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National Rivers and Streams Assessment 2018/19

Field Operations Manual

Non-Wadeable

Version 1.2

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NOTICE

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

National Rivers and Streams Assessment 2018/19: Quality Assurance Project Plan EPA-841-B-17-001

National Rivers and Streams Assessment 2018/19: Site Evaluation Guidelines EPA-841-B-17-002

National Rivers and Streams Assessment 2018/19: Field Operations Manual EPA-841-B-17-003b

National Rivers and Streams Assessment 2018/19: Laboratory Methods Manual EPA 841-B-17-004

This document (*Field Operations Manual (FOM)*) contains a brief introduction and procedures to follow at the base location and on-site, including methods for sampling water chemistry (grabs and *in situ* measurements), periphyton, benthic macroinvertebrates, algal toxins, fish assemblage, fish tissue plugs, 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 (USEPA, 2012), Western Environmental Monitoring and Assessment Program (Baker, et al., 1997), the methods outlined in Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers (Flotemersch, et al., 2006), and methods employed by several key states that were involved in the planning phase of this project. Methods described in this document are to be used specifically in work relating to the NRSA 2018/19. All Project Cooperators must follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Details on specific methods for site evaluation and sample processing can be found in the appropriate companion document.

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ACRONYMS/ABBREVIATIONS

AFDM	Ash-Free Dry mass
ANC	Acid-neutralizing capacity
Ca	Calcium
Cl	Chloride
CN	Cellulose nitrate
CPR	Cardiopulmonary Resuscitation
CW	Channel width(s)
CWA	Clean Water Act
DBH	Diameter at Breast Height
DELT	Deformities, Eroded Fins, Lesions and Tumors
DI	Deionized water
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EPA	Environmental Protection Agency
FOM	Field Operations Manual
GIS	Geographic Information System
GPP	Generator Powered Pulsator
GPS	Global Positioning System
HAB	Harmful Algal Bloom
HDPE	High density polyethylene
IBI	Index of Biotic Integrity
IM	Information Management
K	Potassium
LOM	Lab Operations Manual
LWD	Large Woody Debris
Mg	Magnesium
MMI	Multimetric Index
MSDS	Material Safety Data Sheets
Na	Sodium
NAD	North American Datum
NARS	National Aquatic Resources Survey
NELAP	National Environmental Laboratory Accreditation Program
NHD	National Hydrology Database
NH_3	Ammonia
NH_4	Ammonium
NIST	National Institute of Standards and Technology
NO_2	Nitrite
NO_3	Nitrate
NRC	National Research Council
NRSA	National Rivers and Streams Assessment
O/E	“Observed” over “Expected”
OSHA	Occupational Safety and Health Administration
OW	Office of Water
PBS	phosphate buffered saline
PC	Personal Computer
PETG	polyethylene terephthalate glycol
PFD	Personal Flotation Device
PP	Polypropylene
PPT	Parts per thousand
Psig	pounds per square inch - gauge
PTD	Percent Taxonomic Disagreement

QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QCS	Quality Control Solution
QRG	Quick Reference Guide
RVT	Revisit
SEG	Site Evaluation Guideline
SiO_2	Silica
SO_4	Sulfate
SOP	Standard Operating Procedures
Std	Standard
TL	Total Length
TOC	Total Organic Carbon
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
USDA	United States Department of Agriculture
UNK/RNG	Unknown Range Extension
USGS	United States Geological Survey
WRS	Willamette Research Station
WT	Water Tube

DISTRIBUTION LIST

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

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1 BACKGROUND

This manual describes field protocols and daily operations for crews to use in the Nonwadeable NRSA 2018/19 method. The NRSA is a probability-based survey of our Nation's rivers and streams and is designed to:

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

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

1.1 Survey Design

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

- Resample design applied to NRSA 2008/09 and NRSA 2013/14 sites.
- New site design for NRSA 2018/19.

The survey design is explicitly stratified by state for both designs. The unequal probability categories are specific to the survey design used for the NRSA 2008/09, NRSA 2013/14, and NRSA 2018/19. In all cases the categories are specific combinations of Strahler order categories and nine National Aquatic Resource Survey (NARS) aggregated ecoregions. In addition, a minimum of 20 sites (Resample and New) was guaranteed in each state and a maximum of 75 sites was the limit for an individual state. There are 983 unique sites in the Resample Design and 825 unique sites in the New Site Design. Approximately 10% of the total NRSA sites are scheduled for repeated sampling (revisit sites) in the same year of the two year NRSA field cycle.

The sample frame was derived from the medium resolution National Hydrography Dataset (NHD), in particular NHDPlus V2. Additional details on the NRSA survey design are found in the National Rivers and Streams Assessment Survey Design: 2018/19 documents.

1.2 Target Population and Index Period

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

The target population excludes:

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

The study index period extends from:

- Beginning of June through end of September for most ecoregions.
- 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-17-002* and the NRSA Web site http://www.epa.gov/national-aquatic-resource-surveys/nrsa) for more detailed information on the target population and exclusion criteria.

1.3 Replacing Sites

All base sites must be evaluated for sampling. If a stream or river site is determined to be unsampleable, it must be replaced by another site within the same state and panel. The five panels are:

- NRS18_08TS3R2: sites from NRSA 2008/09 that were sampled twice in 2008/09 and then sampled twice again in 2013/14 (with a few exceptions).
- NRS18_08TS3: sites from NRSA 2008/09 that were sampled once in 2008/09 and then sampled again in 2013/14.
- NRS18_13TS2R2: sites from NRSA 2013/14 that were sampled twice in 2013/14.
- NRS18_13TS2: sites from NRSA 2013/14 that were sampled once in 2013/14 and will be sampled again in 2018/19.
- NRS18_18: new sites selected for NRSA 2018/19 that will be sampled once in 2018/19.

If the site is from the New Site Design (panel NRS18_18), then the replacement site must also be within the same ecoregion and in the same size category. The four general categories are:

- Small streams (SS): 1-2 Strahler order sites.
- Large streams (LS): 3-4 Strahler order sites.
- Major rivers (RM): 5 and above Strahler order sites.
- Other rivers (RO): 5 and above Strahler order sites not considered Major Rivers.

Please refer to the *Site Evaluation Guidelines (EPA-841-B-17-002)* for more detailed information.

1.4 Selection of NRSA Indicators

As part of the indicator selection process, EPA worked with state, tribal, and other partners through technical conferences and indicator teleconferences. The EPA formed a National Rivers and Streams Assessment Steering Committee with state, tribal, and regional representatives to provide feedback and evaluate core and supplemental indicators to be included in the 2018/19 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 and NRSA 2013/14. 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 2018/19 are briefly described in **Table 1.1**.

Table 1.1 Summary Table of Indicators for all NRSA 2018-2019 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_3-N, NO_3-NO_2, NO_3, basic anions and cations, silica, alkalinity [Acid-neutralizing capacity (ANC)], DOC, TOC, TSS, conductivity, pH, turbidity, true color)	Core Indicator	Collected at Transect A at mid-channel; 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 and Cylindrospermopsin	Supplemental Indicator	Collected at Transect A at mid-channel; from a depth of 0.5 m, or mid-depth if water depth is less than 1 meter.
Periphyton composite and periphyton metagenomic	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 and 0.3 m depth
Fish Tissue Plug	Supplemental Indicator	Target species collected throughout the sampling reach as part of fish assemblage sampling
Whole Fish Tissue	Supplemental Indicator at select sites	Target species collected throughout the sampling reach as part of fish assemblage sampling

1.5 Supplemental Material to the Field Operations Manual

The FOM 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 information includes a

road map and set of directions to the site, topographic maps, landowner access forms, sampling permits (if needed), site evaluation forms, and other information necessary to ensure an efficient and safe sampling day.

Field crews may request to receive a printed Quick Reference Guide (QRG) that contains tables and figures summarizing field activities and protocols from the 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 QRG will also be made available to all crews in electronic (Adobe© PDF) format. The field crews are also required to keep the FOM available in the field for reference and for possible protocol clarification.

Quality Assurance (QA) is a required element of all EPA-sponsored studies that involve the collection of environmental data (USEPA 2000a, 2000b). Field crews will be provided a digital copy of the integrated QAPP. The QAPP contains more detailed information regarding quality assurance/quality control (QA/QC) activities and procedures associated with general field operations, sample collection, measurement data collection for specific indicators, and data reporting activities. For more information on the QA procedures, refer to the *National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA 841-B-17-001).*

Related NRSA documents include the following: National Rivers and Streams Assessment: Quality Assurance Project Plan (EPA 841-B-17-001), National Rivers and Streams Assessment: Site Evaluation Guidelines (EPA 841-B-17-002), and National Rivers and Streams Assessment: Laboratory Methods Manual (EPA 841-B-17-004). These documents are available at: http://www.epa.gov/national-aquatic-resource-surveys/nrsa.

1.6 Recording Data and Other Information

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

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**. At the end of each sampling day, the field crew lead is responsible for reviewing each field form for completeness and legibility. The field crew lead must initial each field form after reviewed or if using the App, hit the reviewed button at the top of each electronic form.

Table 1.2 Guidelines for Recording Field Measurements and Tracking Information

Activity	Guidelines
Field Measurements	
Data Recording	• Record measurement values and observations within the NRSA 2018/19 app developed for use on EPA-issued tablet devices. • If unable to record data within the NRSA 2018/19 app then record on data forms preprinted by the IM Team 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 year. ○ 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. Fill in all data even if there is repetition between subsequent lines. ○ 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; re-measurement not possible. U = Suspect measurement; re-measurement 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 sample ID numbers and follow the standard recording format for each type of sample. • Use a fine point indelible marker to record information on label. Cover the completed label with clear tape. • Record sample ID number from label and associated collection information in the app or 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.

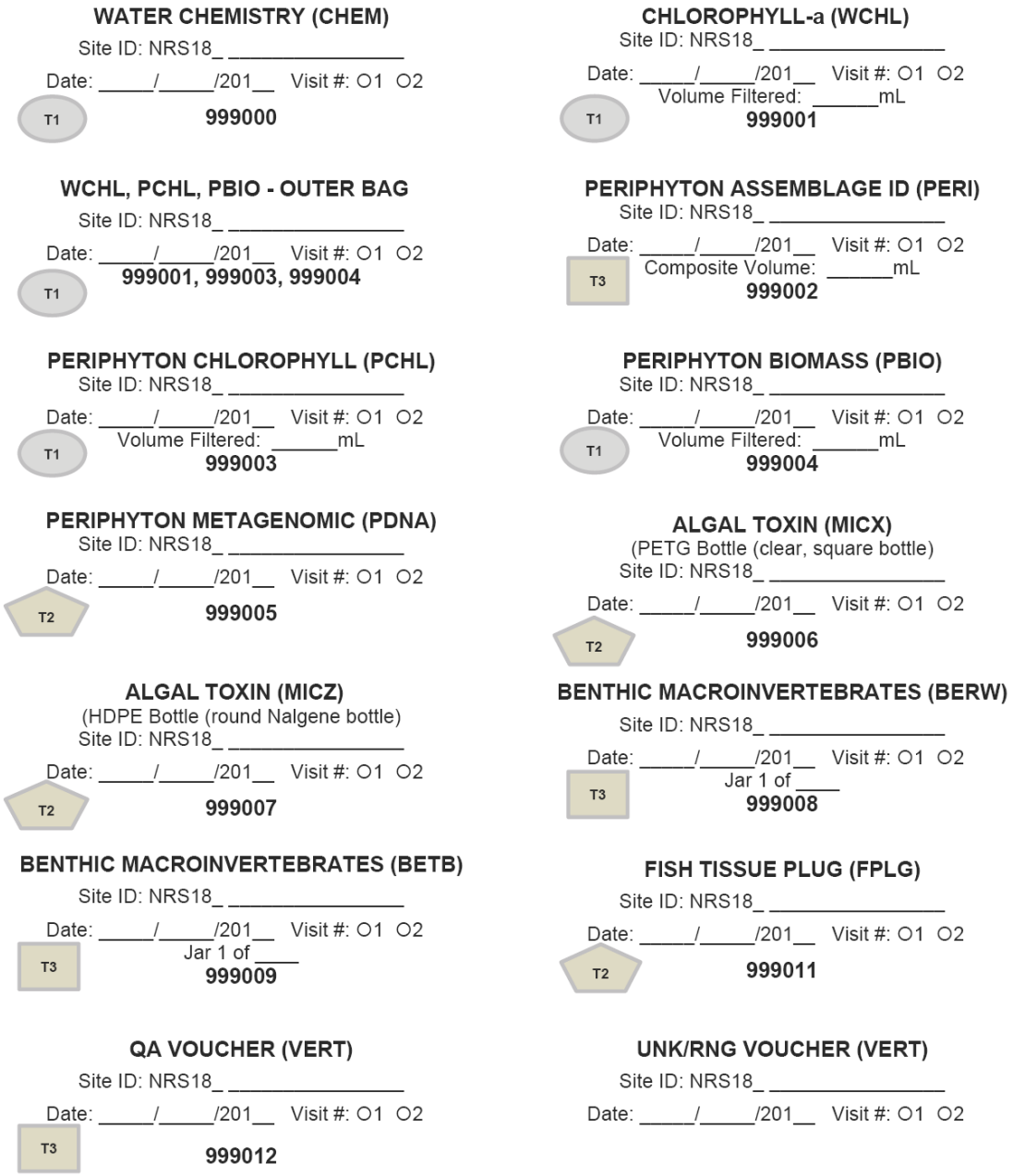


Figure 1.1 Example Sample Labels for Sample Tracking and Identification

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 site will typically consist of four or five people in two 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, at 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. For example, at sites where physical habitat assessments are expected to take longer than fish collections, Group B crew members may collect Group A Tasks including *in situ* measurements and water collections. However, these water quality tasks will need to be completed before fish collections begin.

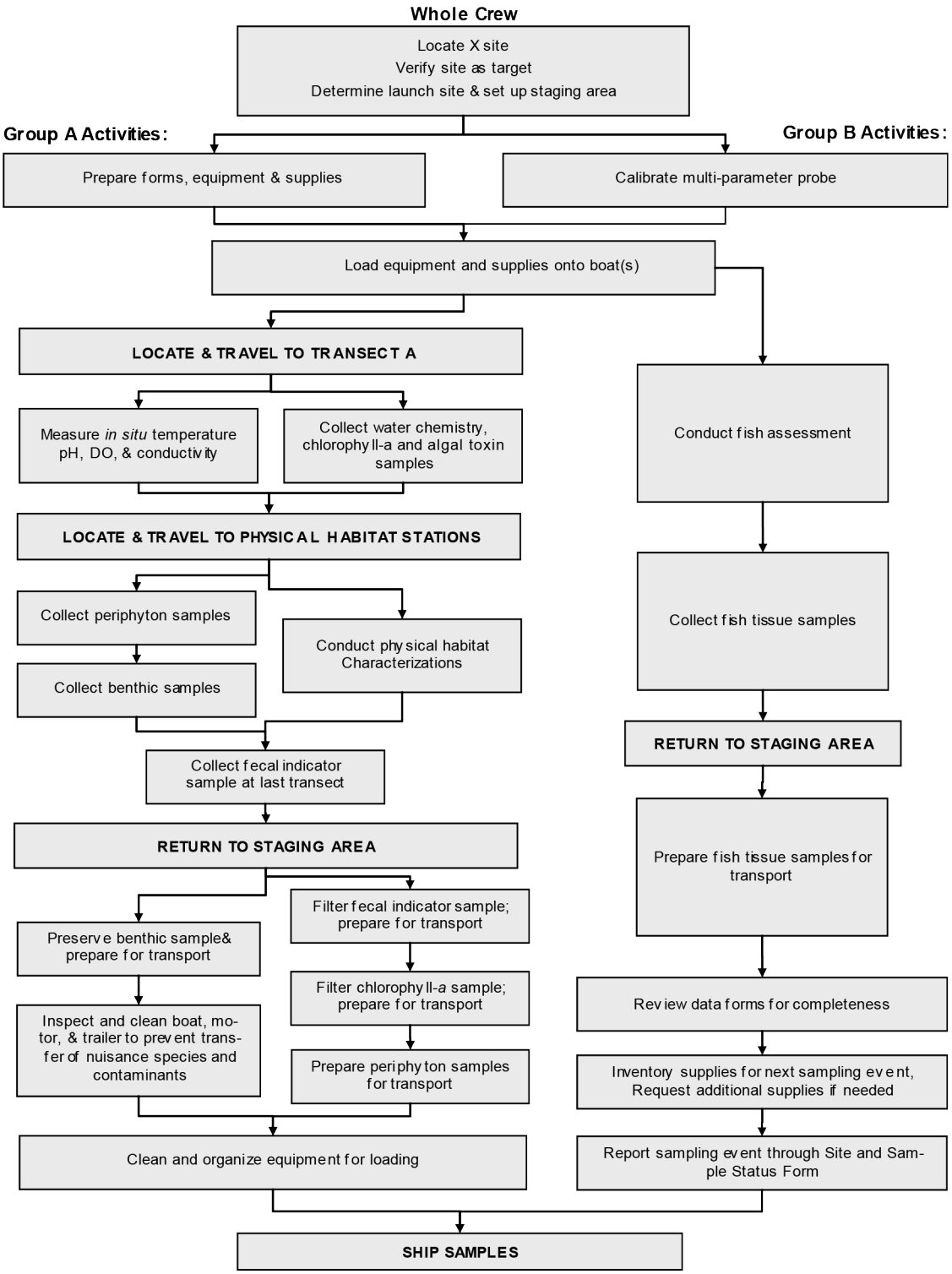


Figure 2.1 Field Sampling Scenario for Non-Wadeable Sites

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.

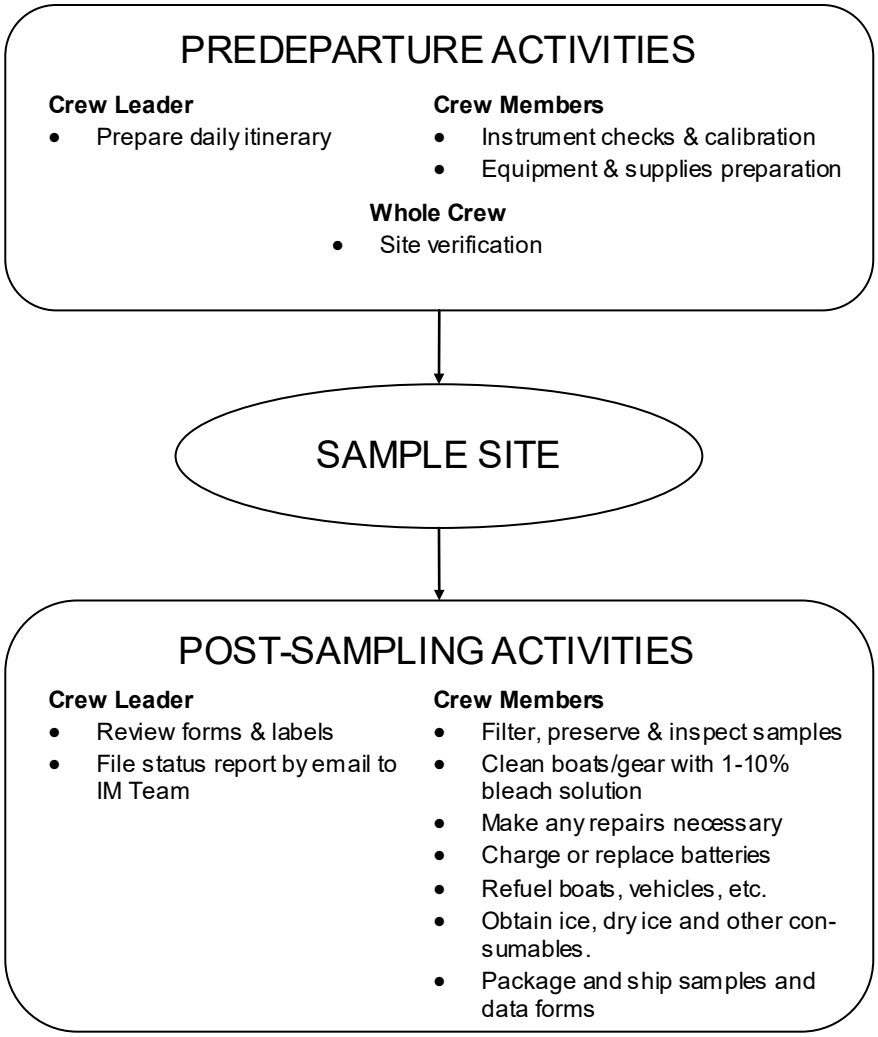


Figure 2.2 Overview of Base Site Activities

2.2.1 Pre-departure Activities

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

2.2.1.1 Daily Itineraries

The Field Crew Leaders are responsible for developing daily itineraries. This entails compiling maps, contact information, copies of permission letters, and access instructions (a "site

packet"). Additional activities include confirming the best access routes, calling the landowners or local contacts, confirming lodging plans, and coordinating rendezvous locations with individuals who must meet with field crews prior to accessing a site.

2.2.1.2 Instrument Checks and Calibration

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

- Turn on the GPS receiver and check the batteries. Replace batteries immediately if a battery warning is displayed.
- Test and calibrate the multi-probe meter. Each field crew should have a copy of the manufacturer's calibration and maintenance procedures. All meters should be calibrated according to manufacturer specifications provided along with the meter.
- Turn on the electrofishing unit and check the oil and gas supply.

2.2.1.3 Equipment and Supply Preparation

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

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

Table 2.1 Stock Solutions, Uses, and Methods for Preparation

Solution	Use	Preparation
Bleach (1%)	Clean nets, other gear, and boat.	Add 40 mL bleach to 4 L distilled water.
Bleach (10%)	Clean periphyton sampling equipment.	Add 40 mL bleach to 400 mL distilled water.
10% Buffered Formalin†	Preservation of periphyton ID sample and fixing Fish Vouchers	Formaldehyde (37-40%) 100 ml Distilled water 900 ml NaH_2PO_4 4.0 g Na_2HPO_4 (anhydrous) 6.5 g Mix to dissolve
95% Ethanol	Preservative for benthic invertebrate samples and fish vouchers.	No preparation needed (use stock solution as is).

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

2.2.2 Post Sampling Activities

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

2.2.2.1 Review Data Forms and Labels

The Field Crew Leader is ultimately responsible for reviewing all data forms and labels for accuracy, completeness, and legibility. Ensure that comments use no “shorthand” or abbreviations. The data forms must be thoroughly reviewed by the Field Crew Leader. Upon completing the review, the Field Crew Leader must initial paper 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 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 the vehicle, boat, and trailer and rinse thoroughly (hot water pressurized rinse with no soap). Rinse equipment and boat with 1% - 10% bleach solution or other specialized disinfectant to prevent the spread of exotics. Note that many organizations now recommend ***against*** using felt-soled wading boots in affected areas due to the difficulty in removing myxospores and mudsnails.

2.2.2.4 Supply Inventory

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

Table 2.2 Post-sampling Equipment Care

Equipment Care after Sampling
1. Clean for biological contaminants.
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 is provided below:
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.

Equipment Care after Sampling
If the stream is listed as "positive" for any of the organisms, or no information is available, *avoid using felt-soled wading boots*, and, after sampling, disinfect **all** fish and benthos sampling gear and other equipment that came into contact with water or sediments (i.e., waders, boots, etc.) by one of the following procedures:
Option A:
1. Soak gear in a 10% household bleach solution for at least 10 minutes, or wipe or spray on a 50% household bleach solution and let stand for 5 minutes. 2. Rinse with clean water (do not use stream water), and remove remaining debris. 3. Place gear in a freezer overnight or soak in a 50% solution of Formula 409® antibacterial cleaner for at least 10 minutes, or soak gear in 120°F (49°C) water for at least 1 minute. 4. Dry gear in direct sunlight (at least 84 °F) for at least 4 hours.
Option B:
1. Soak gear in a solution of Sparquat® (4-6 oz. per gallon of water) for at least 10 minutes (Sparquat is especially effective at inactivating whirling disease spores). 2. Place gear in a freezer overnight or soak in 120°F (49°C) water for at least 1 minute. 3. Dry gear in direct sunlight (at least 84 °F) for at least 4 hours.
2. Clean and dry other equipment prior to storage.
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 all containers used to collect water chemistry samples three times with deionized water. Place beakers in a 1-gallon sealable plastic bag with a cube container for use at the next stream. Check nets for holes and repair or locate replacements.
3. Inventory equipment and supply needs and relay orders to the IM Team via the supply request form. **4. Remove GPS, multi-probe meter, and set up for predeparture checks and calibration. Examine the oxygen membranes for cracks, wrinkles, or bubbles. Replace if necessary, allowing sufficient time for equilibration.** **5. Recharge/replace batteries as necessary.** **6. Replenish fuel and oil**; if a commercial car wash facility is available, thoroughly clean vehicle and boat (hot water pressurized rinse and no soap).

2.3 Safety and Health

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

2.3.1 General Considerations

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

Red Cross (1979), the National Institute for Occupational Safety and Health (1981), U.S. Coast Guard (1987) and Ohio EPA (1990).

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

Primary responsibility for safety while electrofishing rests with the Field Crew Leader. Electrofishing units may deliver a fatal electrical shock, and should only be used by qualified, experienced operators. If using a backpack shocker as a secondary sampling method, 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. Do not make any modifications to the electrofishing unit that would hinder safety. Avoid electrofishing near unprotected people, pets, or livestock. Discontinue activity during thunderstorms or rain. Crew members should keep each other in constant view or communication while electrofishing. For each site, know the location of the nearest emergency care facility. Although the Field Crew Leader has authority, each crew member has the responsibility to question and modify an operation or decline participation if it is unsafe.

Table 2.3 General Health and Safety Considerations

Recommended Training
• 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 PFD and suitable footwear must be worn at all times while on board a boat.

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

2.3.2 Safety Equipment

Appropriate safety apparel such as waders, linesman gloves, safety glasses, etc. must be available and used when necessary. First aid kits, fire extinguishers, and blankets must be readily available in the field. Cellular or satellite telephones and/or portable radios should be provided to field crews working in remote areas in case of an emergency. Supplies (e.g., clean water, antibacterial soap, ethyl alcohol) must be available for cleaning exposed body parts that may have been contaminated by pollutants in the water.

2.3.3 Safety Guidelines for Field Operations

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

During the course of field research activities, field crews may observe violations of environmental regulations, discover improperly disposed hazardous materials, or observe or be

involved with an accidental spill or release of hazardous materials. In such cases it is important that the proper actions be taken and that field personnel do not expose themselves to something harmful. The following guidelines should be applied:

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

Table 2.4 General Safety Guidelines for Field Operations

- Two crew members must be present during all sample collection activities, and no one should be left alone while in the field. Boats should proceed together down the river.
- Use caution when sampling on foot in swift or deep water. Wear a suitable PFD and consider using a safety tether held by an assistant.
- Use extreme care walking on riprap. Rocks can shift unexpectedly and serious falls are possible.
- Field crew members using electrofishing equipment must be insulated from the water, boat, and electrodes via non-breathable waders and linesman gloves. Use chest waders with nonslip soles.
- Electrofishing units may deliver a fatal electrical shock, and should only be used by qualified, experienced operators.
- Professional quality breathable waders with a belt are recommended for littoral sampling only, and at a safe distance from the electrofishing sampling. Neoprene boots are an alternative, but should have sturdy, puncture resistant soles.
- Exposure to water and sediments should be minimized as much as possible. Use gloves if necessary, and clean exposed body parts as soon as possible after contact.
- All electrical equipment must bear the approval seal of Underwriters Laboratories and must be properly grounded to protect against electric shock.
- Use heavy gloves when hands are used to agitate the substrate during collection of benthic macroinvertebrate samples.
- Use appropriate protective equipment (e.g., gloves, safety glasses) when handling and using hazardous chemicals.
- Crews working in areas with venomous snakes must check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a venomous snake.
- Any person allergic to bee stings, other insect bites, or plants (i.e., poison ivy, oak, sumac, etc.) must take proper precautions and have any needed medications handy.
- Field personnel should also protect themselves against deer or wood ticks because of the potential risk of acquiring pathogens that cause Rocky Mountain spotted fever, Lyme disease, and other illnesses.
- Field personnel should be familiar with the symptoms of hypothermia and know what to do in case symptoms occur. Hypothermia can kill a person at temperatures much above freezing (up to 10°C or 50°F) if he or she is exposed to wind or becomes wet.

- Field personnel should be familiar with the symptoms of heat/sun stroke and be prepared to move a suffering individual into cooler surroundings and hydrate immediately.
- Handle and dispose of chemical wastes properly. Do not dispose any chemicals in the field.

2.4 Forms (Paper or Electronic)

Forms are the key to data collection and tracking for the NRSA 2018/19. Electronic forms have been developed as well as paper forms. These electronic forms should streamline data collection. Field crews are encouraged to use electronic forms whenever possible, but will have the option of using paper forms when necessary.

2.4.1 Field Forms

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

- Electronic Field Forms: This form of data collection can be collected through an Apple iOS portable electronic device (tablets). This method of data collection 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.
- Paper Field Forms: A paper field form packet (wadeable or non-wadeable) for each site will be provided by the NARS Information Management (IM) Coordinator if crews have elected to use paper field forms for data collection. Crews will need to add these forms to the site packet prior to going in the field. After a site is sampled, the completed NRSA 2018/19 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 Coordinator and are accompanied by a Tracking form to track which data packets have been shipped. Extra paper field forms will always be provided to field crews to serve as backup copies in case of lost forms or problems with electronic field forms.

2.4.2 Tracking Forms

Tracking forms describe the status and location of all samples collected during NRSA 2018/19. Field Crew Leaders will transmit these forms electronically (through App submissions, by emailing scans of paper forms or by emailing a fillable PDF form) to the NARS IM Coordinator at specified times and crews will pack hard copies of the tracking forms 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 (including immediate shipments and batch shipments).
- Tracking – Packets: Accompany paper data packets that are batched together from multiple sites and shipped every 1 or 2 weeks. These are sent to the NARS IM Coordinator.

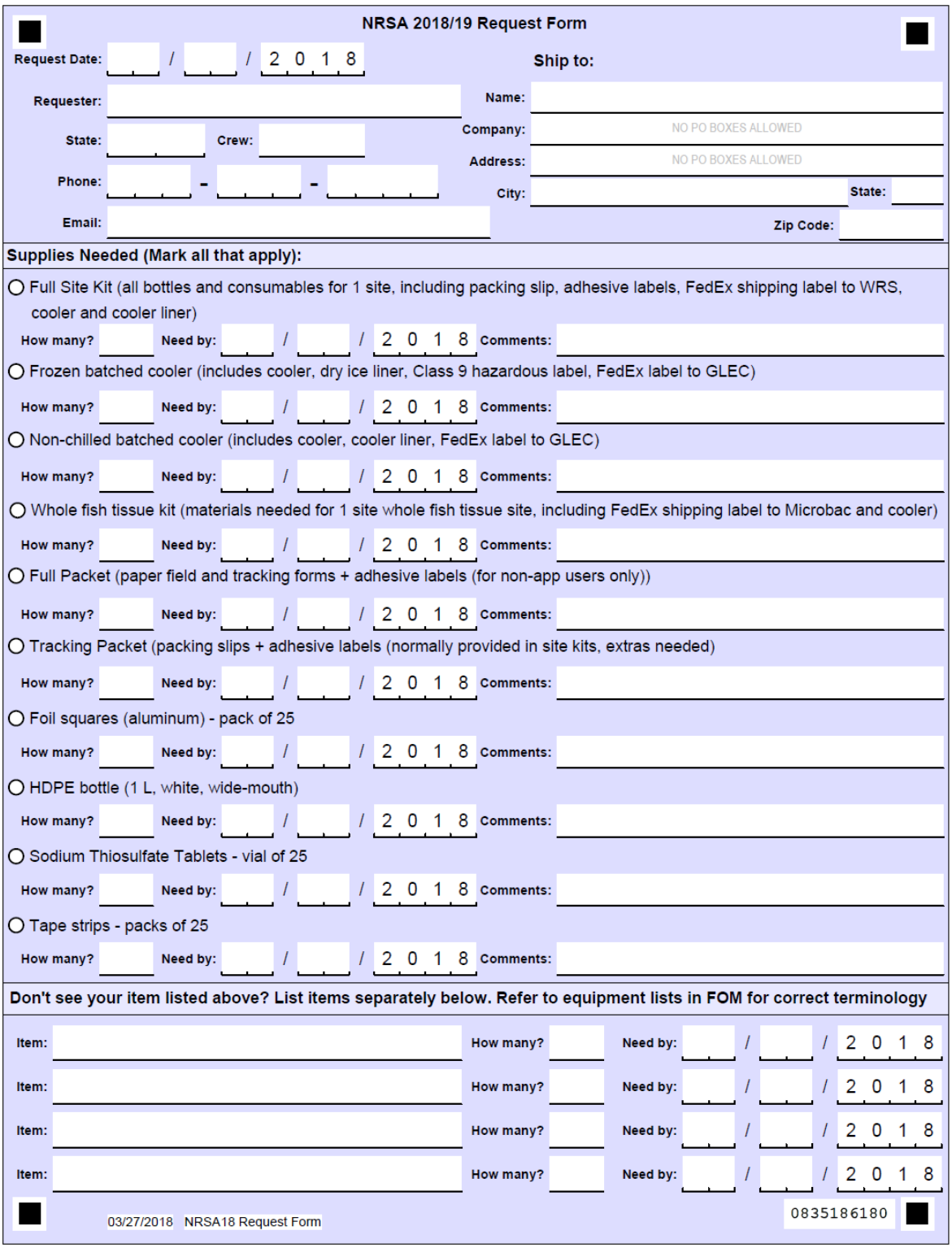
- Tracking –Samples: Submitted at the time of shipping samples that are batched together from multiple sites and shipped every 1 or 2 weeks. Whenever batched samples are shipped to their designated lab for analysis/storage, the appropriate tracking form, which lists the Sample ID numbers for all samples packed in a shipping container, is also transmitted electronically to the NARS IM Coordinator. Separate forms exist for the tracking of frozen batched samples, non-chilled batched samples and whole fish samples.
- Packing Slips: Postcard sized slips pre-populated with sample IDs that match the sample labels. Packing slips are included in the site kit with sample labels. Packing slips are to accompany samples sent to any of the national labs.

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 (**Figure 2.3**). This form will be submitted to the NARS IM Coordinator who will ensure that the request reaches the appropriate entity. Crews should submit the Request Form at least two weeks prior to their desired sampling date. In addition to typing in specific requests, crews may select one or more of the pre-populated items listed below:

- Site kit: contains all consumable supplies for one site, sample labels, packing slips, FedEx shipping label to WRS, cooler (for sending immediate samples to WRS), and cooler liner.
- Whole fish tissue kit (for crews collecting whole fish tissue samples): contains all consumable supplies for each of the 477 river sites designated for whole fish tissue sample collection, additional information for whole fish tissue sampling, cooler, Class 9 hazardous label, and FedEx shipping label to the designated laboratory for interim storage. All whole fish tissue forms and labels will be provided in the paper form packets and tracking packets.
- Frozen batched cooler: contains cooler, dry ice liner and pad, Class 9 hazardous label, FedEx label to GLEC.
- Non-chilled batched cooler: contains cooler, cooler liner, FedEx label to GLEC
- Tracking Packets: Crews using the NARS App, may request tracking packets as backups or replacements to the tracking packets included in the site kit. A tracking packet contains sample labels and packing slips for one site.
- Paper Form Packets: Crews not using the NARS App will need to request paper form packets for their use at a site. Paper form packets will contain the appropriate number of forms for one site and will also contain tracking forms that will need to be completed by hand and scanned at the time of shipping samples.



NRSA 2018/19 Request Form

Request Date: ___ / ___ / 2018

Requester: _____

State: _____ Crew: _____

Phone: ___ - ___ - _____

Email: _____

Ship to:

Name: _____

Company: _____ NO PO BOXES ALLOWED

Address: _____ NO PO BOXES ALLOWED

City: _____ State: _____

Zip Code: _____

Supplies Needed (Mark all that apply):

- ○ Full Site Kit (all bottles and consumables for 1 site, including packing slip, adhesive labels, FedEx shipping label to WRS, cooler and cooler liner)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Frozen batched cooler (includes cooler, dry ice liner, Class 9 hazardous label, FedEx label to GLEC)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Non-chilled batched cooler (includes cooler, cooler liner, FedEx label to GLEC)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Whole fish tissue kit (materials needed for 1 site whole fish tissue site, including FedEx shipping label to Microbac and cooler)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Full Packet (paper field and tracking forms + adhesive labels (for non-app users only))
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Tracking Packet (packing slips + adhesive labels (normally provided in site kits, extras needed)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Foil squares (aluminum) - pack of 25
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ HDPE bottle (1 L, white, wide-mouth)
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Sodium Thiosulfate Tablets - vial of 25
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____
- ○ Tape strips - packs of 25
  How many? _____ Need by: ___ / ___ / 2018 Comments: _____

Don't see your item listed above? List items separately below. Refer to equipment lists in FOM for correct terminology

Item: _____ How many? _____ Need by: ___ / ___ / 2018

Item: _____ How many? _____ Need by: ___ / ___ / 2018

Item: _____ How many? _____ Need by: ___ / ___ / 2018

Item: _____ How many? _____ Need by: ___ / ___ / 2018

03/27/2018 NRSA18 Request Form 0835186180

Figure 2.3 Electronic Request Form

2.4.3.2 Base Kit

The Base Kit is comprised of the subset of durable equipment and supplies needed for NRSA 2018/19 sampling that is provided by EPA through the Contract Field Logistics Coordinator. Typically, one Base Kit is provided to each Field Crew and contains some of the equipment that is used throughout the field season. See **APPENDIX A**: EQUIPMENT & SUPPLIES for a list of the items provided by EPA in the Base Kit.

2.4.3.3 Site Kit

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

2.4.3.4 Field Crew Supplied Items

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

3 INITIAL SITE PROCEDURES

When you arrive at a site, you must first confirm that you are at the correct site, and then determine if the site meets the criteria for sampling and data collection activities (See Site Evaluation Guidelines EPA-841-B-17-002). 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 medium resolution National Hydrography Dataset (NHD), in particular NHD-Plus V2, following a systematic randomized selection process (Stevens and Olsen, 2004). Each point is referred to as the "X-site." The "X-site" is the mid-point of the sampling reach, and it will determine the location and extent for the rest of the sampling reach. The latitude/longitude of the "X-site" is listed on the site evaluation spreadsheet that was distributed to each Field Crew Leader. **Table 3.1** provides the equipment and supplies needed for site verification.

Note that the coordinates provided on the site evaluation spreadsheet may not be located in the middle of the stream or river; and in some cases, the coordinates may be on dry land next to the stream or river. In these cases, it is important for crews to locate the X-site at a point that is in the middle of the stream or river (e.g. midway between the left and right banks). To do this, simply measure the distance between banks and move the point perpendicular to the nearest bank until it is half-way between the left and right banks. Record these coordinates as the X-site on the verification form. If the provided coordinates are located on dry land near a stream, move the coordinates to the nearest blue line in NHD-Plus 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: 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 2018/19 Community Fact Sheet. GPS unit (preferably one capable of recording waypoints) with manual, reference card, extra battery pack. Surveyor's flagging tape (to mark transects). Laser rangefinder. 50 m or 100 m measuring tape with reel (if not using rangefinder).
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 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 20 channel-widths upstream and downstream of the X-site – for small systems, 150m is the minimum reach length that can be sampled, so the upstream and downstream lengths would be 75 meters each. If there are isolated pools of water within the reach that equal greater than 50% of the reach length, proceed to sample using the modified procedures outlined in **Section 3.1.1**. Do not drop the site if it is dry at the X site, as long as there is greater than 50% water throughout the channel. If less than 50% of the reach has water, classify the site as "Dry-visited" on the verification form. 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 Site 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 available oversample site on the state list within the same category based on Strahler category, new/resample status, and ecoregion (**Section 1.3**).

NRSA 2018/19 VERIFICATION (Front)

Reviewed by (initial): ____

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

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

O This is a special State site

STREAM/RIVER VERIFICATION INFORMATION

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

O Other (Describe Here):

Coordinates	Latitude	Longitude	# of Satellites	Elevation at transect A
GPS Decimal Degrees NAD 83	____.____	–____.____	O ≤3 O ≥4	____
Location: O X-Site (wadeable) O Transect A (non-wadeable)				O ft O m

DID YOU SAMPLE THIS SITE?

O YES If Yes, check one below

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

ADDITIONAL SITE CHARACTERISTICS

O Tidally Influenced O Blackwater O Not Applicable

O NO If No, check one below

NON-SAMPLEABLE-PERMANENT
- O Dry - Visited
- O Dry - Not visited
- O Wetland (No Definable Channel)
- O Map Error (No evidence channel/waterbody ever present)
- O Impounded (> 7 day residence time)
- O Tidal (exceeds salinity threshold)
- O Other (explain in comments)

NON-SAMPLEABLE-TEMPORARY
- O Not boatable - Need a different crew - Reschedule regardless of year
- O Not wadeable - Need a different crew - Reschedule regardless of year
- O Other (explain in comments)

NO ACCESS
- O Access Permission Denied
- O Permanently Inaccessible (Unable/Unsafe to Reach Site)
- O Temporarily Inaccessible-Fire, etc. - Reschedule regardless of year
- O Other (explain in comments)

GENERAL COMMENTS

DIRECTIONS TO SITE

02/28/2018 NRSA18 Verification 7734319701

Figure 3.1 Verification Form (front)

Table 3.2 Procedure: Site Verification

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 = NAD83). Record these on the Verification Form.
3. **Use all available means to ensure you are at the correct stream/river** as marked on the map, including 1:24,000 United States Geological Society (USGS) maps, topographic landmarks, road maps, signs, local contacts, etc.
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 20 channel-widths upstream and downstream of the X-site (150 m is minimum sampling reach length, so in small systems the upstream and downstream lengths would be 75 meters each). Assign one of the following sampling status categories to the stream. Record the category on the Verification Form.

Sampleable Categories

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” in the site evaluation spreadsheet.
Wetland: Standing water present, but no definable stream channel. If wetland is surrounding a stream channel, define the site as Target but restrict sampling to the stream channel.
Map Error: No evidence that a water body or stream channel was ever present at the X-site.
Impounded stream: Stream is submerged under a lake or pond due to manmade or natural (e.g., beaver dam) impoundments. If the impounded stream is still wadeable, record it as “Altered” and sample.
Other: Examples would include underground pipelines, or a non-target canal. A sampling site must meet both of the following criteria to be classified as a non-target canal:
The channel is constructed where no natural channel has ever existed.
The sole purpose/usage of the reach is to transfer water. There are no other uses of the waterbody by humans (e.g., fishing, swimming, and boating).

TEMPORARY

Not Boatable: need a different crew.

Not Wadeable: need a different crew.

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

No Access to Site Categories

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

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

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

6. Do not sample non-target or "Non-sampleable" or "No Access" sites. Fill in the "NO" bubble for "Did you sample this site?" and mark the appropriate bubble in the "Non-Sampleable" or "No Access" section of the Verification Form; provide a detailed explanation in comments section.

3.1.3 Elevation at Transect A

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

3.1.4 Sampling During or After Rain Events

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

Table 3.3 Guidelines to Determine the Influence of Rain Events

- If it is running at bank full discharge or the water seems much more turbid than typical for the class of stream do not sample it that day.
- Do not sample that day if it is unsafe to be in the water.
- Keep an eye on the weather reports and rainfall patterns. Do not sample a stream during periods of prolonged heavy rains.

- If the stream seems to be close to normal summer flows, and does not seem to be unduly influenced by storm events, sample it even if it has recently rained or is raining.

3.1.5 Site Photographs

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

3.2 Laying out the sampling reach

Many of the biological and habitat structure measures require sampling a certain length of a stream to get a representative picture of the ecological community. **A length of 40 times the wetted width is necessary to characterize the habitat and several biotic assemblages associated with the sampling reach.** Establish the sampling reach about the X-site using the procedures described in **Table 3.4** (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 Geographic Information System (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 the Verification Form (back) as shown in **Figure 3.2**.

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 site, complete a rough sketch map of the reach you sampled on page 2 of the Verification Form (**Figure 3.2**). In addition to any other interesting features that should be marked on the map, note any landmarks/directions that can be used to find the X-site for future visits.

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

Laying out the sampling reach at the base site (recommended at non-wadeable sites)

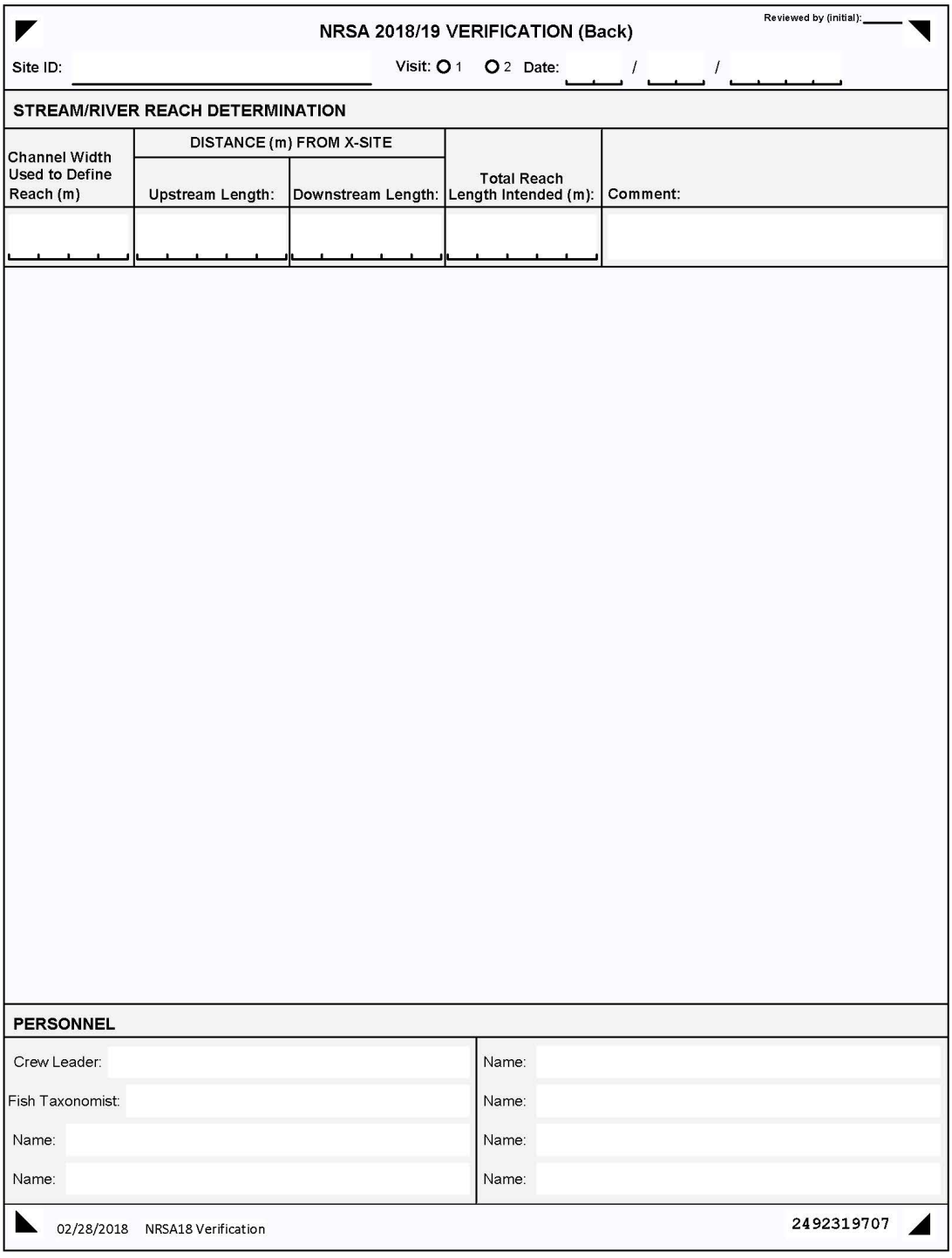
1. Using GIS, an aerial photo or a 1:100:000 topographic map; **locate the X-site using the coordinates provided for the site**.
2. **Determine the average wetted width of the channel at the X-site** using GIS, available maps, and/or aerial photographs. To get an average, determine the wetted width of the channel at five places of "typical" width within approximately five channel widths upstream and downstream from the X-site. **Average the 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. Record both the average channel width and total stream length on page 2 of the Verification Form

4. **From the X-site, measure a distance of one-half the reach length 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 one-half the reach length 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: 0 - 4 = Left Bank, 5 – 9 = Right Bank). From here, three stations will be on the first (randomly selected) side of the river, then two on the other, then two 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 **Table 3.5** 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 five 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 5), measure a distance of one-half the reach length upstream and 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: 0 - 4 = Left Bank, 5 – 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 two on the other, then two 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 nine 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 2018/19 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:		

PERSONNEL

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

02/28/2018 NRSA18 Verification 2492319707

Figure 3.2 Verification Form (back)

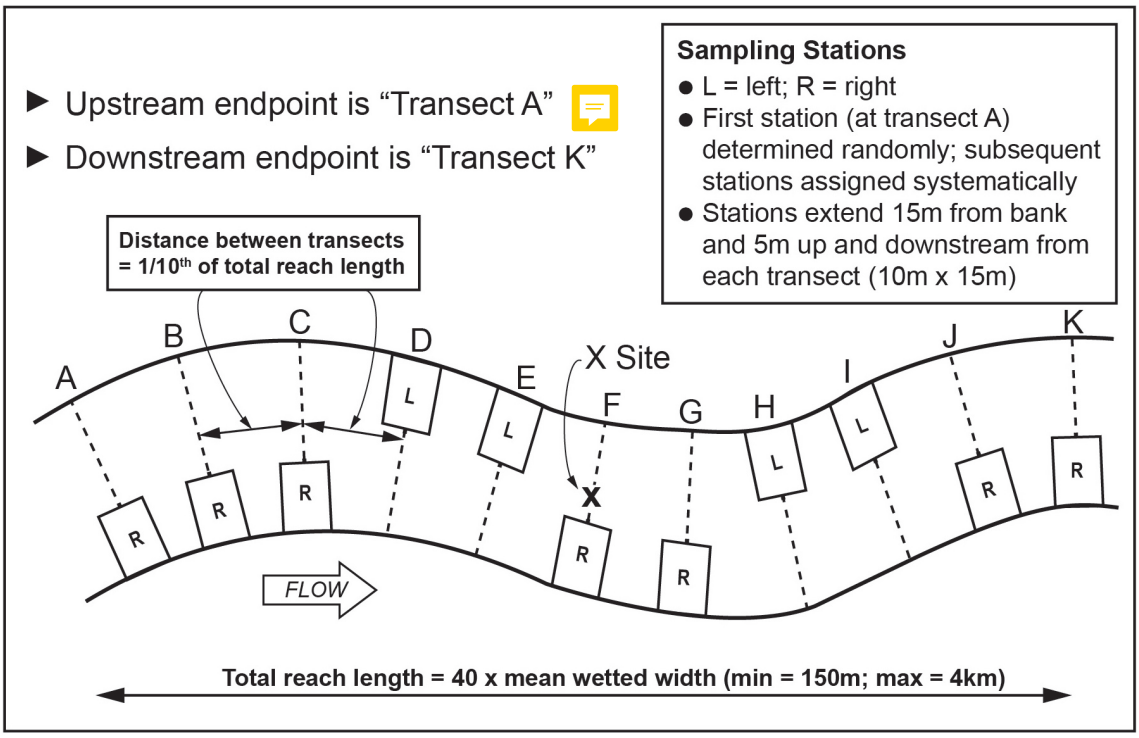


Figure 3.3 Sampling Reach Features (Non-Wadeable Sites)

3.2.1 Sliding the Reach

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

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

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

Table 3.5 Procedure: Sliding the Sampling Reach

1. Slide the reach if you run into an **impoundment** (lake, pond, or reservoir), so that the lake/stream confluence is at one end.
2. Slide the reach if you run into an **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 indicators along the entire sample reach from interrupted streams, regardless of the amount of water present at each of the transects. Obtain depth measurements along the deepest part of the channel (the "thalweg") along the entire sampling reach to provide a record of the "water" status of the stream for future comparisons (e.g., the percent of length with intermittent pools or no water). Other measurements associated with characterizing riparian condition, substrate type, etc., are useful to help infer conditions in the stream when water is flowing.

Table 3.6 Reach Layout Modifications for Interrupted Streams

Streams with less than 50% of the reach length containing water (not necessarily continuous) are considered dry and are not sampled. If more than 50% of the channel has water and if Transect A 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 Transect A. Note that the sample wasn't collected at the Transect A 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. If using the App, sketches on paper can be scanned via CamScanner (software preloaded on iPads) and submitted to EPA SharePoint. 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 two to three meander cycles (about six pool riffle habitat sequences). In the case of braided systems, the objective of this protocol modification is to avoid sampling an excessively long stretch of stream. In a braided system where there is a 100 m wide active channel (giving a 4 km reach length based on the standard procedure) and only 10 m of wetted width (say five, 2 m wide

braids), a 400 m long sample reach length is likely to be sufficient, especially if the system has fairly homogenous habitat throughout its length.

Table 3.7 Procedure: Modifications for Braided Rivers and Streams

1. Estimate the mean width as the bankfull channel width as defined in the physical habitat protocol.
    - If the mean width is ≤15 m, set up a 40 x channel width sample reach in the normal manner, using the mean bankfull width for your calculations.
    - If >15 m, sum up the actual wetted width of all the braids and use that as the width for calculating the 40 x channel width reach length. Remember the minimum reach length is always 150 m.
    - If the reach length seems too short for the system in question, set up a longer sample reach, taking into consideration that the objective is to sample a long enough stretch of a stream to include at least two to three meander cycles (about six pool riffle habitat sequences).
2. Make detailed notes 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 *in situ* DO, pH, water temperature, and conductivity using a calibrated multi-parameter water quality meter (or sonde). Take the measurements mid-channel at Transect A. 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 (e.g., ambient air saturated with water) prior to launching the boat. Note that DO should always be calibrated at the site and should never be calibrated at your base location. Follow your manufacturer's guidelines for calibration of the DO probe.

pH Meter

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

Temperature Meter

Check the accuracy of the sensor against a thermometer that is traceable to the National Institute of Standards and Technology (NIST) at least once per sampling season and record the NIST thermometer reading and the sensor reading on the Field Measurement Form. These same

values can be recorded each time for the Field Measurement Form unless the accuracy check is conducted again. The entire temperature range encountered in the NRSA should be incorporated in the testing procedure and a record of test results kept on file.

Conductivity Meter

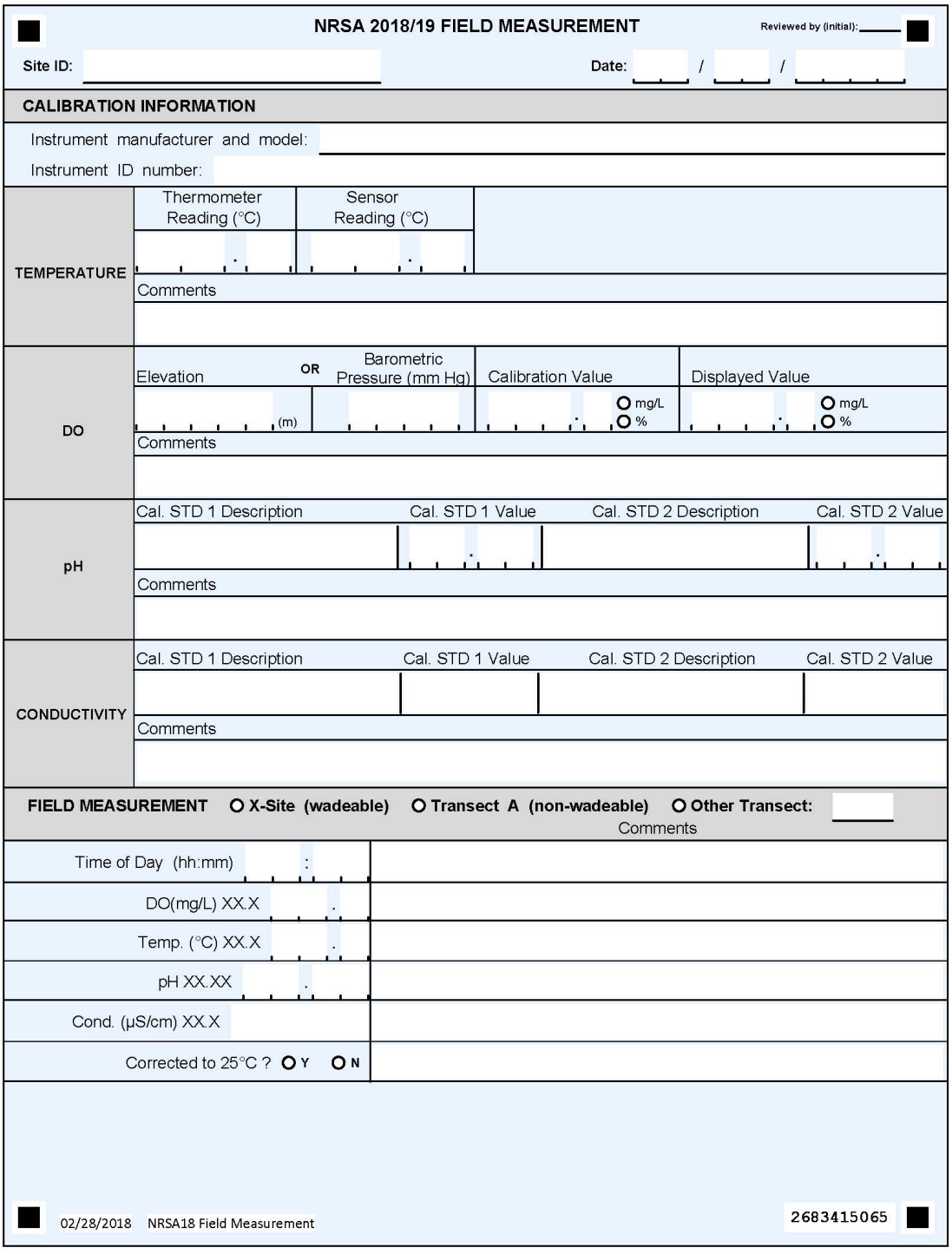
Calibrate the conductivity meter prior to each sampling event. Calibrate the meter in accordance with the manufacturer's instructions. Ideally, a QCS solution should be used that incorporates the entire conductivity range encountered in the NRSA and a record of test results kept on file.

4.1.3 Sampling Procedure

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

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

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



NRSA 2018/19 FIELD MEASUREMENT

Reviewed by (initial): ____

Site ID: ____ Date: ____ / ____ / ____

CALIBRATION INFORMATION

Instrument manufacturer and model: ____

Instrument ID number: ____

TEMPERATURE

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

Comments

DO

Elevation (m)	OR	Barometric Pressure (mm Hg)	Calibration Value	Displayed Value
____		____	____ . ____ O mg/L O %	____ . ____ O mg/L O %

Comments

pH

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

Comments

CONDUCTIVITY

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

Comments

FIELD MEASUREMENT O **X-Site (wadeable)** O **Transect A (non-wadeable)** O **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 ? O Y O N	

02/28/2018 NRSA18 Field Measurement

2683415065

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 ammonium (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). Using a 3 L Nalgene beaker, collect a grab sample into one 4 L cube container (for water chemistry) and one 2 L amber Nalgene bottle (for chlorophyll-*a*) from Transect A at the midpoint of the reach. After collection, store all samples on ice in a closed cooler. After you filter the chlorophyll-*a* sample, the filters must be kept frozen until ready to ship.

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

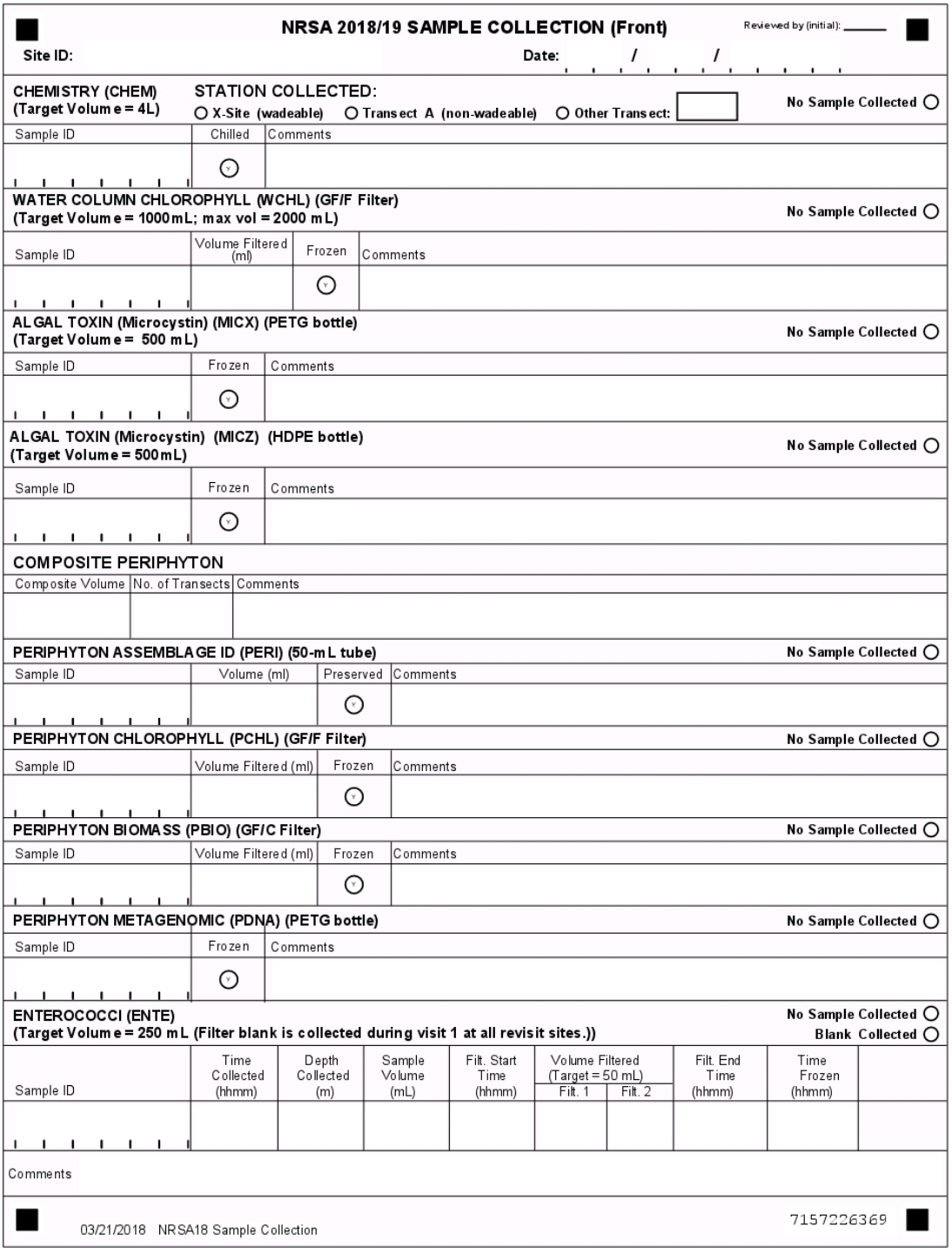
For collecting samples	Nitrile gloves 4 L cube container 2 L amber Nalgene bottle 3 L Nalgene beaker Cooler with ice Plastic electrical tape DI water (for cleaning beaker and 2 L amber bottle between sites) Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Sample Collection Form Water Chemistry sample label with pre-printed Sample ID Clear tape strips Sample Collection 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 Procedure: Water Chemistry and Chlorophyll-*a* Sample Collection (Non-Wadeable Sites)

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 or implement measures to reduce contamination by such chemicals, if applied, such as washing, wearing long gloves, etc. 4. Rinse the 3 L Nalgene beaker three times with water, and discard the rinse downstream. 5. Remove the cube container lid and expand the cube container by pulling out the sides if needed (the weight of the water alone while filling will often open the container sufficiently). NOTE: DO NOT BLOW into the cube container or place your fingers inside the opening to expand it because will cause contamination. 6. Fill the 3 L beaker with water and slowly pour 30 - 50 mL into the cube container. Cap the cube container and rotate so that the water contacts all the surfaces. Discard the water downstream. Repeat this rinsing procedure two more times. 7. Fill the beaker with water and pour into the cube container. Repeat as necessary to fill the cube container. Let the weight of the water expand the cube container. Pour the water slowly as the cube container expands. Completely fill the cube container. Rinse the cube container lid with water. Eliminate any air space from the cube container by squeezing the closed container and opening the cap slightly to allow air to escape, and cap it tightly. Make sure the cap is tightly sealed and not on at an angle. 8. Seal the cap with plastic electrical tape before shipping.
Chlorophyll-*a*
9. Fill the 3 L beaker with water and slowly pour 30 - 50 mL into the 2 L amber Nalgene bottle. Cap the bottle and rotate so that the water contacts all the surfaces. Discard the water downstream. Repeat this rinsing procedure two more times. 10. Fill the beaker with water and pour into the 2 L amber Nalgene bottle, filling the bottle. Cap the bottle tightly. This sample will be filtered later and the bottle will be reused at future sites, therefore it is not necessary to label this bottle.
Storage
Place the cube container and Nalgene bottle in a cooler (on ice or water) and shut the lid. If a cooler is not available, place the cube container in an opaque garbage bag and immerse it in the stream. Once the water chemistry sample is placed on ice, mark the "Chilled" bubble on the sample collection form. **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. If sample(s) are not collected, fill in the "No Sample Collected" bubble on the data form(s) and indicate the reason why targeted sample(s) were not collected in the comments section.



NRSA 2018/19 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 (WCHL) (GF/F Filter) (Target Volume = 1000mL; max vol = 2000 mL) ○ No Sample Collected

Sample ID	Volume Filtered (ml)	Frozen	Comments
		○	

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

Sample ID	Frozen	Comments
	○	

ALGAL TOXIN (Microcystin) (MICZ) (HDPE bottle) (Target Volume = 500mL) ○ No Sample Collected

Sample ID	Frozen	Comments
	○	

COMPOSITE PERIPHYTON

Composite Volume	No. of Transects	Comments

PERIPHYTON ASSEMBLAGE ID (PERI) (50-mL tube) ○ No Sample Collected

Sample ID	Volume (ml)	Preserved	Comments
		○	

PERIPHYTON CHLOROPHYLL (PCHL) (GF/F Filter) ○ No Sample Collected

Sample ID	Volume Filtered (ml)	Frozen	Comments
		○	

PERIPHYTON BIOMASS (PBIO) (GF/C Filter) ○ No Sample Collected

Sample ID	Volume Filtered (ml)	Frozen	Comments
		○	

PERIPHYTON METAGENOMIC (PDNA) (PETG bottle) ○ No Sample Collected

Sample ID	Frozen	Comments
	○	

ENTEROCOCCI (ENTE) (Target Volume = 250 mL (Filter blank is collected during visit 1 at all revisit sites.)) ○ 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

03/21/2018 NRSA18 Sample Collection 7157226369

Figure 4.2 Sample Collection Form (front)

5 ALGAL TOXINS (MICROCYSTINS and CYLINDROSPERMOPSIN)

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

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

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

5.1 Summary of Method

The algal toxin (microcystin and cylindrospermopsin) samples are grab samples taken from Transect A. All field crews must collect a grab sample using the 3 L beaker to fill two 500 ml bottles. Collect these samples 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 the algal toxin samples at Transect A. 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 PETG bottle (500 mL, clear, square) – algal toxins (MICX) HDPE bottle (500 mL white, round) – algal toxins (MICZ) Plastic electrical tape Cooler with ice Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Sample Collection Form Algal toxin sample labels with pre-printed Sample ID Clear tape strips Sample Collection 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 algal toxin (microcystin and cylindrospermopsin) samples at non-wadeable sites.

Table 5.2 Procedure: Algal Toxin (Microcystin and Cylindrospermopsin) Collection (Non-Wadeable Sites)

Microcystin Sample Collection

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

Storage

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

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

Upon returning to your base site (hotel, lab, office, etc.), freeze both samples and keep frozen until shipping. Mark the "Frozen" bubbles on the form to verify samples have been frozen.

If sample(s) are not collected, fill in the "No Sample Collected" bubble on the data form(s) and indicate the reason why targeted sample(s) were not collected in the comments section.

6 BENTHIC MACROINVERTEBRATES

6.1 Summary of Method

Collect a benthic macroinvertebrate composite sample using a D-frame net with 500 µm mesh openings. Take individual samples from the sampling stations at the 11 transects equally distributed along the targeted reach (**Figure 3.3**). Multiple habitats will be encountered and sampled using this approach. Habitats will include various types of bottom substrate as well as woody debris, macrophytes, and leaf packs. Composite all sample material from all 11 sampling locations and field preserve with ~95% ethanol.

6.2 Equipment and Supplies

Table 6.1 shows the checklist of equipment and supplies required to complete the collection of benthic macroinvertebrates. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the site. Record collection data on the back of Sample Collection Form (**Figure 6.1**).

Table 6.1 Equipment and Supplies: Benthic Macroinvertebrate Collection at (Non Wadeable Sites)

For collecting samples	Modified kick net (D-frame with 500 µm mesh) and 52" handle Watch with timer or stopwatch Sieve bucket with 500 µm mesh openings (U.S. std No. 35) 5 gallon bucket Watchmakers' forceps Wash bottle, 1 L capacity labeled "STREAM WATER" Funnel, with large bore spout	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 2018/19 SAMPLE COLLECTION (Back)

Site ID: _____ Date: ____ / ____ / ____

BENTHIC MACROINVERTEBRATES (BERW) - WADEABLE No Sample Collected ○

Sample ID	Number of jars	Preserved (ETOH)	No. of Transects	Comments
		○ Y		

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)	No. of Transects	Comments
		○ Y		

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 ○L ○R	B ○L ○R	C ○L ○R	D ○L ○R	E ○L ○R	F ○L ○R	G ○L ○R	H ○L ○R	I ○L ○R	J ○L ○R	K ○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

02/28/2018 NRSA18 Sample Collection

8904226360

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 designated sampling station at each transect.

The procedure for collecting samples at each transect is described in **Table 6.2**. Take a 1 linear meter sweep from within 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.

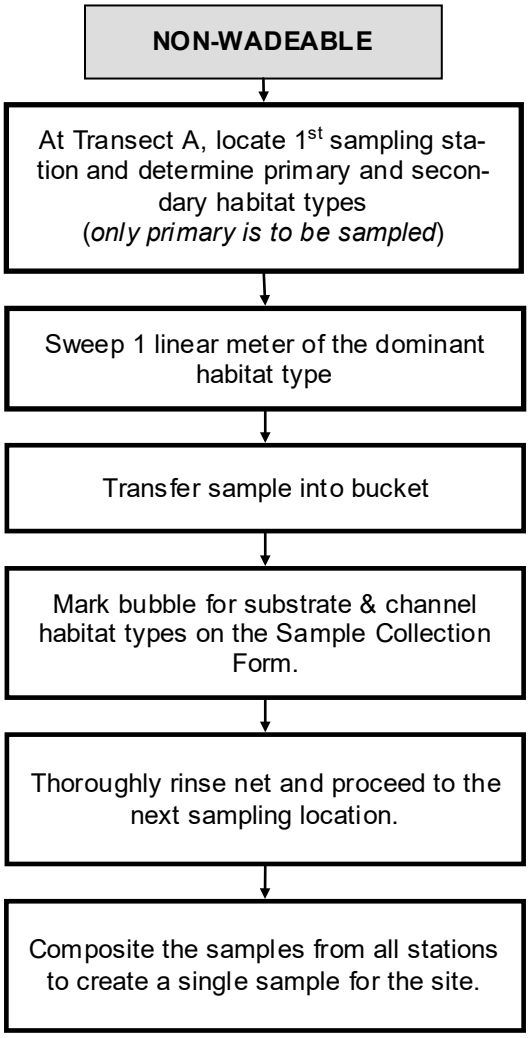


Figure 6.2 Benthic Macroinvertebrate Collection at Non-Wadeable Sites

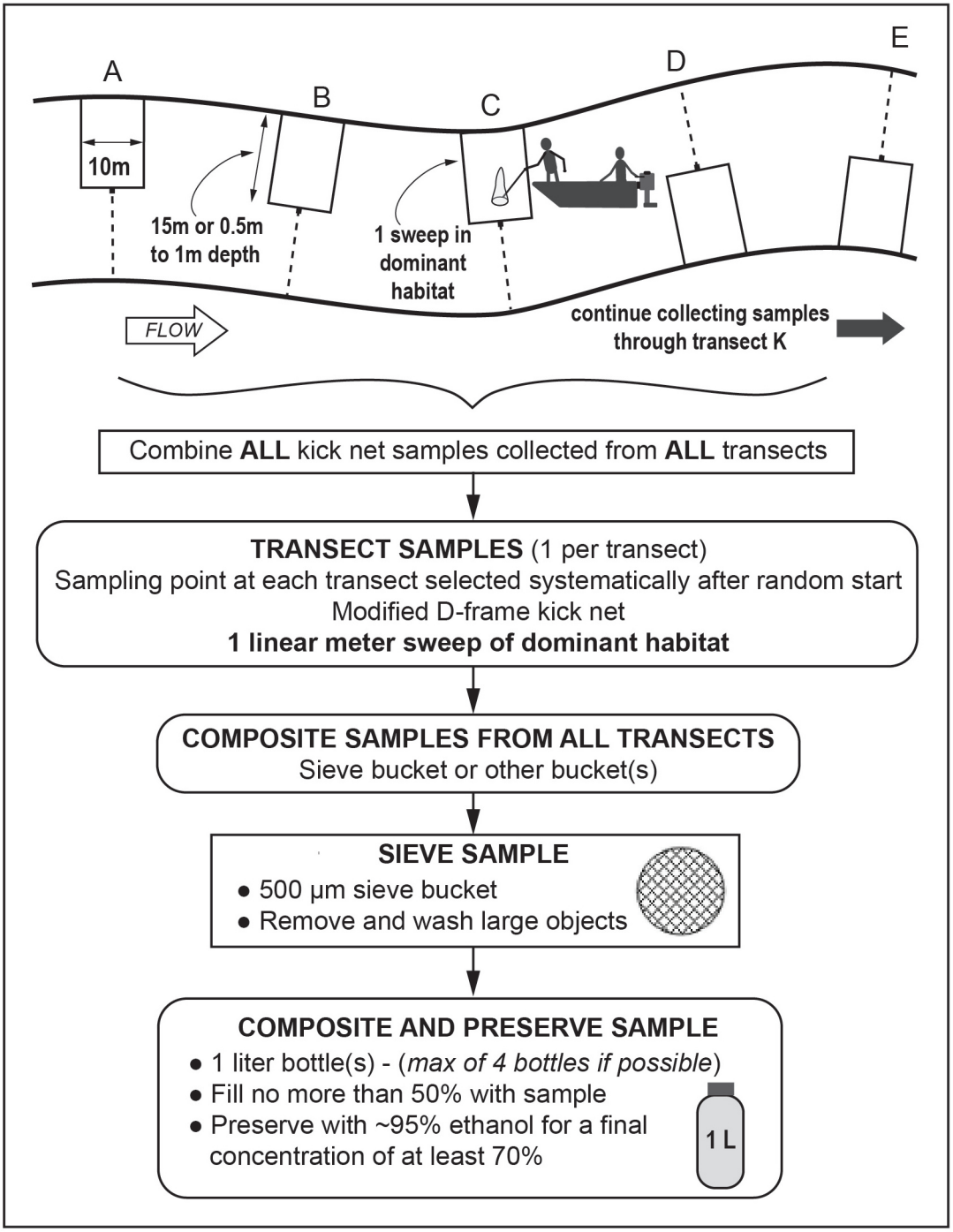


Figure 6.3 Transect Sample Design for Collecting Benthic Macroinvertebrates (Non-Wadeable Sites)

Table 6.2 Procedure: Benthic Macroinvertebrate Sampling (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, facing downstream).
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.

   NOTE: If there are co-dominant substrate types, you may indicate more than one substrate type; note the co-dominants in the comments section of the form.
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. Between each station remove any predacious macroinvertebrates and preserve immediately to prevent loss of organisms. Also, ensure that sample is kept wet between transects. 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. Record the number of transects that were sampled throughout the reach.

6.4 Sample Processing in Field

Use a 500 μm mesh sieve bucket placed inside a larger bucket full of site water while sampling to carry the composite sample as you travel around the site. Once the composite sample from all stations is sieved and reduced in volume, store in a 1 L jar and preserve with 95% ethanol. Do not fill jars more than ½ full of material. Multiple jars may be required if detritus is heavy (**Table 6.3**). If there is a large amount of organic material in the sample, or there are adverse field conditions (i.e. hot, humid weather), place sample in a 1 L jar with ethanol after each station.

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

Record information for each composite sample on the 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: Compositing Samples for Benthic Macroinvertebrates (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 four jars per site. 4. Fill in a sample label with the Sample ID, date of collection, and jar number (i.e., Jar 1 of 2). Attach the completed label to the jar and cover it with a strip of clear tape. 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).* 6. Place a waterproof label inside each jar with the following information written with a number 2 lead pencil: Site ID — Collector(s) 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

7. Completely fill the jar with 95% ethanol (no headspace). It is very important that sufficient ethanol be used, or the organisms will not be properly preserved. Existing water in the jar should not dilute the concentration of ethanol below 70%.
   - *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.*
8. Replace the cap on each jar. Slowly tip the jar to a horizontal position, then gently rotate the jar to mix the preservative. Do not invert or shake the jar. After mixing, seal each jar with plastic electrical tape.
9. Store labeled composite samples in a container with absorbent material that is suitable for use with 70% ethanol until transport or shipment to the laboratory.
10. If sample(s) are not collected, fill in the "No Sample Collected" bubble on the data form(s) and indicate the reason why targeted sample(s) were not collected in the comments section.

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 same 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 reach. At the completion of the day's sampling activities, but before leaving the site, **prepare four types of laboratory samples** (an ID/enumeration sample to determine taxonomic composition and relative abundances, a metagenomics sample, a chlorophyll-*a* sample, and a biomass sample (for ash-free dry mass [AFDM])) from the composite periphyton sample.

7.2 Equipment and Supplies

Table 7.1 is a checklist of equipment and supplies required to conduct periphyton sample collection and processing activities. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the site.

Table 7.1 Equipment and Supplies: Periphyton (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 (20 mL) Timer or stopwatch Cooler (small soft-sided preferred) Wet ice 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 site) with the sample ID number Clear tape strips for covering labels
For cleaning equipment	10% Bleach solution

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 entire 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 into one bottle to produce one composite sample for each site. Record the number of transects sampled and the total volume of the composite sample on the Sample Collection Form as shown in **Figure 4.2**.

Table 7.2 Procedure: Collecting Composite Index Samples of Periphyton (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 or over 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 DI water. Using water from this bottle, wash the dislodged periphyton from the funnel into the 500 mL bottle. Use an amount of water (~45 mL) that brings the composite volume up to the next graduation mark on the bottle. (d) Put the bottle in a cooler on ice while you travel between transects and collect the subsequent samples. (The sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite). **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 clear tube, attached to the tip of the syringe, in a scraping motion. (b) While dislodging periphyton with the tube, simultaneously pull back to 25 mL on the syringe plunger to draw the dislodged periphyton into the syringe. The 25 mL in the syringe combined with the 20mL in the tube equals the target volume of 45 mL. (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 sample needs 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 cm^2 area of soft sediments in a shallow area of the sampling station. (b) Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60 mL syringe with attached clear tube up to the 25mL line of 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 sample needs to be kept cool and dark because a chlorophyll sample will be filtered from the composite.)

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

Storage

3. After samples have been collected from all 11 transects (or as many transects as possible), thoroughly mix the 500 mL bottle regardless of substrate type. Record the total volume of the composite sample in the periphyton section of the Sample Collection Form.
4. Record the number of transects sampled. If all 11 transects are not sampled, record the reason(s) for any missed transects on the field form.
5. If sample(s) are not collected at all, fill in the "No Sample Collected" bubble on the data form(s) and indicate the reason why targeted sample(s) were not collected in the comments section.

Clean up

6. After preparing the four types of laboratory samples (see **Section 13.3**), thoroughly clean each of the pieces of periphyton equipment (delimiter, brush, funnel, syringe, and composite bottle) with a 10% Bleach solution and rinse with tap or DI water.

7.4 Sample Processing in the Field

You will prepare four different types of laboratory samples from the composite samples: an **ID/enumeration sample** (to determine taxonomic composition and relative abundances), a **metagenomic sample**, **chlorophyll-*a* sample**, and a **biomass sample** (for AFDM). All of the methods for processing the four samples are found in the Final Site Activities (**Section 13**) portion of the manual. All the sample containers required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination (e.g., dust, dirt, or mud) that are present at site shorelines.

8 PHYSICAL HABITAT CHARACTERIZATION

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

8.1 Equipment and Supplies

Table 8.1 lists the equipment and supplies required to conduct all the activities described for characterizing physical habitat. This checklist is similar to the checklist presented in **Appendix A**, which is used at the base location to ensure that all of the required equipment is brought to the river. Use this checklist to ensure that equipment and supplies are organized and available at the river site in order to conduct the activities efficiently.

Table 8.1 Equipment and Supplies: Physical Habitat

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 (optional) 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 (optional) 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 mean wetted width near the X-site**, **with a minimum of 150 m and maximum of 4 km**, 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 in 11 sampling locations placed evenly throughout the sampling reach and the presence of snags is recorded along the thalweg between transects. 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 using the NARS App or 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 sounding 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 1 m above waterline (dominant and subdominant size class).

   In 20 m long Littoral Plot extending streamward 10 m 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 20 m long Riparian Plot extending 10 m 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 Physical Habitat Characterization (Non-Wadeable)

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. Use a GPS to determine the coordinates where the transect intersects the chosen bank and record them in the Transect (Bank) space on the field form.
3. Record the dominant and subdominant substrate of the shoreline 1 meter above the water's edge, based on visual observations.
4. Move boat in a "loop" within the 10 x 20 m littoral plot, measuring and recording 5 littoral depths and probing substrate. Use the 'sonar' or 'pole' columns based on the method used.
5. Estimate dominant and subdominant littoral substrate, based on probing the 5 locations wherever possible. If probing is not possible due to depth, flow, or other conditions, mark the 'judgement' bubble.
6. Tally LWD within or partially within the 10 x 20 meter littoral plot that is all or partially in the wetted channel.
7. Tally LWD within or partially within the 10 x 20 meter littoral plot that is entirely out of water but at least partially within the bankfull channel.
8. Choose bank angle class, estimate bankfull height, width and channel incision. (Note that width and incision estimates incorporate both left and right banks.).
9. 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 10 m back from the bankfull line).
10. 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.
11. Estimate areal cover of fish concealment features in the 10 x 20 meter littoral plot.

12. Estimate and record distance to riparian vegetation on the chosen bank.
13. Indicate the level of channel constraint present in the area of the transect and indicate whether you could readily see over the bank.
14. Collect densiometer measurements at bank (facing upstream, downstream, left, right).
15. Proceed to a midstream point on the transect and record the GPS coordinates in the Transect (Midstream) space on the field form.
16. 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 note the presence of snags and characterize mid-channel substrate. The float is interrupted by stops at 11 transect locations for littoral/riparian observations. Crews 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 the 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 to use in some 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 log jams or other obstacles 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.

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 length is 40 times the wetted width at the X-site, with a 150 m 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 length 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** (**Table 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 established transect locations).
- If the thalweg is too deep or it is not physically possible to measure the thalweg, estimate the depth to the best of your ability and flag it on the field form.

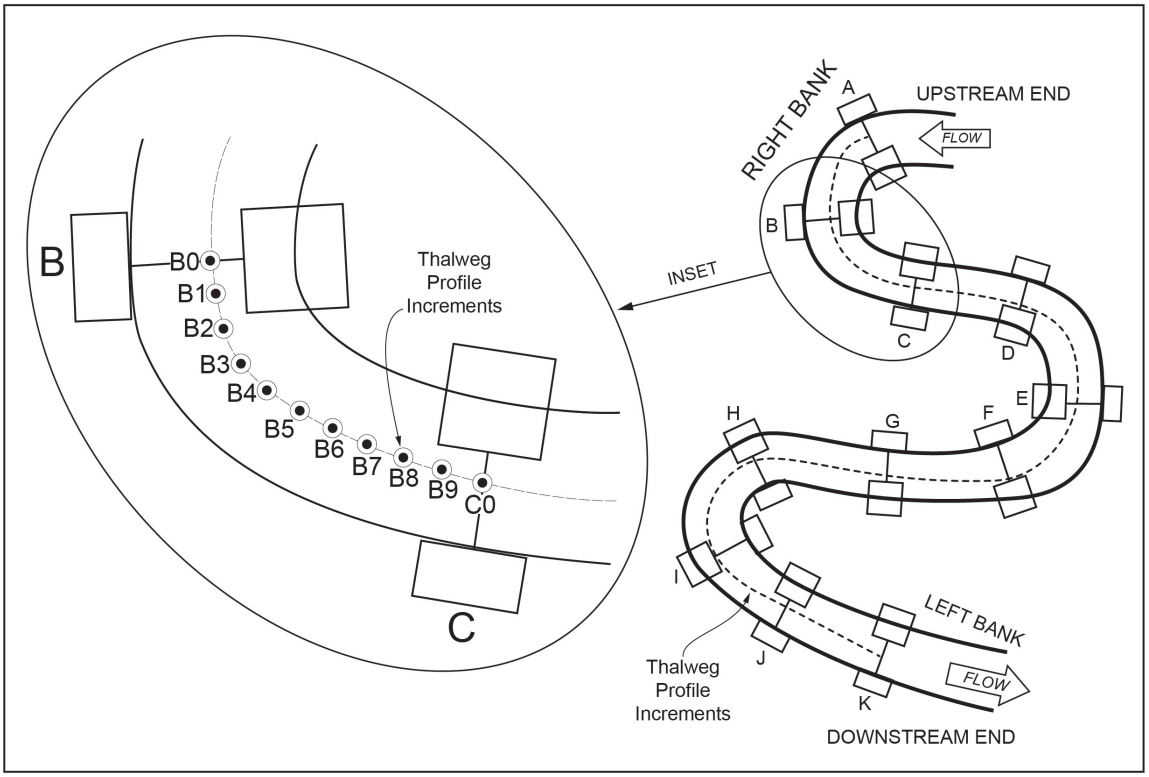


Figure 8.1 Littoral Riparian Plots for Characterizing Riparian Vegetation, human influences, fish cover, littoral substrate, and littoral depths

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 or the water is too rough to safely measure depth, estimate the depth to the best of your ability and record it, with descriptive notes on the comments form.

8.5.2.2 Snags and Substrate Characteristics

The procedure for identifying snags by either dragging the thalweg pole to detect underwater snags or observing snags throughout the wetted channel and substrate characteristics are 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, or other) 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. In addition to noting snags during the thalweg measurements, crewmember will note the presence of snags throughout the wetted channel.

8.5.2.3 Channel Habitat Classification

Classify and record channel habitat types shown in **Table 8.5** and check for the presence of off-channel and backwater habitat at a spatial resolution of about 0.4 channel-width increments. 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 the channel habitat type as dry channel (DR) in places where no water is present in the channel.

Table 8.4 Procedure: Thalweg Profile

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 spacing; thalweg depth, snags, off-channel habitats and other downstream longitudinal profile observations are recorded at intervals of 0.4 channel-widths.
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 at which 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, evenly-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.

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 upstream end 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 in the thalweg, plus the dominant substrate type sensed by dragging the rod along the bottom. Additionally, record the presence of snags outside of the thalweg, within the wetted channel.

d) Fill in the bubble for appropriate "SUBSTRATE" type and record the presence/absence of "SNAGS" (filled "Y" bubbles indicate presence, unfilled bubbles indicate absence). If snags are encountered between stations, mark snags as present on the line for the next station assessed.

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").

Reviewed by (initial): _____

NRSA 2018/19 PHAB: THALWEG PROFILE - NONWADEABLE ONLY

Site ID: _____ Date: ___ / ___ / _____

TRANSECT: O A-B O B-C O C-D O D-E O E-F O F-G O G-H O H-I O I-J O 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 (Flag and write comment below)	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: O ft O m SONAR XX.X	DEPTH (Either) UNITS: O ft O 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	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
1	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
2	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
3	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
4	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
5	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
6	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
7	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
8	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
9	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
10	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	
11	O			O BH O BL O CB O GR O SA O FN O OT	O PO O GL O RI O RA O CA O FA O DR	O	

Flag	Comments

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

03/28/2018 NRSA18 Phab Thalweg Profile - Nonwadeable 8425487570

Figure 8.2 Thalweg Profile Form

Table 8.5 Channel Unit Categories Used on Thalweg Form

Class (Code)[a]	Description
Pool (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.	

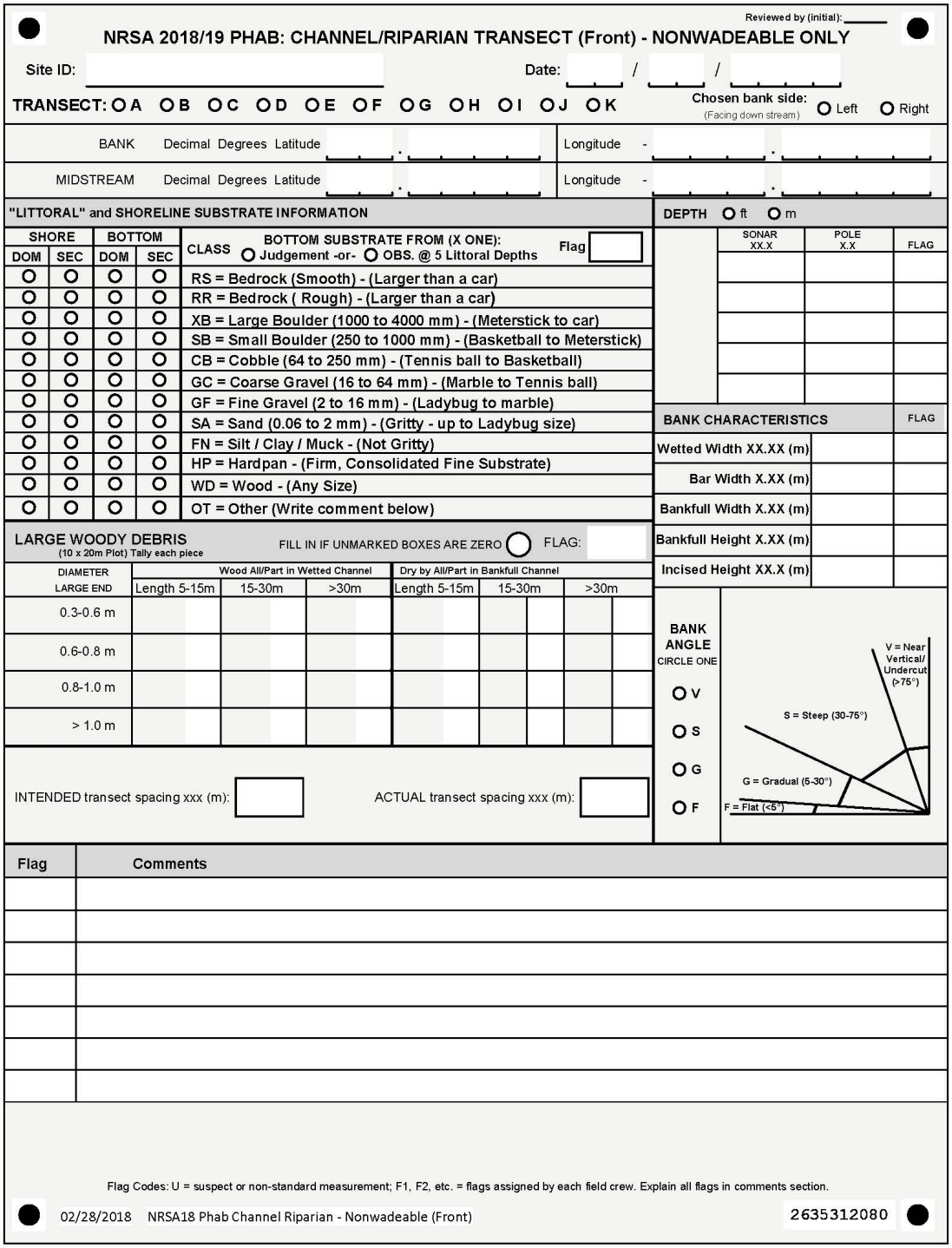
Mid-channel bars, islands, and side channels within a thalweg profile require some guidance. Mid-channel bars are defined as channel features below the bankfull flow level that are dry during baseflow conditions (**Section 8.6.3** defines bankfull channel). Islands are channel features that are dry even 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 that 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 2018/19 PHAB: CHANNEL/RIPARIAN TRANSECT (Front) - NONWADEABLE ONLY

Reviewed by (initial): ____

Site ID: _____ Date: ___ / ___ / _____

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

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

BANK Decimal Degrees Latitude ___.____ Longitude - ___.____

MIDSTREAM Decimal Degrees Latitude ___.____ Longitude - ___.____

"LITTORAL" and SHORELINE SUBSTRATE INFORMATION

SHORE DOM	SHORE SEC	BOTTOM DOM	BOTTOM SEC	CLASS — BOTTOM SUBSTRATE FROM (X ONE): O Judgement -or- O OBS. @ 5 Littoral Depths — Flag
O	O	O	O	RS = Bedrock (Smooth) - (Larger than a car)
O	O	O	O	RR = Bedrock (Rough) - (Larger than a car)
O	O	O	O	XB = Large Boulder (1000 to 4000 mm) - (Meterstick to car)
O	O	O	O	SB = Small Boulder (250 to 1000 mm) - (Basketball to Meterstick)
O	O	O	O	CB = Cobble (64 to 250 mm) - (Tennis ball to Basketball)
O	O	O	O	GC = Coarse Gravel (16 to 64 mm) - (Marble to Tennis ball)
O	O	O	O	GF = Fine Gravel (2 to 16 mm) - (Ladybug to marble)
O	O	O	O	SA = Sand (0.06 to 2 mm) - (Gritty - up to Ladybug size)
O	O	O	O	FN = Silt / Clay / Muck - (Not Gritty)
O	O	O	O	HP = Hardpan - (Firm, Consolidated Fine Substrate)
O	O	O	O	WD = Wood - (Any Size)
O	O	O	O	OT = Other (Write comment below)

DEPTH O ft O m

SONAR XX.X	POLE X.X	FLAG

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

LARGE WOODY DEBRIS (10 x 20m Plot) Tally each piece — FILL IN IF UNMARKED BOXES ARE ZERO O 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: O V O S O G O F

V = Near Vertical/ Undercut (>75°)
S = Steep (30-75°)
G = Gradual (5-30°)
F = Flat (<5°)

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

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.

02/28/2018 NRSA18 Phab Channel Riparian - Nonwadeable (Front) 2635312080

Figure 8.3 Channel/Riparian Transect Form (front)

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**.

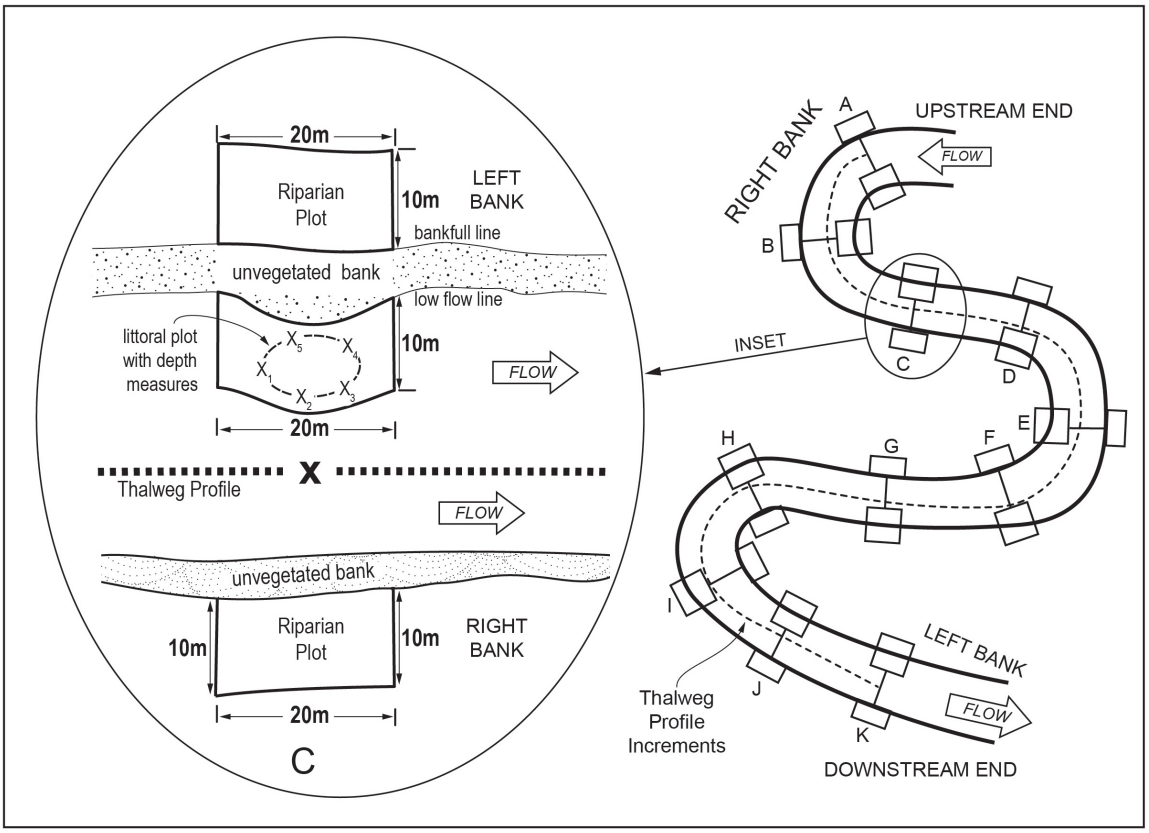


Figure 8.4 Riparian Zone and Instream Fish Cover Plots for a River Cross-Section Transect

Table 8.6 Procedure: Channel Margin Depth and Substrate

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. 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	Yard/meter stick to Car size
SB	Small Boulders	>250 to 1000	Basketball to yard/meter stick size
CB	Cobbles	>64 to 250	Tennis ball to basketball size
GC	Gravel (Coarse)	>16 to 64	Marble to tennis ball size
GF	Gravel (Fine)	> 2 to 16	Ladybug to marble size
SA	Sand	>0.06 to 2	Smaller than ladybug; gritty between fingers
FN	Fines	<0.06	Silt Clay Muck (not gritty between fingers)
HP	Hardpan	>4000	Firm, consolidated fine substrate
WD	Wood	Regardless of Size	Wood & other organic particles
OT	Other	Regardless of Size	Concrete, metal, tires, car bodies, etc. (describe in comments)

3. 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).
4. 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 a small end diameter of ≥30 cm (1 ft) and a length of ≥5 m (15 ft). This includes any portion of woody material that meets those minimum size categories (e.g., include any LWD piece with at least 5 meters of its length having a diameter of 30 cm or greater). 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 wholly or partially 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 >0.3 m (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: 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 (10 m up, 10 m down from the transect). They are positioned along the chosen bank and extend from the shore 10 m 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 (**any portion with a diameter of at least 30 cm [1 ft.] and a length of at least 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 5 m to <15 m, 15 m to <30 m, or >30 m.
   - If the piece is not cylindrical, visually estimate what the diameter would be for a piece of wood with circular cross-section that would have the same volume.
   - When estimating length, include only the part of the LWD piece that has a diameter >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 <15 m, 15 m to <30 m, or >30 m.
6. When entering data via the NARS app, numbers can be types directly into tally boxes, or the "+" and "-" buttons can be used to incrementally change the number. When using paper forms, 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 in the gray box, write the total number of pieces for each diameter × length class in the white box to the right of the gray 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. 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: 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. 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 *bankfull height 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.
4. 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.
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. (i.e., the first valley depositional surface is the active floodplain). However, if the channel is incised greatly, the bankfull level will be below the level of the first terrace of the valley floodplain, making "Bankfull 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 8.11** for a discussion of constraint concepts and assessment procedures.

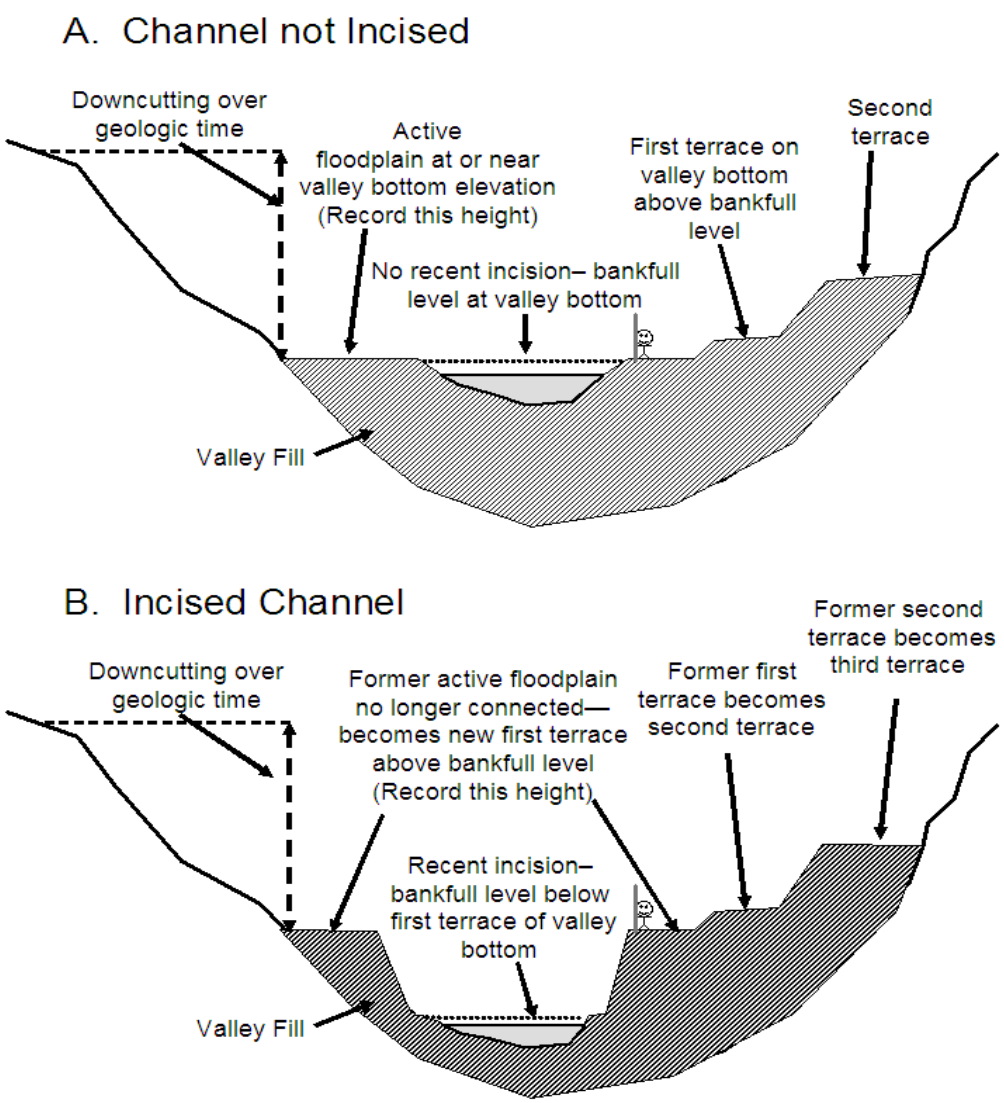


Figure 8.5 Schematic Showing Bankfull Channel and Incision for Channels
(A) not recently incised, and (B) recently incised into valley bottom. Note level of bankfull stage relative to elevation of first terrace on valley bottom (stick figure included for scale)

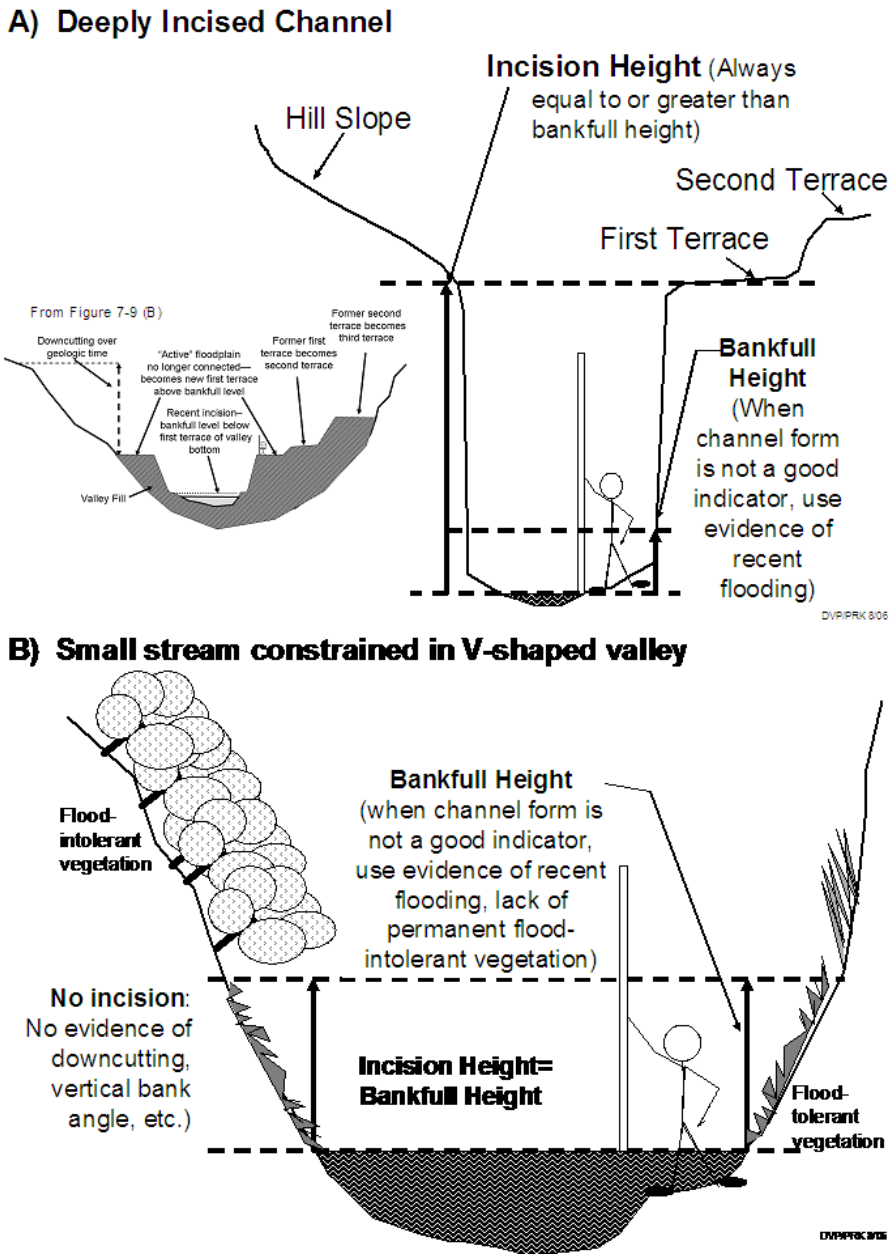
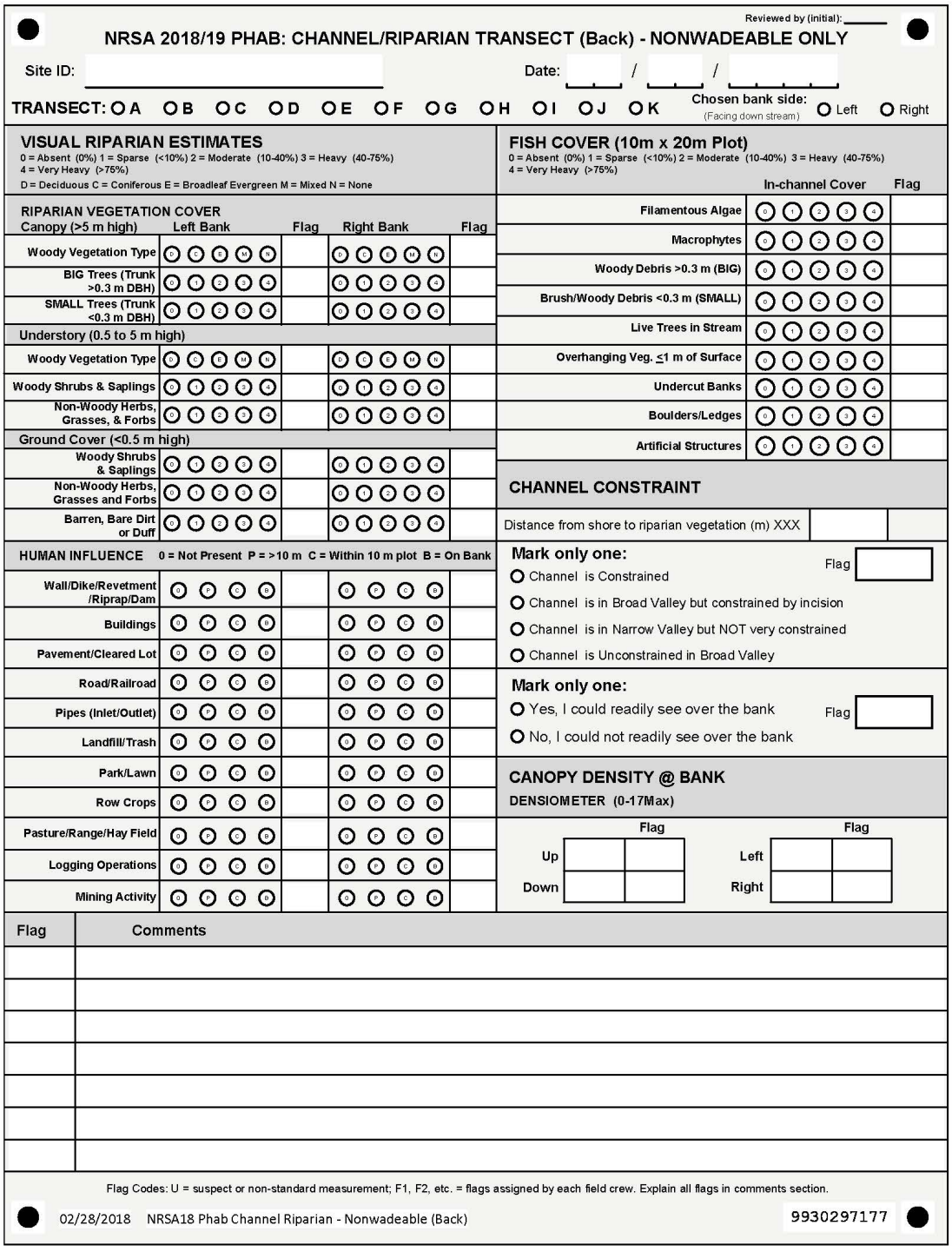


Figure 8.6 Determining Bankfull and Incision Heights for
(A) Deeply Incised Channels, and (B) Streams in Deep V Shaped Valleys (Stick figure included for scale)



Reviewed by (initial): _____

NRSA 2018/19 PHAB: CHANNEL/RIPARIAN TRANSECT (Back) - NONWADEABLE ONLY

Site ID: _____ Date: ___ / ___ / _____

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

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

RIPARIAN VEGETATION COVER Canopy (>5 m high)	Left Bank	Flag	Right Bank	Flag
Woody Vegetation Type	O D C E M N		O D C M N	
BIG Trees (Trunk >0.3 m DBH)	O 1 2 3 4		O 1 2 3 4	
SMALL Trees (Trunk <0.3 m DBH)	O 1 2 3 4		O 1 2 3 4	
Understory (0.5 to 5 m high)				
Woody Vegetation Type	O D C E M N		O D C M N	
Woody Shrubs & Saplings	O 1 2 3 4		O 1 2 3 4	
Non-Woody Herbs, Grasses, & Forbs	O 1 2 3 4		O 1 2 3 4	
Ground Cover (<0.5 m high)				
Woody Shrubs & Saplings	O 1 2 3 4		O 1 2 3 4	
Non-Woody Herbs, Grasses and Forbs	O 1 2 3 4		O 1 2 3 4	
Barren, Bare Dirt or Duff	O 1 2 3 4		O 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	O P C O		O C O B	
Buildings	O P C O		O C O B	
Pavement/Cleared Lot	O P C O		O C O B	
Road/Railroad	O P C O		O C O B	
Pipes (Inlet/Outlet)	O P C O		O C O B	
Landfill/Trash	O P C O		O C O B	
Park/Lawn	O P C O		O C O B	
Row Crops	O P C O		O C O B	
Pasture/Range/Hay Field	O P C O		O C O B	
Logging Operations	O P C O		O C O B	
Mining Activity	O P C O		O C O B	

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

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

CHANNEL CONSTRAINT

Distance from shore to riparian vegetation (m) XXX _____

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

Mark only one: Flag _____
- O Yes, I could readily see over the bank
- O 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.

02/28/2018 NRSA18 Phab Channel Riparian - Nonwadeable (Back) 9930297177

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 10 m X 20 m 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.

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*.

Estimate the areal cover separately in each of the three vegetation layers. Note that the areal cover can be thought of as the amount of shadow cast by a particular layer alone when the sun is directly overhead. *The maximum cover in each layer is 100%, so the sum of the areal covers for the combined three layers could add up to 300%.* The four areal cover classes are *Absent*, *Sparse* (<10%), *Moderate* (10 to 40%), *Heavy* (40 to 75%), and *Very Heavy* (>75%). These cover classes and their corresponding codes are shown on the field data form (**Figure 8.7).** When rating vegetation cover types for a single vegetation layer, mixtures of two or more subdominant classes might all be given *Sparse* (*1*), *Moderate* (*2*), or *Heavy* (*3*) rankings. One *Very Heavy* cover class with no clear subdominant class might be rated *4* with all the remaining classes rated as either *Moderate* (*2*), *Sparse* (*1*) or *Absent* (*0*). Note that within a given vegetation layer, two cover types with 40-75% cover can both be rated *3*, but no more than one cover type could receive a rating of *4*..

Table 8.9 Procedure: 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 canopy layer contains no vegetation or 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 understory layer contains no vegetation or 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.

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 10 m x 20 m 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 8.7.1**).

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 8.6.2**]). 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. Overhanging vegetation includes tree branches, brush, twigs, or other small debris that is not in the water but is close to the stream (within 1 m of the surface) and provides potential cover. For ephemeral channels, estimate the proportional cover of these trees that would be 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: 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 **Table 8.11.** 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 (B), contained within the 10 m $\times$ 20 m riparian plot but not in the stream or on the bank (C), present outside of the riparian plot (P), and absent (0). 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., present in riparian plot "C" takes precedence over outside of riparian plot "P").

You may mark "P" more than once for the same human influence observed outside of more than one riparian observation plot (e.g., at both Transects D and E). **The rule is that you count human disturbance items as often as you see them, BUT NOT IF you have to site through an another transect or its 10x20 meter riparian plot.**

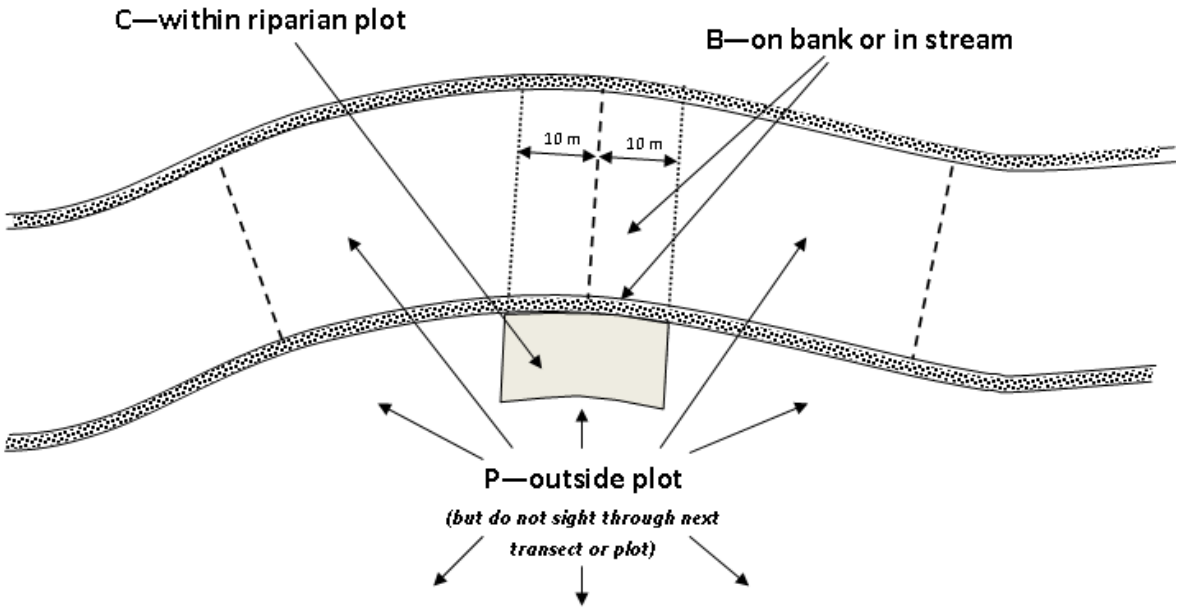


Figure 8.8 Proximity Classes for Human Influences in Non-Wadeable Rivers

Table 8.11 Procedure: 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 10 m 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 (*Contained*) — 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 on the near bank 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). 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**.

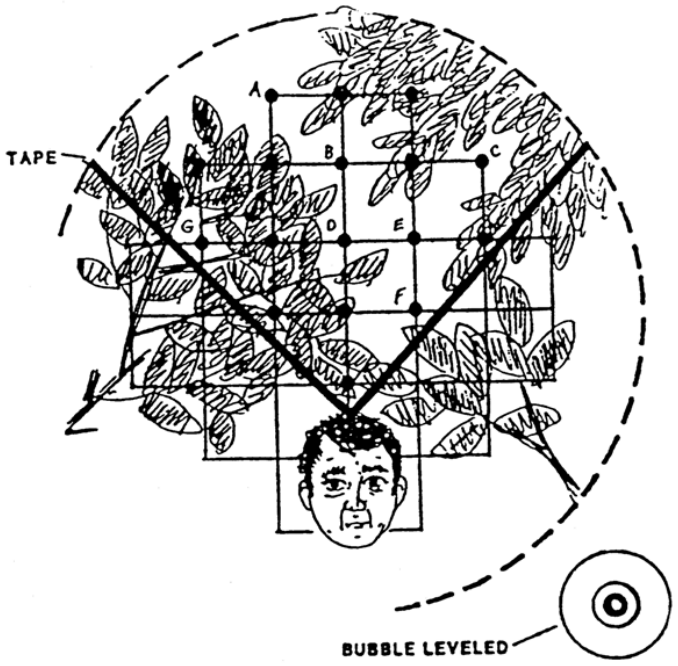


Figure 8.9 Schematic of Modified Convex Spherical Canopy Densiometer
(From Mulvey et al., 1992). In this example, 10 of the 17 intersections show canopy cover, giving a densiometer reading of 10. Note proper positioning with the bubble leveled and face reflected at the apex of the "V."

Table 8.12 Procedure: 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 Procedure: 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 2018/19 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)

- 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%) ------->

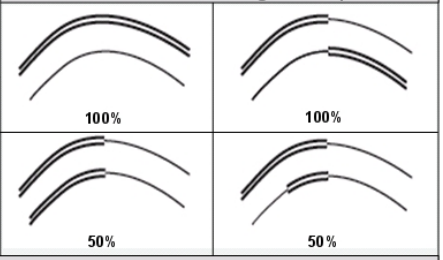
Bankfull width: ____ (m)

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

03/28/2018 NRSA18 Channel Constraint 7734623125

Figure 8.10 Channel Constraint Form

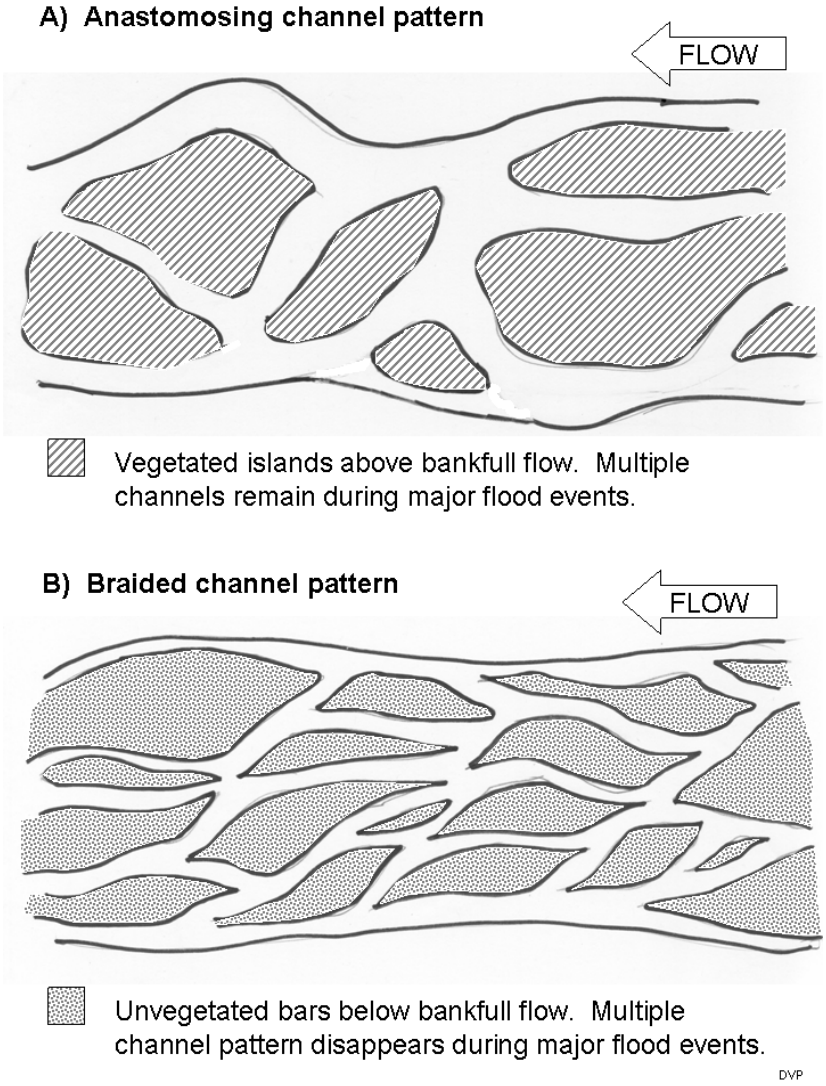


Figure 8.11 Types of Multiple Channel Patterns

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 2018/19 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	

10/04/2017 NRSA18 Torrent Evidence 5543621376

Figure 8.12 Torrent Evidence Form

8.12 Elevation at Transect K

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

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 six hours of collection**. Use a pre-sterilized, 250 ml bottle and collect the sample approximately 1 m off the bank at about 0.3 meter (12 inches) below the water. Following collection, place the sample in a cooler and maintain on ice prior to filtration of two 50 mL volumes. Again, samples must be filtered and frozen on dry ice within six hours of collection. In addition to collecting the sample, look for signs of disturbance throughout the reach that would contribute to the presence of fecal contamination to the waterbody. Record these disturbances on the Site Assessment Form (**Figure 13.3**).

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: Fecal Indicator Sampling (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 Pencils (for data forms)

9.3 Sampling Procedure

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

Table 9.2 Procedure: Fecal Indicator (Enterococci) Sample Collection (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 0.3 m 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 (0.3 m) 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 0.3 m along the 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 (down to the 250 mL mark on the bottle).
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 six hours of collection.

9.4 Sample Processing in the Field

You will need to process two separate filters for the *Enterococci* sample. All the filters required for an individual site should be sealed in plastic bags until use to avoid external sources of contamination. Please refer to **Section 13.3** for information regarding processing the *Enterococci* samples.

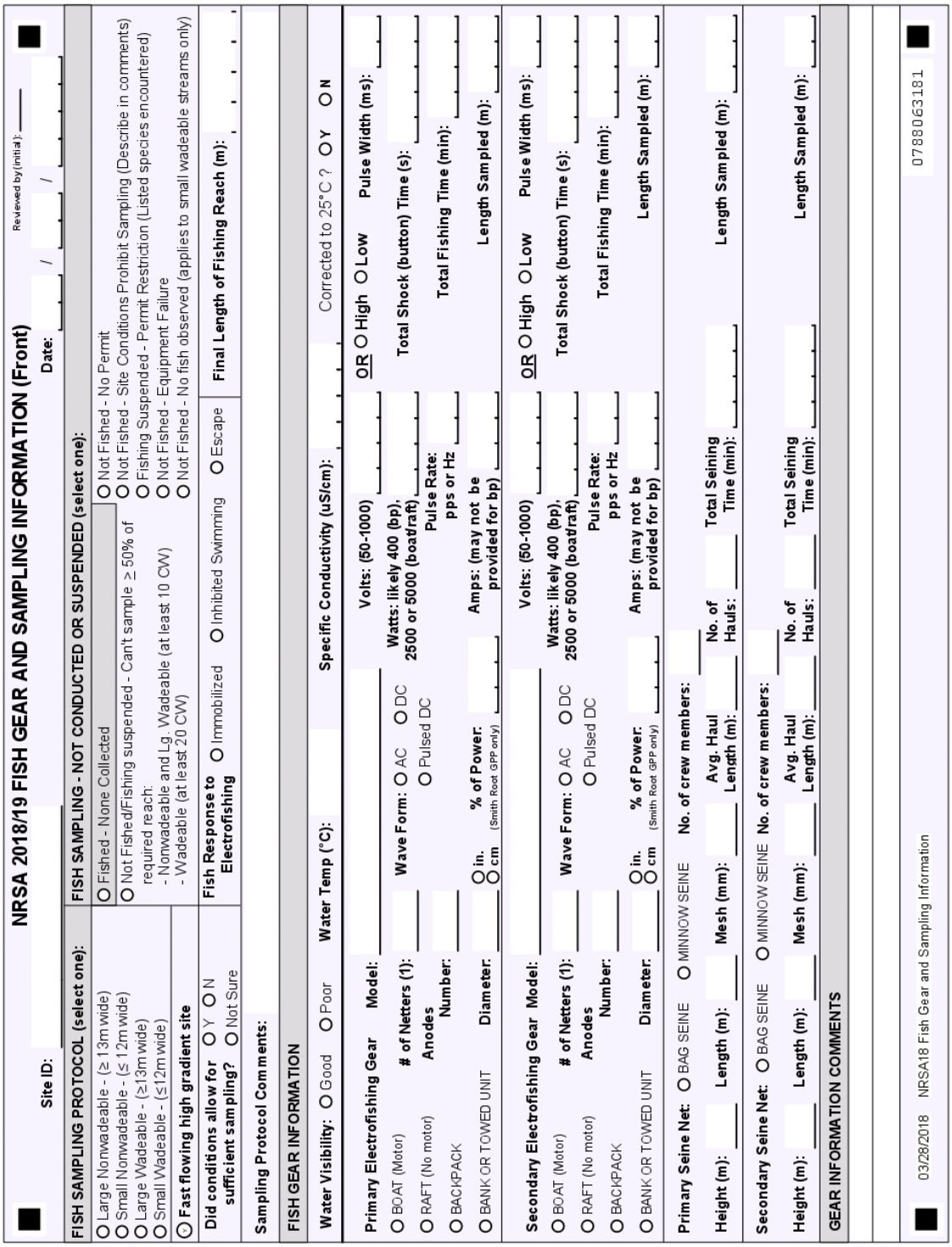
10 FISH ASSEMBLAGE

10.1 Summary of Method

The fish sampling method is designed to provide a representative sample of the fish community, collecting all but the rarest fish taxa inhabiting the site. It is intended to accurately represent species richness, species guilds, relative abundance, size, and presence of anomalies. The intended uses of the fish assemblage data are to calculate predictive models of multimetric indicators (MMIs; similar to an Index of Biotic Integrity [IBI]; Pont et al. 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 fish sampling reach (which will be between 20 and 40 times the mean channel width [CW] recorded on the stream verification form). The fish sampling reach is less than or equal to the sampling reach established for the site (See **Section 3.2**). Use secondary fish collection methods in habitat that cannot be adequately sampled by boat (or raft). Secondary methods may include backpack or some type of towed (e.g., a towed barge, small watercraft, or float tube) electrofishing unit, using your boat (or raft) as a towed barge, or seining as a last option (only if conductivity is too high or too low for electrofishing). Conduct sampling in a downstream direction, allocating effort (button time) within *subreaches* (areas between the cross-section transects). If you have not collected 500 individuals at the end of the minimum fish sampling reach (20 CW; 5 subreaches), sample additional subreaches in their entirety until you obtain at least 500 individuals, or sample the entire sampling reach (40 CW; 10 subreaches). 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 2013/14 effort. There is now a place on the fish gear form for the crew to note the sufficiency of the sampling effort and the general response of fish to electrofishing. The primary and secondary electrofishing sections of the fish gear form now allow for users of certain types of electrofishing systems to record voltage as High or Low, and to record the percent of power. There is now a place on the collection form for a crew to note that they did not evaluate the native/introduced status of any fish collected. This clarifies that blank values for the introduced fish field on the collection form are truly missing, rather than being presumed to be native.



NRSA 2018/19 FISH GEAR AND SAMPLING INFORMATION (Front)

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

FISH SAMPLING PROTOCOL (select one):

- O Large Nonwadeable - (≥ 13m wide)
- O Small Nonwadeable - (≤ 12m wide)
- O Large Wadeable - (≥13m wide)
- O Small Wadeable - (≤12m wide)
- O Fast flowing high gradient site

Did conditions allow for sufficient sampling? O Y O N O Not Sure

FISH SAMPLING - NOT CONDUCTED OR SUSPENDED (select one):

- O Fished - None Collected
- O Not Fished/Fishing suspended - Can't sample ≥ 50% of required reach:
  - Nonwadeable and Lg. Wadeable (at least 10 CW)
  - Wadeable (at least 20 CW)

Fish Response to Electrofishing: O Immobilized O Inhibited Swimming O Escape

- O Not Fished - No Permit
- O Not Fished - Site Conditions Prohibit Sampling (Describe in comments)
- O Fishing Suspended - Permit Restriction (Listed species encountered)
- O Not Fished - Equipment Failure
- O Not Fished - No fish observed (applies to small wadeable streams only)

Final Length of Fishing Reach (m): _____

Sampling Protocol Comments:

FISH GEAR INFORMATION

Water Visibility: O Good O Poor Water Temp (°C): Specific Conductivity (uS/cm): _____ Corrected to 25°C ? O Y O N

Primary Electrofishing Gear Model: _____

O BOAT (Motor) # of Netters (1): _____ Wave Form: O AC O DC O Pulsed DC Volts: (50-1000) _____ OR O High O Low Pulse Width (ms): _____

O RAFT (No motor) Anodes Number: _____ Watts: likely 400 (bp), 2500 or 5000 (boat/raft) _____ Pulse Rate: pps or Hz _____ Total Shock (button) Time (s): _____

O BACKPACK Total Fishing Time (min): _____

O BANK OR TOWED UNIT Diameter: O in. O cm % of Power: (Smith Root GPP only) _____ Amps: (may not be provided for bp) _____ Length Sampled (m): _____

Secondary Electrofishing Gear Model: _____

O BOAT (Motor) # of Netters (1): _____ Wave Form: O AC O DC O Pulsed DC Volts: (50-1000) _____ OR O High O Low Pulse Width (ms): _____

O RAFT (No motor) Anodes Number: _____ Watts: likely 400 (bp), 2500 or 5000 (boat/raft) _____ Pulse Rate: pps or Hz _____ Total Shock (button) Time (s): _____

O BACKPACK Total Fishing Time (min): _____

O BANK OR TOWED UNIT Diameter: O in. O cm % of Power: (Smith Root GPP only) _____ Amps: (may not be provided for bp) _____ Length Sampled (m): _____

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

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

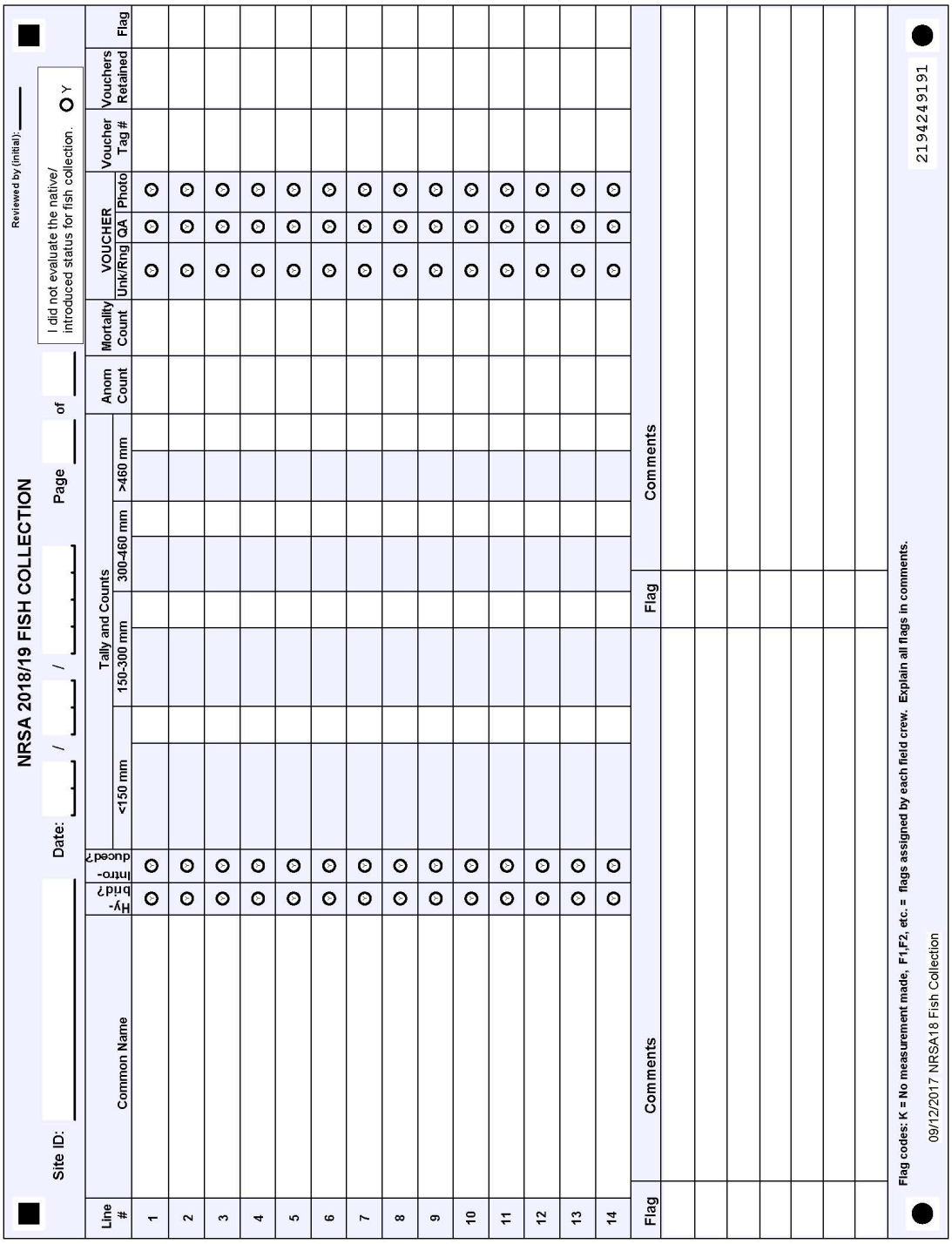
Secondary Seine Net: O BAG SEINE O MINNOW SEINE No. of crew members: _____

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

GEAR INFORMATION COMMENTS

03/28/2018 NRSA18 Fish Gear and Sampling Information 0788063181

Figure 10.1 Fish Gear and Sampling Information (front)



NRSA 2018/19 FISH COLLECTION

Site ID: _____ Date: ___/___/___ Page ___ of ___

Reviewed by (initial): ____

I did not evaluate the native/introduced status for fish collection. ○ Y

Line #	Common Name	Hy-brid?	Intro-duced?	Tally and Counts <150 mm	150-300 mm	300-460 mm	>460 mm	Anom Count	Mortality Count	VOUCHER Unk/Rng	VOUCHER QA	VOUCHER Photo	Voucher Tag #	Vouchers Retained	Flag
1		○	○							○	○	○			
2		○	○							○	○	○			
3		○	○							○	○	○			
4		○	○							○	○	○			
5		○	○							○	○	○			
6		○	○							○	○	○			
7		○	○							○	○	○			
8		○	○							○	○	○			
9		○	○							○	○	○			
10		○	○							○	○	○			
11		○	○							○	○	○			
12		○	○							○	○	○			
13		○	○							○	○	○			
14		○	○							○	○	○			

Flag	Comments	Flag	Comments

Flag codes: K = No measurement made, F1,F2, etc. = flags assigned by each field crew. Explain all flags in comments.

09/12/2017 NRSA18 Fish Collection

21942491 91

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 (Non-Wadeable Sites)

For collecting fish	Boat, motor, and trailer (and necessary safety equipment) Gasoline and oil (if using a 2 cycle motor or generator) Boat electrofishing equipment Pulsator Control Box Foot Pedal Anode Droppers Generator Linesman's Gloves Hearing Protection Towed electrofishing electrofishing equipment (e.g., towed barge, or mounted on a small watercraft or float tube), if needed for subreaches too shallow to sample from a boat or raft used as a towed unit. 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 Buckets
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 sampling reach defined for the site (See **Section 3.2**) and proceed downstream. See **Appendix E** for example starting settings for electrofishing using backpack, towed barge, and

boat (Temple 2018). These are only suggestions; the final determination of settings is decided by the lead fish taxonomist.

The reach length sampled for fish varies based on the mean width of the river used to establish the sampling reach (which has been rounded to the nearest meter as per the directions in **Section 3.2**) and on the number of individuals collected (**Figure 10.3**). In very small rivers (mean channel width [CW] recorded on the stream verification form is ≤ 12 meters), conduct sampling in the same fashion as for small wadeable streams, sampling the entire sampling reach (150 m minimum or 40 CW; 10 subreaches) and moving the boat (or raft) within each subreach to sample both shorelines as well as the mid-channel.

In large rivers (mean channel width [CW] recorded on the stream verification form ≥ 13 meters) the minimum length for fish sampling is 20 CW (5 subreaches). If a minimum of 500 fish are not collected after sampling the minimum fishing reach, sample additional subreaches in their entirety until 500 fish are collected or all 10 subreaches have been sampled. **Table 10.2** summarizes the fishing protocols for each of two sizes of non-wadeable rivers.

For rivers where the CW is ≥ 13 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 or too low). 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 to minimize mortality and stress to fish. You can use multiple lines per species on the fish collection form if necessary (e.g., you collect a large number of individuals and need additional space for tallying.

If seining is used, record all fish collected with seining protocols on separate lines of the fish collection form.

At the end of the minimum 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 and in their entirety) until at least 500 individuals are captured. 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). Stop sampling when you reach Transect K (the end of the sampling reach), regardless of the number of individuals collected.

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

10.3.1 Irruptive Species

For the purposes of NRSA, the term *irruptive species* will be used to describe fish species which are found in locally abundant "patches" in one or two small places within the sampling reach. These are distinct from dominant species which are in abundance throughout most of the reach. As such, irruptive species may artificially skew necessary effort to reach 500 individuals; and, if

included the overall assemblage counts, may artificially skew the calculations of relative abundance of fish species in the reach. To avoid the impact of irruptive species, move quickly through large isolated schools of a single species (e.g., shad, certain shiners, etc.). Also, when tallying total fish at the end of the designated fish sampling reach, calculate the percentage of irruptive species to total individuals captured. If any single irruptive species comprises greater than or equal to 50% of the total sample, continue fishing one or more additional subreaches until the percentage of the irruptive species decreases to less than 50%.

Table 10.2 Summary of Non-wadeable Fishing Protocols

<u>Small Non-Wadeable Rivers (mean channel width [CW] from stream verification form ≤ 12 meters)</u>

- Fish Sampling reach will be between 150 and 480 meters
- Subreaches will be between 15 and 48 meters each
  - ✓ Sample all 10 subreaches in their entirety from bank to bank starting at Transect A
    - If islands are encountered, crews should still fish bank to bank, including both sides of the island.
  - ✓ Total button time will range from 500-700 seconds per subreach
    - You do not have to expend equal button time among the 10 subreaches—you can devote more button time to subreaches with more complex habitat.
  - ✓ No minimum fish number

<u>Large Non-Wadeable Rivers (CW ≥13 meters)</u>

- Fish Sampling reach will be between 260 and 4000 meters
- Subreaches will be 1/10 of the sampling reach
- Minimum fishing length = 5 subreaches
  - ✓ Fish each subreach along bank in pairs of subreaches starting at the random PHab bank at Transect A
    - If islands are encountered, crews will only fish the edge of the island that occurs along the main channel.
  - ✓ Button time is roughly 700 seconds per subreach
    - Depending upon the habitat complexity, you can vary the distance actively fished to allocate the available button time throughout the subreach.
  - ✓ Minimum fish number is 500 unless all 10 subreaches have been fished.
    - ➢ After fishing 5 subreaches, if 500 fish have not been collected, add subreaches one at a time (but fish them in their entirety) until 500 fish are collected or all 10 subreaches (40 CW) have been fished.

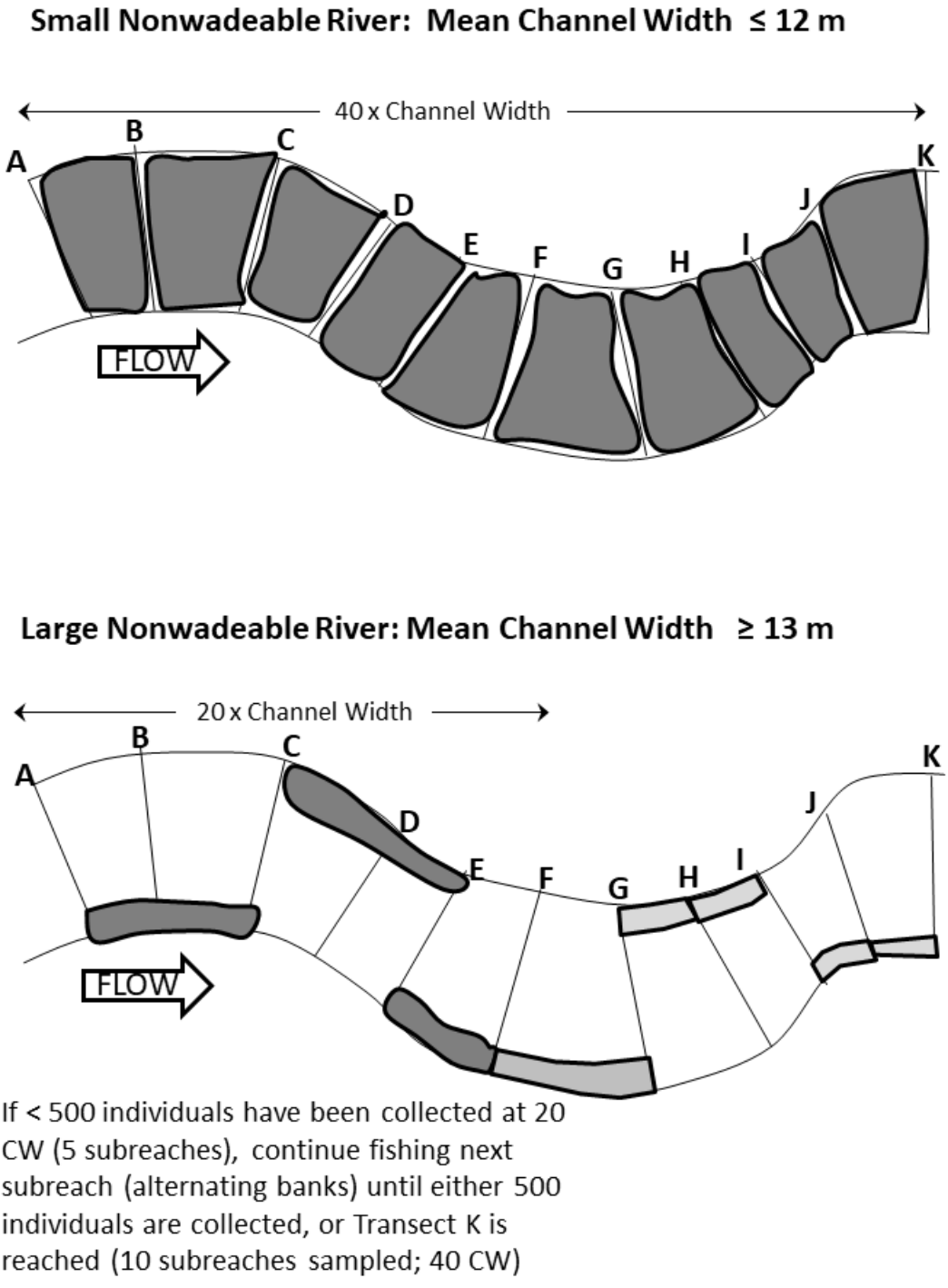


Figure 10.3 Reach Layouts for Fish Sampling at Non-Wadeable Sites
Dark shaded areas indicate the minimum length of the fish sampling reach. Light shaded areas are sampled as needed to meet the required 500 individuals.

Table 10.3 Procedure: Electrofishing (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 for fish.
   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, ambient 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) In a non-wadeable river, you should always attempt to sample; never mark *Not Fished – No Fish Observed.*
   f) If you cannot sample for any other reason, note the reason in the *Sampling Protocol Comments* field.

3) If you can begin to sample, mark *Nonwadeable* in the *Fish Sampling Protocol* section. For safety, everyone must wear PFDs, 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 sampling reach for the river (rounded to the nearest meter and recorded on the stream verification form).
   a) If the mean channel width (CW) from the stream verification form is ≤12 m, the fish sampling reach length is 150 m or 40 CW (10 subreaches).
   b) If the CW is ≥13 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 (note whether the conductivity value is corrected to 25 °C).

6) Mark either *Boat* or *Raft* as the *Primary Electrofishing Gear*. Boats use outboard motors and can travel upstream if needed, 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. Diameter refers to the diameter of a hoop or sphere or the distance between the outer edge of the shape formed by an array of droppers (which is usually round).
   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. See Tables in **Appendix E** for example starting settings. 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.

i) If the electrofishing system only lets you select High and Low voltage (rather than a specific voltage), record the setting used on the fish gear form.

ii) If the electrofishing system only lets you adjust the percent of power, record the value on the fish gear form.

iii) If your conductivity meter cannot measure ambient conductivity, you can "uncorrect" specific conductance at 25 °C to ambient conductivity using the following equation:

(1) Ambient conductivity=Specific conductance x (1+([water temp-25 °C] x 0.02)).

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 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 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 ≥ 13m 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 ~500 -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. You do not have to expend equal time among the subreaches.

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

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

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 or too low 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 or as often as necessary to reduce mortality. Release fish in a location that eliminates the likelihood of recapture.

12) Repeat Steps 7 through 11 until you have sampled the minimum reach length determined in Step 4.

13) If you have sampled the required minimum fishing reach length (20 CW; 5 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 (40 CW). 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; 40 CW, 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) sampled with the primary gear type in the *Primary Electrofishing Gear* section of the fish gear form. a) If you suspend fish sampling before completing the minimum distance (20 CW; 5 subreaches), record the length that was sampled in the Primary Electrofishing Gear section, and mark the reason for the suspension in the *Fish sampling – Not Conducted or Suspended* section of the fish gear form.
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 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 length of the total fish sampling reach sampled by the secondary electrofishing method (if applicable). 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 total fish sampling reach sampled for each type of seine in the *Primary* and *Secondary Seine Net* sections of the fish gear form.
16) Indicate whether conditions allowed for sufficient sampling on the fish gear form (*Yes, No, Not Sure*). If you marked *No* or *Not Sure*, explain why in the Sampling Protocol Comments field.
17) Note the general response behavior of fish to your final electrofishing settings on the fish gear form (source: https://nctc.fws.gov/courses/CSP/CSP2C01/resources/1_EF Effects (Chapter 8).ppt) as either: a) *Immobilized* (no swimming motions due to electrical field). Includes narcosis (slack muscles) and tetany (rigid muscles). b) *Inhibited Swimming* (unbalanced swimming induced by the electrical field). Includes taxis (movement, usually towards the anode), pseudo-taxis (movement, but fish are unconscious and belly-up), and oscillotaxis (movement without orientation). c) *Escape* (upright avoidance swimming).
18) Record the total length of the river that was sampled for fish on the fish gear form. This total length should coincide with the end of a subreach.

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 your boat as a wadeable fishing unit (by attaching an anode pole to the box) if your electrofishing unit is larger than 5000 V (e.g., Generator Powered Pulsator [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 length of the total fish sampling reach that was sampled with

the secondary method. The total button time for boat and wadeable electrofishing combined should be ~700 seconds per subreach. See **Appendix E** for example starting settings for electrofishing using backpack, towed barge, and boat (Temple 2018). These are only suggestions; the final determination of settings is decided by the lead fish taxonomist.

Table 10.4 Procedure: Secondary Electrofishing Methods for Wadeable Areas (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 your boat as a wadeable fishing unit (by attaching an anode pole to the box) if your electrofishing unit is larger than 5000 V (e.g., GPP 5.0).**

2) Mark either *Backpack* or *Bank or Towed Unit* in the *Secondary Electrofishing Gear* section of the fish gear form.
   a) Only one netter and one anode should be used. Record this along with the anode diameter (mark the units as either inches or centimeters) on the fish gear form.

3) **Operation of Backpack Electrofisher**
   a) **For backpack electrofishing** there may be from 2 to 3 people involved (depending upon the crew size).
   b) Set unit to pulsed DC and mark it in the Wave Form section of the fish gear form.
   c) Select the initial voltage setting based on the **ambient** conductivity of the river.
      i) See Tables in **Appendix E.**
   d) Select the initial pulse rate and width.
      i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec.
      ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz.
   e) Turn the electrofisher on, set the timer, and depress the switch to begin fishing. If fishing success is poor, increase the pulse width first and then the voltage. Increase the pulse rate last to minimize mortality or injury to large fish. If mortalities occur, first decrease pulse rate, then voltage, then pulse width.
   f) Once you have determined the appropriate settings, record them on the fish gear form. Start cleared clocks and resume fishing.
   g) Note: some electrofishers do not meter all the requested settings; provide what you can.
   h) If button time is not metered, estimate it with a stop watch and flag the data.

4) **Operation of Towed Electrofisher**
   a) ***When using a towed electrofishing unit, the minimum crew size for electrofishing is three***.
      i) The operator must remain actively at the control box and navigate the towed electrofishing unit.
      ii) The probe operator will use one probe.
   b) Set unit to pulsed DC and mark it in the Wave Form section of the fish gear form.
   c) Select the initial voltage setting based on the **ambient** conductivity of the river (i.e., not corrected to 25 °C).
      i) See Tables in **Appendix E.**
      ii) If the electrofishing system only lets you select High and Low voltage (rather than a specific voltage), record the setting used on the fish gear form.
   d) Select the initial pulse rate and width.

i) In waters with strong swimming fish (length >200 mm), use a pulse rate of 30 Hz with a pulse width of 2 m/sec. ii) If you expect mostly small fish, use a pulse rate of 60-120 Hz. e) If the electrofishing system only lets you adjust the percent of power, record the value on the fish gear form. f) 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. g) Once you have determined the appropriate settings, record them on the fish gear form. Start cleared clocks and resume fishing. h) Note: some electrofishers do not meter all the requested settings; provide what you can. i) If button time is not metered, estimate it with a stop watch and flag the data.
5) Once the settings on the electrofisher are adjusted properly to sample effectively and minimize injury and mortality, begin sampling 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 (e.g., available cut bank and snag habitats), move the anode wand into cover with the current off, turn the anode on when in the cover, and then remove the wand quickly to draw fish out. c) In fast, shallow water, sweep the anode and fish downstream into a net. d) In stretches with deep pools, fish the margins of the pool as much as possible, being extremely careful not to step or slide into deep water. e) Keep the cathode near the anode if fish catch is low.
6) The netter holds the net 1 to 2 ft from the anode, follows the operator, nets stunned individuals, and places them in a bucket. a) **Use a 6 mm (1/4 inch) mesh dip net to** collect stunned fish. Actively capture stunned fish from the electric field and ***immediately place them into the live well. Devote special attention to net small and benthic fishes*** as well as fishes that may respond differently to the electric current.
7) The total button time for boat and wadeable electrofishing combined should be ~700 seconds per subreach.
8) After you finish sampling the wadeable area, return to Step 11 of **Table 10.2**. a) 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, and the length of the fish sampling reach that was sampled by wading in the ***Secondary Electrofishing Gear section*** of the fish gear form.

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.

Record all fish collected with seining protocols on separate lines on the field collection form from those lines used for fish collected by electrofishing.

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**).

Table 10.5 Procedure: Secondary Seining Methods for Wadeable Areas (Non-Wadeable Rivers)

1) Use secondary 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 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 PFDs 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. Note whether the conductivity value is corrected to 25 °C.
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.
   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.

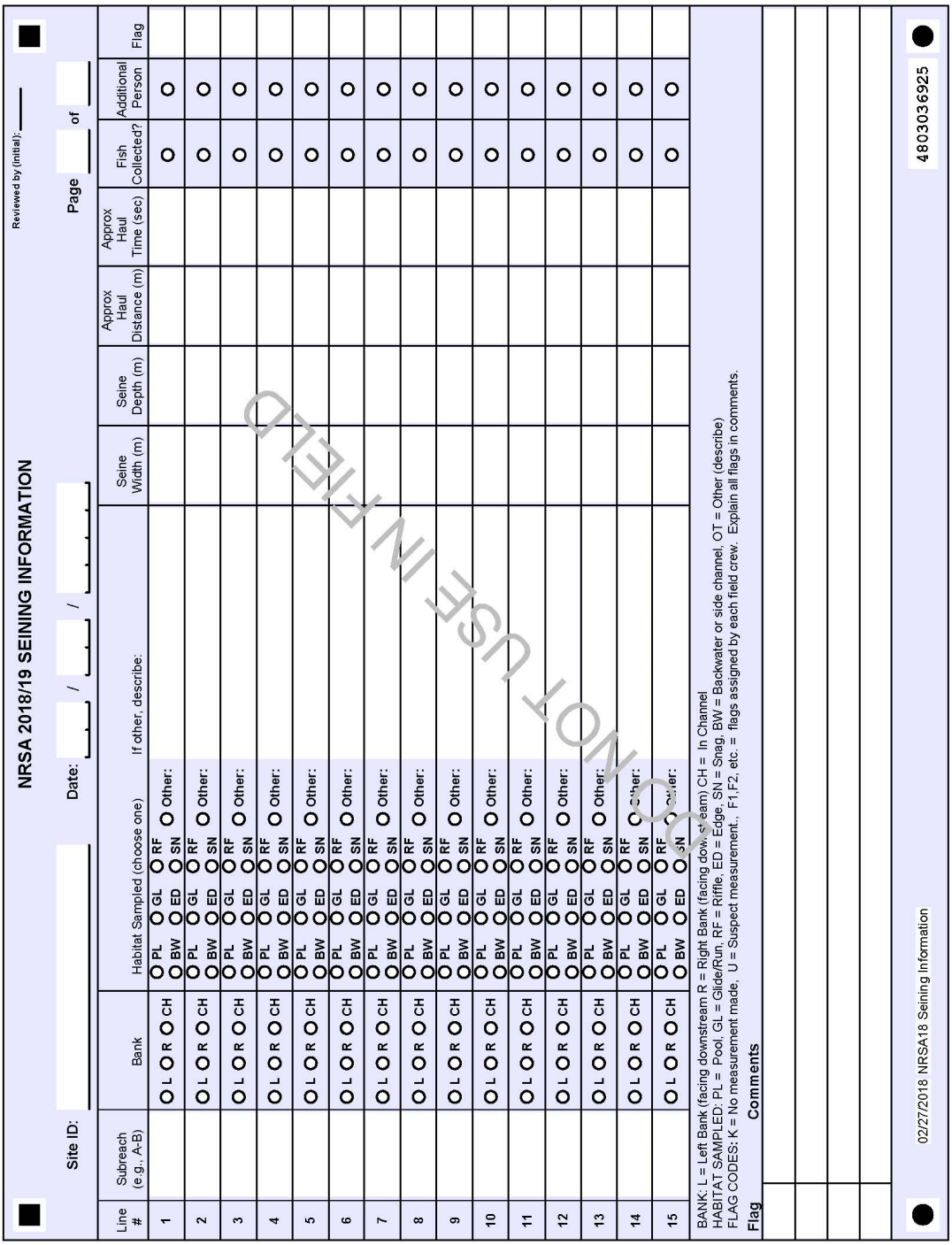
a) Record identifications, tallies, and voucher information on the fish collection form. You may use separate lines on the fish collection form to record species information from separate seine hauls.

i) For each seine haul, record seine characteristics and haul length, habitat, and time on the seining information form (**Figure 10.4**).

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**.

a) 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 total fish sampling reach length sampled for each type of seine. Record the totals in the *Primary* and *Secondary Seine Net s*ection of the fish gear form.



NRSA 2018/19 SEINING INFORMATION

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

Line #	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
1		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
2		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
3		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
4		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
5		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
6		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
7		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
8		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
9		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
10		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
11		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
12		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
13		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
14		O L O R O CH	O PL O GL O RF O BW O ED O SN	O Other:					O	O	
15		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

DO NOT USE IN FIELD

02/27/2018 NRSA18 Seining Information

4803036925

Figure 10.4 Seining Information Form

10.4 Processing Fish

Process the fish at the end of each subreach or pairs of subreaches, as described in **Table 10.6**. However, if fish show signs of stress (e.g., loss of righting response, gaping, gulping air, excessive mucus) in the middle of a subreach, change the water in the live well or stop fishing and initiate

processing. Always process and release individuals of State or Federally listed threatened or endangered species or large game fish immediately after collection. After processing fish, release them in a location that prevents the likelihood of their recapture.

If you use a seine to collect fish, please record the information for each haul on a separate line on the seining information form.

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 may 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 (15 cm [6 inch] intervals), and examine them for the presence of DELT (Deformities, Eroded Fins, Lesions and Tumors) anomalies. Use common names of species established by the American Fisheries Society *Common and Scientific Names of Fishes from the United States, Canada and Mexico* (Nelson, et al. 2004, Page et al. 2013). **Appendix D** provides a list of species names to be used, based on the current cumulative taxa list developed for NRSA.

If you believe a specimen is nonindigenous to the site, mark it as *Introduced* on the 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 10.4.6**).

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 **Section 10.4.6**) 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: Processing Fish (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 may record a single species on multiple lines of the collection form (e.g., use separate lines for individuals collected in multiple 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 > 25 mm (1 inch) in total length (TL). Ideally handle specimens only once. Although not required, you may note amphibians and reptiles captured on the fish collection form.
4) Identify each individual to the lowest possible taxonomic level: 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 and 2013/14. (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., G*reen sunfish x Bluegill*, C*utthroat 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?* If you cannot evaluate the native/introduced status of fish collected, mark the bubble at the top of the collection form. This will confirm that blank values for the *Introduced?* field are missing, rather than being presumed as native.

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 of individuals observed with one or more anomaly in the *Anom Count* column of the collection form.
   a) NOTE: Do not include injuries from collecting, handling, or processing fish, or from parasites in the external anomaly tally.

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.
      ii) 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) If you collect fish via seining, record he information on a separate line on the field form.

13) At the end of sampling, follow the appropriate procedure to prepare the preserved voucher samples (UNK/RNG and/or QA) and/or select specimens for tissue samples.

a) For all voucher samples, use a sufficient volume of 10% buffered formalin—the volume of formalin solution used must exceed the volume of specimens. Use additional jars if necessary. Slit large individuals (TL > 200 mm [~8 in.]) along the right side in the lower abdominal cavity to allow penetration of the formalin solution.

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

15) If you did not prepare a QA voucher sample, mark *No Voucher Preserved* on the back of the fish gear form (this is akin to the 'no sample collected' bubble associated with other sample types).

a) Otherwise complete a sample jar label for the QA voucher sample. Attach it to the sample jar and cover it with clear tape.

b) Record QA voucher sample label information on the back of the fish gear form.

16) Record the file names of any photovouchers taken on the back of the fish gear form.

a) Use only one line per voucher tag, even if you took multiple photos (record the beginning and end of the sequence in the *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., NRS18_WY_10001_V1_tag01a).

17) 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). Ideally, take photos of all species collected a site (that are not preserved) to provide a minimal level of documentation of occurrence. Take photographs of entire specimens and additional specific morphological features that are appropriate and necessary for an independent taxonomist to accurately identify the specimen. Additional detail for these guidelines is provided in Stauffer et al. (2001), which is provided to all field crews in electronic format.

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

Save images in medium to high quality jpeg format. It is important that time and date stamps are accurate, as this information can also be useful in tracking the origin of photographs. Transfer images stored in the camera to a personal computer or external storage device (e.g., thumb drive or flash memory card) at the first available opportunity. At this time, rename the original files to include the site ID, visit number, voucher specimen tag number, and photo sequence (e.g., NRS18_WY_10001_V1_tag01a.jpg). Record the file names on the back of the fish gear form (**Figure 10.6**). You should review your photos to confirm that they provide sufficient details to allow someone else to confidently confirm your identification using only your image files.

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

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

In addition to a UNK/RNG voucher sample (if needed), you will prepare an additional *QA voucher sample (***Section 10.4.7**). 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.

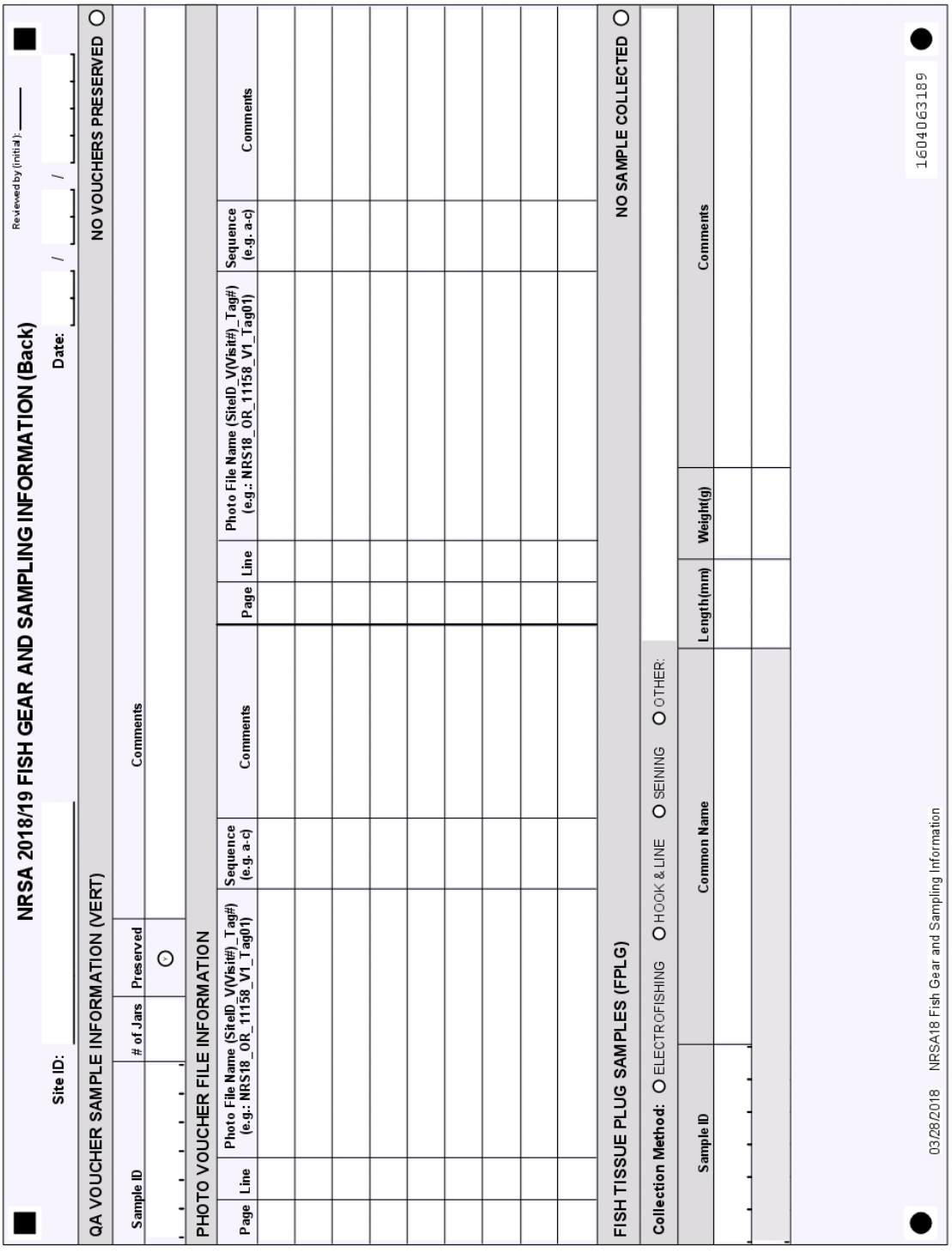
10.4.5 Preserving Voucher Specimen Samples

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 and cover with clear shipping tape. Two jar labels are provided for each type of voucher collection. If you have > 2 jars of either type of sample, use the extra jar labels provided to prepare a label for each additional jar. For the QA voucher sample, write the unique sample ID number on the extra jar label (this is found on the pre-printed QA voucher labels). On each jar label, use the spaces provided to record “Jar N of X”, where “N” is the individual jar number, and “X” is the total number of jars for the sample.

FISH VOUCHER – UNK/RNG Site ID: NRS18_ _____ ___ / ___ / 201__		FISH VOUCHER – UNK/RNG Site ID: NRS18_ _____ ___ / ___ / 201__	
FISH – BAG TAG: 41	FISH – BAG TAG: 42	FISH – BAG TAG: 43	FISH – BAG TAG: 44
FISH – BAG TAG: 37	FISH – BAG TAG: 38	FISH – BAG TAG: 39	FISH – BAG TAG: 40
FISH – BAG TAG: 33	FISH – BAG TAG: 34	FISH – BAG TAG: 35	FISH – BAG TAG: 36
FISH – BAG TAG: 29	FISH – BAG TAG: 30	FISH – BAG TAG: 31	FISH – BAG TAG: 32
FISH – BAG TAG: 25	FISH – BAG TAG: 26	FISH – BAG TAG: 27	FISH – BAG TAG: 28
FISH – BAG TAG: 21	FISH – BAG TAG: 22	FISH – BAG TAG: 23	FISH – BAG TAG: 24
FISH – BAG TAG: 17	FISH – BAG TAG: 18	FISH – BAG TAG: 19	FISH – BAG TAG: 20
FISH – BAG TAG: 13	FISH – BAG TAG: 14	FISH – BAG TAG: 15	FISH – BAG TAG: 16
FISH – BAG TAG: 09	FISH – BAG TAG: 10	FISH – BAG TAG: 11	FISH – BAG TAG: 12
FISH – BAG TAG: 05	FISH – BAG TAG: 06	FISH – BAG TAG: 07	FISH – BAG TAG: 08
FISH – BAG TAG: 01	FISH – BAG TAG: 02	FISH – BAG TAG: 03	FISH – BAG TAG: 04

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



NRSA 2018/19 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.: NRS18_OR_11158_V1_Tag01)	Sequence (e.g. a-c)	Comments	Page	Line	Photo File Name (SiteID_V(Visit#)_Tag#) (e.g.: NRS18_OR_11158_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

03/28/2018 NRSA18 Fish Gear and Sampling Information

1604063189

Figure 10.6 Fish Gear and Sampling Information Form (back)

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 specimens using whatever resources are necessary (magnification, literature, reference collections/specimens, including dissected anatomical features or in-house colleagues).

Following positive laboratory identification, use the fish collection revision form for the sample (**Figure 10.8**) to reconcile the unknown records to reflect revisions to the actual species identifications counts, and any other information recorded on the original collection form. **It is important to update counts and identifications by voucher tag; do not combine multiple samples of the same unknown before updating.**

The fish collection revision form for a site will be provided with all of the original information that was recorded on the original collection form. The form is a fillable Portable Document Format (pdf) form, and corrections can be made using the Adobe Reader© software. For each line, make any corrections on page 1 of the revision form, and provide an explanation of the changes made on the corresponding line number on the page 2 of the revision form (**Figure 10.9**), and mark the YES button under *Changes Made?*. If no corrections are necessary, mark the NO button on the back side of the form under *Changes Made?* Provide your contact information in the space provided on the back side of the form.

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

If you use a non-standard name (i.e., one that is not listed in Appendix D), enter the page, line number, tag number, and taxonomic reference for the name in the Comments section on page 2 of the revision form (**Figure 10.9**). Submit your completed revision forms to the NARS IM staff as soon as possible after completing the laboratory identifications. Retain the preserved UNK/RNG voucher samples from each site– contact your regional EPA coordinator if you cannot store the samples at your facility.

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

10.4.7 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 representative specimens of ALL species (including unknowns and common species) collected from the site. Each unique species (including unknowns) should have a unique QA voucher specimen tag number assigned (**Figure 10.7**). Record information about the preserved QA voucher sample on the back side of the fish gear form (**Figure 10.6**).

Retain all of your QA voucher samples (including digital image files) until given direction by EPA regarding where to send them. When you are ready to ship the samples, complete a sample tracking form as described in **Appendix C**. QA voucher samples may require shipping as "dangerous goods," and packing and documentation requirements will differ depending on whether the samples contain formalin or ethanol, the size of individual bottles, and on the particular shipping service used.

QA FISH VOUCHER (VERT) Site ID: NRS18_ _____ ___ / ___ / 201__ **999012**		QA FISH VOUCHER (VERT) Site ID: NRS18_ _____ ___ / ___ / 201__ **999012**	
FISH – BAG TAG: 41 **999012**	FISH – BAG TAG: 42 **999012**	FISH – BAG TAG: 43 **999012**	FISH – BAG TAG: 44 **999012**
FISH – BAG TAG: 37 **999012**	FISH – BAG TAG: 38 **999012**	FISH – BAG TAG: 39 **999012**	FISH – BAG TAG: 40 **999012**
FISH – BAG TAG: 33 **999012**	FISH – BAG TAG: 34 **999012**	FISH – BAG TAG: 35 **999012**	FISH – BAG TAG: 36 **999012**
FISH – BAG TAG: 29 **999012**	FISH – BAG TAG: 30 **999012**	FISH – BAG TAG: 31 **999012**	FISH – BAG TAG: 32 **999012**
FISH – BAG TAG: 25 **999012**	FISH – BAG TAG: 26 **999012**	FISH – BAG TAG: 27 **999012**	FISH – BAG TAG: 28 **999012**
FISH – BAG TAG: 21 **999012**	FISH – BAG TAG: 22 **999012**	FISH – BAG TAG: 23 **999012**	FISH – BAG TAG: 24 **999012**
FISH – BAG TAG: 17 **999012**	FISH – BAG TAG: 18 **999012**	FISH – BAG TAG: 19 **999012**	FISH – BAG TAG: 20 **999012**
FISH – BAG TAG: 13 **999012**	FISH – BAG TAG: 14 **999012**	FISH – BAG TAG: 15 **999012**	FISH – BAG TAG: 16 **999012**
FISH – BAG TAG: 09 **999012**	FISH – BAG TAG: 10 **999012**	FISH – BAG TAG: 11 **999012**	FISH – BAG TAG: 12 **999012**
FISH – BAG TAG: 05 **999012**	FISH – BAG TAG: 06 **999012**	FISH – BAG TAG: 07 **999012**	FISH – BAG TAG: 08 **999012**
FISH – BAG TAG: 01 **999012**	FISH – BAG TAG: 02 **999012**	FISH – BAG TAG: 03 **999012**	FISH – BAG TAG: 04 **999012**

Figure 10.7 QA Voucher Sample Labels and Voucher Specimen Tags

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

Processing UNK/RNG Voucher Samples
1) Following fixation for 5 to 7 days, decant and properly discard the formalin solution. Formalin is a potential carcinogen and should be used with extreme caution, as vapors and solution are highly caustic and may cause severe irritation on contact with skin, eyes, or mucus membranes. Wear vinyl or nitrile gloves and safety glasses, and always work in a well-ventilated area. 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) The NARS IM staff will send you a Fish Revision Form for each fish collection form you completed at a site. This form lists all records from the original collection form, including any that were marked as being part of the UNK/RNG voucher sample. It will be provided as a fillable form in a portable document format (pdf) file. 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, as well as the voucher specimen tag number you recorded on the collection form.
5) Make any corrections to the original collection form on the revision form using software that can edit a fillable pdf file (e.g., Adobe Reader©). For every line where you make a correction on page 1 of the revision form, provide an explanation on the corresponding line on page 2 of the 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 on page 2 of the revision 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 (the most abundant) on the line of the original unknown. b) Record the revised name and count for the second species as a new record on the revision 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) Fill in the appropriate button under the *Changes Made?* section on page 2 of the revision form to indicate whether or not any changes were made. Provide your contact information on page 2 of the revision form.
7) After reconciling all of the unknowns, and correcting any other information from the original collection forms, submit the completed revision forms to the NARS IM staff in Corvallis. Retain the preserved UNK/RNG voucher samples. Contact your regional EPA Regional Coordinator if you cannot store the samples at your facility.

NRSA 2018/19 FISH COLLECTION REVISIONS

Site ID, Visit, Date Collected (Chem), Crew

Fish Collection Date (Actual Date)

Page

I did not evaluate the native/ introduced status for fish collection. ○ Y

Line #	Common Name	Hy-brid?	Intro-duced?	Counts <150 mm	150-300 mm	300-460 mm	>460 mm	Anom Count	Mortality Count	Vouchers Unk/Rng	Vouchers QA	Vouchers Photo	Voucher Tag #	Vouchers Retained	Flag
1		○	○							○	○	○			
2		○	○							○	○	○			
3		○	○							○	○	○			
4		○	○							○	○	○			
5		○	○							○	○	○			
6		○	○							○	○	○			
7		○	○							○	○	○			
8		○	○							○	○	○			
9		○	○							○	○	○			
10		○	○							○	○	○			
11		○	○							○	○	○			
12		○	○							○	○	○			
13		○	○							○	○	○			
14		○	○							○	○	○			

The above filled data represents your fish file when these records were pulled from the database. We need you to confirm that we have accurate information regarding all aspects of your fishing event. Please make updates here as needed to correctly identify species, update counts and other information. If you make any changes to fish data on this side, please provide a reason for change on the back of this form corresponding to the line number. Your reasons for change are very helpful when reviewing the data and documenting changes in the database for QA purposes.

For instance, if you change unknown darter on line 12 to Johnny Darter - please tell us that on the backside of this page, line 12. If an unknown species turns out be >1 species, record the most abundant species on the line replacing the unknown, and revise the count and any other information. Add the name and information for any new species to an empty line (or use the blank form provided to you). If the flag field is filled, please review your flag and comment data **provided to you separately in csv format**. If your comment is no longer applicable, remove the flag here, open the csv file and delete the flag and comment there. Fill in reason for change on both the revision form and the csv file.

This form is your final attempt at correcting your fish data. **Do not make corrections to fish collection via app submissions. Please return all pages of record – whether or not there were changes.** Indicate if changes were made on the back and give us your contact information should we have any follow-up questions. Thank you in advance for your attention to detail.

02/15/2018 NRSA18 Fish Collection Revisions

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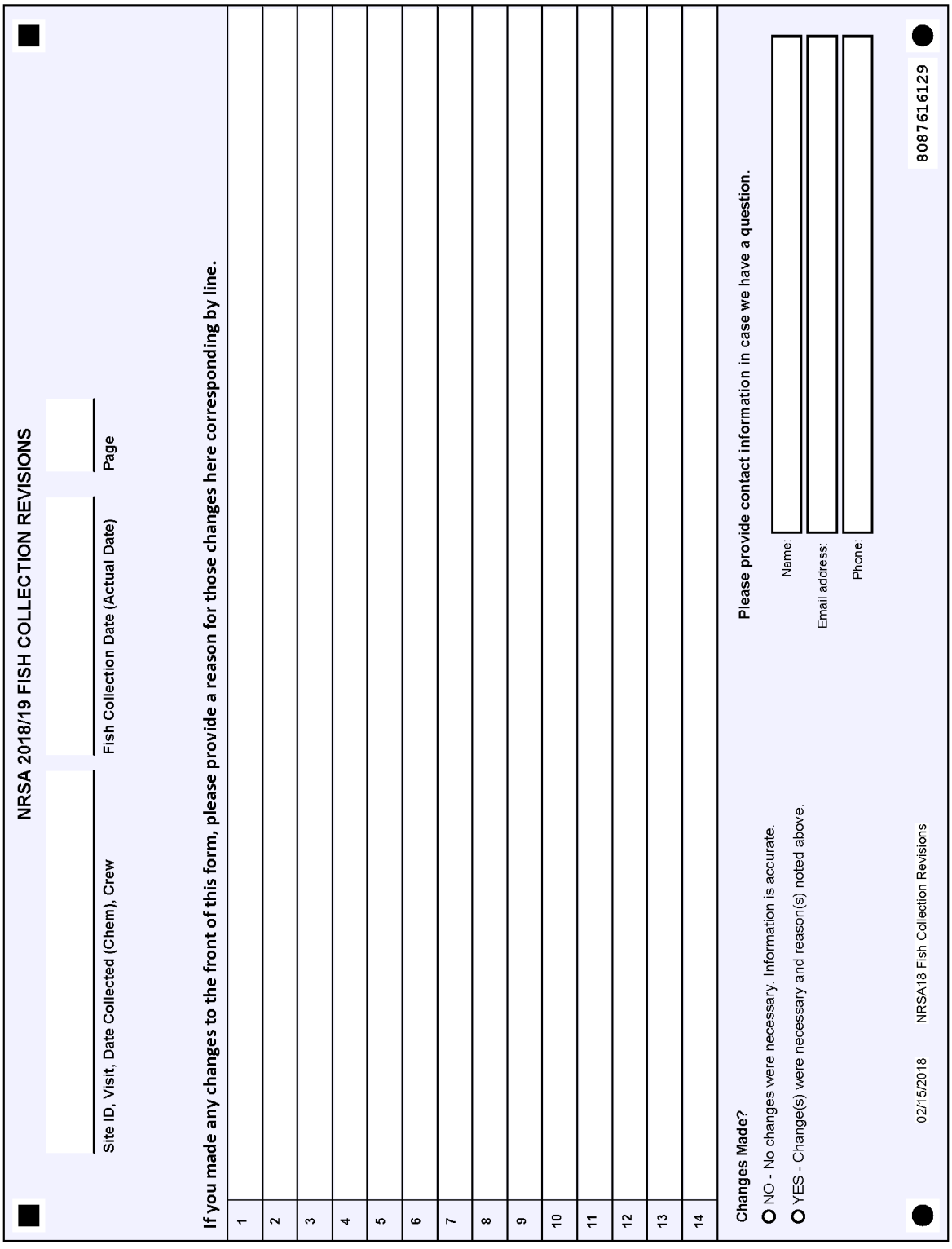
Figure 10.8 Fish Collection Revision Form (Page 1)



NRSA 2018/19 FISH COLLECTION REVISIONS

Site ID, Visit, Date Collected (Chem), Crew | Fish Collection Date (Actual Date) | Page

If you made any changes to the front of this form, please provide a reason for those changes here corresponding by line.

1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	

Changes Made?

- NO - No changes were necessary. Information is accurate.
- YES - Change(s) were necessary and reason(s) noted above.

Please provide contact information in case we have a question.

Name:

Email address:

Phone:

02/15/2018 NRSA18 Fish Collection Revisions

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Figure 10.9 Fish Collection Revision Form (Page 2)

Table 10.8 Procedure: Processing QA Voucher Samples

1)	Ensure that all species collected at a site are represented by either preserved voucher specimens or photovouchers. There should be a unique QA voucher specimen tag number assigned to every species recorded on the fish collection form.
2)	Before submitting the QA Voucher sample, ensure that all specimens have been positively identified. If your attempts at identification do not yield a positive identification for 100% of the fish you retained, contact the Contract Field Logistics Coordinator for further guidance (Chris Turner, cturner@glec.com, 715-829-3737).
3)	After preparing the preserved QA voucher sample, check that the sample ID number recorded on the fish gear form matches the preprinted label attached to each sample jar, and that the number of jars recorded on the fish gear form is correct.
4)	Retain the QA voucher samples in appropriate storage space for formalin until you receive information regarding where to send them from the NRSA staff at EPA Office of Water 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 Regional 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)	Post all photovoucher files for each QA voucher sample to SharePoint. 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 for a single contaminant of concern (mercury), which uses a tissue plug instead of a skin-on fillet. A plug sample consisting of two fish tissue plugs for mercury analysis will be collected from two fish of the same species (one plug per fish) from the target list (below) at all sites where suitable fish species and lengths are available ***except*** during any site visit where whole fish tissue samples are collected (see **Section 12**). These fish are collected during the fish assemblage sample collection effort (**Section 10**). A plug tissue sample is collected by inserting a biopsy punch into a de-scaled thicker area of dorsal muscle section of a live fish. After collection, antibiotic salve is placed over the wound and the fish is released. Fish tissue plugs will not be removed in the field from whole fish tissue samples collected at the 477 designated river sites (**Section 12**). Instead, those plug samples will be extracted in the laboratory by the fish sample preparation lab personnel.

11.2 Equipment and Supplies

Table 11.1 lists the equipment and supplies necessary for field crews to collect fish tissue plug samples. This list is comparable to the checklist presented in **Appendix A**, which provides information to ensure that field crews bring all of the required equipment to the site. Record the fish tissue plug sampling data on the Fish Gear and Voucher/Tissue Sample Information Form (**Figure 10.6**).

Table 11.1 Equipment and Supplies: Fish Tissue Plug Sample

For fish tissue plug samples	Fish measuring board Fish weigh scale Plastic bags Sterile 20 mL glass scintillation vial Coolers with ice Cooler with dry ice Nitrile gloves 8 millimeter disposable biopsy punch (Acuderm brand Acu-Punch or equivalent) Sterile disposable scalpel Sterile forceps Laboratory pipette bulb. Antibiotic salve. Fish collection gear (electrofisher, nets, livewell, etc.) Dip net Field Operations Manual and laminated Quick Reference Guide
For recording measurements	Fish tissue plug sample labels Fish Gear and Sampling Form Soft (#2) lead pencils for recording data on field forms Fine-tipped indelible markers for filling out sample labels Clear tape strips for covering labels

11.3 Sample Collection Procedures

Collection of individual fish specimens for the fish tissue plug indicator occurs in the sample reach during the fish assemblage sampling effort, using the same gear used to collect the fish assemblage samples. Fish tissue plug samples should be taken from the species listed in the target list found in **Table 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 plug sample. 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	MINIMUM LENGTH GUIDELINE
Target Species	Centrarchidae	Spotted bass	*Micropterus punctulatus*	The minimum length guideline is ≥190 mm for all species at sites where only fish plugs are collected. See the table below for length guidelines for whole fish tissue sites (where plugs will be collected in the laboratory).
		Largemouth bass	*Micropterus salmoides*	
		Smallmouth bass	*Micropterus dolomieu*	
		Black crappie	*Pomoxis nigromaculatus*	
		White crappie	*Pomoxis annularis*	
	Ictaluridae	Channel catfish	*Ictalurus punctatus*	
		Blue catfish	*Ictalurus furcatus*	
		Flathead catfish	*Pylodictis olivaris*	
	Percidae	Sauger	*Sander canadensis*	
		Walleye	*Sander vitreus*	
		Yellow perch	*Perca flavescens*	
	Moronidae	White bass	*Morone chrysops*	
	Esocidae	Northern pike	*Esox lucius*	
		Chain pickerel	*Esox niger*	
		Muskellunge	*Esox masquinongy*	
	Salmonidae	Brown trout	*Salmo trutta*	
		Cutthroat trout	*Oncorhynchus clarkii*	
		Rainbow trout	*Oncorhynchus mykiss*	
		Brook trout	*Salvelinus fontinalis*	
Alternates [B]	Catostomidae[A]	Largescale sucker	*Catostomus macrocheilus*	
		Quillback	*Carpiodes cyprinus*	
		River carpsucker	*Carpiodes carpio*	
		River redhorse	*Moxostoma carinatum*	
		Smallmouth buffalo	*Ictiobus bubalus*	
		White sucker	*Catostomus commersonii*	
	Centrarchidae	Bluegill	*Lepomis macrochirus*	
		Rock bass	*Ambloplites rupestris*	
		Redbreast sunfish	*Lepomis auritus*	
		Redear sunfish	*Lepomis microlophus*	
	Cyprinidae	Northern pikeminnow	*Ptychocheilus oregonensis*	
		Sacramento pikeminnow	*Ptychocheilus grandis*	
		Common carp	*Cyprinus carpio*	
	Ictaluridae	Black bullhead	*Ameiurus melas*	
		Brown bullhead	*Ameiurus nebulosus*	
		White catfish	*Ameiurus catus*	
		Yellow bullhead	*Ameiurus natalis*	
	Lepisosteidae	Longnose gar	*Lepisosteus osseus*	
	Moronidae	Striped bass	*Morone saxatilis*	
		White perch	*Morone americana*	
	Percidae	Saugeye	*Sander vitreus x Sander canadensis*	
	Salmonidae	Mountain whitefish	*Prosopium williamsoni*	
	Sciaenidae	Freshwater drum	*Aplodinotus grunniens*	

[A] These are only a few examples of the many sucker species that would be acceptable alternative species.
[B] Only send if preferred species are not available.

Table 11.3 Procedure: Fish Tissue Plug Samples

Fish Tissue Plug Methods
1. Put on clean nitrile gloves before handling the fish. Do not handle any food, drink, sunscreen, or insect repellant until after the composite sample has been collected, measured, and wrapped.

Fish Tissue Plug Methods

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 for the sampled river, or at least be of consumable size if no legal harvest requirements are in effect
    - are of similar size, so that the smaller individual is no less than 75% of the total length of the larger individual.
4. Remove one fish 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.
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 NRSA 2008/09 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 NRSA 2018/19 allows consistency with fish tissue methods of previous NRSAs (2008/09 and 2013/14) and provides sufficient tissue for analysis of multiple chemical contaminants of concern (e.g., mercury, polychlorinated biphenyls or PCBs, and perfluorinated compounds or PFCs). Continued analysis of fillet tissue also allows for temporal analysis of probability-based national fish contamination trends in U.S. rivers. Collecting fish at locations sampled during previous NRSAs will reduce the variability in data for trends analysis.

Whole fish tissue sampling procedures are described in detail in Table 12.3. The objective is to collect one whole fish sample from each of the 477 designated target river sites. The focus is on obtaining fish species that are commonly consumed by humans, that satisfy legal requirements of harvestable size for each river site (or at least consumable size if no legal harvest requirements exist), 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 (i.e., the smallest individual in the sample is no less than 75% of the total length of the largest individual). Collection occurs anywhere in the fish assemblage sampling reach (Section 10). Whole fish samples are shipped to the laboratory designated for interim storage of the samples. Fish sample preparation laboratory staff fillet the fish and homogenize the fillet tissue for analysis of mercury and other contaminants (e.g., PCBs and PFCs).

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 PFDs 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 Composite (Tyvek) tag	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 Black ink pen Fine tipped indelible markers
For shipping the whole fish tissue samples	Preaddressed FedEx airbill Coolers	Tracking Form Chain-of-custody labels Packing/strapping tape Hazard Class 9 shipping labels Dry ice

12.3 Sampling Procedures

The whole fish tissue samples will be collected with the same gear used to collect the fish assemblage samples. Collection of individual specimens for whole fish samples occurs anywhere 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 fillet tissue requirement for chemical analysis and archived tissue (refer to Frequently Asked Questions in the whole fish tissue kits). Recommended target species are given in **Table 12.2.** If the target species are unavailable, the fisheries biologist will select an alternative species to obtain a whole fish sample (i.e., a species that is commonly consumed by humans, with specimens that are of harvestable or consumable size and are in sufficient numbers to yield a fish sample with adequate tissue for analysis). If sufficient fish are not collected during the fish assemblage sampling, sample for up to one additional hour (collections can occur in areas/subreaches not otherwise sampled if desired). If no fish can be collected, record “no sample collected” on the whole fish tissue collection form, along with the reason in the comments section of the form. 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[A]
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
		Muskellunge	*Esox masquinongy*	≥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[C]	Catostomidae[B]	Largescale sucker	*Catostomus macrocheilus*	≥330 mm
		Quillback	*Carpiodes cyprinus*	≥300 mm
		River carpsucker	*Carpiodes carpio*	≥300 mm
		River redhorse	*Moxostoma carinatum*	≥330 mm
		Smallmouth buffalo	*Ictiobus bubalus*	≥300 mm
		White sucker	*Catostomus commersonii*	≥330 mm
	Centrarchidae	Bluegill	*Lepomis macrochirus*	≥200 mm
		Rock bass	*Ambloplites rupestris*	≥200 mm
		Redbreast sunfish	*Lepomis auritus*	≥200 mm
		Redear sunfish	*Lepomis microlophus*	≥200 mm
	Cyprinidae	Northern pikeminnow	*Ptychocheilus oregonensis*	≥300 mm
		Sacramento pikeminnow	*Ptychocheilus grandis*	≥300 mm
		Common carp	*Cyprinus carpio*	≥300 mm
	Ictaluridae	Black bullhead	*Ameiurus melas*	≥300 mm
		Brown bullhead	*Ameiurus nebulosus*	≥300 mm
		White catfish	*Ameiurus catus*	≥300 mm
		Yellow bullhead	*Ameiurus natalis*	≥300 mm
	Lepisosteidae	Longnose gar	*Lepisosteus osseus*	≥530 mm
	Moronidae	Striped bass	*Morone saxatilis*	≥280 mm
		White perch	*Morone americana*	≥300 mm
	Percidae	Saugeye	*Sander vitreus x Sander canadensis*	≥380 mm
	Salmonidae	Mountain whitefish	*Prosopium williamsoni*	≥330 mm
	Sciaenidae	Freshwater drum	*Aplodinotus grunniens*	≥300 mm

[A] Minimum acceptable length is 190 mm (total length).
[B] These are only a few examples of the many sucker species that would be acceptable alternative species.
[C] Only send if preferred species are not available.

Table 12.3 Procedure: Whole Fish Tissue Samples

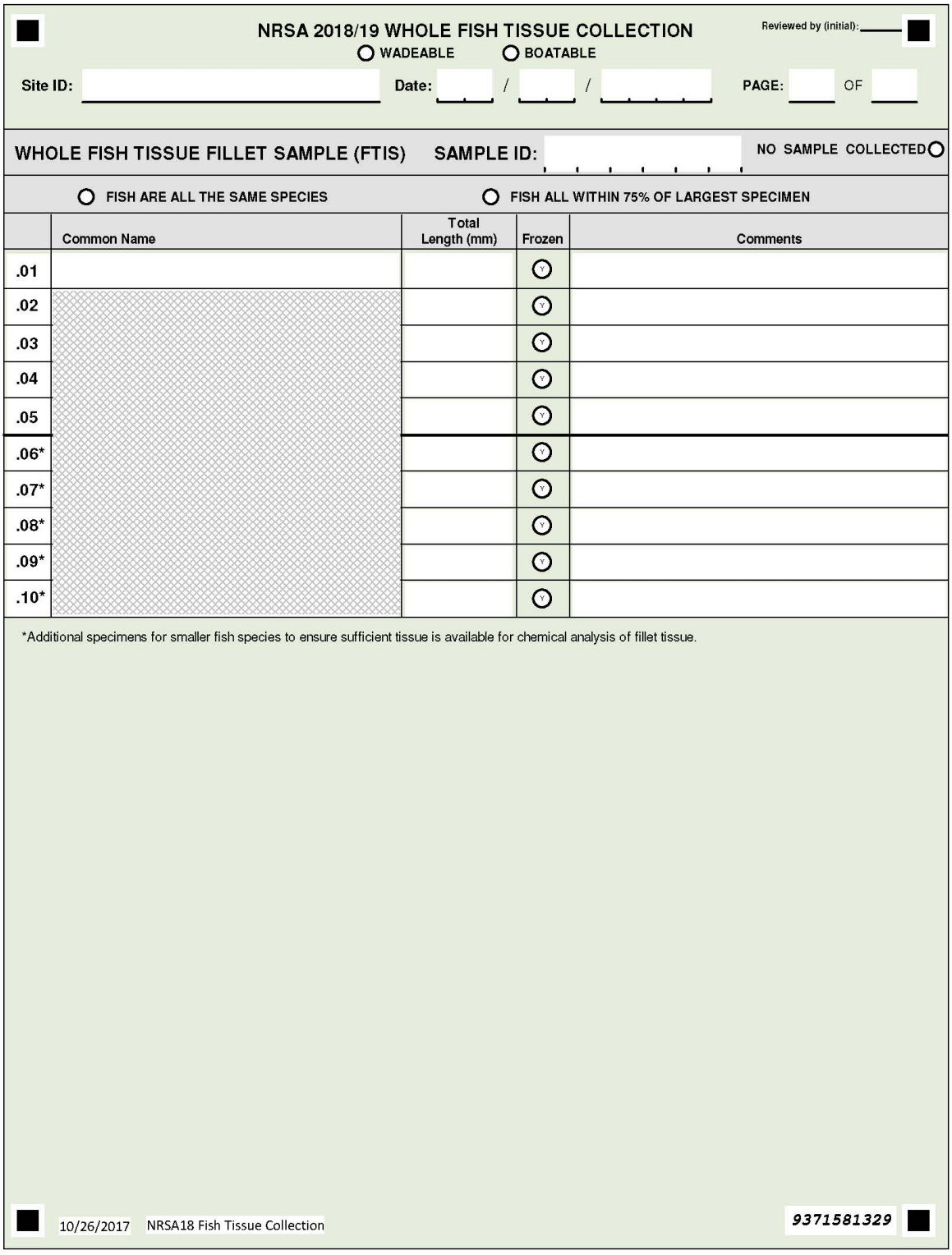
Whole Fish Tissue Method

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 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 as close to the same time as possible, but no more than one week apart (Note: Individual fish may have to be frozen until all fish to be included in the sample are available for delivery to the designated laboratory).

    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/lengths that are recorded on the collection form match the corresponding information on each individual specimen label.
6. Remove each fish selected for analysis from the clean holding container(s) (e.g., livewell) using clean nitrile gloves. Dispatch each fish using a clean wooden bat (or equivalent wooden device).
7. Wrap each fish in extra heavy-duty aluminum foil with the dull side in (foil provided by EPA as solvent-rinsed, oven-baked sheets).
8. Prepare a Sample Identification Label for each sample, ensuring that the label information matches the information recorded on the Whole Fish Tissue Collection Form.
9. Cut a length of food grade tubing (provided by EPA) that is long enough to contain each individual fish and to allow extra length on each end to secure with cable ties. Place each foil wrapped specimen separately into an 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 whole fish composite sample from each river site in a large plastic composite sample bag, complete the bag label, and tape it to the Tyvek composite tag, then seal the composite bag with a cable tie with the composite sample tag attached.
11. After the 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.

Whole Fish Tissue Method

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 Microbac Laboratories (Baltimore, MD) 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 Microbac Laboratories (Baltimore, MD) via priority overnight delivery service).
14. Ship fish tissue samples to the designated laboratory for interim sample storage on Monday through Thursday (no Saturday delivery to the laboratory).



NRSA 2018/19 WHOLE FISH TISSUE COLLECTION

Reviewed by (initial): ____

○ WADEABLE ○ BOATABLE

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

WHOLE 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			Y	
.02			Y	
.03			Y	
.04			Y	
.05			Y	
.06*			Y	
.07*			Y	
.08*			Y	
.09*			Y	
.10*			Y	

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

10/26/2017 NRSA18 Fish Tissue Collection 9371581329

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 IM Coordinator, and ship or store the samples. Activities described in this section are summarized in **Figure 13.1**.

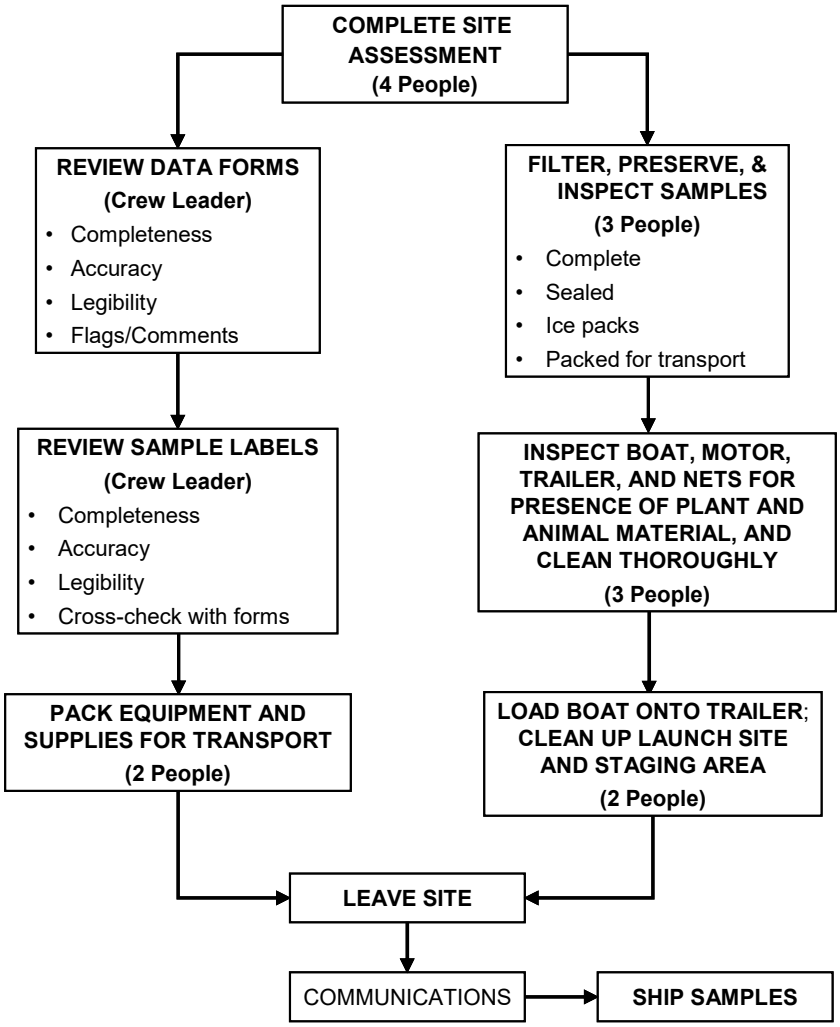


Figure 13.1 Final Site Activities Summary

13.2 General Site Assessment

Complete the Site Assessment Form (**Figure 13.3**) after sampling, recording all observations from the site that were noted during the course of the visit. This Site Assessment Form is designed as a template for recording pertinent field observations. It is by no means comprehensive, and any additional observations should be recorded in the General Assessment section.

13.2.1 Elevation at Transect K

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

13.2.2 Watershed Activities and Disturbances Observed

Record any of the sources of potential stressors listed in the "Watershed Activities and Disturbances Observed" section on the Site Assessment Form (**Figure 13.3**). Include those that were observed while on the site, while driving or walking through the site catchment, or while flying over the site and catchment. For activities and stressors that you observe, rate their abundance or influence as low (L), moderate (M), or heavy (H) on the line next to the listed disturbance. Leave the line blank for any disturbance not observed and be sure to verify that blank field indicate absence by filling in the bubble at the top of the section. The distinction between low, moderate, and heavy will be subjective. For example, if there are two to three houses on a site, fill in "L" for low next to "Residences." If the site is ringed with houses, rate it as heavy (H). Similarly, a small patch of clear-cut logging on a hill overlooking the site would rate a low ranking. Logging activity right on the site shore, however, would get a heavy disturbance ranking. This section includes residential, recreational, agricultural, industrial, and stream management categories.

13.2.3 Site Characteristics

Record observations regarding the general characteristics of the site on the Site Assessment Form (**Figure 13.3**). When assessing these characteristics, look at a 200 m riparian distance on both banks. Rank the site between "pristine" and "highly disturbed", and between "appealing" and "unappealing." Document any signs of beaver activity and flow modifications. Record the dominant land use and forest age class. Document the weather conditions on the day of sampling and any extreme weather conditions in the days prior to sampling.

13.2.4 General Assessment

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

NRSA 2018/19 ASSESSMENT (Front)

Reviewed by (initial): _____

Site ID: _____ Date: ____ / ____ / ____

Elevation at transect K: _____ ○ 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 Dredgong
○ 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. ○ 26 - 75 yrs. ○ > 75 yrs.

WEATHER

CONDITIONS AND LOCAL CONTACTS

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

09/13/2017 NRSA18 Assessment 4803036903

Figure 13.2 Site Assessment Form (front)

Reviewed by (initial): _____

NRSA 2018/19 ASSESSMENT (Back)

Site ID: _____ **Date:** ____ / ____ / ____

GENERAL ASSESSMENT AND COMMENTS

INVASIVE OR NUISANCE SPECIES OF LOCAL INTEREST

Record species of plants and animals that were observed but are not on the invasive plant form. Examples would be Zebra Mussel or New Zealand Mud Snail, or invasive plants or animals of concern to a particular state. Indicate your level of confidence in your identification, and provide some idea of how prevalent it is in the sampling reach or adjacent riparian area.

Species (Common Name)	Confidence	Prevalence	Comments
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	
	O LOW O HIGH	O DOMINANT O SPARSE O COMMON	

09/13/2017 NRSA18 Assessment

4860036906

Figure 13.3 Site Assessment Form

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

13.3.1 Equipment and Supplies (Fecal Indicator Filtering)

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

Table 13.1 Equipment and Supplies: Fecal Indicator (Enterococci) 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 Bubble bag Zip-top bag 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

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

The fecal indicator sample **must** be filtered **before** the chlorophyll-*a* and periphyton samples, since the filtering apparatus needs to be sterile for this sample. The procedures for processing the fecal indicator sample are presented in **Table 13.2**. The sample must be filtered and frozen within six hours of collection.

Table 13.2 Procedure: Processing Fecal Indicator (*Enterococci*) Sample

Filtering for the fecal indicator (Enterococci) 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, two bottles of chilled phosphate buffered saline (PBS), Osmotics 47 mm polycarbonate sterile filter box, and two filter forceps. 3. Chill Filter Extraction tubes with beads on dry ice.

4. Aseptically transfer two 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 sterile PP tube and pour into filter funnel.
9. Replace cover on filter funnel and pump to generate a vacuum (do not generate more than 7 inches of Hg of vacuum [3.44 psig]). Keep pumping until all liquid is in filtrate collection flask.
10. If the first 25 mL volume passes readily through the filter, add another 25 mL and continue filtration. If it was very difficult to filter the first 25 mL, proceed to step 11. If the filter clogs before completely filtering the first or second 25 mL volume, discard the filter and repeat the filtration using a lesser volume.
11. Pour approx. 10 mL of the chilled phosphate buffered saline (PBS) into the graduated PP tube used for the sample. Cap the tube and shake 5 times. Remove the cap and pour rinsate into filter funnel to rinse filter.
12. Filter the rinsate and repeat with another 10 mL of phosphate buffered saline (PBS).
13. Remove filter funnel from base without disturbing filter. Using sterile disposable forceps remove the filter (touching only the filter edges) and fold it in half, in quarters, in eighths, and then in sixteenths (filter will be folded four times).
14. Insert filter into chilled filter extraction tube (with beads). Filter should be inserted open end down (pointed side up) into the tube. Replace and tighten the screw cap.
15. Record the volume of sample filtered through the filter on the small yellow label and apply the label to the extraction tube (DO NOT cover with clear tape).
16. Record the volume of sample filtered through the filter on the outer bag label and apply the label to the bubble bag (DO NOT cover with clear tape).
17. Insert tube(s) into bubble bag and zip-top bag on dry ice for preservation during transport and shipping.
18. Record the volume of water sample filtered through each filter and the volume of buffer rinsate each filter was rinsed with on the Sample Collection Form, Side 1. Record the filtration start time and finish time for the sample as well as the time the filters were frozen.
19. Repeat steps 6 to 15 for the remaining 50 mL sub-sample volume to be filtered. Make every effort to filter the same volume of sample through each of the two filters.

Processing procedure—fecal indicator (*Enterococci*) filter blank

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

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

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

Table 13.3 Equipment and Supplies: Chlorophyll-*a* Processing

For filtering chlorophyll-*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 Zip-top bag
For recording measurements	Sample Collection Form Sample labels #2 pencils Fine-tipped indelible markers Clear tape strips

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

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

Table 13.4 Procedure: Chlorophyll-a Sample Processing

Filtering for the chlorophyll 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 [3.44 psig]). If 250 mL of site water will not pass through the filter, change the filter, rinse the apparatus with DI water, and repeat the procedures using 100 mL of site water. • *NOTE: IF the water is green or turbid, use a smaller volume to start.*
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 labelled with the completed chlorophyll outer bag label. Cover the outer label with clear tape. 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
	125 mL sterile PETG bottle
	60 mL syringe with tip removed

	Aluminum foil squares Forceps Surgical gloves 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
For cleaning equipment	10% Bleach solution

13.3.6 Procedures for Processing the Periphyton Samples

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

13.3.6.1 ID/Enumeration Sample

Prepare the ID/Enumeration sample as a 50 mL aliquot from the composite periphyton sample, following the procedure presented in **Table 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 composite 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: 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. Thoroughly mix the bottle containing the composite sample.
4. Immediately after mixing, pour 50 mL of sample into pre-labeled 50 mL centrifuge tube.
5. Use a syringe or bulb pipette to add 2 mL of 10% formalin to the tube. Cap the tube tightly and seal with plastic electrical tape. Tighten the cap as tightly as possible. The cap will seal tightly after an additional ¼ turn past the point at which initial resistance is met.
6. Shake gently to distribute preservative.

7. 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 Metagenomic Sample

Prepare the periphyton metagenomic sample as a 100 mL aliquot from the composite index sample, following the procedure presented in **Table 13.7**.

Table 13.7 Procedure: Preparing Metagenomic Sample of Periphyton

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

13.3.6.3 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 13.8**. Chlorophyll *a* can degrade rapidly when exposed to bright light. If possible, prepare the samples in subdued light (or shade), filtering as quickly as possible after collection to minimize degradation. If using the same filtration chamber that was used for *Enterococci* and index site chlorophyll-*a* samples, rinse it with deionized water prior to filtering the periphyton chlorophyll-*a* sample. If you are reusing a filtration chamber from a previous site, you should rinse it with DI water each day before use at the base site and then seal in a plastic bag until use at the stream (be sure to use a new chamber at each site for the *Enterococci* sample as it needs to be filtered in a sterile chamber). Keep the glass fiber filters in a dispenser inside a sealed plastic bag until use.

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

Table 13.8 Procedure: Preparing Chlorophyll Samples of Periphyton

Periphyton Chlorophyll *a* Sample Processing Procedure
1. Rinse the sides of the filter funnel and the filter with a small volume of deionized water to prevent contamination from the previously filtered sample 2. Using clean forceps, place a Whatman GF/F 0.7 µm glass fiber filter on the filter holder gridded side down. If needed, use a small amount of deionized water from a wash bottle to help settle the filter properly. Attach the filter funnel to the filter holder and filter chamber, and then attach the vacuum pump to the filter flask. 3. Rinse a 25 mL or 50 mL graduated cylinder three times with small volumes of deionized water and discard. 4. Mix the composite sample bottle thoroughly. *5.* Measure 25 mL (±1 mL) of sample into the graduated cylinder. *NOTE: For a composite sample containing fine sediment, allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.* *6.* Pour the 25 mL aliquot into the filter funnel, replace the cap, and pull the sample through the filter using the vacuum pump. Vacuum pressure from the pump should not exceed 7 inches of Hg (3.44 psig) to avoid rupture of fragile algal cells. *NOTE: If 25 mL of sample will not pass through the filter, discard the filter and rinse the chamber thoroughly with deionized water. Collect a new sample using a smaller volume of sample, measured to ±1 mL. Be sure to record the actual volume sampled on the sample label and the Sample Collection Form.* 7. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump. Remove the bottom portion of the apparatus and pour off the water from the bottom as often as needed. 8. 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. Seal the cap with plastic electrical tape. 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 the self-sealing plastic bag with the water column chlorophyll sample. 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.

13.3.6.4 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 13.9**. Using the same filtration chamber that was used for Enterococci and chlorophyll-a samples, rinse it with deionized water prior to filtering the periphyton biomass sample. If you are reusing a filtration chamber from a previous site, you should rinse it with DI water each day before use at the base site and then seal in a plastic bag

until use at the stream (be sure to use a new chamber at each site for the Enterococci sample as it needs to be filtered in a sterile chamber). Keep the glass fiber filters in a dispenser inside a sealed plastic bag until use.

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

Table 13.9 Procedure: Preparing Ash-Free Dry Mass (AFDM) Samples of Periphyton

Periphyton AFDM Sample Processing Procedures
1. Rinse the sides of the filter funnel and the filter with a small volume of deionized water to prevent contamination from the previously filtered sample.
2. Using clean forceps, place a Whatman 47 mm 1.2 micron GF/C glass fiber filters on the filter holder gridded side down. If needed, use a small amount of deionized water from a wash bottle to help settle the filter properly. Attach the filter funnel to the filter holder and filter chamber, then attach the hand vacuum pump to the filter flask.
3. Rinse a 25 mL or 50 mL graduated cylinder three times with small volumes of deionized water and discard.
4. Mix the composite sample bottle thoroughly.
5. Measure 25 mL (±1 mL) of sample into the graduated cylinder. ***NOTE:*** *For a composite sample containing fine sediment, allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.*
6. Pour the 25 mL aliquot into the filter funnel, replace the cap, and pull the sample through the filter using the vacuum pump. Vacuum pressure from the pump should not exceed 7 inches of Hg (3.44 psig) to avoid rupture of fragile algal cells. ***NOTE:*** *If 25 mL of sample will not pass through the filter, discard the filter and rinse the chamber thoroughly with deionized water. Collect a new sample using a smaller volume of sample, measured to ±1 mL. Be sure to record the actual volume sampled on the sample label and the Sample Collection Form.*
7. Monitor the level of water in the lower chamber to ensure that it does not contact the filter or flow into the pump. Remove the bottom portion of the apparatus and pour off the water from the bottom as often as needed.
8. 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. Seal the cap with plastic electrical tape.
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 the self-sealing plastic bag with the water column and periphyton chlorophyll samples.
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.

13.3.6.5 Cleaning of Periphyton Equipment

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

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 are no markings 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 (with the exception of *Enterococci* labels). Compare sample label information with the information recorded on the corresponding field data forms (e.g., the Sample Collection Form) to ensure accuracy. Make sure that all sample containers are properly sealed.

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 NRSA.

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**.

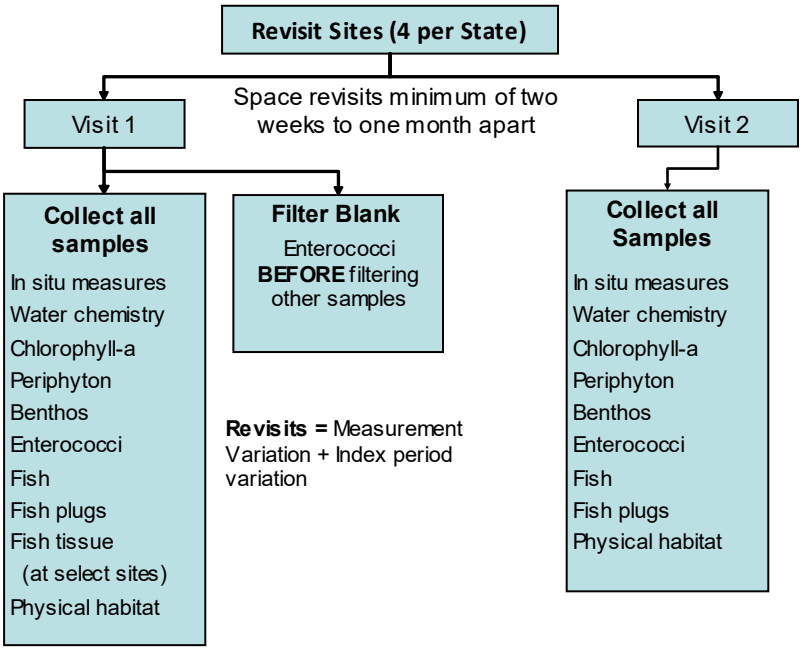


Figure 14.1 Summary of the Revisit Sampling Design

As described **in Sections 11** and **12**, a plug sample consisting of two fish tissue plugs for mercury analysis will be collected from two fish of the same species (one plug per fish) at all sites where suitable fish species and lengths are available ***except*** during any site visit where whole fish tissue samples are collected. Additionally, whole fish tissue samples are to be collected at only ***one*** of the two visits to a revisit site (ideally Visit 1). **Figure 14.2** describes the decision making process regarding the collection of fish plugs versus whole fish tissue.

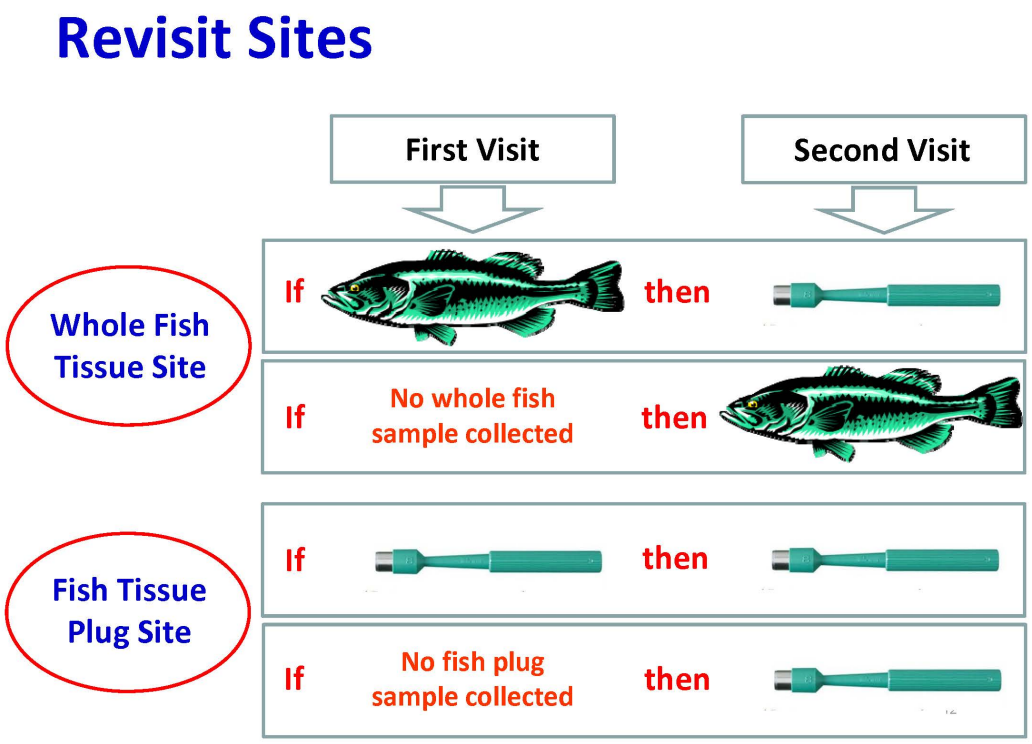


Figure 14.2 Summary of Fish Tissue Protocol for Revisit Sites

14.2 Revisit Sampling Sites

A total of 200 (approximately 10%) of the target sites visited will be revisited during the same field season year by the same field crew that initially sampled the site. Revisit samples and measurements are taken from the same reach as the first visit. **Each state has four revisit sites;** two 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 and NRSA 2013/14 (1-4th order; labeled as Strahler categories Large Stream (LS) or Small Stream (SS)). The base sites are labeled as _08TS3R2 and _13TS2R2, respectively, and are located in the Base/Oversample panel of the Resampled Streams tab within the site evaluation spreadsheet.

Non-Wadeable Revisit sites:

- The two non-wadeable revisit sites are resamples from the NRSA 2008/09 and NRSA 2013/14 (5th order and above, labeled as Strahler categories RiversOther (RO) or RiversMajor (RM)). The base sites are labeled as _08TS3R2 and _13TS2R2, respectively, and are located in the Base/Oversample panel of the Resampled Rivers tab within the site evaluation spreadsheet.

If a site selected for revisit sampling is dropped, then the oversample site (which will also carry the _R2 designation) assigned to replace it should be revisited. If there are no _R2 oversample sites remaining in the panel, re-designate the next _R2 Base site in the panel as a Revisit site AND replace the base site with an appropriate oversample 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 the second sampling event at a revisit site. 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. (**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 QAPP for the NRSA. The following sections will focus only on the field evaluation and assistance visits.

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

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 FOM. A plan and checklist for field evaluation and assistance visits have been developed to detail the methods and procedures. The plan and checklist are included in the QAPP. **Table 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 FOM, 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	• EPA Logistics Coordinator will arrange the field evaluation visit with each Field Crew, ideally within the first two weeks of sampling. • The Evaluator will observe the performance of a crew through one complete set of sampling activities. • If the Crew misses or incorrectly performs a procedure, the Evaluator will note it on the checklist and immediately point it out so the mistake can be corrected on the spot. • The Evaluator will review the results of the evaluation with the Field Crew before leaving the site, noting positive practices and problems.
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.

15 REFERENCES

American Red Cross. 1979. *Standard First Aid and Personal Safety*. American National Red Cross. 269 pp.

Baker, J.R., D.V. Peck, and D.W. Sulton (editors). 1997. *Environmental Monitoring and Assessment Program Surface Waters Field Operations Manual for Lakes*. EPA/620/R-97/001. U.S. Environmental Protection Agency, Washington DC.

Dunne, T., and L.B. Leopold. 1978. *Water in environmental planning*. W.H. Freeman, New York. 818 p.

Flotemersch, J.E.1, B.C. Autrey2, and S.M. Cormier1 (editors). 2000. *Logistics of Ecological Sampling on Large Rivers*. U.S. Environmental Protection Agency, Cincinnati OH.

Flotemersch, J.E., J.B.Stribling, and M.J. Paul. 2006. Concepts and Approaches for the Bioassessment of Non-wadeable Streams and Rivers. EPA 600-R-06-127. U.S. Environmental Protection Agency, Cincinnati, OH.

Harrelson, C.C., C.L. Rawlins, and J.P. Potyondy. 1994. *Stream channel reference sites: an illustrated guide to field technique*. USDA Forest Service, General Technical Report RM-245, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado. 61p.

Hosea, R. C., and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails on wading gear. California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005–02, Sacramento.

Kaufmann, P.R., P. Levine, E.G. Robinson, C. Seeliger, and D. Peck. 1999. *Quantifying Physical Habitat in Wadeable Streams.* EPA/620/R-99/003. U.S. Environmental Protection Agency, Washington, D.C.

Lemmon, P.E. 1957. A new instrument for measuring forest overstory density. Journal of Forestry 55:667-669.

Leopold, L.B. 1994. *A view of the river*. Harvard University Press, Cambridge, Massachusetts. 298 p.

Mulvey, M., L. Caton, and R. Hafele. 1992. *Oregon nonpoint source monitoring protocols: stream bioassessment field manual for macroinvertebrates and habitat assessment.* Oregon Department of Environmental Quality, Laboratory Biomonitoring Section. Portland, Oregon.

National Institute for Occupational Safety and Health. 1981. *Occupational Health Guidelines for Chemical Hazards* (Two Volumes). NIOSH/OSHA Publication No. 81-123.

Nelson, J.S., E.J. Crossman, H. Espinosa-Perez, L.T. Findley, C.R. Gilbert, R.N. Lea, and J.D. Williams. 2004. Common and Scientific Names of Fishes from the United States, Canada, and Mexico. American Fisheries Society, Special Publication 29, Bethesda, Maryland.

Ohio EPA. 1990. *Ohio EPA Fish Evaluation Group Safety Manual.* Ohio Environmental Protection Agency, Ecological Assessment Section, Division of Water Quality Planning and Assessment, Columbus, Ohio.

Stauffer, Dr. Jay R., J. Karish and T.D. Stecko. 2001. Guidelines for Using Digital Photos as Fish Vouchers for Pennsylvania Fishes. The Pennsylvania State University and National Park Service.

Stevens, D. L., Jr., and A. R. Olsen. 2004. Spatially-balanced sampling of natural resources in the presence of frame imperfections. Journal of American Statistical Association:99:262-278.

U.S. Coast Guard. 1987. *Federal Requirements for Recreational Boats.* U.S. Department of Transportation, United States Coast Guard, Washington, D.C.

USDA Forest Service, 1995. *A guide to field identification of bankfull stage in the western United States*. Rocky Mountain Forest and Range Experiment Station, Stream Systems Technology Center, Fort Collins, Colorado (31 minute video, closed captioned).

USDA Forest Service, 2002. *Identifying bankfull stage in forested streams in the eastern United States*. Rocky Mountain Forest and Range Experiment Station, Stream Systems Technology Center, Fort Collins, Colorado (46 minute video, closed captioned).

USEPA. 2000a. EPA Quality Manual for Environmental Programs 5360A1. May 2000. http://www.epa.gov/quality/qs-docs/5360.pdf

USEPA. 2000b. EPA Order 5360.1 A2 CHG2, Policy and Program Requirements for Mandatory Agency-wide Quality System, May 5, 2000. http://www.epa.gov/quality/qs-docs/5360-1.pdf

USEPA. 1986. Occupational Health and Safety Manual. Office of Planning and Management U.S. Environmental Protection Agency, Washington, D.C.

Web Pages:

US EPA Aquatic Monitoring Research: http://www.epa.gov/nheerl/arm

NHD Plus: http://www.horizon-systems.com/nhdplus