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Office of Water  
Washington, DC  
EPA 841-B-22-004

# National Rivers and Streams Assessment 2023/24 Quality Assurance Project Plan

Version 1.0  
February 2023



U.S. Environmental Protection Agency  
Office of Wetlands, Oceans, and Watersheds  
1200 Pennsylvania Avenue, NW  
4503T  
Washington, DC 20460

## Approval Page

Management Approvals: Signature indicates approval for the National Rivers and Streams Assessment (NRSA) 2023/24 Quality Assurance Project Plan (QAPP), related Field Operations Manuals and Laboratory Operations Manual.

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Richard Mitchell	Date
National Rivers and Streams Assessment Project Leader	
U.S. EPA Office of Water	

---

Sarah Lehmann	Date
National Rivers and Streams Assessment Project Quality Assurance Coordinator	
U.S. EPA Office of Water	

---

Susan Holdsworth	Date
Chief, Monitoring Branch	
U.S. EPA Office of Water	

---

Bernice L. Smith	Date
Watershed Restoration, Assessment, and Protection Division Quality Assurance Coordinator	
U.S. EPA Office of Water	

---

Cynthia N. Johnson	Date
Office of Wetlands, Oceans, and Watersheds Quality Assurance Officer	
U.S. EPA Office of Water	

## Quality Assurance Project Plan Review & Distribution Acknowledgement & Commitment to Implement the National Rivers and Streams Assessment 2023/24

I/We have read the Quality Assurance Project Plan (QAPP) and the methods manuals for the National Rivers and Streams Assessment listed below. Our agency/organization agrees to abide by its requirements for work performed under the National Rivers and Streams Assessment. Please check the boxes for the appropriate documents.

- Quality Assurance Project Plan*
- Field Operations Manuals*
- Site Evaluation Guidelines*
- Laboratory Operations Manual*

**Field Crew leads:** *I also certify that I attended a NRSA 2023/24 training and that all members of my crew have received training in NRSA protocols*

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Print Name

---

Title

(Cooperator's Principal Investigator)

---

Organization

---

Signature

Date

**Field Crews:** Please return the signed original to the Logistics Contractor. The Logistics Contractor ensures all parties have signed the QA forms, compiles them and submits them to the EPA Project QA Coordinator. Send your forms to: Contract Logistics Coordinator, Chris Turner, Great Lakes Environmental Center, [cturner@glec.com](mailto:cturner@glec.com).

**Labs and others:** Please return the signed original to Kendra Forde who ensures all parties have signed the QA forms, compiles them, and submits them to the EPA QA Coordinator. Send your forms to: Kendra Forde, [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov). US EPA; 1200 Pennsylvania Ave, NW (4503T); Washington, DC 20460.

Retain a copy for your files.

## Version History

QAPP Version	Date Approved	Changes Made
1.0		

## NOTICE

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

*National Rivers and Streams Assessment 2023-2024: Quality Assurance Project Plan EPA-841-B-22-004*

*National Rivers and Streams Assessment 2023-2024: Site Evaluation Guidelines EPA-841-B-22-005*

*National Rivers and Streams Assessment 2023-2024: Wadeable Field Operations Manual*

*EPA-841-B-22-006*

*National Rivers and Streams Assessment 2023-2024: Non-Wadeable Field Operations Manual*

*EPA-841-B-22-007*

*National Rivers and Streams Assessment 2023-20242023/24: Laboratory Operations Manual EPA-841-B-22-008*

This document, the NRSA 2023/24 Quality Assurance Project Plan (QAPP), contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for NRSA 2023/24. The complete QAPP includes this document and its associated Field Operations Manual (FOM), Laboratory Operations Manual (LOM), and Site Evaluation Guidelines (SEG), which together comprise the integrated set of QAPP documents. Methods described in this document are to be used specifically in work relating to the NRSA 2023/24. All project cooperators should follow these guidelines. Mention of trade names or commercial products in this document does not constitute endorsement or recommendation for use. Reference to “FOM” means both Field Operations Manuals —Wadeable, and Non-wadeable – if the associated text applies to both.

The suggested citation for this document is:

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## Acronyms/Abbreviations

A	Absorbance
AFDM	Ash-Free Dry mass
Ascii	American Standard Code for Information Interchange
ASTM	American Society of Testing and Materials
Ca	Calcium
CAS	Chemical Abstract Service
Cl	Chloride
Ct	Threshold Cycle
Cp	Crossing Point
CSDGM	Content Standards for Digital Geospatial Metadata
CSV	Comma separated values
CV	Coefficient of Variation
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DQO	Data Quality Objective
EMAP	Environmental Monitoring and Assessment Program
ENT	Enterococcus
EPA	Environmental Protection Agency
FGDC	Federal Geographic Data Committee
FOIA	Freedom of Information Act
FOM	Field Operations Manual
FR	Federal Registry
FTP	File Transfer Protocol
GIS	Geographic Information System
GPS	Global Positioning Device
HQ	Head Quarters
IBD	Ionic Balance Difference
IQG	Information Quality Guideline
IM	Information Management
ITIS	Integrated Taxonomic Information System
K	Potassium
LDL	Lower Detection Limit
LIMS	Laboratory Information management System
LOM	Lab Operations Manual
LRL	Lower Reporting Limit
LT	Long Term
ISO	International Organization for Standardization
Mdb	a file-extension used in certain versions of Microsoft Access databases
MDL	Method Detection Levels (limit)
Mg	Magnesium
MMI	Multimetric Index
MQO	Measurement Quality Objective
MRLC	Multi-Resolution Land Characteristics
Na	Sodium
NABS	North American Benthological Society
NACEC	North American Commission for Environmental Cooperation
NAD	North American Datum
NAPA	National Association of Public Administration
NARS	National Aquatic Resource Surveys
NAWQA	National Water-Quality Assessment Program

NCCA	National Coastal Condition Assessment
NELAC	National Environmental Laboratory Accreditation Conference
NELAP	National Environmental Laboratory Accreditation Program
NERL	National Exposure Regional Laboratory
ND	Not Detected
NHD	National Hydrology Database
NH <sub>3</sub>	Ammonia
NH <sub>4</sub>	Ammonium
NIST	National Institute of Standards
NLA	National Lakes Assessment
NLCD	National Land Cover Dataset
NO <sub>2</sub>	Nitrite
NO <sub>3</sub>	Nitrate
NRC	National Research Council
NRSA	National Rivers and Streams Assessment
NTU	Nephelometric Turbidity Units
NWCA	National Wetland Condition Assessment
O/E	“Observed” over “Expected”
OMB	Office of Management and Budget
ORD	Office of Research and Development
OW	Office of Water
PBT	Persistent Bioaccumulative Toxic Chemical
PC	Personal Computer
PctDiff	Percent Difference
PD	Percent Difference
PDE	Percent Difference in Enumeration
PE	Performance Evaluation
PFCs	Perfluorinated chemicals
PPT	Parts per thousand
PRISM	Parameter-elevation Regressions on Independent Slopes Model
PTD	Percent Taxonomic Disagreement
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QA/QC	Quality Assurance/Quality Control
QC	Quality Control
QPCR	Quantitative Polymerase Chain Reaction
QCCS	Quality Control Sample Check
QRG	Quick Reference Guide
R	Statistical software and graphics package
RBS	Relative Bed Stability
RL	Reporting Limit
RSD	Relative Standard Deviation
RTE	Rare, Threatened and Endangered
SAS	Statistical Analysis System
SDTD	Spatial Data Transfer Standard
SEG	Site Evaluation Guideline
SiO <sub>2</sub>	Silica
SO <sub>4</sub>	Sulfate
SOPs	Standard Operating Procedures
SQL	Standard Query Language
SRM	Standard Reference Material
Std	Standard

STORET	Storage and Retrieval Data Warehouse
TOC	Total Organic Carbon
TP	Total Phosphorus
TSS	Total Suspended Solids
USGAO	United States General Accounting Office
USGS	United States Geological Survey
PESD	Western Ecology Division
WSA	Wadeable Streams Assessment
WQX	Water Quality Exchange



## DISTRIBUTION LIST

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

Title	Affiliation	EPA Region/ Office	First Name	Last Name	Email	Phone
<b>EPA Project Lead</b>	EPA	OW	Richard	Mitchell	<a href="mailto:mitchell.richard@epa.gov">mitchell.richard@epa.gov</a>	202-566-0644
<b>EPA Project QA Coordinator</b>	EPA	OW	Sarah	Lehmann	<a href="mailto:lehmann.sarah@epa.gov">lehmann.sarah@epa.gov</a>	202-566-1379
<b>EPA Division QA Coordinator</b>	EPA	OW	Bernice L.	Smith	<a href="mailto:smith.bernicel@epa.gov">smith.bernicel@epa.gov</a>	202-566-1244
<b>EPA OWOW QA Officer</b>	EPA	OW	Cynthia N.	Johnson	<a href="mailto:johnson.cynthian@epa.gov">johnson.cynthian@epa.gov</a>	202-566-1679
<b>EPA Logistics Lead</b>	EPA	OW	Brian	Hasty	<a href="mailto:hasty.brian@epa.gov">hasty.brian@epa.gov</a>	202-564-2236
<b>EPA Laboratory Review Coordinator</b>	EPA	OW	Kendra	Forde	<a href="mailto:forde.kendra@epa.gov">forde.kendra@epa.gov</a>	202-566-0417
<b>NRSA Endangered Species Act Lead</b>	EPA	OW	Lareina	Guenzel	<a href="mailto:Guenzel.lareina@epa.gov">Guenzel.lareina@epa.gov</a>	202-566-0455
<b>Contract Logistics Coordinator</b>	GLEC	--	Chris	Turner	<a href="mailto:cturner@glec.com">cturner@glec.com</a>	715-829-3737
<b>NARS IM Coordinator</b>	EPA	ORD	Karen	Blocksom	<a href="mailto:blocksom.karen@epa.gov">blocksom.karen@epa.gov</a>	541-754-4470
<b>NARS IM Coordinator (Contractor)</b>	GDIT	--	Michelle	Gover	<a href="mailto:gover.michelle@epa.gov">gover.michelle@epa.gov</a>	541-754-4793
<b>ORD Technical Advisor</b>	EPA	ORD	Amanda	Nahlik	<a href="mailto:Nahlik.Amanda@epa.gov">Nahlik.Amanda@epa.gov</a>	541-224-1375

Title	Affiliation	EPA Region/ Office	First Name	Last Name	Email	Phone
<b>Regional Coordinators</b>	EPA	Region 1	Tom	Faber	<a href="mailto:faber.tom@epa.gov">faber.tom@epa.gov</a>	617-918-8672
	EPA	Region 2	Emily	Nering	<a href="mailto:Nering.Emily@epa.gov">Nering.Emily@epa.gov</a>	732-321-6764
	EPA	Region 3	Lou	Reynolds	Reynolds.Louis@epa.gov	304-533-9913
	EPA	Region 3	Leah	Ettema	<a href="mailto:ettema.leah@epa.gov">ettema.leah@epa.gov</a>	304-234-0245
	EPA	Region 4	Elizabeth	Belk	<a href="mailto:belk.elizabeth@epa.gov">belk.elizabeth@epa.gov</a>	404-562-9377
	EPA	Region 4	Jerry	Ackerman	<a href="mailto:Ackerman.Jerry@epa.gov">Ackerman.Jerry@epa.gov</a>	706-355-8721
	EPA	Region 5	Mari	Nord	<a href="mailto:nord.mari@epa.gov">nord.mari@epa.gov</a>	706-355-8721
	EPA	Region 6	Rob	Cook	<a href="mailto:Cook.Robert@epa.gov">Cook.Robert@epa.gov</a>	214-665-7141
	EPA	Region 7	Laura	Webb	<a href="mailto:Webb.Laura@epa.gov">Webb.Laura@epa.gov</a>	913-375-4932
	EPA	Region 8	Liz	Rogers	<a href="mailto:Rogers.Liz@epa.gov">Rogers.Liz@epa.gov</a>	303-312-6974
	EPA	Region 8	Shera	Reems	<a href="mailto:Reems.Shera@epa.gov">Reems.Shera@epa.gov</a>	303-312-6888
	EPA	Region 9	Matt	Bolt	<a href="mailto:Bolt.Matthew@epa.gov">Bolt.Matthew@epa.gov</a>	415-972-3578
	EPA	Region 10	Lil	Herger	<a href="mailto:Herger.Lillian@epa.gov">Herger.Lillian@epa.gov</a>	206-533-1074



# 1 EXECUTIVE SUMMARY

## 1.1 Background

The National Rivers and Streams Assessment (NRSA) 2023/24 effort will provide important information to states and the public about the condition of the nation's river and stream resources and key stressors on a national and regional scale. The United States Environmental Protection Agency (EPA) developed this Quality Assurance Project Plan (QAPP) to support project participants and to ensure that the final assessment is based on data of high quality information. The QAPP contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for NRSA 2023/24. This QAPP is supported by several other NRSA 2023/24 documents listed in the Notice section of this document. They describe in detail the methods for sampling and analysis for all core indicators that are part of the NRSA, and detailed quality control measures are described throughout the QAPP. While NRSA protocols have remained relatively consistent since the first 2008/09 survey, there were a few updates and/or changes made to the field and laboratory protocols for NRSA 2023/24:

- Tissue analysis of mercury, PCBs, and PFAS from fish filets will not be collected.
- Stream discharge measurements in wadeable stream will not be collected.
- Algal toxins will now be collected in a single bottle (PETG) instead of two bottles (PETG and HPED)

All other field and laboratory protocols will be the same as those from NRSA 2018/19.

## 1.2 Quality Assurance Project Plan

The purpose of this QAPP is to document the project data quality objectives and quality assurance/quality control measures that will be implemented in order to ensure that the data collected meets those needs. The plan contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for the NRSA 2023/24 and identifies where these elements are described in detail. This QAPP and its associated documents; the Field Operations Manual, Laboratory Operations Manual and Site Evaluation Guidelines, are interdependent, integrated and collectively make up the full QAPP for the NRSA 2023/24.

## 1.3 Survey Design

Sample collection for NRSA 2023/24 is designed to be completed during the index period of June through the end of September of 2023 and 2024. EPA used an unequal probability design to select approximately 1800 streams and rivers (both wadeable and non-wadeable) from across the 48 conterminous United States. To improve our ability to assess changes, the design includes 790 resample sites that were sampled during the NRSA 2008-2009, NRSA 2013-2014, and/or NRSA 2018-2019. In addition, approximately 200 hand-picked reference sites will be sampled using the same techniques as the probabilistic sites.

## 1.4 Information Management

Environmental monitoring efforts that amass large quantities of information from various sources present unique and challenging data management opportunities. To meet these challenges, the NRSA 2023/24 employs a variety of well-tested information management (IM) strategies to aid in the functional organization and ensured integrity of stored electronic data. IM is integral to all aspects of the

NRSA 2023/24 from initial selection of sampling sites through the dissemination and reporting of final, validated data.

A technical workgroup convened by the Environmental Protection Agency (EPA) Project Leader is responsible for the development of a data analysis plan that includes a verification and validation strategy. These processes are summarized in the data analysis plan section of this QAPP. Validated data are transferred to the central database managed by NARS information management support staff located at the PESD facilities in Corvallis. This database is known as the National Aquatic Resource Surveys Information Management System (NARS IM). All validated measurement and indicator data from the NRSA 2023/24 are eventually transferred to EPA's Water Quality Exchange (WQX) for archival in the [Water Quality Portal](#) for public accessibility. NRSA 2023/24 IM staff provides support and guidance to all program operations in addition to maintaining NARS IM.

## 1.5 Field Operations

Field data acquisition activities are implemented in a consistent manner across the entire country. Each site is assigned a unique ID which identifies it throughout the pre-field, field, laboratory, analysis, and data management phases of the project. Specific procedures for evaluating each sampling location and for replacing non-sampleable sites are documented in NRSA 2023/24 Site Evaluation Guidelines (SEG, *EPA-841-B-22-005*).

NRSA 2023/24 indicators include: in-situ, water chemistry and chlorophyll a, algal toxins (microcystins and cylindrospermopsin), periphyton (ID/enumeration and meta-genomics), benthic macroinvertebrates, fish assemblage, physical habitat, fecal indicators (Enterococci), fish tissue plugs, and antimicrobials. Field measurements and sampling methods are outlined in the NRSA 2023/24 FOMs (*EPA-841-B-22-006* and *EPA-841-B-22-007*). Field crews are trained on these methods at a required EPA-sponsored training session. Field sampling assistance visits will be completed for each field crew for quality assurance purposes.

## 1.6 Laboratory Operations

NRSA 2023/24 laboratory analyses are conducted either by state/tribal-selected laboratories or "National Laboratories" set up by EPA to conduct analyses for any state/tribe which so elects. The designated National Laboratories and state/tribal laboratories must comply with the Quality Assurance/Quality Control (QA/QC) requirements described in this document and in the NRSA 2023/24: Laboratory Operations Manual (LOM, *EPA-841-B-22-008*). Any laboratory selected to conduct analyses with NRSA 2023/24 samples must demonstrate that it can meet the quality standards presented in this NRSA 2023/24 QAPP and LOM.

## 1.7 Peer Review

The NARS program, including the NRSA, utilizes a three-tiered approach for peer review of the Survey.

- internal and external review by USEPA, states, other cooperators and partners;
- external scientific peer review (when applicable); and
- public review (when applicable).

Cooperators have been actively involved in the development of the overall project management, design, indicator selection, and methods. Outside scientific experts from universities, research centers, and other federal agencies have been instrumental in indicator development and will continue to play an important role in data analysis.

## 1.8 Project Overview and Management

Several publications have identified the need for improved water quality monitoring and analysis at multiple scales. In 2000, the General Accounting Office (USGAO 2000) reported that EPA, states, and tribes collectively cannot make statistically valid inferences about water quality (via 305[b] reporting) and lack data to support key management decisions. In 2001, the National Research Council (NRC 2000) recommended EPA, states, and tribes promote a uniform, consistent approach to ambient monitoring and data collection to support core water quality programs. In 2002, the H. John Heinz III Center for Science, Economics, and the Environment (Heinz Center 2002) found that there is not adequate data for national reporting on fresh water, coastal and ocean water quality indicators. The National Association of Public Administrators (NAPA 2002) stated that improved water quality monitoring is necessary to help states and tribes make more effective use of limited resources. EPA's Report on the Environment 2003 (USEPA 2003) states that there is insufficient information to provide a national answer, with confidence and scientific credibility, to the question, 'What is the condition of U.S. waters?'

In response to this need, the Office of Water (OW), in partnership with states and tribes, has begun a program to assess the condition of the nation's waters via a statistically valid approach. The current assessment, the National Rivers and Streams Assessment 2023/24 (referred to as NRSA 2023/24 throughout this document), builds upon the National Rivers and Streams Assessment 2013-2014, the National Rivers and Streams Assessment 2008-2009, the Wadeable Streams Assessment (WSA) implemented by EPA in 2004 to monitor and assess the condition of the nation's wadeable stream resources, as well as other National Aquatic Resource Surveys (NARS) surveys such as the National Coastal Condition Assessment (NCCA), the National Lakes Assessment (NLA) and the National Wetland Condition Assessment (NWCA). The NRSA 2023/24 effort will provide important information to states and the public about the condition of the nation's river and stream resources and key stressors on a national and regional scale. It will also provide trends and a change analysis from the previous assessments.

EPA developed this QAPP to support project participants and to ensure that the final assessment is based on high quality data. The QAPP contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management for NRSA 2023/24. EPA recognizes that states and tribes may add elements to the survey, such as supplemental indicators, that are not covered in the scope of this integrated QAPP. EPA requires that any supplemental elements are addressed by the states, tribes, or their designees, in a separate approved QAPP. This document covers all core NRSA 2023/24 QA activities. The NRSA 2023/24 participants have agreed to follow this QAPP and the protocols and design laid out in this document, and its associated documents – the NRSA 2023/24 Field Operations Manuals (FOMs), Lab Operations Manual (LOM), and Site Evaluation Guidelines (SEG).

This cooperative effort between states, tribes, and federal agencies makes it possible to produce a broad-scale assessment of the condition of the Nation's rivers and streams with both a known confidence and scientific credibility. Through this survey, states and tribes have the opportunity to collect data that can be used to supplement their existing monitoring programs or to begin development of new programs.

The NRSA 2023/24 has three main objectives:

- Estimate the current status, trends, and changes in selected trophic, ecological, and recreational indicators of the condition of the nation's rivers and streams with known statistical confidence;

- Seek associations between selected indicators of natural and anthropogenic stresses and indicators of ecological condition; and
- Assess trends and changes from the earlier river and stream assessments under NARS.

The NRSA Steering Committee, comprised of EPA, state, tribal and other partners, decided on a few improvements and changes to the NRSA indicators. Additions in the 2023/24 survey include an antimicrobial supplement research indicator that will be collected via water grab samples to document the extent of antibiotic resistant microbes and genes at a national regional scale. The following indicator from NRSA 2018-2019 are not being sampled or analyzed for the current survey: whole fish samples for fish filet analysis of human health contaminants. This indicator will be evaluated for inclusion in the next survey expected in 2028-2029. Finally, crews will only collect the microcystin sample in a polyethylene terephthalate (PETG) sample container, which is the recommended sampling container by Abraxis, and will not be collecting samples in the high density polyethylene (HDPE) sample containers used in previous survey. EPA determined this is an appropriate change because a comparison study in which EPA and partners collected/analyzed microcystins using both types of bottles in 2018-2019 did not show differences in results between the two types.

### 1.8.1 Project Organization

EPA began planning the NRSA 2023/24 with state, tribal, and other federal partners in 2021 and is continuing this partnership effort. EPA expects to report the results in December 2026 in compliance with the Data Quality Act. The responsibilities and accountability of the various principals and cooperators are described here and illustrated in (Figure 1.1). Overall, the project will be coordinated by the Office of Water (OW) in Washington, DC, with support from ORD's Pacific Ecological Systems Division (PESD) in Corvallis, Oregon. Each EPA Regional Office has identified a Regional EPA Coordinator who is part of the EPA team providing a critical link with state and tribal partners who conduct the field collection of samples and data following NRSA 2023/24 protocols. Cooperators work with their Regional EPA Coordinator to address any technical issues. The NRSA team implements a comprehensive quality assurance (QA) program to ensure data integrity and provide support for the reliable interpretation of the findings from this project. The Project Lead convenes the NRSA Steering Committee and Technical Experts Workgroups to provide the team with support for determining the best and most appropriate approaches for key technical issues, such as: (1) the selection and establishment of reference conditions based on least-disturbed sites and expert consensus for characterizing benchmarks for assessment of ecological condition; (2) selection and calibration of ecological endpoints and attributes of the biota and relationship to stressor indicators; (3) a data analysis plan for interpreting the data and addressing the objectives in a nationwide assessment; and (4) a framework for the reporting of the condition assessment and conveying the information on the ecological status of the nation's rivers and streams.

Contractor support is provided for all aspects of this project. Contractors will provide support ranging from implementing the survey, sampling and laboratory processing, data management, data analysis, and report writing. Cooperators will interact with their Regional EPA Coordinator and the EPA Project Leader regarding contractual services.

The primary responsibilities of the principals and cooperators are as follows:

#### ***EPA NRSA Project Leader: Richard Mitchell, EPA Office of Water***

- Provides overall coordination of the project and makes decisions regarding the proper functioning of all aspects of the project.

- Makes assignments and delegates authority, as needed to other parts of the project organization.
- Leads the NRSA Steering Committee and establishes needed technical workgroups.
- Interacts with EPA Project Team on technical, logistical, and organizational issues on a regular basis.
- Interacts with the EPA Project Team on technical, logistical, and organizational issues on a regular basis.

***EPA NRSA Project QA Coordinator: Sarah Lehmann, EPA Office of Water***

- Provides leadership, development, and oversight of project-level quality assurance for NARS.
- Assembles and provides leadership for a NRSA 2023/24 Quality Team.
- Maintains official, approved QAPP.
- Maintains all training materials and documentation.
- Maintains all laboratory accreditation files.

***EPA Office of Wetlands, Oceans, and Watersheds, QA Coordinator (QAC): Bernice L. Smith, EPA Office of Water***

- Oversees the quality management activities of the Monitoring Branch
- Serves as the contact person for the technical staff and branch chief on quality management activities including reviewing and approving quality assurance review forms, quality management plans, and quality assurance project plans.

***EPA Office of Wetlands, Oceans, and Watersheds, QA Officer (QAO): Cynthia N. Johnson, EPA Office of Water***

- Functions as an independent officer overseeing all quality assurance (QA) and quality control (QC) activities.
- Responsible for ensuring that the QA program is implemented thoroughly and adequately to document the performance of all activities.

***EPA NRSA Field Logistics Coordinator: Brian Hasty, EPA Office of Water***

- EPA employee who functions to support implementation of the project based on technical guidance established by the EPA Project Leader and serves as point-of-contact for questions from field crews and cooperators for all activities.
- Tracks progress of field sampling activities.

***QA Assistance Visit Coordinator: Brian Hasty, EPA Office of Water***

- The EPA employee who will supervise the implementation of the QA audit program; and
- Directs the field audits and ensures the field are adequately trained to correct errors immediately to avoid erroneous data and the eventual discarding of information from the assessment.

***EPA Technical Advisor: Steven Paulsen, EPA ORD***

- Advises the Project Leader on the relevant experiences and technology developed within the Office of Research and Development (ORD) that may be used in this project.
- Facilitates consultations between NRSA personnel and ORD scientists.

***EPA Study Design Manager: Tony Olsen and Mike Dumelle, EPA ORD***

- Provides leadership and oversight of Design Team
- Coordinates w/ Project Manager and Field Logistics Coordinator to develop and manage the Sampling Frame, select sampling locations, and track field evaluation and site reconnaissance.

***EPA Laboratory Oversight Coordinator: Kendra Forde, EPA Office of Water***

- Ensures participating laboratories complete sample analysis following LOM.
- Ensures participating laboratories follow QA activities.
- Ensures data submitted within the specified timelines.
- Coordinates activities of individual lab Task Order Project Officers to ensure methods are followed and QA activities take place.

***National Laboratory Task Order Managers – one for each contract***

- Responsible for managing activities of the national contract laboratories.
- Provides direction to national and State labs on methods, timelines and QA activities to ensure all actions are followed.
- Provides updates to the Project Leader and EPA Laboratory Review Coordinator on the sample processing status of labs and any questions or concerns raised by participating labs in regard to timelines and deliverables.

***Information Management Coordinator: Michelle Gover, GDIT***

- A contractor who functions to support implementation of the project based on technical guidance established by the EPA Project Leader and Alternate EPA Project Leader.
- Oversees all sample shipments and receives data forms from the Cooperators.
- Oversees all aspects of data entry and data management for the project.

***NRSA Endangered Species Act (ESA) Lead: Lareina Guenzel, EPA OW***

- Primary ESA contact for the U.S. Fish and Wildlife Service (FWS) and National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA/NMFS).
- Works with the EPA Project Lead to ensure that survey manuals and protocols include appropriate responses and reporting requirements in the event that a crew encounters federally listed species when conducting field work.
- Prepares the Biological Evaluation to support Section 7 consultations.
- Works with the survey logistics lead to implement the conservation measures, reasonable and prudent measures, and reporting requirements identified in the Biological Opinion.
- Maintains library of NRSA ESA documents.

***Regional EPA Coordinators***

- Assists EPA Project Leader with regional coordination activities.
- Serves on the Technical Experts Workgroup and interacts with Project Facilitator on technical, logistical, and organizational issues on a regular basis.
- Serves as primary point-of-contact for the Cooperators.

***Steering Committee (Technical Experts Workgroup): States, EPA, academics, other federal agencies***

- Provides expert consultation on key technical issues as identified by the EPA Coordination crew and works with Project Facilitator to resolve approaches and strategies to enable data analysis and interpretation to be scientifically valid.

***Cooperator(s): States, Tribes, United States Geological Survey (USGS), others***

- Under the scope of their assistance agreements, plans and executes their individual studies as part of the cross jurisdictional NRSA 2023/24 and adheres to all QA requirements and standard operating procedures (SOPs).
- Interacts with the Grant Coordinator, Project Facilitator and EPA Project Leader regarding technical, logistical, organizational issues.

***Field Sampling Crew Leaders***

- Functions as the senior member of each Cooperator's field sampling crew and the point of contact for the Field Logistics Coordinator.
- Responsible for overseeing all activities of the field sampling crew and ensuring that the Project field method protocols are followed during all sampling activities.

***Field Logistics Coordinator (FLC): Chris Turner, Great Lakes Environmental Center***

- A contractor who functions to support implementation of the project based on technical guidance established by the EPA Field Logistics Coordinator and the Project Leader.
- Serves as point-of-contact for questions from field crews and cooperators for all activities.
- Tracks progress of field sampling activities.

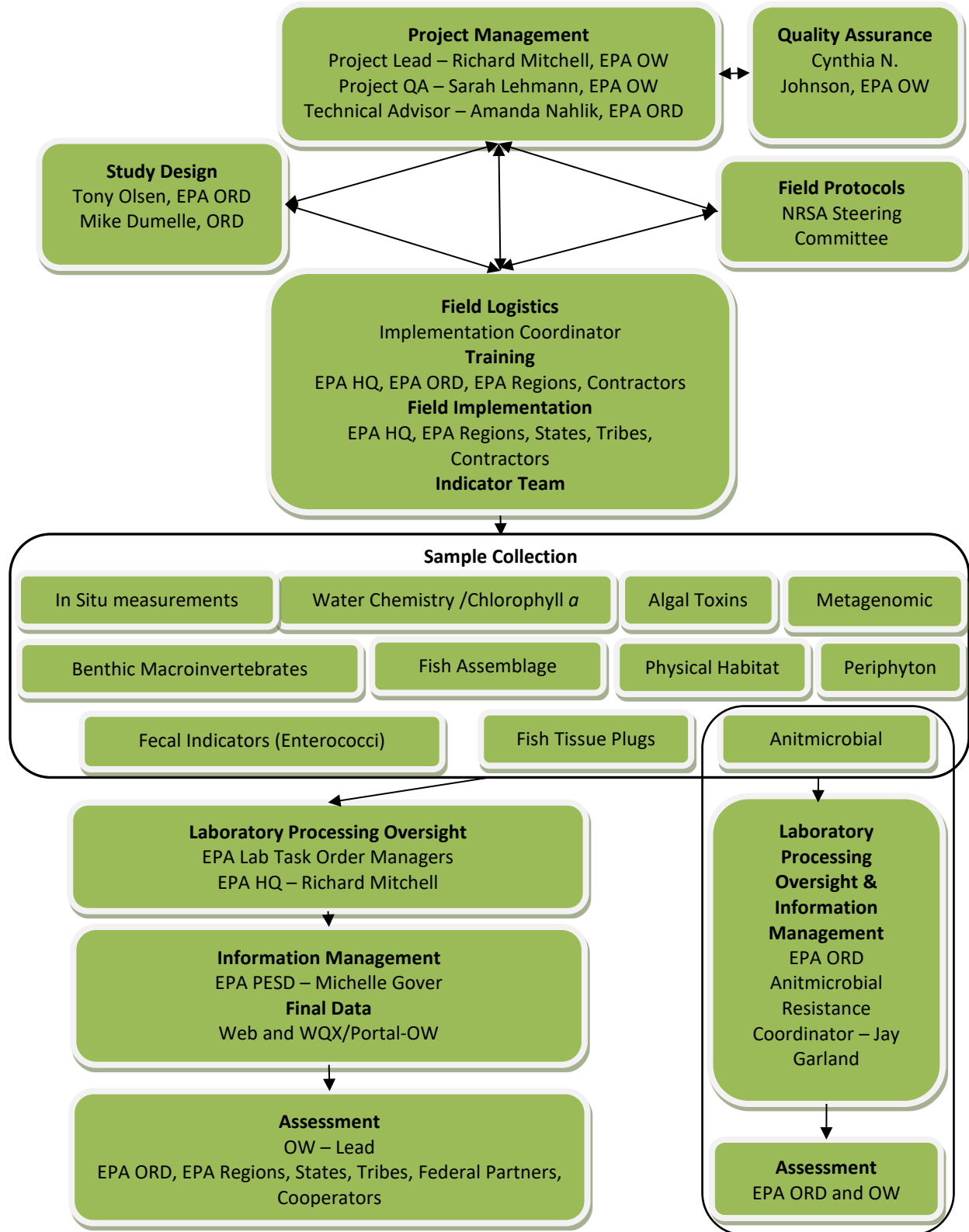


Figure 1.1 Project organization



### 1.8.2 Project Schedule

Training and field sampling will be conducted in 2023 and 2024. Sample processing and data analysis will be completed by 2025 to support a published report in 2026. **Figure 1.2** gives an overview of the major tasks leading up to the final report.

	2021	2022	2023/2024	2024-2025	2026
	research	design	field	lab / data	report
survey planning	- - - -	- -			-
pilot studies		-			
select indicators		- -			
design frame		-			
select sites		-			
implementation			- - - -		
manuals		- -	-		
field training			- -		
sampling season			- -		
sample processing			- - - -	- -	
data analysis				- - -	
draft report					-
peer review					-
final report					- -

**Figure 1.2** Schedule

### 1.8.3 Objectives

The objectives, or design requirements, for the NRSA are to produce:

- Estimates of the 2023/24 status of flowing waters nationally and regionally (9 aggregated Omernik ecoregions);
- Estimates of the 2023/24 status of wadeable streams and non-wadeable rivers nationally and regionally (9 aggregated Omernik ecoregions); and
- Estimates of the change in status in wadeable streams between 2023/24, 2018/19, 2013/14, 2008/09 and 2004, nationally and regionally (9 aggregated Omernik ecoregions) and estimates of the changes in status of all rivers/streams between 2023/2024, 2018/2019 and 2013/14, 2008/09, nationally and regionally (9 aggregated Omernik ecoregions).

Omernik Ecoregions: Ecoregions are areas with generally similar ecosystems and with similar types, qualities, and quantities of environmental resources. The Omernik ecoregion system is hierarchical and considers the spatial patterns of both the living and non-living components of the region. It is broken into 4 levels currently. Ecoregion boundaries were determined by examining patterns of vegetation, animal life, geology, soils, water quality, climate, and human land use, as well as other living and non-living ecosystem components.

#### **1.8.4 Target population**

The target population consists of all streams and rivers within the 48 contiguous states that have flowing water during the study index period excluding portions of tidal rivers up to head of salt defined as 0.5 parts per thousand (ppt) measured in the field. The study index period extends from the beginning of June to the end of September and is characterized by low flow or base flow conditions. State crews that request an early start due to the condition of streams in their area can be granted permission to begin in May with direction from the EPA Project Lead. The target population includes the Great Rivers (i.e. main stem of the Mississippi River). Run-of-the-river ponds and pools are included while reservoirs are excluded (those that have greater than 7 day retention period).

#### **1.8.5 Sample Frame**

The sample frame was derived from the National Hydrography Dataset (NHD), in particular NHD-Plus. Attributes from NHD-Plus and additional attributes added to the sample frame that are used in the survey design include: (1) state, (2) EPA Region, (3) USGS National Water Quality Assessment (NAWQA) Mega Region, (4) Omernik Ecoregion Level 3 (North American Commission for Environmental Cooperation (NACEC) version), (4) WSA aggregated ecoregions (nine and three regions), (5) Strahler order, (6) Strahler order categories (1st, 2nd, ..., 7th and 8th +), (6) FCode, (7) Urban, and (8) Frame07.

#### **1.8.6 Expected sample size**

Expected sample size is 1808 flowing water sites: 790 resample sites and 1018 new sites. The study is designed to sample 1808 probabilistic, 10% of which will be repeat sampled, and 200 hand-picked (potential reference) (approximately 2200 total) river and stream sites across the country.

#### **1.8.7 Oversample**

For the NRSA 2023/24 design, the over sample list of sites is nine times the expected sample size within each state. The large over sample size was done to accommodate those states who may want to increase the number of sites sampled within their state for a state-level design and to provide an adequate number of replacement sites.

#### **1.8.8 Field Protocol Development**

The field sampling protocols for ecological indicators are based on protocols used in the WSA (USEPA 2006b) and the NRSA 2008/09 (USEPA 2016). These protocols were developed by ORD for use in the EMAP<sup>1</sup> program and were developed with the purpose of providing consistent and representative information across the country. During the initial design phase of the project, collaborators and partners worked to refine those protocols for use in NRSA 2008/09, 2013/14 and 2023/24. This involved modifications to the original protocols used in the EMAP program for use in the Great Rivers, tidal systems, and sites that were in between a wadeable and a Non-Wadeable system. Field protocols for collection of fish for fish tissue are based on protocols from EPA's Office of Science and Technology (USEPA 2000).

#### **1.8.9 Information Management**

The first stage of data processing will be to take the input from each of the responsible laboratories and enter them into a common database for final verification and validation. Once the final data sets are

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<sup>1</sup> Environmental Monitoring and Assessment Program (EMAP.) <http://www.epa.gov/emap/>.

made available for the assessment, copies of the data will be transferred to EPA’s Water Quality Exchange /Storage and Retrieval Data Warehouse (WQX/ STORET) and EPA’s NARS Information Management (NARS IM) dataset for long-term storage and access. Working copies of the final data sets will be distributed to the States and Cooperators and maintained at PESD for analysis leading to the assessment.

### 1.8.10 Assessment

The final assessment will be developed by a team, led by OW, which will include several ORD research facilities, EPA Regional Monitoring Coordinators, interested States/Tribes, and Cooperators. All States/Tribes will be invited to participate in a collaborative process to interpret results and shape the data assessment and report. The final assessment will include an appendix describing the quality of the data used in the assessment.

## 1.9 Scope of QA Project Plan

This QAPP addresses all aspects of the data acquisition efforts of the NRSA, which focuses on the 2023 and 2024 sampling of approximately 2200 river and stream sampling events in the contiguous United States. This QA plan also addresses the data integration necessary to create one complete report on the ecological and human health status of the Nation’s rivers and streams based on selected indicators.

Relevant Companion documents to this QAPP are: NRSA 2023/24: Site Evaluation Guidelines, NRSA 2023/24: Field Operations Manuals, and NRSA 2023/24: Laboratory Operations Manual (See introductory pages for citation information for each document).

### 1.9.1 Overview of Field Operations

Field data acquisition activities are implemented for the NRSA (**Table 1.1**), based on guidance developed for earlier Environmental Monitoring and Assessment Program (EMAP) studies (Baker and Merritt 1990). Survey preparation is initiated with selection of the sampling locations by the EMAP Design group (PESD in Corvallis). The list of sampling locations is distributed to the EPA Regional Monitoring Coordinators and all cooperators. With the sampling location list, Cooperator’s field crews can begin site reconnaissance on the primary sites and alternate replacement sites and begin work on obtaining access permission to each site. Specific procedures for evaluating each sampling location and for replacing non target sites are documented in the SEG. Scientific collecting permits from State and Federal agencies will be procured, as needed by the respective State or cooperating organization.

**Table 1.1 Critical logistics elements (from Baker and Merritt, 1990)**

Logistics Plan Component	Required Elements
<b>Project Management</b>	Overview of Logistic Activities Staffing and Personnel Requirements Communications
<b>Access and Scheduling</b>	Sampling Schedule Site Access Reconnaissance
<b>Safety</b>	Safety Plan Waste Disposal Plan
<b>Procurement and Inventory Control</b>	Equipment, Supplies, and Services Requirements Procurement Methods and Scheduling

Logistics Plan Component	Required Elements
<b>Training and Data Collection</b>	Training Program Field Operations Scenario Laboratory Operations Scenarios Quality Assurance Information Management
<b>Assessment of Operations</b>	Field Crew Debriefings Logistics Review and Recommendations

### ***1.9.1.1 Equipment and Supplies***

The field crews will use standard field equipment and supplies which are being provided by EPA and EPA’s Field Logistics Coordinator (FLC). The FLC will work with Regional Monitoring Coordinators, Cooperators, States, and Contractors to make certain the field crews have the equipment and supplies they require in a timely fashion. Detailed lists of equipment required for each field protocol, as well as guidance on equipment inspection and maintenance, are contained in the FOMs.

### ***1.9.1.2 Request Form***

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

### ***1.9.1.3 Base Kit***

The Base Kit is comprised of the subset of durable equipment and supplies needed for NRSA 2023/24 sampling that is provided by USEPA through the FLC. Typically, one Base Kit is provided to each Field Crew and contains some of the equipment that is used throughout the field season. See FOMs for a list of the items provided by USEPA in the Base Kit. We anticipate that this equipment will be available for use in future NRSA efforts.

### ***1.9.1.4 Site Kit***

A Site Kit contains the subset of consumable supplies (i.e., items used up during sampling or requiring replacement after use) provided by USEPA through the FLC. The site kit for core indicators will contain all the sample bottles and labels necessary for sampling a single site. A new Site Kit is provided for each site sampled See FOMs for the consumable items that will be provided by USEPA.

### ***1.9.1.5 Field Crew Supplied Items***

The field crew will also supply particular items for the field sampling day. These are typical field equipment (like a Global Positioning Device (GPS)), or boat equipment and might also include supplies from the previous surveys. See FOMs for the items that the field crew will need to provide.

### ***1.9.1.6 Site Evaluation Guidelines***

The NRSA 2023/24 Site Evaluation Guidelines (SEG) outlines the process to compile the final list of candidate sites for sampling. The process includes locating a candidate river/stream, evaluating the site

to determine if it meets the criteria for inclusion in the target population and is accessible for sampling, and if not, replacing it with an alternate candidate river/stream.

#### **1.9.1.7 Lab Operations Manual**

The methods used for the laboratory sample analysis are available in the NRSA 2023/24 Laboratory Operations Manual (LOM).

#### **1.9.1.8 Field Training**

Field measurements and samples are collected by trained crews. Each Field Crew Leader and the fish taxonomist, must be trained at an EPA-sponsored training session prior to the start of the field season, along with as many crew members as possible. If the Field Crew Leader is also the fish taxonomist for the crew, then it would be preferable that at least one additional crew member attend the training session. EPA will provide the four-day training sessions in a number of locations around the country for cooperators and contractors. It is required that field crews attend all four days of training in their entirety. The training program stresses hands-on practice of methods, comparability among crews, collection of high quality data and samples, and safety. All field crews providing field operational support to NRSA 2023/24 must adhere to the provisions of this integrated QAPP, FOM, and SEG. Trainers will maintain a list of all personnel trained and provide the information to the NRSA Project Lead and the QA Project Lead. Training documentation will be maintained by the NARS QA Lead in NRSA 2023/24 QA files. **Field crews may not operate without a trained field crew leader and another trained field crew member present. Personnel conducting Assistance Visits of field crews must also attend the complete training.**

#### **1.9.1.9 Health and Safety**

Collection and analysis of samples can involve significant risks to personal health and safety. All field crews should develop a safety plan according to the requirements of their organization. Additional information on health and safety can be found in the FOM. It is the responsibility of the group safety officer or project leader from participating organizations, however, to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed.

#### **1.9.1.10 Field Quality Evaluation and Assistance Reviews (auditing)**

Each crew will be visited by a trained person from an EPA Region, Headquarters, or contractor. Evaluation and assistance visits will be conducted with each Field Crew early in the sampling and data collection process, and corrective actions will be conducted in real time. These visits provide EPA with a basis for the uniform evaluation of the data collection techniques, and an opportunity to conduct procedural reviews to minimize data loss due to improper technique or interpretation of program guidance. The field visit evaluations will be based on the uniform training, plans, and checklists.

#### **1.9.1.11 Field Activities**

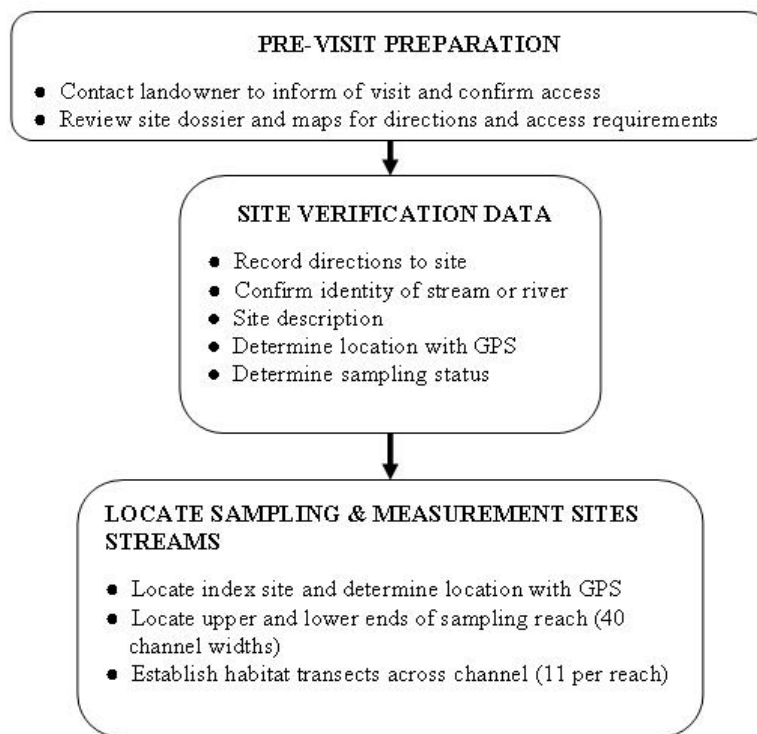
Typically, each field crew is comprised of four to five members. The number and size of crews depends on the duration of the sampling window, geographic distribution of sampling locations, number and complexity of samples and field measurements, and other factors. A variety of methods may be used to access a site. Some sampling locations require crews to hike in, transporting all equipment in backpacks. For this reason, ruggedness and weight are important considerations in the selection of equipment and instrumentation. Crews may need to camp out at the sampling location and may need to provide themselves with the necessary camping equipment.

For each sampling location, a dossier will be prepared by the field crew and contains the following applicable information: road maps, copies of written access permissions, scientific collection permits, coordinates of index sites, information brochures on the program for interested land owners, a topographic map with the index site location marked, and local area emergency numbers. Crew leaders will contact landowners at least two days before the planned sampling date. As the design requires repeat visits to select sampling locations, it is important for the field crews to do everything possible to maintain good relationships with landowners. This includes prior contacts, respect of special requests, closing gates, minimal site disturbance, and removal of all materials including flagging and trash.

The site verification process is shown in **Figure 1.4**. Upon arrival at a site, the location is verified by a GPS receiver, landmark references, and/or local contacts.

Samples and measurements for various indicators are collected in a specified order (see example work flows in **Figure 1.5** and **Figure 1.6**). This order has been set up to minimize the impact of sampling for one indicator upon subsequent indicators; for example, water chemistry samples from rivers and streams are collected before collecting benthic invertebrates as the benthic invertebrate method calls for kicking up sediments which would likely impact the quality of the water sample. Crews may choose to allocate resources as they see fit, but should always be careful not to compromise samples. All methods are fully documented in step-by-step procedures in the NRSA FOMs.

### Site Verification Activities



**Figure 1.3** River and stream field surveys: site verification activities

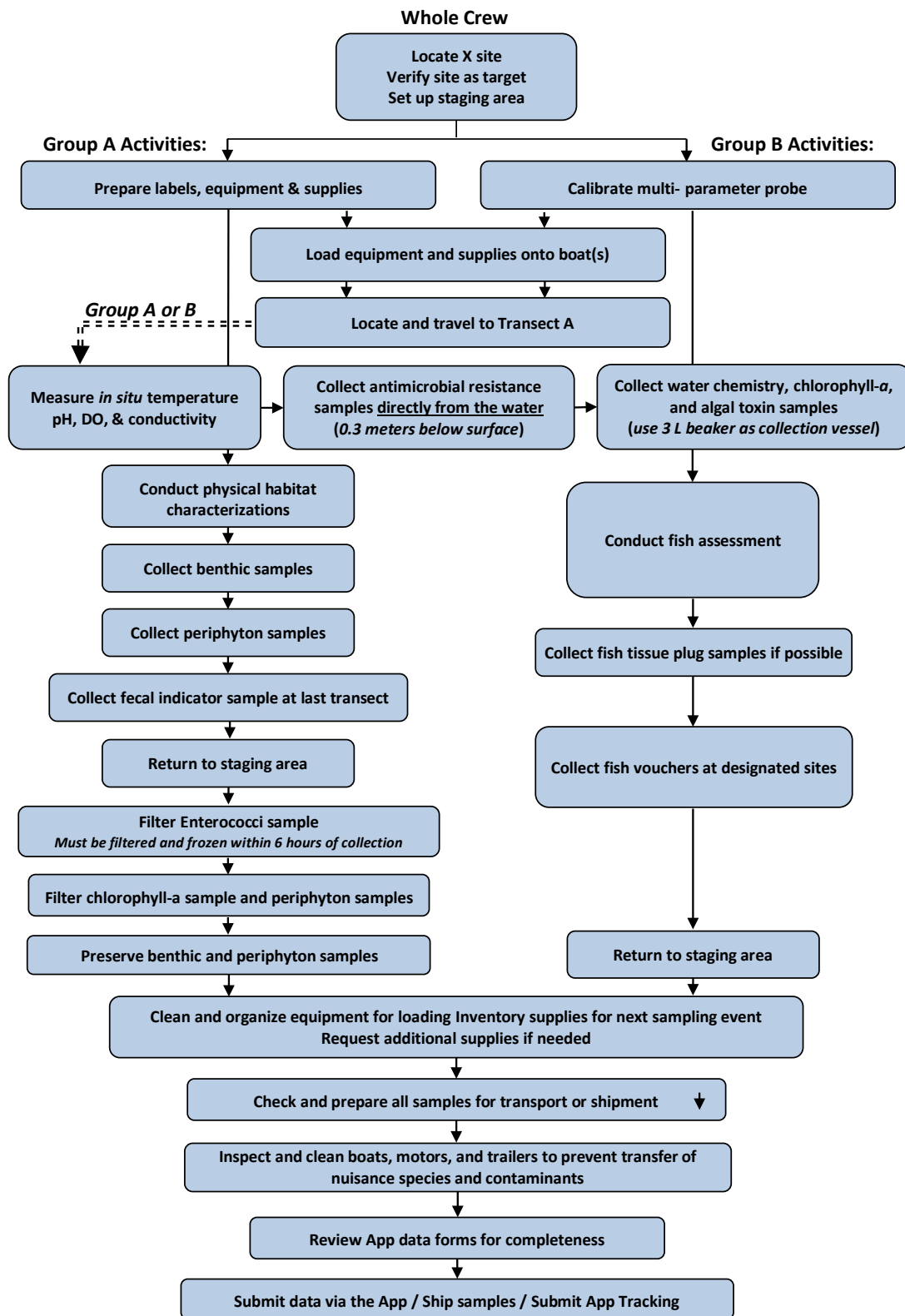


Figure 1.4 Non-Wadeable river and stream sampling: summary of field activities





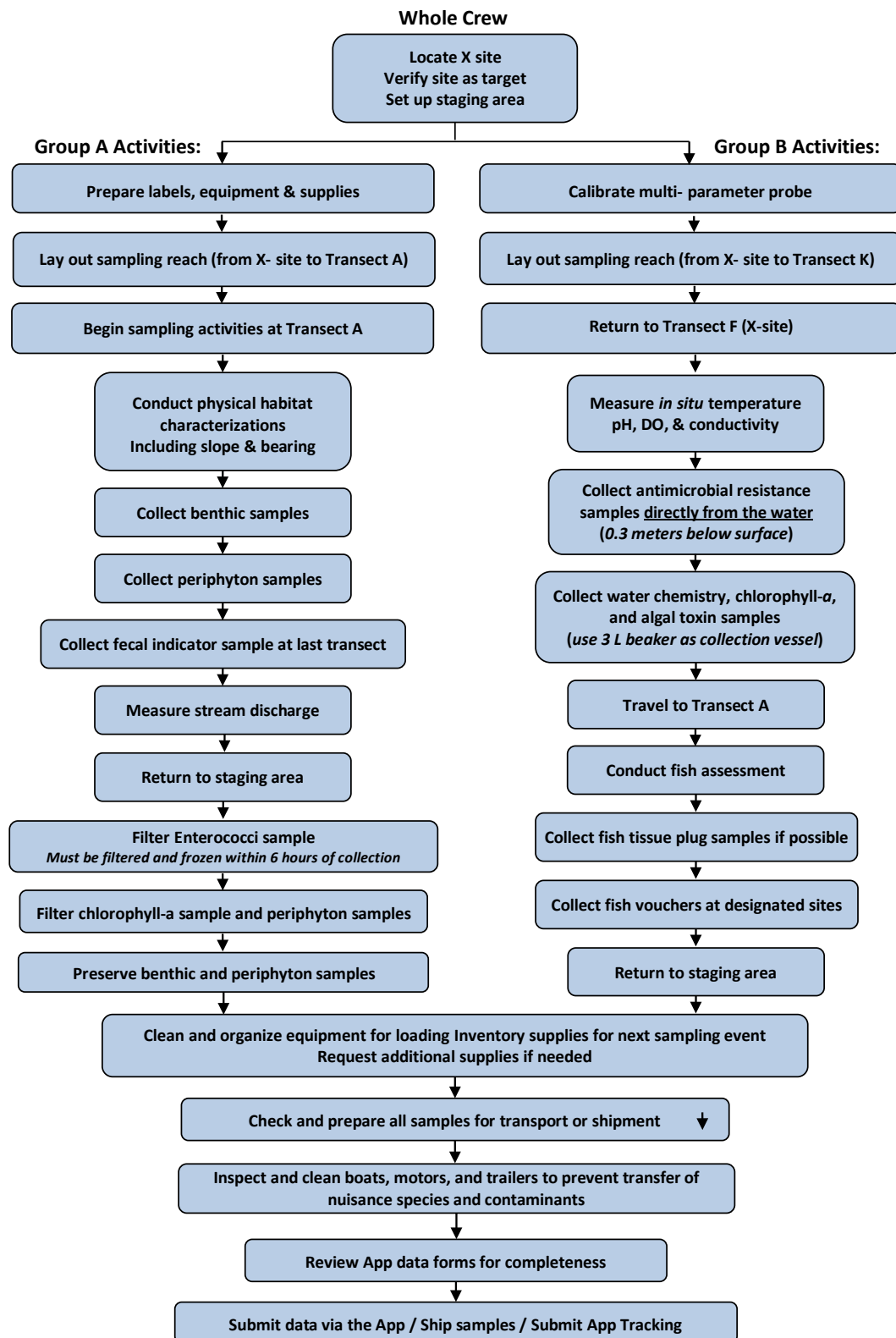


Figure 1.5 Wadeable stream sampling: summary of field activities

The FOMs also contain detailed instructions for completing documentation, labeling samples, any field processing requirements, and sample storage and shipping. Field communications will be through Field Crew Leaders, and will involve regularly scheduled conference calls or contacts with the NRSA 2023/24 Communications Center.

Standardized field data forms are the primary means of data recording. For NRSA 2023/24, crews will have the option to use paper or electronic forms. On completion, the data forms are reviewed by a person other than the person who initially entered the information. Prior to departure from the field site, the field crew leader reviews all forms and labels for completeness and legibility and ensures that all samples are properly labeled and packed. This review process will be done for either form of data collection (paper or electronic).

Upon return from field sampling to the office, field crews using paper forms send completed data forms to the information management staff at PESD in Corvallis, Oregon for entry into a computerized database. Field crews using electronic forms send completed forms via email as soon as they have internet access. At PESD, the IM team review electronic data files independently to verify that values are consistent with those recorded on the field data form or original field data file (Section 4.4.2).

Field crews store or package samples for shipment in accordance with instructions contained in the FOMs, including taking precautions to ensure holding times are not exceeded. Samples which must be shipped are delivered by field crews to a commercial carrier; copies of bills of lading or other documentation are maintained by the crew. Using the pertinent tracking form, crews notify the NARS IM Center about sample shipment; thus, tracking procedures can be initiated quickly in the event samples are not received. Chain-of-custody forms are completed by the crews for all transfers of samples, with copies maintained by the field crew. The FLC or NARS IM team will follow up with field crews about any missing samples and/ or incomplete files.

The field operations phase is completed with collection of all samples or expiration of the sampling window. Following completion of all sampling, a debriefing session will be scheduled (Table 1.1). These debriefings cover all aspects of the field program and solicit suggestions for improvements.

### 1.9.2 Overview of Laboratory Operations

Holding times for surface water samples vary with the sample types and analyte. Field crews begin some analytical measurements during sampling (e.g., *in situ* measurements) while other analytical measurements are not initiated until sampling has been completed (e.g., water chemistry, algal toxins, fecal indicators (Enterococci)). Analytical methods are summarized in the LOM. When available, standard methods are used and are referenced. Where experimental methods are used or standard methods are modified, these methods are documented in the laboratory methods manual or in internal documentation, and the laboratory coordinator will work with appropriate experts to describe them in Standard Operating Procedures (SOPs) developed by the analytical laboratories.

Contractor and/or cooperator laboratories will perform chemical, physical, and biological analyses. National contract labs will process most samples. Where those labs are currently in place, EPA has identified them here. PESD, a lab managed by Judy Greydanus, will analyze water chemistry and chlorophyll-a samples. A national contract lab, GLEC, will analyze algal toxin samples. EPA anticipates that a few pre-approved state labs may opt to analyze samples for algal toxins. A national contract lab, Ecoanalysts, will conduct benthic macroinvertebrate identifications as will a few pre-approved state labs. A national contract lab, GLEC, will conduct periphyton identifications as will a few pre-approved State labs. EPA's National Exposure Research Laboratory (NERL) will analyze samples for enterococci and the periphyton meta-genomics indicators. A national contract lab, Physis Environmental Laboratories,

Inc. will analyze fish tissue plugs, and fish tissue filet samples will be stored at the national contract lab, Microbac Lab, until analytical labs are identified. A national contractor, ESS Group, Inc., will conduct fish identification vouchers for quality control purposes as will a few pre-approved state labs/fish taxonomists. Field crews record the physical habitat measurements in the field on the field data sheets. Field crews send data from the forms either electronically, if using the NRSA electronic form application, or by mail, if using the hard copy forms to the NARS IM team. The NARS IM team uploads data provided electronically or scans field forms into the NARS IM database.

Laboratories providing analytical support must have the appropriate facilities to properly store and prepare samples and appropriate instrumentation and staff to provide data of the required quality within the time period dictated by the project. Laboratories are expected to conduct operations using good laboratory practices. The following are general guidelines for analytical support laboratories:

- A program of scheduled maintenance of analytical balances, water purification systems, microscopes, laboratory equipment, and instrumentation.
- Verification of the calibration of analytical balances using ASTM class 1 or 2 weights which have are National Institute of Standards and Technology (NIST) traceable certificates.
- Verification of the calibration of top-loading balances using NIST-certified ASTM class 3 weights or better.
- Checking and recording the composition of fresh calibration standards against the previous lot of calibration standards. Participating laboratories will keep a percentage of the previous lot of calibration standard to check against the next batch of samples processed. This will ensure that a comparison between lots can occur. Acceptable comparisons are less than or equal to two percent of the theoretical value. (This acceptance is tighter than the method calibration criteria.)
- Recording all analytical data in bound logbooks in ink, or on standardized recording forms.
- Verification of the calibration of uniquely identified daily use thermometers using NIST-certified thermometers.
- Monitoring and recording (in a logbook or on a recording form) temperatures and performance of cold storage areas and freezer units (where samples, reagents, and standards may be stored). During periods of sample collection operations, monitoring must be done on a daily basis.
- An overall program of laboratory health and safety including periodic inspection and verification of presence and adequacy of first aid and spill kits; verification of presence and performance of safety showers, eyewash stations, and fume hoods; sufficiently exhausted reagent storage units, where applicable; available chemical and hazardous materials inventory; and accessible material safety data sheets for all required materials.
- An overall program of hazardous waste management and minimization, and evidence of proper waste handling and disposal procedures (90-day storage, manifested waste streams, etc.).
- If needed, having a source of reagent water meeting American Society of Testing and Materials (ASTM) Type I specifications for conductivity ( $< 1 \mu\text{S}/\text{cm}$  at  $25^\circ\text{C}$ ; ASTM 2011) available in sufficient quantity to support analytical operations.
- Appropriate microscopes or other magnification for biological sample sorting and organism identification.
- Approved biological identification and taxonomic keys/guides for use in biological identification (diatoms, benthic macroinvertebrates) as appropriate.
- Labeling all containers used in the laboratory with date prepared contents, and initials of the individual who prepared the contents.

- Dating and storing all chemicals safely upon receipt. Chemicals are disposed of properly when the expiration date has expired.
- Using a laboratory information management system to track the location and status of any sample received for analysis.
- Reporting results electronically using standard formats and units compatible with NARS IM (see LOM for data templates). These files will be labeled properly by referencing the indicator and/or analyte and date.

All laboratories providing analytical support to NRSA 2023/24 must adhere to the provisions of this integrated QAPP and LOM. Laboratories will provide information documenting their ability to conduct the analyses with the required level of data quality prior to data analysis. Different requirements will be provided based on the type of analysis being completed by the laboratory (i.e. chemistry vs. biological analyses).

Laboratories will send the documentation to the **EPA Laboratory Oversight Coordinator** (or other such designated parties) in NRSA 2023/24 QA files. Such information may include the following, depending on the evaluation by the Project Quality Assurance Coordinator

- Signed Quality Assurance Project Plan by the laboratory performing analysis;
- Signed Laboratory Form;
- Valid Accreditation or Certification;
- Laboratory's Quality Manual and/or Data Management Plan;
- Method Detection Limits (MDL);
- Demonstration of Capability (DOC);
- Results from inter-laboratory comparison studies;
- Analysis of performance evaluation samples; and
- Control charts and results of internal QC sample or internal reference sample analyses to Document achieved precision, bias, accuracy.

Other requirements may include:

- Participation in calls regarding laboratory procedures and processes with participating laboratories;
- Participation in a laboratory technical assessment or audit;
- Participation in performance evaluation studies; and
- Participation in inter-laboratory sample exchange.

See Section 5 of this QAPP for additional information related to laboratory certification. All qualified laboratories shall work with the NARS IM Center to track samples as specified by the NARS IM Lead.

#### ***1.9.2.1 Water Chemistry and Chlorophyll A Lab Quality Evaluation***

Participating laboratories will send requested documentation to the NRSA 2023/24 QA Team for evaluation of qualifications. The NRSA 2023/24 QA Team will maintain these records in the project QA file.

#### ***1.9.2.2 Biological Laboratory Quality Evaluation***

The NRSA 2023/24 Quality Team requested and, whenever possible, reviewed the past performance of biological laboratories. The biological laboratories shall adhere to the quality assurance objectives and requirements as specified for the pertinent indicators in the LOM.

### 1.9.3 Data Analysis and Reporting

A technical data analysis and reporting workgroup convened by the EPA Project Leader is responsible for development of a data analysis plan that includes a verification and validation strategy. These processes are summarized in the data analysis sections of this QAPP.

Validated data are transferred to the central database, the National Aquatic Resource Surveys Information Management database, NARS IM, managed by IM support staff located at PESD in Corvallis. IM activities are discussed further in Section 4. Data in the NARS IM database are available to cooperators for use in development of indicator metrics. All validated measurement and indicator data from NRSA 2023/24 are eventually transferred to EPA's Water Quality Exchange (WQX) and then the National STORET warehouse. The periphyton meta-genomics data, as research data, will not be incorporated into NARS IM.

### 1.9.4 Peer Review

If deemed necessary, the NRSA 2023/24 report will undergo a thorough peer review process, where the scientific community and the public will be given the opportunity to provide comments. Cooperators have been actively involved in the development of the overall project management, design, methods, and standards including the drafting of five key project documents:

- Quality Assurance Project Plan
- Site Evaluation Guidelines
- Field Operations Manuals (Wadeable and Non-wadeable)
- Laboratory Operations Manual

The USEPA NARS program, including the NRSA 2023/24, utilizes a three-tiered approach for peer review of the Survey: (1) internal and external review by EPA, states, other cooperators and partners, (2) external scientific peer review, when applicable, and (3) public review, when applicable.

Once data analysis has been completed, cooperators examine the results. The NRSA team reviews comments and feedback from the cooperators and incorporate such feedback into the draft report, when appropriate. The NRSA Project Team follows Agency and OMB requirements for public and peer review. External scientific peer review and public review is initiated for new analyses or approaches as appropriate. Additionally, following applicable guidance other aspects of the NRSA may undergo public and scientific peer review.

- Follow the Agency's Information Quality Guidelines (IQG) and complete the IQG checklist.
- Develop and maintain a public website with links to standard operating procedures, quality assurance documents, fact sheets, scientific peer review feedback, and final report.
- Conduct technical workgroup meetings composed of scientific experts, cooperators, and EPA to evaluate and recommend data analysis options and indicators.
- Complete data validation on all chemical, physical and biological data.
- Conduct final data analysis with workgroup to generate assessment results.
- Engage peer review contractor to identify external peer review panel (if applicable).
- Develop draft report presenting assessment results.
- Develop final draft report incorporating input from cooperators and results from data analysis group to be distributed for peer a review.
- Issue Federal Register (FR) Notice announcing document availability and hold public comment (30-45 days) (if applicable).
- Consider public comments and produce a final report (if applicable).

The proposed peer review schedule is provided below in **Table 1.2** and is contingent upon timeliness of data validation and schedule availability for regional meetings and experts for data analysis workshop.

**Table 1.2 Proposed schedule**

Proposed Schedule	Activity
May 2024– November 2024	Data validation
December 2024-June 2025	Internal data analysis and review meetings (e.g., web conferences)
June 2025	Draft released for external peer review (if applicable)
December 2025	Draft released for public review (if applicable)

## 2 DATA QUALITY OBJECTIVES

It is a policy of the U.S. EPA that Data Quality Objectives (DQOs) be developed for all environmental data collection activities following the prescribed DQO Process. DQOs are qualitative and quantitative statements that clarify study objectives, define the appropriate types of data, and specify the tolerable levels of potential decision errors that will be used as the basis for establishing the quality and quantity of data needed to support decisions (USEPA 2006). Data quality objectives thus provide the criteria to design a sampling program within cost and resource constraints or technology limitations imposed upon a project or study. DQOs are typically expressed in terms of acceptable uncertainty (e.g., width of an uncertainty band or interval) associated with a point estimate at a desired level of statistical confidence (USEPA 2006). The DQO Process is used to establish performance or acceptance criteria, which serve as the basis for designing a plan for collecting data of sufficient quality and quantity to support the goals of a study (USEPA 2006). As a general rule, performance criteria represent the full set of specifications that are needed to design a data or information collection effort such that, when implemented, it will generate newly-collected data that are of sufficient quality and quantity to address the project's goals (USEPA 2006). Acceptance criteria are specifications intended to evaluate the adequacy of one or more existing sources of information or data as being acceptable to support the project's intended use (USEPA 2006).

### 2.1 Data Quality Objectives for the NRSA

Target DQOs established for the NRSA 2023/24 relate to the goal of describing the current status of selected indicators of the condition of rivers and streams in the conterminous U.S. and ecoregions of interest.

The formal statement of the DQO for national estimates is as follows:

- Estimate the proportion of rivers/streams ( $\pm 5\%$ ) in the conterminous U.S. that fall below the designated threshold for good conditions for selected measures with 95% confidence.

For the ecoregions of interest the DQO is:

- Estimate the proportion of rivers/streams ( $\pm 15\%$ ) in a specific ecoregion that fall below the designated threshold for good conditions for selected measures with 95% confidence.

For estimates of change, the DQOs are:

- Estimate the proportion of rivers/ streams ( $\pm 7\%$ ) in the conterminous U.S. that have changed condition classes for selected measures with 95% confidence.

### 2.2 Measurement Quality Objectives

For each indicator, performance objectives (associated primarily with measurement error) are established for several different attributes of data quality (Smith et al., 1988). Specific objectives for each indicator are presented in the indicator section of this QAPP. The following sections define the data quality attributes and present approaches for evaluating them against acceptance criteria established for the program.

#### 2.2.1 Method Detection Limits

For chemical measurements, requirements for the method detection limit (MDL) are established. The MDL is defined as the lowest level of analyte that can be distinguished from zero with 99% confidence

based on a single measurement (1) (Glase et al., 1981). The MDL for an individual analyte is calculated as:

**Equation 2.1** 
$$MDL = t_{[\alpha=0.01, \nu=n-1]} \times S$$

where  $t$  is a Student's  $t$  value at a significance level ( $\alpha$ ) of 0.01 and  $n-1$  degrees of freedom ( $\nu$ ), and  $s$  is the standard deviation of a set of  $n$  measurements of a standard solution. Standard solutions should contain analyte concentrations between two and three times the MDL objective, and should be subjected to the entire analytical method (including any preparation or processing stages). At least seven non-consecutive replicate measurements of a standard solution are required to calculate a valid estimate of the MDL. Replicate analyses of the standard should be conducted over a period of several days (or several different calibration curves) to obtain a long-term (among-batch) estimate of the MDL.

Laboratories shall periodically monitor MDLs on a per batch basis. Suggested procedures for monitoring MDLs are: (1) to analyze a set of serial dilutions of a low level standard, determining the lowest dilution that produces a detectable response; and (2) repeated analysis (at least seven measurements) of a low-level standard within a single batch.

Laboratories must submit estimates of Reporting Limits (RLs) (and how they are determined) with analytical results. Laboratories must flag analytical results associated with RLs that exceed the objectives as being associated with unacceptable RLs. Laboratories must report analytical data that are below the estimated RLs, but above the laboratory's MDL, but laboratories also flag these as "estimated" values (detected but not quantified). Laboratories should report (if possible), values below the MDL, but the laboratory must flag the value as being below the MDL. If a laboratory has to report values below the MDL as being equal to the MDL, this must be clearly stated in the metadata submitted with any analytical results to avoid the misuse of these results in assessment analyses. Sampling Precision, Bias, and Accuracy

Precision and bias are estimates of random and systematic error in a measurement process (Kirchmer, 1983; Hunt and Wilson, 1986). Collectively, precision and bias provide an estimate of the total error or uncertainty associated with an individual measurement or set of measurements. Systematic errors are minimized by using validated methodologies and standardized procedures. Precision is estimated from repeated measurements of samples. Net bias is determined from repeated measurements of solutions of known composition, or from the analysis of samples that have been fortified by the addition of a known quantity of analyte. For analytes with large ranges of expected concentrations, objectives for precision and bias are established in both absolute and relative terms, following the approach outlined in Hunt and Wilson, 1986. At lower concentrations, objectives are specified in absolute terms. At higher concentrations, objectives are stated in relative terms. The point of transition between an absolute and relative objective is calculated as the quotient of the absolute objective divided by the relative objective (expressed as a proportion, e.g., 0.10 rather than as a percentage, e.g., 10%). Final estimates will be calculated by the analysis staff at PESD.

Precision in absolute terms is estimated as the sample standard deviation when the number of measurements is greater than two:

**Equation 2.2** 
$$SD = \sqrt{\frac{\sum_{i=1}^n (xi - \bar{x})^2}{n-1}}$$

Where:



$x$  is the value of the replicate,  
 $\bar{x}$  is the mean of repeated sample measurements, and  
 $n$  is the number of replicates.

Relative precision for such measurements is estimated as the relative standard deviation (RSD, or coefficient of variation, [CV]):

**Equation 2.3** 
$$RSD = \frac{s}{\bar{x}} \times 100$$

Where:

$s$  is the sample standard deviation of the set of measurements, and  
 $\bar{x}$  equals the mean value for the set of measurements.

Precision based on duplicate measurements is estimated based on the range of measured values (which equals the difference for two measurements). The relative percent difference (RPD) is calculated as:

**Equation 2.4** 
$$RPD = \left( \frac{|A - B|}{(A + B)/2} \right) \times 100$$

Where:

$A$  is the first measured value, and  
 $B$  is the second measured value.

Precision objectives based on the range of duplicate measurements can be calculated as:

**Equation 2.5** 
$$\text{Critical Range} = s \times \sqrt{2}$$

Where:

$s$  represents the precision objective in terms of a standard deviation. Range-based objectives are calculated in relative terms as:

**Equation 2.6** 
$$\text{Critical RPD} = RSD \times \sqrt{2}$$

Where:

$RSD$  represents the precision objectives in terms of a relative standard deviation.

For repeated measurements of samples of known composition, net bias ( $B$ ) is estimated in absolute terms as:

**Equation 2.7** 
$$B = \bar{x} - T$$

Where:

$\bar{x}$  equals the mean value for the set of measurements and  
 $T$  equals the theoretical or target value of a performance evaluation sample.

Bias in relative terms [ $B(\%)$ ] is calculated as:

**Equation 2.8** 
$$B(\%) = \frac{\bar{X} - T}{T} \times 100$$

Where:

$\bar{x}$  equals the mean value for the set of measurements, and

$T$  equals the theoretical or target value of a performance evaluation sample.

Accuracy is estimated for some analytes from fortified or spiked samples as the percent recovery. Percent recovery is calculated as:

**Equation 2.9** 
$$\% \text{ recovery} = \frac{C_{is} - C_i}{C_s} \times 100$$

Where:

$C_{is}$  is the measured concentration of the spiked sample,

$C_i$  is the concentration of the unspiked sample, and

$C_s$  is the concentration of the spike.

### 2.2.2 Taxonomic Precision and Accuracy

For the NRSA, taxonomic precision will be quantified by comparing whole-sample identifications completed by independent taxonomists or laboratories. Accuracy of taxonomy will be qualitatively evaluated through specification of target hierarchical levels (e.g., family, genus, or species); and the specification of appropriate technical taxonomic literature or other references (e.g., identification keys, voucher specimens). To calculate taxonomic precision, 10% of the biological samples from each participating laboratory will be randomly-selected by EPA HQ, and sent to an independent taxonomist for re-identification. Comparison of the results of whole sample re-identifications will provide a Percent Taxonomic Disagreement (PTD) calculated as:

**Equation 2.10** 
$$PTD = \left[ 1 - \left( \frac{comp_{pos}}{N} \right) \right] \times 100$$

Where:

$comp_{pos}$  is the number of agreements, and

$N$  is the total number of individuals in the larger of the two counts.

The lower the PTD, the more similar are taxonomic results and the overall taxonomic precision is better. A measurement quality objective (MQO) of 15% is recommended for taxonomic difference or disagreement (overall mean  $\leq 15\%$  is acceptable based on similar projects) for benthic macroinvertebrates and fish. Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, and the reasons for disagreement investigated. Periphyton and algal samples have a higher PTD due to the variance amongst species (perhaps as much as 50%).

Sample enumeration is another component of taxonomic precision with macroinvertebrates. Sample enumeration agreement will be checked with the same 10% of samples used to check taxonomic precision. Final specimen counts for samples are dependent on the taxonomist, not the rough counts obtained during the sorting activity. Comparison of counts is quantified by calculation of percent difference in enumeration (PDE), calculated as:

**Equation 2.11** 
$$PDE = \left( \frac{|Lab1 - Lab2|}{Lab1 + Lab2} \right) \times 100$$

An MQO of 5% is recommended (overall mean of  $\leq 5\%$  is acceptable) for several biological samples, while others will have higher PDE's. This is based on the laboratory approaches used and the nature of the indicator.

Corrective actions for samples exceeding these MQOs can include defining the taxa for which re-identification may be necessary (potentially even by third party), for which samples (even outside of the 10% lot of QC samples) it is necessary, and where there may be issues of nomenclatural or enumeration problems. Taxa lists will be changed when disagreements are resolved by a third party.

Taxonomic accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts, usually contract or university affiliated persons who have peer-reviewed publications for the taxonomic group they are reviewing. Samples will be identified using the most appropriate technical literature that is accepted by the taxonomic discipline and reflects the accepted nomenclature. The Integrated Taxonomic Information System (ITIS, <http://www.itis.gov/>), Encyclopedia of Life (EOL) or the Catalogue of Life (COL) will be used to verify nomenclatural validity and reporting. A reference collection will be compiled by each lab as the samples are identified. Specialists in several taxonomic groups will verify selected individuals of different taxa, as determined by the NRSA workgroup.

### 2.2.3 Completeness

Completeness requirements are established and evaluated from two perspectives. First, valid data for individual indicators must be acquired from a minimum number of sampling locations in order to make subpopulation estimates with a specified level of confidence or sampling precision. The objective of this study is to complete sampling at 95% or more of the 1800 initial sampling sites and the 200 reference sites. Percent completeness is calculated as:

**Equation 2.12** 
$$\%C = V / T \times 100$$

Where:

$V$  = number of measurements/samples judged valid, and

$T$  = total number of planned measurements/samples.

Within each indicator, completeness objectives are also established for individual samples or individual measurement variables or analytes. These objectives are estimated as the percentage of valid data obtained versus the amount of data expected based on the number of samples collected or number of measurements conducted. Where necessary, supplementary objectives for completeness are presented in the indicator-specific sections of this QAPP.

### 2.2.4 Comparability

Comparability is defined as the confidence with which one data set can be compared to another (Stanley and Verner, 1985; Smith et al., 1988). For all indicators, comparability is addressed by the use of standardized training, sampling procedures, sampling equipment and analytical methodologies by all sampling crews and laboratories. These are also the same used to collect data in EMAP West and WSA studies. Comparability of data within and among indicators is also facilitated by the implementation of

standardized quality assurance and quality control techniques and standardized performance and acceptance criteria. For all measurements, reporting units and format are specified, incorporated into standardized data recording forms, and documented in the information management system. Comparability is also addressed by providing results of QA sample data, such as estimates of precision and bias. If some incomparability between sampling crews comes to light, the data collected by those crews will be evaluated and possibly rejected.

### **2.2.5 Representativeness**

Representativeness is defined as "the degree to which the data accurately and precisely represent a characteristic of a population parameter, variation of a property, a process characteristic, or an operational condition" (Stanley and Verner, 1986, Smith et al., 1988). At one level, representativeness is affected by problems in any or all of the other attributes of data quality.

At another level, representativeness is affected by the selection of the target surface water bodies, the location of sampling sites within that body, the time period when samples are collected, and the time period when samples are analyzed. The probability-based sampling design should provide estimates of condition of surface water resource populations that are representative of the region. The individual sampling programs defined for each indicator attempt to address representativeness within the constraints of the sampling design and index sampling period. Holding time requirements for analyses ensure analytical results are representative of conditions at the time of sampling. Use of QC samples which are similar in composition to samples being measured provides estimates of precision and bias that are applicable to sample measurements.

### 3 SURVEY DESIGN

The survey design for the NRSA 2023/24 is the same as used for the previous NRSA 2018/19 and EMAP-West plus the Great Rivers and the tidal system surveys. The design is a sample survey design (a.k.a. probability design) that ensures a representative set of sample sites from which inferences can be made about the target population. For the NRSA, the target population represents perennial rivers and streams in the conterminous US, excluding sites below the head of salt and reservoirs.

There is a large body of statistical literature dealing with sample survey designs which addresses the problem of making statements about many by sampling the few (e.g., Cochran 1977, Kish 1965, Kish 1987, and Sarndal et al. 1992). Sample surveys have been used in a variety of fields (e.g., election polls, monthly labor estimates, forest inventory analysis, national wetlands inventory) to determine the status of populations (large groups of sites) of interest, especially if the population is too numerous to census or if it is unnecessary to census the population to reach the desired level of precision for describing the population's status. A key point in favor of probability based designs is that they allow lower cost sampling programs because a smaller number of sites are able to support conclusions with known accuracy and precision about status and trends of a region.

The survey designs used in NARS to date have been documented in published reports for each resource group and in the peer reviewed literature. A brief description of the design concepts and the specific application for riverine systems is provided below. Much of this is extracted from various publications and from Stevens (1994) which provides an excellent overview of the design concepts, issues and applications for the entire program. The EMAP sampling design strategy is based on the fundamental requirement for a probability sample of an explicitly defined regional resource population, where the sample is constrained to reflect the spatial dispersion of the population.

#### 3.1 Probability-Based Sampling Design and Site Selection

##### 3.1.1 Target Population

The target population for NRSA 2023/24 includes perennial stream and river channels (natural and constructed) mapped at 1:100,000 scale within the conterminous U.S, excluding sites below the head of salt and reservoirs.

##### 3.1.2 Sample Frame

The NRSA 2023/24 sample frame was derived from the National Hydrography Dataset-Plus (NHD), in particular NHDPlus V2. <sup>2</sup>The National Hydrography Dataset (NHD) is the surface water component of The National Map. The NHD is a digital vector dataset used by geographic information systems (GIS). It contains features such as lakes, ponds, streams, rivers, canals, dams and streamgages. These data are designed to be used in general mapping and in the analysis of surface-water systems. NHDPlus is an integrated suite of application-ready geospatial data sets that incorporate many of the best features of the [National Hydrography Dataset \(NHD\)](#), the [National Elevation Dataset \(NED\)](#), and the [Watershed Boundary Dataset \(WBD\)](#). Further information about the codes used within the NHD-Plus can be found on the NHD webpage (<http://www.horizon-systems.com/NHDPlus/index.php>).

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<sup>2</sup> These refer to the old digital line graph file codes used in the NHD. These codes are: rapid, stream, braided stream, aqueduct, and canal.

This frame is subdivided into two major parts: (1) all National Hydrography Database (NHD)-Plus stream, river and canal segments coded as perennial, and (2) all NHD-Plus stream, river and canal segments coded as non-perennial, i.e., all other stream, river and canal segments. The purpose of subdividing the frame is to allow a sampling focus on systems that have an exceedingly high probability of being flowing waters during the index sampling period.

Sites were selected for the NRSA project using a hierarchical randomization design process described by Stevens and Olsen (1999, 2003, 2004). The NHD served as the frame representing streams and rivers in the US. Data from approximately 1800 river and stream sites in the United States will be used in the assessment and sampled over a two year index period. This total sample size will allow national reporting as well as regional reporting at the scale of 9 aggregated Omernik Level III ecoregions, the ten EPA Regions and 10-15 major drainage basins. Several States have added additional sites to be able to report on the condition of streams and/or rivers within their boundaries.

Key features of the approach are (1) utilizing survey theory for continuous populations within a bounded area, (2) explicit control of the spatial dispersion of the sample through hierarchical randomization, (3) unequal probability of selection by Strahler order, and (4) nested subsampling to incorporate intensified sampling in special study regions.

### **3.1.3 Revisit and Resample Sites**

Of the sites visited in the field and found to be target sites, a total of 10% will be revisited. The 10% are designated by the EPA for each State - two wadeable and two non-wadeable per State. The primary purpose of this revisit set of sites is to allow variance estimates that would provide information on the extent to which the population estimates might vary over the sampling season.

In addition, 790 sites from the NRSA 2018/19 will be resampled during the 2023 and 2024 sampling season to evaluate change from the previous NRSAs and the WSA.

### **3.1.4 Evaluation of Sites**

The number of sites that must be evaluated to achieve the expected number of field sites that can be sampled can only be estimated based on assumptions concerning expected error rates in Reach File version 3.0, percent of landowner refusals, and percent of physically inaccessible sites. Based on the estimates gained in previous studies, a list of alternate sites was selected at the same time as the base sites. These alternate sites will be used in order until the desired sample size designated for the state has been achieved.

## **3.2 Hand-picked Site Selection**

EPA selected a set of potential reference sites to sample in NRSA 2023/24. This hand-picked set of candidate sites comes from the previous pool of reference sites sampled in previous NRSA and EMAP surveys. There will be approximately 100 sites selected with 10 site for each of the 10 EPA regions. All sites will be sampled in both years for a total of 200 sampling events. The purpose of these sites will be to set up a core set of reference sites that will be sampled during each future NRSA survey to track changes in reference condition at a national and regional scale.

## 4 INFORMATION MANAGEMENT

Environmental monitoring efforts that amass large quantities of information from various sources present unique and challenging data management opportunities. To meet these challenges, the NRSA 2023/24 employs a variety of well-tested information management (IM) strategies to aid in the functional organization and ensured integrity of stored electronic data. IM is integral to all aspects of the NRSA 2023/24 from initial selection of sampling sites through the dissemination and reporting of final, validated data. And, by extension, all participants in the NRSA 2023/24 have certain responsibilities and obligations which also make them a part of the IM system. This “inclusive” approach to managing information helps to:

- Strengthen relationships among NRSA 2023/24 cooperators;
- Increase the quality and relevance of accumulated data; and
- Ensure the flexibility and sustainability of the NRSA 2023/24 IM structure.

This IM strategy provides a congruent and scientifically meaningful approach for maintaining environmental monitoring data that will satisfy both the scientific and technological requirements of the NRSA 2023/24.

### 4.1 Roles and Responsibilities

At each point where data and information are generated, compiled, or stored, the NRSA 2023/24 IM team must manage the information (**Table 4.1**). Thus, the IM system includes all of the data-generating activities, all of the means of recording and storing information, and all of the processes that use data. The IM system also includes electronic means of generating, storing, organizing and archiving data, and the effort to achieve a functional IM process is all encompassing. *To that end, all participants in the NRSA 2023/24 play an integral part within the IM system.* The following table provides a summary of the IM responsibilities identified by NRSA 2023/24 group. Specific information on the field crew responsibilities for tracking and sending information is found in the FOMs.

**Table 4.1 Summary of IM responsibilities.**

NRSA 2023/24 Group	Contact	Primary Role	Responsibility
<b>Field Crews</b>	State/tribal partners and contractor or other field crews (regional EPA, etc.)	Acquire in-situ measurements and prescribed list of biotic/abiotic samples at each site targeted for the survey	<p>Complete and review field data forms and sample tracking forms for accuracy, completeness, and legibility.</p> <p>Email/Ship/fax field and sample tracking forms to NARS IM Center so information can be integrated into the central database</p> <p>Work with the NARS IM Center staff to develop acceptable file structures and electronic data transfer protocols should there be a need to transfer and integrate data into the central database</p> <p>Provide all data as specified in FOM, SEG or as negotiated with the NRSA Project Leader.</p> <p>Maintain open communications with NARS IM Center regarding any data issues</p>
<b>Analytical Laboratories</b>	State/tribal partners and contractors	Analyze samples received from field crews in the manner appropriate to acquire biotic/abiotic indicators/measurements requested.	<p>Review all electronic data transmittal files for completeness and accuracy (as identified in the QAPP).</p> <p>Work with the NARS IM Center staff to develop file structures and electronic data transfer protocols for electronically-based data.</p> <p>Submit completed sample tracking forms to NRSA 2023/24 IM Center so information can be updated in the central database</p> <p>Provide all data and metadata as specified in the laboratory transmittal guidance section of the LOM, with specific templates for each indicator or as negotiated with the NRSA Project Leader.</p> <p>Maintain open communications with NRSA 2023/24 IM Center regarding any data issues.</p>
<b>IM Center staff</b>	USEPA ORD Pacific Ecology Systems Division, (PESD), Contractors	Provides support and guidance for all IM operations related to maintaining a central data management system for NRSA 2023/24	<p>Develop/update field data forms (electronic and paper versions).</p> <p>Plan and implement electronic data flow and management processes.</p> <p>Manage the centralized database and implement related administration duties.</p> <p>Receive, scan, and conduct error checking of field data forms.</p> <p>Monitor and track samples from field collection, through shipment to appropriate laboratory.</p> <p>Receive data submission packages (analytical results and metadata) from each laboratory.</p> <p>Run automated error checking, e.g., formatting differences, field edits, range checks, logic checks, etc.</p> <p>Receive verified, validated, and final indicator data files (including record changes and reason for change) from QA reviewers. Maintain history of all changes to data records from inception through delivery to WQX.</p> <p>Organize data in preparation for data verification and validation analysis and public dissemination.</p> <p>Implement backup and recovery support for central database.</p> <p>Implement data version control as appropriate.</p>



NRSA 2023/24 Group	Contact	Primary Role	Responsibility
<b>Project Quality Assurance Coordinator</b>	USEPA Office Of Water	Review and evaluate the relevancy and quality of information/data collected and generated through the NRSA 2023/24 surveys.	<p>Monitor quality control information.</p> <p>Evaluate results stemming from field and laboratory audits.</p> <p>Investigate and take corrective action, as necessary, to mitigate any data quality issues.</p> <p>Issue guidance to NRSA 2023/24 Project Leader and IM Center staff for qualifying data when quality standards are not met or when protocols deviate from plan.</p>
<b>Steering Committee</b>	NRSA Project Lead and other team members, EPA Regional and ORD staff, States, tribes, other federal agencies	Provide technical recommendations related to data analysis, reporting and overall implementation	<p>Provide feedback and recommendations related to QA, data management, analysis, reporting and data distribution issues</p> <p>Review and comment on QA and information management documentation (QAPP, data templates, etc.).</p>
<b>Data Analysis and Reporting Team</b>	USEPA Office of Water, ORD PESD, Partners	Provide the data analysis and technical support for NRSA 2023/24 reporting requirements	<p>Provide data integration, aggregation and transformation support as needed for data analysis.</p> <p>Provide supporting information necessary to create metadata.</p> <p>Investigate and follow-up on data anomalies using identified data analysis activities.</p> <p>Produce estimates of extent and ecological condition of the target population of the resource.</p> <p>Provide written background information and data analysis interpretation for report(s).</p> <p>Document in-depth data analysis procedures used.</p> <p>Provide mapping/graphical support.</p> <p>Document formatting and version control.</p> <p>Develops QA report for management.</p>
<b>Data Finalization Team</b>	TBD	Provides data librarian support	<p>Prepare NRSA 2023/24 data for transfer to USEPA public web-server(s).</p> <p>Generate data inventory catalog record (Science Inventory Record).</p> <p>Ensure all metadata is consistent, complete, and compliant with USEPA standards.</p>

#### 4.1.1 State/ Tribe-Based Data Management

Some state and tribal partners will be managing activities for both field sampling and laboratory analyses and may prefer to handle data management activities in-house. While the NARS program encourages states and tribes to use these in-house capabilities, it is imperative that NRSA 2023/24 partners understand their particular role and responsibilities for executing these functions within the context of the national program. If a state or tribe chooses to do IM in-house, the state or tribe must perform all of the functions associated with the following roles:

- Field Crew—including shipping/faxing of field data forms to the IM Coordinator (NRSA 2023/24 paper or electronic field forms must be used and the original field forms must be sent to the NARS IM Center as outlined in the NRSA 2023/24 FOM).
- Quality Control Team for laboratory data.
- NRSA QA Project Coordinator for ensuring that laboratory results meet specified QA requirements.
- All data will flow from the state or tribe to the NARS IM Center. Typically, the state or tribe will provide a single point of contact for all things related to NRSA 2023/24 data. However, it may be advantageous for the NARS IM Center staff to have direct communication with the state or tribe participating laboratories to facilitate the transfer of data, a point that may be negotiated between the primary state or tribal contact, the regional coordinator and the NRSA 2023/24 Project Leader (with input from the NARS IM Center staff).
- Data transfers to the NARS IM Center must be timely. States and tribes must submit all initial laboratory results (i.e., those that have been verified by the laboratory and have passed all internal laboratory QA/QC criteria) in the appropriate format to NARS IM Center by May 2024 (for 2023 data) and May 2025 (for 2024 data), in order to meet NRSA 2023/24 product deadlines.
- Data transfers must be complete. For example, laboratory analysis results submitted by a state or tribe must be accompanied by related quality control and quality assurance data, qualifiers code definitions, contaminant/parameter code cross-references/descriptions, test methods, instrumentation information and any other relevant laboratory-based assessments or documentation related to specific analytical batch runs.
- The state or Tribe will ensure that data meet minimum quality standards and that data transfer files meet negotiated content and file structure standards.

The NARS IM Center will provide the necessary guidance for IM requirements. Each group that will perform in-house IM functions will incorporate these guidelines as is practicable or as previously negotiated.

## 4.2 Overview of System Structure

In its entirety, the NARS IM system includes site selection and logistics information, sample labels and field data forms, tracking records, mapping and analytical data, data validation and analysis processes, reports, and archives. NARS IM staff provides support and guidance to all program operations in addition to maintaining a central database management system for the NRSA data.

The central repository for data and associated information collected for use by NRSA 2023/24 is a secure, access-controlled server located at PESD-Corvallis. This database is known as the NARS IM. Data are stored and managed on this system using the Structured Query Language (SQL). Data review (e.g., verification and validation) and data analysis (e.g., estimates of status and extent) are accomplished primarily using programs developed in either Statistical Analysis System (SAS) or 'R' language software packages.

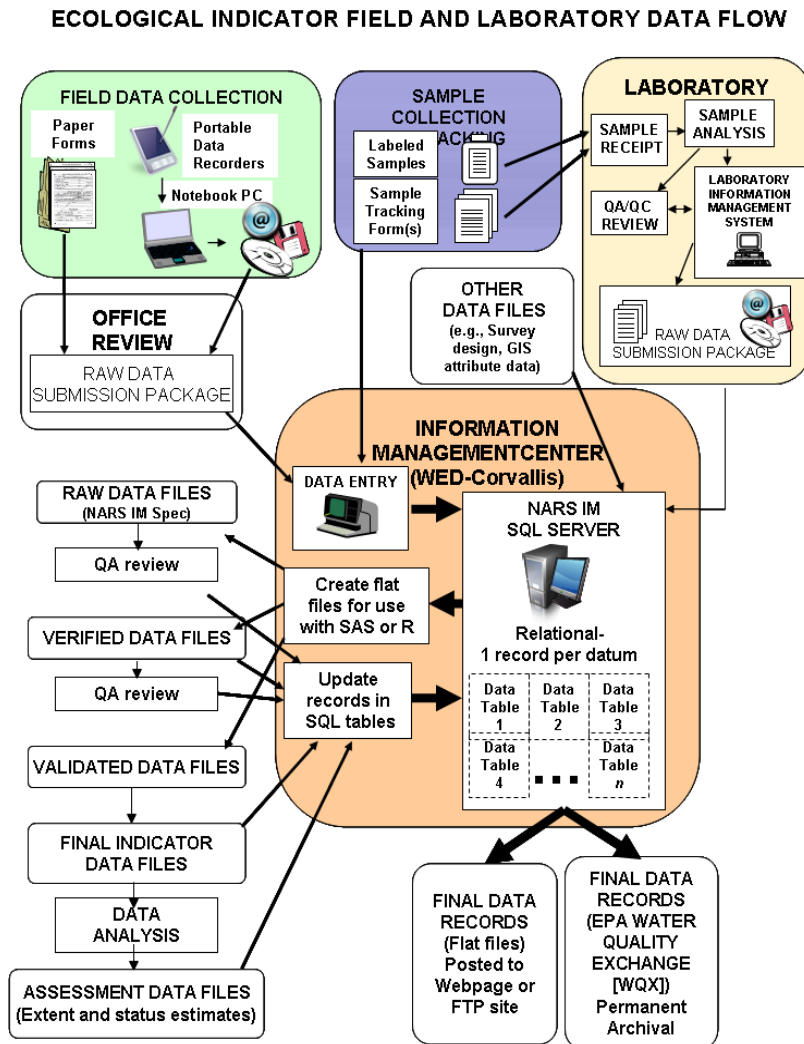
### 4.2.1 Data Flow

The NRSA 2023/24 will accumulate large quantities of observational and laboratory analysis data. To manage this information appropriately, it is essential to have a well-defined data flow model and documented approach for acquiring, storing, and summarizing the data. This conceptual model (**Figure 4.1**) helps focus efforts on maintaining organizational and custodial integrity, ensuring that data available for analyses are of the highest possible quality.

#### 4.2.2 Simplified Description of Data Flow

There are several components associated with the flow of information, these are:

- Communication between the NARS IM Center and the various data contributors (e.g., field crews, laboratories and the data analysis and reporting team) is vital for maintaining an organized, timely, and successful flow of information and data.
- Data are captured or acquired from four basic sources; field data transcription, laboratory analysis reporting, automated data capture, and submission of external data files (e.g., Geographic Information Systems (GIS) data) encompassing an array of data types (site characterization, biotic assessment, sediment and tissue contaminants, and water quality analysis). Data capture generally relies on the transference of electronic data, e.g., optical character readers and email, to a central data repository. However, some data must be transcribed by hand in order to complete a record.
- Data repository or storage provides the computing platform where raw data are archived, partially processed data are staged, and the “final” data, assimilated into a final, user-ready data file structure, are stored. The raw data archive is maintained in a manner consistent with providing an audit trail of all incoming records. The staging area provides the IM Center staff with a platform for running the data through all of its QA/QC paces as well as providing data analysts a first look at the incoming data. This area of the data system evolves as new data are gathered and user-requirements are updated. The final data format becomes the primary source for all statistical analysis and data distribution.
- Metadata—a descriptive document that contains information compliant with the Content Standards for Digital Geospatial Metadata (CSDGM) developed by the Federal Geographic Data Committee (FGDC).



**Figure 4.1 Conceptual model of data flow into and out of the master SQL**

The following sections describe core information management standards, data transfer protocols, and data quality and results validation. Additionally, Section 4.4 describes the major data inputs to the central database and the associated QA/QC processes used to record, enter, and validate measurement and analytical data collected.

### 4.2.3 Core Information Management Standards

The development and organization of the NARS IM system is compliant with current EPA guidelines and standards. Areas addressed by these policies and guidelines include, but are not limited to, the following:

- Taxonomic nomenclature and coding;
- Locational data;
- Sampling unit identification and reference;
- Hardware and software; and
- Data catalog documentation.

NRSA 2023/24 is committed to compliance with all applicable regulations and guidance concerning hardware and software procurement, maintenance, configuration control, and QA/QC. To that end, the NRSA 2023/24 team has adopted several IM standards that help maximize the ability to exchange data within the study and with other aquatic resource surveys or similar large-scale monitoring and assessment studies (e.g. NARS, past EMAP and R-EMAP studies). Specific information follows.

#### **4.2.4 Data Formats**

##### **4.2.4.1 Attribute Data**

- SQL Tables;
- SAS Data Sets;
- R Data Sets<sup>3</sup>; and
- American Standard Code for Information Interchange (Ascii) Files: Comma-Separated values, or space-delimited, or fixed column.

##### **4.2.4.2 GIS Data**

- ARC/INFO native and export files; compressed .tar file of ARC/INFO workspace

##### **4.2.4.3 Standard Coding Systems**

- Sampling Site: (EPA Locational Data Policy; EPA 1991);
- Coordinates: Latitude and Longitude in decimal degrees ( $\pm 0.002$ );
- Datum: NAD83;
- Chemical Compounds: Chemical Abstracts Service (CAS 1999) (<http://www.cas.org/>) ;
- Species Codes: Integrated Taxonomic Information System when possible; and
- Land cover/land use codes: Multi-Resolution Land Characteristics; National Hydrography Dataset Plus Version 2.0.

#### **4.2.5 Public Accessibility**

While any data created using public funds are subject to the Freedom of Information Act (FOIA), some basic rules apply for general public accessibility and use. Briefly, those rules are:

- Program must comply with Data Quality Act before making any data available to the public and person generating data must fill out and have a signed Information Quality Guidelines package before any posting to the Web or distribution of any kind.
- Data and metadata files are made available to the contributor or participating group for review or other project-related use from NARS IM or in flat files before moving to an EPA-approved public website.
- Data to be placed on a public website will undergo QA/QC review according to the approved QAPP.
- Only “final” data are readily available through an EPA-approved public website.

As new guidance and requirements are issued, the NARS IM staff will assess the impact upon the IM system and develop plans for ensuring timely compliance.

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<sup>3</sup> R is a [free software programming language](#) and a software environment for [statistical computing](#) and graphics. The R language is widely used among [statisticians](#) and [data miners](#) for developing statistical software and data analysis.

### 4.3 Data Transfer Protocols

Field crews are expected to submit all field collected data using the using the iPad provided electronic field forms containing *in situ* measurement and event information to the NARS IM Center defined in the FOM for submission. Laboratories will submit electronic data files. Field crews and laboratories must submit all sample tracking and analytical results data to the NARS IM Center in electronic form using a standard software package to export and format data. Data submission templates for laboratories are included in the LOM. Examples of software and the associated formats are:

**Table 4.2 Summary of software**

Software	Export Options (file extensions)
Microsoft Excel®	xls, xlsx, csv, formatted txt delimited
SAS®	csv, formatted txt delimited
R	csv, formatted txt delimited, R workspaces (.Rdata)

All electronic files must be accompanied by appropriate documentation (e.g., metadata, laboratory reports, QA/QC data and review results). This documentation must contain sufficient information to identify field contents, field formats, qualifier codes, etc. It is very important to keep EPA informed of the completeness of the analyses. Labs may send files periodically, before all samples are analyzed, but EPA must be informed that more data are pending if a partial file is submitted. All data files sent by the labs must be accompanied by text documentation describing the status of the analyses, any QA/QC problems encountered during processing, and any other information pertaining to the quality of the data. Following is a list of general transmittal requirements each laboratory, state, or tribal based IM group should consider when packaging data for electronic transfer to the IM Center:

- Provide data in row/column data file/table structure – see Appendix D in LOM for templates. All cooperators and contractors should further consider the following:
  - a. Include NRSA site and sample ID provided on the sample container label in a field for each record (row) to ensure that each data file/table record can be related to a site visit.
  - b. Use a consistent set of column labels.
  - c. Use file structures consistently.
  - d. Use a consistent set of data qualifiers.
  - e. Use a consistent set of units.
  - f. Include method detection limit (MDL) as part of each result record.
  - g. Include reporting limit (RL) as part of each result record for water chemistry.
  - h. Provide a description of each result/QC/QA qualifier.
  - i. Provide results/measurements/MDL/RL in numeric form.
  - j. Maintain result qualifiers (e.g., <, Not Detected (ND)) in a separate column.
  - k. Use a separate column to identify record-type. For example, if QA or QC data are included in a data file, there should be a column that allows the IM staff to readily identify the different result types.
  - l. Include laboratory sample identifier.
  - m. Include batch numbers/information so results can be paired with appropriate QA/QC information.
  - n. Include “true value” concentrations, if appropriate, in QA/QC records.

- o. Include a short description of preparation and analytical methods used to analyze samples (where appropriate) either as part of the record or as a separate description for the test(s) performed on the sample. For example, EPAxxxx.x, ASTMxxx.x, etc. Provide a broader description (e.g., citation) if a non-standard method is used.
- p. Include a short description of instrumentation used to acquire the test result (where appropriate). This may be reported either as part of the record or as a separate description for each test performed on the sample. For example, GC/MS-ECD, ICP-MS, etc.
- q. Ensure that data ready for transfer to NARS IM are verified and validated, and results are qualified to the extent possible (final verification and validation are conducted by EPA).
- r. Data results must meet the specified requirements for each indicator found in the LOM as specified by contract or agreement.
- s. Identify and qualify missing data (why are the data missing?).
- t. Submit any other associated quality assurance assessments and relevant data related to laboratory results (i.e., chemistry, nutrients). Examples include summaries of QC sample analyses (blanks, duplicates, check standards, matrix spikes) standard or certified reference materials, etc.), results for external performance evaluation or proficiency testing samples, and any internal consistency checks conducted by the laboratory. For requirements, please see specific indicator sections of this QAPP and LOM.

Laboratories will work with the NARS IM Coordinator to establish a data load process into NARS IM.

## 4.4 Data Quality and Results Validation

Data quality is integrated throughout the life cycle of the data. This includes development of appropriate forms, labels etc. for capturing data as well as verifying data entry, results, and other assessments. Indicator workgroup experts, the data analysis and reporting team submit any recommended changes to the Project QA Coordinator who recommends and submits any changes (deletions, additions, corrections) to the NARS IM data center for inclusion in the validated data repository. All explanation for data changes is included in the record history.

### 4.4.1 Design and Site Status Data Files

The site selection process described in Section 3 produces a list of candidate sampling locations, inclusion probabilities, and associated site classification data (e.g., target status, ecoregion, etc.). The Design Team provides this file to the NRSA 2023/24 Project Leader, who in turn distributes to the IM staff, and field coordinators. Field coordinators determine ownership and contacts for acquiring permission to access each site, and conduct site evaluation and reconnaissance activities. Field Crews document information from site evaluation and reconnaissance activities following the SEG and the FOM. The site evaluation spreadsheets are submitted to the Project Lead by the field crews. The NARS IM Center compiles all information such as ownership, site evaluation, and reconnaissance information for each site into a “site status” data file. Any missing information from the site status data file is identified and a request is made by the NARS IM Center to the field crew (or site evaluator) to complete the record.

#### 4.4.2 Sample Collection and Field Data

Field crews record sampling event observational data in a standard and consistent manner using field data collection forms via iOS NRSA application as discussed in the NRSA 2023/24 FOM). Prior to initiation of field activities, the NARS IM staff works with the indicator leads and analytical support laboratories to develop standardized field data forms and sample labels. Adhesive labels, completed by the field crews, have a standard recording format and are affixed to each sample container. Field protocols include precautions to ensure that label information remains legible and the label remains attached to the sample.

NRSA 2023/24 provides a iOS developed application for completing field forms: electronic data entry using pre-developed e-forms. Instructions for completing each e-form are documented in the NRSA 2023/24 FOM. Recorded data whether through e-forms or paper are reviewed upon completion of data collection and recording activities by the Field Crew Leader. Field crews check completed data forms and sample labels before leaving a sampling site to ensure information and data were recorded legibly and completely. Errors are corrected by field crews if possible, and data considered as suspect are qualified using a flag variable. The field sampling crew enters explanations for all flagged data in a comments section. Field crews transmit e-forms to the NARS IM Staff by selecting the “submit” button as described in the FOM.

All samples are tracked from the point of collection. Tracking of samples refers to the documentation of the specified location of each sample in the centralized NARS IM Center database. This is done by requiring that field crews ensure that copies of the shipping and custody record accompany all sample transfers; other copies are transmitted to the IM Center. Each sample has a custody record that laboratory manager is required to enter into NARS IM Center upon receipt of sample. The IM Center tracks samples to ensure that they are delivered to the appropriate laboratory, that lost shipments can be quickly identified and traced, and that any problems with samples observed when received at the laboratory are reported promptly so that corrective action can be taken, if necessary. Detailed procedures on shipping and sample tracking can be found in the FOMs.

Procedures for completion of sample labels and field data forms and use of personal computers (PCs) are covered extensively in training sessions. General QC checks and procedures associated with sample collection and transfer, field measurements, and field data form completion for most indicators are listed in **Table 4.3**. Additional QA/QC checks or procedures specific to individual indicators are described in the LOM.

**Table 4.3 Summary sample and field data quality control activities: sample tracking**

Quality Control Activity	Description and/or Requirements
<b>Contamination Prevention</b>	All containers for individual site sealed in plastic bags until use; specific contamination avoidance measures covered in training
<b>Sample Identification</b>	Pre-printed labels with unique ID number on each sample
<b>Data Recording</b>	Data recorded on pre-printed forms of water-resistant paper; field sampling crew reviews data forms for accuracy, completeness, and legibility
<b>Data Qualifiers</b>	Defined qualifier codes used on data form; qualifiers explained in comments section on data form
<b>Sample Custody Records</b>	Unique sample ID and tracking form information entered in LIMS; sample shipment and receipt confirmed



Quality Control Activity	Description and/or Requirements
<b>Sample Tracking</b>	Sample condition inspected upon receipt and noted on tracking form with copies sent to NRSA Field Logistics Coordinator and/or IM
<b>Data Entry</b>	Data entered using customized entry screens that resemble the data forms; entries reviewed manually or by automated comparison of double entry
<b>Data Submission</b>	Standard format defined for each measurement including units, significant figures, and decimal places, accepted code values, and required field width
<b>Data Archival</b>	All data records, including raw data, archived in an organized manner. For example, following verification/validation of the last submission into the NARS database, it is copied to a terabit external hard drive and sent to the Project Leader for inclusion in his project file, scheduled as 501, permanent records.  Processed samples and reference collections of taxonomic specimens submitted for cataloging and curing at an appropriate museum facility

### 4.4.3 Laboratory Analyses and Data Recording

Upon receipt of a sample shipment, analytical laboratory receiving personnel check the condition and identification of each sample against the sample tracking record. Each sample is identified by information written on the sample label. The lab reports any discrepancies, damaged samples, or missing samples to the NARS IM staff and NRSA 2023/24 Project Lead electronically.

Most of the laboratory analyses for the NRSA 2023/24 indicators, particularly chemical and physical analyses, follow or are based on standard methods. Standard methods generally include requirements for QC checks and procedures. General laboratory QA/QC procedures applicable to most NRSA 2023/24 indicators are described in Section 5. Additional QA/QC procedures specific to individual indicator and parameter analyses are described in the LOM and the QAPP. Biological sample analyses are generally based on current acceptable practices within the particular biological discipline. Some QC checks and procedures applicable to most NRSA 2023/24 biological samples are described in the LOM and the QAPP. **Table 4.4** provides a summary of the lab data QC activities for NRSA 2023/24.

**Table 4.4 Summary laboratory data quality control activities**

Quality Control Activity	Description and/or Requirements
<b>Instrument Maintenance</b>	Follow manufacturer’s recommendations and specific guidelines in methods; maintain logbook of maintenance/repair activities
<b>Calibration</b>	Calibrate instruments according to manufacturer’s recommendations for each specific indicator; recalibrate or replace before analyzing any samples
<b>QC Data</b>	Maintain control charts, determine LT-MDLs and achieved data attributes; include QC data summary (narrative and compatible electronic format) in submission package
<b>Data Recording</b>	Use software compatible with NARS IM system. Check all data entered against the original bench sheet to identify and correct entry errors.  Review other QA data (e.g., condition upon receipt, etc.) for possible problems with sample or specimen.
<b>Data Qualifiers</b>	Use defined qualifier codes; explain all qualifiers

Quality Control Activity	Description and/or Requirements
<b>Data Entry</b>	Automated comparison of double entry or 100% manual check against original data form
<b>Submission Package</b>	Includes: <ul style="list-style-type: none"> <li>▪ Letter by laboratory manager</li> <li>▪ Data</li> <li>▪ Data qualifiers and explanations</li> <li>▪ Electronic format compatible with NARS IM</li> <li>▪ Documentation of file and database structures</li> <li>▪ Metadata: variable descriptions and formats</li> <li>▪ Summary report of any problems and corrective actions implemented</li> </ul>

A laboratory's IM system may consist of only hardcopy records such as bench sheets and logbooks, an electronic laboratory information management system (LIMS), or some combination of hardcopy and electronic records. Laboratory data records are reviewed at the end of each analysis day by the designated laboratory onsite QA coordinator or by supervisory personnel. Errors are corrected by laboratory personnel if possible, and data considered as suspect by laboratory analysts are qualified with a flag variable. All flagged data are explained in a comments section. Private contract laboratories generally have a laboratory Quality Assurance Project Plan and established procedures for recording, reviewing, and validating analysis data.

Once analytical data have passed all of the laboratory's internal review procedures, the lab prepares and transfers a submission package using the prescribed templates in the LOM. The contents of the submission package are largely dictated by the type of analysis (physical, chemical, or biological).

Remaining sample material and voucher specimens may be transferred to EPA's designated laboratory or facilities as directed by the NRSA 2023/24 Project Lead. All samples and raw data files (including logbooks, bench sheets, and instrument tracings) are to be retained by the laboratory for 3 years or until authorized for disposal, in writing, by the EPA Project Leader. Deliverables from contractors and cooperators, including raw data, are permanent as per EPA Record Schedule 258 (<http://www.epa.gov/records/policy/schedule/sched/258.htm>). EPA's project records are scheduled 501 (<http://www.epa.gov/records/policy/schedule/sched/501.htm>) and are also permanent.

#### **4.4.4 Data Review, Verification, and Validation Activities**

Raw data files are created from entry of field and analytical data, including data for QA/QC samples and any data qualifiers noted on the field forms or analytical data package.

##### **4.4.4.1 Electronic Forms**

The NARS IM Center directly uploads information from the electronic field collection forms into their database. During the upload process, incoming data are subjected to a number of automated error checking routines. Omissions and errors are automatically noted in an email message to the field crew lead.

##### **4.4.4.2 Additional Review**

Additional validation is accomplished by the NARS IM Center staff using a specific set of guidelines and executing a series of programs (computer code) to check for: correct file structure and variable naming

and formats, outliers, missing data, typographical errors and illogical or inconsistent data based on expected relationships to other variables. Data that fail any check routine are identified in an “exception report” that is reviewed by an appropriate scientist for resolution.

The NARS IM Center brings any remaining questionable data to the attention of the EPA Project QA Coordinator and individuals responsible for collecting the data for resolution. The EPA Project QA Coordinator reviews all data to determine completeness and validity. Additionally, the data are run through a rigorous inspection using SQL queries or other computer programs such as SAS or R to check for anomalous data values that are especially large or small, or are noteworthy in other ways. Focus is on rare, extreme values since outliers may affect statistical quantities such as averages and standard deviations.

The EPA Project QA Coordinator examines all laboratory quality assurance (QA) information to determine if the laboratory met the predefined data quality objectives - available through the QAPP. Some of the typical checks made in the processes of verification and validation are described in **Table 4.5**.

Automated review procedures may be used. The primary purpose of the initial checks is to confirm that each data value present in an electronic data file is accurate with respect to the value that was initially recorded on a data form or obtained from an analytical instrument. In general, these activities focus on individual variables in the raw data file and may include range checks for numeric variables, frequency tabulations of coded or alphanumeric variables to identify erroneous codes or misspelled entries, and summations of variables reported in terms of percent or percentiles. In addition, associated QA information (e.g., sample holding time) and QC sample data are reviewed to determine if they meet acceptance criteria. Suspect values are assigned a data qualifier. They will either be corrected, replaced with a new acceptable value from sample reanalysis, or confirmed suspect after sample reanalysis. For biological samples, species identifications are corrected for entry errors associated with incorrect or misspelled codes. Errors associated with misidentification of specimens are corrected after voucher specimens have been confirmed and the results are available. Files corrected for entry errors are considered to be raw data files. Copies of all raw data files are maintained in the centralized NARS IM System. Any suspect data will be flagged for data qualification.

The NARS IM staff, with the support of the NRSA 2023/24 Quality Team, correct and qualify all questionable data. Copies of the raw data files are maintained in NARS IM, generally in active files until completion of reporting and then in archive files. Redundant copies of all data files are maintained and all files are periodically backed up to the EPA HQ shared G drive system.

**Table 4.5 Data review, verification, and validation quality control activities**

Quality Control Activity	Description and/or Requirements
<b>Review any qualifiers associated with variable</b>	Determine if value is suspect or invalid; assign validation qualifiers as appropriate
<b>Determine if Measurement Quality Objective (MQOs) and project DQOs have been achieved</b>	Determine potential impact on achieving research and/or program objectives
<b>Exploratory data analyses (univariate, bivariate, multivariate) utilizing all data</b>	Identify outlier values and determine if analytical error or site-specific phenomenon is responsible
<b>Confirm assumptions regarding specific types of statistical techniques being utilized in development of metrics and indicators</b>	Determine potential impact on achieving research and/or program objectives

In the final stage of data verification and validation, exploratory data analysis techniques may be used to identify extreme data points or statistical outliers in the data set. Examples of univariate analysis techniques include the generation and examination of box-and-whisker plots and subsequent statistical tests of any outlying data points. Bivariate techniques include calculation of Spearman correlation coefficients for all pairs of variables in the data set with subsequent examination of bivariate plots of variables having high correlation coefficients. Multivariate techniques have also been used in detecting extreme or outlying values in environmental data sets (Meglen, 1985; Garner et al., 1991; Stapanian et al., 1993).

The Quality Team reviews suspect data to determine the source of error, if possible. If the error is correctable, the data set is edited to incorporate the correct data. If the source of the error cannot be determined, the Quality Team qualifies the data as questionable or invalid. Data qualified as questionable may be acceptable for certain types of data analyses and interpretation activities. The decision to use questionable data must be made by the individual data users. Data qualified as invalid are considered to be unacceptable for use in any analysis or interpretation activities and will generally be removed from the data file and replaced with a missing value code and explanatory comment or flag code. After completion of verification and validation activities, a final data file is created, with copies transmitted for archival and for uploading to the centralized IM system.

Once verified and validated, data files are made available for use in various types of interpretation activities; each activity may require additional restructuring of the data files. These restructuring activities are collectively referred to as "data enhancement." In order to develop indicator metrics from one or more variables, data files may be restructured so as to provide a single record per site.

## **4.5 Data Transfer**

Field crews may transmit all data electronically. Copies of raw, verified, and validated laboratory data files are transferred from the Project QA Coordinator to the IM staff for inclusion in the central IM system. All transfers of data are conducted using a means of transfer, file structure, and file format that has been approved by the EPA IM Project lead. Data files that do not meet the required specifications will not be incorporated into the centralized data access and management system.

### **4.5.1 Database Changes**

The NARS IM Center staff complete data corrections at the lowest level to ensure that any subsequent updates will contain only the most correct data. The NARS IM Center sends back laboratory results found to be in error to the originator (e.g., analysis laboratory) for correction. After the originator makes any corrections, the entire batch or file is resubmitted to the NARS IM Center. The NARS IM Center uses these resubmissions to replace any previous versions of the same data.

The NARS IM Center uses a version control methodology when receiving files. This methodology is explained in the following sentences. Incoming data are not always immediately transportable into a format compatible with the desired file structures. When this situation occurs, the IM staff creates a copy of the original data file, which then becomes the working file in which any formatting changes will take place. The original raw data will remain unchanged. This practice further ensures the integrity of the data and provides an additional data recovery avenue, should the need arise.

All significant changes are documented by the NARS IM Center staff. The NARS IM Center includes this information in the final summary documentation for the database (metadata).

After corrections have been applied to the data, the NARS IM Center will rerun the validation programs to re-inspect the data.

If requested by the NARS Project QA Coordinator and funds are available, the NARS IM Center will implement database auditing features to track changes.

## **4.6 Metadata**

All metadata will be kept according to the Federal Geographic Data Committee, Content standard for digital geospatial metadata, version 2.0. FGDC-STD-001-1998 (FGDC 1998).

### **4.6.1 Parameter Formats**

The following parameter formats will be used:

- Sampling Site (EPA Locational Data Policy (USEPA 1991))
- Latitude and Longitude in decimal degrees (+/- 7.4), Negative longitude values (west of the prime meridian),
- Datum: NAD83;
- Date: YYYYMMDD (year, month, day)
- Hour: HHMMSS (hour, minute, second), Greenwich mean time, Local time
- Data loaded to STORET will take on the STORET formats upon loading.

### **4.6.2 Standard Coding Systems**

The following standard coding systems will be used:

- Chemical Compounds: Chemical Abstracts Service (CAS 1999)
- Taxonomic Names: USGS BioData (<https://aquatic.biodata.usgs.gov/landing.action>)
- Land cover/land use codes: Multi-Resolution Land Characteristics (MRLC 1999)

## **4.7 Information Management Operations**

### **4.7.1 Computing Infrastructure**

Electronic data are collected and maintained within a central server housed at PESD using a Windows Server (current configuration) or higher computing platform in SQL native tables for the primary data repository and SAS® native data sets or R datasets for data analysis. Official IM functions are conducted in a centralized environment.

### **4.7.2 Data Security and Accessibility**

The NARS IM Center ensures that all data files in NARS IM are protected from corruption by computer viruses, unauthorized access, and hardware and software failures. Guidance and policy documents of EPA and management policies established by the IM Technical Coordination Group for data access and data confidentiality are followed. Raw and verified data files are accessible only to the NRSA 2023/24 collaborators. Validated data files are accessible only to users specifically authorized by the NRSA 2023/24 Project Leader. Data files in the central repository used for access and dissemination are marked as read-only to prevent corruption by inadvertent editing, additions, or deletions.

Data generated, processed, and incorporated into the IM system are routinely stored as well as archived on redundant systems by the NARS IM Center. This ensures that if one system is destroyed or

incapacitated, IM staff can reconstruct the databases. Procedures developed to archive the data, monitor the process, and recover the data are described in IM documentation.

Data security and accessibility standards implemented for NRSA 2023/24 IM meet EPA's standard security authentication (i.e., username, password) process in accordance to EPA's *Information Management Security Manual* (1999; EPA Directive 2195 A1) and EPA Order 2195.1 A4 (2001D). Any data sharing requiring file transfer protocol (FTP) or internet protocol is provided through an authenticated site.

#### **4.7.3 Life Cycle**

Data may be retrieved electronically by the NRSA 2023/24 team, partners and others throughout the records retention and disposition lifecycle or as practicable (Section 4.4).

#### **4.7.4 Data Recovery and Emergency Backup Procedures**

The NARS IM Center maintains several backup copies of all data files and of the programs used for processing the data. Backups of the entire system are maintained off-site by the NARS IM Center. The IM process used by the NARS IM Center for NRSA 2023/24 uses system backup procedures. The NARS IM Center backs up and archives the central database according to procedures already established for EPA PESD and NARS IM. All laboratories generating data and developing data files are expected to establish procedures for backing up and archiving computerized data.

#### **4.7.5 Long-Term Data Accessibility and Archive**

All data are transferred by OW's Water Quality Exchange (WQX) team working with the NARS IM Team to U.S. EPA's agency-wide WQX data management system for archival purposes. WQX is a repository for water quality, biological, and physical data and is used by state environmental agencies, EPA and other federal agencies, universities, and private citizens. Data from the NRSA 2023/24 project will be run through an Interface Module in an Excel format and uploaded to WQX by the WQX team. Once uploaded, states and tribes and the public will be able to download data (using Oracle software) from their region. Data will also be provided in flat files on the NARS website.

### **4.8 Records Management**

Removable storage media (i.e., CDs, USB Drives) and paper records are maintained in a centrally located area at the NARS IM Center. Paper records will be returned to OW once the assessment is complete. The IM Team identifies and maintains files using standard divisional procedures as established by EPA PESD. Records retention and disposition comply with U.S. EPA directive 2160 Records Management Manual (July, 1984) in accordance with the Federal Records Act of 1950.

## 5 INDICATORS

A description of the NRSA indicators is found in **Table 5.1**.

**Table 5.1 Indicators and collection location**

Indicator	Description	Specs/Location in Sampling Reach
<b>In Situ measurements (pH, DO, temperature, conductivity)</b>	Measurements for temperature, pH, dissolved oxygen (DO), and conductivity taken to detect extremes in condition that might indicate impairment.	One set of measurements taken at the index site (Wadeable) or Transect A (Non-Wadeable); readings are taken at 0.5 m depth, or at mid-depth if depth is less than one meter.
<b>Water chemistry (TP, TN, NH<sub>3</sub>-N, NO<sub>3</sub>-NO<sub>2</sub>, NO<sub>3</sub>), basic anions and cations, silica, alkalinity [ANC], dissolved organic carbon (DOC), TOC, TSS, conductivity, pH, turbidity, true color)</b>	Water chemistry measurements will be used to determine the acidic conditions and nutrient enrichment, as well as classification of water chemistry type.	Collected from a depth of 0.5 m at the index site (Wadeable) or Transect A (Non-Wadeable)
<b>Chlorophyll-a</b>	Chlorophyll-a is used to determine algal biomass in the water.	Collected as part of water chemistry and periphyton samples
<b>Algal Toxins (Microcystin and Cylindrospermopsin)</b>	Measurement used to determine the harmful algal bloom biomass in the water.	Collected from a depth of 0.5 m at the index site (Wadeable) or Transect A (Non-Wadeable)
<b>Periphyton</b>	Periphyton community information is used to assess the biological health of rivers and streams algal community. The NRSA will measure attributes of the overall structure and function of the periphyton community, diversity and abundance to evaluate biological integrity.	Collected from 11 locations systematically placed at each site and combined into a single composite sample. Sub-sampled for taxonomy, chlorophyll-a, Ash Free Dry Mass (AFDM), and metagenomics.
<b>Benthic macroinvertebrate assemblage (Littoral)</b>	Benthic macroinvertebrate community information is used to assess the biological health of rivers and streams. The NRSA will measure attributes of the overall structure and function of the benthic macroinvertebrate community, diversity, abundances, etc to evaluate biological integrity.	Collected from 11 locations systematically placed at each site and combined into a single composite sample
<b>Fish Assemblage</b>	The assessment will measure specific attributes of the overall structure and function of the ichthyofaunal community to	Sampled throughout the sampling reach at specified locations

Indicator	Description	Specs/Location in Sampling Reach
	evaluate biological integrity and water quality.	
<b>Physical habitat assessment</b>	The physical habitat assessment of the sampling reach and the riparian zone (the region lying along a bank)	Measurements collected throughout the sampling reach at specified locations
<b>Fecal indicator (<i>Enterococci</i>)</b>	<i>Enterococci</i> are bacteria that are endemic to the guts of warm blooded creatures. These bacteria, by themselves, are not considered harmful to humans but often occur in the presence of potential human pathogens (the definition of an indicator organism).	Collected at the last transect one meter off the bank
<b>Fish Tissue Plug</b>	Fish Tissue plugs will provide information on the national distribution of mercury, a bioaccumulative and toxic chemical in fish species.	Target species collected throughout the sampling reach at every site where suitable fish species and lengths are available
<b>Antimicrobial Resistance</b>	Antimicrobial resistance samples will provide information on the national distribution of both antimicrobial resistant microbes as well as genes associated with antimicrobial resistance.	Collected at the last transect at the center of the stream channel.

## 5.1 Water Chemistry and In-situ Measurements (Including chlorophyll-*a*-)

### 5.1.1 Introduction

Ecological indicators based on field and laboratory collected river and stream water chemistry information attempt to evaluate stream condition with respect to stressors such as acidic deposition and other types of physical or chemical contamination. Data are collected for a variety of physical and chemical constituents to provide information on the acid-base status of each stream, water clarity, primary productivity, nutrient status, mass balance budgets of constituents, color, temperature regime, and presence and extent of anaerobic conditions. Data are collected for chlorophyll-*a* to provide information on the algal loading and gross biomass of cyanobacteria and other algae within each stream and river.

Detailed sample collection and handling procedures are described in the FOMs.



### 5.1.2 Pertinent QA/QC Procedures

A single analytical laboratory and some State laboratories will analyze the water chemistry samples. The specific quality control procedures used by each laboratory are implemented to ensure that:

- Objectives established for various data quality indicators being met.
- Results are consistent and comparable among all participating laboratories.

The analytical laboratory demonstrated in previous studies that it can meet the required Laboratory Reporting Levels (RLs) (USEPA 2004). All laboratories will follow the QA/QC procedures outlined in the QAPP and the LOM will be followed to ensure these Laboratory RLs are met. A summary and diagram of the QA processes related to water chemistry samples for the NRSA 2023/24 is found in **Figure 5.2**.

#### 5.1.2.1 Laboratory Performance Requirements

**Table 5.2** summarizes the pertinent laboratory performance requirements for the water chemistry indicators.

**Table 5.2 Laboratory method performance requirements: water chemistry**

Analyte	Units	Potential Range of Samples <sup>4</sup>	Lower Reporting Limit <sup>5</sup>	Transition Value <sup>6</sup>	Precision Objective <sup>7</sup>	Bias Objective <sup>8</sup>
<b>Conductivity</b>	µS/cm at 25°C	1 to 77,000	2.0	20	± 1 or ±10%	± 1 or 5%
<b>pH (laboratory)</b>	Standard (Std) Units	3.5 to 10	N/A	5.75, 8.25	>5.75 or <8.25 = ±0.15 ≤5.75 or >8.25 = ±0.07	>5.75 and <8.25 = ±0.10 ≤5.75 or >8.25 = ±0.05
<b>Turbidity</b>	Nephelometric Turbidity Units (NTU)	0 to 44,000	2.0	10	± 1 or ±10%	± 1 or ±10%

1. *Estimated from samples analyzed at the WED-Corvallis laboratory between 1999 and 2005 for TIME, EMAP-West, and WSA streams from across the U.S.*
2. *The lower reporting limit is the lowest value that needs to be quantified (as opposed to just detected), and represents the value of the lowest nonzero calibration standard used. It is set to 2 times the long-term method detection limit, following USGS Open File Report 99-193 New Reporting Procedures Based on Long-Term Method Detection Levels and Some Considerations for Interpretations of Water-Quality Data Provided by the U.S. Geological Survey National Water Quality Laboratory.*
3. *Value at which performance objectives for precision and bias switch from absolute (E transition value) to relative > transition value). Two-tiered approach based on Hunt, D.T.E. and A.L. Wilson. 1986. The Chemical Analysis of Water: General Principles and Techniques. 2nd ed. Royal Society of Chemistry, London, England.*
4. *For standard samples, precision is estimated as the standard deviation of repeated measurements across batches at the lower concentration range, and as percent relative standard deviation of repeated measurements across batches at the higher concentration range.*  
  
*For pH precision, the looser criteria applies to more highly alkaline samples. For NRSA, that is less of a concern than the ability to measure acidic samples accurately and precisely.*
5. *Bias (systematic error) is estimated as the difference between the mean measured value and the target value of a performance evaluation and/or internal reference samples at the lower concentration range measured across sample batches, and as the percent difference at the higher concentration range.*

Analyte	Units	Potential Range of Samples <sup>4</sup>	Lower Reporting Limit <sup>5</sup>	Transition Value <sup>6</sup>	Precision Objective <sup>7</sup>	Bias Objective <sup>8</sup>
<b>Acid Neutralizing Capacity (ANC)</b>	µeq/L (1 mg/L as CaCO <sub>3</sub> =20 µeq/L)	-300 to +75,000 (-16 to 3,750 mg as CaCO <sub>3</sub> )	N/A	±50	± 5 or ±10%	± 5 or ±10%
<b>Dissolved Organic Carbon (DOC)</b>	mg C/L	0.1 to 109	0.20	≤ 2 > 2	± 0.10 or ±10%	± 0.10 or ±10%
<b>Ammonia-N (NH<sub>3</sub>-N)</b>	mg N/L	0 to 17	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
<b>Nitrate-Nitrite (NO<sub>3</sub>-NO<sub>2</sub>)</b>	mg N/L	0 to 360 (as nitrate)	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
<b>Total Nitrogen (TN)</b>	mg/L	0.1 to 90	0.02	0.10	± 0.01 or ±10%	± 0.01 or ±10%
<b>Total Phosphorus (TP)</b>	µg P/L	0 to 22,000	4	20	± 2 or ±10%	± 2 or ±10%
<b>Sulfate (SO<sub>4</sub>)</b>	mg SO <sub>4</sub> /L	0 to 33,500	0.50	2.5	± 0.25 or ±10%	± 0.25 or ±10%
<b>Chloride (Cl)</b>	mg Cl/L	0 to 19,000	0.20	1	± 0.10 or ±10%	± 0.10 or ±10%
<b>Nitrate (NO<sub>3</sub>)</b>	mg N/L	0 to 360	0.02	0.1	± 0.01 or ±10%	± 0.01 ±10%
<b>Calcium (Ca)</b>	mg Ca/L	0.04 to 5,000	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
<b>Magnesium (Mg)</b>	mg Mg/L	0-5000	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
<b>Sodium (Na)</b>	mg Na/L	0-22,500	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
<b>Potassium (K)</b>	mg K/L	0-170	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
<b>Silica (SiO<sub>2</sub>)</b>	mg SiO <sub>2</sub> /L	0.01 to 100	0.10	0.5	± 0.05 or ±10%	± 0.05 or ±10%
<b>Total Suspended Solids (TSS)</b>	mg/L	0 to 27,000	2	10	± 1 or ±10%	± 1 or ±10%
<b>True Color</b>	PCU	0 to 1000	5	50	±5 or ±10%	±5 or ±10%
<b>Chlorophyll a</b>	µg/L (in extract)	0.7 to 11,000	0.5	15	± 1.5 or ±10%	± 1.5 or ±10%

Laboratory Quality Control Samples **Table 5.3** summarizes the pertinent laboratory quality control samples for the water chemistry indicators.

**Table 5.3 Laboratory quality control samples: water chemistry**

QC Sample Type and Description	Analytes	Description	Frequency	Acceptance Criteria	Corrective Action
<b>Laboratory/ Reagent Blank</b>	All except TSS (For TSS, the lab will filter a known volume of reagent water and process the filters per method)		Once per analytical batch prior to sample analysis	Control limits $\leq$ LRL	Prepare and analyze new blank. Determine and correct problem (e.g., reagent contamination, instrument calibration, or contamination introduced during filtration) before proceeding with any sample analyses. Reestablish statistical control by analyzing three blank samples.
<b>Filtration Blank</b>	All dissolved analytes	ASTM Type II reagent water processed through filtration unit	Prepare once per week and archive Prepare filter blank for each box of 100 filters, and examine the results before any other filters are used from that box.	Measured concentrations $<$ LRL	Measure archived samples if review of other laboratory blank information suggest source of contamination is sample processing.
<b>LT-MDL Limit Quality Control Check Sample (QCCS)</b>	All analyses except true color and turbidity	Prepared so concentration is four to six times the LT-MDL objective	Once per day	Target LT-MDL value (which is calculated as a 99% confidence interval)	Confirm achieved LRL by repeated analysis of LT-MDL QCCS. Evaluate affected samples for possible re-analysis.

QC Sample Type and Description	Analytes	Description	Frequency	Acceptance Criteria	Corrective Action
<b>Initial and Continuing Calibration Verification</b>			Analyze ICV after calibration. Analyze CCV after every 10 samples and at the end of analytical batch.	±10% or method criteria	Perform corrective action and repeat all associated samples since last successful CCV. Alternatively, recalibrate and re-analyze all samples since last successful CCV.
<b>Analytical Duplicate Sample</b>	All analyses		One per 10 samples	Within precision objective	If results are below LRL: Prepare and analyze duplicate from different sample (volume permitting). Review precision of batch. Check preparation of duplicate sample.
<b>Standard Reference Material (SRM)</b>	When available for a particular analyte		One analysis in a minimum of five separate batches	Manufacturers certified range	Analyze standard in next batch to confirm suspected imprecision or bias. Evaluate calibration and QCCS solutions and standards for contamination and preparation error. Correct before any further analyses of routine samples are conducted. Reestablish control by three successive reference standard measurements that are acceptable. Qualify all sample batches analyzed since the last acceptable reference standard measurement for possible reanalysis.

QC Sample Type and Description	Analytes	Description	Frequency	Acceptance Criteria	Corrective Action
<b>Matrix Spike Samples</b>	Only prepared when samples with potential for matrix interferences are encountered		One per batch	Control limits for recovery cannot exceed 100±20%	Select two additional samples and prepare fortified subsamples. Reanalyze all suspected samples in batch by the method of standard additions. Prepare three subsamples (unfortified, fortified with solution approximately equal to the endogenous concentration, and fortified with solution approximately twice the endogenous concentration).

### 5.1.2.2 Data Reporting, Review, and Management

Checks made of the data in the process of review and verification is summarized in **Table 5.4**. Data reporting units and significant figures are summarized in **Table 5.5**.

The Project QA Officer is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members.

**Table 5.4 Data validation quality control: water chemistry**

Activity or Procedure	Requirements and Corrective Action
<b>Range checks, summary statistics, and/or exploratory data analysis (e.g., box and whisker plots)</b>	Correct reporting errors or qualify as suspect or invalid.
<b>Review holding times</b>	Qualify value for additional review
<b>Ion balance: Calculate percent ion balance difference (%IBD) using data from cations, anions, pH, and ANC.</b>	<p>If total ionic strength ≤100 µeq/L %IBD ≤ ±25%.</p> <p>If total ionic strength &gt; 100 µeq/L %IBD ≤±10%.</p> <p>Determine which analytes, if any, are the largest contributors to the ion imbalance. Review suspect analytes for analytical error and reanalyze. Flag = unacceptable %IBD</p> <p>If analytical error is not indicated, qualify sample to attribute imbalance to unmeasured ions. Reanalysis is not required. Flag = %IBD outside acceptance criteria due to unmeasured ions</p>

Activity or Procedure	Requirements and Corrective Action
<p><b>Conductivity check:</b> Compare measured conductivity of each sample to a calculated conductivity based on the equivalent conductance of major ions in solution (Hillman et al., 1987).</p>	<p>If measured conductivity <math>\leq 25 \mu\text{S}/\text{cm}</math>,  <math>([\text{measured} - \text{calculated}] \div \text{measured}) \leq \pm 25\%</math>.                      If measured conductivity <math>&gt; 25 \mu\text{S}/\text{cm}</math>,  <math>([\text{measured} - \text{calculated}] \div \text{measured}) \leq \pm 15\%</math>.                      Determine which analytes, if any, are the largest contributors to the difference between calculated and measured conductivity. Review suspect analytes for analytical error and reanalyze. If analytical error is not indicated, qualify sample to attribute conductivity difference to unmeasured ions. Reanalysis is not required.</p>
<p>Review data from QA samples (laboratory Performance evaluation (PE) samples, and inter-laboratory comparison samples)</p>	<p>Indicator QC Coordinator determines impact and possible limitations on overall usability of data based on the specific issue.</p>

Table 5.5 Data reporting criteria: water chemistry

Measurement	Units	No. Significant Figures	Minimum No. Decimal Places
DO	mg/L	2	1
Temperature	°C	2	1
pH	pH units	3	2
Carbon, total & dissolved organic	mg/L	3	1
ANC	$\mu\text{eq}/\text{L}$	3	1
Conductivity	$\mu\text{S}/\text{cm}$ at 25 °C	3	1
Calcium, magnesium, sodium, potassium, ammonium, chloride, nitrate, and sulfate	$\mu\text{eq}/\text{L}$	3	1
Silica	mg/L	3	2
Total phosphorus	$\mu\text{g}/\text{L}$	3	0
Total nitrogen	mg/L	3	2
Nitrate-Nitrite	mg/L	3	2
Ammonia	mg/L	3	2
Turbidity	NTU	3	0
True color	PCU	2	0
TSS	mg/L	3	1
Chlorophyll a	$\mu\text{g}/\text{l}$	3	2

The ion balance for each sample is computed using the results for major cations, anions, and the measured acid neutralizing capacity. The percent ion difference (%IBD) for a sample is calculated as:

### Percent ion difference (%IBD)

$$\text{Equation 5.1} \quad \%IBD = \frac{(\sum \text{cations} - \sum \text{anions}) - ANC}{ANC + \sum \text{anions} + \sum \text{cations} + 2[H^+]}$$

Where:

ANC is the acid neutralization capacity; cations are the concentrations of calcium, magnesium, sodium, potassium, and ammonium (converted from mg/L to  $\mu\text{eq/L}$ ); anions are the concentrations of chloride, nitrate, and sulfate (converted from mg/L to  $\mu\text{eq/L}$ ), and  $H^+$  is the hydrogen ion concentration calculated from the antilog of the sample pH. Factors to convert major ions from mg/L to  $\mu\text{eq/L}$  are presented in **Table 5.6**. For the conductivity check, equivalent conductivities for major ions are presented in

**Table 5.7**.

**Table 5.6 Constants for converting major ion concentration from mg/L to  $\mu\text{eq/L}$**

Analyte	Conversion from mg/L to $\mu\text{eq/L}$ <sup>9</sup>
Calcium	49.9
Magnesium	82.3
Potassium	25.6
Sodium	43.5
Ammonia-N	71.39
Ammonium	55.4
Chloride	28.2
Nitrate-N	71.39
Nitrate	16.1
Sulfate	20.8

**Table 5.7 Factors to calculate equivalent conductivities of major ions<sup>10</sup>**

Ion	Equivalent Conductance per mg/L ( $\mu\text{S/cm}$ at 25 °C)	Ion	Equivalent Conductance per mg/L ( $\mu\text{S/cm}$ at 25 °C)
Calcium	2.60	Nitrate	1.15
Magnesium	3.82	Sulfate	1.54
Potassium	1.84	Hydrogen	$3.5 \times 10^5$ <sup>11</sup>
Sodium	2.13	Hydroxide	$1.92 \times 10^5$

<sup>9</sup> Measured values are multiplied by the conversion factor. For ammonia and nitrate, two factors are provided, one if results are reported as mg N/L, the other if the ion is reported directly.

<sup>10</sup> From Hillman et al. (1987).

<sup>11</sup> Specific conductance per mole/L, rather than per mg/L.

<b>Ammonium</b>	4.13	<b>Bicarbonate</b>	0.715
<b>Chloride</b>	2.14	<b>Carbonate</b>	2.82

### 5.1.3 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities.

Crews will measure water chemistry field measurements with a calibrated multiprobe. The crews will calibrate the DO, pH, and conductivity prior to each sampling event in the field. Crews will test the temperature meter against a thermometer that is traceable to the National Institute of Standards (NIST) at least once per sampling season. Field crews will verify that all sample containers are uncontaminated and intact, and that all sample labels are legible and intact. A summary of field quality control procedures for water chemistry is presented in **Table 5.8** and a visual description is laid out in **Figure 5.1**.

Before leaving the field, the crews will:

- Check the label to ensure that all written information is complete and legible.
- Place a strip of clear packing tape over the label, covering the label completely.
- Record the sample ID number assigned to the water chemistry sample on the Sample Collection Form.
- Enter a flag code and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity.
- Store the sample on wet ice in a cooler.
- Recheck all forms and labels for completeness and legibility.

**Table 5.8 Field quality control: water chemistry**

Check Description	Frequency	Acceptance Criteria	Corrective Actions
<b>Check calibration of multiprobe</b>	Prior to each sampling day	Specific to instrument	Adjust and recalibrate, redeploy gear
<b>Check calibrated sounding rod</b>	Each site	Depth measurements for all sampling points	Obtain best estimate of depth where actual measurement not possible
<b>Check integrity of sample containers and labels</b>	Each site	Clean, intact containers and labels	Obtain replacement supplies



**FIELD MEASUREMENT PROCESS: WATER CHEMISTRY INDICATOR**

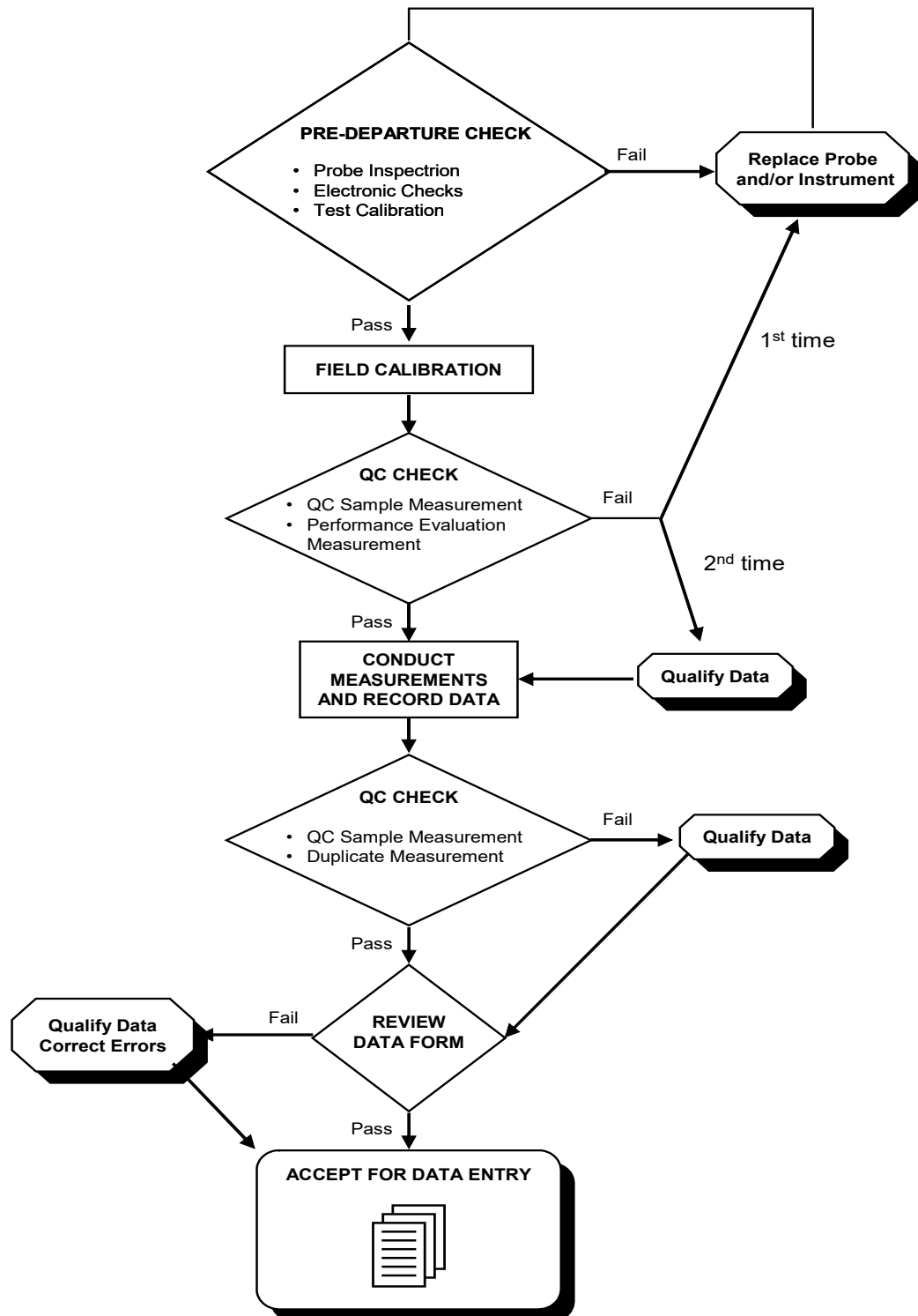


Figure 5.1 Field measurement process: water chemistry samples

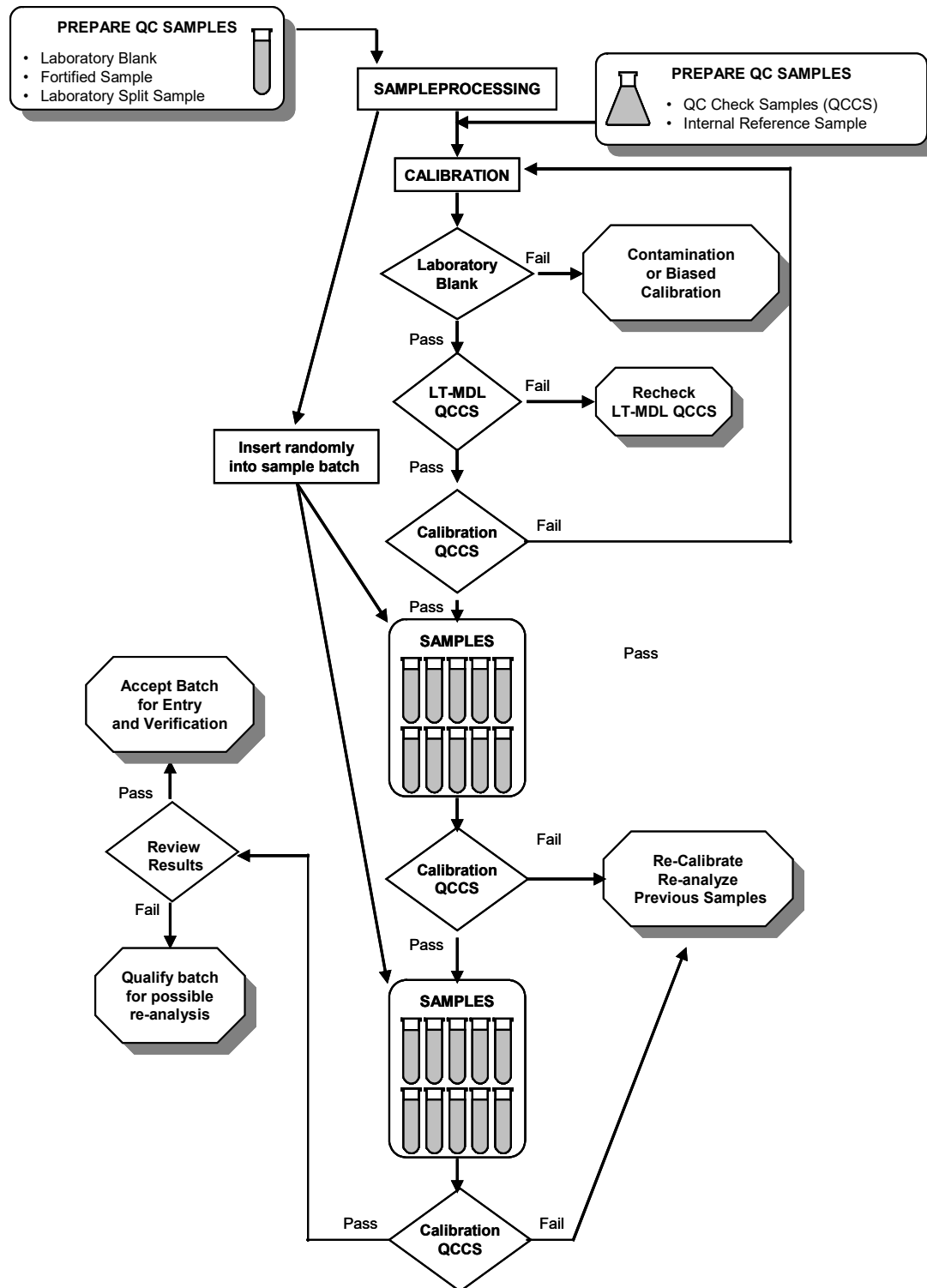


Figure 5.2 Analysis activities: water chemistry samples

## 5.2 Algal Toxins: Microcystin and Cylindrospermopsin

### 5.2.1 Sample Design and Methods

Detailed sample collection and handling procedures are found in the FOMs.

### 5.2.2 Pertinent QA/QC Procedures

#### 5.2.2.1 Quality Assurance Objectives

MQOs for absorbances are given in **Table 5.9**. General requirements for comparability and representativeness are addressed in **Section 2**.

**Table 5.9 Measurement data quality objectives: microcystin and cylindrospermopsin**

Variable or Measurement	Precision	Accuracy	Completeness
Algal Toxin Indicator	±15% <sup>12</sup>	±25% <sup>13</sup>	NA

#### 5.2.2.2 QA Values and Objectives

Quality control for the microcystin and cylindrospermopsin indicators are listed in **Table 5.10**.

**Table 5.10 Sample analysis quality control activities: microcystin and cylindrospermopsin**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Kit – Shelf Life</b>	Is within its expiration date listed on kit box.	If kit has expired, then discard or set aside for training activities.
<b>Kit - Contents</b>	All required contents must be present and in acceptable condition. This is important because Abraxis has calibrated the standards and reagents separately for each kit.	If any bottles are missing or damaged, discard the kit.
<b>Calibration</b>	All of the following must be met: <ul style="list-style-type: none"> <li>Standard curve must have a correlation coefficient of <math>\geq 0.99</math>;</li> <li>Average absorbance value, <math>\bar{A}_0</math>, for S0 must be <math>&gt; 0.80</math>; and</li> <li>Standards S0-S5 (S6 for cylindrospermopsin) must have decreasing average absorbance values. That is, if <math>\bar{A}_i</math> is the average of the absorbance values for <math>S_i</math>, then the absorbance average values must be:</li> </ul>	If any requirement fails: <ul style="list-style-type: none"> <li>Results from the analytical run are not reported.</li> <li>All samples in the analytical run are reanalyzed until calibration provides acceptable results.</li> </ul>

<sup>12</sup> For algal toxins, the precision for a sample is reported in terms of the percent coefficient of variation (%CV) of its absorbance values. For the %CV calculation, see the Laboratory Operations Manual. Relative Standard Deviation (RSD) is the same as the %CV. Because many of the plate reader software programs provides the CV in their outputs, the procedure presents the quality control requirement in terms of %CV instead of RSD.

<sup>13</sup> For algal toxins, accuracy is calculated by comparing the average concentration of the kit control with the required range (0.75 +/- 0.185).

	$\bar{A}_0 > \bar{A}_1 > \bar{A}_2 > \bar{A}_3 > \bar{A}_4 > \bar{A}_5 > \bar{A}_6$ (for cylindrospermopsin only)	
<b>Kit Control</b>	The average concentration value of the duplicates (or triplicate) must be within the range of 0.75 +/- 0.185 µg/L for microcystin kits and 0.75 +/- 0.15 µg/L for cylindrospermopsin. That is, results must be between 0.565 and 0.935 for microcystin and between 0.60 and 0.90 for cylindrospermopsin .	<p>If either requirement fails:</p> <ul style="list-style-type: none"> <li>• Results from the analytical run are not reported</li> <li>• The lab evaluates its processes, and if appropriate, modifies its processes to correct possible contamination or other problems.</li> <li>• The lab reanalyzes all samples in the analytical run until the controls meet the requirements.</li> </ul>
<b>Negative Control</b>	<p>The values for the negative control replicates must meet the following requirements:</p> <ul style="list-style-type: none"> <li>○ All concentration values must be &lt; 0.1 µg/L (i.e., the reporting limit) for cylindrospermopsin and all concentration values must be &lt; 0.15 µg/L (i.e., the reporting limit) for microcystin; and</li> <li>○ One or more concentration results must be nondetectable (i.e., &lt;0.10 µg/L for microcystin and &lt;0.05 µg/L for cylindrospermopsin)</li> </ul>	
<b>Sample Evaluations</b>	All samples are run in duplicate. Each duplicate pair must have %CV≤15% between its absorbance values.	<p>If %CV of the absorbances for the sample&gt;15%, then:</p> <ul style="list-style-type: none"> <li>• Record the results for both duplicates.</li> <li>• Report the data for both duplicate results as Quality Control Failure “QCF”; and</li> <li>• Re-analyze the sample in a new analytical run. <b>No samples are to be run more than twice.</b></li> </ul> <p>If the second run passes, then the data analyst will exclude the data from the first run. If both runs fail, the data analyst will determine if either value should be used in the analysis (e.g., it might be acceptable to use data if the CV is just slightly over 15%).</p>
<b>Results Within Calibration Range</b>	All samples are run in duplicate. If both of the values are less than the upper calibration range (i.e., 5.0 µg/L for undiluted microcystin	If one or both duplicates register as ‘HIGH,’ then the sample must be diluted and re-run until both

	samples, 2.0 µg/L for cylindrospermopsin samples), then the requirement is met.	results are within the calibration range. <b>No samples are to be run more than twice.</b> If samples are re-run, do not enter concentration information of the first run.
<b>External Quality Control Sample</b>	External QC Coordinator, supported by QC contractor, provides 1-2 sets of identical samples to all laboratories and compares results.	Based upon the evaluation, the External QC Coordinator may request additional information from one or more laboratories about any deviations from the Method or unique laboratory practices that might account for differences between the laboratory and others. With this additional information, the External QC Coordinator will determine an appropriate course of action, including no action, flagging the data, or excluding some or all of the laboratory's data.

### 5.3 Periphyton

#### 5.3.1 Introduction

Periphyton are diatoms and soft-bodied algae, as well as fungi and bacteria, that are attached or otherwise associated with channel substrates. Periphyton, in general, can contribute to the physical stability of inorganic substrate particles, and provide habitat and structure. Periphyton are useful indicators of environmental condition because they respond rapidly and are sensitive to a number of anthropogenic disturbances, including habitat destruction, contamination by nutrients, metals, herbicides, hydrocarbons, and acidification.

#### 5.3.2 Sampling Design and Methods

Detailed sample collection and handling procedures are described in FOM. Field collected periphyton samples will be subdivided into four samples for diatom identification, chlorophyll a, ash free dry mass, and metagenomic analysis.

*Analysis:* Diatom identification samples are preserved, processed, enumerated, and organisms identified to the lowest possible taxonomic level (genus/species) using specified standard keys and references. Processing and archival methods are based on a modified USGS NAWQA method (Charles et al. 2003). Detailed procedures are contained in the LOM.

Chlorophyll *a* sub-samples will be filtered in the field and analyzed in the laboratory according to the procedures outlined in the LOM.

AFDM subsamples will be filtered in the field and analyzed in the laboratory according to the procedures outlined in the laboratory operations manual.

The periphyton metagenomic sample will be collected in the field and shipped to the lab as described in the FOMs. These samples will be analyzed at an EPA ORD lab, and the ORD lab is developing a separate QAPP for this work.

### 5.3.3 Quality Assurance Objectives

MQOs are given in **Table 5.11**. The MQOs refer to the diatom ID samples. The QA procedures for periphyton chlorophyll a and AFDM are identical to the water chemistry chlorophyll a procedures. The water chemistry labs will perform the sample analysis for all of these samples and follow the same QA procedures laid out in **Section 5.1**. General requirements for comparability and representativeness are addressed in **Section 2**. The MQOs for the periphyton meta-genomics subsample will be found in the ORD QAPP. Three different measurement data quality objectives are used in evaluating diatom data:

For diatoms – Percent Taxonomic Disagreement (PTD) and Percent Difference in Enumeration (PDE)). Targets are shown in

**Table 5.11**

**Table 5.11 Measurement data quality objectives: diatom periphyton**

Variable or Measurement	Precision	Accuracy	Completeness
Enumeration	75% <sup>a</sup>	85% <sup>b</sup>	99%
Identification	75% <sup>a</sup>	85% <sup>b</sup>	99%

<sup>a</sup> As measured by (100%-PTD); <sup>b</sup> As measured by (100%-PDE)

### 5.3.4 Pertinent QA/QC Procedures for ID Periphyton Sample

Quality control activities and data validation are summarized in **Table 5.12** and **Table 5.13**. Equations used are presented below. **Percent disagreement in enumeration (PDE)**: measure of taxonomic precision for diatoms comparing the number of organisms,  $n_1$ , counted in a sample by the primary taxonomist with the number of organisms,  $n_2$ , counted by the secondary taxonomist.

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100$$

**Percent taxonomic disagreement (PTD)**: measure of taxonomic precision for diatoms comparing the number of agreements (positive comparisons,  $comp_{pos}$ ) of the primary taxonomist and internal or external QC taxonomists. In the following equation,  $N$  is the total number of organisms in the larger of the two counts.

$$PTD = \left[ 1 - \frac{comp_{pos}}{N} \right] \times 100$$

#### 5.3.4.1 Internal Taxonomic QC

Before samples are counted and identified, a lead taxonomist will develop pre-count regional voucher flora for the diatom taxa. This process is described in detail in section 10.7 of the NRSA LOM.

The internal QC taxonomist will randomly select 10% of the diatom slides for an independent count and identification by another Internal QC Taxonomist. As appropriate, calculate the PctDiff, PDE, and PTD. If any do not meet the QA requirements, perform a third count and reidentification for the sample. The

process for selecting, at random, which samples will be re-identified and counted is described in 10.7.3 in the LOM.

**Table 5.12 Quality control: all activities**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>Internal QC Taxonomist verifies that diatom slide is appropriate for diatom analysis</b>	All samples	No obvious problems such as bubbles under the coverslip	Slide is discarded and replaced with a new slide
<b>Duplicate identification for Internal QC</b>	10% of samples per taxonomist will be re-analyzed by a second taxonomist. This process randomly selects those samples to be re-identified.	PctDiff ≤ 50% (soft algae) PDE ≤ 15% (diatoms) PTD ≤ 25% (diatoms)	If any criterion is exceeded, perform a third count and reidentification for the sample.
<b>Use of widely/commonly accepted taxonomic references by all NRSA labs</b>	For all identifications	All keys and references used by each lab must be on bibliography prepared by one or more additional NRSA labs or in BioData (see Section 10.7 in the LOM for retrieval instructions). This requirement demonstrates the general acceptance of the references by the scientific community.	If a lab proposes to use other references, the lab must identify them in the database.
<b>Prepare reference collection</b>	Each new taxon per laboratory	Complete reference collection to be maintained by each individual laboratory	Internal Taxonomy QC Officer periodically reviews data and reference collection to ensure reference collection is complete and identifications are accurate

**Table 5.13 Data validation: diatom**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>Taxonomic "reasonable-ness" checks</b>	All data	Taxa known to occur in given rivers or streams or geographic area	Second or third identification by expert in that taxon

### 5.3.5 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOM. That quality is enhanced by the training and experience of project staff and documentation of sampling activities. Flag codes are recorded and comments provided on the Sample Collection Form to denote any problems encountered in collecting the sample or the presence of any conditions that may affect sample integrity. A summary of field quality control procedures for periphyton samples is presented in **Table 5.14**.

**Table 5.14 Sample collection and field processing quality control: periphyton**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Check integrity of sample containers and labels</b>	Clean, intact containers and labels	Obtain replacement supplies
<b>Sample Storage (field)</b>	Store samples on wet ice and in a dark place (cooler)	Discard and recollect sample
<b>Homogenize composite</b>	Thoroughly mix samples before processing to ensure that the sample material is evenly distributed throughout the composite.	Discard and recollect sample
<b>Processing samples in the field</b>	Use the appropriate filter or preservative for each type of sample prepared from the composite.	Discard and prepare a replacement subsample from the composite
<b>Holding times</b>	The frozen chlorophyll and AFDM filters are shipped immediately on wet ice. The ID sample preserved with formalin solution is held in a refrigerator and must be shipped on wet ice within 2 weeks of collection. <i>The FROZEN periphyton meta-genomic samples must be shipped within 1 week of collection on dry ice.</i>	Qualify samples

## 5.4 Benthic Macroinvertebrates

### 5.4.1 Introduction

The benthic macroinvertebrate assemblage found in sediments and on substrates of streams and rivers reflect an important aspect of the biological condition of the stream or river. The response of benthic communities to various stressors can often be used to determine the type of stressor and to monitor trends (Klemm et al., 1990). The overall objectives of the benthic macroinvertebrate indicators are to detect stresses on community structure in rivers and streams and to assess and monitor the relative severity of those stresses. The benthic macroinvertebrate indicator procedures are based on various bioassessment literatures (Barbour et al. 1999, Hawkins et al. 2000, Peck et al. 2003).

### 5.4.2 Sampling Design and Methods

Detailed sample collection and handling procedures are described in the FOM.

*Analysis:* Community identification samples are preserved, processed, enumerated, and organisms identified to the lowest possible taxonomic level (generally genus) using specified standard keys and references. Detailed procedures are contained in the LOM.

### 5.4.3 Quality Assurance Objectives

Measurement quality objectives (MQOs) are given in

**Table 5.15**, Section 2.2. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in Section 2.2 represents the maximum allowable criteria for statistical control purposes. Precision is calculated as percent efficiency, estimated from examination of randomly selected sample residuals by a second analyst and independent identifications of organisms in randomly



selected samples. The MQO for sorting and picking accuracy (defined and procedure in LOM Section 5) is estimated from examinations (repicks) of randomly selected residues by experienced taxonomists.

**Table 5.15 Measurement data quality objectives: benthic macroinvertebrates**

Variable or Measurement	Precision	Accuracy	Completeness
Sort and Pick	90% <sup>14</sup>	90% <sup>15</sup>	99% <sup>16</sup>
Identification	85% <sup>17</sup>	95% <sup>13</sup>	99%

The completeness objectives are established for each measurement per site type (e.g., probability sites, revisit sites, etc.). Failure to achieve the minimum requirements for a particular site type results in regional population estimates having wider confidence intervals. Failure to achieve requirements for repeat and annual revisit samples reduces the precision of estimates of index period and annual variance components, and may impact the representativeness of these estimates because of possible bias in the set of measurements obtained.

#### 5.4.4 Pertinent QA/QC Procedures

##### 5.4.4.1 Sorting and Subsampling QC

- A QC Analyst will use 6-10X microscopes to check all sorted grids from the first five samples processed by a sorter to ensure that each meets the acceptable criteria for percent sorting efficiency (PSE), which is 90%. This will not only apply to inexperienced sorters, but also to those initially deemed as “experienced.” Qualification will only occur when sorters achieve PSE ≥ 90% for five samples consecutively.
- The QC Officer will calculate PSE for each sample as follows:

**Equation 5.2 Percent sorting efficiency (PSE).**

$$PSE = \frac{A}{A + B} \times 100$$

Where: A = number of organisms found by the primary sorter, and B = number of recoveries (organisms missed by the primary sort and found during the QC check).

- If the sorting efficiency for each of these five consecutive samples is ≥ 90% for a particular individual, this individual is considered “experienced” and can serve as a QC Officer. In the event that an individual fails to achieve ≥ 90% sorting efficiency, he or she will be required to sort an additional five samples to continue to monitor their sorting efficiency. However, if he or she shows marked improvement in sorting efficiency prior to completion of the next

<sup>14</sup> Taxonomic accuracy and sorting accuracy as calculated using equation 2.11 in Section 2.2

<sup>15</sup> Taxonomic accuracy and sorting accuracy as calculated using equation 2.11 in Section 2.2

<sup>16</sup> Sample completeness as calculated using equation 2.12 in Section 2.2

<sup>17</sup> Taxonomic precision as calculated using equation 2.10 in Section 2.2

- five samples, achieving  $\geq 90\%$  sorting efficiency, the QA Officer may, at his/her discretion, consider this individual to be “experienced”. Do not calculate PSE for samples processed by more than one individual.
- After individuals qualify, 10% (1 out of 10, randomly selected) of their samples will be checked.
  - If an “experienced” individual fails to maintain a  $\geq 90\%$  PSE as determined by QC checks, a QC Officer will perform QC checks on every grid of five consecutive samples until a  $\geq 90\%$  sorting efficiency is achieved on all five. During this time, that individual will not be able to perform QC checks.

### 5.4.5 Taxonomic QC

#### 5.4.5.1 Internal Taxonomic QC

As directed by the EPA QA Coordinator, an in-house QC Analyst will conduct an internal 10% re-identification of all samples identified by that laboratory to ensure that each meets the acceptable criteria for percent identification efficiency which is 85%.

If the individual fails to maintain a  $\geq 85\%$  identification as determined by QC checks, previous samples will be re-counted and identified.

#### 5.4.5.2 External Taxonomic QC

- Upon receipt of the data, the EPA QA Coordinator for macroinvertebrates will randomly select 10% of the samples. The EPA QA Coordinator will then have the original laboratory send those samples to a QC taxonomist (another experienced taxonomist who did not participate in the original identifications). The original laboratory will complete a chain-of-custody form and send with the samples and notify NARS IM
- The QC taxonomist will perform whole-sample re-identifications, taking care to ensure inclusion of all slide-mounted specimens and completing another copy of the Benthic Macroinvertebrate Taxonomic Bench Sheet for each sample. The QC taxonomist will label each bench sheet with the term “QC Re-ID.” As each bench sheet is completed, the QC taxonomist will fax it to the NARS QA Coordinator.
- The EPA QA Coordinator will compare the taxonomic results (counts AND identifications) generated by the primary and QC taxonomists for each sample and calculate percent difference in enumeration (PDE) and percent taxonomic disagreement (PTD) as measures of taxonomic precision (Stribling et al. 2003) as follows:

**Equation 5.3 Percent difference in enumeration (PDE).**

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100$$

Where:  $n_1$  is the number of specimens counted in a sample by the first taxonomist and  $n_2$  is the number of specimens counted by the QC taxonomist.

- The recommendation for PDE is 5% or less.

**Equation 5.4 Percent taxonomic disagreement (PTD).**

$$PTD = \left[ 1 - \frac{comp_{pos}}{N} \right] \times 100$$

Where:  $comp_{pos}$  is the number of agreements (positive comparisons) and  $N$  is the total number of specimens in the larger of the two counts.

- A PTD of 15% or less is recommended for taxonomic difference (overall mean  $\leq 15\%$  is acceptable). The NRSA QA Officer will examine individual samples exceeding 15% for taxonomic areas of substantial disagreement, and investigate the reasons for disagreement. A reconciliation call between the primary and secondary taxonomist will facilitate this discussion of samples that do not meet specified criteria.. The NRSA QA officer, along with the QC taxonomist and the primary taxonomist, will investigate results greater than this value and they will note them for indication of error patterns or trends.
- Corrective actions include determining problem areas (taxa) and consistent disagreements and addressing problems through taxonomist interactions. These actions help to rectify disagreements resulting from identification to a specific taxonomic level.

#### 5.4.6 Taxonomic QC Review & Reconciliation

The EPA QA Coordinator prepares a report or technical memorandum to quantify aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report is submitted to the HQ Project Management Team, with copies sent to the primary and QC taxonomists. Another copy is maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist.

Each laboratory prepares reference/ voucher samples. These samples will be identified and digitally referenced (a photograph with taxonomic information superimposed on the photograph and in the file name) and will be included in an electronic file folder on the NARS Sharefile. All samples are stored at the laboratory until the Project Lead notifies the lab regarding disposition.

**Table 5.16 Laboratory quality control: benthic macroinvertebrates**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>SAMPLE PROCESSING AND SORTING</b>			
<b>Sample pickate examined by different analyst within lab</b>	10% of all samples completed per analyst	PSE $\geq 90\%$	If $< 90\%$ , examine all residuals of samples by that analyst and retrain analyst
<b>Sorting QC Officer counts number of organisms not found in sorted grids</b>	All samples	Sorter achieves PSE $\geq 90\%$ in 5 consecutive samples. Sorter is now considered "experienced"	Sorting QC Officer checks all samples until acceptance criteria met
<b>Sorting QC Officer counts number of organisms not found in sorted grids for experienced sorters</b>	1 in 10 samples completed per sorter	Sorter achieves PSE $\geq 90\%$	If $< 90\%$ , examine all sorted grids in samples assigned to sorter since last achieving proficiency (i.e., PSE $\geq 90\%$ ). Sorter loses "experienced" status and must again show proficiency by achieving PSE $\geq 90\%$ in 5 consecutive samples. If the sorter

			shows marked improvement in their sorting efficiency prior to completion of the next five samples, the Sorting QC Officer may, at his/her discretion, consider this individual to be “experienced” and check only 1 in the next 10 samples.
<b>External QC Coordinator evaluates grid and quarter data to determine if the sample was well mixed as demonstrated by consistency in counts between grids (or quarters)</b>	All grids and quarters within each sample	Sorter demonstrates relative consistency for 90% of assigned samples	If <90%, evaluate whether: 1) the sorter’s consistency is similar to other sorters; or 2) few samples were assigned the sorter. If neither explanation applies, EPA’s External QC Coordinator contacts the laboratory to discuss possible corrective action (e.g., resorting of sorter’s samples)
<b>IDENTIFICATION</b>			
<b>Duplicate identification by Internal Taxonomy QC Officer</b>	1 in 10 samples per taxonomist	PTD ≤15%	If PTD >15%, reidentify all samples completed by that taxonomist since last meeting the acceptance criteria, focusing on taxa of concern
<b>Independent identification by outside, expert, taxonomist</b>	All uncertain taxa	Uncertain identifications to be confirmed by expert in particular taxa	Record both tentative and independent IDs
<b>External QC</b>	10% of all samples completed per laboratory	PDE ≤ 5% PTD ≤ 15%	If PDE > 5%, implement recommended corrective actions. If PTD > 15%, implement recommended corrective actions.
<b>Use of widely/commonly accepted taxonomic references by all NRSA labs</b>	For all identifications	All keys and references used by each lab must be on bibliography prepared by one or more additional NRSA labs. This requirement demonstrates the general acceptance of the references by the scientific community.	If a lab proposes to use other references, the lab must obtain prior permission from Project QA Officer before submitting the data with the identifications based upon the references.
<b>Prepare reference collection</b>	Each new taxon per laboratory	Complete reference collection to be maintained by each individual	Internal Taxonomy QC Officer periodically reviews data and reference collection to ensure reference collection is complete

		laboratory	and identifications are accurate
<b>DATA VALIDATION</b>			
<b>Taxonomic "reasonableness" checks</b>	All data sheets	Taxa known to occur in given rivers or streams or geographic area	Second or third identification by expert in that taxon

### 5.4.7 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities. Field Crews enter a flag code and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Specific quality control measures for field operations are listed in **Table 5.17**.

**Table 5.17 Sample collection and field processing quality control: benthic macroinvertebrates**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Check integrity of sample containers and labels</b>	Clean, intact containers and labels	Obtain replacement supplies
<b>Sample Collection</b>	Keep the individual benthic macroinvertebrate subsamples wet while in the sieve bucket as each subsequent subsample is collected.	Discard and recollect sample if sample is not preserved
<b>Sample Collection</b>	Carry a small amount of ethanol to immediately preserve larger predaceous invertebrates to reduce the chance that other specimens will be consumed or damaged.	Qualify samples
<b>Sample Processing (field)</b>	Preserve with 95% ethanol. Fill jars 1/3 full of material to reduce the chance of organisms being damaged.	Qualify sample. If sample is deteriorated, discard sample and recollect.
<b>Sample Storage (field)</b>	Store benthic samples in a cool, dark place until shipment to analytical lab	Discard and recollect sample
<b>Holding time</b>	Preserved samples can be stored indefinitely; periodically check jars and change the ethanol if sample material appears to be degrading.	Qualify samples

## 5.5 Fish Assemblage

### 5.5.1 Introduction

Monitoring of the fish assemblage is an integral component of many water quality management programs. The assessment will measure specific attributes of the overall structure and function of the ichthyofaunal community to evaluate biological integrity and water quality.

### 5.5.2 Sampling Design and Methods

Detailed sample collection and handling procedures are described in the FOMs.

*Analysis:* Community identification samples are preserved, processed, enumerated, and organisms identified to the lowest possible taxonomic level (generally genus) using specified standard keys and references. Detailed procedures are contained in the LOM.

### 5.5.3 Quality Assurance Objectives

MQOs are given in **Table 5.18**. General requirements for comparability and representativeness are addressed in Section 2. Precision is calculated as percent efficiency, estimated from independent identifications of organisms in randomly selected samples. The MQO for accuracy is evaluated by having individual specimens representative of selected taxa identified by recognized experts.

**Table 5.18 Measurement data quality objectives: fish community**

Variable or Measurement	Precision	Accuracy	Completeness
Identification	85%	85% <sup>18</sup>	99%

### 5.5.4 Pertinent QA/QC Procedures

- The EPA Project QA Officer will randomly select 10% of the samples for QA analysis. The EPA Project QA Officer will then have the field crews voucher samples in the field and send them to a QC taxonomist (another experienced taxonomist who did not participate in the original identifications). The field crew and laboratory will complete a chain-of-custody form and send with the samples.
- The QC taxonomist will perform whole-sample re-identifications, taking care to ensure inclusion of all samples and completing fish voucher Taxonomic Bench Sheet for each sample. As each bench sheet is completed, email it to the Project Lead.
- The EPA Project QA officer will compare the taxonomic results (counts AND identifications) generated by the field crews and QC taxonomists for each sample and calculate percent difference in enumeration (PDE) and percent taxonomic disagreement (PTD) as measures of taxonomic precision (Stribling et al. 2003) as follows:

**Equation 5.5 Percent difference in enumeration (PDE).**

$$PDE = \frac{|n_1 - n_2|}{n_1 + n_2} \times 100$$

Where:  $n_1$  is the number of specimens counted in a sample by the field taxonomist and  $n_2$  is the number of specimens counted by the QC taxonomist.

**Equation 5.6 Percent taxonomic disagreement (PTD).**

$$PTD = \left[ 1 - \frac{comp_{pos}}{N} \right] \times 100$$

<sup>18</sup> Taxonomic accuracy as calculated as described in 3.2.3

Where:  $comp_{pos}$  is the number of agreements (positive comparisons) and  $N$  is the total number of specimens in the larger of the two counts.

- The recommendation for PDE is 5% or less.
- A PTD of 15% or less is recommended for taxonomic difference (overall mean  $\leq 15\%$  is acceptable). Individual samples exceeding 15% are examined for taxonomic areas of substantial disagreement, and the reasons for disagreement investigated. A reconciliation call between the primary and secondary taxonomist will facilitate this discussion. Results greater than this value are investigated and logged for indication of error patterns or trends.
- Corrective actions include determining problem areas (taxa) and consistent disagreements and addressing problems through taxonomist interactions. These actions help to rectify disagreements resulting from identification to a specific taxonomic level.

### 5.5.5 Taxonomic QC Review & Reconciliation

The EPA Project QA Officer prepares a report or technical memorandum to quantify aspects of taxonomic precision, assess data acceptability, highlight taxonomic problem areas, and provide recommendations for improving precision. This report is submitted to the HQ Project Management Team, with copies sent to the field and QC taxonomists. Another copy is maintained in the project file. Significant differences may result in the re-identification of samples by the primary taxonomist and a second QC check by the secondary taxonomist.

Each laboratory prepares reference/ voucher samples. These samples will be identified and digitally referenced (a photograph with taxonomic information superimposed on the photograph and in the file name) and will be included in an electronic file folder on the NARS Sharefile. All samples are stored at the laboratory until the Project Lead notifies the lab regarding disposition.

### 5.5.6 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities.

An experienced fish taxonomist will identify the collected fish specimens in the field. All specimens must be identified by common name as listed in Appendix D of the FOMs. The biologist may choose to retain certain specimens for identification or verification in the laboratory. These samples are retained at the discretion of the fish taxonomist and are separate from the official voucher specimens that must be collected at 10% of each field crews' sites to be re-identified by an independent taxonomist.

A summary of field quality control procedures for the fish community indicator is presented in **Table 5.19**.

**Table 5.19 Sample collection and field processing quality control: fish community**

Quality Control Activity	Description and Requirements	Corrective Action
Check integrity of sample containers and labels	Clean, intact containers and labels	Obtain replacement supplies

Quality Control Activity	Description and Requirements	Corrective Action
<b>Set up electrofishing equipment</b>	An experienced fisheries biologist sets up the unit. Determine if appropriate fish capture results are achieved.	If results are poor, adjustments are made to the pulse width and voltage to sample effectively and minimize injury/mortality.  Determine if electroshocker is functioning properly; if not use backup (e.g. generator).
<b>Comparable effort</b>	Reset unit clock to document button time (700 seconds per transect).	If button time is not metered, estimate it with a stop watch and flag the data.
<b>Comparable effort</b>	No more than 1 person is netting at any one time.	Limit number of crew members with nets.
<b>Field Processing</b>	Fish should be released in a location that prevents the likelihood of their recapture.	Flag data if fish were released in area where recapture was possible
<b>Field Processing</b>	The fisheries biologist will identify specimens in the field using a standardized list of common names.	Indicator lead will contact crews to resolve discrepancies in names.
<b>Sample Collection</b>	The biologist may retain uncertain specimens for ID or verification in the laboratory. These samples are retained at the discretion of the biologist and are separate from the official voucher specimens that must be collected at 10% of each field crews' sites to be re-identified by an independent taxonomist.	Flag data. If crew does not collect voucher at specified site, NRSA QA Officer will identify additional QA sites for collection.
<b>Sample Collection - Taxonomic QC samples</b>	EPA selected sites designated for independent, taxonomic confirmation of fish assemblage taxonomy. A minimum of 1 complete voucher is required for each field taxonomist and will consist of either preserved specimen(s) or digital images representative of all species in the sample, even common species.	If crew does not collect voucher at specified site, EPA Project QA Officer will identify additional QA sites for collection.
<b>Sample Preservation</b>	Fish retained for laboratory ID or vouchers are preserved with 10% buffered formalin. All personnel must read and should follow the appropriate guidelines for handling formalin in the field. An MSDS can be found at the following website. <a href="http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10076&amp;p_table=standards">http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10076&amp;p_table=standards</a>	If vouchers are not adequately preserved, new vouchers must be collected at the next field site.



### **5.5.6.1 Voucher Specimens**

Approximately 10% of each field crews' sites will be randomly pre-selected for re-identification by an independent QA/QC taxonomist. These samples will be selected in coordination from the EPA Project QA Officer. A minimum of one complete voucher is required for each person performing field taxonomy and will consist of either preserved specimen(s) or digital images representative of all species in the sample, even common species. Multiple specimens per species can be used as vouchers, if necessary (i.e., to document different life or growth stages, or sexes). Note that a complete sample voucher does not mean that all individuals of each species will be vouchered, only enough so that independent verification can be achieved.

For species that are retained, specimen containers should be labeled with the sample number, site ID number, site name, and collection date. There should be no taxonomic identification labels in or on the container.

Digital images should be taken as voucher documentation for species that are recognized as Rare, Threatened, or Endangered (RTE) they should not be harmed or killed. Very common and well-known, or very large-bodied species may also be recorded by digital images; however, these can be preserved at the discretion of the taxonomist. Labeling, within the image, should be similar to that used for preserved samples and not include taxonomic identification. Guidance for naming photo files is provided below in the photovouchering section.

### **5.5.6.2 Photovouchering**

Digital imagery should be used for fish species that cannot be retained as preserved specimens (e.g., RTE species; very large bodied; or very common). Views appropriate and necessary for an independent taxonomist to accurately identify the specimen should be the primary goal of the photography. Additional detail for these guidelines is provided in Stauffer et al. (2001), and the Field Logistics Coordinator will distributed to all field crews electronically via the sharefile site. 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 should occupy as much of the field of view as possible, and the use of a fish board is recommended to provide a reference to scale (i.e., ruler or some calibrated device) and an adequate background color for photographs. Information on Station ID, Site Name, Date and a unique species ID (i.e., A, B, C, etc.) should also be captured in the photograph, so that photos can be identified if file names become corrupted. All photovouchered species should have at least a full-body photo (preferably of the left side of the fish) and other zoom images as necessary for individual species, such as lateral line, ocular/oral orientation, fin rays, gill arches, or others. It may also be necessary to photograph males, females, or juveniles.

Images should be saved in medium- to high-quality jpeg format, with the resulting file name of each picture noted on the Fish Collection Form. It is important that time and date stamps are accurate as this information can also be useful in tracking the origin of photographs. It is recommended that images stored in the camera be transferred to a PC or storage device 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). Field crews should maintain files for the duration of the sampling season. Notification regarding the transfer of all images to the existing database will be provided at the conclusion of the sampling.

## 5.5.7 Quality Control Procedures: Laboratory Operations (Voucher Specimens)

### 5.5.7.1 Sample Receipt and Processing

QC activities associated with sample receipt and processing are presented in

**Table 5.20.** The communications center and information management staff is notified of sample receipt and any associated problems as soon as possible after samples are received.

**Table 5.20 Sample receipt and processing quality control: fish community**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Sample Log-in</b>	Upon receipt of a sample shipment, laboratory personnel check the condition and identification of each sample against the sample tracking record.	Discrepancies, damaged, or missing samples are reported to the IM staff and indicator lead
<b>Sample Storage</b>	Samples Stored in formalin in dark room or photovouchers kept on external hard drive	Qualify sample as suspect for all analyses
<b>Holding time</b>	Not Applicable	Qualify samples
<b>Preservation</b>	Vouchers are stored on formalin	Qualify samples

### 5.5.7.2 Analysis of Samples

Specific quality control measures for laboratory operations are listed in **Table 5.21** and

**Table 5.22.**

**Table 5.21 Laboratory quality control: fish voucher taxonomic identification**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>Use widely/commonly accepted taxonomic references</b>	All identifications	All keys and references used must be on bibliography prepared by the field and QC taxonomists	For all field crew identifications, EPA will convert field crew's use of common names to taxonomic references
<b>Independent identification by outside, expert, laboratory fish taxonomist ("QC taxonomist")</b>	When field taxonomist cannot identify specimen	Identification by QC taxonomist (who must be a different individual than the field taxonomist)	Replace field crew's "unknown" identification with determination by QC taxonomist
<b>External QC</b>	Approximately 10% of all sites sampled by each field taxonomist	PTD ≤ 15%	If PTD > 15%, review data for possible explanations; otherwise, insert data qualifier for field crew identifications

<b>Calculate average PTD for field taxonomist</b>	Each sample submitted to the QC taxonomist	PTD ≤ 15%	If PTD > 15%, consult with NARS QA Officer for appropriate action.
<b>Conduct assistance visit</b>	EPA may choose to visit any laboratory	Checklist will be developed if EPA chooses to conduct a laboratory visit.	Performance and any recommended improvements described in debrief with laboratory staff

**Table 5.22 Data validation: fish voucher taxonomic identification**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>Data Validation: Taxonomic "reasonable-ness" checks</b>	All data sheets	Genera known to occur in given rivers/streams or geographic area	Data qualifiers on data that fail reasonableness check. No further corrective action steps.

## 5.6 Physical Habitat Quality

### 5.6.1 Introduction

Naturally occurring differences in physical habitat structure and associated hydraulic characteristics among surface waters contributes too much of the observed variation in species composition and abundance within a zoogeographic province. Structural complexity of aquatic habitats provides the variety of physical and chemical conditions to support diverse biotic assemblages and maintain long-term stability. Anthropogenic alterations of riparian physical habitat, such as channel alterations, wetland drainage, grazing, agricultural practices, weed control, and streambank modifications such as revetments or development, generally act to reduce the complexity of aquatic habitat and result in a loss of species and ecosystem degradation.

For the NRSA, indicators derived from data collected on physical habitat quality will be used to help explain or characterize stream and river conditions relative to biological response and trophic state indicators. Specific groups of physical habitat attributes important in stream and river ecology include: channel dimensions, gradient, substrate; habitat complexity and cover; riparian vegetation cover and structure; anthropogenic alterations; and channel-riparian interaction (Kaufmann, 1993). Overall objectives for this indicator are to develop quantitative and reproducible indices, using both multivariate and multimetric approaches, to classify streams and rivers and to monitor biologically relevant changes in habitat quality and intensity of disturbance.

### 5.6.2 Sampling Design and Methods

As the physical habitat indicator is based on field measurements and observations, there is no sample collection associated with this indicator. At NRSA sites, eleven cross-sectional measurement transects are spaced at equal intervals proportional to baseflow channel width, thereby scaling the sampling reach length and resolution in proportion to stream and river size. A systematic spatial sampling design is used to minimize bias in the selection of the measurement sites. Additional measurements are made at equally spaced intervals between the cross-sectional sites.

Field measurements, observations, and associated methodology for the protocol are summarized in

**Table 5.23.** Detailed procedures for completing the protocols are provided in the FOM.

There are no sample collections or laboratory analyses associated with the physical habitat measurements.

**Table 5.23 Field measurement methods: physical habitat**

Variable or Measurement	Units	Summary of Method	References
<b>THALWEG PROFILE</b>			
Thalweg depth	cm	Measure max depth at 100-150 points for wadeable or 200 points for non-wadeable along reach with surveyor's rod or sonar equipment	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Wetted width	0.1m	Measure wetted width with range finder or measuring tape on perpendicular line to mid-channel line	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Habitat class	none	Visually estimate channel habitat using defined class descriptions	Frissell et al., 1986
<b>WOODY DEBRIS TALLY</b>			
Large woody debris	# of pieces	Use pole drag and visually estimate amount of woody debris in baseflow channel using defined class descriptions	Robison and Beschta, 1990
<b>CHANNEL AND RIPARIAN CROSS-SECTIONS</b>			
Slope and bearing	%/ degrees	Backsight between cross-section stations using clinometer, rangefinder, compass, surveyor's level & tripod	Robison & Kaufmann, in prep.; Stack, 1989
Substrate size	mm	At 5 points on cross section, estimate size of one selected particle using defined class descriptions	Wolman, 1954; Bain et al., 1985; Plafkin et al., 1989
Bank angle	degrees	Use clinometer and surveyors rod to measure angle	Platts et al., 1983
Bank incision	0.1m	Visually estimate height from water surface to first terrace of floodplain	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Bank undercut	cm	Measure horizontal distance of undercut	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Bankfull width	0.1m	Measure width at top of bankfull height	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Bankfull height	0.1m	Measure height from water surface to estimated water surface during bankfull flow	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Canopy cover	points of intersection	Count points of intersection on densiometer at specific points and directions on cross-section	Lemmon, 1957; Mulvey et al., 1992

Variable or Measurement	Units	Summary of Method	References
Riparian vegetation structure	percent	Observations of ground cover, understory, and canopy types and coverage of area 5 m on either side of cross section and 10 m back from bank	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Fish cover, algae, macrophytes	percent	Visually estimate in-channel features 5 m on either side of cross section	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
Human influence	none	Estimate presence/absence of defined types of anthropogenic features	US EPA <a href="http://www.epa.gov/emap/">http://www.epa.gov/emap/</a>
<b>STREAM DISCHARGE</b>			
Discharge	m/s or L/min.	Velocity-Area method, Portable Weir method, timed bucket discharge method	Linsley et al., 1982

### 5.6.3 Quality Assurance Objectives

Measurement data quality objectives (measurement DQOs or MQOs) are given in **Table 5.24**. General requirements for comparability and representativeness are addressed in Section 2. The MQOs given in **Table 5.24** represent the maximum allowable criteria for statistical control purposes. Precision is determined from results of revisits by a different crew (field measurements) and by duplicate measurements by the same crew on a different day.

The completeness objectives are established for each measurement *per site type* (e.g., NRSA sites, revisit sites, state comparability sites). Failure to achieve the minimum requirements for a particular site type results in regional population estimates having wider confidence intervals. Failure to achieve requirements for repeat and annual revisit samples reduces the precision of estimates of index period and annual variance components, and may impact the representativeness of these estimates because of possible bias in the set of measurements obtained.

**Table 5.24 Measurement data quality objectives: physical habitat**

Variable or Measurement	Precision	Accuracy	Completeness
<b>Field Measurements and Observations</b>	±10%	NA	90%
<b>Map-Based Measurements</b>	±10%	NA	100%

NA = not applicable

### 5.6.4 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities. Specific quality control measures are listed in **Table 5.25** for field measurements and observations.

**Table 5.25 Field quality control: physical habitat**

Check Description	Frequency	Acceptance Criteria	Corrective Actions
Check totals for cover class categories (vegetation type, fish cover)	Each transect	Sum must be reasonable (best professional judgment)	Repeat observations
Check completeness of thalweg depth measurements	Each site	Depth measurements for all sampling points	Obtain best estimate of depth where actual measurement not possible

## 5.7 Fecal Indicator: Enterococci

### 5.7.1 Introduction

The primary function of collecting water samples for Pathogen Indicator Testing is to provide a relative comparison of fecal pollution indicators for national rivers and streams. The concentration of Enterococci (the current bacterial indicator for fresh and marine waters) in a water body correlates with the level of more infectious gastrointestinal pathogens present in the water body. While some Enterococci are opportunistic pathogens among immuno-compromised human individuals, the presence of Enterococci is more importantly an indicator of the presence of more pathogenic microbes (bacteria, viruses and protozoa) associated with human or animal fecal waste.

### 5.7.2 Sampling Design and Methods

Detailed sample collection and handling procedures are described in the FOMs.

### 5.7.3 Pertinent QA/QC Procedures

#### 5.7.3.1 Quality Assurance Objectives

Measurement quality objectives (MQO) are given in **Table 5.26**. General requirements for comparability and representativeness are addressed in Section 2.

**Table 5.26 Measurement data quality objectives: pathogen-indicator DNA sequences**

Variable or Measurement <sup>19</sup>	Method Precision	Method Accuracy	Completeness
SPC & ENT DNA sequence numbers of Calibrators & Standards by AQM	RSD=30%	<u>80%</u>	95%
ENT CCEs by dCt RQM	RSD = 40%	40%	95%
ENT CCEs by ddCt RQM	RSD = 55%	50%	95%

<sup>19</sup> AQM = Absolute Quantitation Method; dCt=delta (change) of control treated; RQM = Relative Quantitation Method; SPC = Sample Processing Control (Salmon DNA/Sketa) (note – Sketa is a reagent); CCEs = Calibrator Cell Equivalents; RSD= Relative Standard Distribution; ENT=Enterococci

### 5.7.3.2 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities. Specific quality control measures are listed in

**Table 5.27** for field measurements and observations.

**Table 5.27 Sample collection and field processing quality control: fecal indicator**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Check integrity of sample containers and labels</b>	Clean, intact containers and labels	Obtain replacement supplies
<b>Sterility of sample containers</b>	Sample collection bottle and filtering apparatus are sterile and must be unopened prior to sampling. Nitrile gloves must be worn during sampling and filtering	Discard sample and recollect in the field.
<b>Sample Collection</b>	Collect sample at the last transect to minimize holding time before filtering and freezing	Discard sample and recollect in the field.
<b>Sample holding</b>	Sample is held in a cooler on wet ice until filtering	Discard sample and recollect in the field.
<b>Field Processing</b>	Sample is filtered and filters are frozen on dry ice within 6 hours of collection	Discard sample and recollect in the field
<b>Field Blanks</b>	Field blanks must be filtered at 10% of sites	Review blank data and flag sample data.

### 5.7.3.3 Quality Control Procedures: Laboratory Operations

Specific quality control measures for laboratory operations are listed in **Table 5.28**.

**Table 5.28 Laboratory quality control: pathogen-indicator DNA sequences**

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
<b>SAMPLE PROCESSING</b>			
Re-process sub-samples (Lab Duplicates)	10% of all samples completed per laboratory	Percent Congruence <70% RSD	If >70%, re-process additional sub-samples
<b>qPCR ANALYSIS</b>			
Duplicate analysis by different biologist within lab	10% of all samples completed per laboratory	Percent Congruence ≤30% RSD	If >30%, determine reason and if cause is systemic, re-analyze all samples in question.

Check or Sample Description	Frequency	Acceptance Criteria	Corrective Action
Use single stock of <i>E. faecalis</i> calibrator	For all qPCR calibrator samples for quantitation	All calibrator sample <i>C<sub>p</sub></i> ( <i>C<sub>t</sub></i> ) must have an RSD $\leq$ 30%.	If calibrator <i>C<sub>p</sub></i> ( <i>C<sub>t</sub></i> ) values exceed an RSD value of 30% a batch's calibrator samples shall be re-analyzed and replaced with new calibrators to be processed and analyzed if RSD not back within range.
DATA PROCESSING & REVIEW			
100% verification and review of qPCR data	All qPCR amplification traces, raw and processed data sheets	All final data will be checked against raw data, exported data, and calculated data printouts before entry into LIMS and upload to Corvallis, OR database.	Second tier review by contractor and third tier review by EPA.

#### 5.7.4 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validations are summarized in **Table 5.29**. All raw data (including all standardized forms and logbooks) are retained in an organized fashion for seven years or until written authorization for disposition has been received from the NRSA Project Coordinator. Once data have passed all acceptance requirements, data is submitted to NARS IM and coordinated with the NRSA data Information Coordinator.

**Table 5.29 Data validation quality control: fecal indicator**

Check Description	Frequency	Acceptance Criteria	Corrective Action
Duplicate sampling	Duplicate composite samples collected at 10% of sites	Measurements should be within 10 percent	Review data for reasonableness; determine if acceptance criteria need to be modified
Field filter blanks	Field blanks filtered at 10% of sites	Measurements should be within 10 percent	Review data for reasonableness; determine if acceptance criteria need to be modified

### 5.8 Antimicrobial Supplemental Research Indicator

Information about this indicator is contained and maintained by EPA's Office of Research and Development Cincinnati Laboratory, and can be obtained by contacting Jay Garland, EPA's ORD Antimicrobial Resistance Coordinator.



## 5.9 Fish Tissue Plugs

### 5.9.1 Introduction

Fish are time-integrating indicators of persistent pollutants, and contaminant bioaccumulation in fish tissue has important human health implications for people who consume fish. The objective for fish plug sampling is to collect one plug sample for mercury analysis at all river and stream sites where suitable fish species and lengths are available **except** the 478 river sites selected for whole fish tissue sampling. A plug sample consists of two fish tissue plugs collected from two fish of the same species (one plug per fish). Analysis of the NRSA fish tissue plug samples will provide information on the national distribution of mercury in fish from streams and rivers of the contiguous United States.

### 5.9.2 Sampling Design and Methods

Detailed fish tissue plug sample collection and handling procedures are described in the FOMs.

Collection of individual fish specimens for the fish tissue plug samples 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 identified in the target list found in **Table 5.34**. 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. Some recommended alternative species are included in **Table 5.34**.

**Table 5.30 Recommended target and alternate species: fish tissue plug collection**

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

		Cutthroat trout	<i>Oncorhynchus clarkii</i>	~300 mm
		Rainbow trout	<i>Oncorhynchus mykiss</i>	~300 mm
		Brook trout	<i>Salvelinus fontinalis</i>	~330 mm
<b>Alternates</b>	Cyprinidae	Northern pikeminnow	<i>Ptychocheilus oregonensis</i>	~300 mm
	Centrarchidae	Bluegill	<i>Lepomis macrochirus</i>	~200 mm
		Rock bass	<i>Ambloplites rupestris</i>	~200 mm
		Redbreast sunfish	<i>Lepomis auritus</i>	~200 mm

### 5.9.2.1 Sampling and Analytical Methodologies for Field Operations and Laboratory Analyses

Detailed sampling methods and procedures for handling and shipping fish plug samples are found in the FOMs. The laboratory method for mercury analysis of fish plug samples is performance based. Example standard operating procedures are provided in Appendix F of the LOM.

## 5.9.3 Pertinent QA/QC Procedures

### 5.9.3.1 Quality Assurance Objectives

The relevant quality objectives for fish tissue plug sample collection activities are primarily related to sample handling issues. Types of field sampling data needed for the fish tissue plugs are listed in **Table 5.35**. Methods and procedures described in this QAPP and the FOMs are intended to reduce the magnitude of the sources of uncertainty (and their frequency of occurrence) by applying:

- standardized sample collection and handling procedures, and
- use of trained scientists to perform the sample collection and handling activities.

**Table 5.31 Field data types: fish tissue plug**

Variable or Measurement	Measurement Endpoint or Unit
Fish specimen	Species-level taxonomic identification
Fish length	Millimeters (mm), total length
Fish weight	Grams (g)

### 5.9.3.2 Quality Control Procedures: Field Operations

Field data quality is addressed, in part, by application and consistent performance of valid procedures documented in the standard operating procedures detailed in the FOMs. That quality is enhanced by the training and experience of project staff and documentation of sampling activities. Specific quality control measures are listed in **Table 5.36** for field measurements and observations.

**Table 5.32 Field quality control: fish tissue plug**

Quality Control Activity	Description and Requirements	Corrective Action
<b>Check integrity of sample containers and labels</b>	Clean, intact containers and labels	Obtain replacement supplies
<b>Set up electrofishing equipment</b>	An experienced fisheries biologist sets up the unit. If results are poor, adjustments are made to the pulse width and voltage to sample effectively and minimize injury/mortality.	Adjust voltage in field
<b>Field Processing</b>	The fisheries biologist will identify specimens in the field using a standardized list of common names (App. D of the FOMs).	Labs verify. If not same species, sample not composited
<b>Sample Collection</b>	The fisheries biologist will retain 2 specimens of the same species to form the composite sample	If not the same species, sample not composited
<b>Sample Collection</b>	The length of the smallest fish must be at least 75% of the length of the longest fish.	If fish out of length range requirement, EPA contacted for instructions

#### 5.9.4 Data Management, Review, and Validation

Checks made of the data in the process of review, verification, and validation is summarized in **Table 5.37**. The Indicator Lead is ultimately responsible for ensuring the validity of the data, although performance of the specific checks may be delegated to other staff members. All raw data (including all standardized forms and logbooks) are retained in an organized fashion for seven years or until written authorization for disposition has been received from the NRSA Project Coordinator. Once data have passed all acceptance requirements, data submitted to EPA Coordinator.

**Table 5.33 Data validation quality control: fish tissue plug**

Check Description	Frequency	Acceptance Criteria	Corrective Action
<b>75% rule</b>	All composites	Length of smallest fish in the composite must be at least 75% of the length of the longest fish.	Indicator lead will review composite data and advise the lab before processing begins

#### 5.9.5 Quality Control Procedures: Laboratory Operations

**Table 5.34 Measurement data quality objectives: fish tissue plug**

Variable or Measurement	MDL	Quantitation Limit
<b>Mercury</b>	0.47 ng/g	5.0 ng/g

**Table 5.35 Lab quality control: fish tissue plug**

Activity	Evaluation/Acceptance Criteria	Corrective Action
<b>Demonstrate competency for analyzing fish samples to meet the performance measures</b>	Demonstration of past experience with fish tissue samples in applying the laboratory SOP in achieving the method detection limit	EPA will not approve any laboratory for NRSA sample processing if the laboratory cannot demonstrate competency. In other words, EPA will select another laboratory that can demonstrate competency for its NRSA samples.
<b>Check condition of sample when it arrives.</b>	Sample issues, such as punctures or rips in wrapping; missing label; temperature; adherence to holding time requirements; sufficient volume for test. All samples should arrive at the laboratory frozen.	Assign appropriate condition code identified in Appendix G.
<b>Store sample appropriately. While stored at the laboratory, the sample must be kept at a maximum temperature of -20° C.</b>	Check the temperature of the freezer per laboratory's standard operating procedures.	Record temperature of sample upon arrival at the laboratory. If at any other time, samples are warmer than required, note temperature and duration in comment field.
<b>Analyze sample within holding time</b>	The test must be completed within the holding time (i.e., 1 year). If the original test fails, then the retest also must be conducted within the holding time.	Perform test, but note reason for performing test outside holding time. EPA expects that the laboratory will exercise every effort to perform tests before the holding time expires.
<b>Maintain quality control specifications from selected method/SOP (that meets the measurement data quality objectives)</b>	Data meet all QC specifications in the selected method/SOP.	If data do not meet all QC requirements, data must be flagged. If the lab believes the data are to be qualified without rerunning sample, the lab must consult with the EPA Survey QA Lead before proceeding.
<b>Maintain the required MDL</b>	Evaluate for each sample	If MDL could not be achieved, then provide dilution factor or QC code and explanation in the comment field.
<b>Use consistent units for QC samples and field samples</b>	Verify that all units are consistently provided in wet weight units	If it is not possible to provide the results in the same units as most other analyses, then assign a QC code and describe the reason for different units in the comments field of the database.
<b>Maintain completeness</b>	Completeness objective is 95% for all parameters.	Contact the EPA Survey QA Lead immediately if issues affect laboratory's ability to meet completeness objective.

## 6 FIELD AND BIOLOGICAL LABORATORY QUALITY EVALUATION AND ASSISTANCE VISITS

### 6.1 National Rivers and Streams Assessment Field Quality Evaluation and Assistance Visit Plan

Evaluation and assistance visits (AV) will be conducted with each field crew early in the sampling and data collection process, if possible, and corrective actions will be conducted in real time. These visits provide both a quality 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 visit also provides the field crews with an opportunity to clarify procedures and offer suggestions for future improvements based on their sampling experience preceding the visit. The field evaluations, while performed by a number of different supporting collaborator agencies and participants, will be based on the uniform training, plans, and checklists. This review and assistance task will be conducted for each unique field crew collecting and contributing data under this program; hence no data will be recorded to the project database that was produced by an 'unaudited' process or individual. The field evaluations will be based on the evaluation plan and field evaluation checklist.

One or more designated EPA or Contractor staff members who are qualified (i.e. have completed training) in the procedures of the NRSA 2023/24 field sampling operations will visit trained state, tribal, contractor, and EPA field sampling crews during sampling operations on site. If membership of a field crew changes, and at least two of the members have not been evaluated previously, the field crew must be evaluated again during sampling operations as soon as possible to ensure that all members of the field crew understand and can perform the procedures. If a deviation is needed from the process described here, the staff member conducting the AV must contact the NRSA Project Lead. The NRSA Project Lead will contact the NRSA Project QA Officer to determine an acceptable course of action.

The purpose of this on-site visit will be to identify and correct deficiencies during field sampling operations. The process will involve preparation activities, field day activities and post field day activities as described in the following sections. Additionally, conference calls with crews may be held approximately every two weeks to discuss issues as they come up throughout the sampling season.

#### 6.1.1 Preparation Activities

- Each Field Crew Evaluator will schedule an assistance visit with their designated crews in consultation with the Contractor Field Logistics Coordinator, Regional NRSA Coordinator, and respective Field Sampling Crew Leader. Ideally, each Field Crew will be evaluated within the first two weeks of beginning sampling operations, so that procedures can be corrected or additional training provided, if needed.
- Each Evaluator is responsible for providing their own field gear sufficient to accompany the Field Sampling Crews during a complete sampling cycle. Schedule of the Field visits will be made by the Evaluator in consultation with the respective Field Crew Leader. **Evaluators should be prepared to spend additional time in the field if needed (see below).**
- Each Field Crew Evaluator will ensure that field crews are aware of their visit plans and all capacity and safety equipment will be provided for the Field Crew Evaluator.
- Each Field Crew Evaluator will need to bring the items listed in **Table 6.1**.

**Table 6.1 Equipment and supplies: field evaluation and assistance visits**

Type	Item	Quantity
<b>Form</b>	Appendix B (see FOM 2023/24)	1
<b>Documentation</b>	NRSA 2023/24 Field Operations Manuals	1
	NRSA 2023/24 Quality Assurance Project Plan	1
	Clipboard	1
	Pencils (#2, for data forms)/Pen (or computer for electronic versions)	1
	Field notebook (optional)	1
<b>Gear</b>	Field gear (e.g., protective clothing, sunscreen, insect repellent, hat, water, food, backpack, cell phone)	As needed

### 6.1.2 Field Day Activities

- The Field Crew Evaluator will review the Field Evaluation & Assistance Visit Checklist with each crew during the field sampling day and establish and plan and schedule for their evaluation activities for the day.
- The Field Crew Evaluator will view the performance of a field crew through one complete set of sampling activities as detailed on the checklist.
- Scheduling might necessitate starting the evaluation midway on the list of tasks at a site, instead of at the beginning. In that case, the Field Crew Evaluator will follow the crew to the next site to complete the evaluation of the first activities on the list.
- If the field crew misses or incorrectly performs a procedure, the Field Crew 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 Field Crew 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.
- When the sampling operation has been completed, the Field Crew 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]; deficiencies [would adversely affect data quality]). The Field Crew Evaluator will ensure that the field crew understands the findings and will be able to perform the procedures properly in the future.
- The Field Crew Evaluator will review the list and record responses or concerns from the field crew, if any; on the checklist (this may happen throughout the field day).
- The Field Crew Leader will sign the checklist after this review.

### 6.1.3 Post Field Day Activities

- The Field Crew Evaluator will review the checklist that evening and provide a summary of findings, including lessons learned and concerns.
- If the Field Crew Evaluator finds major deficiencies in the field crew operations (e.g., less than two members, equipment, or performance problems) the Field Crew Evaluator must contact the EPA NRSA 2023/24 Project Lead. The EPA NRSA 2023/24 Project Lead will contact the EPA NRSA 2023/24 QA Project Coordinator 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.

- The Field Crew Evaluator will retain a copy of the checklist and submit to the EPA NRSA Logistics Lead either via Fed-Ex or electronically.
- The EPA NRSA 2023/24 Project Lead and the EPA Logistics Lead or authorized designee will review the returned Field Evaluation and Assistance Visit Checklist, note any issues, and check off the completion of the evaluation for each field crew.

### 6.1.4 Summary

Table 6.2 summarizes the plan, checklist, and corrective action procedures.

**Table 6.2 Summary: field evaluation and assistance visits**

<b>Field Evaluation Plan</b>	<p>The Field Crew Evaluator:</p> <ul style="list-style-type: none"> <li>• Arranges the field evaluation visit in consultation with the QA Officer, Regional NRSA Coordinator, and respective Field Sampling Crew Leader, ideally within the first two weeks of sampling</li> <li>• Observes the performance of a crew through one complete set of sampling activities</li> <li>• Takes note of errors the field crew makes on the checklist and immediately point these out to correct the mistake</li> <li>• Reviews the results of the evaluation with the field crew before leaving the site, noting positive practices, lessons learned, and concern</li> </ul>
<b>Field Evaluation Checklist</b>	<p>The Field Crew Evaluator:</p> <ul style="list-style-type: none"> <li>• Observes all pre-sampling activities and verifies that equipment is properly calibrated and in good working order, and protocols are followed</li> <li>• 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</li> <li>• Confirms that the field crew has followed NRSA protocols for locating the river/stream X point</li> <li>• Observes the index site sampling, confirming that all protocols are followed</li> <li>• Observes the littoral sampling and habitat characterization, confirming that all protocols are followed</li> <li>• Records responses or concerns, if any, on the Field Evaluation and Assistance Checklist</li> </ul>
<b>Corrective Action Procedures</b>	<ul style="list-style-type: none"> <li>• If the Field Crew 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.</li> <li>• If the Field Crew Evaluator finds major deficiencies in the Field Crew operations the Evaluator must contact the EPA NRSA 2023/2024 Project Lead.</li> </ul>

## 6.2 National Rivers and Streams Assessment Laboratory Quality Evaluation and Assistance Visit Plan

As part of the NRSA 2023/24, field samples will be collected at each assessment site. These samples will be sent to laboratories cooperating in the assessment. To ensure quality, each Project Cooperator laboratory analyzing samples from the NRSA 2023/24 will receive an evaluation from an NRSA Lab Evaluator. All Project Cooperator laboratories will follow these guidelines.

No national program of accreditation for laboratory processing for many of our indicators currently exists. For this reason, a rigorous program of laboratory evaluation has been developed to support the NRSA 2023/24.

Given the large number of laboratories participating in the NRSA 2023/24, it is not feasible to perform an assistance visit<sup>20</sup> (AV) on each of these laboratories. An AV would include an on-site visit to the laboratory lasting at least a day. As a result, the EPA Headquarters Project Management Team will conduct remote review of laboratory certifications and accreditations of all laboratories. Additionally, EPA will include an inter-laboratory comparison between some laboratories (mainly for biological indicators). If issues arise from the remote review or inter-laboratory comparison that cannot be resolved remotely then the EPA Quality Team and/or contractors may perform an on-site visit to the laboratory. This process is in keeping with EPA's *Policy to Assure Competency of Laboratories, Field Sampling, and Other Organizations Generating Environmental Measurement Data under Agency-Funded Acquisitions*.

### 6.2.1 Remote Evaluation/Technical Assessment

A remote evaluation procedure has been developed for performing assessment of all laboratories participating in the NRSA 2023/24.

The NRSA QA Team will conduct laboratory evaluation prior to data analysis to ensure that the laboratories are qualified and that techniques are implemented consistently across the multiple laboratories generating data for the program. The EPA National Aquatic Resource Surveys team has developed laboratory evaluation plans to ensure uniform interpretation and guidance in the procedural reviews.

The NRSA Quality Team is using a procedure that requests the laboratory to provide documentation of its policies and procedures. For the NRSA 2023/24 project, the Quality Team is requesting that each participating laboratory provide the following documentation:

- The laboratory's Quality Manual, Quality Management Plan or similar document.
- Standard Operating Procedures (SOPs) for each analysis to be performed.
- Long term Method Detection Limits (MDLs) for each instrument used and Demonstration of Capability for each analysis to be performed.
- A list of the laboratory's accreditations and certifications, if any.
- Results from Proficiency Tests for each analyte to be analyzed under the NRSA 2023/24 project.

If a laboratory has clearly documented procedures for sample receiving, storage, preservation, preparation, analysis, and data reporting; has successfully analyzed Proficiency Test samples (if required by EPA, EPA will provide the PT samples); has a Quality Manual that thoroughly addresses laboratory quality including standard and sample preparation, record keeping and QA non-conformance; participates in a nationally recognized or state certification program; and has demonstrated ability to perform the testing for which program/project the audit is intended, then the length of an on-site visit will be minimum, if not waived entirely. The QA Team will make a final decision on the need for an actual on-site visit after the review and evaluation of the documentation requested.

If a laboratory meets or exceeds all of the major requirements and is deficient in an area that can be corrected remotely by the lab, suggestions will be offered and the laboratory will be given an opportunity to correct the issue. The QA Team will then verify the correction of the deficiency remotely.

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*19 The evaluation of the labs is being considered an Assistance Visit rather than an audit because the evaluation is designed to provide guidance to the labs rather than as "inspection" as in a traditional audit.*



In addition, all labs must sign a Lab Signature Form (see NRSA 2023/24 LOM) indicating that they will abide by the following:

- Utilize procedures identified in the NRSA 2023/24 LOM (or equivalent). If using equivalent procedures, please provide procedures manual to demonstrate ability to meet the required MQOs.
- Read and abide by the NRSA 2023/24 Quality Assurance Project Plan (QAPP) and related Standard Operating Procedures (SOPs).
- Have an organized IT system in place for recording sample tracking and analysis data.
- Provide data using the template provided in the Lab Operations Manual.
- Provide data results in a timely manner. This will vary with the type of analysis and the number of samples to be processed. Sample data must be received no later than May 1, 2024 for samples collected in 2023 and May 1, 2025 for samples collected in 2024 or as otherwise negotiated with EPA.
- Participate in a lab technical assessment or audit if requested by EPA NRSA staff (this may be a conference call or on-site audit).

If a lab is participating in biology analyses, they must, in addition, abide by the following:

- Use taxonomic standards outlined in the NRSA 2023/24 Lab Manual.
- Participate in taxonomic reconciliation exercises during the field and data analysis season, which include conference calls and other lab reviews (see more below on Inter-laboratory comparison).

### **6.2.2 Water Chemistry Laboratories**

The water chemistry laboratory approval process which is outlined on in the previous paragraphs of this section is deemed appropriate because many laboratories participate in one or more national laboratory accreditation programs such as the National Environmental Laboratory Accreditation Program (NELAP), International Organization for Standardization (ISO-17025) as well as various state certification programs which include strict requirements around documentation and procedures as well as site visits by the accrediting authority. It is built off of the process used by the NLA 2012. The laboratories participating in NRSA 2023/24 meet these qualifications and as such have demonstrated their ability to function independently. This process is one that has been utilized in Region 3 for many years and is designed around the national accrediting programs listed above.

### **6.2.3 Inter-laboratory Comparison**

The NRSA QA plan includes an inter-laboratory investigation for the laboratories performing analysis on benthic macroinvertebrates, and periphyton data for the NRSA 2023/24. This process is defined as an inter-laboratory comparison since the same protocols and method will be used by both laboratories as described in this manual. The QA plan also includes an independent taxonomist (EPA Contractor) to re-identify 10% of the samples from each laboratory.

Assistance Visits will be used to:

- Confirm the NRSA 2023/24 Laboratory Operations Manual (LOM) methods are being properly implemented by cooperator laboratories.
- Assist with questions from laboratory personnel.
- Suggest corrections if any errors are made in implementing the lab methods.

#### **6.2.4 NRSA 2023/24 Document Request Form Chemistry Laboratories**

EPA and its state and tribal partners will conduct a survey of the nation's rivers and streams. This National River and Streams Assessment (NRSA), is designed to provide statistically valid regional and national estimates of the condition of rivers and streams. Consistent sampling and analytical procedures ensure that the results can be compared across the country. As part of the NRSA 2023/24, the Quality Assurance Team will conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform chemistry analyses under this project. Our review will assess your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's NRSA 2023/24.

The first step of this assessment process will involve the review of your laboratory's certification and/or documentation. Subsequent actions may include (if needed): reconciliation exercises and/or a site visit. All laboratories will need to complete the following forms:

##### **If your lab has been previously approved within the last 5 years for the specific parameters:**

- A signature on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for chemistry laboratories conducting analyses for the NRSA 2023/24. A signature on the QAPP and the LOM Signature Form indicates that you will follow both the QAPP and the LOM.

##### **If you have not been approved within the last 5 years for the specific parameters in order for us to determine your ability to participate as a laboratory in the NRSA, we are requesting that you submit the following documents (if available) for review:**

- Documentation of a successful quality assurance audit from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years (if you need assistance with this please contact the individual listed below).
- Documentation showing participation in a previous NARS for Water Chemistry for the same parameters/methods.

##### **Additionally, we request that all laboratories provide the following information in support of your capabilities, (these materials are required if neither of the two items above are provided):**

- A copy of your Laboratory's accreditations and certifications if applicable (i.e. NELAC, ISO, state certifications, North American Benthological Society (NABS), etc.).
- An updated copy of your Laboratory's QAPP.
- Standard Operating Procedures (SOPs) for your laboratory for each analysis to be performed (if not covered in 2023/24 NRSA LOM).
- Documentation attesting to experience running all analytes for the 2023/24 NRSA, including chlorophyll a and Ash Free Dry Mass (AFDM).

##### **See LOM for copies of the Document Request form used for both the Biological laboratories and the Chemical laboratories.**

This documentation may be submitted electronically via e-mail to [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov). Questions concerning this request can be submitted [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov) (202-566-0417) or [mitchell.richard@epa.gov](mailto:mitchell.richard@epa.gov) (202-566-0644).

#### **6.2.5 NRSA 2023/24 Document Request Form Biology Labs**

EPA and its state and tribal partners will conduct a survey of the nation's rivers and streams. This National River and Streams Assessment (NRSA), is designed to provide statistically valid regional and

national estimates of the condition of rivers and streams. Consistent sampling and analytical procedures ensure that the results can be compared across the country. As part of the 2023/24 NRSA, the Quality Assurance Team will conduct a technical assessment to verify quality control practices in your laboratory and its ability to perform biology analyses under this project. Our review will assess your laboratory's ability to receive, store, prepare, analyze, and report sample data generated under EPA's 2023/24 NRSA.

The first step of this assessment process will involve the review of your laboratory's certification and/or documentation. Subsequent actions may include (if needed): reconciliation exercises and/or a site visit. All laboratories will need to complete the following forms:

- If your laboratory has been previously approved within the last 5 years for the specific parameters: A signature on the attached Laboratory Signature Form indicates that your laboratory will follow the quality assurance protocols required for biology laboratories conducting analyses for the 2023/24 NRSA. A signature on the QAPP and the LOM Signature Form indicates you will follow both the QAPP and the LOM.

**If you have not been approved within the last 5 years for the specific parameters, in order for us to determine your ability to participate as a laboratory in the NRSA, we are requesting that you submit the following documents (if available) for review:**

- Documentation of a successful quality assurance audit from a prior National Aquatic Resource Survey (NARS) that occurred within the last 5 years (if you need assistance with this please contact the individual listed below).
- Documentation showing participation in previous NARS for this particular indicator.

**Additionally, we request that all laboratories provide the following information in support of your capabilities, (these materials are required if neither of the two items above are provided):**

- A copy of your Laboratory's accreditations and certifications if applicable (i.e. NELAC, ISO, state certifications, NABS, etc.).
- Documentation of NABS (or other) certification for the taxonomists performing analyses (if applicable).
- An updated copy of your Laboratory's QAPP.
- Standard Operating Procedures (SOPs) for your lab for each analysis to be performed (if not covered in NRSA 2023/24 LOM).

**See LOM for copies of the Document Request form used for both the Biological laboratories and the Chemical laboratories.**

This documentation may be submitted electronically via e-mail to [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov). Questions concerning this request can be submitted [forde.kendra@epa.gov](mailto:forde.kendra@epa.gov) (202-566-0417) or [mitchell.richard@epa.gov](mailto:mitchell.richard@epa.gov) (202-566-0644).

## 7 DATA ANALYSIS PLAN

The Data Analysis Plan describes the general process used to evaluate the data generated from the survey. It outlines the steps taken to assess the condition of the nation's rivers and streams and identify the relative impact of stressors on this condition. Results from the analysis will be included in the final report and used in future analyses. The data analysis plan will likely be refined and clarified as the data are analyzed by EPA and states.

### 7.1 Data Interpretation Background

The basic intent of data interpretation is to evaluate the occurrence and distribution of parameters throughout the population of rivers and streams in the conterminous United States within the context of regionally relevant expectations for least disturbed reference conditions. This is analyzed using a cumulative distribution function (CDF). Based on information from the cumulative distribution function, the analysis will also categorize the condition of water for most indicators as good, fair, poor, and unassessed (for various reasons such as samples not collected, quality assurance issues, etc.). Because of the large-scale and multijurisdictional nature of this effort, the key issues for data interpretation are unique and include: the scale of assessment, selecting the best indicators, defining the least impacted reference conditions, and determining thresholds for judging condition.

#### 7.1.1 Scale of assessment

This will be the fourth national report on the ecological condition of the nation's rivers and streams (and the fourth for wadeable systems) using comparable methods. EPA selected the sampling locations for the assessment using a probability based design, and developed rules for selection to meet certain distribution criteria, while ensuring that the design yielded a set of rivers and streams that would provide for statistically valid conclusions about the condition of the population of rivers/streams across the nation. A challenge that this mosaic of waterbodies poses is developing a data analysis plan that allows EPA and other partners to interpret data and present results at a large, aggregate scale. Additional information on data analysis procedures used for previous NRSA surveys NRSA 2008/09 and proposed for NRSA 2023/24 can be found in the NRSA 2018-2019 Technical Report (<http://water.epa.gov/type/rsl/monitoring/riverssurvey/index.cfm>).

#### 7.1.2 Selecting the best indicators

Indicators should be applicable across all reporting units, and must be able to differentiate a range of conditions. The Agency formed a steering committee for these discussions. Starting with the NRSA 2008/09 indicators, the Committee, comprised of EPA, state and other representatives provided advice and recommendations to the Agency on indicator selection/refinement.

EPA developed screening and evaluation criteria which included indicator applicability on a national scale, the ability of an indicator to reflect various aspects of ecological condition, and cost-effectiveness.

#### 7.1.3 Defining least impacted reference condition

Reference condition data are necessary to describe expectations for biological conditions under least disturbed setting. The NRSA 2023/24 project team will use an approach similar to that used in NRSA 2008/09, which is described in detail in the NRSA 2008-2009 Technical Appendix <http://water.epa.gov/type/rsl/monitoring/riverssurvey/index.cfm>.

#### **7.1.4 Determining thresholds for judging condition**

This reference site approach is used to set expectations and benchmarks for interpreting the data on river/stream condition. The range of conditions found in the reference sites for an ecoregion describes a distribution of those biological or stressor values expected for least disturbed condition. The benchmarks used to define distinct condition classes (e.g., good, fair, poor / least disturbed, moderately disturbed, most disturbed) will be drawn from this reference distribution. Typically, EPA's approach is to examine the range of values for a biological or stressor indicator in all of the reference sites in a region, and to use the 5<sup>th</sup> percentile of the reference distribution for that indicator to separate the most disturbed of all sites from moderately disturbed sites. (Note: depending on the indicator, data analysis groups and indicator leads may recommend alternative percentiles which will be reviewed by EPA). Using the 5<sup>th</sup> percentile means that rivers/streams in the most disturbed category are worse than 95% of the best sites used to define reference condition. Similarly, the 25<sup>th</sup> percentile of the reference distribution can be used to distinguish between moderately disturbed sites and those in least disturbed condition. This means that rivers/streams reported as least disturbed are as good as 75% of the sites used to define reference condition.

### **7.2 Geospatial Data**

Geospatial data is an integral part of data analysis for the NRSA 2023/24, as it has been for all other surveys. The following activities are anticipated: review of coordinate data and corrections, watershed delineations, and computing landscape metrics. Through the site evaluation process, rivers/streams that have changed or are inaccurately represented in the National Hydrography Dataset (NHD) will be noted and provided to EPA's NHD team.

### **7.3 Datasets Used for the Report**

The datasets used to report on condition of the nation's river and stream, as well as trends and change over time will include data collected during 2023/2024 along with all previous NRSA surveys. Additionally, threshold values based on EPA water quality criteria and World Health Organization values will be applied to the NRSA 2023/24 data for the human health related indicators. Geospatial files will include river/stream coverage and watershed delineations based on NHD+, the National Land Cover Dataset (NLCD), and Parameter-elevation Regressions on Independent Slopes Model (PRISM).

The survey will use indicators to assess ecological integrity; extent of stressors impacting integrity; and the recreational value of rivers/streams.

#### **7.3.1 Ecological Integrity**

Ecological integrity describes the ecological condition of rivers/streams based on different assemblages of the aquatic community and their physical habitat. The indicators include benthic macroinvertebrates, periphyton and fish assemblages.

#### **7.3.2 Stressor Status/Extent**

Stressor indicators describe the extent of key parameters on the condition of rivers/streams as well as the relative risk and attributable risk associated with stressors. The indicators include nutrients, physical habitat (the riparian and instream zones) and excess sediments among others.

### **7.3.3 Recreational value**

Recreational indicators address the ability of the population to support recreational uses such as swimming, fishing and boating. The protection of these uses is one of the requirements in the Clean Water Act under 305(b). The extent of algal toxins (microcystin and cylindrospermopsin), the extent of fish tissue concentrations above screening values for protection of human health, and Enterococci levels will serve as the primary indicators of recreational value.

## **7.4 Indicator Data Analysis**

### **7.4.1 Water Chemistry and Chlorophyll a**

A wide array of water chemistry parameters will be measured, including DO, pH, total N, total P, ANC, DOC, NH<sub>4</sub>, NO<sub>3</sub>-NO<sub>2</sub>, SO<sub>4</sub>, Cl, NO<sub>3</sub>, Ca, Mg, Na, K, SiO<sub>2</sub>, TSS, True Color, and chlorophyll-*a*. Values for these parameters and their distribution will be reported. Water chemistry analysis is critical for interpreting the biological indicators. Chlorophyll-*a*, and nutrient measurements will be used to determine the extent of these key stressors on aquatic life and to assess relative risk/attributable risk.

### **7.4.2 Algal Toxins**

Cyanobacteria (blue-green algae) blooms are common midsummer to late fall events that occur in many waters throughout the United States. Algal toxin production has been identified as a significant potential human health problem that has been associated with many of these bloom events. However, little is known about the general occurrence of algal toxins in the pelagic zones of these water bodies, where extensive blooms are less likely to occur than in near-shore areas.

The data analysis team will analyze the total (whole water) concentrations of microcystins and cylindrospermopsin in rivers/streams throughout the United States using a standardized immunoassay test. In addition, the data analysis team will analyze and interpret the data for microcystin and cylindrospermopsin occurrence and concentration in the context of other environmental data that is collected as part of the NRSA assessment (e.g. nutrients, chlorophyll, turbidity, specific conductance, pH).

### **7.4.3 Benthic Macroinvertebrate, Diatom, and Fish assemblages**

Benthic macroinvertebrate and fish assemblage will be analyzed using both multimetric indices (MMI) (modeled for all assemblages; and potentially modeled and traditional for benthic macroinvertebrates) and observed/expected indices (O/E) models. The MMI approach summarizes various assemblage attributes, such as composition, tolerance to disturbance, trophic and habitat preferences, as individual metrics or measures of the biological community. Candidate metrics are evaluated for aspects of performance and a subset of the best performing metrics are combined into an index known as a Macroinvertebrate Index of Biotic Condition. This index is then used to rank the condition of the resource.

The predictive model or O/E approach estimates the expected taxonomic composition of an assemblage in the absence of human stressors, using a set of least-disturbed sites and other variables related to natural gradients, such as elevation, stream size, latitude and longitude. The resulting models are then used to estimate the expected taxa composition (taxa richness) at each site sampled. The number of expected taxa actually observed at a site is compared to the number of expected taxa as an Observed Expected ratio or index. Departure from a ratio of one indicate that the taxonomic composition in the sample differs from that expected under least -disturbed conditions. The greater the departure from one, the greater the sample differs from the least disturbed condition.

EPA scientists will develop a separate data analysis plan for research related to the periphyton meta-genomics indicator.

#### **7.4.4 Physical Habitat**

An assessment of river and stream (fluvial) physical habitat condition is a major component of the NRSA. The assessment focuses on streambed stability and excess fine sediments, instream habitat cover complexity, riparian vegetation, and riparian human disturbances. These four indicators are generally important throughout the U.S. Furthermore, the project team had reasonable confidence in factoring out natural variability to determine expected values and the degree of anthropogenic alteration of the habitat attributes represented by these indicators.

##### ***7.4.4.1 Relative Bed Stability and Excess Fines***

Streambed characteristics (e.g., bedrock, cobbles, silt) are often cited as major controls on the species composition of macroinvertebrate, periphyton, and fish assemblages in streams (e.g., Hynes 1970, Cummins 1974, Platts et al. 1983, Barbour et al. 1999, Bryce et al., 2008, 2010). Along with bedform (e.g., riffles and pools), streambed particle size influences the hydraulic roughness and consequently the range of water velocities in a stream channel. It also influences the size range of interstices that provide living space and cover for macroinvertebrates and smaller vertebrates. Accumulations of fine substrate particles (excess fine sediments) fill the interstices of coarser bed materials, reducing habitat space and its availability for benthic fish and macroinvertebrates (Hawkins et al. 1982 Platts et al. 1983, Rinne 1988). In addition, these fine particles impede circulation of oxygenated water into hyporheic habitats reducing egg-to-emergence survival and growth of juvenile salmonids (Suttle et al. 2004). Streambed characteristics are often sensitive indicators of the effects of human activities on streams (MacDonald et al. 1991, Barbour et al. 1999, Kaufmann et al. 2009). Decreases in the mean particle size and increases in streambed fine sediments can destabilize stream channels (Wilcock 1997, 1998) and may indicate increases in the rates of upland erosion and sediment supply (Lisle 1981, Dietrich et al. 1998).

The scaled median streambed particle size is expressed as Relative Bed Stability (*RBS*), calculated as the ratio of the geometric mean diameter,  $D_g$ , divided by  $D_{cbf}$ , the critical diameter (maximum mobile diameter) at bankfull flow (Gordon et al., 1992), where  $D_g$  is based on systematic streambed particle sampling (“pebble counts”) and  $D_{cbf}$  is based on the estimated streambed shear stress calculated from slope, channel dimensions, and hydraulic roughness during bankfull flow conditions

##### ***7.4.4.2 Instream Habitat Cover Complexity***

Although the precise mechanisms are not completely understood, the most diverse fish and macroinvertebrate assemblages are usually found in streams that have complex mixtures of habitat features: large wood, boulders, undercut banks, tree roots, etc. (Kovalenko et al. 2011). When other needs are met, complex habitat with abundant cover should generally support greater biodiversity than simple habitats that lack cover (Gorman and Karr 1978, Benson and Magnuson 1992). Human use of streams and riparian areas often results in the simplification of this habitat, with potential effects on biotic integrity (Kovalenko et al., 2011). For this assessment, EPA proposes to continue the use of a measure (XFC\_NAT in Kaufmann et al., 1999) that sums the amount of instream habitat consisting of undercut banks, boulders, large pieces of wood, brush, and cover from overhanging vegetation within a meter of the water surface, all of which are estimated visually by NRSA field crews.

### **7.4.4.3 Riparian Vegetation**

The importance of riparian vegetation to channel structure, cover, shading, inputs of nutrients and large wood, and as a wildlife corridor and buffer against anthropogenic disturbance is well recognized (Naiman et al. 1988, Gregory et al. 1991). Riparian vegetation not only moderates stream temperatures through shading, but also increases bank stability and the potential for inputs of coarse and fine particulate organic material. Organic inputs from riparian vegetation become food for stream organisms and provide structure that creates and maintains complex channel habitat.

EPA proposes to continue evaluating the cover and complexity of riparian vegetation based on the metric *XCMGW*, which is calculated from visual estimates made by field crews of the areal cover and type of vegetation in three layers: the ground layer (<0.5m), med-layer (0.5-5.0 m) and upper layer (>5.0 m). The separate measures of large and small diameter trees, woody and non-woody mid-layer vegetation, and woody and non-woody ground cover are all visual estimates of areal cover. *XCMGW* sums the cover of *woody* vegetation summed over these three vegetation layers, expressing both the abundance of vegetation cover and its structural complexity. Its theoretical maximum is 3.0 if there is 100% cover in each of the three vegetation layers. *XCMGW* gives an indication of the longevity and sustainability of perennial vegetation in the riparian corridor (Kaufmann et al. 1999).

### **7.4.4.4 Riparian Human Disturbances**

Agriculture, roads, buildings, and other evidence of human activities in and near the stream and river channel may exert stress on aquatic ecosystems and may also serve as indicators of overall anthropogenic stress. EPA's 1992 stream monitoring workshop recommended field assessment of the frequency and extent of both in-channel and near-channel human activities and disturbances (Kaufmann 1993). The vulnerability of the stream network to potentially detrimental human activities increases with the proximity of those activities to the streams themselves. NRSA follow Stoddard et al. (2005b) and U.S. EPA (2006) in using a direct measure of riparian human disturbance that tallies 11 specific forms of human activities and disturbances (walls, dikes, revetments or dams; buildings; pavement or cleared lots; roads or railroads; influent or effluent pipes; landfills or trash; parks or lawns; row crop agriculture; pasture or rangeland; logging; and mining) at 22 separate locations along the stream reach, and weights them according to how close to the channel they are observed (*W1\_HALL* in Kaufmann et al. 1997). Observations within the stream or on its banks are weighted by 1.5, those within the 10 × 10 meter plots are weighted by 1.0, and those visible beyond the plots are weighted by 0.5. The index *W1\_HALL* ranged from 0 (no observed disturbance) to ~7 (e.g., equivalent to four or 5 types of disturbance observed in the stream, throughout the reach; or seven types observed within all 22 riparian plots bounding the stream reach). Although direct human activities certainly affect riparian vegetation complexity and layering measured by the Riparian Vegetation Index (previous paragraph), the Riparian Disturbance Index is more encompassing, and differs by being a *direct* measure of observable human activities that are presently or potentially detrimental to streams.

### **7.4.5 Enterococci**

Enterococci are bacteria that live in the intestinal tracts of warm-blooded animals, including humans, and therefore indicate possible contamination of streams and rivers by fecal waste. Epidemiological studies conducted at beaches affected by human sources of fecal contamination have established a relationship between the density of enterococci in ambient waters and the elevated incidence of gastrointestinal illness in swimmers. For the NRSA, water samples are analyzed using a process known as quantitative polymerase chain reaction, or qPCR, a methodology that facilitates the detection of DNA



sequences unique to these bacteria. Analysts compare the NRSA results to a new EPA qPCR threshold for protecting human health in ambient waters designated for swimming.

#### **7.4.6 Fish Tissue Plug Indicator**

Mercury is widely distributed in the environment, due to both natural processes and human activities. Measuring mercury levels in fish tissue is critical because about 80% of all fish consumption advisories currently involve mercury. Analysts compare mercury results for the fish tissue plug indicator analyses to EPA's human health screening value for mercury of 300 ppb that, if exceeded, can be harmful to human health.

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## 9 Appendix A: FOM and LOM Revision History

Wadeable Field Operations Manual (FOM) Version	Date Approved	Changes Made