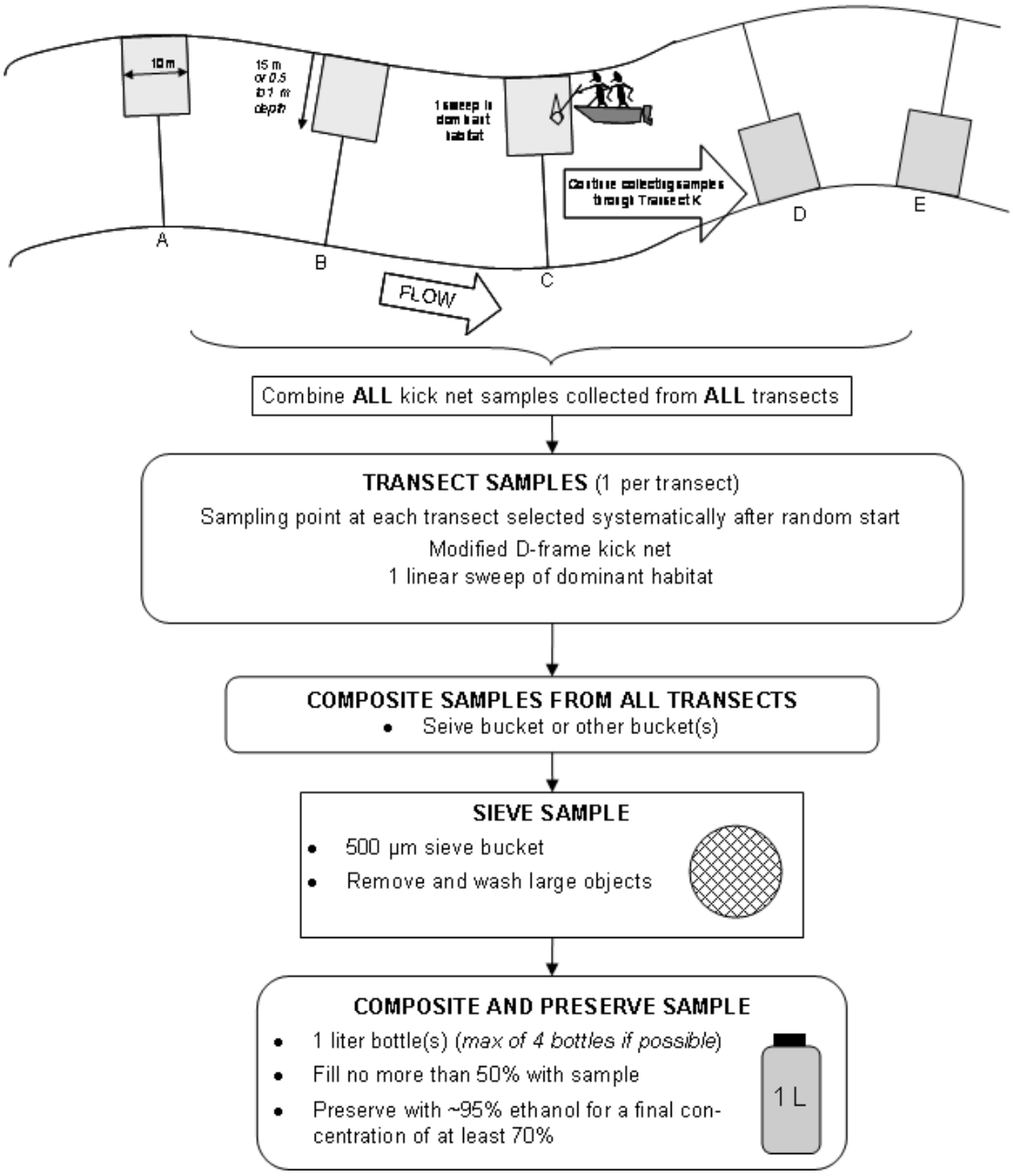


Figure 6.3 Transect Sample Design for Collecting Benthic Macroinvertebrates at Non-Wadeable Sites

Table 6.2 Procedure for Benthic Macroinvertebrate Sampling at Non-Wadeable Sites

Collecting the Benthic Macroinvertebrate Sample

1. After locating the sampling station at Transect A according to procedures described in the reach layout section, identify the dominant and secondary habitat type within the plot:
   - Rocky/cobble/gravel/large woody debris
   - Organic fine mud or sand
   - Macrophyte beds
   - Leaf Pack
2. Use the D-frame dip net (equipped with 500 µm mesh) to sweep through **1 linear meter** of the most dominant habitat type within the 10m x 15m sampling station, making sure to disturb the substrate enough to dislodge organisms.

   a) If the dominant habitat is rocky/cobble/large woody debris it may be necessary to exit the boat and disturb the substrate (e.g., overturn rocks, logs) using your feet while sweeping the net through the disturbed area.
   b) Because a dip-net is being used for sampling, the maximum depth for sampling will be approximately 0.5 to 1 m; therefore, in cases in which the depth of the river quickly drops off it may be necessary to sample in the nearest several meters to the shore.
3. After completing the 1 linear meter sweep, remove all organisms and debris from net and place them in a bucket following sample processing procedures described in the following section.
4. Record the side of the river on which the sampling station is located (left or right).
5. Record the sampled habitat type (Dominant) as well as the Secondary habitat type on the Sample Collection Form.
   - **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.
6. Identify the channel habitat type where the sampling station 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.
7. Proceed to the next sampling station and repeat steps 1-6. The organisms and detritus collected at each station on the river should be combined in a single bucket to create a single composite sample for the river.

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). If there is lots of organic material in the sample, or there are adverse field conditions (i.e. hot, humid weather), place sample in a 1-L jar with ethanol after each station.

Try to use no more than 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).

Table 6.3 Procedure for Compositing Samples for Benthic Macroinvertebrates at Non-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 is not more than ½ full, 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. ▪ *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).* **Place a waterproof label inside each jar with the following information written with a #2 lead pencil:** 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"

Compositing Benthic Macroinvertebrate Sample

6. 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%.
   - *NOTE: Composite samples can be transported back to the vehicle before adding ethanol if necessary. In this case, fill the jar with stream water, then drain using the net (or sieve) across the opening to prevent loss of organisms, and replace with ethanol at the vehicle.*
7. 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.
8. 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 near shore shallows at each of the sampling stations located on the 11 cross section transects ("A" through "K") established within the sampling reach. Collect periphyton samples at the transect location as the benthic macroinvertebrate samples (Section 6), directly after the benthic macroinvertebrate samples have been collected. Prepare one composite 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 river.

Table 7.1 Equipment and Supplies List for Periphyton at Non-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 with marked volume gradations 60-mL plastic syringe with tip removed, and length of tubing Field Operations Manual or 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 sample labels Sample labels (4 per set) with the 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 (left or right). Collect the substrate selected for sampling from a depth no deeper than 0.5 m. If you cannot collect a sample 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 for Collecting Composite Index Samples of Periphyton at Non-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-cm^2 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-cm^2 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 *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.

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 Checklist of Equipment and Supplies for Physical Habitat

For making measurements	▪ 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 ▪ SONAR unit ▪ Laser rangefinder (400 ft. distance range) and clear waterproof bag ▪ Clinometer ▪ Binoculars ▪ Surveyor's telescoping leveling rod ▪ Sounding rod ▪ Field Operations Manual and/or laminated quick reference guide
For recording data	▪ 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 ▪ 1+ extras field Form: Visual Assessment Form

8.2 Summary of Methods Approach

Physical habitat in rivers includes all those physical attributes that influence or provide sustenance to organisms within the stream. The physical habitat of a river varies naturally, thus expectations differ even in the absence of anthropogenic disturbance. River sample reach lengths are defined as **40 times the wetted width at the x-site**, **with a minimum of 150m and maximum of 4k**, as described in Section 3. Measurement points are systematically placed to statistically represent the entire reach. River depth and wetted width are measured at very tightly spaced intervals, whereas channel cross-section profiles, substrate, bank characteristics and riparian vegetation structure are measured at larger intervals. Woody debris is tallied along the full length of the sampling reach. The tightly spaced depth and width measures allow calculation of indices of channel structural complexity, objective classification of channel units such as pools, and quantification of residual pool depth, pool volume, and total stream volume.

8.3 Components of the Field Habitat Assessment

Field data collection for the physical habitat assessment is accomplished in a single float down each sampling reach.. The physical habitat methods are made up of the following components: Thalweg profile, Littoral/Riparian Cross-Sections, and assessments of the entire reach. Table 8.2 describes the components of physical habitat in non-wadeable systems and gives an overview of how the data is collected. Measurements are recorded on 11 copies of a two-sided field form, and separate forms for assessing the degree of channel constraint, and recording evidence of debris torrents or recent major flooding.

Table 8.2 Components of Non-Wadeable River Physical Habitat Protocol

1. **Thalweg Profile:**

   At 10 equally spaced intervals between each of 11 transects (100 along entire reach):

   - Classify habitat type, record presence of backwater and off-channel habitats.
   - Determine dominant substrate visually or using sounding rod.
   - Record the presence of mid-channel snags
   - Measure thalweg (maximum) depth using Sonar or rod

2. **Littoral/Riparian Cross-Sections:** at 11 transects at equal intervals along reach length:

   Measure/estimate from one chosen bank on 11 transects :

   - Wetted width and Mid-channel bar width (laser range finder).
   - Bankfull width (laser) and height (pole and clinometer used as level).
   - Incision height (pole and clinometer used as level).
   - Bank angle (estimate)
   - Riparian canopy cover (densiometer) in four directions from chosen bank.
   - Shoreline Substrate in the first 1m above waterline (dominant and subdominant size class).

   In 20m long Littoral Plot extending streamward 10m from chosen bank : [1]

   - Littoral depth at 5 locations systematically-spaced within plot (Sonar or sounding rod).
   - Dominant and Subdominant substrate size class at 5 systematically-spaced locations (visual or sounding rod).
   - Tally large woody debris in littoral plot and in bankfull channel by size and length class.
   - Areal cover class of fish concealment and other features, including:

filamentous algae	overhanging vegetation	aquatic macrophytes
undercut banks	large woody debris	boulders and rock ledges
brush/small woody debris	live trees or roots	artificial structures

In 20m long Riparian Plot extending 10m landward starting at bankfull margin--both sides of river:[1]

- Estimate areal cover class and type (e.g., woody) of riparian vegetation in Canopy, Mid-Layer, and Ground Cover layers
- Observe and record human activities and disturbances and their proximity to the channel.

For the whole sampling reach, after completing thalweg and littoral/riparian measurements:

- Classify channel type and degree of constraint, identify features causing constraint, estimate the percentage of constrained channel margin for the whole reach, and estimate the bankfull and valley widths.

[1]Note: Boundaries for visual observations are estimated by eye.

8.4 Summary of Workflow

Table 8.3 lists the activities performed at and between each transect for the physical habitat characterization. The activities are performed along the chosen river bank and mid-channel (thalweg profile).

Table 8.3 Summary of Workflow Non-Wadeable Physical Habitat Characterization

A. At the chosen bank on first transect (farthest upstream):

1. Complete the header on the front of the Channel/Riparian Transect form including the Site ID, Date, Transect (A-K) and bank (left or right) that was assigned during reach layout.
2. Read the GPS coordinates and record them in the Transect (Bank) space on the field form.
3. Record the dominant and subdominant littoral substrate, based on visual observations.
4. Move boat in a "loop" within the 10 x 20 m littoral plot, measuring 5 littoral depths and probing substrate.
5. Estimate dominant and subdominant littoral substrate, based on probing the 5 locations.
6. Estimate areal cover of fish concealment features in the 10 x 20 meter littoral plot.
7. Tally LWD within or partially within the 10 x 20 meter littoral plot.
8. Collect densiometer measurements at bank (facing upstream, downstream, left, right).
9. Choose bank angle class, estimate bankfull height, width and channel incision. (Note that width and incision estimates incorporate both left and right banks.).
10. Tally LWD entirely out of water but at least partially within the bankfull channel.
11. Estimate and record distance to riparian vegetation on the chosen bank.
12. Make visual riparian vegetation cover estimates for the 10 x 20 meter riparian plot on both sides of the channel. (Riparian plot starts where perennial vegetation begins or at bankfull channel margin, whichever is closest to the wetted river margin. The plot continues 10m back from the bankfull line).
13. Make visual human disturbance tally on both sides of the river. Use the same plot dimensions as for riparian vegetation -- except that if a disturbance item is observed in the river or within the bankfull channel, the proximity code is "B", use the close rating; "C" if disturbance is present within the riparian plot. If the item is only observed beyond (outside) the riparian plot, the proximity code is "P". If the disturbance is not present in or adjacent to the plot, mark as "0", not present.
14. Proceed to a midstream point on the transect and record the GPS coordinates in the Transect (Midstream) space on the field form

15. If the next transect has not already been marked during reach layout, get out far enough from the bank so you can see downstream. Then use the laser rangefinder to sight and record the distance to the intended position of the next downstream transect.

B Thalweg Profile:

1. As soon as you get out from the bank after doing transect activities, take the first of 10 thalweg depth measurements and substrate/snag probes using sonar and pole -- also classify habitat type and record presence of side-channels and backwaters.
2. **Estimate thalweg measurement distance increments using the GPS course-tracking and trip-meter functions.** Alternatively, estimate these distances by keeping track of boat lengths or channel-width distances traversed; each thalweg measurement is 1/10th the distance between transects, which can help you keep track of your downstream progress).

C. Repeat the Whole Process (for the remaining 10 transects and subreaches).

D. Channel Constraint Assessment

After completing the Thalweg Profile and Littoral-Riparian measurements and observations at all 11 Transects, complete the classification and estimation of channel constraint type, frequency of contact with constraining features, and the width ratio of bankfull channel divided by valley width. You may wish to refer to the individual transect assessments of incision and constraint.

8.5 Work Flow and Reach Marking

In a single midstream float down the 40 channel-width reach, the 2-person habitat crew accomplishes a reconnaissance, a sonar/pole depth profile, and a pole-drag to tally snags and characterize mid-channel substrate. The float is interrupted by stops at 11 transect locations for littoral/riparian observations. They determine (and mark – optional, but recommended) the intended position of each successive downstream transect using a global positioning system (GPS) and/or a laser range finder. The crew then floats downstream along the thalweg to the new transect location, making thalweg profile measurements and observations at 10 evenly-spaced increments along the way. When they reach the new downstream transect location, they stop to perform cross-section, littoral, and riparian measurements, recording the actual GPS latitude/longitude of the transect position (bank and midstream). They will also collect biological samples at each transect.

GPS coordinates are determined for the actual locations of each transect stop. **If GPS unit also has course tracking, trip-meter (accumulated distance and bearing), and waypoint setting/navigation features,** we recommend using it to locate thalweg measurement points (use course tracking and trip meter). Equipping the boat with a bow or stern anchor to stop at transect locations can greatly ease the shore marking operation and shoreline measurement activities, though such equipment can be dangerous in rivers.

8.5.1 Reconnaissance for Physical Habitat Data Collection

The habitat crew will also record reconnaissance and safety notes at this time. They will inform the second boat of the route, craft, and safety precautions needed during its subsequent electrofishing activities. They also assist the electrofishing boat crew over jams and help to conduct shuttles (this can take considerable time where put-ins and take-outs are distant). As the crew floats downstream, they may choose and communicate to the electrofishing crew the most practical path to be used when fishing with a less maneuverable boat, taking into consideration multiple channels, blind channels, backwaters, alcoves, impassible riffles, rapids, jams, and hazards such as dams, bridges and power lines. 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.
The sampling reach is 40 times the wetted width at the X- site, with a 500m minimum and 4 kilometer maximum reach. Section 3.1.1 describes the procedures for locating the X-site, or the midpoint of the sample reach. Section 3.2 describes the protocol for delineating a sample reach that is 40 times its width. Those sections also describe the protocol for measuring out (with a laser range finder or GIS software) and locating the **11 littoral/riparian stations where many habitat measurements will be made** (Figure 8.1).

The thalweg profile measurements are spaced as evenly as practicable over the entire sample reach length. They must also be sufficiently close together to not "miss" deep areas and habitat units that are in a size range of about 1/3 to 1/2 of the average channel width. To set the interval between thalweg profile measurements:

- Divide the reach length by 100 to set the thalweg increment distance. You will be making 100 evenly-spaced thalweg profile measurements, 10 between each detailed channel cross-section where littoral/riparian observations are made (the 11 transect locations already established).
- If the thalweg is too deep or not physically possible to be measured, estimate the depth to the best of your ability and flag it on the field form.

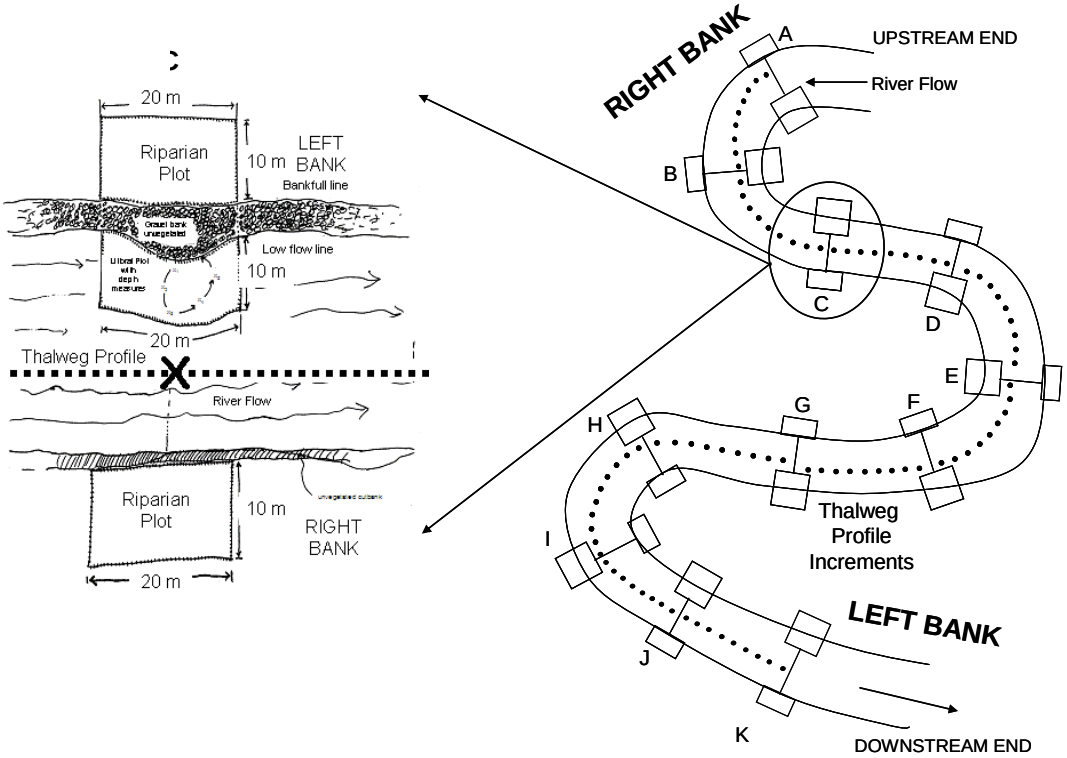


Figure 8.1 Littoral Riparian Plots for Characterizing Riparian Vegetation, human influences, fish cover, littoral substrate, and littoral depths

8.5.2 Thalweg Profile

"Thalweg" refers to the flow path of the deepest water in a river channel. The *thalweg profile* is a longitudinal survey of maximum depth and several other selected characteristics at 100 near-equally spaced points along the centerline of the river between the two ends of the river reach (Figure 8.1). For practical reasons, field crews will approximate a thalweg profile by sounding along the river course that they judge is deepest, but also safely navigable. **Locations for observations and measurements along the path of this profile are determined using the GPS course-tracking and trip-meter features (recommended)**, or by visually estimating distances based upon the river width. Data from the thalweg profile allows calculation of indices of residual pool volume, river size, channel complexity, and the relative proportions of habitat types such as riffles and pools. The procedure for obtaining thalweg profile measurements is presented Table 8.4. Record data on the Thalweg Profile Form as shown in Figure 8.2.

8.5.2.1 Thalweg Depth Profile

A thalweg depth profile of the entire 40 channel-width reach is approximated by a sonar or sounding rod while floating downstream along the deepest part of the channel (or closest navigable path). In the absence of a recording fathometer (sonar depth sounder with strip-chart output or electronic data recorder), the crew records depths at frequent, relatively evenly-spaced downstream intervals while observing a sonar display and holding a surveyor's rod off the side of the boat. The sonar screen is mounted so that the crewmember can read depths on the sonar and the rod at the same time. The sonar sensor may need to be mounted at the opposite end of the boat to avoid mistaking the rod's echo for the bottom, though using a narrow beam (16 degree) sonar transducer minimizes this problem. It is easy to hold the sounding rod vertically if you are going at the same speed as the water. If the thalweg is too deep to safely be recorded, estimate the depth and note on comments form.

8.5.2.2 Pole Drag for Snags and Substrate Characteristics

The procedure for dragging the thalweg pole to detect underwater snags and substrate characteristics is presented in Table 8.4. While floating downstream, one crewmember holds a calibrated PVC sounding rod or surveying rod down vertically from the gunwale of the boat, dragging it lightly on the bottom to simultaneously "feel" the substrate, detect snags, and measure depth with the aid of sonar. The crewmember shall record the dominant substrate type sensed by dragging the rod along the bottom (bedrock/hardpan, boulder, cobble, gravel, sand, silt & finer) on the Thalweg Profile Form (Figure 8.2). Substrate characteristics are recorded at every thalweg depth measurement (e.g., 10 determinations between each transect). In shallow, fast-water situations, where pole-dragging might be hazardous, crews will estimate bottom conditions the best they can visually and by using paddles and oars. If unavoidable, suspend measurements until out of whitewater situations, but make notes and appropriately flag observations concerning your best judgments of depth and substrate.

8.5.2.3 Channel Habitat Classification

Classify and record channel habitat types shown in Table 8.5 at a spatial resolution of about 0.5 channel-widths and check presence of off-channel and backwater habitat at every 0.4 channel-width increment. The procedures for classifying channel habitat are presented in Table 8.5. Designate side channels, backwaters and other off-channel areas independent of the main-

channel habitat type. Main channel habitat units are at least half as long as the channel is wide. (e.g., if there is a small, deep, pool-like area at the thalweg within a large riffle area, don't record it as a pool unless it occupies an area about half as wide or long as the channel is wide). For dry and intermittent rivers, record zeros for depth and wetted width in places where no water is in the channel. Record habitat type as dry channel (DR).

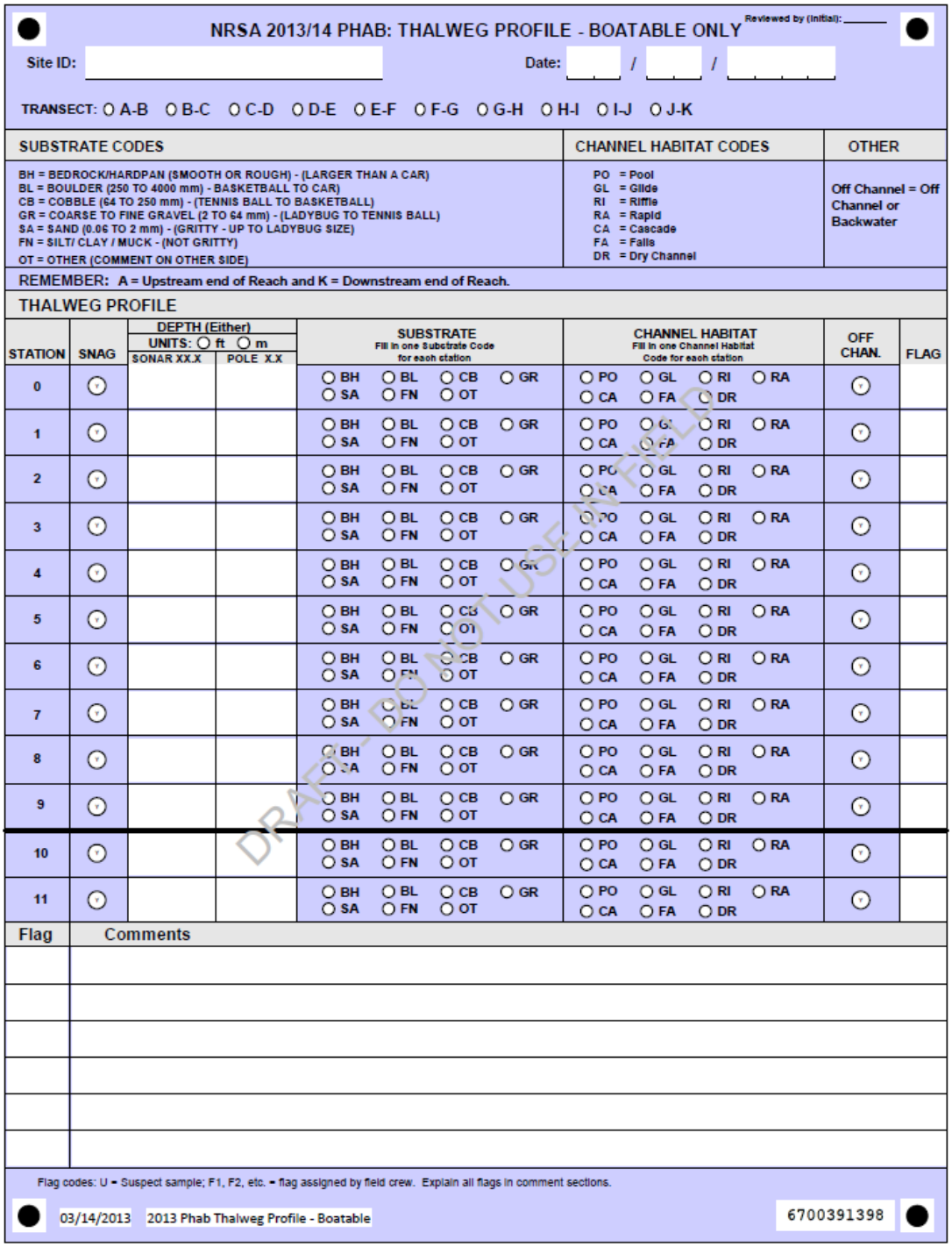
Table 8.4 Thalweg Profile Procedure

Taking the Thalweg Profile
1. Record GPS coordinates (Lat/Long) midstream and at shoreline location on the Channel/Riparian Transect Form at the transect located at the upstream end of the thalweg subreach about to be assessed.
2. Determine the interval between transects based on the mean wetted width used to determine the reach length. Transects are at 4 channel-width spacings; thalweg depth, snags, off-channel habitats and other downstream longitudinal profile observations are recorded at intervals of 0.4 channel-width.
3. Complete header information on the Thalweg Profile Form, noting transect pair (up- to downstream).
4. Begin at the upstream transect (station "0" of "10"). Determine the locations to take measurements using the course-tracking and trip-meter functions of the GPS. Alternatively, estimate your position.
Thalweg Depth Profile a) While floating downstream along the thalweg, record depths at frequent, even-spaced intervals while observing a sonar display and holding a surveyor's rod off the side of the boat. b) A depth recording every 0.4 channel-width distance is required, yielding 10 measurements between channel/riparian cross-section transects. c) If the depth is >0.5 meters, or contains a lot of air bubbles, the sonar fathometer may not give reliable depth estimates. In this case, record depths using a calibrated sounding rod. In shallow, fast-water situations depths may have to be visually estimated to the nearest 0.5 m. d) Measure depths to nearest 0.1 m and record in the "SONAR" or "POLE" column.
Pole Drag for Snags and Substrate Characteristics a) From the gunwale of the boat, hold a surveying rod or calibrated PVC sounding rod down vertically into the water. **(CAUTION: Hold the rod over the side or stern of the raft; otherwise it could be jerked out of your hands if it catches on an obstruction in fast water.)** b) Lightly drag the rod on the river bottom to "feel" the substrate and detect snags. c) Record the presence of snags hit by the rod or seen visually, plus the dominant substrate type sensed by dragging the rod along the bottom. d) Circle the appropriate "SUBSTRATE" type and record the presence/absence of "SNAGS". e) If it is too deep to safely measure the substrate type, estimate the type based on knowledge and surrounding measurements and flag the data.
Channel Habitat Classification a) Classify and record the channel habitat type at increments of every 0.4 channel width. b) Check for off-channel and backwater habitat at increments of every 0.4 channel width. c) If channel is split by a bar or island, navigate and survey the channel with the most flow. d) When a side channel is encountered, fill in "Y" in the "OFF-CHANNEL" column beginning with the point of divergence from the main channel, continuing downriver until the side channel converges with the main channel.
5. Proceed downriver to the next station, and repeat the above procedures.
6. Repeat the above procedures until you reach next transect. Set a waypoint location for the transect location midstream and at the adjacent bank. Record waypoints that you set for transect mid-stream, and

transect shoreline locations on the Channel/Riparian Transect Form corresponding to the downstream end of the thalweg sub-reach you just traversed.

NOTE: if you have taken measurements at all 10 primary thalweg stations and there is still a significant amount of river before the next transect, you should evenly space 1 or 2 additional thalweg profile stations in the remaining area to maintain the stations as evenly spaced as possible and not miss thalweg data in the downstream end of the subreach. Record these additional stations in the data rows labeled 10 and/or 11 on the form.

After completing activities at the shoreline, prepare a new Thalweg Profile Form, then repeat the above procedures for each of the reach segments, until you reach the downriver end of the reach (Transect "K").



NRSA 2013/14 PHAB: THALWEG PROFILE - BOATABLE ONLY

Reviewed by (Initial): _____

Site ID: _____ Date: ___ / ___ / _____

TRANSECT: ○ A-B ○ B-C ○ C-D ○ D-E ○ E-F ○ F-G ○ G-H ○ H-I ○ I-J ○ J-K

SUBSTRATE CODES	CHANNEL HABITAT CODES	OTHER
BH = BEDROCK/HARDPAN (SMOOTH OR ROUGH) - (LARGER THAN A CAR) BL = BOULDER (250 TO 4000 mm) - BASKETBALL TO CAR) CB = COBBLE (64 TO 250 mm) - (TENNIS BALL TO BASKETBALL) GR = COARSE TO FINE GRAVEL (2 TO 64 mm) - (LADYBUG TO TENNIS BALL) SA = SAND (0.06 TO 2 mm) - (GRITTY - UP TO LADYBUG SIZE) FN = SILT/ CLAY / MUCK - (NOT GRITTY) OT = OTHER (COMMENT ON OTHER SIDE)	PO = Pool GL = Glide RI = Riffle RA = Rapid CA = Cascade FA = Falls DR = Dry Channel	Off Channel = Off Channel or Backwater

REMEMBER: A = Upstream end of Reach and K = Downstream end of Reach.

THALWEG PROFILE

STATION	SNAG	DEPTH (Either) UNITS: ○ ft ○ m — SONAR XX.X	DEPTH (Either) UNITS: ○ ft ○ m — POLE X.X	SUBSTRATE (Fill in one Substrate Code for each station)	CHANNEL HABITAT (Fill in one Channel Habitat Code for each station)	OFF CHAN.	FLAG
0	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
1	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
2	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
3	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
4	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
5	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
6	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
7	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
8	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
9	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
10	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	
11	○			○ BH ○ BL ○ CB ○ GR ○ SA ○ FN ○ OT	○ PO ○ GL ○ RI ○ RA ○ CA ○ FA ○ DR	○	

Flag	Comments

Flag codes: U = Suspect sample; F1, F2, etc. = flag assigned by field crew. Explain all flags in comment sections.

03/14/2013 2013 Phab Thalweg Profile - Boatable 6700391398

DRAFT - DO NOT USE IN FIELD

Figure 8.2 Thalweg Profile Form

Table 8.5 Channel Unit Categories Used on Thalweg Form

Class (Code)[a]	Description
Pools (PO):	Still water, low velocity, smooth, surface, deep compared to other parts of channel
Glide (GL)	Water moving slowly, with a smooth, unbroken surface. Low turbulence.
Riffle (RI)	Water moving, with small ripples, waves and eddies—waves not breaking, surface tension not broken. Sound: "babbling", "gurgling".
Rapid (RA)	Water movement rapid and turbulent, surface with intermittent whitewater with breaking waves. Sound: continuous rushing, but not as loud as cascade.
Cascade (CA)	Water movement rapid & very turbulent over steep channel bottom. Most of the water surface is broken in short, irregular plunges, mostly whitewater. Sound: roaring.
Falls (FA)	Free falling water over vertical or near vertical drop into plunge, water turbulent and white over high falls. Sound: splash to roar. (Don't navigate raft over a waterfall!).
Dry channel (DR)	No water in the channel.
Off-channel	Side-channels, sloughs, backwaters, and alcoves separated from the main channel.

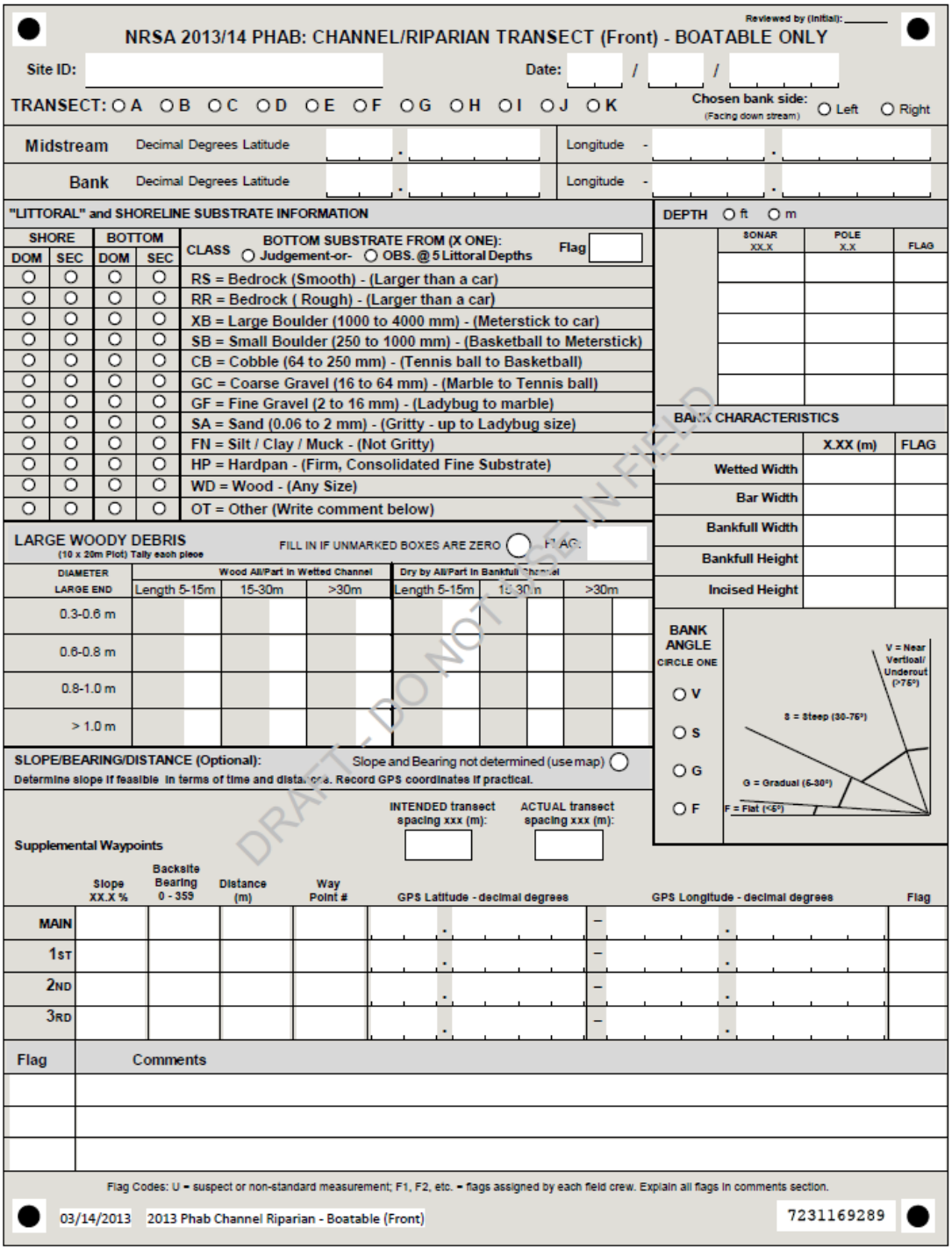
[a] In order for a channel habitat unit to be distinguished, it must be at least as wide or long as the channel is wide.

Mid-channel bars, islands, and side channels within a thalweg profile require some guidance. Mid-channel bars are defined as channel features below the bankfull flow level that are dry during baseflow conditions (Section 8.6.3 defines bankfull channel). Islands are channel features that are dry even when the river is at bankfull flow. If a mid-channel feature is as high as the surrounding flood plain, it is considered an island. Both mid-channel bars and islands cause the river to split into side channels. If a bar or island is encountered along the thalweg profile, navigate and survey the channel that carries the most flow. Note side channels are present but do not sample them.

When side channels are present, fill in the "Y" bubble on the Thalweg Profile form in the "Off-Channel" column. These notations will begin at the point of divergence from the main channel, continuing downstream to the point of convergence with the main channel. In the case of a slough or alcove, the "off-channel" notation should continue from the point of divergence downstream to where the off-channel feature is no longer evident. When major side channels occur, flag the "Off-Channel" notations and indicate in the comments section that the feature is a side channel.

8.6 Channel Margin ("Littoral") and Riparian Measurements

This section covers channel margin depth and substrate, large woody debris, bank angle, channel cross-section morphology, canopy cover, riparian vegetation structure, fish cover, and human influences. Record measurements on the Channel/Riparian Transect Form (Figure 8.3 and Figure 8.7).



NRSA 2013/14 PHAB: CHANNEL/RIPARIAN TRANSECT (Front) - BOATABLE ONLY

Reviewed by (initial): _____

Site ID: _____ Date: ___ / ___ / ___

TRANSECT: ○ A ○ B ○ C ○ D ○ E ○ F ○ G ○ H ○ I ○ J ○ K

Chosen bank side: (Facing down stream) ○ Left ○ Right

Midstream	Decimal Degrees Latitude ___ . ___	Longitude - ___ . ___
Bank	Decimal Degrees Latitude ___ . ___	Longitude - ___ . ___

"LITTORAL" and SHORELINE SUBSTRATE INFORMATION

SHORE DOM	SHORE SEC	BOTTOM DOM	BOTTOM SEC	CLASS — BOTTOM SUBSTRATE FROM (X ONE): ○ Judgement-or- ○ OBS. @ 5 Littoral Depths — Flag ___
○	○	○	○	RS = Bedrock (Smooth) - (Larger than a car)
○	○	○	○	RR = Bedrock (Rough) - (Larger than a car)
○	○	○	○	XB = Large Boulder (1000 to 4000 mm) - (Meterstick to car)
○	○	○	○	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) - (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)
○	○	○	○	FN = Silt / Clay / Muck - (Not Gritty)
○	○	○	○	HP = Hardpan - (Firm, Consolidated Fine Substrate)
○	○	○	○	WD = Wood - (Any Size)
○	○	○	○	OT = Other (Write comment below)

DEPTH ○ ft ○ m

SONAR XX.X	POLE X.X	FLAG

BANK CHARACTERISTICS

	X.XX (m)	FLAG
Wetted Width		
Bar Width		
Bankfull Width		
Bankfull Height		
Incised Height		

LARGE WOODY DEBRIS (10 x 20m Plot) Tally each piece — FILL IN IF UNMARKED BOXES ARE ZERO ○ — FLAG: ___

DIAMETER LARGE END	Wood All/Part in Wetted Channel: Length 5-15m	15-30m	>30m	Dry by All/Part in Bankfull Channel: Length 5-15m	15-30m	>30m
0.3-0.6 m						
0.6-0.8 m						
0.8-1.0 m						
> 1.0 m						

BANK ANGLE CIRCLE ONE

○ V
○ S
○ G
○ F

SLOPE/BEARING/DISTANCE (Optional): Slope and Bearing not determined (use map) ○

Determine slope if feasible in terms of time and distance. Record GPS coordinates if practical.

INTENDED transect spacing xxx (m): ___ ACTUAL transect spacing xxx (m): ___

Supplemental Waypoints

	Slope XX.X %	Backsite Bearing 0 - 359	Distance (m)	Way Point #	GPS Latitude - decimal degrees	GPS Longitude - decimal degrees	Flag
MAIN					.	– .	
1ST					.	– .	
2ND					.	– .	
3RD					.	– .	

Flag	Comments

Flag Codes: U = suspect or non-standard measurement; F1, F2, etc. = flags assigned by each field crew. Explain all flags in comments section.

03/14/2013 2013 Phab Channel Riparian - Boatable (Front) 7231169289

DRAFT - DO NOT USE IN FIELD

Figure 8.3 Channel/Riparian Transect Form, page 1 (front side)

8.6.1 Channel Margin Depth and Substrate

Channel margin depths are measured along the designated shoreline at each transect within a 10m x 20m littoral plot that is centered on the transect (Figure 8.4). Dominant and sub-dominant bottom substrates are determined and recorded at 5 systematically-spaced locations that are located by eye within the 10m x 20m plot. The procedure for obtaining channel margin depth and substrate measurements is described in more detail in Table 8.6. Record these measurements on the Channel/Riparian Transect Form as shown in Figure 8.3. Identify the dominant and subdominant substrate present along a shoreline swath 20 meters long and 1 meter back from the waterline. The substrate size class choices are as shown in Table 8.6.

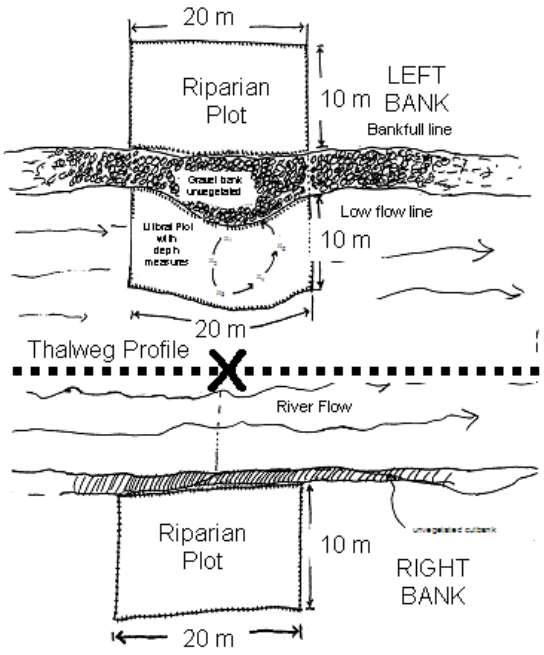


Figure 8.4 Riparian Zone and Instream Fish Cover Plots for a River Cross-Section Transect

Table 8.6 Channel Margin Depth and Substrate Procedure

1. Fill in the header information on page 1 of a Channel/Riparian Transect Form. Be sure to indicate the letter designating the transect location. Indicate the assessed bank (left or right) as designated during reach layout activities. Also ensure that GPS coordinates have been recorded for each transect at the bank and midstream locations.
2. Measure depth and observe bottom substrates within the 10m x 20 m littoral plot that is centered on each transect location.
3. Determine and record the depth and the dominant and subdominant substrate size class at 5 systematically-spaced locations estimated by eye within this 10m x 20m plot as well as on the shoreline 1m back from the waterline. **If the substrate particle is "artificial" (e.g. concrete, asphalt), choose the appropriate size class, flag the observation and note that it is artificial in the comment space.**

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
XB	Large Boulders	>1000 to 4000	Meter stick to Car size
SB	Small Boulders	>250 to 1000	Basketball to 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	Gritty – up to ladybug size,
FN	Fines	<0.06	Silt Clay Muck (not gritty between fingers)
HP	Hardpan		Firm, consolidated fine substrate
WD	Wood	Regardless of Size	Wood & other organic particles
OT	Other	Regardless of Size	Concrete, metal, tires, etc. (note in comments)

4. On page 1 of the Channel/Riparian Transect Form, indicate the appropriate shore and bottom substrate type and record the depth measurements ("SONAR" or "POLE" columns).
5. Repeat Steps 1 through 4 at each new cross-section transect.

8.6.2 Large Woody Debris

Large Woody Debris (LWD) is defined as woody material with small end diameter of ≥30 cm (1ft) and length of ≥5 m (15 ft). These size criteria are larger than those used in wadeable streams because of the lesser role that small wood plays in controlling velocity and morphology of larger rivers. The procedure for tallying LWD is presented in Table 8.7. For each tally (Wood All/Part in Wetted Channel and Dry but All/Part in Bankfull Channel), the field form (Figure 8.3) provides 12 entry boxes for tallying debris pieces visually estimated within three length and four diameter class combinations. Tally each LWD piece in only one box. Tally all LWD that is within each 10x20 littoral plot. Do not tally woody debris in the area between channel cross-sections, but the presence and location of large debris dams and accumulations should be mapped (sketched) and noted in the thalweg profile comments.

For each LWD piece, first visually estimate its length and its large and small end diameters and place it in one of the diameter and length categories. The diameter classes on the field form (Figure 8.3) refer to the large end diameter. Sometimes LWD is not cylindrical, so it has no clear "diameter". In these cases visually estimate what the diameter would be for a piece of wood with circular cross-section that would have the same volume. When evaluating length, include only the part of the LWD piece that has a diameter >0.3m (1 ft). Count each of the LWD pieces as one tally entry and include the whole piece when assessing dimensions, even if part of it is outside of the bankfull channel. If you encounter massive, complex debris jams, estimate their length, width, and height. Estimate the diameter and length of large "key" pieces and judge the average diameter and length of the other pieces making up the jam. Record this information in the comments section of the form.

Table 8.7 Procedure for Tallying Large Woody Debris

Note: Tally pieces of large woody debris (LWD) within the 11 transect littoral plots of the river reach at the same time the shoreline measurements are being determined. Include all pieces whose large end is located within the littoral plot in the tally. Tally wood that is at least partially within the wetted channel separately from that that is not presently wetted, but still within or directly above (bridging) the bankfull channel

1. LWD is tallied in 11 "plots" systematically spaced over the entire length of the sampling reach. These plots are each 20 m long in the upstream-downstream direction (10m up, 10m down from the transect). They are positioned along the chosen bank and extend from the shore 10m towards mid-channel and then all the way to the bankfull margin.
2. Tally all LWD pieces within the plot that are at least partially within the presently wetted (baseflow) channel. First, determine if a piece is large enough to be classified as LWD (**small end diameter 30 cm [1 ft.]; length 5 m [15 ft.]**)
3. For each piece of LWD, determine its diameter class **based on the diameter of the large end** (0.3 m to < 0.6 m, 0.6 m to <0.8 m, 0.8 m to <1.0 m, or >1.0 m), and the **length class of the LWD pieces based on the part of its length that has diameter ≥30 cm.** Length classes are 5m to <15m, 15m to <30m, or >30m.
   - 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 >0.3 m (1 ft.)
4. Place a tally mark in the appropriate diameter × length class tally box in the "**WOOD ALL/PART IN WETTED CHANNEL**" section of the Channel/Riparian Transect Form.
5. Tally all shoreline LWD pieces along the littoral plot that are at least partially within or above (bridging) the bankfull channel, but not in the wetted channel. For each piece, determine the diameter class based on the diameter of the **large end** (0.3 m to < 0.6 m, 0.6 m to <0.8 m, 0.8 m to <1.0 m, or >1.0 m), and the **length class based on the length of the piece that has diameter ≥30 cm.** Length classes are 5m to <15m, 15m to <30m, or >30m.
6. Place a tally mark for each piece in the appropriate diameter × length class tally box in the "**DRY BUT ALL/PART IN BANKFULL CHANNEL**" section of the Channel/Riparian Transect Form.
7. After all pieces within the segment have been tallied, write the total number of pieces for each diameter × 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 river transect, using a new Channel/Riparian Transect Form.

8.6.3 Bank Angle and Channel Cross-Section Morphology

Bank angles of undercut, vertical, steep, and gradual are visually estimated as defined on the field form (Figure 8.3). Observations are made from the wetted channel margin up 5 m (a canoe's length) into the bankfull channel margin on the previously chosen side of the stream.

You will measure or estimate the wetted width, mid-channel bar width, bankfull height and width, the amount of incision, and the degree of channel constraint. These are assessed for **the whole channel (left and right banks)** at each of the 11 cross-section transects. Record each on the Channel/Riparian Transect Form (Figure 8.3). The procedures for obtaining bank angle and measurements of channel cross-section morphology are presented in Table 8.8.

Wetted width is the width of the channel containing free-standing water; if >15 m, it can be measured with a laser rangefinder. **Mid-channel bar width**, the width of exposed mid-channel gravel or sand bars, is included within the wetted width, but is also recorded separately. In channel cross-section measurements, the wetted and bankfull channel boundaries include mid-

channel bars. Therefore, the wetted width is measured as the distance between wetted left and right banks. Measure across and over mid-channel bars and boulders. If **islands** are present, treat them like bars, but flag these measurements and indicate in the comments that the "bar" is an island. If you are unable to see across the full width of the river when an island separates a side channel from the main channel, record the width of the main channel, flag the observation, and note in the comments section that the width pertains only to the main channel.

Table 8.8 Procedure for Bank Angle and Channel Cross-Section

Bank Angle and Cross Section Methods
1. Record the *wetted width* of the river and the *width of exposed mid-channel bars* (if present) in the *BANK CHARACTERISTICS* section of the field data form. Also determine the *bankfull channel width*.
2. Visually estimate the bank angle (undercut, vertical, steep, gradual), as defined on the field form. Bank angle observations refer to the area from the wetted channel margin up 5 m (canoe's length) into the bankfull channel margin on the previously chosen side of the river. Mark the angle in the "BANK ANGLES" section of the Channel/Riparian Transect Form.
3. Hold the surveyor's rod vertically, with its base planted at the water's edge. Examine both banks, then determine the channel *incision* as the *height up from the water surface to elevation of the **first terrace** of the valley floodplain* (Note this is at or above the bankfull channel height). Whenever possible, use the clinometer as a level (positioned so it reads 0% slope) to measure this height by transferring (backsighting) it onto the surveyor's rod. Record this value in the *INCISED HEIGHT* field of the bank characteristics section on the field data form.
4. 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.
5. Repeat Steps 1 through 4 at each cross-section transect. Record data for each transect on a separate field data form.

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. Estimates of the bankfull dimensions of stream channels are extremely important in the NRSA. They are used to calculate shear stress and bed stability (see Kaufmann et al., 1999). 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 year's 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 8.5) as a measure of the degree of *incision* or *downcutting* of the stream below the general level of its valley. This value is recorded in the **incised height** field. It may not be evident at the time of sampling whether the channel is downcutting, stable, or aggrading (raising its bed by depositing sediment). However, by recording incision heights measured in this way and monitoring them over time, we will be able to tell if streams are incising or aggrading.

If the channel is not greatly incised, bankfull channel height and incision height will be the same. 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 Height" smaller than "Incision" (Figure 8.6). **Bankfull height is never greater than incision height**. Look for evidence of recent flows (within about 1 year) to distinguish bankfull and incision heights, though recent flooding of extraordinary magnitude may be misleading. 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 determine incision height and bankfull height. Remember that incision height is measured as **vertical distance to the first terrace above bankfull; if terrace heights differ on left and right banks,**

choose the lower of the two terraces. Even when quite constrained by their valley sideslopes, large rivers often have flood terraces above bankfull height. In some cases, though, your sample reach may be in a steep "V" shaped valley or gorge formed over eons, and the slopes of the channel banks simply extend 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 comments that no terrace is evident. Similarly, when the river is extremely incised below an ancient terrace or plateau,(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.

Finally, assess the **local degree of river channel constraint** (i.e., at the transect) by following the guidelines on the back of the Channel/Riparian Transect form (Figure 8.7) regarding the relationships among channel incision, valley sideslope, and width of the valley floodplain. Mark whether you could or could not readily see over the bank. You will also do an overall assessment of channel constraint for the whole river reach; see Section 8.12 for a discussion of constraint concepts and assessment procedures.

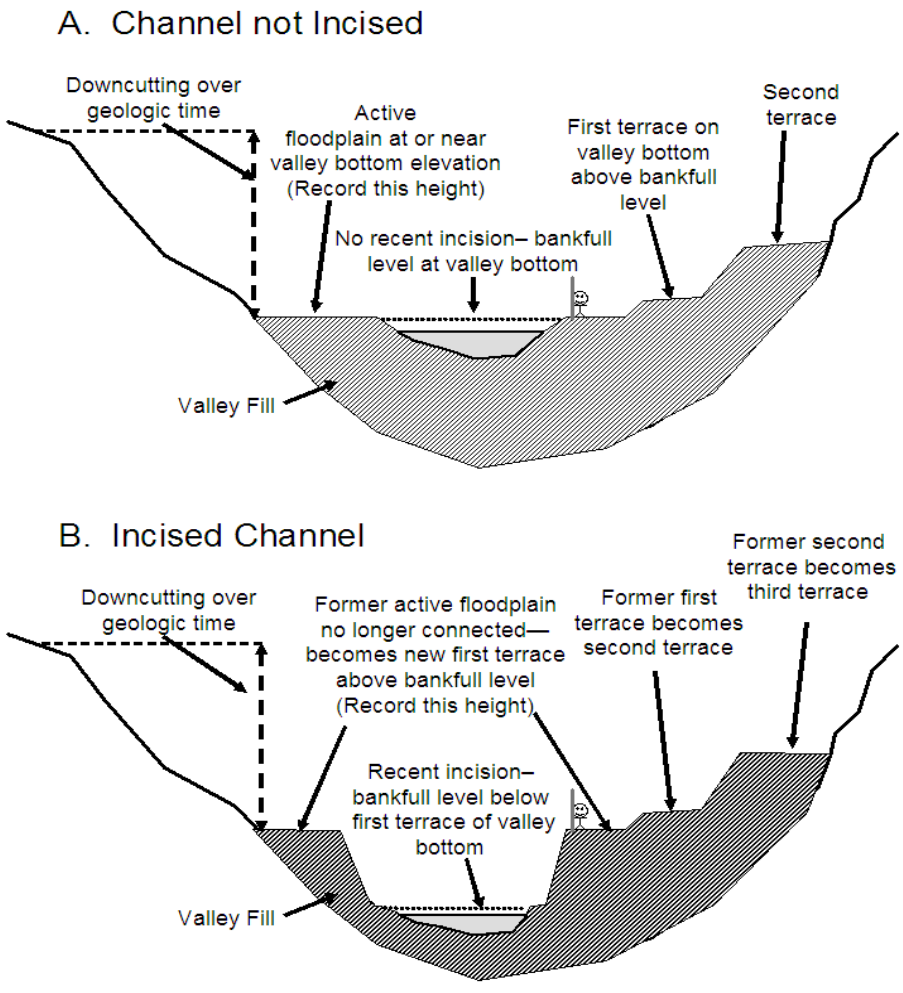


Figure 8.5 Schematic Showing Bankfull Channel and Incision for Channels

(A) not recently incised, and (B) recently incised into valley bottom. Note level of bankfull stage relative to elevation of first terrace on valley bottom (stick figure included for scale)

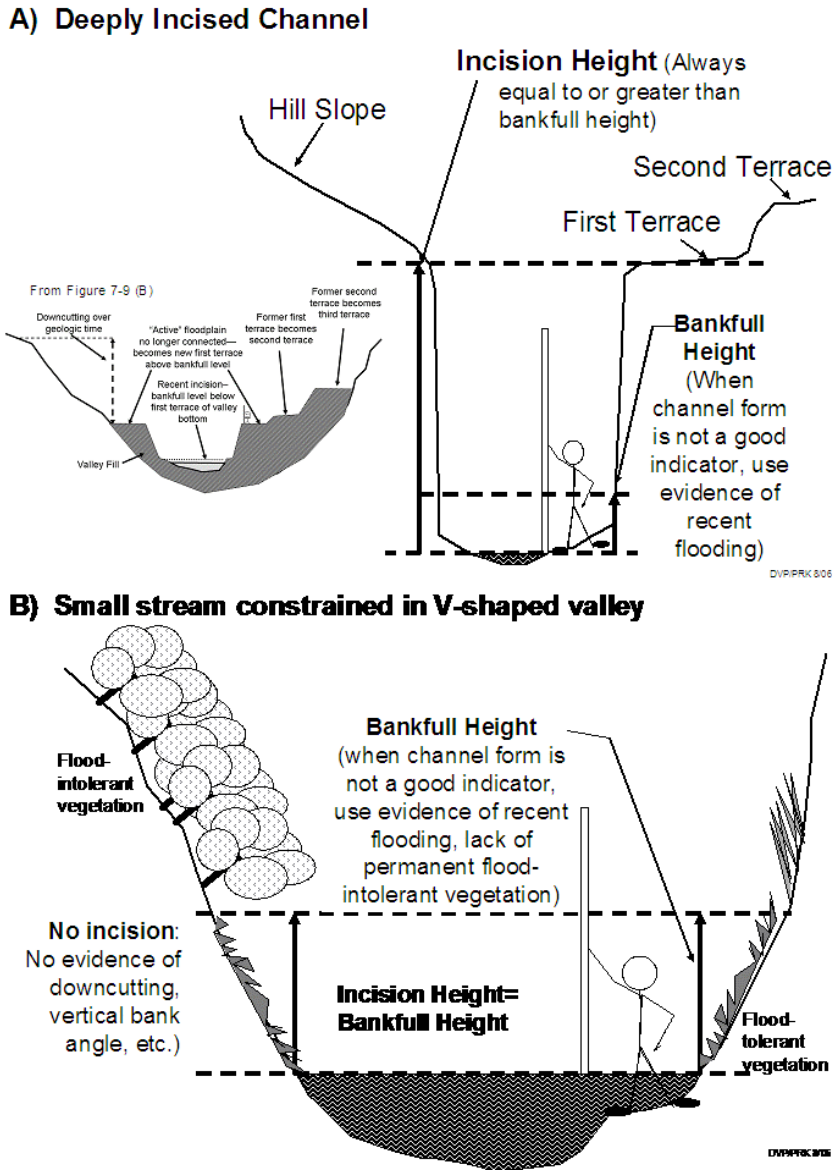
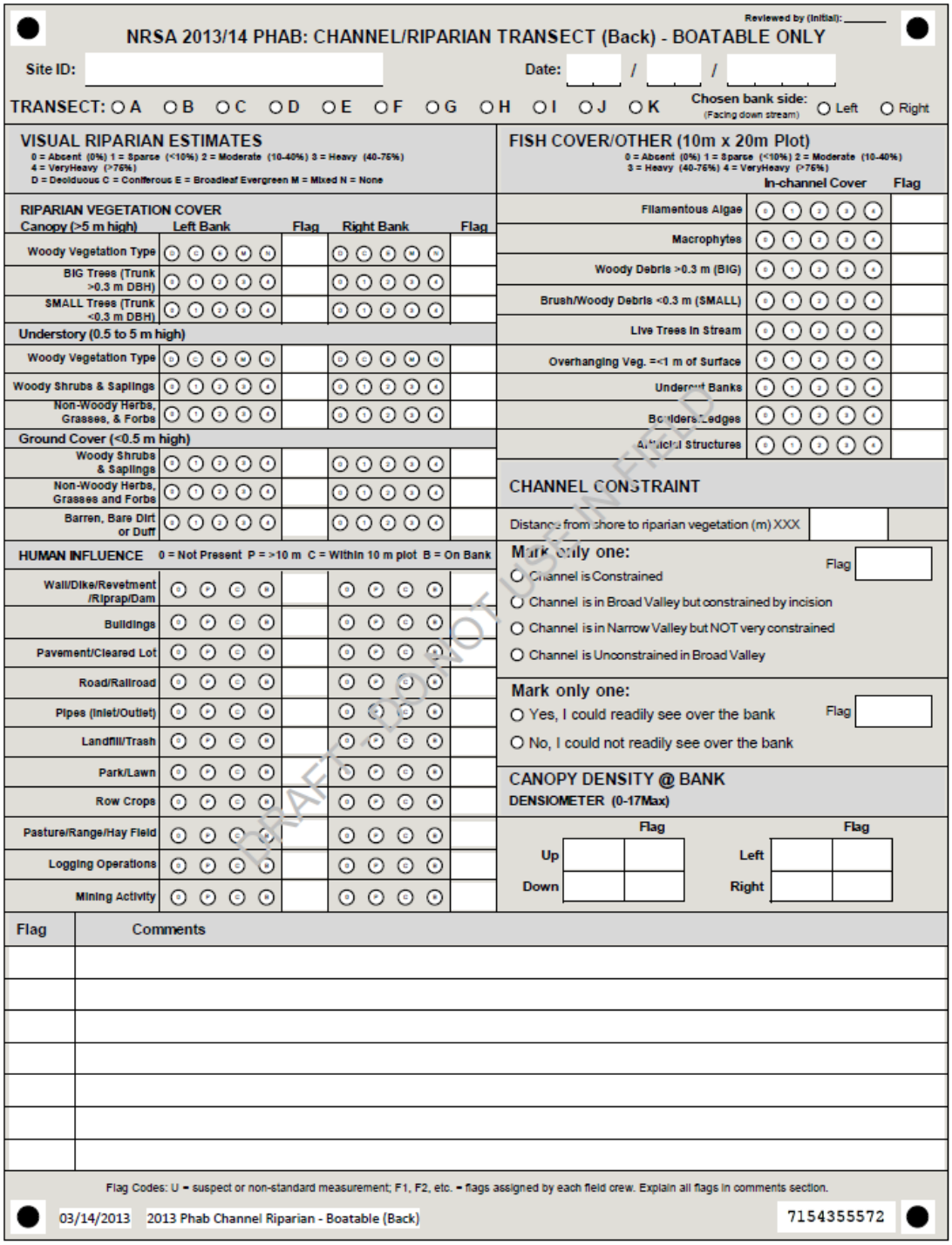


Figure 8.6 Determining Bankfull and Incision Heights for (A) Deeply Incised Channels, and (B) Streams in Deep V Shaped Valleys (Stick figure included for scale)



Reviewed by (initial): _____

NRSA 2013/14 PHAB: CHANNEL/RIPARIAN TRANSECT (Back) - BOATABLE ONLY

Site ID: _____ Date: ____ / ____ / _____

TRANSECT: ○ A ○ B ○ C ○ D ○ E ○ F ○ G ○ H ○ I ○ J ○ K Chosen bank side: (Facing down stream) ○ Left ○ Right

VISUAL RIPARIAN ESTIMATES
0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = VeryHeavy (>75%)
D = Deciduous C = Coniferous E = Broadleaf Evergreen M = Mixed N = None

RIPARIAN VEGETATION COVER Canopy (>5 m high)	Left Bank	Flag	Right Bank	Flag
Woody Vegetation Type	○ ○ ○ ○ ○		○ ○ ○ ○ ○	
BIG Trees (Trunk >0.3 m DBH)	○ 1 2 3 4		○ 1 2 3 4	
SMALL Trees (Trunk <0.3 m DBH)	○ 1 2 3 4		○ 1 2 3 4	
Understory (0.5 to 5 m high)				
Woody Vegetation Type	○ ○ ○ ○ ○		○ ○ ○ ○ ○	
Woody Shrubs & Saplings	○ 1 2 3 4		○ 1 2 3 4	
Non-Woody Herbs, Grasses, & Forbs	○ 1 2 3 4		○ 1 2 3 4	
Ground Cover (<0.5 m high)				
Woody Shrubs & Saplings	○ 1 2 3 4		○ 1 2 3 4	
Non-Woody Herbs, Grasses and Forbs	○ 1 2 3 4		○ 1 2 3 4	
Barren, Bare Dirt or Duff	○ 1 2 3 4		○ 1 2 3 4	

HUMAN INFLUENCE 0 = Not Present P = >10 m C = Within 10 m plot B = On Bank				
Wall/Dike/Revetment /Riprap/Dam	○ P C B		○ P C B	
Buildings	○ P C B		○ P C B	
Pavement/Cleared Lot	○ P C B		○ P C B	
Road/Railroad	○ P C B		○ P C B	
Pipes (Inlet/Outlet)	○ P C B		○ P C B	
Landfill/Trash	○ P C B		○ P C B	
Park/Lawn	○ P C B		○ P C B	
Row Crops	○ P C B		○ P C B	
Pasture/Range/Hay Field	○ P C B		○ P C B	
Logging Operations	○ P C B		○ P C B	
Mining Activity	○ P C B		○ P C B	

FISH COVER/OTHER (10m x 20m Plot)
0 = Absent (0%) 1 = Sparse (<10%) 2 = Moderate (10-40%) 3 = Heavy (40-75%) 4 = VeryHeavy (>75%)

	In-channel Cover	Flag
Filamentous Algae	○ 1 2 3 4	
Macrophytes	○ 1 2 3 4	
Woody Debris >0.3 m (BIG)	○ 1 2 3 4	
Brush/Woody Debris <0.3 m (SMALL)	○ 1 2 3 4	
Live Trees in Stream	○ 1 2 3 4	
Overhanging Veg. =<1 m of Surface	○ 1 2 3 4	
Undercut Banks	○ 1 2 3 4	
Boulders/Ledges	○ 1 2 3 4	
Artificial Structures	○ 1 2 3 4	

CHANNEL CONSTRAINT

Distance from shore to riparian vegetation (m) XXX _____

Mark only one: Flag _____
- ○ Channel is Constrained
- ○ Channel is in Broad Valley but constrained by incision
- ○ Channel is in Narrow Valley but NOT very constrained
- ○ Channel is Unconstrained in Broad Valley

Mark only one: Flag _____
- ○ Yes, I could readily see over the bank
- ○ No, I could not readily see over the bank

CANOPY DENSITY @ BANK
DENSIOMETER (0-17Max)

	Flag		Flag
Up		Left	
Down		Right	

Flag	Comments

Flag Codes: U = suspect or non-standard measurement; F1, F2, etc. = flags assigned by each field crew. Explain all flags in comments section.

03/14/2013 2013 Phab Channel Riparian - Boatable (Back) 7154355572

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Figure 8.7 Channel/Riparian Transect Form, page 2 (back side).

8.7 Visual Riparian Estimates

8.7.1 Riparian Vegetation Structure

Riparian vegetation observations apply to the riparian area upstream 10 m and downstream 10 m from each of the 11 transects. They include the visible area from the river bankfull margin back a distance of 10 m (30 ft) shoreward from both the left and right banks, creating a 10m X 20m riparian plot on each side of the river (Figure 8.1). The riparian plot dimensions are estimated, not measured. Table 8.9 presents the procedure for characterizing riparian vegetation structure and composition. Figure 8.7Figure 8.7 illustrates how measurement data are recorded in the "Visual Riparian Estimates" section of the Channel/Riparian Transect Form, side 2.

Before estimating the areal coverage of the vegetation layers, record the type of *woody* vegetation (*broadleaf **D**eciduous*, ***C**oniferous*, *broadleaf **E**vergreen*, ***M**ixed*, or ***N**one*) 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*.

Table 8.9 Procedure for Characterizing Riparian Vegetation Structure

Riparian Vegetative Cover
1. Anchor or tie up at the river margin at a cross-section transect; then make the following observations to characterize riparian vegetation structure.
2. Estimate the distance from the shore to the edge of the riparian vegetation plot; record it just below the title "Channel Constraint" on the Channel/Riparian Transect Form, side 2.
3. Facing the left bank (left as you face downstream), estimate a distance of 10 m back into the riparian vegetation, beginning at the bankfull channel margin. Estimate the cover and structure of riparian vegetation within an estimated 10 m x 20 m plot centered on the transect, and starting where perennial vegetation begins or at the bankfull river margin (whichever is closest to the river shoreline). On steeply-sloping channel margins, estimate the riparian plot dimensions as if they were projected down from an aerial view.
4. Within this 10 m × 20 m area, conceptually divide the riparian vegetation into 3 layers: a CANOPY (>5m high), an UNDERSTORY (0.5 to 5 m high), and a GROUND COVER layer (<0.5 m high).
5. Within this 10 m × 20 m area, determine the dominant **woody** vegetation type for the CANOPY LAYER (vegetation > 5 m high) as either Deciduous, Coniferous, broadleaf Evergreen, Mixed, or None. Consider the layer "Mixed" if more than 10% of the areal coverage is made up of the alternate vegetation type. If the dominant vegetation type in the canopy layer is not woody, record the vegetation type as "None". Indicate the appropriate vegetation type in the "VISUAL RIPARIAN ESTIMATES" section of the Channel/Riparian Transect Form.
6. 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%).
7. 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 5 for the canopy layer. If the dominant vegetation type in the understory is not woody (e.g., herbaceous), record the vegetation type as "None".
8. Determine the areal cover class for woody shrubs and saplings separately from non-woody

vegetation within the understory, as described in Step 6 for the canopy layer.

9. 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 or duff (dead organic material) present as described in Step 6 for large canopy trees.
10. Repeat Steps 1-9 for all transects, using a separate field data form for each transect.

You will 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%. When rating vegetation cover types, mixtures of two or more subdominant classes might all be given sparse ("1") moderate ("2") or heavy ("3") rankings. One very heavy cover class with no clear subdominant class might be ranked "4" with all the remaining classes either moderate ("2"), sparse ("1") or absent ("0"). Two heavy classes with 40-75% cover can both be ranked "3", but no more than one cover type could receive a rating of *4*.

8.8 Instream Fish Cover, Algae, and Aquatic Macrophytes

Over a defined length and distance from shore at the sampling locations (Figure 8.4), 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 fish cover is outlined in Table 8.10. Record data in the "Fish Cover/Other" section of the Channel/Riparian Transect Form as shown in Figure 8.8. Crews will estimate the areal cover of all of the fish cover and other listed features that are in the water and on the banks within the 10m x 20m plot only on the side of the river previously chosen for assessment during reach layout (Figure 8.1). The areal cover classes of fish concealment and other features are the same as those described for riparian vegetation (Section 8.8.1)

Filamentous algae pertains to long streaming algae that often occur in slow moving waters. Aquatic macrophytes are water loving plants in the river, including mosses, which could provide cover for fish or macroinvertebrates. If the river channel contains live wetland grasses, include these as macrophytes. Woody debris are the larger pieces of wood that can provide cover and influence stream morphology (i.e., those pieces that would be included in the large woody debris tally [Section 8.7.2]). Brush/woody debris pertains to the smaller wood that primarily affects cover but not morphology. The entry for trees or brush within one meter of the surface is the amount of brush, twigs, small debris etc. that is not in the water but is close to the stream and provides cover. "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. For ephemeral channels, estimate the proportional cover of these trees that is inundated during bankfull flows. Boulders are typically basketball to car sized particles. Many streams contain artificial structures designed for fish habitat enhancement. Streams may also have in-channel structures discarded (e.g., cars or tires) or purposefully placed for diversion, impoundment, channel stabilization, or other purposes. Record the cover of these structures on the form

Table 8.10 Procedure for Estimating Fish Cover

Fish Cover
1. Stop at the designated shoreline at a cross-section transect and estimate a 10 m distance upstream and downstream (20 m total length), and a 10 m distance out from the banks to define a 20 m x 10 m littoral plot.
2. Examine the water and the banks within the 20 m x 10 m littoral plot for the following features and types of fish cover: *filamentous algae, aquatic macrophytes, large woody debris, in-channel live trees or roots, brush and small woody debris, overhanging vegetation, undercut banks, boulders, and artificial structures.*
3. For each cover type, estimate its areal cover by eye and/or by sounding with a pole. Record the appropriate cover class in the "FISH COVER/OTHER" section of the Channel/Riparian Transect Form: *0=absent*: zero cover, *1=sparse*: <10%, *2=moderate*: 10-40%, *3=heavy*: >40-75%, or *4=very heavy*: >75%).
4. Repeat Steps 1 through 3 at each cross-section transect, recording data from each transect on a separate field data form.

8.9 Human Influences

For the left and right banks at each of the 11 detailed Channel/Riparian Cross-Sections, evaluate the presence/absence and the proximity of 11 categories of human influences outlined in Figure 8.8. Record human influences on the Channel/Riparian Transect Form (Figure 8.7). Relate your observations and proximity evaluations to the river and riparian area within 10 m upstream and 10 m downstream from the transect (Figure 8.8). Four proximity classes are used: In the river or on the bank within 10 m upstream or downstream of the cross-section transect, present within the 10 m $\times$ 20 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 as shown in Figure 8.7. 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 10x20 meter riparian plot.**

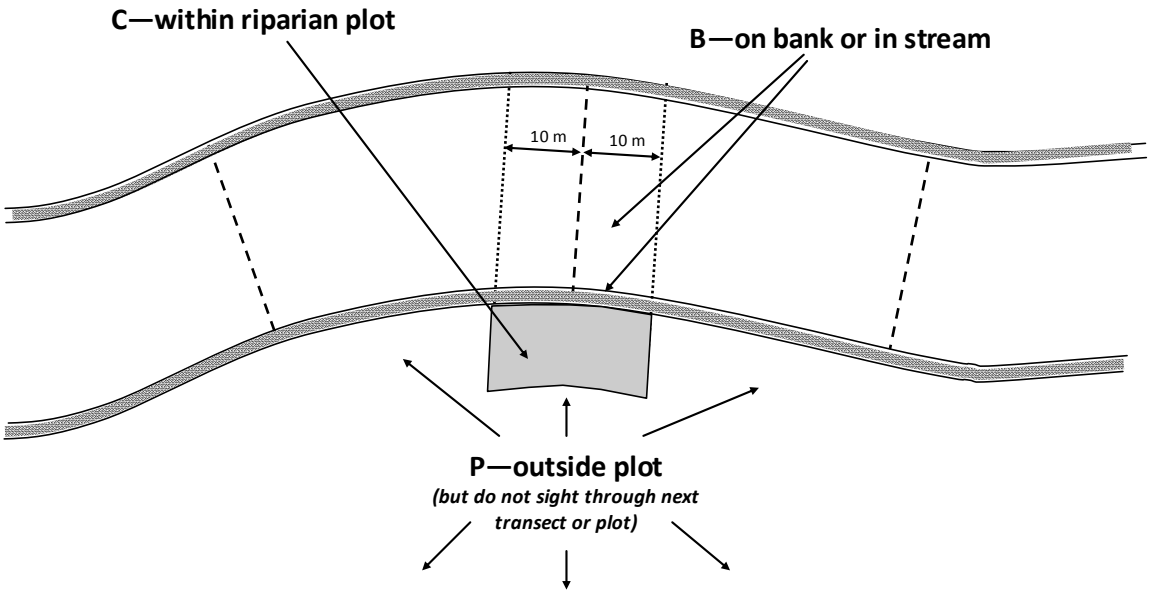


Figure 8.8 Proximity Classes for Human Influences in Non-Wadeable Rivers

Table 8.11 Procedure for Estimating Human Influence

Human Influence	
1.	Stop at the designated shoreline at a cross-section transect, look toward the left bank (left when facing downstream), and estimate a 10m distance upstream and downstream (20 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 river segment for the following human influences: (1) walls, dikes, revetments, riprap, & dams; (2) buildings; (3) cleared lot, pavement (e.g., paved, graveled, dirt parking lot, foundation); (4) roads or railroads, (5) inlet or outlet pipes; (6) landfills or trash (e.g., cans, bottles, trash heaps); (7) parks or maintained lawns; (8) row crops; (9) pastures, rangeland, or hay fields; (10) logging; and (11) mining (include gravel mining).
3.	For each type of influence, determine if it is present and what its proximity is to the river 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 site through another transect or its 10 m × 20 m riparian plot.
4.	For each type of influence, record the proximity class in the "HUMAN INFLUENCE" part of the "VISUAL RIPARIAN ESTIMATES" section of the Channel/Riparian Transect Form. Proximity classes are:
	B (*Bank*) — Present within the defined 10 m stream segment and located in the stream or on the stream bank.
	C (*Close*) — Present within the 10 × 20 m riparian plot area, but away from the bank.
	P (*Present*) — Present, but outside the riparian plot area.
	0 (*Absent*) — Not present within or adjacent to the 20 m stream segment or the riparian plot area at the transect
5.	Repeat Steps 1 through 4 for the opposite bank.
6.	Repeat Steps 1 through 5 for each cross-section transect, recording data for each transect on a separate field form.

8.10 Canopy Cover Measurements

Canopy cover over the river is determined at each of the 11 cross-section transects. A spherical densiometer (model *A* **convex type**) is used (Lemmon 1957) and is provided in the base kit to each crew. Mark the densiometer with a permanent marker or tape exactly as shown in Figure 8.9 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). Measure vegetative cover over the reach at the chosen bank at each of the 11 transects (A-K). Four measurements are obtained at each cross-section transect (upriver, downriver, left, and right).

The procedure for obtaining canopy cover data is presented in Table 8.12. 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 8.9. 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 four measurement points, record the number of intersection points (maximum=17) that have vegetation covering them in the *CANOPY DENSITY @ BANK* section of the Channel/Riparian Cross-section Form as shown in Figure 8.7.

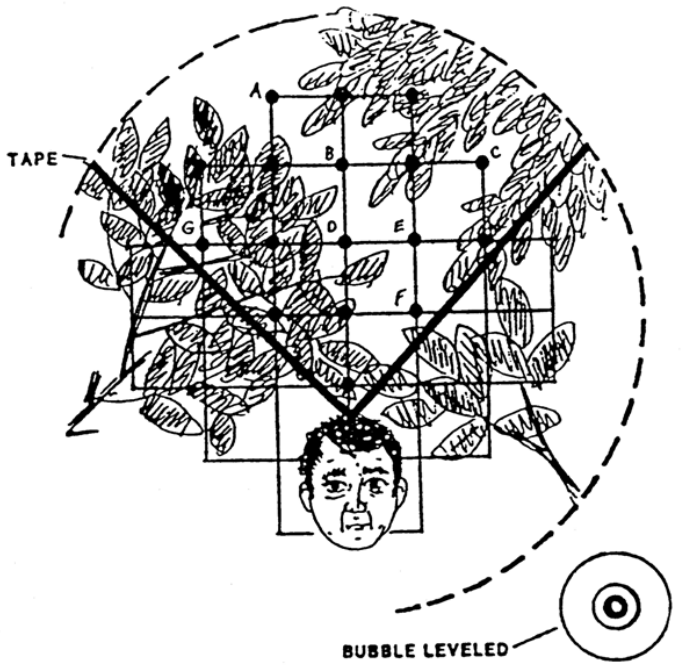


Figure 8.9 Schematic of Modified Convex Spherical Canopy Densiometer

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

Table 8.12 Procedure for Canopy Cover Measurements

Canopy Cover Methods
1. Take densiometer readings at a cross-section transect while anchored or tied up at the river margin. 2. Hold the densiometer 0.3 m (1 ft) above the surface of the river. Holding the densiometer level using the bubble level, move it in front of you so your face is just below the apex of the taped "V". 3. At the channel margin measurement locations, count the number of grid intersection points within the "V" that are covered by either a tree, a leaf, a high branch, or the bank itself. 4. Take 1 reading each facing upstream (UP), downstream (DOWN), left bank (LEFT), and right bank (RIGHT). Right and left banks are defined with reference to an observer facing downstream. 5. Record the UP, DOWN, LEFT, and RIGHT values (0 to 17) in the "CANOPY COVER @ BANK" section of the Channel/Riparian Transect Form. 6. Repeat Steps 1 through 5 at each cross-section transect. Record data for each transect on a separate field data form.

8.11 Channel Constraint Assessment, Debris Torrents and Recent Floods

8.11.1 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 8.13. Record data on the Channel Constraint Assessment Form (Figure 8.10). First, classify the stream reach channel pattern as predominantly a *single channel*, an *anastomosing channel*, or a *braided channel (*Figure 8.11*)*:

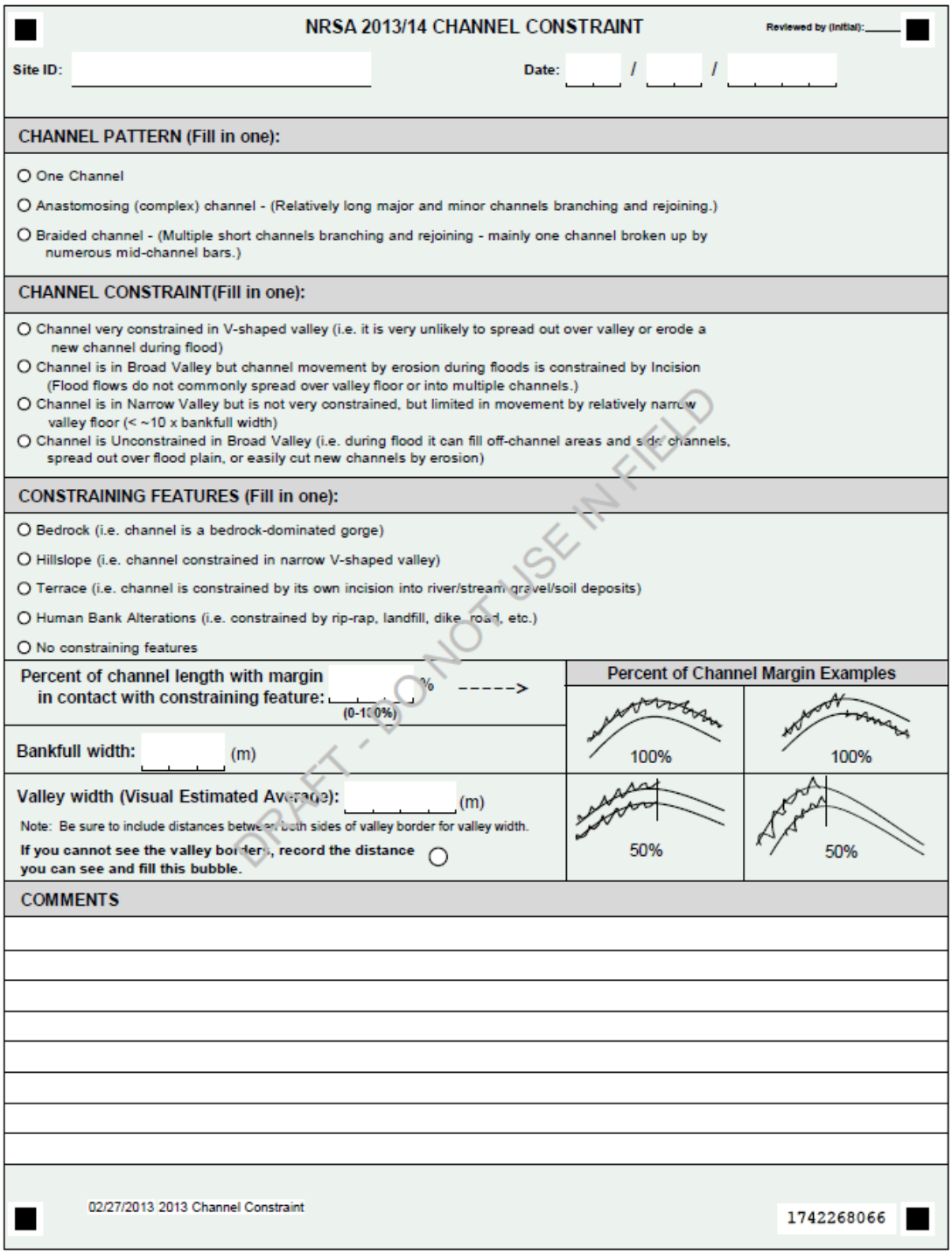
1. Single channels may have occasional in-channel bars or islands with side channels, but feature a predominant single channel, or a dominant main channel with a subordinate side channel.
2. Anastomosing channels have relatively long major and minor channels (but no predominant channel) in a complex 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 8.13 Procedures for Assessing Channel Constraint

Channel Constraint
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. Channel Constraint: Determine the degree, extent, and type of channel constraint based on envisioning the stream at bankfull flow. 1. Classify the stream reach channel pattern as predominantly a single channel, an anastomosing channel, or a braided channel. • 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. • Anastomosing channels have relatively long major and minor channels branching and rejoining in a complex network separated by vegetated islands, with no obvious dominant channel. • Braided channels also have multiple branching and rejoining channels, separated by unvegetated bars. Subchannels are generally small, short, and numerous, often with no obvious dominant channel. 2. After classifying the channel pattern, determine whether the channel is constrained within a narrow valley, constrained by local features within a broad valley, unconstrained and free to move about within a broad floodplain, or free to move about, but within a relatively narrow valley floor. 3. Then examine the channel to ascertain the bank and valley features that constrain the stream. Entry choices for the type of constraining features are bedrock, hillslopes, terraces/alluvial fans, and human land use (e.g., a road, a dike, landfill, rip-rap, etc.). 4. Based on your determinations from Steps 1 through 3, select and record one of the constraint classes shown on the Channel Constraint Form. 5. Estimate the percent of the channel margin in contact with constraining features (for unconstrained channels, this is 0%). Record this value on the Channel Constraint Form. 6. Finally, estimate the "typical" bankfull channel width, and visually estimate the average width of the valley floor. Record these values on the Channel Constraint Form. NOTE: To aid in this estimate, you may wish to refer to the individual transect assessments of incision and constraint that were recorded on the 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.



NRSA 2013/14 CHANNEL CONSTRAINT

Reviewed by (initial):____

Site ID: _____ Date: ____ / ____ / _____

CHANNEL PATTERN (Fill in one):

- O One Channel
- O Anastomosing (complex) channel - (Relatively long major and minor channels branching and rejoining.)
- O Braided channel - (Multiple short channels branching and rejoining - mainly one channel broken up by numerous mid-channel bars.)

CHANNEL CONSTRAINT(Fill in one):

- O Channel very constrained in V-shaped valley (i.e. it is very unlikely to spread out over valley or erode a new channel during flood)
- O Channel is in Broad Valley but channel movement by erosion during floods is constrained by Incision (Flood flows do not commonly spread over valley floor or into multiple channels.)
- O Channel is in Narrow Valley but is not very constrained, but limited in movement by relatively narrow valley floor (< ~10 x bankfull width)
- O Channel is Unconstrained in Broad Valley (i.e. during flood it can fill off-channel areas and side channels, spread out over flood plain, or easily cut new channels by erosion)

CONSTRAINING FEATURES (Fill in one):

- O Bedrock (i.e. channel is a bedrock-dominated gorge)
- O Hillslope (i.e. channel constrained in narrow V-shaped valley)
- O Terrace (i.e. channel is constrained by its own incision into river/stream gravel/soil deposits)
- O Human Bank Alterations (i.e. constrained by rip-rap, landfill, dike, road, etc.)
- O No constraining features

Percent of channel length with margin in contact with constraining feature: ____ % (0-100%) ----->

Bankfull width: ____ (m)

Valley width (Visual Estimated Average): ____ (m)

Note: Be sure to include distances between both sides of valley border for valley width.

If you cannot see the valley borders, record the distance you can see and fill this bubble. O

Percent of Channel Margin Examples

COMMENTS

02/27/2013 2013 Channel Constraint

1742268066

DRAFT - DO NOT USE IN FIELD

Figure 8.10 Channel Constraint Form

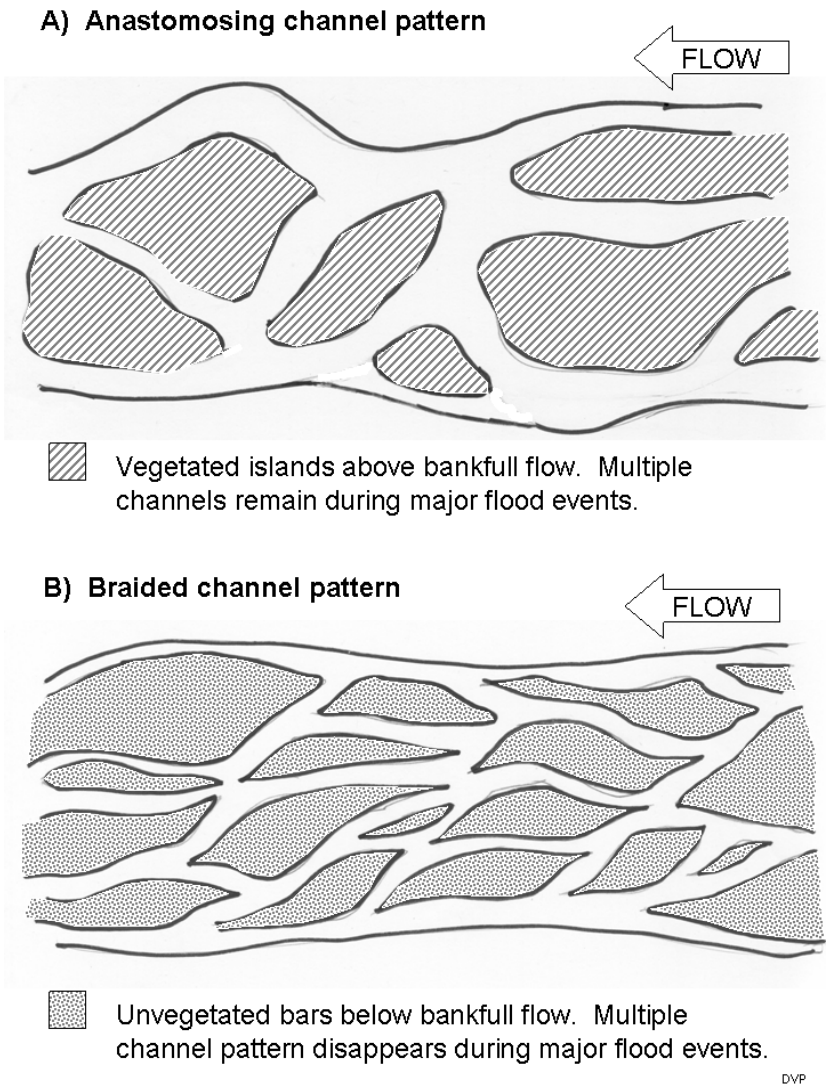


Figure 8.11 Types of Multiple Channel Patterns

8.11.2 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 8.12). 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.

NRSA 2013/14 TORRENT EVIDENCE ASSESSMENT

Reviewed by (Initial): _____

Site ID: _____ Date: ___/___/_____

Please fill in any of the following that are evident.

EVIDENCE OF TORRENT SCOURING:	
○	01 - Stream channel has a recently devegetated corridor two or more times the width of the low flow channel. This corridor lacks riparian vegetation with possible exception of fireweed, even-aged alder or cottonwood seedlings, grasses, or other herbaceous plants.
○	02 - Stream substrate cobbles or large gravel particles are NOT IMBRICATED. (Imbricated means that they lie with flat sides horizontal and that they are stacked like roof shingles -- imagine the upstream direction as the top of the "roof.") In a torrent scour or deposition channel, the stones are laying in unorganized patterns, lying "every which way." In addition many of the substrate particles are angular (not "water-worn.")
○	03 - Channel has little evidence of pool-riffle structure. (For example, could you ride a mountain bike down the channel?)
○	04 - The stream channel is scoured down to bedrock for substantial portion of reach.
○	05 - There are gravel or cobble berms (little levees) above bankfull level.
○	06 - Downstream of the scoured reach (possibly several miles), there are massive deposits of sediment, logs, and other debris.
○	07 - Riparian trees have fresh bark scars at many points along the stream at seemingly unbelievable heights above the channel bed.
○	08 - Riparian trees have fallen into the channel as a result of scouring near their roots.
EVIDENCE OF TORRENT DEPOSITS:	
○	09 - There are massive deposits of sediment, logs, and other debris in the reach. They may contain wood and boulders that, in your judgement, could not have been moved by the stream at even extreme flood stage.
○	10 - If the stream has begun to erode newly laid deposits, it is evident that these deposits are "MATRIX SUPPORTED." This means that the large particles, like boulders and cobbles, are often not touching each other, but have silt, sand, and other fine particles between them (their weight is supported by these fine particles -- in contrast to a normal stream deposit, where fines, if present, normally "fill in" the interstices between coarser particles.)
NO EVIDENCE:	
○	11 - No evidence of torrent scouring or torrent deposits.
COMMENTS	

DRAFT - DO NOT USE IN FIELD

02/27/2013 2013 Torrent Evidence 3362134147

Figure 8.12 Torrent Evidence Form

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 (Figure 9.1).

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 (Figure 4.2).

Table 9.1 Equipment and Supplies List for Fecal Indicator Sampling at Non-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 for Fecal Indicator (Enterococci) Sample Collection at Non-Wadeable Sites

Enterococci Sample
1. **Put on sterile, nitrile gloves**. 2. Select a sampling location at transect K that is a**pproximately 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.

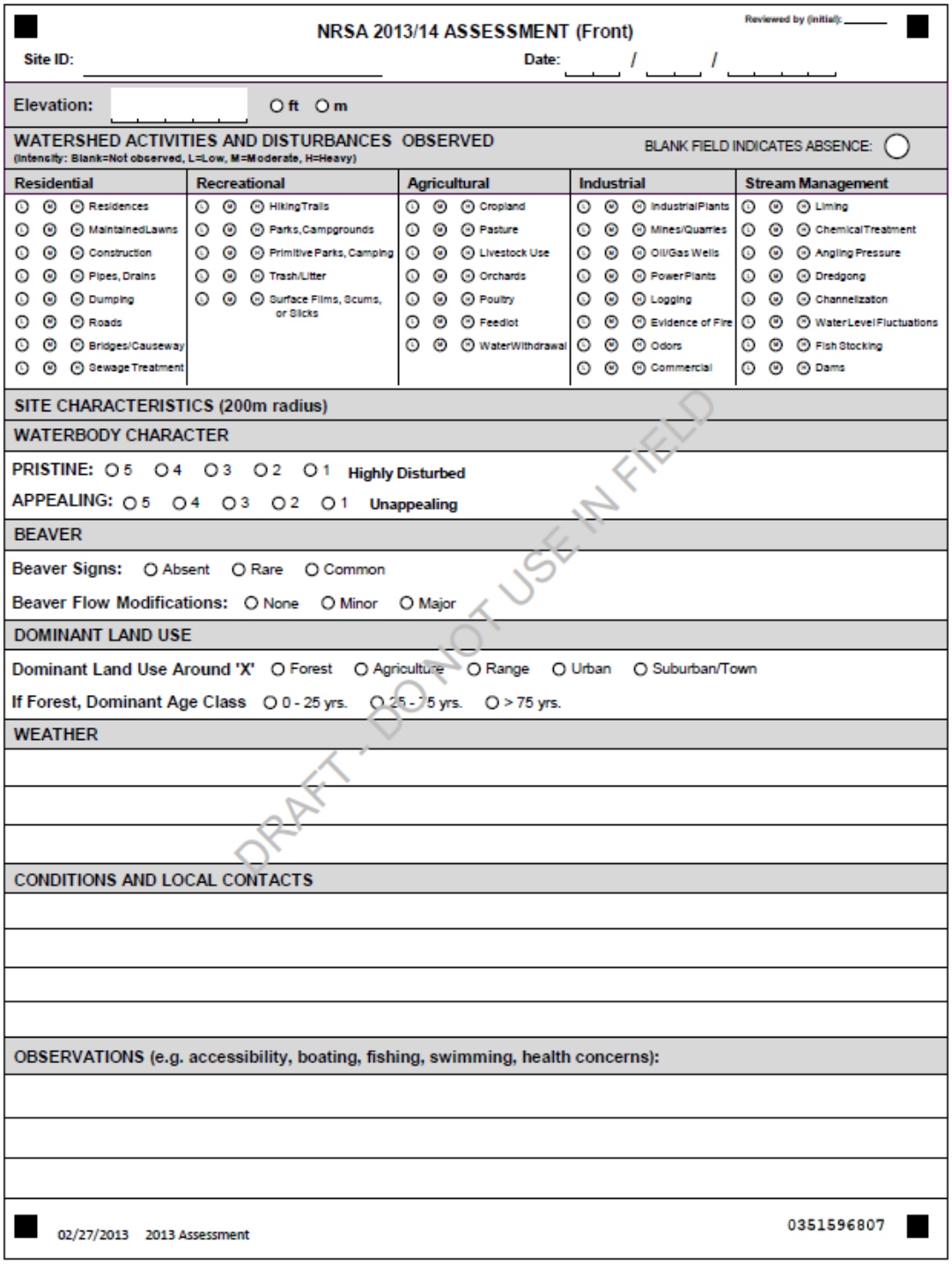
7. **Add the sodium thiosulfate tablet, cap, and shake bottle 25 times**.

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.



NRSA 2013/14 ASSESSMENT (Front)

Reviewed by (initial): _____

Site ID: _____ Date: ____ / ____ / ____

Elevation: _____ ○ ft ○ m

WATERSHED ACTIVITIES AND DISTURBANCES OBSERVED
(Intensity: Blank=Not observed, L=Low, M=Moderate, H=Heavy)

BLANK FIELD INDICATES ABSENCE: ◯

Residential	Recreational	Agricultural	Industrial	Stream Management
Residences	Hiking Trails	Cropland	Industrial Plants	Liming
Maintained Lawns	Parks, Campgrounds	Pasture	Mines/Quarries	Chemical Treatment
Construction	Primitive Parks, Camping	Livestock Use	Oil/Gas Wells	Angling Pressure
Pipes, Drains	Trash/Litter	Orchards	Power Plants	Dredging
Dumping	Surface Films, Scums, or Slicks	Poultry	Logging	Channelization
Roads		Feedlot	Evidence of Fire	Water Level Fluctuations
Bridges/Causeway		Water Withdrawal	Odors	Fish Stocking
Sewage Treatment			Commercial	Dams

SITE CHARACTERISTICS (200m radius)

WATERBODY CHARACTER

PRISTINE: ○ 5 ○ 4 ○ 3 ○ 2 ○ 1 **Highly Disturbed**

APPEALING: ○ 5 ○ 4 ○ 3 ○ 2 ○ 1 **Unappealing**

BEAVER

Beaver Signs: ○ Absent ○ Rare ○ Common

Beaver Flow Modifications: ○ None ○ Minor ○ Major

DOMINANT LAND USE

Dominant Land Use Around 'X' ○ Forest ○ Agriculture ○ Range ○ Urban ○ Suburban/Town

If Forest, Dominant Age Class ○ 0 - 25 yrs. ○ 25 - 75 yrs. ○ > 75 yrs.

WEATHER

CONDITIONS AND LOCAL CONTACTS

OBSERVATIONS (e.g. accessibility, boating, fishing, swimming, health concerns):

DRAFT - DO NOT USE IN FIELD

02/27/2013 2013 Assessment 0351596807

Figure 9.1 Site Assessment Form

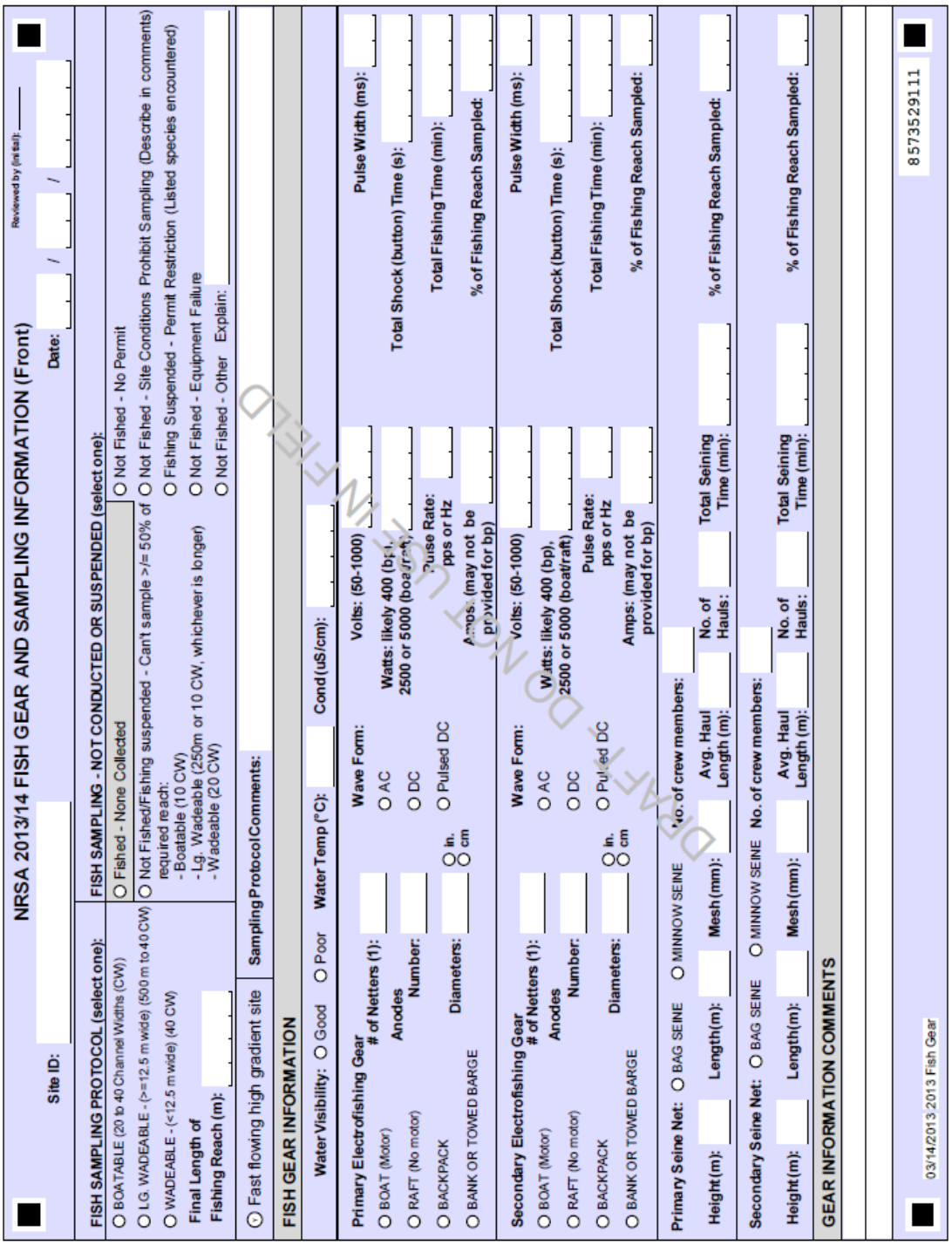
10 FISH ASSEMBLAGE

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 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. 2009, 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.

In non-wadeable rivers, collect fish using boat (or raft) electrofishing over a defined sampling reach within the support reach established for the site. Use secondary fish collection methods in habitat that cannot be adequately sampled by boat. Secondary methods may include backpack or tote barge electrofishing, using your boat as a barge, or seining as a last option only if conductivity is too high for electrofishing. Conduct sampling in a downstream direction, 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 in their entirety until you obtain at least 500 individuals. 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 numerous revisions and clarifications to the non-wadeable sampling protocol from that used in the NRSA 2008/09. Separate collection forms for each subreach are no longer required. The layout of the fish sampling reach and how to allocate effort across the reach is clarified. Guidance is provided to use supplemental gear to more effectively sample areas inaccessible to boats or rafts. Guidance is provided to deal with irruptive species (e.g., shad, certain shiners, etc.) that may artificially skew count and relative abundance results. The fish gear form is modified to include the specific protocol used to sample (boatable, large wadeable, or wadeable), clarify reasons for not sampling, and include information for any secondary gear used. The collection form is modified to include information about introduced species 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 at a site. The procedure is clarified for preparing voucher specimens to distinguish *unknown/range extension vouchers* (those taken back to a local facility for identification) and *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.



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

Site ID: _____ Date: ___/___/___ Reviewed by (initial): _____

FISH SAMPLING PROTOCOL (select one):
- ○ BOATABLE (20 to 40 Channel Widths (CW))
- ○ LG. WADEABLE - (>=12.5 m wide) (500 m to 40 CW)
- ○ WADEABLE - (<12.5 m wide) (40 CW)

Final Length of Fishing Reach (m): _____

FISH SAMPLING - NOT CONDUCTED OR SUSPENDED (select one):
- ○ Fished - None Collected
- ○ 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)
- ○ Not Fished - No Permit
- ○ Not Fished - Site Conditions Prohibit Sampling (Describe in comments)
- ○ Fishing Suspended - Permit Restriction (Listed species encountered)
- ○ Not Fished - Equipment Failure
- ○ Not Fished - Other Explain: _____

○ Fast flowing high gradient site

Sampling Protocol Comments: _____

FISH GEAR INFORMATION

Water Visibility: ○ Good ○ Poor Water Temp (°C): _____ Cond (uS/cm): _____

Primary Electrofishing Gear
- # of Netters (1): _____
- Anodes Number: _____
- Diameters: _____ ○ in. ○ cm

Wave Form: ○ AC ○ DC ○ Pulsed DC

Volts: (50-1000) _____
Watts: likely 400 (bp), 2500 or 5000 (boat/raft) _____
Pulse Rate: pps or Hz _____
Amps: (may not be provided for bp) _____

Pulse Width (ms): _____
Total Shock (button) Time (s): _____
Total Fishing Time (min): _____
% of Fishing Reach Sampled: _____

○ BOAT (Motor)
○ RAFT (No motor)
○ BACKPACK
○ BANK OR TOWED BARGE

Secondary Electrofishing Gear
- # of Netters (1): _____
- Anodes Number: _____
- Diameters: _____ ○ in. ○ cm

Wave Form: ○ AC ○ DC ○ Pulsed DC

Volts: (50-1000) _____
Watts: likely 400 (bp), 2500 or 5000 (boat/raft) _____
Pulse Rate: pps or Hz _____
Amps: (may not be provided for bp) _____

Pulse Width (ms): _____
Total Shock (button) Time (s): _____
Total Fishing Time (min): _____
% of Fishing Reach Sampled: _____

○ BOAT (Motor)
○ RAFT (No motor)
○ BACKPACK
○ BANK OR TOWED BARGE

Primary Seine Net: ○ BAG SEINE ○ MINNOW SEINE No. of crew members: _____

Height (m): _____ Length (m): _____ Mesh (mm): _____ Avg. Haul Length (m): _____ No. of Hauls: _____ Total Seining Time (min): _____ % of Fishing Reach Sampled: _____

Secondary Seine Net: ○ BAG SEINE ○ MINNOW SEINE No. of crew members: _____

Height (m): _____ Length (m): _____ Mesh (mm): _____ Avg. Haul Length (m): _____ No. of Hauls: _____ Total Seining Time (min): _____ % of Fishing Reach Sampled: _____

GEAR INFORMATION COMMENTS

DRAFT DO NOT USE IN FIELD

03/14/2013 2013 Fish Gear

8573529111

Figure 10.1 Fish Gear and Sampling Information, Side 1

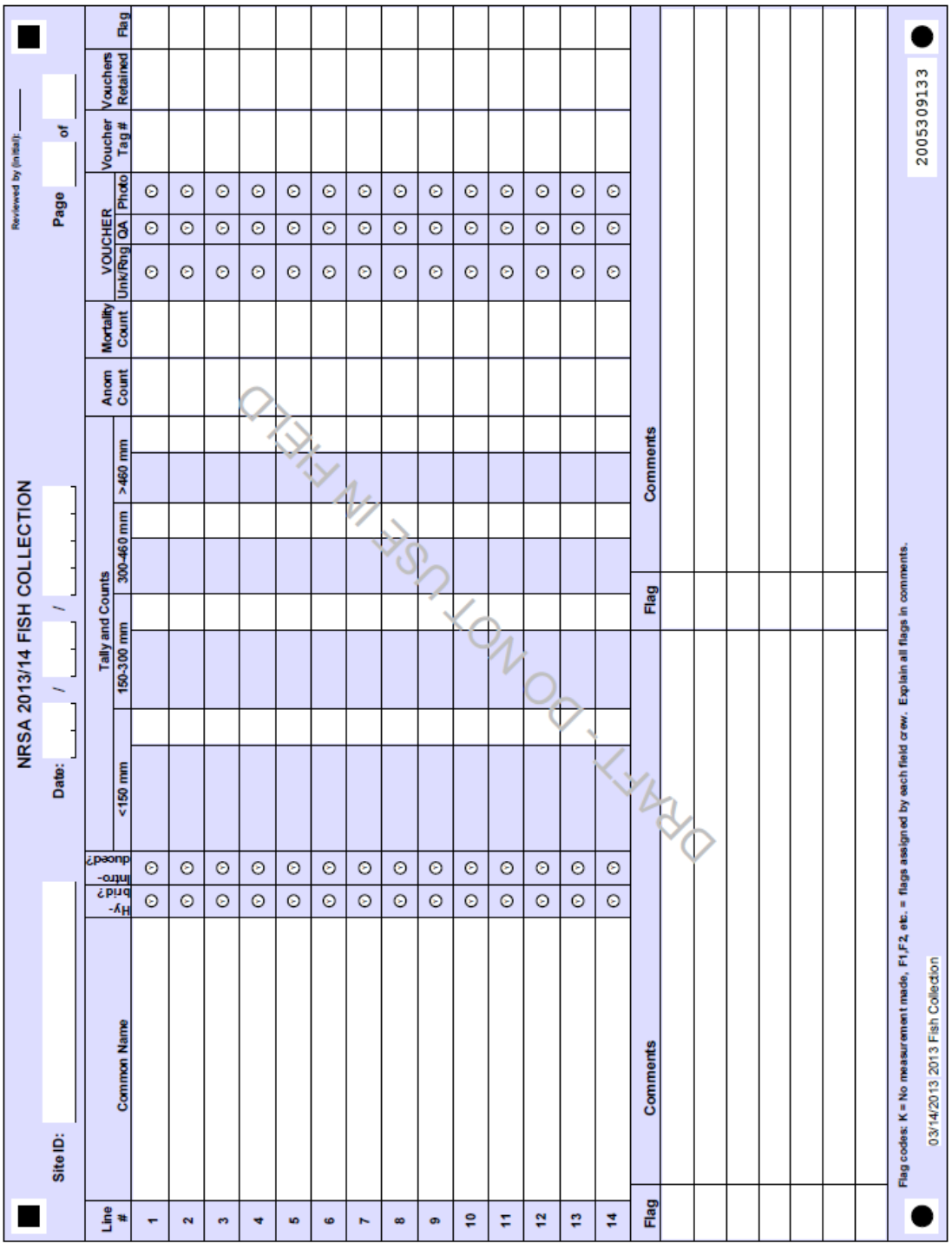


Figure 10.2 Fish Collection Form

10.2 Equipment and Supplies

Table 10.1 shows the checklist of equipment and supplies required to complete the non-wadeable fish sampling. 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 Sampling at Non-Wadeable Sites

For collecting fish	Boat, motor, and trailer (and necessary safety equipment) Gasoline and oil (if using a 2 cycle generator) Boat electrofishing equipment Pulsator Control Box Foot Pedal Anode Droppers Generator Linesman's Gloves Hearing Protection Tow barge electrofishing equipment Probes with extensions. Appropriate switching box Pulsator control box (5.0 GPP or less) Backpack electrofisher (as used for wadeable streams) Dip nets (non-conductive handles) ¼" mesh Scientific collection permit(s) 10 ft x 6 ft minnow or bag seine with ¼ in. mesh (additional 4 ft depth seine may also be used)	GPS with transect waypoints preloaded Several Leak proof HDPE jars for fish voucher specimens (various sizes from 250 mL – 4 L) 1 scalpel for slitting open large fish before preservation 1 container of 10% buffered formalin 1 aquarium net for dipping small fish from live well 2 measuring boards (3 cm size classes) (optional; needed only if quantitative length data are needed) 1 set Fish ID keys Field Operations Manual and/or laminated Quick Reference Guide Digital camera with extra memory card & battery 20 ft x 6 ft minnow or bag seine with ¼ inch mesh (additional 4 ft depth seine may also be used) Polarized sunglasses and hats
For recording measurements	Sheet of sample labels and voucher specimen tags (for unknown/range extension voucher samples) Sheet of sample labels and voucher specimen tags (for QA voucher samples) Fish Gear and Information form Fish collection forms (several per site, depending on expected species richness) Seining information form Clear tape strips	Soft (#2) lead pencils Fine-tip indelible markers

10.3 Sampling Procedures

Table 10.2 describes the procedure for collecting fish in non-wadeable streams. The sampling crew should consist of one boat operator (who also controls the electrofishing unit) and one dip netter (equipped with a 1/4" mesh dip net) situated at the bow. Begin sampling at the upstream end of the support reach defined for the site and proceed downstream.

The reach length sampled for fish varies based on the width of the river used to establish the length of the support reach and on the number of individuals collected (Figure 10.3). In very small rivers (< 12.5 m wide), conduct sampling in the same fashion as for small wadeable streams, sampling the entire support reach and moving the boat (or raft) within each subreach to sample both shorelines as well as the mid-channel.

In medium rivers (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 rivers (mean CW > 25 meters), the minimum length for fish sampling is 5 of the 10 subreaches. In both medium and large rivers, 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 non-wadeable rivers.

For rivers ≥ 12.5 m wide, restrict sampling to shoreline habitats within each subreach. Start on the same bank as the habitat crew, and move to the opposite bank after every two subreaches. Within each subreach, sample for ~700 seconds of "button time." Prior to sampling each subreach, determine the most appropriate gear for the subreach (e.g., boat or raft vs. barge or backpack electrofishing units, or seines if the conductivity is too high). When electrofishing, proceed downstream at a pace equal to or slightly greater than the prevailing current to maximize capture efficiency. Maneuver the electrofishing unit in and around complex habitat when necessary; however, use discretion in sampling these habitats to maintain equal effort among subreaches.

Whenever possible, process fish at the end of each subreach. 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).

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 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 Non-wadeable Fishing Protocols

Small Non-Wadeable (less than 12.5 meters wide)

- Sampling reach will be between 150 and 500 meters
- Subreaches will be between 15 and 50 meters each
  - ✓ Sample all 10 subreaches in their entirety from bank to bank starting at Transect A
  - ✓ Button time is roughly 700 seconds per subreach
  - ✓ No minimum fish number

Medium Non-Wadeable (12.5 to 25 meters wide)

- 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 between 5 and 10 subreaches
- If needed, extend fishing length to end at a transect
  - ✓ Fish each subreach fish along bank in pairs of subreaches starting at the random PHab bank at Transect A
  - ✓ Button time is roughly 700 seconds per subreach
  - ✓ Minimum fish number is 500 unless all 10 subreaches have been fished.
    - ➢ 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.

Large Non-Wadeable (25 + meters wide)

- 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 fish along bank in pairs of subreaches starting at the random PHab bank at Transect A
  - ✓ Button time is roughly 700 seconds per 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.

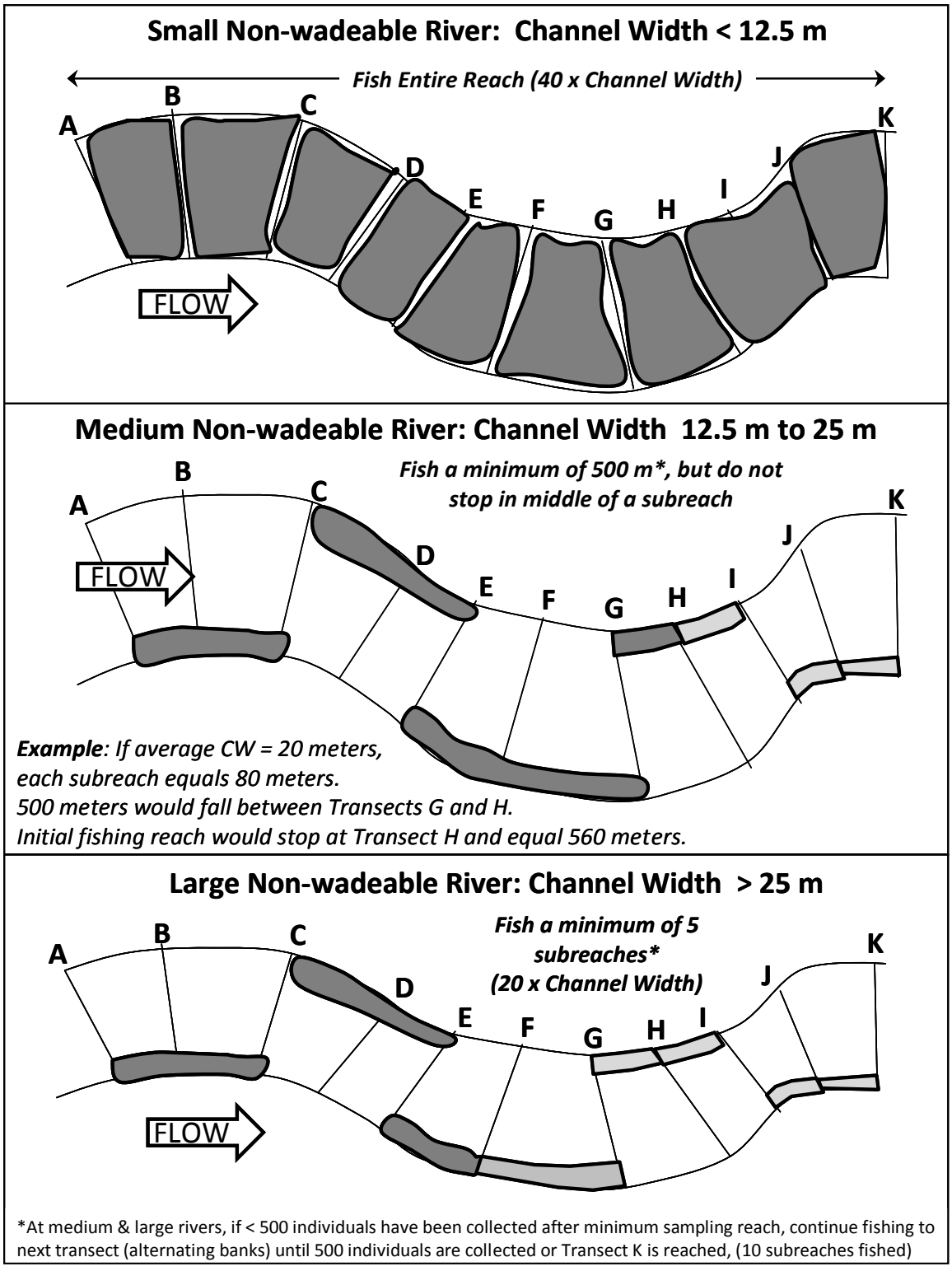


Figure 10.3 Reach Layouts for Fish Sampling at Non-Wadeable Sites

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.

Table 10.3 Procedure for Electrofishing at Non-Wadeable Sites

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. 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 *Not Fished - No Permit*. b) If site conditions prevent boat or raft electrofishing (e.g., no access for boat or raft, safety concerns, conductivity is too high or too low to use a boat electrofishing unit), determine if you can sample with the use of secondary methods (backpack or barge shocker, or by seining). i) If yes, follow the procedures presented in Section 10.3.2 and/or Section 10.3.3. **ii)** If not, mark *Not Fished – Site Conditions Prohibit Sampling*. Note the conditions in the *Sampling Protocol Comments.* c) If you determine that > 50% of the required fish sampling reach is physically inaccessible or otherwise unsampleable by any means, mark *Not Fished/Fishing Suspended – Can't sample >=50% of required reach.* d) If you cannot sample because of equipment problems, mark *Not Fished – Equipment Failure.* e) If you cannot sample for any other reason, mark *Not Fished – Other* and note the reason in the *Explain* field.
3) If you can begin to sample, mark *Boatable* in the *Fish Sampling Protocol* section. For safety, everyone must wear personal floatation devices, foot protection, and insulated linesman's gloves a) To aid vision while netting fish, wear polarized sunglasses and a hat or visor.
4) Determine the minimum length for the fish sampling reach based on the width used to define the support reach for the river (recorded on the stream verification form). a) If the width is < 12.5 m, the fish sampling reach length is the same as the support reach (40 channel widths). b) If the width is between 12.5 and 25 m, the minimum fish sampling reach length is ~500 m. If 500 m falls within a subreach, extend the length to the end of the subreach. c) If the width is > 25 m, the minimum fish sampling reach length is 20 channel widths (5 subreaches).
5) Mark the appropriate *Water Visibility* conditions on the form. *Poor* implies that your ability to electrofish effectively is compromised because of poor visibility. Record the water temperature and conductivity.
6) Mark either *Boat* or *Raft* as the *Primary Electrofishing Gear*. Boats use outboard motors and can travel upstream, while rafts are oar-powered and generally cannot move upstream. a) Only one netter should be used. Record this along with the number of anodes, and their diameter (mark the units as either inches or centimeters) on the fish gear form.

b) Set the boat electrofishing unit to pulsed DC and mark it in the *Wave Form* section of the fish gear form.

c) Test the other settings outside of the sampling area. Start the electrofisher, set the timer, and depress the switch to begin fishing. Typical settings are: 500-1000 V DC; 8-20 A; and 120 Hz. 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 the voltage, then the pulse width.

d) Record the final settings that will be used for sampling on the fish gear form.

7) Begin sampling at the upstream end of the support reach (Transect A). Start on the bank (right or left as you face downstream) selected at random to begin the physical habitat sampling.

a) For small rivers (< 12.5 m wide), move the boat (or raft) within each subreach to sample both shorelines as well as the mid-channel areas (similar to what is done for a small wadeable stream using a backpack electrofishing unit).

b) For rivers > 12.5 m wide, sample along the shoreline only. Move to the opposite bank after every two subreaches. Within each subreach, proceed downstream in close proximity to the bank and at a pace equal to or slightly greater than the prevailing current to maximize capture efficiency. You can "nose in" your boat or raft into shoreline habitat to effectively sample but do not remain in that habitat for too long.

8) Generally effort (i.e., button time) should be ~700 seconds per subreach.

a) At sites with maximum reach length (4000 m) it is likely that the entire length within each subreach (400 m) will not be fished. Depending upon the habitat complexity, you can vary the distance actively fished to allocate the available button time throughout the subreach.

b) **Avoid the temptation to focus sampling in the richest habitat types**.

9) Use a 6mm (1/4 inch) mesh dip net to collect stunned fish. Actively capture stunned fish from the electric field and immediately place them into the live well. Devote special attention to net small and benthic fishes as well as fishes that may respond differently to the electric current.

a) **Irruptive species:** If you encounter a large school of a **single species** (e.g., shad, certain shiners, etc.), quickly move through it to ensure you can sample the entire subreach within the allotted button time.

10) **Sampling with Secondary Gear**: If shallow habitat exists within a subreach that is inaccessible to your boat or raft, use secondary collection methods to sample the habitat thoroughly. If you use a secondary electrofishing gear to sample some portion of a subreach, follow the directions provided in Table 10.4. As a last option, if the conductivity is too high to use a backpack or barge electrofishing units, seine those areas you cannot access with a boat, following the directions presented in Table 10.5.

11) Process fish at the completion of each transect to reduce mortality and track sampling effort. Release fish in a location that eliminates the likelihood of recapture.

12) Repeat Steps 7 through 10 until you have sampled the minimum reach length determined in Step 4.

13) If you have sampled the required minimum fishing reach length but have not sampled 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 14.

b) If you collect > 500 individuals, determine if a single irruptive species comprises > 50% of the total number of individuals.

   i) If 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 14.

   ii) If not, go to Step 14.

c) If you have sampled all 10 subreaches (i.e., you have reached Transect K), go to Step 14.

14) After sampling all the required subreaches, record the final length of the fish sampling reach (as tracked by GPS or measured by range finder) in the *Fish Sampling Protocol* section of the fish gear form.

a) If you suspend sampling before completing the minimum distance, record the 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.

b) It is acceptable to take >1 day to complete the fish sampling.

15) In the *Primary Electrofishing Gear* section of the fish gear form, record the total button time expended for boat electrofishing, the total time spent sampling, and the percentage of the total fish sampling reach sampled by the boat electrofishing unit.

a) In the *Secondary Electrofishing Gear* section of the fish gear form, record the total button time expended for secondary electrofishing, the total time spent sampling, and the percentage of the total fish sampling reach sampled by the secondary electrofishing method

b) If seining was used as an additional collection method, record 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 *Fish Sampling Protocol* section of the fish gear form) for each type of seine.

10.3.2 Secondary Electrofishing

If shallow habitat exists within a subreach that is inaccessible to your boat or raft, it will be necessary to use secondary collection methods to sample the habitat thoroughly. Table 10.4 presents the procedure for collecting fish from large wadeable areas of subreaches through the use of secondary electrofishing techniques. The intent of the secondary electrofishing methods is to provide comparable data to boat electrofishing. Do not use secondary methods to sample "microhabitats" within a subreach. Do not use wadeable electrofishing if your electrofishing unit is larger than 5000 V (e,g,. a GPP 5.0). Record information about the gear used in the *Secondary Electrofishing Gear* section of the fish gear form (Figure 10.1). At the end of sampling, record the button time of the secondary gear, the total amount of time spent using the secondary method, and the proportion of the total fish sampling reach that was sampled with the secondary method. The total button time for boat and wadeable electrofishing should be ~700 seconds per subreach.

Table 10.4 Secondary Electrofishing Methods for Wadeable Areas of Non-Wadeable Rivers

1) Use secondary electrofishing as a collection method whenever an area within a subreach is inaccessible by boat (e.g., a large riffle area). The area must be safe to wade. Do not use wadeable electrofishing if your electrofishing unit is larger than 5000 V (e.g., GPP 5.0).

2) Mark either *Backpack* or *Bank or Towed Barge* in the *Secondary Electrofishing Gear* section of the fish gear form.
   a) Only one netter should be used. Record this along with the number of anodes, and their diameter (mark the units as either inches or centimeters) on the fish gear form.
   b) **For backpack electrofishing** there may be from 2 to 3 people involved (depending upon the crew size). ***When using a barge or pram, the minimum crew size for electrofishing is three***. The barge operator must remain at the control box and navigate the barge. Use only one probe operator, but there may be 1-2 additional crewmembers involved (depending upon crew size).

3) **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 µS/cm).
      ii) 500-800 V for medium conductivity (100 to 300 µS/cm).
      iii) 900-1100 V for low conductivity (< 100 µS/cm).
   c) Select the initial pulse rate and width.
      i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.
      ii) If you expect mostly small fish, use a pulse rate of 60-70 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) 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. f) If button time is not metered, estimate it with a stop watch and flag the data.
4) **Operation of Tote 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. iv) 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.
5) Once the settings on the electrofisher are adjusted properly to sample effectively and minimize injury and mortality, begin sampling at the wadeable area at the downstream end and work upstream. **a)** Depress the switch and slowly sweep the electrode from side to side sampling the wadeable area. **b)** In slack water areas, 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. **c)** In fast, shallow water, sweep the anode and fish downstream into a net. Keep the cathode near the anode if fish catch is low**.**
6) **Use a 6mm (1/4 inch) mesh dip net to** collect stunned fish. Actively capture stunned fish from the electric fie***ld and immediately place them into the live well. Devote special attention to net small and benthic fishes*** as well as fishes that may respond differently to the electric current.
7) The total button time for boat and wadeable electrofishing should be ~700 seconds per subreach.
8) After you finish sampling the wadeable area, return to Step 11 of Table 10.2.
9) You need to track the button time, total fishing time, and the length of stream sampled by wading in each subreach. At the end of all sampling, record the total button time and total fishing time expended for wadeable electrofishing in the ***Secondary Electrofishing Gear section*** of the fish gear form. Estimate the proportion of the fish sampling reach (recorded in the *Fish Sampling Protocol* section of the fish gear form) that was sampled by wadeable electrofishing.

10.3.3 Secondary Seining

In waters where high conductivity would require the use of an electrofishing unit larger than 5000 V (e.g., a 7.5 or 9.0 GPP system), use seining as a secondary method in large wadeable areas that are inaccessible to a boat. **Use seining only as the last option for collecting fish at a non-wadeable site.** 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 large wadeable areas of subreaches. The intent of the supplemental seining is to provide comparable data to boat electrofishing. Do not use seining to sample small "microhabitats" within a subreach.

Although wadeable electrofishing techniques typically work best in an upstream direction, seining may work best moving downstream. Depending upon habitat types and complexity, use 2 to 3 crew members. Two crewmembers move the seine; a third person creates and maintains a bag in the seine in areas with higher velocities, or agitates rocks in riffles or snags. To avoid mortality, process fish after each seine haul. Use additional lines on the fish collection form (Figure 10.2) to record species collected by seining (i.e., do not combine results for a single species from boat electrofishing and seining on the same line). To track effort, seine characteristics and haul length, habitat, and time should be tallied after each seine haul.

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 and Secondary Seine Net sections 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 Secondary Seining Methods for Wadeable Areas of Non-Wadeable Rivers

Procedures for Seining at All Sites with Wadeable Areas
1) Use supplemental seining as a last option only (e.g., when electrofishing is ineffective due to high conductivity or extremely high turbidity). Area must be safely wadeable. a) If site conditions are such that only seining is used, note the reason in the *Sampling Protocol Comments* 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 *Fish Sampling Protocol* section. a) Proceed to the downstream end of the support reach (Transect A). i) At some sites, seining may be more effective while working downstream (from Transect K) instead of upstream. (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 *Water Visibility* conditions on the form. *Poor* 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 (*Bag Seine* or *Minnow Seine*) in the *Primary Seine Net* section of the fish gear form. This is the seine that will be used for sampling the majority of the fish sampling reach. a) Record the number of crewmembers (2-3), and the net dimensions (height, total length, and mesh size) on the fish gear form. b) If you have to use a second type of seine for parts of the sampling reach, Mark the type and record the dimensions in the Secondary Seine Net section of the fish gear form.
6) Determine the length of the fish sampling reach and the number of subreaches that should be

sampled (refer to Table 10.2)

7) To maximize capture efficiency, please do the following:
   a) Always use 10 and 20 ft. seines. When necessary, reduce the width by rolling seine poles and floats into the net.
   b) When narrowing seines, always keep lead line outside of the pole.
   c) When working edge habitats, only roll the inner side of the seine, while keeping the near bank pole extended.
   d) As a default, use seines that are 2 meters in depth. A 1.25 meter seine may be used in shallow habitats.
   e) Keep the float line above the surface (avoid dragging it below the surface while pulling).
   f) Maintain the lead line along the river bottom.
   g) Either tie the seine to the poles tightly, or roll the seine into the poles.
   h) Always maintain the bag behind the poles.

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.
   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.
      i) Avoid extended seine hauls that collect hundreds of individuals.
      ii) Seine as many available habitat types as possible within each subreach (one haul each).
      iii) Total time spent seining a site should be comparable to what would have been spent electrofishing.
   b) **Riffle Habitats**
      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.
      ii) Starting no more than 3 m upstream, a third crewmember kicks the substrate and overturns rocks, proceeding quickly downstream toward the net.
      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.
      iv) Record seine characteristics and haul length, habitat, and time on the seining information form
   c) **Pool, Backwater, and Bar Habitats (Slack water)**
      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.
      ii) In areas with current, pull the net downstream and then sweep toward the bank with one or both poles, or post one pole on the bank and sweep the other end in a wide arc from midstream to the same bank.
      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.
      iv) Pull the bag completely to shore at a predesignated point.
   d) **Glide or Run Habitats (noticeable current)**

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.

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.

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.

iv) Pull the bag completely to shore at a predesignated point.

e) **Edge Habitats**

i) Edge habitats may be shallow too deep with complex to uniform habitat, and may include undercut banks.

ii) Use two crewmembers, each tending a seine pole. Seine along the near shore area.

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.

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.

f) **Snag Habitats**

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.

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, and 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.

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.

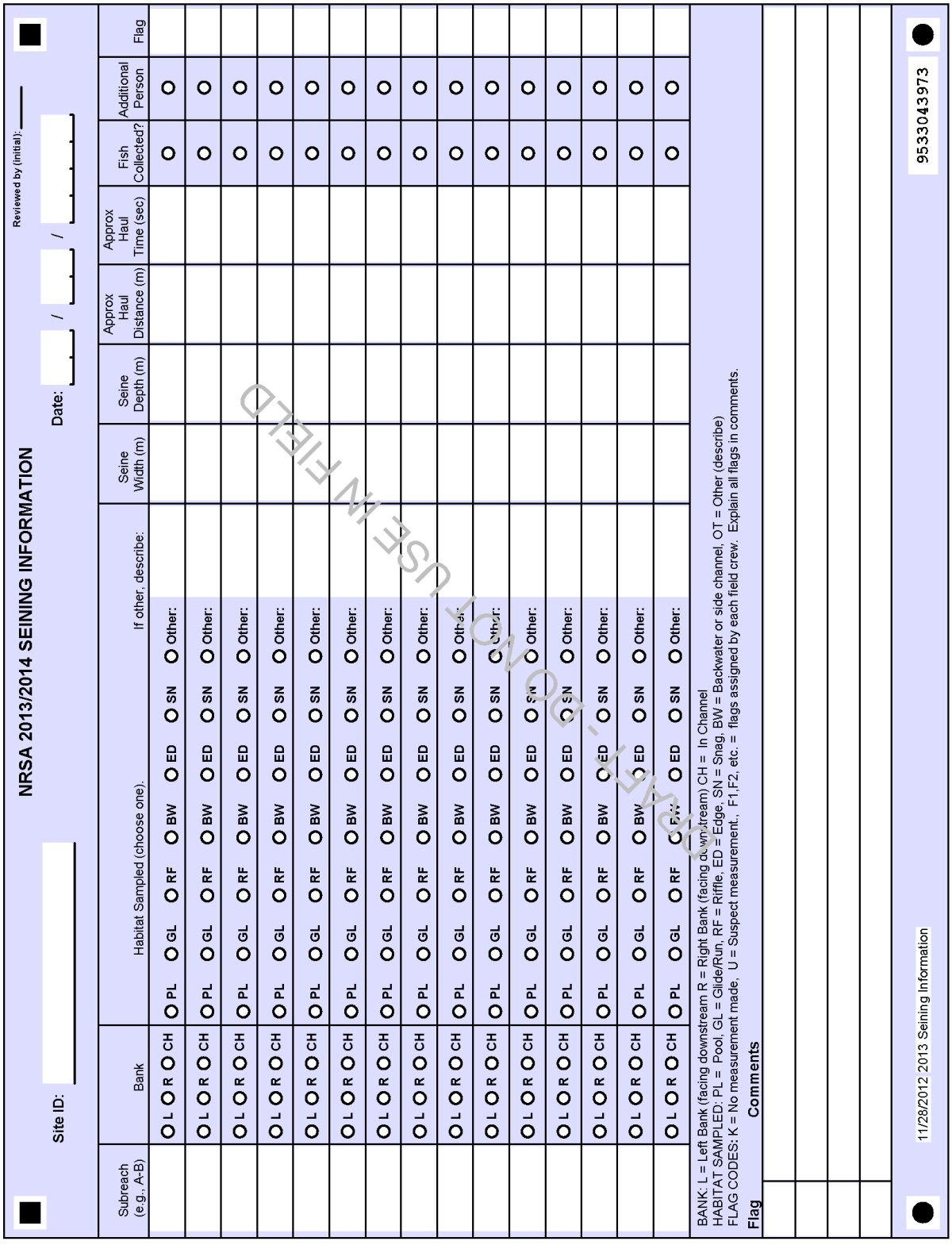
9) To minimize mortality, process fish (i.e., identify, count, and prepare preserved voucher specimens or photovoucher images) after each seine haul (rather than at the end of a subreach.

a) Record identifications, tallies, and voucher information on the fish collection form. You may use separate lines on the fish collection form to record species information from separate seine hauls.

b) Tally seine characteristics and haul length, habitat, and time on the seining information form.

10) After you finish seining the wadeable area of the subreach, return to Step 11 of Table 10.3.

11) 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 *Fish Sampling Protocol* section of the fish gear form) for each type of seine. Record the totals in the *Primary* and *Secondary Seine Net s*ection of the fish gear form.



NRSA 2013/2014 SEINING INFORMATION

Site ID: _____ Date: ___/___/___ Reviewed by (initial): _____

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
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
	O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	

BANK: L = Left Bank (facing downstream R = Right Bank (facing downstream) CH = In Channel
HABITAT SAMPLED: PL = Pool, GL = Glide/Run, 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

11/28/2012 2013 Seining Information

9533043973

DRAFT - DO NOT USE IN FIELD

Figure 10.4 Seining Information Form

10.4 Processing Fish

Processing the fish at the end of each subreach is 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.

10.4.1 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 10.4.2).

10.4.2 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 Section10.4.6) or take good digital photographs (Section 10.4.3) 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 for Processing Fish at Non-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) 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.). a) Process species listed as threatened and endangered first as described in Step 4. i) Photograph specimens for voucher purposes if conditions permit and stress to individuals will be minimal. Mark as *Photo* in the *Voucher* section of the collection form. ii) If individuals die due to sampling, prepare them as part of the local voucher sample and preserve them in the field. Comply with the conditions of your collection permit in regards to mortality of listed species). iii) Return individuals to the river immediately after processing.
3) Only identify and process individuals > 25mm (1 inch) in total length (TL). Ideally handle specimens only once. Although not required, you may note amphibians and reptiles captured on the fish collection form.
4) Identify each individual to the lowest possible taxonomic level: a) If you can confidently identify the individual to species, record the common name on the first blank line in the *Common Name* field of the fish collection form. 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. (1) Record the complete common name. Avoid using shortened names (e.g., *stoneroller*, *carp*, *bass*, etc.). (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 *Comments* section of the collection form. b) If you cannot positively identify an individual to the species level: 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., *UNKNOWN CATOSTOMID A*, *UNKNOWN MOXOSTOMA A*) in the *Common Name* column of the collection form. ii) If you are permitted to retain the specimen, assign it the next available sequential tag number (starting with *01*) in the *Voucher Tag Number* column and see Step 9. c) If you believe the individual is a hybrid: i) Mark as *Hybrid?* on the collection form. ii) If the hybrid has an accepted standard common name (e.g., *Tiger muskellunge, Saugeye, Wip*er, etc), record that name. For other hybrids record the common name of both species (e.g., *Green sunfish x Bluegill*, *Cutthroat trout x Rainbow trout*). Avoid using non-specific

terms such as *Hybrid sunfish*.

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 *Voucher Tag Number* column and see Step 9.

5) If you know the species is not native to this location, mark as *Introduced?*

6) Visually estimate the total length of each individual (a measuring board is not necessary). Keep a running tally in the appropriate *Tally and Counts* 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 *Anom Count* 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 *Mortality/Count* 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 *Unk/Rng* in the *Voucher* section of the collection form.

b) Assign the species the next available voucher specimen tag, and record the number in the *Voucher Tag #* 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 *Voucher Tag #* column of the collection form. Include the specimen tag in all photos of the species. Mark *Photo* in the *Voucher* 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 *Vouchers Retained* 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 *Tally and Counts* 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 *QA* in the *Voucher* 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 *Voucher Tag #* 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 *Voucher Tag #* column of the collection form. Include the specimen tag in all photos of the species. Mark *Photo* in the *Voucher* section of the collection form.

c) Record the number of individuals retained for the preserved voucher sample in the *Vouchers Retained* 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 *Tally and Counts* section. The retained voucher specimens represent a subsample of the total count.

ii) Place the specimens in a fine mesh bag (or separate jar) along with the voucher specimen tag that matches the number recorded on the collection form. You can have multiple bags (or jars) of the same species, but each bag (or jar) will have a separate voucher tag number (i.e., one tag per line on the collection form).

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) 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.

13) Complete a sample jar label for the UNK/RNG voucher sample. Attach it to the sample jar and cover it with clear tape.

14) If you did not prepare a QA voucher sample, mark *No Voucher Preserved* 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.

15) 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 *Sequence* 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).

16) If you did not collect any fish from the entire fish sampling reach, mark *Fished – None Collected* in the *Fish Sampling – Not Conducted or Suspended* section of the fish gear form.

10.4.3 Photovouchering

Use digital imagery for fish species that cannot be preserved as voucher specimens (e.g., rare, threatened, and endangered species; very large bodied or very common species). 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 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 IM staff. You will also send copies of your image files as part of your QA voucher samples.

10.4.4 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 phototvouchers) 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 10.4.7). 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.

10.4.5 Preserving Voucher Specimen Samples

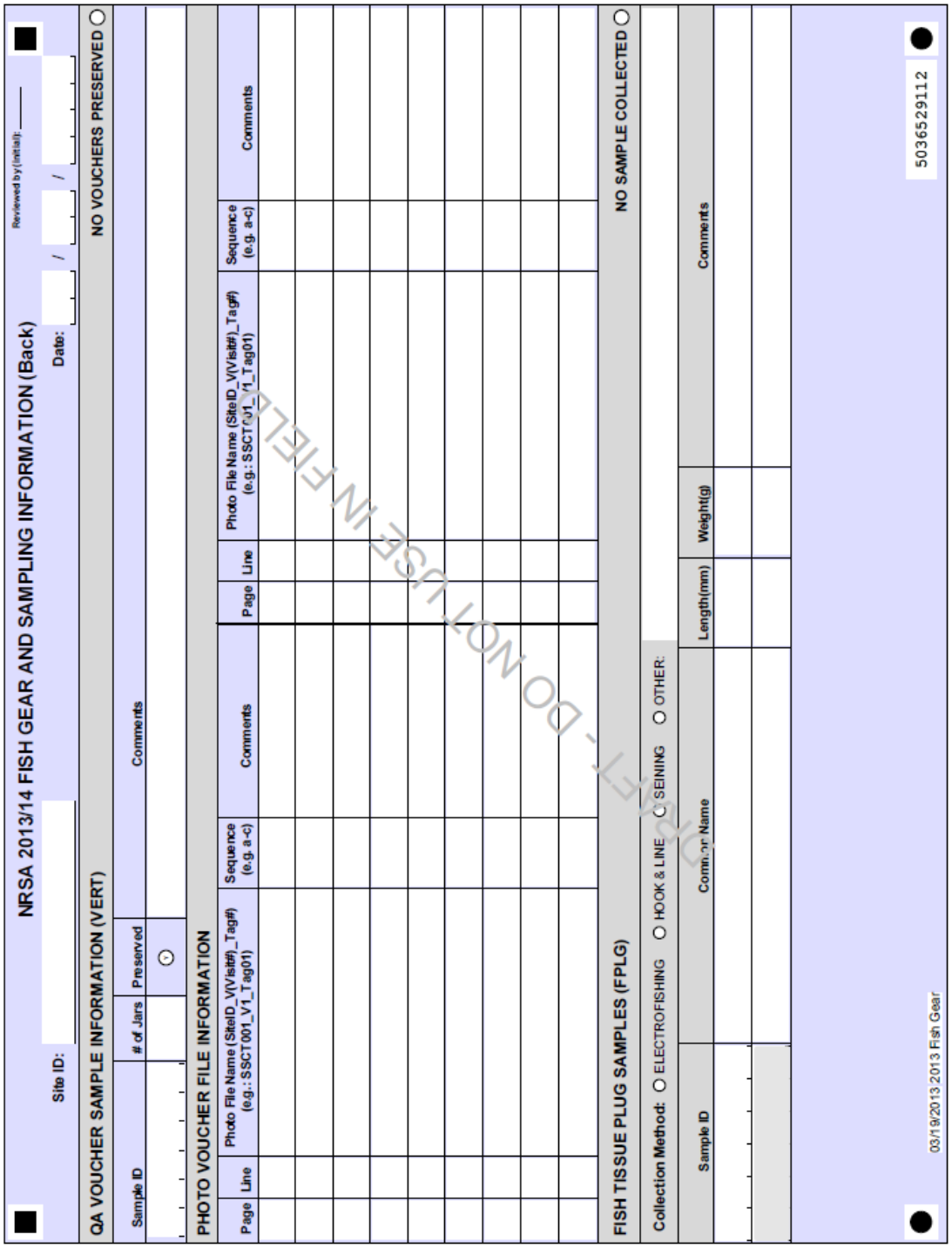
Preserve UNK/RNG and QA voucher specimens in the field with a 10% buffered formalin solution. The volume of formalin must be equal to or greater than the total volume of specimens. Use additional jars if necessary to ensure proper preservation. For individuals having a total length larger than 200 mm (~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 10.7 for QA voucher samples). Attach the completed label to the jar 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

DRAFT - DO NOT USE IN FIELD

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



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

Site ID: _____ Date: __/__/__ Reviewed by (initial): _____

QA VOUCHER SAMPLE INFORMATION (VERT) NO VOUCHERS PRESERVED ○

Sample ID	# of Jars	Preserved	Comments
		○	

PHOTO VOUCHER FILE INFORMATION

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

DRAFT - DO NOT USE IN FIELD

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) NO SAMPLE COLLECTED ○

Collection Method: ○ ELECTROFISHING ○ HOOK & LINE ○ SEINING ○ OTHER:

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

03/19/2013 2013 Fish Gear

5036529112

Figure 10.6 Fish Gear and Sampling Information Form, Side 2

10.4.6 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 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 10.8) 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/RE 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.

10.4.7 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 (**Error! Reference source not found.**). Record nformation 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.

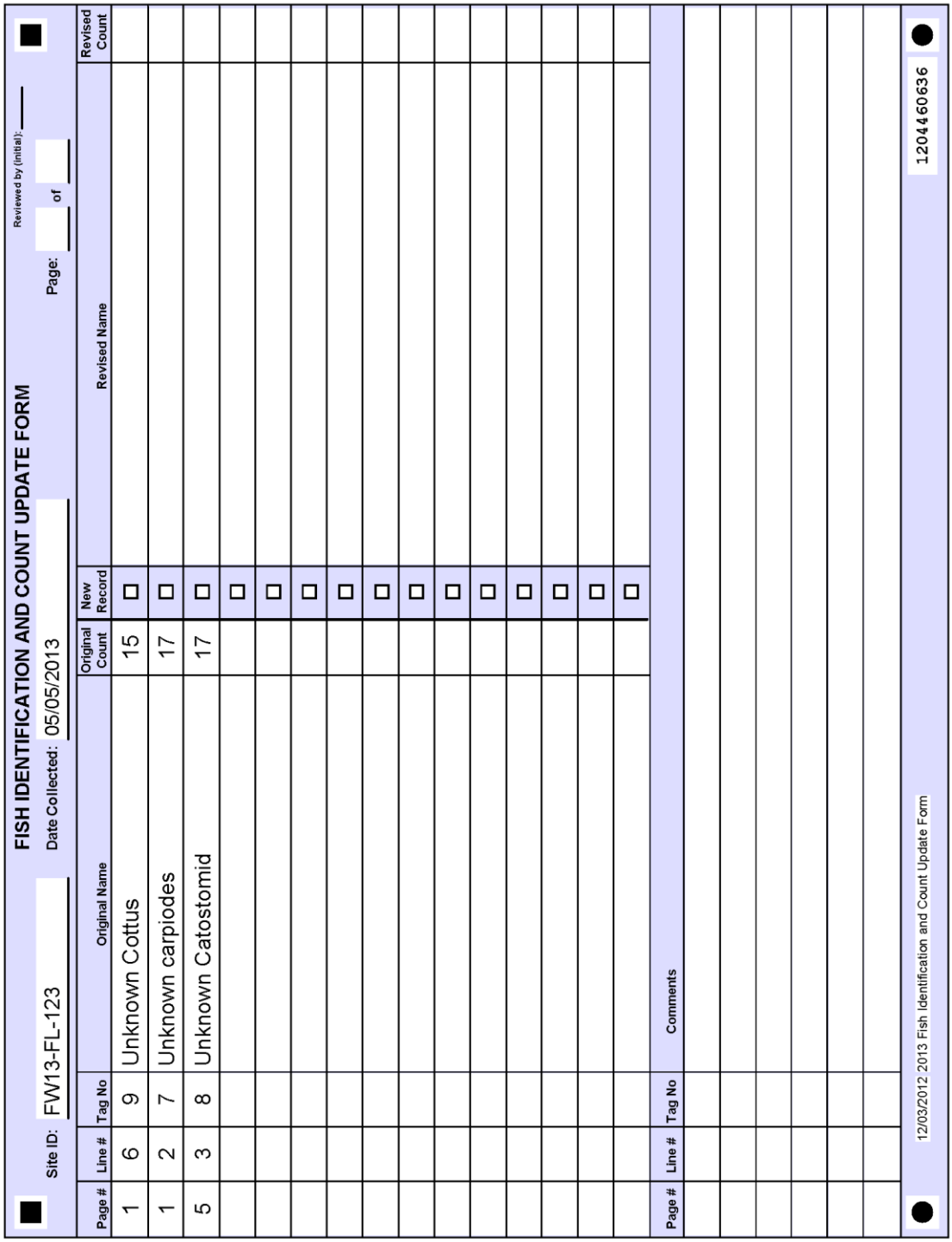
QA FISH VOUCHER (VERT) Site ID: _____ ___ / ___ / 201__ 990011		QA FISH VOUCHER (VERT) Site ID: _____ ___ / ___ / 201__ 990011	
FISH – BAG TAG: 41 990011	FISH – BAG TAG: 42 990011	FISH – BAG TAG: 43 990011	FISH – BAG TAG: 44 990011
FISH – BAG TAG: 37 990011	FISH – BAG TAG: 38 990011	FISH – BAG TAG: 39 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

DRAFT - DO NOT USE IN FIELD

Figure 10.7 QA Voucher Sample Labels and Voucher Specimen Tags

Table 10.7 Processing Unknown/Range Extension (UNK/RNG) Voucher Samples

Processing UNK/RNG Voucher Samples
1) Following fixation for 5 to 7 days, decant and properly discard the formalin solution. Formalin is a potential carcinogen and should be used with extreme caution, as vapors and solution are highly caustic and may cause severe irritation on contact with skin, eyes, or mucus membranes. Wear vinyl or nitrile gloves and safety glasses, and always work in a well-ventilated area. a) Formalin must be disposed of properly. Contact your regional EPA coordinator if your laboratory does not have the capability of handling waste formalin.
2) Replace the formalin with tap water and soak specimens over a 4-5 day period. Soaking may require periodic water changes and should continue until the odor of formalin is barely detectable.
3) Decant the tap water. Use 45%-50% isopropyl alcohol or 70% ethanol as a final preservative for specimens.
4) 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. 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 *Revised Name* and *Revised Count* columns of the update form. 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 *Comments* 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. 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. 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 *New Record.* Leave the *Original Name* and *Original Count* Columns blank. iii) Record the revised name and count in the *Revised Name* and *Revised Count* 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.



FISH IDENTIFICATION AND COUNT UPDATE FORM

Site ID: FW13-FL-123 Date Collected: 05/05/2013 Page: ___ of ___ Reviewed by (initial): ____

Page #	Line #	Tag No	Original Name	Original Count	New Record	Revised Name	Revised Count
1	6	9	Unknown Cottus	15	☐		
1	2	7	Unknown carpiodes	17	☐		
5	3	8	Unknown Catostomid	17	☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		
					☐		

Page #	Line #	Tag No	Comments

12/03/2012 2013 Fish Identification and Count Update Form 1204460636

Figure 10.8 Fish Identification and Count Update Form

Table 10.8 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, cturner@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 Project Manager or EPA Regional Coordinator. .
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 Section 3 and 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 METHOD

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 0). 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 List for 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	*Micropterus punctulatus*	~280 mm
		Largemouth bass	*Micropterus salmoides*	~280 mm
		Smallmouth bass	*Micropterus dolomieu*	~300 mm
		Black crappie	*Pomoxis nigromaculatus*	~330 mm
		White crappie	*Pomoxis annularis*	~330 mm
	Ictaluridae	Channel catfish	*Ictalurus punctatus*	~300 mm
		Blue catfish	*Ictalurus furcatus*	~300 mm
		Flathead catfish	*Pylodictis olivaris*	~300 mm
	Percidae	Sauger	*Sander canadensis*	~380 mm
		Walleye	*Sander vitreus*	~380 mm
		Yellow perch	*Perca flavescens*	~330 mm
	Moronidae	White bass	*Morone chrysops*	~330 mm
	Esocidae	Northern pike	*Esox lucius*	~430 mm
		Chain pickerel	*Esox niger*	~430 mm
	Salmonidae	Brown trout	*Salmo trutta*	~300 mm
		Cutthroat trout	*Oncorhynchus clarkii*	~300 mm
		Rainbow trout	*Oncorhynchus mykiss*	~300 mm
		Brook trout	*Salvelinus fontinalis*	~330 mm
Alternates	Cyprinidae	Northern pikeminnow	*Ptychocheilus oregonensis*	~300 mm
	Centrarchidae	Bluegill	*Lepomis macrochirus*	~200 mm
		Rock bass	*Ambloplites rupestris*	~200 mm
		Redbreast sunfish	*Lepomis auritus*	~200 mm

Table 11.3 Sampling Procedures for Fish Tissue Plug Samples

Fish Tissue Plug Methods
1. Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellant 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: • 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 scalpel **11.** Wearing clean nitrile gloves, insert the 8 millimeter biopsy punch into the dorsal muscle of the fish through the scale-free area. The punch is inserted with a slight twisting motion cutting the skin and muscle tissue. Once full depth of the punch is achieved a slight bending or tilting of the punch is needed to break off the end of the sample. Remove biopsy punch taking care to ensure sample remains in the punch. **Note: The full depth of the punch should be filled with muscle tissue, which should result in collecting a minimum of 0.25 to 0.35 grams of fish tissue for mercury analysis.** 12. Apply a generous amount of antibiotic salve to the plug area and gently return the fish to the water.

Fish Tissue Plug Methods

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.
17. Keep the samples frozen on dry ice or in a freezer at ≤-20°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 12.1 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 12.1).

Table 12.1 Equipment and Supplies - Whole Fish Tissue Sample Collection

For collecting whole fish tissue sample	• 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)	• 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
For storing and preserving whole fish tissue sample	• Aluminum foil (solvent rinsed and • baked) • Heavy-duty food grade polyethylene • tubing • Large plastic (composite) bags	• Knife or scissors • Dry Ice • Plastic cable ties • Coolers
For documenting the whole fish tissue sample	• Whole Fish Tissue Collection Form • Clipboard • Clear tape strips	• Sample Identification Labels • #2 pencils • Fine tipped indelible markers
For shipping the whole fish tissue samples	• Preaddressed FedEx airbill • Coolers	• Tracking Form • Chain-of-custody labels • Packing/strapping tape

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 12.2. The procedures for collecting and processing whole fish tissue samples are presented in Table 12.3.

Table 12.2 Recommended Target Species for Whole Fish Tissue Collection

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

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Table 12.3 Sampling Procedures for Whole Fish Tissue Samples

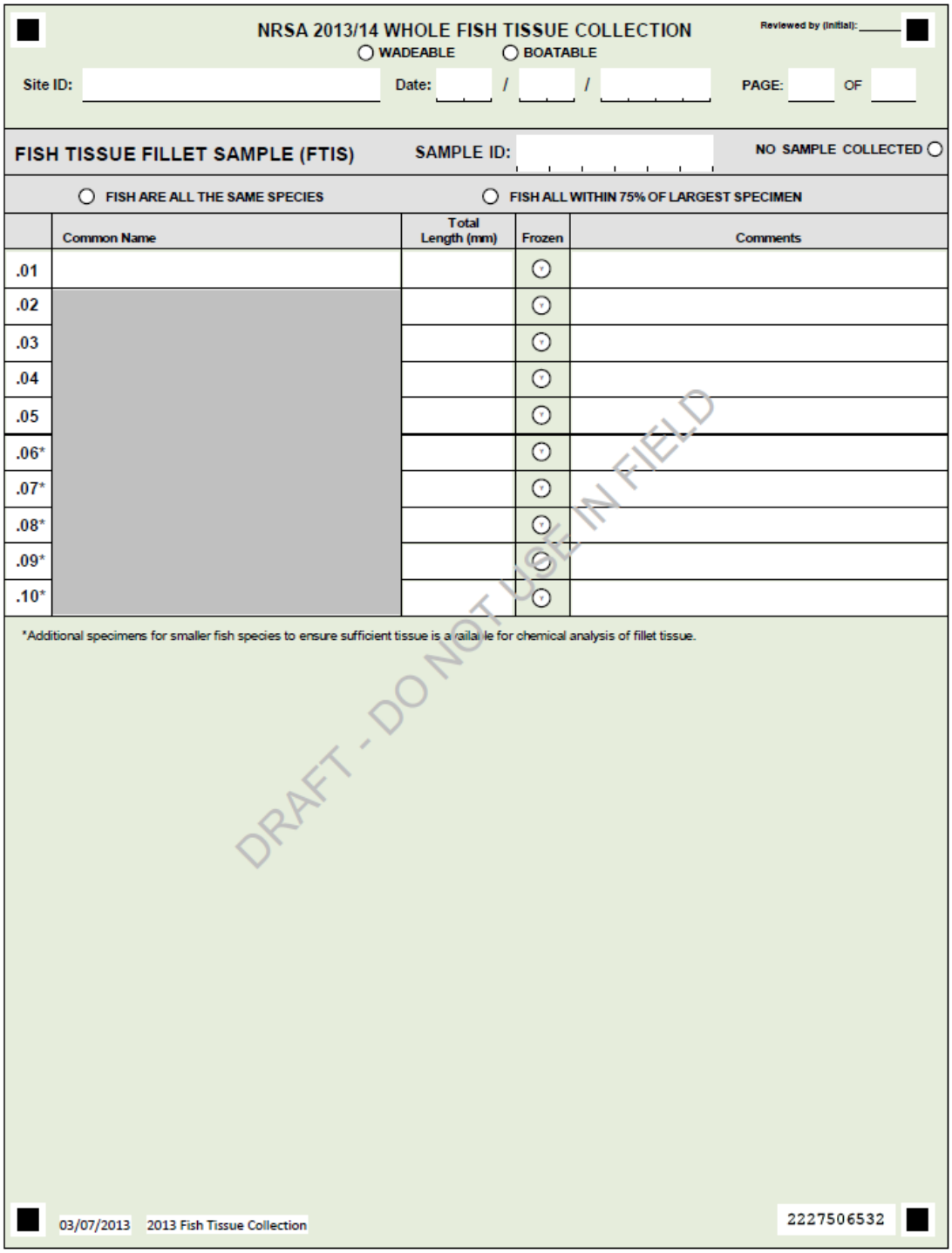
Whole Fish Tissue Method
1. Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellant until after the whole fish sample has been collected, measured, and wrapped.
2. Rinse potential target species/individuals in ambient water to remove any foreign 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 12.2 for minimum species length guidelines). Select fish for each sample based on the following criteria: • 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

Whole Fish Tissue Method

fish to be included in the sample are available for delivery to the designated laboratory).

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.

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 12.1) 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/length s 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 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 ≤-20°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

Reviewed by (Initial): _____

○ WADEABLE ○ BOATABLE

Site ID: _____ Date: ____ / ____ / _____ PAGE: ____ OF ____

FISH TISSUE FILLET SAMPLE (FTIS) SAMPLE ID: _____ NO SAMPLE COLLECTED ○

○ FISH ARE ALL THE SAME SPECIES ○ FISH ALL WITHIN 75% OF LARGEST SPECIMEN

	Common Name	Total Length (mm)	Frozen	Comments
.01			○	
.02			○	
.03			○	
.04			○	
.05			○	
.06*			○	
.07*			○	
.08*			○	
.09*			○	
.10*			○	

*Additional specimens for smaller fish species to ensure sufficient tissue is available for chemical analysis of fillet tissue.

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03/07/2013 2013 Fish Tissue Collection 2227506532

Figure 12.1 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.

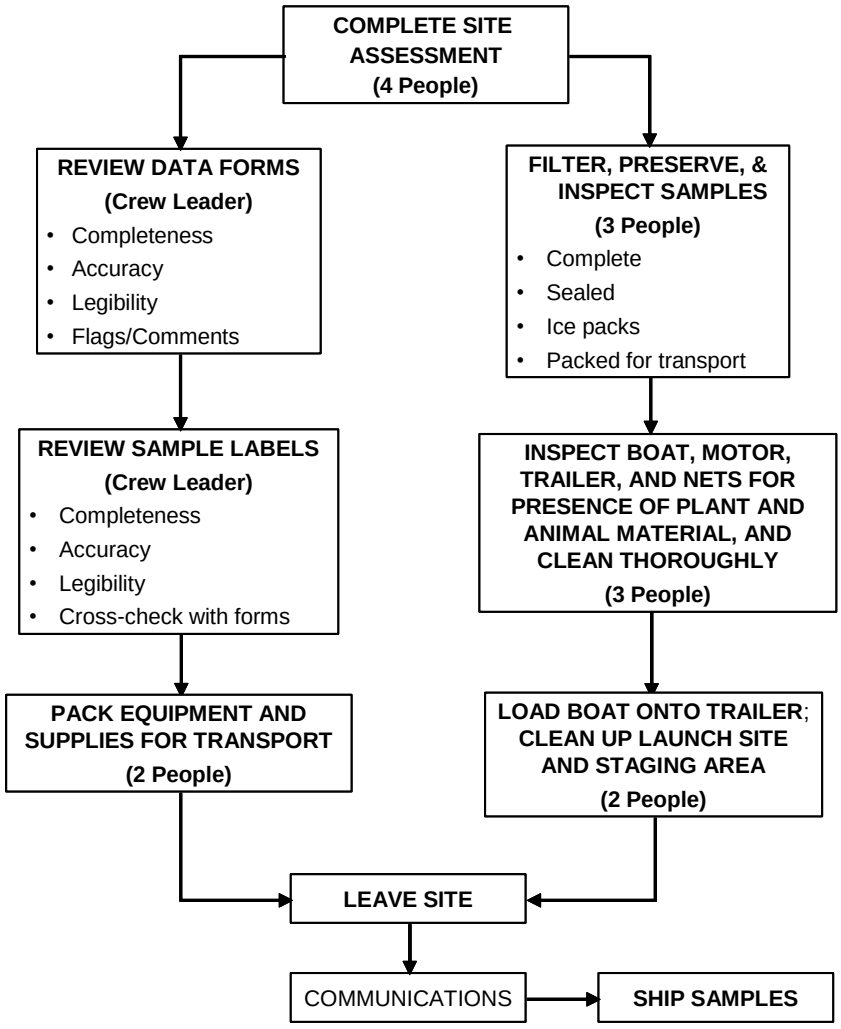


Figure 13.1 Final Site Activities Summary

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 Watershed Activities and Disturbances Observed

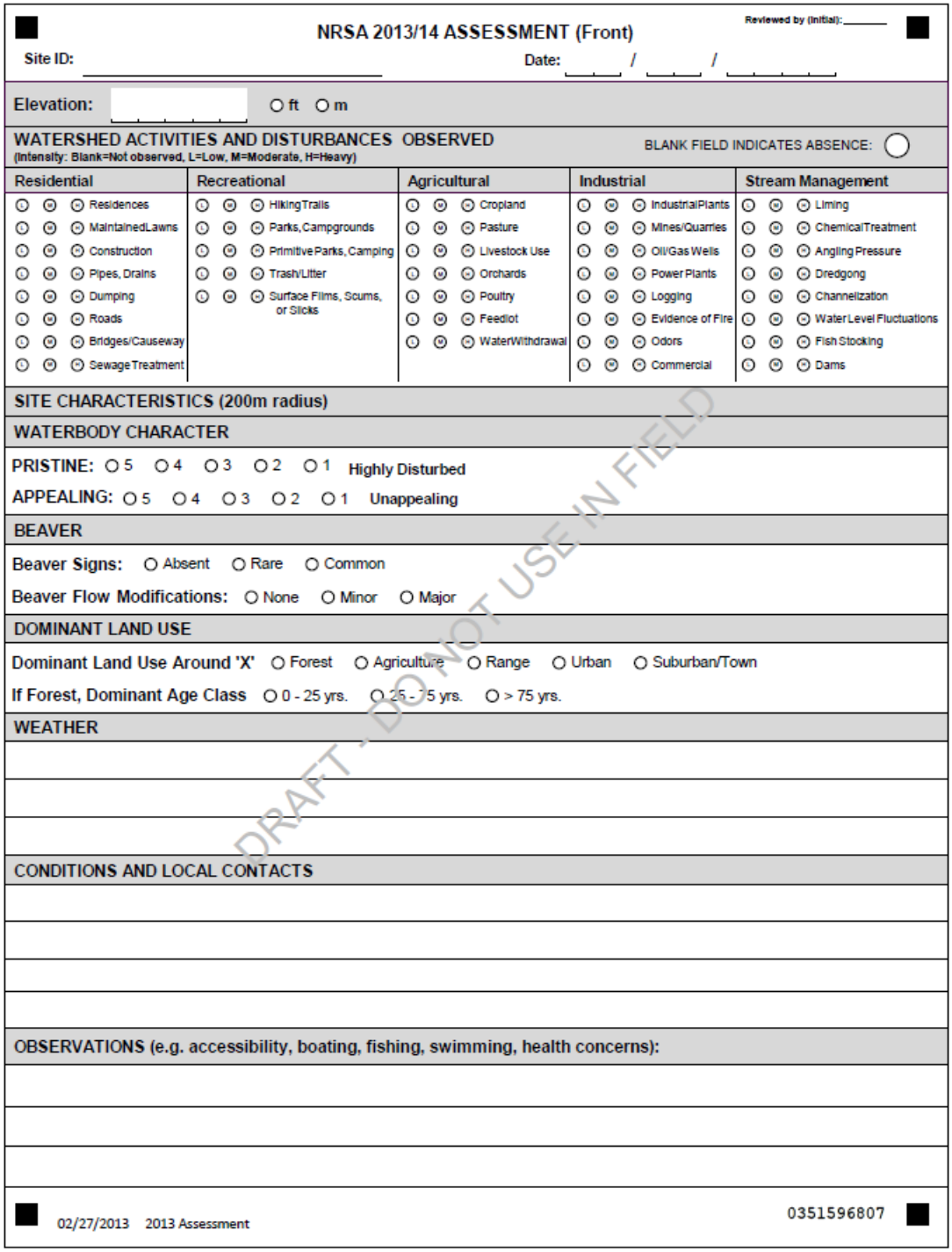
Record any of the sources of potential stressors listed in the "Watershed Activities and Disturbances Observed" section on the Site Assessment Form (Figure 13.2). Include those that were observed while on the site, while driving or walking through the site catchment, or while flying over the site and 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.

13.2.2 Site Characteristics

Record observations regarding the general characteristics of the site on the Site Assessment Form (Figure 13.2). When assessing these characteristics, look at a 200 m riparian distance on both banks. Rank the site between "pristine" and "highly disturbed", and between "appealing" and "unappealing." Document any signs of beaver activity and flow modifications. Record the dominant land use and forest age class. Document the weather conditions on the day of sampling and any extreme weather conditions in the days prior to sampling.

13.2.3 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: _____ ○ ft ○ m

WATERSHED ACTIVITIES AND DISTURBANCES OBSERVED
(Intensity: Blank=Not observed, L=Low, M=Moderate, H=Heavy)

BLANK FIELD INDICATES ABSENCE: ○

Residential	Recreational	Agricultural	Industrial	Stream Management
○ L ○ M ○ H Residences	○ L ○ M ○ H Hiking Trails	○ L ○ M ○ H Cropland	○ L ○ M ○ H Industrial Plants	○ L ○ M ○ H Liming
○ L ○ M ○ H Maintained Lawns	○ L ○ M ○ H Parks, Campgrounds	○ L ○ M ○ H Pasture	○ L ○ M ○ H Mines/Quarries	○ L ○ M ○ H Chemical Treatment
○ L ○ M ○ H Construction	○ L ○ M ○ H Primitive Parks, Camping	○ L ○ M ○ H Livestock Use	○ L ○ M ○ H Oil/Gas Wells	○ L ○ M ○ H Angling Pressure
○ L ○ M ○ H Pipes, Drains	○ L ○ M ○ H Trash/Litter	○ L ○ M ○ H Orchards	○ L ○ M ○ H Power Plants	○ L ○ M ○ H Dredging
○ L ○ M ○ H Dumping	○ L ○ M ○ H Surface Films, Scums, or Slicks	○ L ○ M ○ H Poultry	○ L ○ M ○ H Logging	○ L ○ M ○ H Channelization
○ L ○ M ○ H Roads		○ L ○ M ○ H Feedlot	○ L ○ M ○ H Evidence of Fire	○ L ○ M ○ H Water Level Fluctuations
○ L ○ M ○ H Bridges/Causeway		○ L ○ M ○ H Water Withdrawal	○ L ○ M ○ H Odors	○ L ○ M ○ H Fish Stocking
○ L ○ M ○ H Sewage Treatment			○ L ○ M ○ H Commercial	○ L ○ M ○ H Dams

SITE CHARACTERISTICS (200m radius)

WATERBODY CHARACTER

PRISTINE: ○ 5 ○ 4 ○ 3 ○ 2 ○ 1 Highly Disturbed

APPEALING: ○ 5 ○ 4 ○ 3 ○ 2 ○ 1 Unappealing

BEAVER

Beaver Signs: ○ Absent ○ Rare ○ Common

Beaver Flow Modifications: ○ None ○ Minor ○ Major

DOMINANT LAND USE

Dominant Land Use Around 'X' ○ Forest ○ Agriculture ○ Range ○ Urban ○ Suburban/Town

If Forest, Dominant Age Class ○ 0 - 25 yrs. ○ 25 - 75 yrs. ○ > 75 yrs.

WEATHER

CONDITIONS AND LOCAL CONTACTS

OBSERVATIONS (e.g. accessibility, boating, fishing, swimming, health concerns):

02/27/2013 2013 Assessment 0351596807

DRAFT - DO NOT USE IN FIELD

Figure 13.2 Site Assessment Form

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 List for Fecal Indicator Sample

For processing samples	▪ 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
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 ▪ Fecal Indicator sample labels (2 vial labels and 1 bag label) ▪ Filter blank label if collecting filter blank ▪ Clear tape strips for covering labels

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 Processing procedure - Fecal Indicator Sample

Filtering for the Enterococci (fecal indicator) sample
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.
16. 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 List for Chlorophyll-a Processing

For filtering chlorophyll-*a* 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 Processing Procedure - Chlorophyll-a Sample

Filtering for the chlorophyll a water sample
1. Put on nitrile gloves.
2. Use clean forceps to place a Whatman GF/F 0.7 µm glass fiber filter in the graduated filter holder apparatus with the gridded side of the filter facing down.
3. Retrieve the 2 liter chlorophyll sample bottle from the cooler and 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. • *NOTE: IF the water is green or turbid, use a smaller volume to start with.*
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 2 mL of 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 Figure 4.2. Store the preserved samples upright in a container containing absorbent material.

Table 13.6 Procedure for 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 2 mL of 10% formalin to the tube. Cap the tube tightly and seal with plastic electrical tape. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met.
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 for 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 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 ¼ 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. 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 for 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 ¼ 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 Crew Leader reviews all of the data forms and sample labels for accuracy, completeness, and legibility. The other crew members inspect all sample containers and package them in preparation for transport, storage, or shipment. Refer to Appendix C for details on preparing samples for shipping.

Ensure that all required data forms for the site have been completed. Confirm that the SITE-ID, the visit number, and date of visit are correct on 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 revisit sampling design is provided in Figure 14.1.

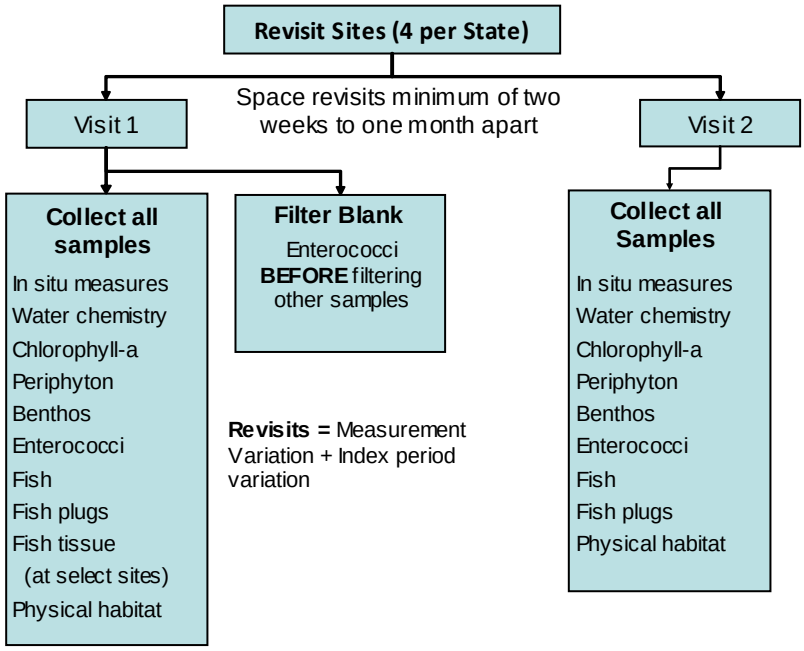


Figure 14.1 Summary of the Revisit Sampling Design

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 an 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 revisits sites. We will not be collecting replicate data on whole fish tissue. Whole fish tissue samples 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

Field Evaluation Plan	• Field Logistics Coordinator will arrange the field evaluation visit with each Field Crew, ideally within the first two weeks of sampling. • The Evaluator will observe the performance of a crew through one complete set of sampling activities. • If the Crew misses or incorrectly performs a procedure, the Evaluator will note it on the checklist and immediately point it out so the mistake can be corrected on the spot. • The Evaluator will review the results of the evaluation with the Field Crew before leaving the site, noting positive practices and problems.
Field Evaluation Checklist	• The Evaluator observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and NRSA protocols are followed. • The Evaluator checks the sample containers to verify that they are the correct type and size, and checks the labels to be sure they are correctly and completely filled out. • The Evaluator confirms that the Field Crew has followed NRSA protocols for locating the site. • The Evaluator observes the complete set of sampling activities, confirming that all protocols are followed. • The Evaluator will record responses or concerns, if any, on the Field Evaluation and Assistance Check List.
Corrective Action Procedures	• If the Evaluator's findings indicate that the Field Crew is not performing the procedures correctly, safely, or thoroughly, the Evaluator must continue working with this Field Crew until certain of the Crew's ability to conduct the sampling properly so that data quality is not adversely affected. • If the Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact a NRSA QA Project Coordinator.

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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