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National Rivers and Streams Assessment 2018–2019 Technical Support Document

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The *National Rivers and Streams Assessment 2018-2019 Technical Support Document* details methods and analysis approaches used in the National Rivers and Streams Assessment 2018-2019 conducted by the United States Environmental Protection Agency (USEPA) and partner organizations. This document supports the results presented in *National Rivers and Streams Assessment: The Third Collaborative Survey* (EPA-841-R-22-004).

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Version History:

Version 1.1 – September 2024
Changes occurred to section 9.4 on PFAS fish tissue analysis and results and section 9.5 on calculations of PFAS fish tissue screening levels for human health protection. These changes were related to updates in analysis and screening levels for PFAS in fish tissue.

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LIST OF ACRONYMS

ANC	Acid Neutralizing Capacity
CCE	Calibrator Cell Equivalent
CPL	Coastal Plain ecoregion
DII	Dam Influence Index
DOC	Dissolved Organic Carbon
EMAP	EPA's Environmental Monitoring and Assessment Program
EPA	U.S. Environmental Protection Agency
FFG	Functional feeding group
FMMI	Fish Multimetric Index
HUC	Hydrologic Unit Codes
IBI	Index of Biotic Integrity
IQR	Interquartile Range
Km	kilometers
MAHA	Mid-Atlantic Highlands Assessment
MAIA	Mid-Atlantic Integrated Assessment
NAP	Northern Appalachians ecoregion
NARS	National Aquatic Resource Surveys
NAWQA	National Ambient Water Quality Assessment
NLCD	National Land Cover Dataset
MAHA	Mid-Atlantic Highlands
NPL	Northern Plains ecoregion
NRSA	National Rivers and Streams Assessment
O/E	Ratio of Observed to Expected
OTU	Operational Taxonomic Unit
PCA	Principal Component Analysis
QA	Quality Assurance
QC	Quality Control
RBS	Relative Bed Stability
RF	Random Forest
RMSE	Root Mean Squared Error
S:N	Signal to Noise (Signal:Noise) ratio
SAP	Southern Appalachians ecoregion
SD	Standard Deviation
SPL	Southern Plains ecoregion
TPL	Temperate Plains ecoregion
UMW	Upper Midwest ecoregion
WMT	Western Mountains ecoregion
WSA	Wadeable Streams Assessment
XER	Xeric ecoregion

1 INTRODUCTION

National Rivers and Streams Assessment: The Third Collaborative Survey is the third in a series of National Rivers and Streams Assessment (NRSA) reports that utilize a randomized statistical survey design to assess the quality of the nation's perennial rivers and streams. The NRSA is one of the National Aquatic Resource Surveys (NARS), a set of collaborative programs between EPA, states, and tribes designed to assess the quality of the nation's waters using a statistical survey design. The survey data underlying this NRSA report were collected in the summers of 2018 and 2019; as such, the findings presented in the report show a snapshot in time.The key goals of the NRSA report are to describe the ecological and recreational quality of the nation's perennial river and stream resources, how those conditions are changing, and the key stressors affecting those waters. Clean Water Act (CWA) Sections 104(a) and (b) collectively grant the Administrator authority to investigate and report on water quality across the country. NARS data also inform and benefit the national water quality inventory report that EPA prepares for Congress pursuant to CWA Section 305(b)(2).

This technical support document provides information about the analytical approaches used for the NRSA 2018-19. National results from NRSA are included in the *National Rivers and Streams Assessment: The Third Collaborative Survey* report and results for subpopulations, including EPA regions and ecological regions, are presented in the online data dashboard (https://riverstreamassessment.epa.gov/dashboard).

1.1 ADDITIONAL RESOURCES FOR SURVEY OPERATIONS

A series of protocols were used to ensure consistency throughout the survey operations. The following documents provide the field sampling methods, laboratory procedures, quality measures, and site selection for the NRSA 2018-19. Data from the survey are available to download at https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

- U.S. EPA. 2018. National Rivers and Streams Assessment: Field Operations Manual. EPA-841-B-12-009a and EPA-841-B-12-009b. Washington, D.C.
- U.S. EPA. 2018. National Rivers and Streams Assessment: Laboratory Operations MethodsManual. EPA 841-B-12-010. Washington, D.C.
- U.S. EPA. 2018. National Rivers and Streams Assessment: Quality Assurance Project Plan. EPA 841-B-12-007. Washington, D.C.
- U.S. EPA. 2012. National Rivers and Streams Assessment: Site Evaluation Guidelines. EPA841-B-12-008. Washington, D.C.

2 QUALITY ASSURANCE

EPA implemented and assessed the quality of its operations and data throughout the NRSA 2018-19 survey. This chapter documents the NRSA's adherence to the requirements of EPA's quality system implemented by the Office of Water (OW) as explained in the introduction section below. The following sections describe the quality aspects of the statistical design, field operations, laboratory assessments, data management, and report writing.

2.1 INTRODUCTION

The EPA quality system incorporates a national consensus standard for quality systems authorized bythe American National Standards Institute (ANSI) and developed by the American Society for Quality Control (ASQC), ANSI/ASQC E4-2004, *Quality Systems for Environmental Data and Technology Programs – Requirements with Guidance for Use*. EPA Order CIO 2105.0, dated May 5, 2000, requires all component organizations to participate in an agency-wide quality system. The EPA Order also requires quality assurance project plans or "equivalent documents" for all projects and tasks involvingenvironmental data.

In accordance with the EPA Order, the OW's developed the Office of Water Quality Management Plan (QMP; USEPA 2021) to describe OW's quality system that applies to all water programs and activities, including the NRSA, collecting or using environmental data. As required by the EPA Order and OW QMP, the NRSA team developed and abided by its QAPP throughout the survey. One significant challenge encountered was application of the quality control procedures for periphyton. As a result, EPA did not include periphyton in the NRSA 2018-19 reportand continues to work with the United States Geological Survey (USGS) and other experts to improve periphyton (specifically diatom) taxonomy through development of tools and training materials. The NRSA QAPP contains elements of the overall project management, data quality objectives, measurement and data acquisition, and information management. The QAPP also deals with the dataintegration necessary between the Wadeable Streams Assessment (WSA), the NRSA, and EPA's Environmental Monitoring and Assessment Program (EMAP) Western Pilot Study (2001-2004) to create a comprehensive report on the status of the nation's rivers and streams.

The following companion documents to the QAPP present detailed procedures for implementing the field and lab work for the NRSA 2018-19 survey:

- National Rivers and Streams Assessment 2018-19: Site Evaluation Guidelines EPA 841-B-17-002
- National Rivers and Streams Assessment 2018-19: Field Operations Manual (Wadeable and Boatable) (FOM), EPA-841-B-17-003a and EPA-841-B-17-003b
- National Rivers and Streams Assessment: Laboratory Operations Manual (LOM), EPA 841-B-17-0004

The four documents together address all aspects of the NRSA's data acquisition and evaluation. The LOM also lists measurement quality objectives (MQOs) which were used to evaluate the level of quality attainment for individual survey metrics. Every person involved in the NRSA was

responsiblefor abiding by the QAPP and adhering to the procedures specified in its companion documents. NRSA participants were instructed and/or trained in the requirements applicable to the person's role in the survey. For example, field crews attended a combined classroom and hands-on training in field procedures. Laboratory personnel provided appropriate SOPs and certifications; and attended calls to discuss implementation of the lab procedures.

2.2 SURVEY DESIGN

The NRSA's survey design was based upon statistical concepts that are well accepted by the scientific community. As described in the following sections, the survey design objectives were met by requirements of the statistical design, completeness of implementing the design, and consistency with established procedures.

2.2.1 STATISICAL DESIGN

There is a large body of statistical literature dealing with sample survey designs which addresses the challenge of making statements about many by sampling the few (Kish 1965). Sample surveys have been used in a variety of fields (e.g., monthly labor estimates) to determine the status of populations 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. In natural resource fields, probability sampling surveys have been consistently used to estimate the conditions of the entire population. For example, the National Agricultural Statistics Survey (NASS) conducted by the U.S. Department of Agriculture and the Forest Inventory Analysis (FIAT) conducted by the U.S. Forest Service (Bickford et al., 1963, Hazard and Law 1989) have both used probability-based sampling concepts to monitor and estimate the condition and productivity of agricultural and forest resources from a commodity perspective. The sampling design strategy for NRSA is based on the fundamental requirement for a probability sample of an explicitly defined resource population, where the sample is constrained to reflect the spatial dispersion of the population. This design has been documented in peer reviewed literature (Stevens 1994, Stevens and Olsen 1999). By applying the statistical concepts of this design, the survey was able to meet the following overarching data quality objectives:

- In the conterminous U.S., estimate the proportion of perennial river and stream length (± 5 percent) in good/fair/poor condition (or above/below criteria, etc.) for selected indicators with 95 percent confidence based on NRSA benchmarks[1].
- For each of the aggregated Omernik Level III Ecoregions, estimate the proportion of perennial river and stream length (±15 percent) in good/fair/poor condition (or above/below criteria, etc.) for selected indicators with 95 percent confidence based on NRSA benchmarks[1].

[1] The NRSA assessment benchmarks have no legal effect and are not equivalent to individual state water quality standards. NRSA condition categories also may not correspond to the categories states and tribes use when they assess water quality relative to their specific water quality standards under the Clean Water Act. For example, a rating of poor condition under NRSA does not necessarily mean a site is "impaired" as defined by state and tribal water quality standards assessment protocols.

2.2.2 COMPLETENESS

To ensure that the implementation of the NRSA 2018-19 sample design resulted in adequate measurements, the survey included completeness requirements for field sampling and laboratory analyses. The QAPP requires that valid data for individual indicators must be acquired from a minimum number of sampling locations to make subpopulation estimates with a specified level of confidence or sampling precision. As the starting place for selecting field sites, EPA used the National Hydrography Dataset (NHD; https://www.usgs.gov/core-science-systems/ngp/national-hydrography) as the frame representing streams and rivers in the US because it was the most complete source of stream hydrology available at the national scale. The data completeness requirements were achieved, and sites where data for an indicator could not be collected were classified as "Not Assessed" in the population estimates.

2.2.3 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, NRSA ensured comparability by using standardized sampling procedures, sampling equipment, and analytical methodologies by all sampling crews and laboratories. For all measurements, reporting units and format are specified, incorporated into standardized data recording forms, and securely transferred into a centralized information management system. Because NRSA 2018-19 used the same or comparable methods to collect data in EMAP West and WSA studies, the data also can be compared across the studies. The following sections on field and laboratory operations describe additional measures to ensure consistency in NRSA.

2.3 QUALITY ASSURANCE IN FIELD OPERATIONS

The requirements and methods presented in the Field Operations Manuals (FOM) ensured that quality objectives were attainable and survey activities were manageable. As described below, NRSA tested its FOM, trained crews using the FOM, visited crews during the field season, and confirmed fish specimen identifications.

2.3.1 FIELD METHOD PILOT TESTING

Representatives from the NRSA team, logistics and data management contractors, and state partners tested sampling methods, paper and electronic field forms, and equipment described in the FOM. The test run assessed the accuracy and clarity of the FOM's instructions for executing the procedures and quality steps. The test run also evaluated sampling logistics, sample preparation, and sample shipping instructions. As a result of lessons learned during the test run, NRSA staff correctedand improved the FOM prior to field crew training.

2.3.2 TRAINING OF FIELD TRAINERS AND ASSISTANCE VISITORS

Before training field crews, members of the NRSA team, oversight staff, contractor trainers, and other experts tested the training materials during a 3-day period that included classroom and hands

on training sessions. This "train-the-trainer" event serves two primary purposes. First, the event is designed to make sure that all trainers understand the methods and are providing consistent instruction to field crews. Second, it provides another opportunity to ensure that the field documents and forms are clear and accurate. During this training event, the attendees tested the materials to ensure that the instructions were correct and easy to execute and practiced actually training the methods. The training materials included the FOM, iOS App forms, and PowerPoint presentations. As a result of the training, practice training sessions and expert discussions, NRSA staff revised and improved training materials, the FOM and QRG before the field crew trainings began.

2.3.3 FIELD CREW TRAINING

To ensure consistency across field crews, all field crews were required to attend a 4-day training session in 2018 prior to visiting any field site. In 2019 field crews attended either a 2 or 3-day training to demonstrate their ability to perform the field methods properly. At a minimum, the field crew leader and the fish taxonomist from each crew were required to attend each year. NRSA trainers led regional field crew training sessions consisting of classroom and field-based lessons. The training included sessions on conducting site reconnaissance, recording field observations and *in situ* data, collecting field samples, preparing, packing and shipping sample containers, and use of the standardized field forms. The field crew leaders were taught to review every form and verify that all hand-entered data were complete and correct.

2.3.4 FIELD ASSISTANCE VISITS

To further assist the crews in correctly implementing the field procedures and quality steps, a trained NRSA team member or contractor visited every NRSA field crew during the field season. These visits, known as assistance visits (AV), provided an opportunity to observe field crews in the normal course of a field day, assist in correctly applying the procedures, and document the crew's adherence to sampling procedures. A total of 223 AVs were completed in the summers of 2018 and 2019. If circumstances were noted where a field crew was not conducting a procedure properly, the observer recorded the deficiency, reviewed the appropriate procedure with field team, and assisted the field crew until the procedure was completed correctly.

2.3.5 REVISITS OF SELECTED FIELD SITES

To evaluate within-year sampling variability, the NRSA design called for crews to revisit 10 percent of the sites selected in the design. These sites were sampled twice in the NRSA index period during a single year (visit 1 and visit 2). Useful metrics and indicators tend to have high repeatability, that is among site variability will be greater than sampling variability based on repeat sampling at a subset of sites. To quantify repeatability, NARS uses Signal:Noise (S:N), or the ratio of variance associated with sampling site (signal) to the variance associated with repeated visits to the same site (noise) (Kaufmann et al., 1999). All sites are included in the signal, whereas only revisit sites contribute to the noise component.

Metrics with high S:N are more likely to show consistent responses to human caused disturbance, and S:N values ≤ 1 indicate that sampling a site twice yields as much or more metric variability as

sampling two different sites (Stoddard et al., 2008). The S:N values were used by analysts in the process of selecting metrics and evaluating indicators.

2.3.6 EVALUATION OF FISH IDENTIFICATIONS

To ensure consistent naming conventions, field taxonomist and laboratory taxonomist were required to use commonly accepted taxonomic references to identify fish vouchers. To evaluate their identifications, field taxonomists were required to send fish vouchers from one or more site visits to expert ichthyologists for a second, independent identification. The laboratory taxonomists were able to determine the taxa for 1,293 vouchers which came from ~10 percent of the sites where fish were collected for NRSA 2018-19. Overall, there was 79 percent agreement between the field taxonomist and laboratory taxonomists.

2.4 LABORATORY QUALITY ASSURANCE AND QUALITY CONTROL

The NRSA laboratories used standard methods and/or followed the requirements (e.g., performance-based objectives) in the Laboratory Operations Manual (LOM). The QAPP identified the overall quality requirements and the LOM provided methods that could be used to achieve the quality requirements. If a laboratory chose a different method, it still had to meet the QA requirements as described below.

2.4.1 BASIC CAPABILITIES

All laboratories were required to submit documentation of their analytical capabilities prior to analyzing any NRSA 2018-19 sample. NRSA team members reviewed documentation to ensure that the laboratories could meet required measurement quality objectives (MQOs; e.g., reporting limits, detection limits). National Environmental Laboratory Accreditation Conference certification, satisfactory participation in round-robin, or other usual and customary types of evaluations were considered acceptable capabilities documentation.

2.4.2 BENTHIC MACROINVERTEBRATE IDENTIFICATIONS

For benthic macroinvertebrate taxonomy, laboratories were required to use the same taxa lists, conduct regular internal QC checks, and participate in an independent quality check. All participating laboratories identified organisms using the most appropriate technical literature that was accepted by the taxonomic discipline and reflected the accepted nomenclature at the time of the survey. The Integrated Taxonomic Information System (ITIS, https://www.itis.gov/) were also used to verify nomenclatural validity and reporting.

Taxonomic accuracy is evaluated by comparing identifications of the same organisms by primary and secondary, independent laboratories. Each primary laboratory provided organisms from 10 percent of its samples, or at least three samples if they had fewer than 10 samples, to a secondary laboratory for an independent evaluation. EPA, supported by an expert contractor, assessed the primary and secondary identifications, and then held reconciliation calls to allow the taxonomists to discuss organisms that were identified differently. As part of this process, recommendations and

corrective actions were identified to address inaccurate taxonomic identification; and measurement objectives were calculated to ensure the data were of sufficient quality for the NRSA.

Of the 2,186 benthic macroinvertebrate samples, the secondary laboratory identified organisms in 204 samples. The mean percent taxonomic disagreement (PTD) between laboratories was 9.1 percent for both 2018 and 2019 (better than the NRSA measurement objective of 15 percent as identified in the QAPP). The overall percent difference in enumeration (PDE) was 3.1 and 0.9 percent for 2018 and 2019, respectively (better than the NRSA measurement objective of 5 percent as identified in the QAPP).

Even when the measurement objectives were met, laboratories implemented recommendations and corrective steps for the QC samples and all other samples with the same organisms. If, for example, it was evident that empty mollusk shells were being identified and recorded in one or more of the QC samples, the laboratories needed to verify that they had not counted empty mollusk shells in their other samples.

2.4.3 CHEMICAL ANALYSES

For quality assurance of chemical analyses, laboratories used QC samples which are similar in composition to samples being measured. They provide estimates of precision and bias that are applicable to sample measurements. To ensure the ongoing quality of data during analyses, every batch of water samples was required to include QA samples to verify the precision and accuracy of the equipment, reagent quality, and other quality measures. These checks were completed by analyzing blanks or samples spiked with known or unknown quantities of reference materials, duplicate analyses of the same samples, blank analyses, or other appropriate evaluations. The laboratories reported QA results along with each batch of sample results. In addition, laboratories reported holding times. Holding time requirements for analyses ensure analytical results are representative of conditions at the time of sampling. The NARS team reviewed the data and noted any quality failures. The data analysts used the information about quality to determine whether to include or exclude data in the evaluations. As described in the next section, the consolidated NRSA database was further evaluated for quality issues.

2.5 DATA MANAGEMENT AND REVIEW

Information management (IM) is integral to all aspects of the NRSA from initial selection of sampling sites through dissemination and reporting of final, validated data. Quality measures implemented for the IM system are aimed at preventing corruption of data at the time of their initial incorporation into the system and maintaining the integrity of data and information after incorporation into the system.

Reconnaissance, field observation and laboratory analysis data were transferred from NRSA survey participants and collected and managed by the NARS IM center. Data and information were managed using a tiered approach. First, *all* data transferred from a field team or laboratory were physically organized (e.g., system folders) and stored in their original state. Next, NARS IM created a synthesized and standardized version of the data to populate a database that represented the primary

source for all subsequent data requests, uses and needs. All samples were tracked from collection to the laboratory.

The IM staff applied an iterative process in reviewing the database for completeness, transcription errors, formatting compatibility, consistency issues and other quality control-related topics. This first-line data review was performed primarily by NARS IM in consultation with the NRSA QA team. A second-phase data quality review consisted of evaluating the quality of data based on MQOs as described in the QAPP. This QA review was performed by the NRSA QA team using a variety of qualitative and quantitative analytical and visualization approaches. Data that met the MQOs were used without restriction. Data that did not meet the MQOs were qualified and further evaluated to determine the extent to which quality control results deviated from the target MQOs. Minor deviations, such as the field latitude and longitude did not fall on the mapped flow line, were noted and qualified but did not prevent data from being used in analyses. Major deviations were also noted and qualified, but data were excluded from the analyses. An example of a major deviation was insufficient fish assemblage sampling; when this occurred, the fish multimetric index was not calculated for a given site. Data not used for analyses because of quality control concerns account for a subset of the missing data for each indicator analysis and add to the uncertainty in condition estimates.

2.6 MAIN REPORT

The main report provides a summary of the findings of each of the data analyses and EPA's interpretation of them. The main report was extensively reviewed in-house by the NRSA team,its partners, and other EPA experts. Because previous reports using the same analytical procedures were reviewed through an Independent External Review process, it was determined that a letter review was not required for the main report. Note that EPA did conduct a letter peer review of the NRSA nutrient benchmark setting process in 2021. EPA used the comments from the states and EPA's Office of Research and Development to refine the main report and improve the clarity of documentation in this technical support document (TSD). Comments on the nutrient benchmark setting process were used to improve and clarify information in this TSD.

2.7 LITERATURE CITED

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3 SELECTION OF PROBABILITY SITES

Using a statistical survey design, 1,808 sites were selected at random to represent the quality of the larger population (1.5 million miles) of perennial rivers and streams across the conterminous United States, from large rivers to small headwater streams. Sites were selected using a random sampling technique that uses a probability-based design described in this chapter. The following sections describe the statistical objectives, target population, sample frame, survey design, evaluation, and statistical analysis.

3.1 OBJECTIVES

The data quality objects, or design requirements, for the National Rivers and Streams Assessment 2018-19 were:

- to estimate the proportion of rivers and streams with a margin of error of ± 5% in the conterminous U.S. in good/fair/poor condition (or above/below criteria, etc.) for selected indicators with 95 percent confidence based on NRSA benchmarks,
- to estimate the proportion of rivers and streams with a margin of error of ± 15% in each of nine ecological reporting regions in good/fair/poor condition (or above/below criteria, etc.) for selected indicators with 95 percent confidence based on NRSA benchmarks.
- to estimate the change in proportion of river and streams in the conterminous U.S. between 2008-09, 2013-14 and 2018-19 in good/fair/poor condition (or above/below criteria, etc.) for selected measures based on NRSA benchmarks. Change estimates should have a margin of error of ± 15% at 95% confidence.
- to estimate the change in proportion of river and streams in the conterminous U.S. between 2008-09, 2013-14 and 2018-19 in each of nine ecological reporting regions in good/fair/poor condition (or above/below criteria, etc.) for selected measures based on NRSA benchmarks. Change estimates should have a margin of error of ± 15% at 95% confidence.
- accomplish the above while ensuring that the minimum sample size for a state will be 20 and maximum will be 75.
- Revisit 10% of the sites for variance component estimation and quality assurance.

3.2 TARGET POPULATION

The target population consisted of all streams and rivers within the 48 contiguous states that had flowing water during the study index period (i.e., beginning of June through end of September formost regions). This included major rivers and small streams. Sites had to have > 50% of the reach length with standing water and sites were to be sampled during base flow conditions. Sites with water in less than 50% of the reach length were dropped and replaced. The target population excludes tidal rivers and streams up to head of salt (defined as < 0.5 ppt for this study), as well as run-of-the-river ponds and reservoirs with greater than 7-day residence time.

3.3 SAMPLE FRAME

The sample frame, used to represent the target population, was derived from the medium resolution National Hydrography Dataset (NHD-Plus) V2. Attributes from NHD-Plus and additional attributes added to the sample frame that are used in the survey design are:

- MajorRiver: rivers identified as major rivers or additional rivers in the book: Rivers of North America (Benke and Cushing 2005);
- Strahler order;
- Strahler category where categories are RiversMajor (5th order and higher), RiversOther (5th order and higher), LargeStreams (3rd,4th order), and SmallStreams (1st, 2nd order);
- BorderRiver: rivers and streams that occur on state and country boundaries. Each reach is identified by two-state postal codes such as MO:IL for the portion of the Mississippi River that forms the boundary between Missouri and Illinois. A border river/stream is assigned to one of the two states for the survey design;
- Ecological Reporting Region: Nine aggregated Omernik ecoregions used for reporting;
- Omernik and North American ecoregions Levels I, II, III and IV;
- Postal code (state);
- Urban and non-urban rivers and streams; and
- Landownership as non-federal, Forest Service, BLM, Tribal Land, US Fish and Wildlife Service, US National Park Service, and Department of Defense.

The urban/non-urban attribute was created by intersecting a modified version of the Census Bureau national urban boundary GIS coverage with NHD-Plus. The Census Bureau's boundaries were buffered 100 meters to include a majority of stream features intersecting and coincident with urban areas. Where this buffer did not completely gather all the river features within the urban areas (rivers intersecting cities are excluded from the Census Bureau's urban areas), the NHD-Plus river area (polygon) features were clipped at a three-kilometer buffer around the urban areas and combined with the buffered urban area to create the modified urban database. If a stream or river segment was within this boundary, it is designated as "Urban"; otherwise, it is designated as "NonUrban".

FCODE is directly from NHD-Plus and is used to identify which segments in NHD were included in the sample frame. The FCODEs are a numeric identifier of the channel type. The attribute Frame07 identifies each segment as either "Include" or "Exclude." Frame07 was created so that segments included in the sample frame could be easily identified. All segments chosen to be sampled were evaluated in the field prior to sampling to ensure they met the target population of NRSA (i.e., perennial rivers and streams). Sites that were not perennial were not sampled but were instead replaced by the next perennial segment in the list. FCODE values included in the GIS shapefile:

FCODEs Included in 2018-19 sample frame:

33600	Canal/Ditch
42801	Pipeline: Pipeline Type = Aqueduct; Relationship to Surface = At or Near
46000	Stream/River
46006	Stream/River (Perennial)
58000	Artificial Path (removed from dataset if coded through Lake/Pond and Reservoirs)

FCODEs Excluded in 2018-19 sample frame

33400	Connector
46003	Stream/River (Intermittent)
42800	Pipeline
42802	Pipeline: Pipeline Type = Aqueduct; Relationship to Surface = Elevated
42803	Pipeline: Pipeline Type = Aqueduct; Relationship to Surface = Underground
42804	Pipeline: Pipeline Type = Aqueduct; Relationship to Surface = Underwater
42806	Pipeline: Pipeline Type = General Case; Relationship to Surface = Elevated
4280	Pipeline: Pipeline Type = General Case; Relationship to Surface = Underground
42809	Pipeline: Pipeline Type = Penstock; Relationship to Surface = At or Near
42811	Pipeline: Pipeline Type = Penstock; Relationship to Surface = Underground
42813	Pipeline: Pipeline Type = Siphon
56600	Coastline
58000	Artificial Path if coded through Lake/Pond and Reservoirs

3.4 SURVEY DESIGN

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

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

Five basic panels are used for NRSA 2018-19:

- NRS18_08TS3R2: sites from NRSA 2008-09 that were sampled twice in 2008-09 and then sampled twice again in 2013-14 (a few exceptions). TS3 designates that the site will have been sampled in all three NRSA surveys. R2 designates a site that will be sampled twice in 2018-19.
- NRS18_08TS3: sites from NRSA 2008-09 that were sampled once in 2008-09 and sampled again in 2013-14. TS3 designates that the site will have been sampled in all three NRSA surveys.
- NRS18_13TS2R2: sites from NRSA 2013-14 that were sampled twice in 2013-14. TS2 designates that the site will have been sampled in two NRSA surveys. R2 designates a site that will be sampled twice in 2018-19.
- NRS18_13TS2: sites from NRSA 2013-14 that were sampled once in 2013-14 and will be sampled again in 2018-19. TS2 designates that the site will have been sampled in two NRSA surveys.
- NRS18_18: new sites selected for NRSA 2018-19 that will be sampled once in 2018-19.

3.4.1 RESAMPLE DESIGN

The Resample survey design is a subsample of the NRSA 2008-09 sites and NRSA 2013-14 sites that were target and sampled in NRSA 2008-09 and NRSA 2013-14. The major objective for this design

is change estimation, although all sites sampled in 2013-14 will be used when change is estimated. The resample design has four panels:

- NRS18_08TS3R2 – 96 sites (two per state) from NRSA 2008-09 sites that were sampled twice in 2008-09 and that were also sampled twice in 2013-14 and will be sampled twice in 2018-19. In each state one site is a stream (Strahler order 1-4) and one site is a river (Strahler order 5-10). Note that Arizona sites visited twice are both rivers since no streams were available that were visited twice in prior surveys.
- NRS18_08TS3 – 377 sites that were sampled once in 2008-09, once in 2013-14 and will be sampled once in 2018-19. Approximately 50% of sites in each state will be streams and 50% will be rivers. Sample size for each state is based on sample size used in 2013-14 proportional to achieve 408 sites.
- NRS18_13TS2R2 - 96 sites (two per state) from NRSA 2013-14 sites that were sampled twice in 2013-14 and will be sampled twice in 2018-19. In each state one site is a stream (Strahler order 1-4) and one site is a river (Strahler order 5-10). Note that Vermont sites visited twice are both streams since no rivers were available that were visited twice in prior surveys.
- NRS18_13TS2 – 414 sites that were sampled once in 2013-14 and will be sampled once in 2018-19. Approximately 25% of sites in each state will be Small Streams(1st-2nd), Large Streams (3rd-4th), Rivers Major (5th+) and Rivers Other (5th+). Sample size for each state is based on sample size used in 2013-14 proportional to achieve 408 sites.

This results in 983 unique sites in the Resample Design. Allocation of sites to NARS aggregated ecoregions is proportional to the number sampled in the prior surveys.

3.4.2 NEW SITE DESIGN

The NRSA 2018-19 new site survey design is a new survey design where the expected sample sizes are based on the nine ecological reporting regions and four categories of Rivers Major (5th and greater), Rivers Other (5th and greater), Large Streams (Strahler order 3rd, 4th), and Small Streams (Strahler order 1st, 2nd). Allocation of number of sites to states is proportional to stream length. The New Site Design is explicitly stratified by state. Unequal probability categories are 36 combinations of NARS nine aggregated ecoregions and four Strahler order categories (SS – small streams (1st-2nd), LS – large streams (3rd-4th), RM – major rivers (5th+) and RO – other rivers (5th+). In addition, a minimum of 20 sites (Resample and New) was guaranteed in each state and a maximum of 75 sites (Resample and New) for a state.

Final site distribution: First each state was assigned one site for each unequal probability category of streams and rivers that occur in the state. This allocates 414 sites in the New Site Design. Next the remaining 411 sites were allocated to the states proportional to their stream and river length.

3.4.3 OVERSAMPLE AND SITE REPLACEMENT

Site replacement is based on the 2018-19 panel variable NRS18_PNL. Five basic panels are used for NRSA 2018-19 (**Table 3-1**):

- NRS18_08TS3R2: sites from NRSA 2008-09 that were sampled twice in 2008-09 and then sampled twice again in 2013-14 (a few exceptions). TS3 designates that the site will have been sampled in all three NRSA surveys. R2 designates a site that will be sampled twice in 2018-19.
- NRS18_08TS3: sites from NRSA 2008-09 that were sampled once in 2008-09 and sampled again in 2013-14. TS3 designates that the site will have been sampled in all three NRSA surveys.
- NRS18_13TS2R2: sites from NRSA 2013-14 that were sampled twice in 2013-14. TS2 designates that the site will have been sampled in two NRSA surveys. R2 designates a site that will be sampled twice in 2018-19.
- NRS18_13TS2: sites from NRSA 2013-14 that were sampled once in 2013-14 and will be sampled again in 2018-19. TS2 designates that the site will have been sampled in two NRSA surveys.
- NRS18_18: new sites selected for NRSA 2018-19 that will be sampled once in 2018-19.

Table 3-1. Base Panels and Oversample replacement categories

NRSA 2018-19 panel	Base sites within 2018-19 panel	Over sample sites within 2018-19 panel that will be used as replacement sites within the panel
NRS18_08TS3R2	NRS18_08TS3R2_BaseStream	NRS18_08TS3R2_OverStream
NRS18_08TS3R2	NRS18_08TS3R2_BaseRiver	NRS18_08TS3R2_OverRiver
NRS18_08TS3	NRS18_08TS3_BaseStream	NRS18_08TS3_OverStream
NRS18_08TS3	NRS18_08TS3_BaseRiver	NRS18_08TS3_OverRiver
NRS18_13TS2R2	NRS18_13TS2R2_BaseStream	NRS18_13TS2R2_OverStream
NRS18_13TS2R2	NRS18_13TS2R2_BaseRiver	NRS18_13TS2R2_OverRiver
NRS18_13TS2	NRS18_13TS2_BaseSS	NRS18_13TS2_OverSS
NRS18_13TS2	NRS18_13TS2_BaseLS	NRS18_13TS2_OverLS
NRS18_13TS2	NRS18_13TS2_BaseRO	NRS18_13TS2_OverRO
NRS18_13TS2	NRS18_13TS2_BaseRM	NRS18_13TS2_OverRM
NRS18_18	NRS18_18_BaseSS_XXX	NRS18_18_BaseSS_XXX
NRS18_18	NRS18_18_BaseLS_XXX	NRS18_18_BaseLS_XXX
NRS18_18	NRS18_18_BaseRO_XXX	NRS18_18_BaseRO_XXX
NRS18_18	NRS18_18_BaseRM_XXX	NRS18_18_BaseRM_XXX

XXX designates one of the nine aggregated ecoregions: CPL, NAP, NPL, SAP, SPL, TPL, UMW, WMT, or XER. Sites within each state and above six categories are provided in siteID order, and the replacement must be in siteID order within the panel. Panels with "R2" are sites that will be sampled twice in 2018-19. If no over sample sites are available, or all over sample sites have been used, for an "R2" panel, then the next site in siteID order within the same basic panel is used. For example, if no over sample site is available in panel NRS18_08TS3R2_BaseStream, then use first site in panel NRS18_08TS3_BaseStream.

3.5 EVALUATION PROCESS

The survey design weights in the design file assumed that the survey design was implemented as designed. To achieve the planned sample size, we replaced sites that could not be sampled with oversamples as described above. Because some sites were replaced, the original survey design weights are no longer correct and EPA statisticians had to adjust the weights. This weight adjustment process required the statisticians knowing what happened to each site in the base design and the oversample sites (e.g., was the site sampled or dropped and if dropped why).

EvalStatus (evaluation status) was initially set to "NotEval" to indicate that the site had yet to be evaluated for sampling. When a site was evaluated for sampling, then the EvalStatus for the site was changed. Recommended codes are provided in **Table 3-2**.

Table 3-2. Recommended Codes for Evaluating Sites

EvalStatus Code	Name	Meaning
TS	Target Sampled	Site was a member of the target population and was sampled
LD	Landowner Denial	Landowner denied access to the site
PB	Physical Barrier	Physical barrier prevented access to the site
NT	Non-Target	Site was not a member of the target population
NN	Not Needed	Site was a member of the oversample and was not evaluated forsampling
Other codes		Other codes were often useful. For example, rather than use NT, the status may include specific codes indicating why thesite was non-target.

3.6 IMPLEMENTATION OF THE DESIGN

For NRSA 2018-19, 5,129 design sites were evaluated. Of these 1,909 were evaluated as target and sampled, with 188 sites sampled twice. The remaining sites were dropped and replaced for various reasons (**Table 3-3)**. The margin of error for national estimates was +/- 3% and for ecoregion estimates was +/- 15% with 95% confidence. For the difference analysis, estimates had a margin of error of +/- 5% at the national level and +/- 18% at the ecoregional level with 95% confidence. A minimum of 20 sites were sampled in each state.

Table 3-3. Evaluation Status of Dropped Sites

Category	Number of sites dropped
Impounded	28
Inaccessible	410
Landowner_NoAccess	1045
MapError	51
NonPerennial	729
NonTarget_Other	23

Tidal	340
Wetland	33

3.7 STATISTICAL ANALYSIS

Any statistical analysis of the data must incorporate information about the monitoring survey design. For NRSA, when estimates of characteristics for the entire target population are computed, the statistical analysis must account for the stratifications and unequal probability selection in the design. Procedures for doing this are available from the Aquatic Resource Monitoring Web page (https://archive.epa.gov/nheerl/arm/web/html/index.html). A statistical analysis library of functions to do common population estimates in the statistical software environment R is available from the webpage. In the NRSA 2018-19 Site Information data file, the adjusted weights used to calculate national condition estimates are in the column "WGT_EXT_SP"

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4 SELECTION OF SITES TO ESTABLISH REFERENCE CONDITIONS

One way to assess current quality is to compare data to a benchmark. For a number of indicators, the NARS assessments apply a reference approach for setting benchmarks. For NRSA, the reference approach is one in which least-disturbed sites in ecological regions are used to establish a reference distribution from which benchmarks for assessing quality at other sites are identified. The least-disturbed condition approach attempts to capture the best available chemical, physical and biological habitat conditions given the current state of the landscape (Stoddard et al., 2006). The NRSA reference sites and distribution do not represent pre-Columbian or "pristine" conditions.

The approach described in this chapter was used to select metrics for benthic macroinvertebrate and fish multimetric indices (MMI); and to define the ecoregion-specific benchmarks used in the NARS analyses. This approach was modified for water chemistry and physical habitat analysis. The process for identifying the final set of reference sites for each of the indicators that use them for setting benchmarks is described in each of the indicator chapters: see Chapter 5, Chapter 6, Chapter 7, and Chapter 8 for additional details.

This chapter describes the methodology used to select the reference sites including background and updates to the approach, the sources of candidate reference sites; and the chemical, physical screens, and geospatial screens used for assessing the quality of the benthic macroinvertebrate assemblage. It also describes how analysts used the reference approach to establish benchmarks.

4.1 Background and Updates

The NRSA approach is based on guidance and research for applying the reference approach to assess streams in terms of biological characteristics (i.e., biocriteria) and nutrient concentrations (US EPA 1996, USEPA 2000, Herlihy and Sifenos 2008, Herlihy et al., 2008, Stoddard et al., 2008). The analysis conducted for NRSA builds off the 2004 Wadeable Streams Assessment (WSA; USEPA, 2006), a nationwide assessment that preceded NARS. For the WSA, scientists applied the reference-based approach at an aggregated level III ecoregional scale. As described below, the NRSA analysis updates previously published screening criteria (i.e., Herlihy et al., 2008)[2] for identifying reference sites used in setting benchmarks and developing metrics/indices in aggregated ecoregions.

The NRSA 2008-09 analysis used the reference site data from WSA as well as new reference site data from additional hand-picked and probability sites sampled during NRSA 2008-09. Adding sites from NRSA was necessary so that non-wadeable streams and rivers would be included in the reference selection process (WSA included only wadeable streams). After the addition of the NRSA 2008-09 sites, including non-wadeable systems, the analysts reviewed and ultimately updated some

[2] Although some of the supporting literature for the nutrient reference-based approach used nutrient ecoregions, the WSA and subsequent NRSA reference approach is applied at aggregated level III ecoregions for nutrients as well as other indicators. As a result, the nutrient screening criteria and benchmarks could not be used directly.

of the reference screening criteria[3] originally used for WSA. These updates to the original WSA screening values are shown in red in **Table 4-2**. For the 2008-09 analysis, benchmarks were updated after inclusion of the additional rivers and stream reference sites. Additionally, the fish MMIs and physical habitat indicators were updated using the additional reference sites (for the benthic macroinvertebrates the WSA MMIs were still used).

For the NRSA 2013-14 analysis, potential additional reference sites were identified by filtering the 2013-14 sample for disturbance using the same process described in this chapter. A comparison of existing NRSA 2008-09 benchmarks was made against the benchmarks calculated by adding the new NRSA 2013-14 reference site data. After analyzing the revised benchmarks, the analysts determined that the differences compared to the NRSA 2008-09 benchmarks were large enough for the fish MMI and three of the four physical habitat indicators to warrant revisions to the benchmarks for these indicators. For other indicators (i.e., benthic macroinvertebrate MMI, nutrients), the analysts determined that the differences did not warrant revisions to the benchmarks. For these indicators, the existing NRSA 2008-09 benchmarks were applied.

For the 2018-19 assessment, EPA did not to update the benchmarks for any of the reference-based indicators, thus establishing a consistent baseline against which to measure condition, changes and trends.

4.2 SOURCES OF REFERENCE SITES

The fish, macroinvertebrate, and physical reference sites used in the NRSA came from four major activities:

1. We used sites sampled during the NRSA. These included both sites selected from the probability sample and sites hand-picked by best professional judgment that were sampled and analyzed using NRSA methods as part of the NRSA (number of sites shown in **Table 4-1**, "NRSA-Screened" column).

2. In addition to the sites sampled in the NRSA, we obtained data for potential reference site from USGS' National Water-Quality Assessment Program (NAWQA), EPA Region 7, the State of Wisconsin, and the State of Oklahoma (number of sites shown in **Table 4-1**, "NRSA-External" column). These data included fish and macroinvertebrate assemblage data as well as physical and chemical habitat data.

3. Benthic macroinvertebrate reference site data also came from the 1,655 wadeable stream sites available for use in the EPA WSA. In the WSA, reference sites were obtained from two different approaches: first by screening the WSA survey data for physical and chemical criteria in the same manner described Section **4.3** (number of sites shown in **Table 4-1**, "WSA-Screened" column), and second from macroinvertebrate data provided by other agencies, universities, or states from sites

[3] Screening criteria for selecting least-disturbed reference conditions can be developed iteratively with the goal of establishing the least amount of ambient human disturbance (Stoddard et al 2006) while maintaining sufficient reference sites for setting benchmarks.

that were deemed to be suitable as reference sites by best professional judgment (number of sites shown in **Table 4-1**, "WSA-External" column). These sites either were sampled with the same methodology as the WSA or had field and lab protocols with enough similarities that the data analysis group determined that the data were comparable. The reference sites from this second approach were only used in developing an MMI for benthic macroinvertebrate samples, not for setting the benchmarks.

The WSA reference site screening process and data sources are described in detail in Herlihy et al. (2008). In **Table 4-1**, the first two data columns summarize the number of available WSA benthic macroinvertebrate reference sites by ecoregion.

4. We also included additional reference site data for fish from stream and river sites used by Herlihy et al. (2006) in a national analysis of fish assemblage data. The screening process used to define reference sites is described in Herlihy et al. (2006) and defined in detail in Appendix 1 of that document. The Herlihy et al. (2006) study only used the first two years of data from EMAP (Environmental Monitoring and Assessment Program)-West. For NRSA, reference fish data from the last three years of EMAP-West were also available and were included. Final numbers of reference sites and screening used to refine the fish reference population are outlined in **Chapter 6**.

Table 4-1. Initial set of sites available for use in the NRSA

Ecoregion	WSA Activities		NRSA Activities		Total
	WSA—External	WSA—Screened	NRSA—External	NRSA—Screened	
Northern Appalachians (NAP)	114	27	2	37	180
Southern Appalachians (SAP)	370	35	22	38	465
Coastal Plain (CPL)	112	15	3	46	176
Upper Midwest (UMW)	68	12	38	30	148
Temperate Plains (TPL)	124	38	50	22	234
Northern Plains (NPL)	10	18	3	47	78
Southern Plains (SPL)	56	21	51	34	162
Western Mountains (WMT)	335	129	4	40	508
Xeric Region (XER)	132	39	2	33	206
Total	1,321	334	175	327	2,157

4.3 CHEMICAL AND PHYSICAL SCREENS

To select reference sites from those compiled as described in Section **4.2**, we first used chemical and physical data collected at each site (e.g., nutrients, turbidity, acidity, riparian condition) to determine whether the site is in least-disturbed condition for its ecoregion. In the NRSA, eight physical and chemical parameters were used to screen for reference sites, total nitrogen (total N), total phosphorus (total P), chloride, sulfate, acid neutralizing capacity, turbidity, percent fine substrate, and riparian disturbance index. If a site exceeded the screening value identified in **Table 4-2** for any one stressor it was dropped from reference consideration. As described in Section **4.2**, some screening criteria were updated from those used in WSA.

Given that expectations of least-disturbed condition vary across ecoregions, the criteria values for exclusion varied by ecoregion. The nine aggregate level III ecoregions developed for the WSA assessment were used to regionalize reference conditions. Ecoregional specific screening criteria in the Western Mountains ecoregion was broken into three finer-scale ecoregion subgroups for screening to match EMAP-West's use of a somewhat finer spatial scale.

As noted in Section **4.2**, in addition to the sites sampled in the NRSA, we obtained possible reference site external data from four other agencies. Data from these external surveys were screened for physical and chemical criteria using the same criteria used for NRSA sample sites in **Table 4-2** using whatever screening data were available in each survey.

All sites in the NRSA (both probability and hand-picked, boatable and wadeable) and the added external data that passed all criteria were considered to be candidate reference sites for the NRSA assessment. The number of sites by ecoregion used in the screening of biological reference sites are summarized in **Table 4-1.** These reference sites include both fish and macroinvertebrate data. The NRSA did not use data on the biological assemblages themselves for any screening as these are the primary components of the stream and river ecosystems being evaluated, and to use them would

constitute circular reasoning.

Note that the Rapid Bioassessment Protocol (RBP) physical habitat score was used as a filter in WSA but was not available in the NRSA data to use as a screen. The six ecoregions in the top half of the table were used in WSA and reported in Herlihy et al. (2008); the ecoregions in the bottom half of the table were screened using criteria developed in EMAP-West.

Sites were also screened using the criteria in **Table 4-3** to identify most disturbed sites that could be used to test responsiveness in method and indicator development.

4.4 GEOSPATIAL SCREENS

As a final screen, all sites that passed the chemical and physical screens were then screened using three additional landscape-GIS screening criteria. These screens included a dam influence index, urbanization influence, and agricultural influence.

The dam influence index (DII) was used to assess the influence of upstream dams and the largest reservoir on the current list of potential reference sites. The complete watershed was assessed for any of the sites with a watershed boundary with a maximum distance of less than 200 km upstream of the sampling point. Any site that had a watershed with a distance greater than 200 km upstream of the sample point, had a wedge-shaped area assessed until 200 km upstream was reached. A cut-off distance of 200 km upstream was used because it is unlikely land use activities occurring greater than 200 km upstream will directly influence a given sample reach downstream. For example, a sample reach on the lower Mississippi is more likely to be influenced by a dam located near the sample reach than a dam located in Montana, even though the Missouri River occurring within Montana is part of the upstream watershed of the lower Mississippi. For all watersheds (i.e., full watersheds up to 200 km upstream of a sample reach), a calculation of the volume of the largest reservoir, the number of dams, and an index that weighted the maximum reservoir volume within the watershed or wedge by its proximity to the sample point was conducted. Each upstream reservoir was inversely weighted by its upstreamflow distance from the sample point as:

$$w_i = e^{-\left(\frac{Dflow}{Defolding}\right)}$$

where D_{flow} is the flow distance to the sample site, and $D_{efolding}$ is an e-folding value that determines the rate at which the weight exponentially decreases (here 100 km). DII equals the largest distance-weighted volume within the watershed:

$$DII = \max(w_i * D_i)$$

where D_i = reservoir volume (km^3). The criteria for dropping a potential reference site was a DII value equal to or greater than one.

Percent urbanization and agricultural influence were assessed within a 1 km2 area around the mid-point of the sampled stream segment. To conduct this analysis a 1 km2 radius buffer around the

mid-point was overlaid onto the National Land Cover Database 2006 (USGS 2011) to calculate the percentage of urban land cover and percent row crop, as defined by the NLCD. The criteria used for dropping a potential reference site were if it had greater than a) 5% urban land cover or b)15% agricultural (row crop) land cover. The land cover percentages used for consistent screening of near-reach human influence were based on best professional judgement. While other options for factoring in urbanization and agricultural influences could have been used, such as assessing urban land cover and percent row crops at the watershed scale, the analysts chose the 1 km2 area to focus on proximal land use conditions.

4.5 ESTABLISHING BENCHMARKS

To assess sites using the reference condition approach, we compared information from the probability sites with characteristics observed at least-disturbed sites (reference condition) by establishing benchmarks identified from the reference distribution. As noted above, the approach used in NRSA draws on guidance and research for applying the reference approach to assess streams in terms of biological characteristics (i.e., biocriteria) and nutrient concentrations (US EPA 1996, USEPA 2000, Herlihy and Sifenos 2008, Herlihy et al., 2008, Stoddard et al., 2008).

Using this approach, NRSA used the 5th/25th or the 75th/95th percentiles from each of nine aggregate ecoregional reference distribution to define benchmarks for several indicators that delineate condition between good, fair, and poor[4] (Hughes et al., 1986; USEPA 1996) (see Chapter 5, Chapter 6, Chapter 7, and Chapter 8 for additional details). As noted in Chapter 2, the benchmarks described in this document are not equivalent to state or tribal water quality standards. Instead, they provide a means of interpreting the results in terms of least-disturbed sites in the region. For the biological and nutrient data, the percentiles that are selected can be interpreted in terms of a site's probability of being similar to least-disturbed reference condition. For example, if a site's biological index score is less than the 5th percentile of the reference condition index scores, then the probability that biological condition at the site is similar to reference is less than 5%. The physical habitat analysis, while using regional reference sites, applied other statistical analyses and models then set condition benchmarks (good, fair, and poor) based on the model results (see Chapter 8 for further details).

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[4] The 5th/25th percentiles were used when higher indicator values are better, such as with MMIs. The 75th/95th percentiles were used when higher indicator values are worse, such as with nutrient concentrations.

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Table 4-2. Criteria for eight chemical and physical habitat filters used to identify the least-disturbed reference sites for benthic macroinvertebrate and fish indicators for each of the nine aggregate ecoregions for NRSA. A site must pass all eight filters to be considered a least-disturbed reference site.

Filter criterion	NAP	SAP	CPL	UMW	TPL	SPL	NPL	XER	WMT-SW[e]	WMT-SRock[e]	WMT-Nrock/Pacific[e]
Total P (µg/L)	≤20	≤20	≤75	≤50	≤100	≤150	≤150	≤50	≤50	≤25	≤25
Total N (µg/L)	≤750	≤750	>2500	≤1000	≤3000	≤4500	≤4500	≤1500	≤750	≤750	≤750
Cl^- (µeq/L)	≤250[a]	≤200	–	≤300	≤2000	≤1000	≤1000	≤1000	≤300	≤200	≤200[a]
$SO4^{2-}$ (µeq/L)	≤250	≤400	≤600	≤400	–	–	–	–	–	≤200	≤200
ANC (µeq/L) + DOC (mg/L)[b]	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5	≥50+≥5	≥50 + ≥5	≥50 + ≥5	≥50 + ≥5
Turbidity (NTU)	≤5	≤5	≤10	≤5	≤50	≤50	≤50	≤25	≤5	≤5	≤5
Riparian Disturbance Index[c]	≤2	≤2	≤2	≤2	≤2	≤2	≤2	≤1.5	≤0.5/≤1.5[d]	≤1/≤1.5[d]	≤0.5/≤1.5[d]
% fine substrate	≤25	≤25	≤50	≤40	≤80	≤90	≤90	≤50	≤15	≤15	≤15

Values in red indicate a change from that used in WSA as reported in Herlihy et al. (2008). All screening criteria are based on baseflow conditions.

– indicates that filter was not used in that ecoregion.
ANC = acid neutralizing capacity, DOC = dissolved organic carbon
[a] Cl^- criterion not applied in Northeastern Coastal Zone (ecoregion 59) or Coast Range (ecoregion 1) sites
[b] Filter was specific for inorganic acidity; site had to exceed both criteria to fail
[c] Riparian disturbance index variable name is *W1_HALL* in physical habitat database (see Chapter 7)
[d] Wadeable stream/Boatable river criteria. Different criteria were used by stream size in the Western Mountains.
[e] To match screening criteria to what was done in the EMAP-West component of WSA, the Western Mountains ecoregion was divided into three subgroups: SW = Southwestern Mountains (Omernik level III codes 8 and 23,
Southern California Mts., and Arizona/New Mexico Mts.), SRock = Southern Rockies (Omernik 19 and 21, Southern Rockies and Wasatch/Uintas), and NRock/Pacific = Northern Rockies and Pacific Mountains (all other WMT level IIIecoregions)

Table 4-3. Criteria for eight chemical and physical habitat filters used to identify the most-disturbed[a] sites for each of the nine aggregate ecoregions for NRSA. A site needed to pass one of the eight filters to be considered a most-disturbed site.

Filter criterion	NAP	SAP	CPL	UMW	TPL	SPL	NPL	XER	WMT-SW[e]	WMT-SRock[e]	WMT-Nrock/Pacific[e]
Total P (µg/L)	>100	>100	>250	>150	>500	>500	>500	>150	>150	>100	>100
Total N (µg/L)	>3500	>3500	>8000	>5000	>15000	>10000	>10000	>5000	>1500	>1500	>1500
Cl^- (µeq/L)	>10000	>1000	–	>2000	>5000	>5000	>5000	>5000	>1000	>1000	>1000
$SO4^{2-}$ (µeq/L)	>1000	>1000	>4000	>2000	–	–	–	–	–	>1000	>1000
ANC (µeq/L) + DOC (mg/L)[b]	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5	<0 + <5
Turbidity (NTU)	>10	>20	>50	>30	>100	>100	>100	>75	>10	>10	>10
Riparian Disturbance Index[c]	>4	>4	>4	>4	>4	>3	>3	>3	>3	>3	>3
% fine substrate	>75	>75	>95	>90	≥100	>99	>99	>90	>50	>50	>50

[a] A set of most-disturbed sites in each ecoregion is needed to test metric and MMI responsiveness in discriminating between most- and least-disturbed sites. The criteria in Table 4.3 are the screening factors used to identify a set of most-disturbed sites in each ecoregion as reported in Stoddard et al. (2008). All screening criteria are based on baseflow conditions.

– indicates that filter was not used in that ecoregion.
ANC = acid neutralizing capacity, DOC = dissolved organic carbon
[b] Filter was specific for inorganic acidity; site had to exceed both criteria to fail
[c] Riparian disturbance index variable name is *W1_HALL* in physical habitat database (see Chapter 7).
[e] To match screening criteria to what was done in the EMAP-West component of WSA, the Western Mountains ecoregion was divided into three subgroups: SW = Southwestern Mountains (Omernik level III codes 8 and 23, Southern California Mts., and Arizona/New Mexico Mts.), SRock = Southern Rockies (Omernik 19 and 21, Southern Rockies and Wasatch/Uintas), and NRock/Pacific = Northern Rockies and Pacific Mountains (all other WMT level IIIecoregions)

5 BENTHIC MACROINVERTEBRATES

Benthic macroinvertebrates were collected using a D-frame net with 500 µm mesh openings at 11 transects equally distributed along the targeted reach. Samples were composited from the 11 transects and the material was field preserved with ~95% ethanol. Organisms were enumerated and identified to the lowest possible taxonomic level (generally genus) using specified standard keys and references (see the NRSA 2018-19 Field Operations Manual and Laboratory Operations Manual for additional details). Benthic macroinvertebrate counts, metrics, and multimetric index condition from NRSA are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

The taxonomic composition and relative abundance of different taxa that make up the benthic macroinvertebrate assemblage present in a stream have been used extensively in North America, Europe, and Australia to assess how human activities affect ecological condition (Barbour et al., 1995,1999; Karr and Chu 1999). As explained in general terms in the NRSA 2008-09 Technical Report (USEPA 2016; see Section **5.2**) two principal types of ecological assessment tools to assess conditionbased on benthic macroinvertebrates are currently prevalent: multimetric indices and predictive models of taxa richness. The purpose of these indicators is to present the complex community taxonomic data represented within an assemblage in a way that is understandable and informative to resource managers and the public. The following sections provide an overview of the approaches used to develop an indicator based on benthic macroinvertebrate assemblages, followed by details regarding data preparation and the process used to arrive at a final indicator. The same analyses and benchmarks were used in NRSA 2008-09, NRSA 2013-14, and 2018-19.

5.1 OVERVIEW

Multimetric indicators have been used in the U.S. to assess stream condition based on fish and macroinvertebrate assemblage data (e.g., Karr and Chu, 1999; Barbour et al., 1999; Barbour et al., 1995). The multimetric approach involves summarizing various assemblage attributes (e.g., composition, tolerance to disturbance, trophic and habitat preferences) as individual "metrics" or measures of the biological community. Candidate metrics are then evaluated for various aspects of performance and a subset of the best performing metrics are then combined into an index, referred to as a multimetric index or MMI. For NRSA 2018-19, NRSA 2013-14 and NRSA 2008-09, the benthic macroinvertebrate MMI developed in the WSA was used to generate the population estimates used in the assessment. The WSA MMI is detailed in Stoddard et al. (2008).

The predictive model approach was initially developed in Europe and Australia, and is becoming more prevalent within the U.S. The approach estimates the expected taxonomic composition of an assemblage in the absence of human stressors (Hawkins et al.., 2000; Wright, 2000), using a set of "least-disturbed" sites and other variables related natural gradients (such as elevation, stream size, stream gradient, latitude, longitude). The resulting models are then used to estimate the expected taxa composition (expressed as taxa richness) at each stream site sampled. The number of expected taxa observed at a site is compared to the total number of expected taxa as an observed:expected ratio (O/E index). Departures from a ratio of 1.0 indicate that the taxonomic composition in a stream sample differs from that expected under less disturbed conditions.

5.2 DATA PREPARATION

5.2.1 STANDARDIZING COUNTS

The number of individuals in a sample was standardized to a constant number to provide an adequate number of individuals that was the same for the most samples and that could be used for the multimetric index development. A subsampling technique involving random sampling without replacement was used to extract a true "fixed count" of 300 individuals from the total number of individuals enumerated for a sample (target lab count was 500 individuals). Samples that did not contain at least 300 individuals were used in the assessment because low counts can indicate a response to one or more stressors. Only those sites with at least 250 individuals, however, were used as least-disturbed reference sites.

5.2.2 AUTECOLOGICAL CHARACTERISTICS

Autecological characteristics refer to specific ecological requirements or preferences of a taxon for habitat preference, feeding behavior, and tolerance to human disturbance. These characteristics are prerequisites for identifying and calculating many metrics. A number of state/regional organizations and research centers have developed autecological characteristics for benthic macroinvertebrates in their region. For the WSA and NRSA, a consistent "national" list of characteristics that consolidated and reconciled any discrepancies among the regional lists was needed before certain biological metrics could be developed and calibrated and an MMI could be constructed. The same autecological information used in WSA was used in NRSA 2008-09, 2013-14, and 2018-19.

Members of the data analysis group pulled together autecological information from five existing sources: (1) the EPA Rapid Bioassessment Protocols document; (2) the USGS National Ambient Water Quality Assessment (NAWQA) national and northwest lists; (3) the Utah State University list; (4) the EMAP Mid-Atlantic Highlands (MAHA); and (5) the EMAP Mid-Atlantic Integrated Assessment (MAIA) list. These five were chosen because they were thought to be the most independent of each other and the most inclusive. A single national-level list was developed based on the decision rules described in the following sections.

5.2.2.1 TOLERANCE VALUES

Tolerance value assignments followed the convention for macroinvertebrates, ranging between 0 (least tolerant or most sensitive) and 10 (most tolerant). Foreach taxon, tolerance values from all five sources were reviewed and a final assignment made according to the following rules:

- If values from different lists were all <3 (sensitive), final value = mean.
- If values from different lists were all >3 and <7 (facultative), final value = mean.
- If values from different lists were all >7 (tolerant), final value = mean.
- If values from different lists spanned sensitive, facultative, and tolerant categories, best professional judgment was used, along with alternative sources of information (if available) to assign a final tolerance value.

Tolerance values of 0 to ≤3 were considered "sensitive" or "intolerant." Tolerance values ≥7 to 10 were considered "tolerant," and values in between were considered "facultative."

5.2.2.2 FUNCTIONAL FEEDING GROUP AND HABITAT PREFERENCES

In many cases, there was agreement among the five data sources identified in Section **5.2.3**. When discrepancies in functional feeding group (FFG) or habitat preference ("habit") assignments among the five primary data sources were identified, a final assignment was made based on the most prevalent assignment. In cases where there was no prevalent assignment, the workgroup examined why disagreements existed, flagged the taxon, and used best professional judgment to make the final assignment.

5.3 MULTIMETRIC INDEX DEVELOPMENT

5.3.1 REGIONAL MULTIMETRIC DEVELOPMENT

The same autecology and taxonomic resolution used in WSA was applied to the NRSA macroinvertebrate 300 fixed count data to calculate the community metrics used to calculate the MMI. In the WSA, a best ecoregional MMI was developed by summing the six metrics that performed best in that ecoregion (the national aggregate nine ecoregions). Each of the six metrics was scored on a 0–10 scale by interpolating metrics between a floor and ceiling value. The six metric 0-10 point scaled scores were then summed and normalized to a 0–100 scale by multiplying by 100/60 to calculate the final MMI. Details of this process are described in Stoddard et al. (2008).

The final metrics used in each ecoregion, metric direction, and floor and ceiling values are summarized in **Table 5-1**. Scoring equations are different depending on if the metric responds positively (high values good) or negatively (high values bad) with disturbance. For positive metrics, values above the ceiling get 10 points, and values below the floor get 0 points. For negative metrics, values above the ceiling get 0 points, and values below the floor get 10 points. The interpolation equations for scoring the 0-10 points for metrics between the floor and ceiling values are:

- Positive Metrics: Metric Points = 10 * ((metric value-floor)/(ceiling-floor))
- Negative Metrics: Metric Points = 10 * (1 - ((metric value-floor)/(ceiling-floor))).

The MMI used in the NRSA report is identical to the WSA MMI in terms of metrics and scoring. Based on NRSA revisit data, the MMI had a S:N ratio of 2.8 and a pooled standard deviation of 10.0 (out of 0–100).

Table 5-1. Six benthic community metrics, scoring direction, and floor and ceiling values used in calculating the NRSA and WSA MMI in each of the nine aggregate ecoregions.

Ecoregion	Direction	Metric	Floor	Ceiling
CPL	Negative	Non-Insect % Individuals	0.70	73.0
	Positive	Shannon Diversity	1.62	3.31
	Positive	Shredder Taxa Richness	1	9
	Positive	Clinger % Taxa Richness	14.3	54.8
	Positive	EPT Taxa Richness	1	17
	Negative	Tolerant % Taxa Richness	5.56	50.0
NAP	Positive	EPT % Taxa Richness	9.52	57.6
	Negative	% Individuals in Top 5 Taxa	37.2	76.2
	Positive	Scraper Taxa Richness	3	12
	Positive	Clinger % Taxa Richness	28.6	70.0
	Positive	EPT Taxa Richness	3	24
	Positive	PTV 0-5.9 % Taxa Richness	46.2	86.1
NPL	Positive	EPT % Taxa Richness	3.85	50.0
	Positive	Shannon Diversity	1.10	3.07
	Positive	Scraper Taxa Richness	1	6
	Negative	Burrower % Taxa Richness	6.45	35.3
	Positive	Ephemeroptera Taxa Richness	0	7
	Positive	PTV 0-5.9 Taxa Richness	4	28
SAP	Positive	Ephemeroptera % Taxa Richness	5.41	28.6
	Positive	Shannon Diversity	2.05	3.44
	Positive	Scraper Taxa Richness	3	12
	Negative	Burrower % Taxa Richness	3.45	25.0
	Positive	EPT Taxa Richness	5	25
	Negative	Tolerant % Taxa Richness	2.44	27.6
SPL	Positive	EPT % Individuals	0.67	66.0
	Positive	Shannon Diversity	1.16	3.27
	Positive	Scraper Taxa Richness	1	8
	Negative	Burrower % Taxa Richness	5.0	36.1
	Positive	EPT Taxa Richness	1	16
	Positive	Intolerant Taxa Richness	1	8
TPL	Positive	EPT % Individuals	0.67	80.3
	Positive	Shannon Diversity	1.41	3.17
	Positive	Scraper Taxa Richness	1	9
	Positive	Clinger Taxa Richness	3	20
	Positive	Ephemeroptera Taxa Richness	1	11
	Negative	PTV 8-9.9 % Taxa Richness	4.35	33.3
UMW	Negative	Chironomid % Taxa Richness	11.2	50.8
	Positive	Shannon Diversity	2.01	3.56
	Positive	Shredder Taxa Richness	3	10
	Negative	Burrower % Taxa Richness	3.77	28.6
	Positive	EPT Taxa Richness	4	22
	Negative	PTV 8-9.9 %Taxa Richness	2.51	29.5
WMT	Positive	EPT % Taxa Richness	18.5	62.9
	Negative	% Individuals in Top 5 Taxa	40.6	82.3
	Positive	Scraper Taxa Richness	1	8
	Positive	Clinger % Taxa Richness	27.0	69.6
	Positive	EPT Taxa Richness	6	23
	Negative	Tolerant %Taxa Richness	2.27	25
XER	Negative	Non-Insect % Individuals	3.33	36.0
	Negative	% Individuals in Top 5 Taxa	44.7	92.3
	Positive	Scraper Taxa Richness	0	7
	Positive	Clinger % Taxa Richness	15.8	65.8
	Positive	EPT Taxa Richness	1	18
	Negative	Tolerant % Taxa Richness	3.57	36.4

5.3.2 MODELING OF MMI BENCHMARKS

Previous large-scale assessments have converted MMI scores into classes of assemblage quality by comparing those scores to the distribution of scores observed at least-disturbed reference sites. If a site's MMI score was less than the 5th percentile of the reference distribution, it was classified as "poor" quality; scores between the 5th and 25th percentile were classified as "fair"; and scores in the 25th percentile or higher were classified as "good." This approach assumes that the distribution of MMI scores at reference sites reflects an approximately equal, minimum level of human disturbance across those sites. But this assumption did not appear to be valid for some of the nine WSA regions,which was confirmed by state and regional parties at meetings to review the draft results.

For the WSA, the project team performed a principal components analysis (PCA) of the physical habitat and water chemistry variables (Total P, Total N, pH, Chloride, Sulfate, Turbidity, %Fine Substrate, Riparian Disturbance Index) that had originally been used to screen for biological reference sites as described in Chapter 4**.** The first principal component (Factor 1) of this PCA well represented a generalized gradient of human disturbance. MMI scores at the reference sites, however, were weakly, but significantly, related to this disturbance gradient in some of the aggregate ecoregions. Thus, MMI reference distributions from these regions may be biased downward because they include somewhat disturbed sites which may have lower MMI scores. As part of the WSA, Herlihy et al. (2008) developed a process that used this PCA disturbance gradient to reduce the effects of disturbance on benchmark values within the reference site population. The process uses multiple regression modeling to develop adjusted benchmarks analogous to the 5th and 25th percentiles of reference sites in each ecoregion based on the slope of the MMI-disturbance relationship in each ecoregion.

These adjusted benchmarks were used in the WSA but were based on a small sample size of reference sites. To increase the sample size used in the regression model, the benchmark adjustment process was rerun for NRSA using the original WSA reference sites plus the additional NRSA reference sites identified in Chapter 4. As in the WSA analysis and other benchmark setting, we used a 1.5*interquartile range (IQR) outlier screening test in each ecoregion to drop MMI outliers from the analysis (sites with values outside the range of Q1-1.5*IQR or Q3+1.5*IQR were dropped). This removed 6 sites from the analysis (all low; 3 in WMT, and 3 in XER). There were a grand total of 647 least-disturbed reference sites used for the benchmark regression adjustment modeling and the resulting regression statistics for each ecoregion are shown in **Table 5-2**. The process for calculating these adjusted benchmarks and fitting the regression model is detailed in Herlihy et al. (2008). Briefly, the process involves setting the goal for disturbance to the 25th percentile of the Factor 1 disturbance score for reference sites in each ecoregion. The ecoregion MMI value at that goal is predicted from the MMI-disturbance regression as:

$$MMIpred = (GOAL * SLOPE) + INTERCEPT$$

Then the percentiles to be used as the adjusted benchmarks are calculated assuming there is a normal distribution around this predicted mean using the RMSE of the regression model as the standard error,

$$\text{Good-Fair } 25^{th} \text{ benchmark} = MMIpred - 0.675 * RMSE$$
$$\text{Fair-Poor } 5^{th} \text{ benchmark} = MMIpred - 1.650 * RMSE$$

The resulting adjusted MMI benchmark values for the condition classes in each ecoregion used in the NRSA report are given in **Table 5-3.**

Table 5-2. MMI-Disturbance Regression Model Statistics Used for Setting Benchmarks

Ecoregion	Number of Reference Sites	Factor 1 Goal*	Regression RMSE	Regression Slope	Regression Intercept
CPL	32	-0.1501	14.55	0	64.74
NAP	56	-0.5247	14.55	-7.257	61.06
NPL	65	0.8723	14.55	-14.95	79.66
SAP	64	-0.5531	14.55	-7.257	50.78
SPL	43	0.7637	14.55	-7.257	50.84
TPL	49	1.045	14.55	-7.257	57.75
UMW	39	-0.1138	14.55	0	46.74
WMT	209	-1.326	14.55	-7.257	50.27
XER	90	-0.4628	14.55	-7.257	63.44

* The 25th percentile of Factor 1 score was the "goal" on the PCA factor 1 disturbance gradient for hindcasting ecoregional benchmarks.

Table 5-3. Benchmark Values for the Nine Regional Benthic MMIs.

Ecoregion	Good Benchmark	Poor Benchmark
CPL	≥54.9	<40.7
NAP	≥55.0	<40.9
NPL	≥56.8	<42.6
SAP	≥45.0	<30.8
SPL	≥35.5	<21.3
TPL	≥40.3	<26.2
UMW	≥36.9	<22.7
WMT	≥50.1	<35.9
XER	≥57.0	<42.8

*Any site with an MMI score that was not "good" or "poor" was considered "fair."

5.4 LITERATURE CITED

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6 FISH ASSEMBLAGE

6.1 BACKGROUND

Fish assemblages in streams and rivers offer several unique advantages to assess ecological quality, based on their mobility, longevity, trophic relationships, and socioeconomic importance (Barbour et al., 1999, Roset et al., 2007). For fish assemblages, assessing ecological quality has generally been based on developing and using multimetric indices (MMIs), which are derivations of the original Index of Biotic Integrity (IBI) developed by Karr (Karr 1981). There are numerous examples of MMIs developed for fish assemblages in smaller streams (e.g., McCormick et al., 2001, Hughes et al,. 2004, Bramblett et al., 2005, Roset et al.. 2007) as well as for larger rivers (Lyons et al.. 2001, Emery et al., 2003, Mebane et al., 2003, Pearson et al., 2011).

6.1.1 MULTIMETRIC INDICATOR FOR NRSA 2018-19

For the NRSA 2008-09, we developed fish MMIs using predictive models of metric response (e.g., Oberdorff et al., 2002, Tejerina-Garro et al., 2006, Pont et al., 2007, Pont et al., 2009). This approach essentially provided an estimate of expected quality (in terms of metric values) at individual sites, rather than using a set of regional least-disturbed reference sites to define expected values for a particular metric. For the NRSA 2013-14, we constructed MMIs using a more traditional approach that used regional sets of reference sites to define expected conditions for metrics (e.g., Stoddard et al. ,2008), and adjusted metrics for watershed area using linear regression if the effect was large enough Details of the development and evaluation of the predictive model based MMIs can be found in the technical support documents for the NRSA 2008-09 and 2013-14 (USEPA 2016, 2020).

For the NRSA 2018-19, we used the same MMIs as were used for the 2013-14 assessment. We have retained the details regarding the development and evaluation of these MMIs here for convenience.

6.1.2 REGIONALIZATION

We developed separate traditional fish MMIs for each of the nine NARS reporting regions for the NRSA 2013-14 (**Figure 6-1**).

6.2 METHODS

6.2.1 FIELD METHODS

Collection methods for fish are described in the NRSA 2018-19 field operations manuals (USEPA 2017a, b). Collection methods used for the NRSA 2018-19 were essentially unchanged from those used for the previous NRSA studies (USEPA 2009, USEPA 2018a, b). These minor changes included text changes to help clarify sampling procedures or field forms. Three variants of the basic sampling protocol (using electrofishing) were used depending on the width of the stream and if it was wadeable. For streams less than 12.5 m wide, a reach length equal to 40 channel widths was sampled for fish. For larger streams (> 12.5 m wide), a minimum reach length of 500 m or 20

channel widths was sampled (whichever was longer). If 500 individuals were not collected after sampling the minimum reach length, sampling continued until 500 individuals were collected (or a reach length equal to 40 channel widths was sampled). Larger wadeable streams were sampled using backpack or barge electrofishing units; non-wadeable rivers were sampled using raft or boat electrofishing systems.

For the NRSA 2018-19, 2,110 site visits were initially available for collecting fish. These included 2,039 visits to 1,851 probability sites and to 71 hand-picked sites (including one revisit) that were evaluated as potential least-disturbed reference sites (see Section **4.2**). There were 188 revisits to a subset of the 1,851 probability sites (either within a single year or across the two years of sampling). Fish sampling was attempted at 1,775 site visits (including 175 revisits). A sufficient sample (based on length of reach sampled for fish and the number of individuals collected) was obtained at 1,726 site visits (including 172 revisits). Conditions prevented a sufficient sample from being collected at 55 site visits (including three revisits). Of the sites sampled for fish, no fish were collected at 68 site visits (including one revisit). Seining only was conducted at 29 site visits (including two revisits). No fish data were obtained from 305 site visits (including 12 revisits), due to collection permit restrictions (183 site visits, including 8 revisits), equipment failure (19 site visits, including one revisit), site conditions (92 site visits, including three revisits), loss of data after collection (3 site visits), or other reasons (8 site visits).

6.2.2 COUNTING, TAXONOMY, AND AUTECOLOGY

Fish were tallied and identified in the field, then released alive unless used for fish tissue or vouchers. Voucher specimens were collected if field identification could not be accomplished. Voucher samples of all species collected were also prepared at 10% of sites for each field taxonomist. Voucher samples were sent to an independent taxonomist to evaluate the taxonomic proficiency of each field taxonomist. All names submitted on field data forms were reviewed and revised when necessary to create a listing of nationally consistent common and scientific names. Where possible, taxonomic names (common and scientific) were based on Nelson et al. (2004) and Page et al. (2013). The online database FishBase (http://www.fishbase.org) served as a secondary source of taxonomic names. In rare cases, a journal article of a newly described species was used. Collection maps for each taxon were prepared and compared to published maps in Page and Burr (2011) or alternative web publications for a few rare endemic species. For the 2018-2019 NRSA, 101 new taxa names were added to the 631 unique taxa names from the NRSA 2018-2019 (excluding unknowns, hybrids, and amphibians). Amphibians were not used in the fish MMIs but were retained in the database for potential use by other users of NRSA data.

Each taxon was characterized for several different autecological traits, based on available sources of published information (e.g., McCormick et al., 2001, Goldstein and Meador 2004, Whittier et al., 2007b, Frimpong and Angermeier 2009). Traits included habitat guilds (lotic habitat and temperature), trophic guild, reproductive guild, migration strategy, and tolerance to human disturbance. A file of all fish taxa and their associated autecological assignments is available on the NRSA website.

Assignments of native status were based primarily on shapefiles of individual species distribution from NatureServe (http://www.natureserve.org). Alternative sources included the USGS

Nonindigenous Species database (http://nas.er.usgs.gov), FishBase, published maps in Page and Burr (2011), and relevant state fish publications (if available).

Because fish collected at a site cannot always be confidently identified to species, there is a risk of inflating the number of species actually collected. For each sample, we reviewed the list of taxa to determine whether they were represented at more than one level of resolution. For example, if an "Unknown Catostomus" was collected, and it was the only representative of the genus at the site, we assigned it as a distinct taxon. If any other species of the genus were collected, then we considered the unknown as not distinct. We used only the number of distinct taxa in the sample to calculate any metrics based on species richness.

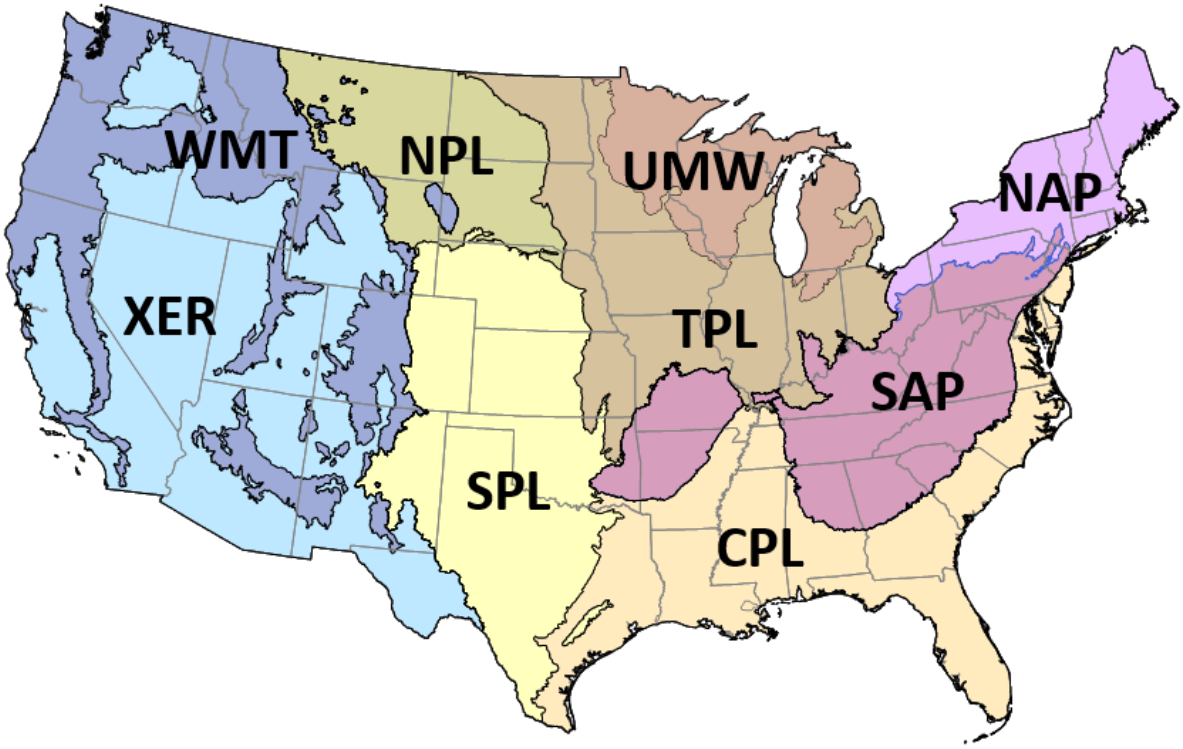

Figure 6-1. Aggregated Omernik ecoregions used to develop traditional fish MMIs for NRSA2018-19. A separate fish MMI was developed for each of the nine aggregated ecoregions. NAP=Northern Appalachians, SAP=Southern Appalachians, CPL=Coastal Plains, TPL=Temperate Plains, UMW=Upper Midwest, SPL=Southern Plains, NPL=NorthernPlains, XER=Xeric West, WMT=Western Mountains.

6.3 FISH MULTIMETRIC INDEX DEVELOPMENT

We used a consistent process to develop a multimetric index for fish for each of the nine aggregated ecoregions. We used the sites from the NRSA 2008-09 to develop and evaluate the fish MMIs, then calculated fish MMI scores for the NRSA 2018-19 data. We evaluated each metric for its

responsiveness to disturbance, i.e., its ability to discern between least-disturbed and most-disturbed sites (following Stoddard et al., 2008). We then selected metrics representing different dimensions of assemblage structure or function to include in the fish MMI based on responsiveness and lack of correlation with other metrics, following Whittier et al. (2007b) and Stoddard et al. (2008).

6.3.1 LEAST-DISTURBED REFERENCE SITES FOR FISH

We modified the base list of least-disturbed reference sites (Chapter 4) determined for NRSA to eliminate additional fish samples that might not be representative of least-disturbed conditions (i.e., excluded sites where < 25 fish were caught or had >50% non-native individuals) (**Table 6-1**). The final set of least-disturbed reference sites are identified in the NRSA database (variable RT_NRSA_FISH=R). No new least-disturbed sites for fish were identified for the 2018-19 NRSA.

To validate the fish MMIs and their component metrics, we identified a random subset of least-disturbed sites (validation sites) within each aggregated ecoregion and excluded them from fish MMI development. We set aside 29 validation sites in the Eastern Highlands (NAP=16, SAP=13), 66 sites in the Plains and Lowlands (CPL=10, NPL=16, SPL=13, TPL=14, UMW=13), and 23 sites in the West region (WMT=13, XER=10). We expected the distribution of fish MMI scores calculated for the validation sites would be similar to the distribution of fish MMI scores calculated for the calibration sites that were used to develop the fish MMIs.

6.3.2 CANDIDATE METRICS

We calculated 162 candidate metrics (Appendix 7.B) representing the following dimensions of fish assemblage structure and function (following Stoddard et al., 2008):

- Nonnative species (ALIEN) based on presence in 8-digit USGS Hydrologic Units
- Taxonomic composition (COMP)
- Species richness (RICH)
- Habitat guild (HABIT)
- Life history/migratory strategy (LIFE)
- Reproductive guild (REPRO)
- Trophic guild (TROPH)
- Tolerance (TOLER) to anthropogenic disturbance

The codes (in uppercase) for each category are used in the NRSA database to identify metric categories. For nearly all metrics, we derived three variants based on all taxa in the sample and for only native taxa in the sample: one based on distinct taxa richness, one based on the percent of individuals in the sample, and one based on the percent of distinct taxa in the sample (potentially yielding 6 different variants). For some trophic metrics, additional variants were derived using only taxa that were not considered tolerant to disturbance. We included only those tolerance metrics based on sensitive and tolerant taxa, because the "intermediate tolerance" assignments included taxa with unknown tolerance.

6.3.3 ADJUSTMENT OF METRIC RESPONSE FOR WATERSHED AREA

We used the set of least-disturbed reference sites in each aggregated ecoregion to evaluate whether metrics should be adjusted for stream size. Many studies have shown that some metrics (especially those based on species richness) vary naturally with stream size (e.g., Fausch et al., 1984, Simon and Lyons 1995, McCormick et al., 2001). We used watershed area (in km2) as our measure of stream size and compared the metric response to watershed area (transformed using log10) using linear regression. We used an R2 value >0.10 (following the rationale of Hawkins et al., 2010a and Vander Laan and Hawkins 2014) in deciding whether to use the model-adjusted responses for a particular metric. For metrics requiring adjustment, we used the residual values from the regression as the adjusted metric response (Stoddard et al., 2008).

Table 6-1. Criteria used to select least-disturbed sites for use in developing the regional NRSA fish multimetric indices (MMIs) based on 2008-09 and 2013-14 data.

Criteria				
Start with the base set of NRSA least-disturbed reference sites				
Keep sites with fish samples				
Drop sites where seining was the only sampling method				
Drop sites with insufficient sampling				
• Wadeable: Reach length sampled was less than 20 channel widths and less than 500 individuals were collected				
• Large Wadeable: Reach length sampled was less than 500 m and less than 500 individuals were collected				
• Boatable: Reach length sampled was less than 20 channel widths sampled				
Drop sites with sufficient sampling where less than 30 individuals were collected				
Drop sites with sufficient sampling where nonnative individuals comprised >50% of total number of individuals collected				
Drop non-wadeable sites hand-selected from the EMAP-Western Pilot Study that were sampled for fish. These sites were sampled using a much larger reach length (100 channel widths) than the reach length used for NRSA (40 channel widths).				
Final Number of Least-Disturbed Reference Sites				
		Calibration Sites	Validation Sites	Total
Northern Appalachians	NAP	43	16	59
Southern Appalachians	SAP	72	13	85
Coastal Plains	CPL	27	10	37
Northern Plains	NPL	33	16	49
Southern Plains	SPL	34	13	47
Temperate Plains	TPL	31	14	45
Upper Midwest	UMW	48	13	61

Western Mountains	WMT	77	13	90
Xeric West	XER	30	10	40
Total		395	118	513

6.3.4 SELECTION OF FINAL CANDIDATE METRICS

We reduced the number of candidate metrics using a series of screening procedures, following Stoddard et al. (2008). The original (i.e., prior to any adjustment for watershed area) metric response values were evaluated for range. To evaluate repeatability, we calculated S:N for each metric following Kaufmann et al. (1999), to compare the variance observed at revisit sites (within the index period) with the total variance observed across all sites. For adjusted metrics, the S:N value was calculated after adjusting for watershed area to remove the effects of natural variability from the "signal", as suggested by Esselman et al. (2013). For both original and adjusted metrics, the mean response values of the set of least-disturbed reference sites and the set of most-disturbed sites were compared with two-sample t-tests (assuming unequal variances). Stoddard et al. (2008) present the advantages of using t values over other statistics as an indicator of metric responsiveness to disturbance. A candidate metric was not generally considered further if it met any of the following conditions:

- A richness metric (NTAX) had a range < 4
- A percentage metrics (PTAX, PIND) had a range < 10%, or had a 90th percentile value=0
- A metric had a S:N value < 1.25
- A metric had an absolute value of t < 1.73
- The set of least-disturbed validation sites was significantly different ($p \leq 0.05$) from the set of least-disturbed calibration sites (two sample t-test)

Exceptions were made if there were no metrics in a category that passed all the screens. In these cases, we chose the metric with the best t value to include in the final set of candidate metrics.

Metrics that passed these screens were then sorted by metric category and t-value. In cases where the "native only" variant was similar in t-value to the "all species" variant, only one was retained (usually the all species variant unless there was a sizable difference in the S:N value, and then both variants were retained in the final list of candidate metrics).

6.3.5 METRIC SCORING

We rescaled response values for each of the final suite of metrics to a score ranging between 0 and 10. For "positive" metrics (those having higher values in least-disturbed sites) we used the 5th percentile of all sites to set the "floor" (below which a score of 0 was assigned), and the 95th percentile of least-disturbed sites to set the "ceiling" (above which a score of 10 was assigned) following Stoddard et al. (2008) and as described by Blocksom (2003). For "negative" metrics (where values were higher in the more disturbed sites), the floor was set at the 5th percentile of least-disturbed sites, and the ceiling was set at the 95th percentile of all sites. We assigned a score to response values between the floor and ceiling using linear interpolation.

We summed the metric scores for each site to derive the fish MMI score. We then multiplied the fish MMI score by (10/number of metrics) to rescale the score to range between 0 and 100.

6.3.6 SELECTION OF FINAL FISH MMIS

For each of the nine aggregated ecoregions, we used the final list of candidate metrics, and calculated thousands of candidate fish MMIs based on all possible combinations of the eight metrics (one from each category), as recommended by Van Sickle (2010). This approach allowed us to evaluate not only the maximum pairwise correlation among a suite of metrics comprising a fish MMI, but also the mean pairwise correlation of the suite itself. Indices having low mean correlations among pairs of metrics may perform better than an index containing component metrics selected to minimize redundancy based on a maximum allowable correlation coefficient (Van Sickle 2010). For each candidate fish MMI, we determined:

1. The *F* value based on comparing the set of least-disturbed vs. the set of more highly disturbed sites. We derived a *t*-value as $\sqrt{F}$.
2. The difference between the 25th percentile of the set of least-disturbed sites and the 75th percentile of the set of more highly disturbed sites. This value (SEPDIFF) is an estimate of the degree of overlap of the respective boxplots, which has been used to evaluate metric andindex performance (Barbour et al., 1996).

To select the "best" fish MMI from the large number of potential candidates, we excluded any candidate fish MMIs that had a maximum pairwise correlation of >0.7, or which had a S:N ratio of <2.5 (**Table 6-2**). We input the t values and the SEPDIFF values for the remaining candidate fish MMIs into a principal components analysis. We selected the candidate fish MMI that had the highest score for the first PCA axis for further evaluation. Combining the values for t and SEPDIFF into a single PCA axis score provided a simple, objective, and repeatable way to select a fish MMI that had optimal responsiveness to anthropogenic alteration.

We examined the performance of the component metrics across the range of stream sizes sampled for NRSA. The potential exists for bias in the fish MMI due to different fish species pools being available for larger rivers versus smaller streams. Differences across the size range might also result from the different sampling protocols that were used (wadeable, large wadeable, and boatable). We used the set of least-disturbed sites to examine patterns in metric response values across Strahler stream order categories. If one of the component metrics in the "best" fish MMI identified for an aggregated ecoregion showed a noticeable pattern of either increasing or decreasing response with Strahler order based on examining boxplots of least-disturbed sites across stream orders, we selected the fish MMI with the next highest PCA axis score.

Table 6-3 presents the regression equations used to adjust metrics that were included in each of the nine regional fish MMIs. The number of adjusted metrics included in a final suite of eight metrics ranged from two (Southern Plains) to six (Northern Plains). For two aggregated ecoregions (Coastal Plain and Temperate Plains), the ALIEN metric performed better after adjusting for watershed area. While it is expected that many richness-based metrics would require adjustment, there are a fair

number of proportional metrics (based on either individuals or taxa) that performed better after adjustment. This may be due to NRSA including a wider range of stream sizes than many other MMI development efforts that are based on a smaller set of streams (either smaller or larger).

Table 6-2. Number of final candidate fish multimetric indices (MMIs) calculated from the final set of passed metrics, before and after screening for maximum pairwise correlation among metrics and S:N ratio.

Aggregated Ecoregion	Number of Candidate fish MMIs calculated	Number of Candidate Fish MMIs remaining after screening
Northern Appalachians (NAP)	33,264	9,472
Southern Appalachians (SAP)	36,288	21,976
Coastal Plains (CPL)	9,072	1,494
Southern Plains (SPL)	8,064	2,084
Northern Plains (NPL)	27,648	5,092
Temperate Plains (TPL)	21,600	3,115
Upper Midwest (UMW)	90,720	25,692
Western Mountains (WMT)	84,000	7,120
Xeric West (XER)	32,400	13,220

Table 6-3. Regression equations for adjusting metrics for watershed area. LWSAREA_NEW is the log10-transformed value of watershed area in km2. Only metrics that were included in the final suite of metrics used to construct one of the nine regional fish MMIs are presented.

Coastal Plain Aggregated Ecoregion (CPL)
ALIENPIND_WS=ALIENPIND-(-0.219734+(0.178533*LWSAREA_NEW));
LOTPIND_WS=LOTPIND-(83.680193+(-5.644243*LWSAREA_NEW));
LITHPIND_WS=LITHPIND-(90.591166+(-21.2575*LWSAREA_NEW));
NAT_TOTLNTAX_WS=NAT_TOTLNTAX-(10.929299+(2.873952*LWSAREA_NEW));
TOLRNTAX_WS=TOLRNTAX-(1.831029+(1.559498*LWSAREA_NEW));

Northern Appalachians Aggregated Ecoregion (NAP)
LITHPTAX_WS=LITHPTAX-(91.493806+(-9.389536*LWSAREA_NEW));
NTOLPTAX_WS=NTOLPTAX-(83.244125+(-5.594874*LWSAREA_NEW));
TOLRNTAX_WS=TOLRNTAX-(-0.072385+(1.002947*LWSAREA_NEW));

Northern Plains Aggregated Ecoregion (NPL)
LOTNTAX_WS=LOTNTAX-(0.878392+(1.759049*LWSAREA_NEW));
MIGRNTAX_WS=MIGRNTAX-(0.438798+(0.39651*LWSAREA_NEW));

LITHPIND_WS=LITHPIND-(81.213041+(-13.064343*LWSAREA_NEW));
NTOLPTAX_WS=NTOLPTAX-(121.656224+(-18.471843*LWSAREA_NEW));
NAT_INTLPIND_WS=NAT_INTLPIND-(84.560234+(-21.788603*LWSAREA_NEW));

NAT_CARNNTAX_WS=NAT_CARNNTAX-(-1.380617+(0.928968*LWSAREA_NEW));

Southern Appalachians Aggregated Ecoregion (SAP)
NAT_CENTNTAX_WS=NAT_CENTNTAX-(-0.017051+(0.776488*LWSAREA_NEW));
NAT_LITHPIND_WS=NAT_LITHPIND-(85.390153+(-10.818128*LWSAREA_NEW));
INVPIND_WS=INVPIND-(26.04262+(11.423482*LWSAREA_NEW));

Southern Plains Aggregated Ecoregion (SPL)
CYPRPTAX_WS=CYPRPTAX-(45.705777+(-9.448293*LWSAREA_NEW));
NAT_MIGRPTAX_WS=NAT_MIGRPTAX-(-0.604356+(0.532868*LWSAREA_NEW));

Temperate Plains Aggregated Ecoregion (TPL)
ALIENNTAX_WS=ALIENNTAX-(-0.22423+(0.200411*LWSAREA_NEW));
NAT_ICTAPIND_WS=NAT_ICTAPIND-(-0.189542+(0.816572*LWSAREA_NEW));
NAT_NTOLNTAX_WS=NAT_NTOLNTAX-(1.946393+(2.107837*LWSAREA_NEW));
CARNNTAX_WS=CARNNTAX-(-0.005878+(1.292597*LWSAREA_NEW));

Upper Midwest Aggregated Ecoregion (UMW)
INTLLOTNTAX_WS=INTLLOTNTAX-(1.09723+(0.659379*LWSAREA_NEW));
NTOLNTAX_WS=NTOLNTAX-(2.216995+(2.870941*LWSAREA_NEW));
TOLRNTAX_WS=TOLRNTAX-(0.398305+(1.755202*LWSAREA_NEW));

Western Mountains Aggregated Ecoregion (WMT)
INTLLOTPTAX_WS=INTLLOTPTAX-(110.962575+(-21.540681*LWSAREA_NEW));
NAT_MIGRPTAX_WS=NAT_MIGRPTAX-(90.991326+(-15.318296*LWSAREA_NEW));
NAT_TOTLNTAX_WS=NAT_TOTLNTAX-(0.748128+(1.104128*LWSAREA_NEW));

Xeric West Aggregated Ecoregion (XER)
MIGRPTAX_WS=MIGRPTAX-(93.412006+(-20.33135*LWSAREA_NEW));
LITHNTAX_WS=LITHNTAX-(-0.265844+(1.369981*LWSAREA_NEW));
TOLRNTAX_WS=TOLRNTAX-(-0.142977+(0.094138*LWSAREA_NEW));
BENTINVPTAX_WS=BENTINVPTAX-(-5.705387+(9.987192*LWSAREA_NEW));

The following subsections provide information on the performance of each of the metrics that were used to construct a regional fish MMI. The information includes the floor and ceiling values that were used to develop a score for each metric (Section **6.3.5**).

6.3.6.1 METRIC PERFORMANCE AND SCORING: COASTAL PLAIN AGGREGATED ECOREGION

Table 6-4 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Coastal Plain aggregated ecoregion (CPL). The final suite included two negative metrics (the alien and tolerance metrics), and five metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 2.05 to 5.04, with only two metrics

having a t-value > 4. Signal to noise ratios ranged from 0.6 to 61.7. The life history metric (percent of migratory taxa that were intolerant to disturbance) had a low S:N ratio, but it was the best-performing of any of the life history metrics in this aggregated ecoregion.

6.3.6.2 METRIC PERFORMANCE AND SCORING: NORTHERN APPALACHIANS AGGREGATED ECOREGION

Table 6-5 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Northern Appalachians aggregated ecoregion (NAP). The final suite included three negative metrics (the alien, tolerance, and trophic metrics), and three metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 2.40 to 8.39, with five metrics having a t-value > 4. Signal to noise ratios ranged from 1.9 to 180. The trophic metric (number of invertivore taxa) did not respond as we expected; it is a negative metric in this fish MMI, indicating that there were more invertivore species in the set of most-disturbed sites than in the set of least-disturbed sites. However, fish MMIs that included trophic metrics that responded as expected did not perform as well as the fish MMI constructed using the metrics in **Table 6-5.**

The INVNTAX metric was the most responsive trophic metric based on the t-value (**Table 6-5**) and had a higher S:N ratio than other trophic metrics with similar t-values.

Table 6-4. Performance information of metrics used to construct the fish MMI for the Coastal Plain aggregated ecoregion. Column name is the field name in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	ALIENPIND_WS	% nonnative individuals (adjusted for watershed area)	-2.88	15.8	NEG	-0.49	14.28
Composition	RBCATONTAX	Number of round-bodied sucker taxa	5.04	2.7	POS	0	3.00
Habitat[d]	LOTPIND_WS	% Lotic individuals (adjusted for watershed area)	3.65	7.4	POS	-73.80	30.66
Life History	INTLMIGRPTAX	% of taxa that are migratory and intolerant to disturbance	2.30	0.6	POS	0	5.88
Reproductive[e]	LITHPIND_WS	% lithophil individuals (adjusted for watershed area)	4.71	61.7	POS	-81.39	33.83
Richness	NAT_TOTLNTAX_WS	Number of native taxa (adjusted for watershed area)	2.60	6.8	POS	-15.49	7.21
Tolerance	TOLRNTAX_WS	Number of tolerant taxa (adjusted for watershed area)	-2.05	11.8	NEG	-3.60	7.12
Trophic	INVPTAX	% of taxa that are invertivores	3.90	7.1	POS	9.09	68.75

[a] Based on comparisons of mean values of least-disturbed and most-disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites.
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor=5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic: Occupies flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-5. Performance information of metrics used to construct the fish MMI for the Northern Appalachians aggregated ecoregion. Column name is the field name in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	ALIENNTAX	Number of nonnative taxa	-4.02	1.9	NEG	0	4
Composition	SALMNTAX	Number of taxa in family Salmonidae	6.06	5.4	POS	0	2
Habitat[d]	NAT_RHEOPIND	% individuals that are native and rheophils	6.37	10.2	POS	0	100
Life History	INTLMIGRPIND	% individuals that are migratory and intolerant to disturbance	2.40	180	POS	0	8
Reproductive[e]	LITHPTAX_WS	% of taxa that are lithophils (adjusted for watershed area)	7.46	14.2	POS	-55.684	23.496
Richness	NTOLPTAX_WS	% of taxa that are not tolerant (adjusted for watershed area)	3.55	3.8	POS	-39.539	22.490
Tolerance	TOLRNTAX_WS	Number of tolerant taxa (adjusted for watershed area)	-8.39	3.1	NEG	-1.611	6.853
Trophic	INVNTAX	Number of taxa that are invertivores	-5.54	4.0	NEG	0	9

[a] Based on comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor= 5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

6.3.6.3 METRIC PERFORMANCE AND SCORING: NORTHERN PLAINS AGGREGATED ECOREGION

Table 6-6 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Northern Plains aggregated ecoregion (NPL). The final suite included two negative metrics (the alien and composition metrics), and six metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 1.24 to 4.59, with only one metric having a t-value > 4. Signal to noise ratios ranged from 0.4 to 332. The most responsive alien metric (number of nonnative taxa, a negative metric) did not meet the criteria for responsiveness or repeatability (Section **6.3.4**). Other alien metrics did not show much response to disturbance, suggesting that the set of least-disturbed sites in this aggregated ecoregion were similar in terms of the number of nonnative taxa, the percent of nonnative individuals, and the percent of nonnative taxa to the set of most-disturbed sites. Because nonnative species typically represent a direct stressor to native fish communities, native/nonnative metrics are commonly used by fishery biologists in assessing fish community health (e.g., Simon and Lyons 1995, McCormick et al., 2000, Hughes et al., 2004, Bramblett et al., 2005, Whittier et al., 2007b). The low values for responsiveness of metrics based on nonnative species or individuals has been observed in other studies (McCormick et al., 2000, Hughes et al., 2004, Bramblett et al., 2005, Whittier et al., 2007b). The number of nonnative taxa metric was included to represent the alien metric category, even though its influence was negligible on the overall MMI for the ecoregion.

6.3.6.4 METRIC PERFORMANCE AND SCORING: SOUTHERN APPALACHIANS AGGREGATED ECOREGION

Table 6-7 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Southern Appalachians aggregated ecoregion (SAP). The final suite included three negative metrics (the composition, life history, and tolerance metrics), and three metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 0.58 to 12.15, with seven metrics having a t-value > 4. Signal to noise ratios ranged from 1.5 to 23.4. The best alien metric, percent of taxa that are native, was not very responsive. However, the percent of native taxa is considered to have a positive influence on a fish assemblage, and thus was included as the alien metric in the regional fish MMI.

6.3.6.5 METRIC PERFORMANCE AND SCORING: SOUTHERN PLAINS AGGREGATED ECOREGION

Table 6-8 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Southern Plains aggregated ecoregion (SPL). The final suite included three negative metrics (the composition, life history, and tolerance metrics), and two metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 0.61 to 4.26, with only one metric having a t-value > 4. Signal to noise ratios ranged from 4.6 to 113.4. The most responsive metrics in three categories (composition, life history, and tolerance) did not meet the criteria for responsiveness (Section **6.3.4**).

Table 6-6. Performance information of metrics used to construct the fish MMI for the Northern Plains aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	ALIENNTAX	Number of nonnative taxa	1.24	-0.4	NEG	0	2
Composition	NAT_CYPRPIND	% individuals that are native and within the family Cyprinidae	-2.54	2.3	NEG	0	100
Habitat[d]	LOTNTAX_WS	Number of lotic taxa (adjusted for watershed area)	4.59	1.5	POS	-5.045	4.352
Life History	MIGRNTAX_WS	Number of migratory taxa (adjusted for watershed area)	2.69	0.7	POS	-1.907	1.579
Reproductive[e]	LITHPIND_WS	% individuals that are lithophils (adjusted for watershed area)	3.14	6.1	POS	-52.180	53.848
Richness	NTOLPTAX_WS	% taxa that are not tolerant (adjusted for watershed area)	2.52	6.3	POS	-66.112	29.110
Tolerance	NAT_INTLPIND_WS	% individuals that are native and intolerant (adjusted for watershed area)	1.82	332.4	POS	-42.369	62.153
Trophic	NAT_CARNNTAX_WS	Number of taxa that are native and carnivores (adjusted for watershed area)	3.81	1.3	POS	-2.091	1.960

[a] Based on comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor= 5th percentile of least-disturbed sites (a metric value ≤ floor is assigneda score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-7. Performance information of metrics used to construct the fish MMI for the Southern Appalachians aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	NAT_PTAX	% of taxa that are native	-0.58	23.4	POS	80	100
Composition	NAT_CENTNTAX_WS	Number of taxa within the family Centrarchidae (adjusted for watershed area)	-4.30	1.5	NEG	-1.535	3.620
Habitat[d]	NAT_NTOLBENTPTAX	% of taxa that are native, benthic and not tolerant	9.19	3.9	POS	0	66.670
Life History	NAT_MIGRNTAX	Number of taxa that are native and migratory	-5.42	2.2	NEG	0	4
Reproductive[e]	NAT_LITHPIND_WS	% of individuals that are native and lithophils (adjusted for watershed area)	5.85	7.7	POS	-56.528	28.448
Richness	NTOLPTAX	% of taxa that are not tolerant	8.72	8.7	POS	31.820	100
Tolerance	TOLRPTAX	% of taxa that are tolerant	-12.15	9.7	NEG	0	66.670
Trophic	INVPIND_WS	% of individuals that are invertivores (adjusted for watershed area)	5.01	8.1	POS	-60.259	38.399

[a] Based on comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor= 5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-8. Performance information of metrics used to construct the fish MMI for the Southern Plains aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	NAT_PIND	% of individuals that are native	1.98	113.4	POS	67.610	100
Composition	CYPRPTAX_WS	% of taxa within the family Cyprinidae (adjusted for watershed area)	-1.71	4.8	NEG	-16.787	62.614
Habitat[d]	RHEOPIND	% of individuals that are rheophils	3.49	51.3	POS	0	92.710
Life History	NAT_MIGRPTAX_WS	% of taxa that are native and migratory (adjusted for watershed area)	-0.61	6.3	NEG	-1.326	31.490
Reproductive[e]	LITHNTAX	Number of taxa that are lithophils	4.26	6.9	POS	0	6
Richness	NAT_NTOLNTAX	Number of taxa that are native and not tolerant	1.85	4.6	POS	1	8
Tolerance	TOLRNTAX	Number of tolerant taxa	-1.54	14.4	NEG	2	14
Trophic	HERBPTAX	% of taxa that are herbivores	3.96	19.0	POS	0	25

[a] Based on comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

6.3.6.6 METRIC PERFORMANCE AND SCORING: TEMPERATE PLAINS AGGREGATED ECOREGION

Table 6-9 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Temperate Plains aggregated ecoregion (TPL). The final suite included three negative metrics (the alien, composition, and life history metrics), and four metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 1.69 to 6.96, with three metrics having a t-value > 4. Signal to noise ratios ranged from 1.2 to 12.6. The most responsive metric in the life history category did not quite meet the criteria for responsiveness (Section **6.3.4**). The life history metric (number of taxa that are migratory and intolerant) also did not respond as we expected; it is a negative metric in this fish MMI, indicating that there were more intolerant migratory species in the set of most-disturbed sites than in the set of least-disturbed sites.

6.3.6.7 METRIC PERFORMANCE AND SCORING: UPPER MIDWEST AGGREGATED ECOREGION

Table 6-10 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Upper Midwest aggregated ecoregion (UMW). The final suite included only one negative metric (the tolerance metric), and three metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 0.22 to 5.91, with four metrics having a t-value > 4. Signal to noise ratios ranged from 2.3 to 12.7. The best alien metric, percent of taxa that are native, was not very responsive. However, the percent of native taxa is considered to have a positive influence on a fish assemblage, and thus was included as the alien metric in the regional fish MMI

6.3.6.8 METRIC PERFORMANCE AND SCORING: WESTERN MOUNTAINS AGGREGATED ECOREGION

Table 6-11 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Western Mountains aggregated ecoregion (WMT). The final suite included three negative metrics (the composition, tolerance, and trophic metrics), and three metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 1.45 to 5.56, with five metrics having a t-value > 4. Signal to noise ratios ranged from 1.3 to 23.2. The most responsive reproductive metric (% of taxa that are lithophils) did not quite meet the criteria for responsiveness (Section **6.3.4**).

Table 6-9. Performance information of metrics used to construct the fish MMI for the Temperate Plains aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	ALIENNTAX_WS	Number of nonnative taxa (adjusted for watershed area)	-4.12	2.1	NEG	-0.298	2.045
Composition	NAT_ICTAPIND_WS	% of individuals that are native and within the family Ictaluridae (adjusted for watershed area)	-2.94	12.6	NEG	-1.940	17.204
Habitat[d]	RHEONTAX	Number of taxa that are rheophils	5.02	1.7	POS	0	4
Life History	INTLMIGRNTAX	Number of taxa that are migratory and intolerant	-1.69	1.2	NEG	0	1
Reproductive[e]	LITHPIND	% of individuals that are lithophils	2.93	1.4	POS	0	97.520
Richness	NAT_NTOLNTAX_WS	Number of taxa that are native and not tolerant (adjusted for watershed area)	6.96	2.7	POS	-9.403	4.824
Tolerance	INTLPTAX	% of taxa that are intolerant	3.19	4.2	POS	0	50
Trophic	CARNNTAX_WS	Number of taxa that are carnivores (adjusted for watershed area)	3.40	2.5	POS	-3.761	2.235

[a] Based on comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-10. Performance information of metrics used to construct the fish MMI for the Upper Midwest aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	NAT_PTAX	% of taxa that are native	-0.22	4.8	POS	85.710	100
Composition	CYPRNTAX	Number of taxa within the family Cyprinidae	2.19	3.6	POS	0	9
Habitat[d]	INTLLOTNTAX_WS	Number of taxa that are lotic and intolerant (adjusted for watershed area)	5.91	2.9	POS	-3.287	2.110
Life History	INTLMIGRPTAX	% of taxa that are migratory and intolerant	3.04	2.3	POS	0	13.330
Reproductive[e]	LITHPIND	% of individuals that are lithophils	4.22	12.7	POS	0	95.350
Richness	NTOLNTAX_WS	Number of taxa that are not tolerant (adjusted for watershed area)	4.54	8.2	POS	-8.389	6.445
Tolerance	TOLRNTAX_WS	Number of tolerant taxa (adjusted for watershed area)	-3.02	3.1	NEG	-3.785	5.549
Trophic	INTLINVPTAX	% of taxa that are invertivores and intolerant	5.40	7.5	POS	0	33.330

[a] Based on the comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigneda score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-11. Performance information of metrics used to construct the fish MMI for the Western Mountains aggregated ecoregion. Column name is the field names in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	NAT_PIND	% of individuals that are native	5.56	10.5	POS	0	100
Composition	NAT_CATOPIND	% of individuals that are native and within the family Catastomidae	-4.20	15.2	NEG	0.000	68
Habitat[d]	INTLLOTPTAX_WS	% of taxa that are lotic and intolerant (adjusted for watershed area)	4.23	7.3	POS	-72.045	27.826
Life History	NAT_MIGRPTAX_WS	% of taxa that are native and migratory (adjusted for watershed area)	2.10	23.2	POS	-74.290	40.433
Reproductive[e]	LITHPTAX	% of taxa that are lithophils	1.45	16.6	POS	25.000	100
Richness	NAT_TOTLNTAX_WS	Number of native taxa (adjusted for watershed area)	2.39	1.5	POS	-3.009	3.272
Tolerance	TOLRNTAX	Number of tolerant taxa	-4.71	1.3	NEG	0	2
Trophic	NAT_HERBPTAX	% of taxa that are native and herbivores	-4.20	8.2	NEG	0	33.330

[a] Based on the comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigneda score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

6.3.6.9 METRIC PERFORMANCE AND SCORING: XERIC WEST AGGREGATED ECOREGION

Table 6-12 presents the performance and scoring information for the eight metrics that were used to construct the fish MMI for the Xeric West aggregated ecoregion (XER). The final suite included two negative metrics (the composition and tolerance metrics), and four metrics that were adjusted for watershed area (**Table 6-3**). Absolute values of t ranged from 1.45 to 5.56, with five metrics having a t-value > 4. Signal to noise ratios ranged from 3.4 to 21.8.

6.4 FISH MMI PERFORMANCE

We evaluated several aspects of performance of the nine regional fish MMIs (**Table 6-13**). We compared the fish MMI scores from a set of validation least-disturbed sites to those of the set of calibration least-disturbed sites to confirm that the models were behaving as anticipated. For all nine regional fish MMIs, the mean values of the validation sites and sites used to evaluate the metrics and construct the fish MMIs were not significantly different (two-sample t-test).

We evaluated the responsiveness of the regional fish MMIs to disturbance using two measures: 1) t-tests to compare the fish MMI scores for the set of least-disturbed sites to those for the set of more highly disturbed sites (Stoddard et al., 2008), and 2) the difference between the 25th percentile of least-disturbed sites and the 75th percentile of the set of most-disturbed sites. Boxplots are presented in **Figure 6-2**. The results of t-tests (two sample tests assuming unequal variances) and the percentile differences are presented in **Table 6-13**. The t-values ranged from 5.71 for the Northern Plains to 15.38 for the Northern Appalachians. The percentile differences were all positive (i.e., the boxes did not overlap), and ranged from 0.75 for the Western Mountains to 22.4 for the Northern Appalachians.

We estimated precision of the fish MMIs by calculating the standard deviation of standardized fish MMI scores (dividing each value by the mean) from all least-disturbed sites. Precision values greater than zero provide an indication of the remaining disturbance signal left in the set of least-disturbed sites, plus measurement error. Precision values ranged from 0.10 in the Xeric West aggregated ecoregion to 0.28 in the Northern Plains and the Upper Midwest aggregated ecoregions. Precision values between 0.10 and 0.25 are comparable to values obtained for other predictive models of taxa loss (Hawkins et al., 2010a).

We evaluated the repeatability of the regional fish MMIs using a set of sites that were visited at least twice during the course of the NRSA 2008-09 project, typically two times in a single year (Kaufmann et al., 1999, Stoddard et al., 2008). We used a general linear model (PROC GLM, SAS v. 9.12) to obtain estimates of among-site and within-site (from repeat visits) variability. PROC GLM was used because of the highly unbalanced design (only a small subset of sites had repeat visits). We used a nested model (sites within year) where both site and year were random effects.

Table 6-12. Performance information of metrics used to construct the fish MMI for the Xeric West aggregated ecoregion. Column name is the field name in the NRSA database.

Metric Category	Column Name	Description	*t*-value[a]	Signal: Noise Value[b]	Scoring Information[c]		
					Direction of Response	Floor	Ceiling
Alien	NAT_PIND	% of individuals that are native	7.75	5.4	POS	0	100
Composition	CENTPTAX	% of taxa that are within the family Centrarchidae	-4.12	3.4	NEG	0	25.000
Habitat[d]	RHEOPIND	% of individuals that are rheophils	5.03	13.1	POS	0	100
Life History	MIGRPTAX_WS	% of taxa that are migratory (adjusted for watershed area)	1.79	7.9	POS	-64.832	38.279
Reproductive[e]	LITHNTAX_WS	Number of taxa that are lithophils (adjusted for watershed area)	8.39	6.4	POS	-6.202	1.649
Richness	NTOLPTAX	% of taxa that are not tolerant	8.02	8.9	POS	0	100
Tolerance	TOLRNTAX_WS	Number of taxa that are tolerant (adjusted for watershed area)	-8.56	3.7	NEG	-0.129	4.670
Trophic	BENTINVPTAX_WS	% of taxa that are benthic invertivores (adjusted for watershed area)	6.43	21.8	POS	-48.740	23.306

[a] Based on the comparison of mean values of least-disturbed and most disturbed sites (validation sites have been excluded).
[b] Based on variability among sites vs. variability within sites (validation sites have been excluded).
[c] Direction: POS=Positive metric (mean value for least-disturbed sites is greater than mean value for most-disturbed sites). NEG=negative metric (mean value for most-disturbed sites is greater than the mean value for least-disturbed sites). For positive metrics, the floor= 5th percentile of all sites (a metric value ≤ floor is assigned a score of 0), and the ceiling=95th percentile of least-disturbed sites (a metric value ≥ ceiling is assigned a score of 10). For negative metrics, the floor=5th percentile of least-disturbed sites (a metric value ≤ floor is assigned a score of 10), and the ceiling=95th percentile of all sites (a metric value ≥ ceiling is assigned a score of 0).
[d] Habitat metrics: Lotic species occupy flowing water habitats; Rheophils occupy fast water habitats.
[e] Reproductive metrics: Lithophils require clean substrate for spawning.

Table 6-13. Performance statistics for the nine regional fish MMIs.

Performance Characteristic	Coastal Plain Fish MMI	Northern Appalachians Fish MMI	Northern Plains Fish MMI	Southern Appalachians Fish MMI	Southern Plains Fish MMI	Temperate Plains Fish MMI	Upper Midwest Fish MMI	Western Mountains Fish MMI	Xeric West Fish MMI
Validation least-disturbed sites vs. least-disturbed sites used in MMI development	*t*=-1.22	*t*=1.00	*t*=1.12	*t*=0.92	*t*=-0.02	*t*=0.41	*t*=1.40	*t*=0.43	*t*=0.86
Least-disturbed sites vs. most-disturbed sites	*t=10.3*	*t=15.38*	*t=5.71*	*t=14.7*	*t=8.07*	*t=9.76*	*t*=7.45	*t*=7.74	*t*=11.42
Difference between 25th percentile of least-disturbed sites and 75th percentile of most-disturbed sites	+6.2	+22.4	+1.6	+8.8	+2.4	+8.5	+1.42	+0.75	+9.5
Model precision (SD of least-disturbed sites)	0.17	0.22	0.28	0.17	0.14	0.15	0.28	0.0.11	0.10
Repeatability (S:N)	6.6	71.2	4.4	6.5	13.5	6.2	4.3	29.1	9.8

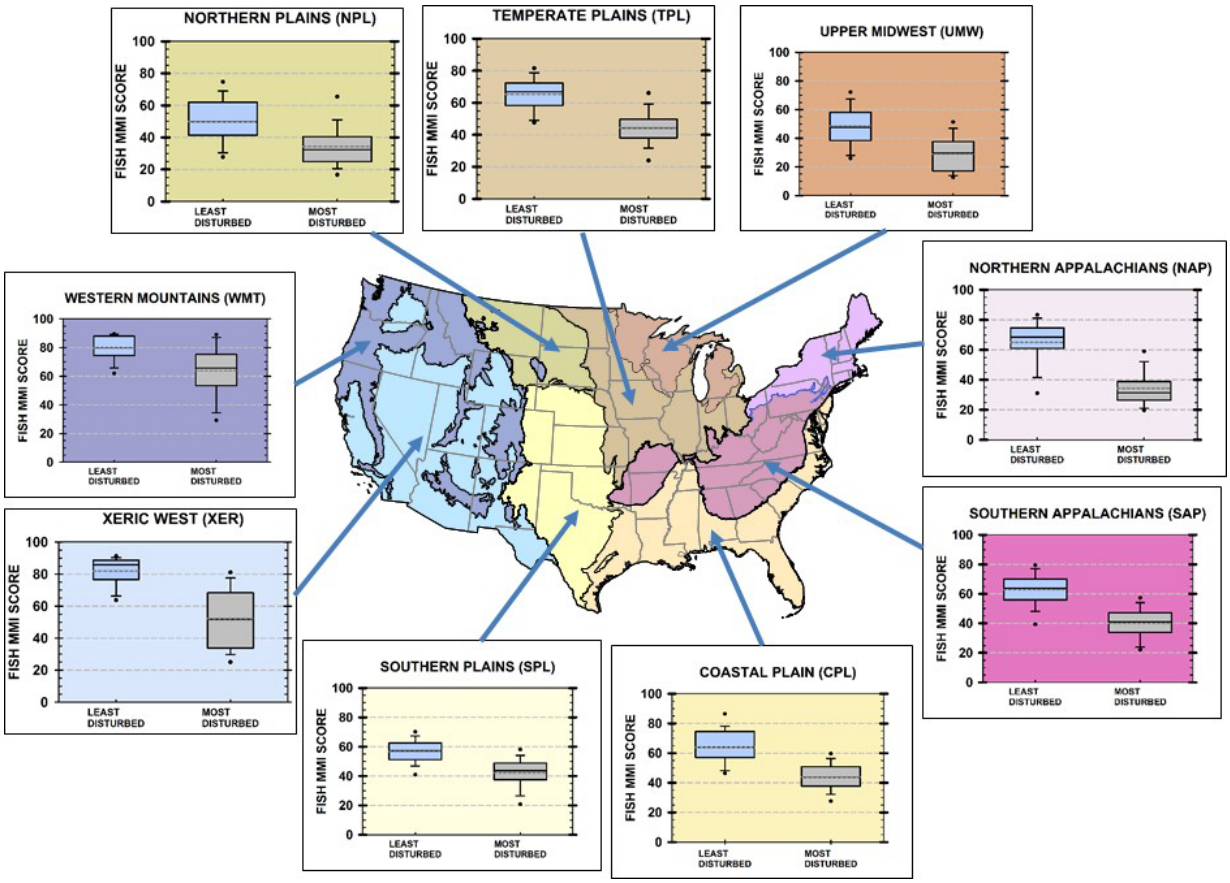

Figure 6-2. Boxplots comparing regional fish MMI scores of least-disturbed sites to most-disturbed sites. Whiskers indicate 10th and 90th percentiles. Points indicate 5th and 95th percentiles.

We estimated repeatability by deriving a S:N ratio as (F – 1)/c, where F is the F-statistic from the ANOVA, and c is a coefficient in the equation used to estimate the expected mean square. If all sites had repeat visits, c would equal 2 (Kaufmann et al., 1999). If no sites had repeat visits, c would equal 1. S:N ratios ranged from 4.3 in the Upper Midwest aggregated ecoregion to 71.2 in the Northern Appalachians aggregated ecoregion. High values of S:N need to be interpreted in the context of the number of repeat visit sites included in the analysis. Artificially high values of S:N can result if there are a small number of repeat visit sites that have little (or no) variance in fish MMI scores among them.

We examined the performance of the fish MMIs across the range of stream sizes sampled for NRSA. The potential exists for bias in the fish MMI due to different fish species pools being available for larger rivers versus smaller streams. Differences across the size range might result from the different sampling protocols used according to river or stream size (wadeable, large wadeable, and boatable). We used the set of least-disturbed sites to examine patterns in fish MMI scores across three size categories based on Strahler order (**Figure 6-3**). In most aggregated ecoregions, there is little difference between the distribution of fish MMI scores among stream size classes. In the Northern Appalachians aggregated ecoregion, fish MMI scores at the least-disturbed sites that are 5th order or larger are significantly different from fish MMI scores in least-disturbed sites that are first or second order (one-way ANOVA with Tukey multiple comparisons test, $p < 0.05$).

We examined the potential effect of the three different fish sampling protocols for streams of different sizes (**Figure 6-4**). The distribution of fish MMI scores in least-disturbed sites were similar among the three protocols for most of the nine aggregated ecoregions. In the Northern Appalachians, this appears to be a tendency for fish MMI scores for least-disturbed sites sampled using the boatable protocol to be lower than fish MMI scores for least-disturbed sites sampled using either the wadeable or large wadeable protocols, but the difference is not significantly different (one-way ANOVA with Tukey multiple comparisons test). In the Northern Plains aggregated ecoregion, fish MMI scores for least-disturbed sites sampled with the boatable protocol are significantly higher than fish MMI scores at least-disturbed sites sampled using either the wadeable or large wadeable protocol (one-way ANOVA with Tukey multiple comparisons test, $p < 0.05$). The effect of sampling protocol in the Upper Midwest and Xeric West aggregated ecoregions are difficult to evaluate, as there was only one least-disturbed site sampled each using the large wadeable and boatable protocols.

The NRSA includes streams of different temperature regimes as well as a broad range of stream sizes. We used the predicted summer (July-August) daily stream temperatures (°C) based on reference condition USGS stream temperature stations (Hill et al.) to estimate the mean summer stream temperature (MSST). We classified the set of least-disturbed streams in each aggregated ecoregion as either cold water (MSST < 17 °C), cool water (MSST between 17 and 20 °C) or warm water (MSST ≥ 20 °C). **Figure 6-5** shows the distribution of fish MMI scores among the three temperature classes for each aggregated ecoregion. The Coastal Plains aggregated ecoregion did not have any least-disturbed sites that were classified as either cold or cool water. The Southern Plains aggregated ecoregion did not have any least-disturbed sites classified as cold water (and only two sites classified as cool water), and the Temperate Plains aggregated ecoregion only had one least-disturbed site classified as cold water (and only two sites classified as cool water). The Upper Midwest aggregated ecoregion only had three least-disturbed sites classified as warm water. The Western Mountains aggregated ecoregion had only one least-disturbed site classified as warm water. In the Northern Appalachians aggregated ecoregion, fish MMI scores for warm water sites are significantly lower than fish MMI scores for either cool water or cold water sites (one-way ANOVA with Tukey multiple comparisons test, $p < 0.001$). In the Northern Plains aggregated ecoregion, fish MMI scores for warm water and cool water sites are significantly lower than fish MMI scores at cold water sites (one-way ANOVA with Tukey multiple comparisons test, $p < 0.001$). In the Xeric West aggregated ecoregion, fish MMI scores for warm water sites are significantly lower than fish MMI scores for either cool water or cold water sites (one-way ANOVA with Tukey multiple comparisons test, $p < 0.01$).

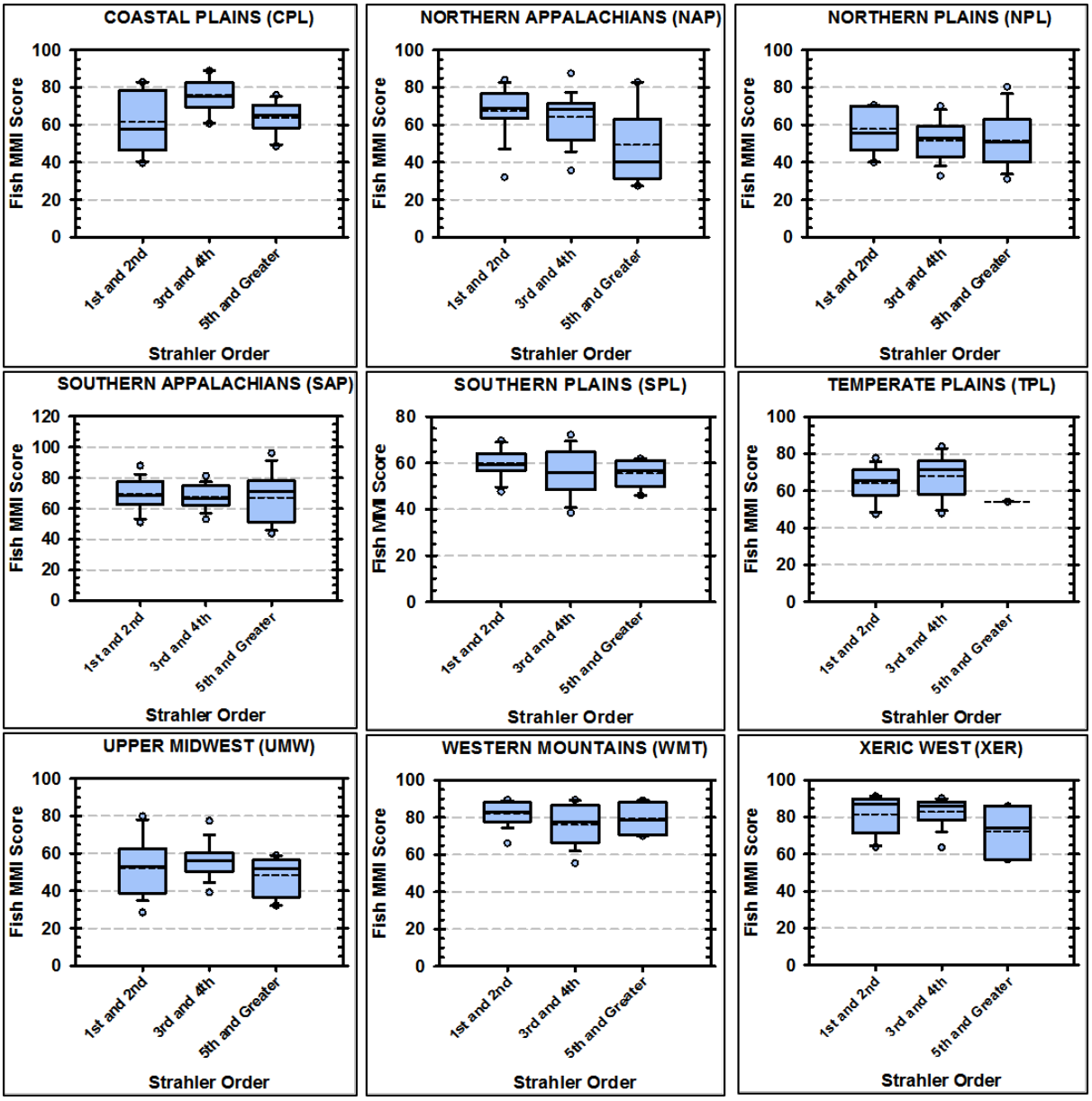

Figure 6-3. Regional fish MMI scores versus Strahler order category (least-disturbed sites).

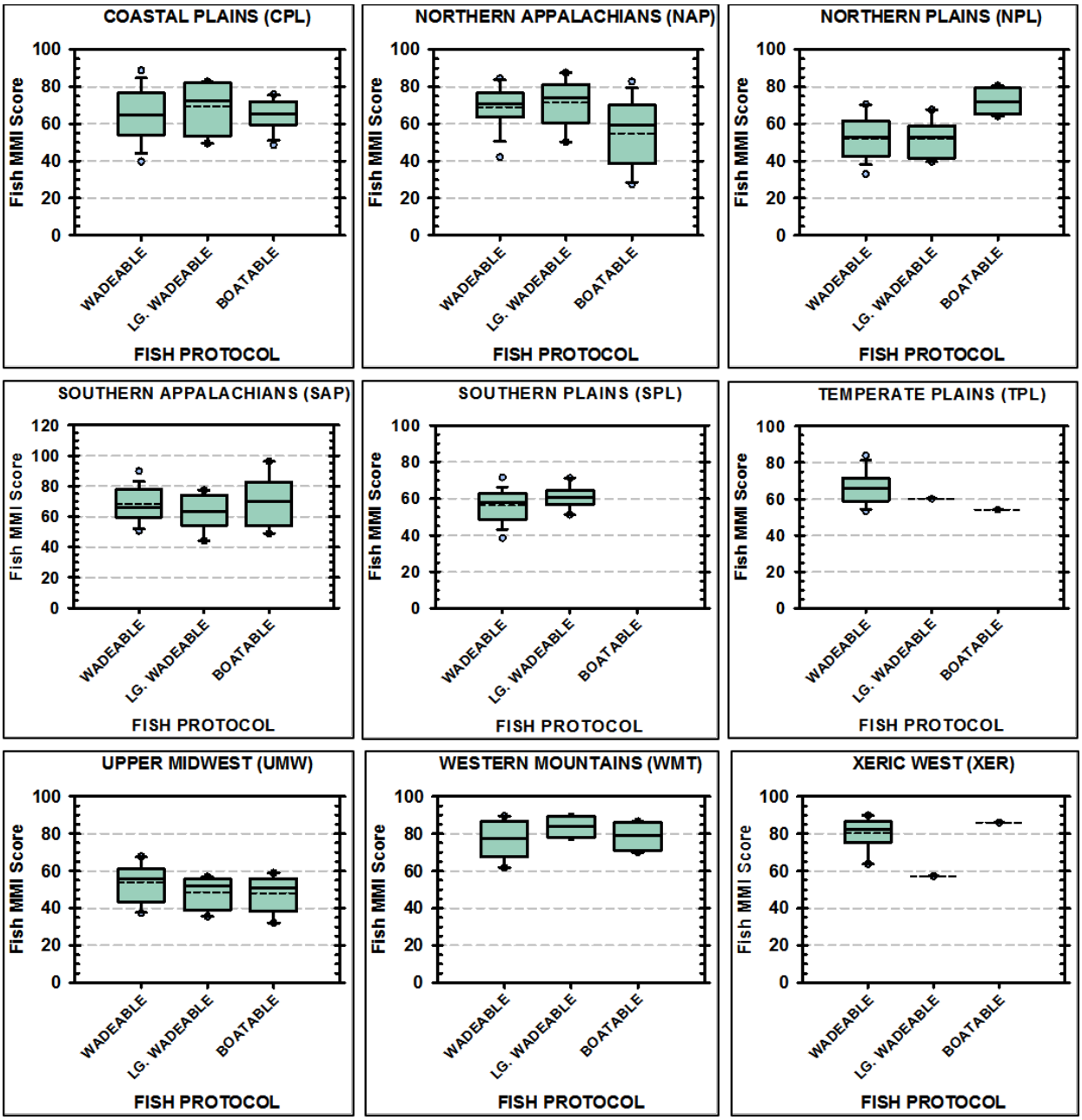

Figure 6-4. Regional fish MMI scores versus fish sampling protocol (least-disturbed sites).

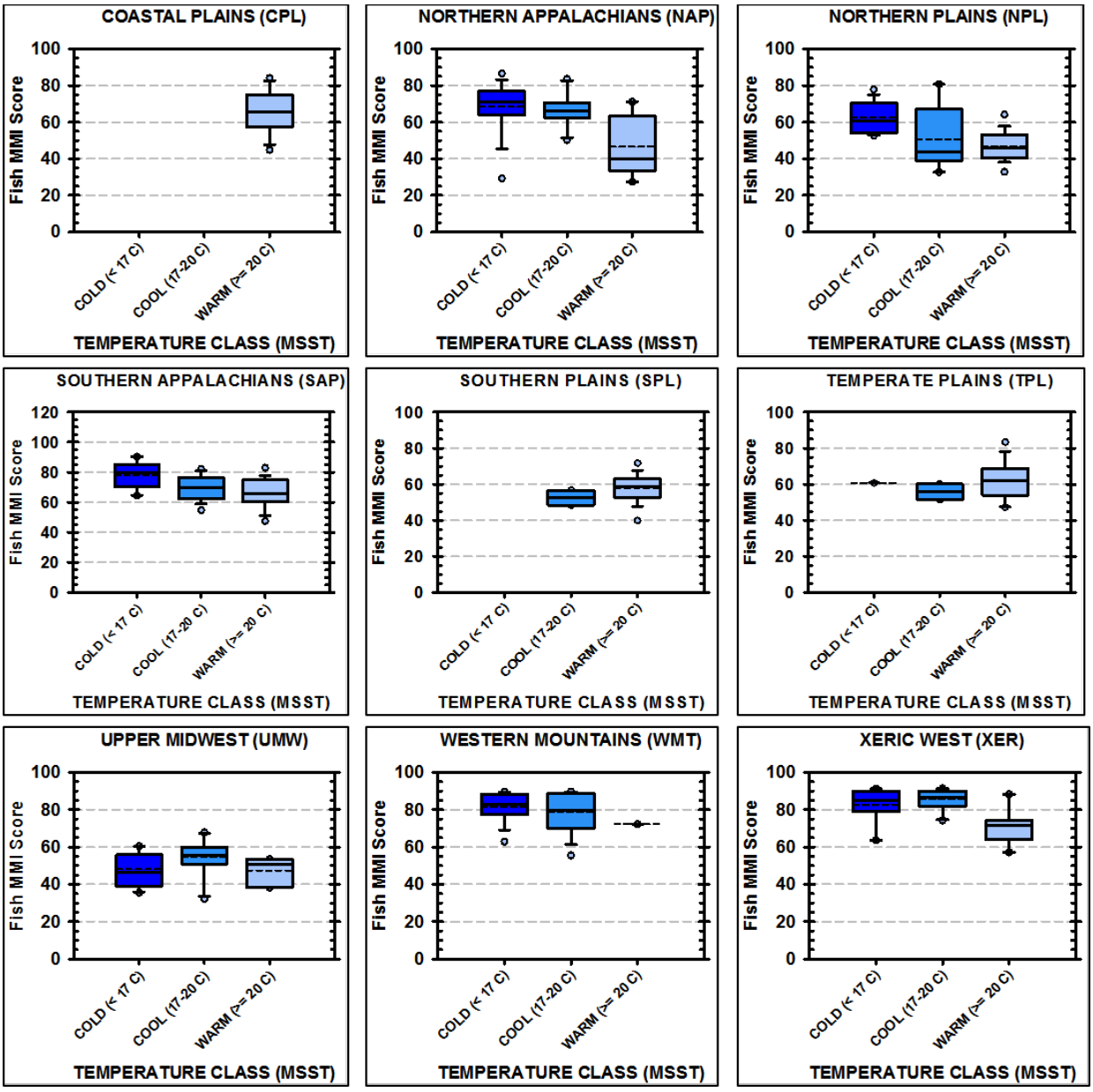

Figure 6-5. Regional fish MMI scores versus stream temperature class (least-disturbed sites). Temperature based on modeled mean summer stream temperature (MSST).

6.5 SITES WITH LOW FISH ABUNDANCE

The target population for NRSA includes small perennial headwater streams. Some very small streams may not contain fish even in the absence of human disturbance. We followed the approach described by McCormick et al. (2001) and used least-disturbed sites to estimate a drainage area below which the probability was high that no fish would be present (**Table 6-14**). This approach uses the relationship between a set of four physical habitat variables that characterize habitat volume and the number of fish collected. This relationship defines a habitat volume value below which nearly all sites sampled were devoid of fish. Then this habitat volume value is related to watershed area to determine the drainage area below which streams are expected to be naturally fishless.

Figure 6-6 shows the results of this analysis. The value for the habitat volume index below which almost all sites were fishless is 0.41. When habitat volume is plotted against watershed area, this value corresponds to a watershed area of approximately 2 km2. For sites with watershed areas less than 2 km2 where no fish were collected, we do not report the fish MMI score. Otherwise, we assign a fish MMI score of zero to sites with no fish collected.

6.6 BENCHMARKS FOR ASSIGNING ECOLOGICAL CONDITION

For the NRSA, ecological condition is based on the deviation from least-disturbed condition (Stoddard et al., 2006, Hawkins et al., 2010b). Within each of the nine aggregated ecoregions regions, benchmarks for defining "good" condition and "poor" condition are based on the distribution of fish MMI scores in least-disturbed sites. For the NRSA 2018-2919, we used the same benchmarks as were used for the NRSA 2013-20142019 (**Table 6-15**).

Benchmarks were set following the same process used for benthic macroinvertebrate condition (see Section **5.3.2**). We combined the least-disturbed sites identified for the NRSA 2008-09 and the NRSA 2013-14 to develop benchmarks that were then applied to the fish MMI scores from both assessments. We used a single visit per site and used the latest visit if a least-disturbed site was sampled in 2008-09 and resampled in 2013-14. We attempted to adjust for differences in the quality of least-disturbed sites across the nine aggregated ecoregions by applying the "hindcasting" approach described in Section **5.3.2** and by Herlihy et al. (2008), and the NRSA 2008-09 technical report (USEPA 2016).

Table 6-14. Determining the minimum drainage area expected to reliably support the presenceof fish (adapted from McCormick et al. (2001)). Variable names are from the NRSA database. Scores for each metric between the upper and lower criteria were estimated by linear interpolation.

SET OF SITES
Use least-disturbed sites only (RT_NRSA_FISH="R") to minimize effects of human disturbance
HABITAT VOLUME INDEX
Percent of support reach length that is dry (PCT_DRS)
If PCT_DR < 1%, score = 1. If PCT-DR ≥ 20%, then score = 0.
Log_{10}[(mean wetted width x mean thalweg depth) +0.001] (LXWXD)
If LXWXD > 1, score=1. If LXWXD ≤ -1.4, then score = 0.
Residual pool depth (RP100)
If RP100 ≥ 20, then score=1. If RP100 ≤ 0, then score = 0.
Mean wetted width
If XWIDTH ≥ 6, then score = 1. If XWIDTH = 0, then score = 0.
HABITAT VOLUME INDEX = (PCT_DR score + LXWXD score + RP100 score + XWIDTH score)/4
PLOT NUMBER OF FISH COLLECTED (TOTLNIND) VS. HABITAT VOLUME INDEX (QVOLX)
Value for QVOLX below which most sites have no fish = 0.41
PLOT HABITAT VOLUME INDEX VS. WATERSHED AREA (WSAREA_KM2)
QVOLX = 0.41 corresponds to a watershed area of ~ 2 km^2

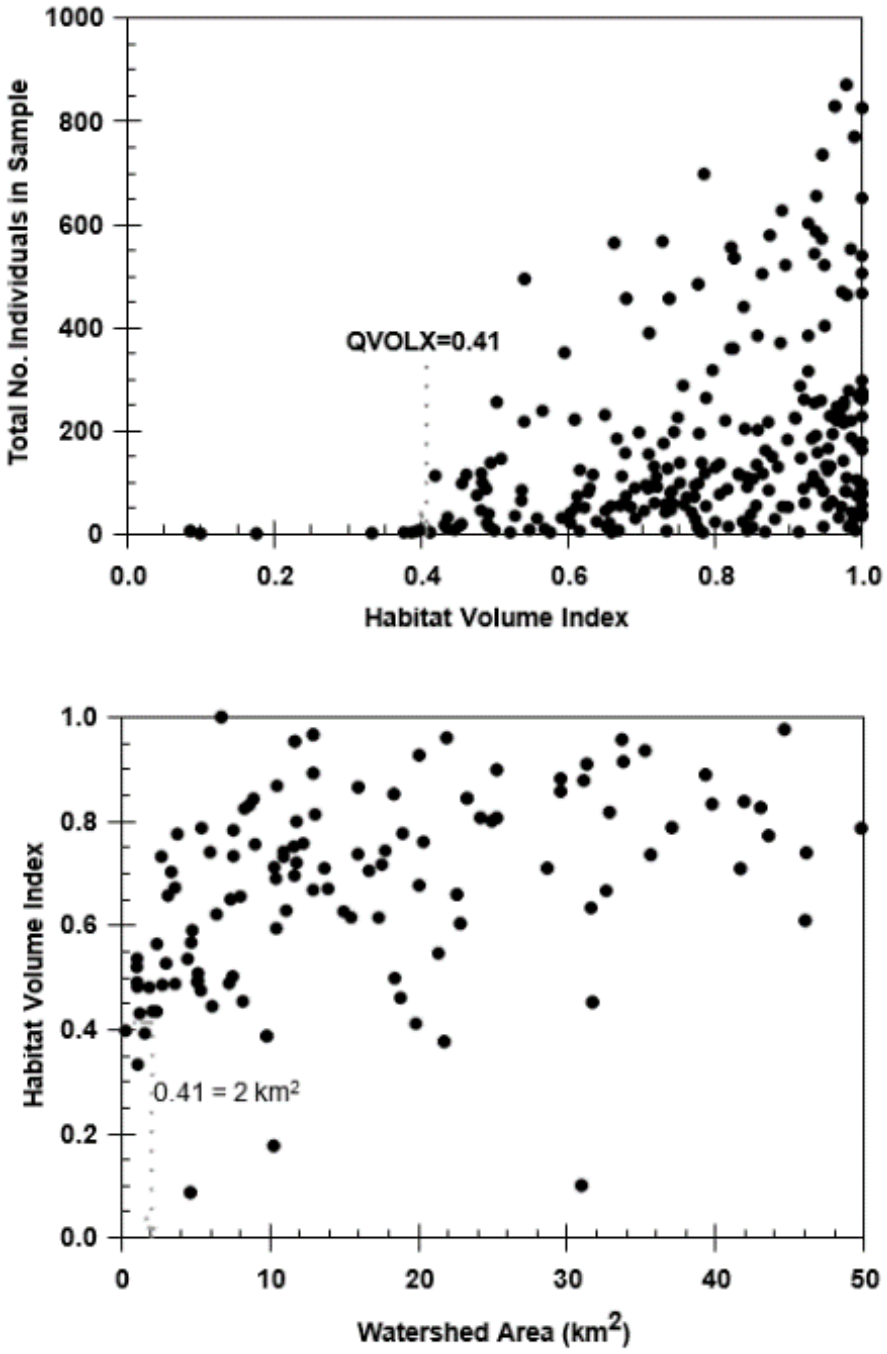

Figure 6-6. Relationship between number of fish collected, reduced habitat volume, and small watershed size at least-disturbed sites. Fish are not likely to be found in streams with a watershed area of < 2 km2. The scales of total number of fish collected and watershed area axes have been truncated for clarity.

The benchmarks for assigning "good" condition range between ≥ 39.8 for the Upper Midwest to ≥ 76.8 for the Xeric West. The benchmarks for assigning "poor" condition range from < 29.3 in the Upper Midwest to <65.4 in the Western Mountains. The hindcasting approach results in the

benchmarks in each aggregated ecoregion differing by 10.5. Note that even though the fish MMI for the Upper Midwest has lower benchmarks than the other aggregated ecoregions, the fish MMI still performs well (**Table 6-13**; **Figure 6-2**).

6.7 DISCUSSION

We developed and evaluated regional fish MMIs based on approaches that have evolved from our experience with other regional-scale assessment efforts (e.g., McCormick et al., 2001, Whittier et al., 2007b, Stoddard et al., 2008, Van Sickle 2010). Using this approach, we constructed a fish MMI that was responsive to disturbance and repeatable for each of the nine aggregated ecoregions (**Table 6-13** and **Figure 6-2**). Our evaluation approach focuses on selecting metrics and fish MMIs that maximize responsiveness to disturbance and have adequate values for other performance criteria such as repeatability. In all nine aggregated ecoregions, the fish MMIs tend to be more responsive to disturbance and repeatable than any of their component metrics (Table 6.4 through Table 6.13).

We calculate candidate metrics based on the percent of taxa, which are not commonly considered for fish. For each of the nine regional fish MMIs, one or more of the final metrics is based on the percent of taxa (Table 6.4 through Table 6.12). We examine the relationship between watershed area and metric response for all candidate metrics (not just richness metrics) and adjust the metric response value when the coefficient of determination (R2) for the linear regression is > 0.10. The fish MMIs for eight of the nine aggregated ecoregions have at least one metric that is not a richness metric where the adjusted metric performs better than the unadjusted metric (**Table 6-3**).

The ability to calculate large numbers of candidate MMIs from a set of metrics that met all our evaluation criteria is an improvement over stepwise selection of metrics based on correlations with metrics already selected (Stoddard et al., 2008, Van Sickle 2010). This approach provides the opportunity to evaluate MMIs based on suites of metrics that might not otherwise be considered and helps to ensure the best-performing MMI is selected. Incorporating the difference between the 25th percentile of least-disturbed and 75th percentile of more disturbed sites (**Table 6-13**) and the F- score (or t-value) provides a quick and reproducible way of selecting a final fish MMI from the tens of thousands of candidate fish MMIs that can be generated (**Table 6-2**). However, within each aggregated ecoregion, there may be several alternative fish MMIs with similar performance (i.e., a slightly lower PCA axis score, t-value, and S:N ratio) to the fish MMI we selected as the final.

Table 6-15. Benchmarks for assigning ecological condition based on the distribution of regional fish MMI scores in least-disturbed sites sampled in NRSA 2008-09 or NRSA 2013-14, adjusted using the hindcasting approach of Herlihy et al. (2008). Aggregated ecoregions are shown in Figure 6-2. Sample sizes are in parentheses.

Aggregated Ecoregion	Good/Fair	Fair/Poor
Eastern Highlands		
Northern Appalachians (60)	≥ 57.6	< 47.1
Southern Appalachian (94)	≥ 60.3	< 49.8
Plains and Lowlands		
Coastal Plains (39)	≥ 57.3	< 46.8
Northern Plains (42)	≥ 46.3	< 35.8
Southern Plains (43)	≥ 50.2	< 39.7

Temperate Plains (28)	≥ 58.0	< 47.5
Upper Midwest (28)	≥ 39.8	< 29.3
West		
Western Mountains (70)	≥ 75.9	< 65.4
Xeric West (25)	≥ 76.8	< 63.7

We did note some potential influence of stream size, sampling protocol, and temperature regime on fish MMI scores in least-disturbed sites in some aggregated ecoregions (Figure 6.4 through Figure 6.6). These patterns were less evident in the original fish MMIs we developed for the three climatic regions (**Figure 6-1**; USEPA 2016). The fish MMIs in these aggregated ecoregions still performed well despite these influences (**Figure 6-3**, **Table 6-13**). At the scale of our aggregated ecoregions, small sample sizes and, in some cases, a limited geographic range of some classes of least-disturbed sites, make developing separate MMIs for different types of streams (e.g., larger streams or warm water streams) impractical.

We can consider several future refinements to the NRSA fish MMI development process as data are acquired from future rounds of NRSA. At present, we cannot develop fish MMIs for those relatively few NRSA sites that are sampled by seining. These sites tend to be confined to certain geographic areas. Once we have acquired seining data from enough sites, we may be able to construct a fish MMI that performs well and is compatible with the fish MMIs developed based on electrofishing data. An increased pool of least-disturbed sites in each of the nine aggregated ecoregions would allow for a more rigorous evaluation of the potential influence of factors such as stream size, protocol, and temperature regime. For larger streams, a national-scale index might be feasible given the advances in available techniques used to construct and evaluate MMIs. We have the data to construct numeric tolerance values for individual fish species based on a national-scale data, which would expand upon previous efforts (Meador and Carlisle 2007, Whittier et al., 2007a) and provide a tool with broad applicability to bioassessment activities. The fish MMIs we developed are tailored to respond to a general measure of disturbance, rather than being comprised of metrics that are responsive to different types of specific stressors. Examining the relationships between metrics and individual stressors would improve the interpretability of the fish MMI and the resultant estimates of risk that are produced as part of the overall assessment in NRSA.

Fish counts, metrics, and multimetric index condition from NRSA are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic- resource-surveys.

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APPENDIX 6.A COMPARISON OF MODEL-BASED AND TRADITIONAL FISH MULTIMETRIC INDICES FOR NRSA 2008-09

We used the data from the NRSA 2008-09 study to compare the performance of fish MMIs developed with predictive models (random forests) to adjust metric responses for natural variability to the performance of fish MMIs developed with a more traditional approach, where metric responses are adjusted for watershed area using linear regression (Sections **6.1.1** and **6.1.2**). The development and evaluation process for both approaches is essentially the same; the objective being that the fish MMIs are the best representation of fish ecology that is responsive to disturbance (Sections **6.4** through **6.6**). Both fish MMIs are developed using the same sets of least-disturbed (LD) and most disturbed (MD) sites. Both fish MMIs are comprised of eight metrics andinclude a combination of adjusted and unadjusted metrics. Condition classes were developed for both fish MMIs in the same manner (Section **6.7**).

We evaluated four aspects of the performance of the fish MMIs: responsiveness to disturbance, precision, repeatability, and sensitivity.

6.A.1 RESPONSIVENESS TO DISTURBANCE AND PRECISION

Figure 6.A.1 compares scores for both types of fish MMIs in least-disturbed and most disturbed sites in each of the nine aggregated ecoregions. The model-based fish MMI was developed for the three large climatic regions (**Figure 6-1**), but the fish MMI scores are broken down for each of the nine aggregated ecoregions. In general, the distributions of least-disturbed and most disturbed sites are similar for both types of fish MMIs.

We used a *t*-test between least-disturbed and most disturbed sites as our performance test for responsiveness to disturbance (**Figure 6.A.2**). Sample sizes of least-disturbed and most disturbed sites within each ecoregion were similar if not identical for both types of fish MMI. For both typesof fish MMI, differences between mean values of least-disturbed and most disturbed sites were highly significant in all aggregated ecoregions ($p < 0.0001$). The traditional fish MMIs had higher values for *t* in all but one aggregated ecoregion (Xeric West).

We used the standard deviation of fish MMI scores in least-disturbed sites, after adjusting scores by dividing by the mean value, as our performance test for precision. Precision is expected to be zero if our adjustments have accounted for natural variability. Precision values greater than zero represent any disturbance signal remaining after adjustment as well as measurement error. Neither approach to constructing the fish MMI completely adjusted for natural variability (**Figure 6.A.2**), but the amount of unexplained variability in both types of fish MMIs did not impact the ability of the fish MMIs to be responsive to disturbance (e.g., in the Northern Appalachians aggregated ecoregion, the traditional fish MMI was comparatively imprecise, but was very responsive to disturbance). The model-based fish MMIs tended to be slightly more precise than the traditional MMIs. Both types of fish MMIs were comparatively imprecise in the aggregated ecoregions that were included in the Plains and Lowlands climatic region.

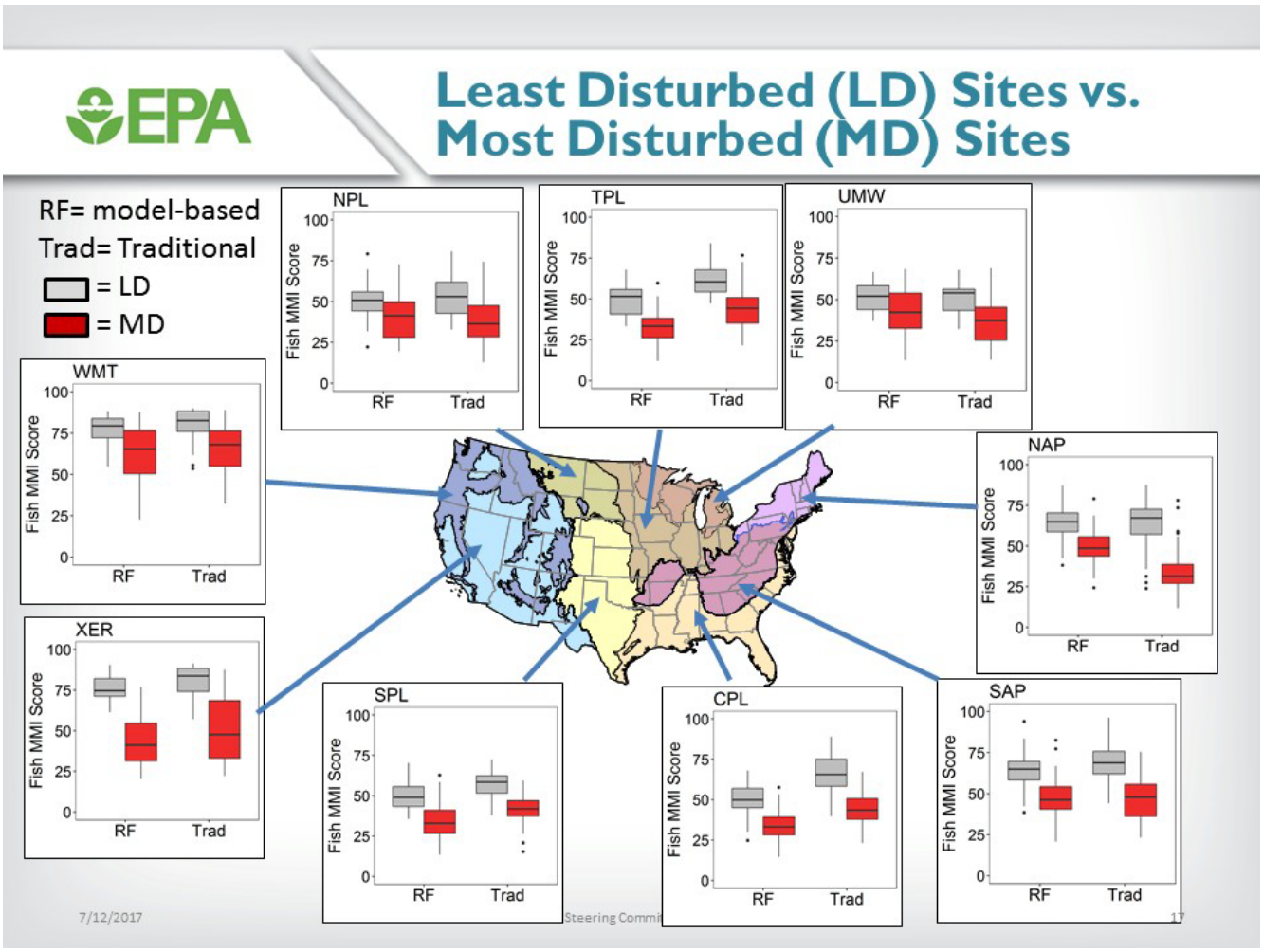


Figure 6.A.1. Distribution of fish MMI scores in least-disturbed vs. most-disturbed sites in NRSA 2008-09. For each aggregated ecoregion (see Figure 6.2), the left-hand pair of boxplots are for the model-based fish MMI (RF), and the right-hand pair are for the "traditional" fish MMI (Trad). Gray boxes=least-disturbed sites and red boxes=most-disturbed sites.

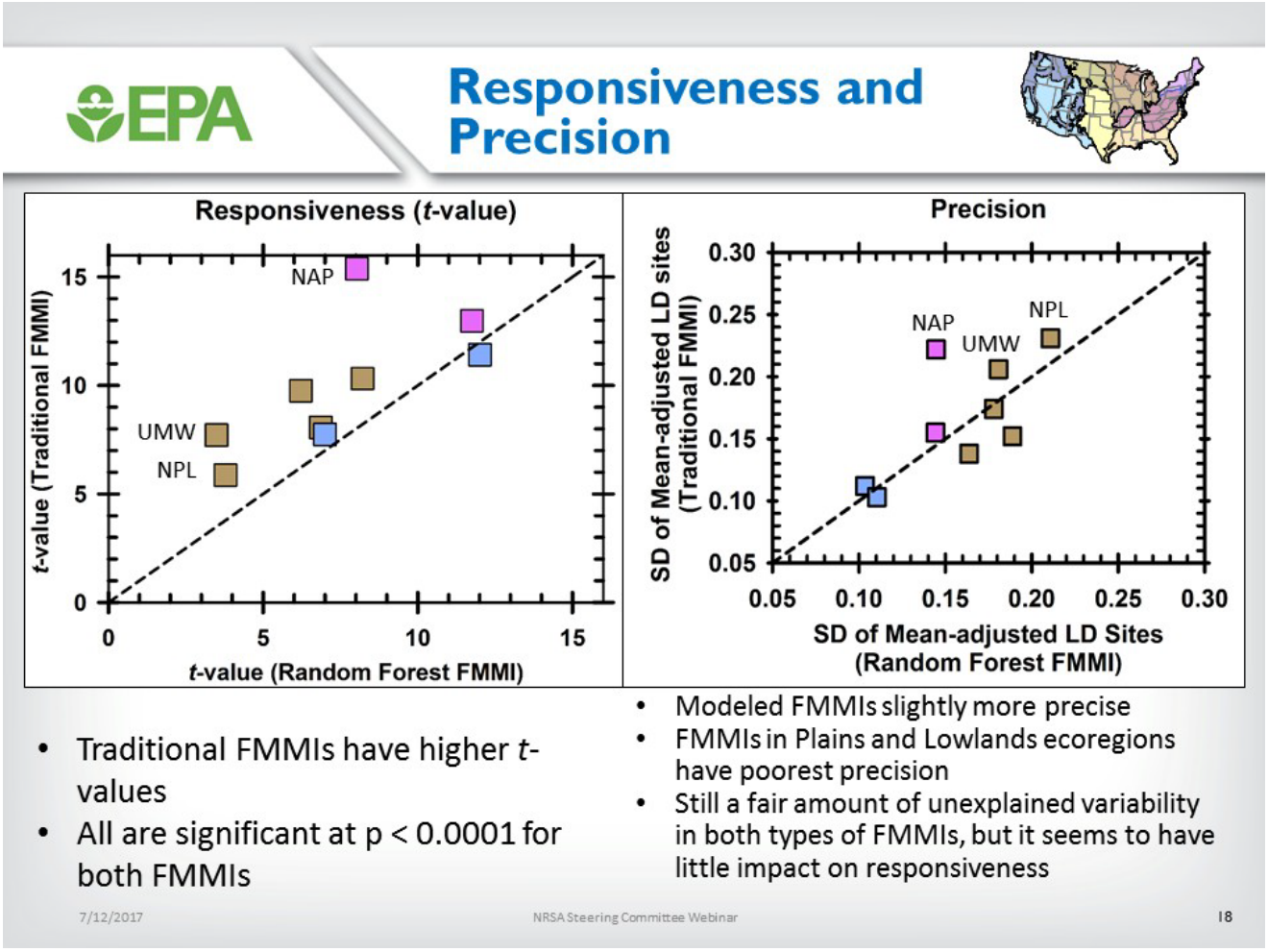


Figure 6.A.2. Comparison of two types of fish MMI scores for responsiveness to disturbance and precision in nine aggregated ecoregions (see Figure 6-2). Y-axis= Traditional fish MMI score; X-axis=model-based fish MMI score; line is a 1:1 line. Colors coincide with regions on map inset (violet=Eastern Highlands, brown=Plains and Lowlands, blue=Western Mountains and Xeric).

6.A.2 REPEATABILITY AND SENSITIVITY

We evaluated repeatability of the fish MMIs by calculating a S:N ratio (S:N; see Section **6.4**), which compared the variance among sites to variance within sites (from repeat visits). We estimated the sensitivity of the fish MMIs based on the proportion of most disturbed sites that were significantly different (using an interval test) from the set of least-disturbed sites. The interval test is more conservative than simply looking at the number of most disturbed sites that are below a single percentile value (e.g., the 5th percentile) of the least-disturbed sites.

Figure 6.A.3 shows the results of the comparisons for repeatability and sensitivity. S:N ratios for both types of fish MMIs are adequate for use in NRSA. The model-based fish MMIs tend to have slightly higher values of S:N than the traditional fish MMIs. In some aggregated ecoregions (e.g., the Northern Appalachians or the Upper Midwest), there is a small number of sites with repeat visits. If

there is little or no variability in the fish MMI scores at these sites between visits, it will result in a very high estimate of S:N that is mostly a function of small sample size.

Sensitivity values are similar for both types of fish MMIs and are nearly identical for those aggregated ecoregions in the Plains and Lowlands climatic regions. The low values (< 40% for all but one aggregated ecoregion) reflect the variability present in both the least-disturbed and most disturbed sites at the scale of the aggregated ecoregions. The lowest sensitivity was seen in the Northern Plains aggregated ecoregion, while the greatest sensitivity was observed in the Xeric West aggregated ecoregion.

6.A.3 Correlation of Fish MMI Scores

We looked at how similar the traditional fish MMI scores were to the model-based fish MMI scores at all sites. For each aggregated ecoregion, we calculated the Pearson correlation coefficient, and calculated the geometric mean functional regression (GMFR) because each fish MMI is measured with error. We used a single index visit for each site and excluded sites where no fish were collected. Correlation coefficients are > 0.7 for all but the Northern Plains aggregated ecoregion (**Figure 6.A.4**). The GFMR analysis indicates that for two aggregated ecoregions (the Upper Midwest and the Western Mountains), the two fish MMIs are identical (slope=1, intercept=0). In the Xeric West aggregated ecoregion, the traditional fish MMI scores are consistently higher by a small amount than the model-based fish MMI scores (slope=1, intercept > 0). For the remaining aggregated ecoregions, slopes are >1 except in the Southern Plains aggregated ecoregion.

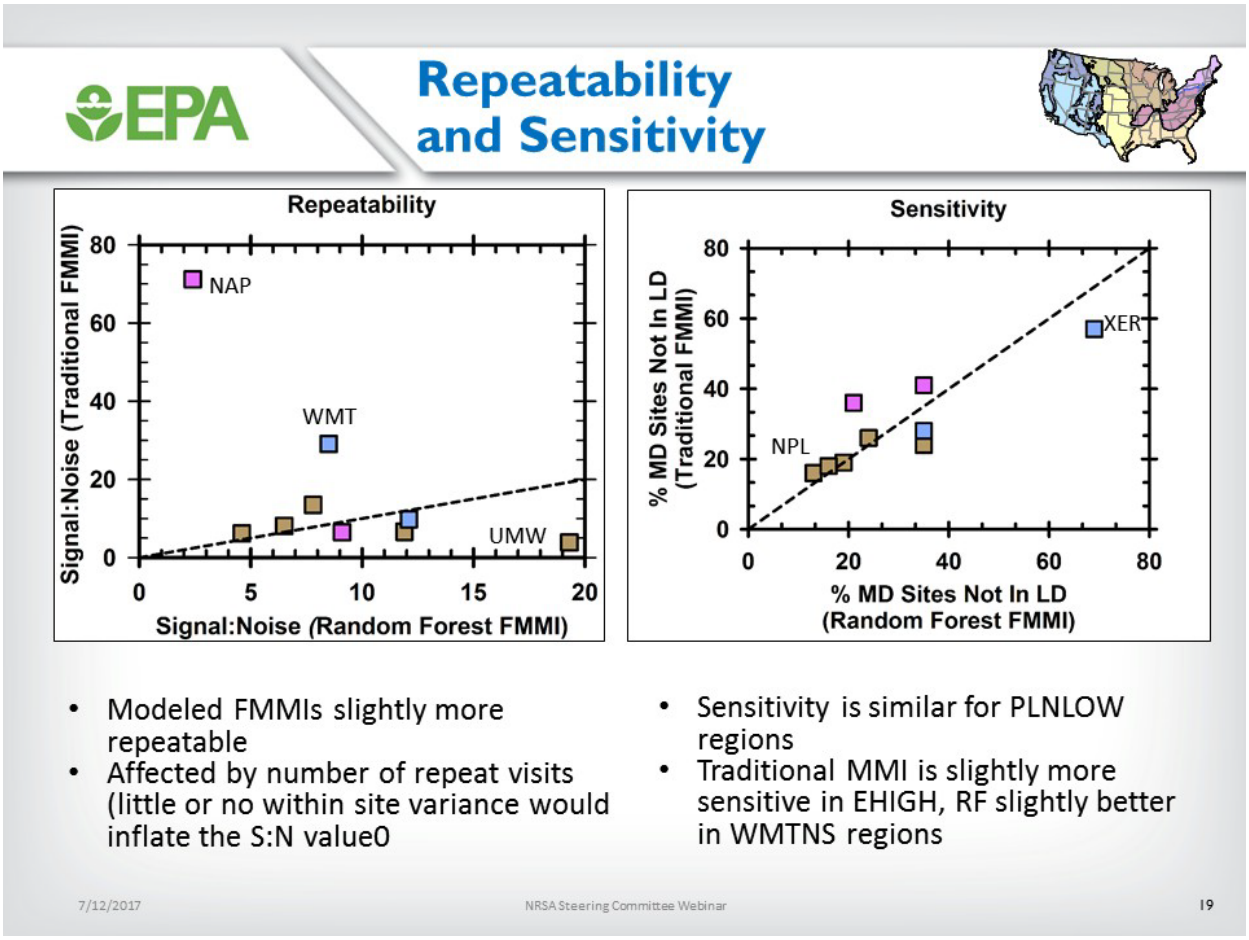


Figure 6.A.3. Comparison of two types of fish MMI scores for repeatability and sensitivity in nine aggregated ecoregions (see Figure 6.2). Y-axis=Traditional fish MMI score; X-axis=model-based fish MMI score; line is a 1:1 line. Colors coincide with regions on map inset (violet=Eastern Highlands, brown=Plains and Lowlands, blue=Western Mountains and Xeric).

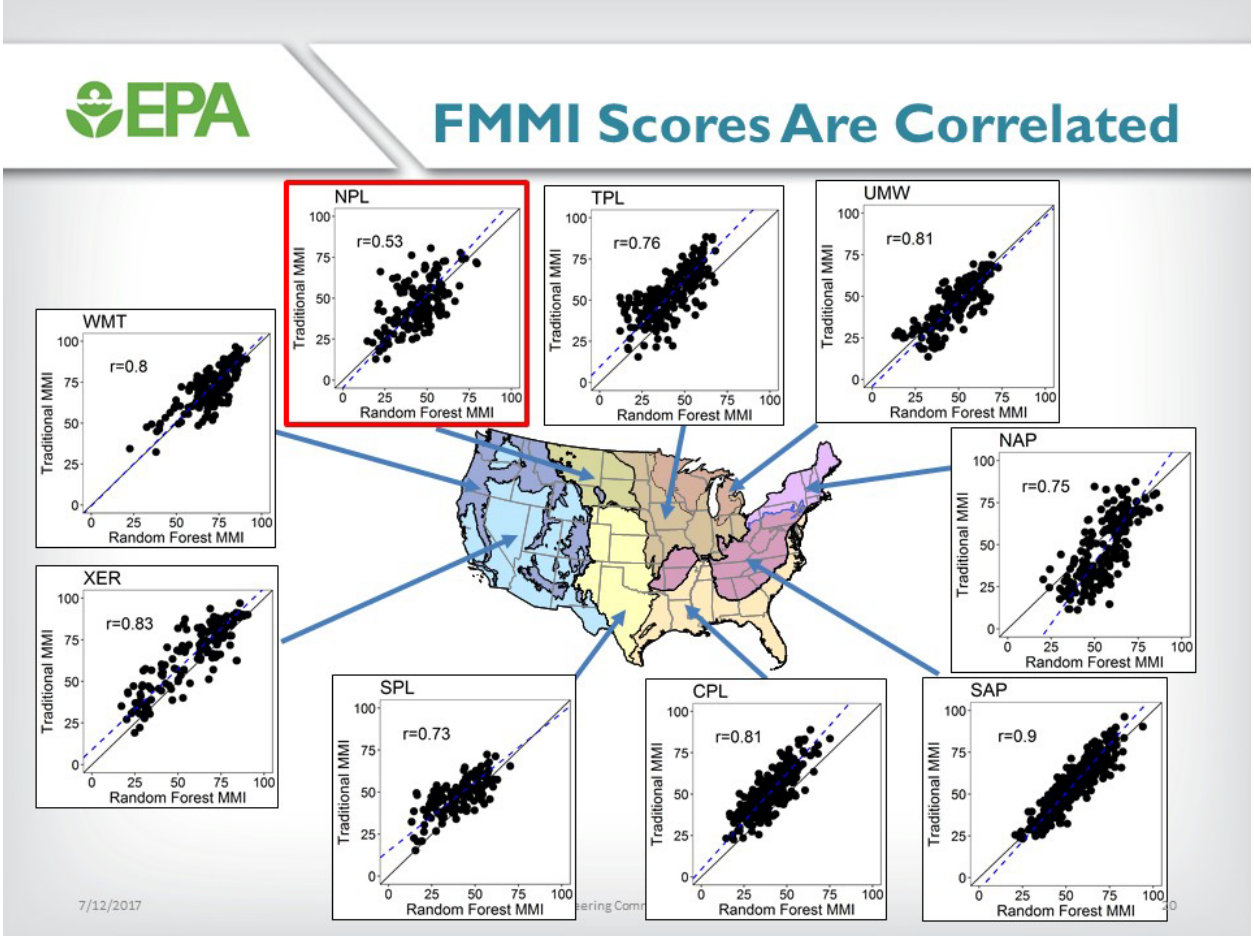


Figure 6.A.4. Comparison of two types of fish MMI scores in nine aggregated ecoregions (see Figure 6.2). Points are index visits only, and siteswhere no fish were collected are excluded. Y-axis=Traditional fish MMI score; X-axis=model-based fish MMI score. Solid black line: 1:1 line; Dashed Blue line: Regression line based on geometric mean functional regression.

6.A.4 POPULATION ESTIMATES

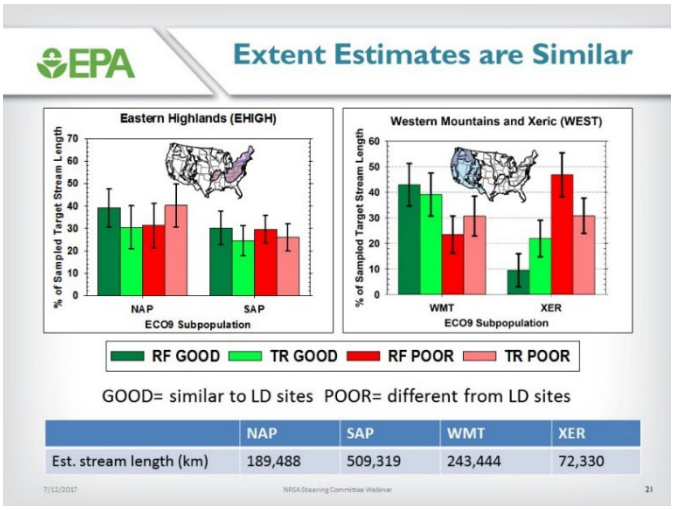
The survey design for NRSA allows us to make inferences from the set of sampled sites to a much larger target population. We wanted to know if the two types of fish MMIs would yield different estimates of biological condition for the target population. We assigned condition classes for each type of fish MMI in each aggregated ecoregion using the approach described in Section **6.5**.

Condition class is assigned for each of the nine aggregated ecoregions based on the deviation from the set of LD sites. For the four aggregated ecoregions in the Eastern Highlands and Western Mountains climatic regions, the two types of fish MMIs yield similar estimates of the percent of stream length in both good and poor condition (**Figure 6.A.5**); the largest differences in length are in the Xeric West aggregated ecoregion. In the Plains and Lowlands climatic region, the two types of fish MMIs yield similar estimates of the percent of stream length in good and poor condition for all aggregated ecoregions except for the Coastal Plains, where the traditional fish MMI produces a smaller percent of stream length in good condition and a larger percent of stream length in poor

condition compared to the model-based fish MMI. One or both types of fish MMIs in the Northern Plains and the Upper Midwest aggregated ecoregions had some performance issues, yet the condition class estimates for the sampled target population were similar.

Based on our evaluations, both types of fish MMIs generally have similar performance and provide similar estimates of biological condition for the samples target population in each aggregated ecoregion. Scores for the two types of fish MMIs are well correlated despite differences in component metrics and the scale at which metric adjustments are made (climatic region for the model-based fish MMI and aggregated ecoregion for the traditional fish MMI). The quality of least-disturbed sites may be less similar among the five aggregated ecoregions that are included in the Plains and Lowlands climatic region than the aggregated ecoregions that are included in either the Eastern Highlands or Western Mountains climatic regions.

We calculated the traditional fish MMI scores for all sites in the NRSA 2008-09 and the NRSA 2013-14. The scale of traditional fish MMI development (i.e., the nine aggregated ecoregions) is consistent with the MMI developed for the benthic macroinvertebrate assemblage in NRSA. We did not use any least-disturbed sites identified in NRSA 2013-14 to develop the fish MMIs, but we did pool the least-disturbed sites from both studies to estimate the benchmark values to assign biological condition.



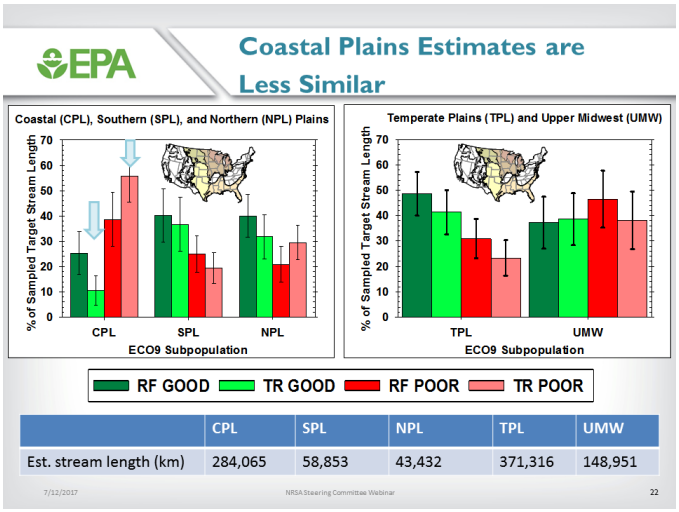


Figure 6.A.5. Biological condition in nine aggregated ecoregions (ECO9) (see Figure 6-2) for the NRSA 2008-09 based on two types of fish MMIs. Left panel shows aggregated ecoregions within the Eastern Highlands and Western Mountains climatic regions. Right panel shows aggregated ecoregions within the Plains and Lowlands climatic regions. Bars represent the percent of the length of the sampled target population inferred from the set of sampled sites; error bars are 95% confidence intervals. RF=model-based fish MMI; TR=traditional fish MMI. Good=similar to least-disturbed sites; Poor=different from least-disturbed sites (see Section 6.5 and Table 6-15). The total estimated length of the sampled target population for each aggregated ecoregion is shown in the table.

APPENDIX 6.B CANDIDATE METRICS CONSIDERED FOR FISH MMI DEVELOPMENT

Table 6.B.1 presents the candidate metrics that were evaluated for potential inclusions in the regional fish MMIs. Metric classes represent different attributes of fish assemblage structure or function. Some metrics are combinations of two different metric classes. Composition metrics generally focus on the family level of taxonomic resolution. Round-bodied suckers include the following genera: Catostomus, Cycleptus, Erimyzon, Hypentelium, Minytrema, Moxostoma, Pantosteus, and Thoburnia. Migratory species include both diadromous and anadromous species. "Not tolerant" metrics include all species not classified as tolerant (and thus include intolerant species, moderately tolerant species, and species that have no tolerance category assigned). We use "intolerant" here in the same context as others use "sensitive."

Table 6.B.1. List of candidate metrics.

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
ALIEN	ALIENNTAX	No. Non-native species
ALIEN	ALIENPIND	% Non-native individuals
ALIEN	ALIENPTAX	% Non-native taxa
ALIEN	NAT_PIND	% Native individuals
ALIEN	NAT_PTAX	% Native taxa
COMPOSITION	CATONTAX	No. Catostomid species
COMPOSITION	CATOPIND	% Catostomid individuals
COMPOSITION	CATOPTAX	% Catostomid taxa
COMPOSITION	NAT_CATONTAX	No. Native catostomid species
COMPOSITION	NAT_CATOPIND	% Native catostomid individuals
COMPOSITION	NAT_CATOPTAX	% Native catostomid taxa
COMPOSITION	RBCATONTAX	No. Round-bodied catostomid species
COMPOSITION	RBCATOPIND	% Round-bodied catostomid individuals
COMPOSITION	RBCATOPTAX	% Round-bodied catostomid taxa
COMPOSITION	NAT_RBCATONTAX	No. Native round-bodied catostomid species
COMPOSITION	NAT_RBCATOPIND	% Native round-bodied catostomid individuals
COMPOSITION	NAT_RBCATOPTAX	% Native round-bodied catostomid taxa
COMPOSITION	CENTNTAX	No. Centrarchid species (excl. *Micropterus* spp.)
COMPOSITION	CENTPIND	% Centrarchid individuals (excl. *Micropterus* spp.)

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
COMPOSITION	CENTPTAX	% Centrarchid taxa (excl. *Micropterus* spp.)
COMPOSITION	NAT_CENTNTAX	No. Native centrarchid species (excl. *Micropterus* spp.)
COMPOSITION	NAT_CENTPIND	% native centrarchid individuals (excl. *Micropterus* spp.)
COMPOSITION	NAT_CENTPTAX	% Native centrarchid taxa (excl. *Micropterus* spp.)
COMPOSITION	CYPRNTAX	No. Cyprinid species (excluding all carps and goldfish)
COMPOSITION	CYPRPIND	% Cyprinid individuals(excluding all carps and goldfish)
COMPOSITION	CYPRPTAX	% Cyprinid individuals(excluding all carps and goldfish)
COMPOSITION	NAT_CYPRNTAX	No. Native cyprinid species(excluding all carps and goldfish)
COMPOSITION	NAT_CYPRPIND	% Native cyprinid individuals(excluding all carps and goldfish)
COMPOSITION	NAT_CYPRPTAX	% Native cyprinid individuals(excluding all carps and goldfish)
COMPOSITION	ICTANTAX	No. Ictalurid species
COMPOSITION	ICTAPIND	% Ictalurid individuals
COMPOSITION	ICTAPTAX	% Ictalurid taxa
COMPOSITION	NAT_ICTANTAX	No. Native ictalurid species
COMPOSITION	NAT_ICTAPIND	% Native ictalurid individuals
COMPOSITION	NAT_ICTAPTAX	% Native ictalurid taxa
COMPOSITION	SALMNTAX	No. Salmonid species
COMPOSITION	SALMPIND	% Salmonid individuals
COMPOSITION	SALMPTAX	% Salmonid taxa
COMPOSITION	NAT_SALMNTAX	No. Native salmonid species
COMPOSITION	NAT_SALMPIND	% Native salmonid individuals
COMPOSITION	NAT_SALMPTAX	% Native salmonid taxa
HABITAT	COLDNTAX	No. Coldwater species
HABITAT	COLDPIND	% Coldwater individuals
HABITAT	COLDPTAX	% Coldwater taxa
HABITAT	NAT_COLDNTAX	No. Native coldwater species
HABITAT	NAT_COLDPIND	% Native coldwater individuals
HABITAT	NAT_COLDPTAX	% Native coldwater taxa
HABITAT	LOTNTAX	No. Lotic species

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
HABITAT	LOTPIND	% Lotic individuals
HABITAT	LOTPTAX	% Lotic taxa
HABITAT	NAT_LOTNTAX	No. Native lotic species
HABITAT	NAT_LOTPIND	% Native lotic individuals
HABITAT	NAT_LOTPTAX	% Native lotic taxa
HABITAT	NAT_NTOLBENTNTAX	No. of Native not tolerant benthic species (BPJ based tolerance assignments)
HABITAT	NAT_NTOLBENTPIND	% Native not tolerant benthic individuals (BPJ based tolerance assignments)
HABITAT	NAT_NTOLBENTPTAX	% Native not tolerant benthictaxa (BPJ based tolerance assignments)
HABITAT	RHEONTAX	No. Rheophilic species
HABITAT	RHEOPIND	% Rheophilic individuals
HABITAT	RHEOPTAX	% Rheophilic taxa
HABITAT	NAT_RHEONTAX	No. Native rheophilic species
HABITAT	NAT_RHEOPIND	% Native rheophilic individuals
HABITAT	NAT_RHEOPTAX	% Native rheophilic taxa
HABITAT	NTOLBENTNTAX	No. Not tolerant benthic species
HABITAT	NTOLBENTPIND	% Not tolerant benthic species
HABITAT	NTOLBENTPTAX	% not tolerant benthic taxa
HABITAT (TOLERANCE)	INTLLOTNTAX	No. Intolerant lotic species
HABITAT (TOLERANCE)	INTLLOTPIND	% Intolerant lotic individuals
HABITAT (TOLERANCE)	INTLLOTPTAX	% Intolerant lotic taxa
HABITAT (TOLERANCE)	NAT_INTLLOTNTAX	No. Native intolerant lotic species
HABITAT (TOLERANCE)	NAT_INTLLOTPIND	% Native intolerant lotic individuals
HABITAT (TOLERANCE)	NAT_INTLLOTPTAX	% Native intolerant lotic taxa
HABITAT (TOLERANCE)	INTLRHEONTAX	No. Intolerant rheophilic species
HABITAT (TOLERANCE)	INTLRHEOPIND	% Intolerant rheophilic individuals
HABITAT (TOLERANCE)	INTLRHEOPTAX	% Intolerant rheophilic taxa
HABITAT (TOLERANCE)	NAT_INTLRHEONTAX	No. Native intolerant rheophilic species
HABITAT (TOLERANCE)	NAT_INTLRHEOPIND	% Native intolerant rheophilic individuals
HABITAT (TOLERANCE)	NAT_INTLRHEOPTAX	% Native intolerant rheophilic taxa
LIFE HISTORY (MIGRATION STRATEGY)	MIGRNTAX	No. Migratory species

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
LIFE HISTORY (MIGRATION STRATEGY)	MIGRPIND	% Migratory individuals
LIFE HISTORY (MIGRATION STRATEGY)	MIGRPTAX	% Migratory taxa
LIFE HISTORY (MIGRATION STRATEGY)	NAT_MIGRNTAX	No. Native migratory species
LIFE HISTORY (MIGRATION STRATEGY)	NAT_MIGRPIND	% Native migratory individuals
LIFE HISTORY (MIGRATION STRATEGY)	NAT_MIGRPTAX	% Native migratory taxa
LIFE HISTORY (TOLERANCE)	INTLMIGRNTAX	No. Intolerant migratory species
LIFE HISTORY (TOLERANCE)	INTLMIGRPIND	% Intolerant migratory individuals
LIFE HISTORY (TOLERANCE)	INTLMIGRPTAX	% Intolerant migratory taxa
LIFE HISTORY (TOLERANCE)	NAT_INTLMIGRNTAX	No. Native intolerant migratory species
LIFE HISTORY (TOLERANCE)	NAT_INTLMIGRPIND	% Native intolerant migratory individuals
LIFE HISTORY (TOLERANCE)	NAT_INTLMIGRPTAX	% Native intolerant migratory taxa
REPRODUCTIVE	LITHNTAX	No. Lithophilic spawner species
REPRODUCTIVE	LITHPIND	% Lithophilic spawner individuals
REPRODUCTIVE	LITHPTAX	% Lithophilic spawner taxa
REPRODUCTIVE	NAT_LITHNTAX	No. Native lithophilic spawner species
REPRODUCTIVE	NAT_LITHPIND	% Native lithophilic spawner individuals
REPRODUCTIVE	NAT_LITHPTAX	% Native lithophilic spawner taxa
RICHNESS	TOTLNTAX	Total no. distinct species collected
RICHNESS	NAT_TOTLNTAX	No. Native distinct species collected
RICHNESS	NTOLNTAX	No. Not tolerant species
RICHNESS	NTOLPIND	% Not tolerant individuals
RICHNESS	NTOLPTAX	% Not tolerant taxa
RICHNESS	NAT_NTOLNTAX	No. Native not tolerant species
RICHNESS	NAT_NTOLPIND	% Native not tolerant individuals
RICHNESS	NAT_NTOLPTAX	% Native not tolerant taxa
TOLERANCE	INTLNTAX	No. Intolerant species (BPJ-based tolerance assignments)

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
TOLERANCE	INTLPIND	% Intolerant individuals (BPJ-based tolerance assignments)
TOLERANCE	INTLPTAX	% Intolerant taxa (BPJ-based tolerance assignments)
TOLERANCE	NAT_INTLNTAX	No. Native intolerant species(BPJ-based tolerance assignments)
TOLERANCE	NAT_INTLPIND	% Native intolerant individuals(BPJ-based tolerance assignments)
TOLERANCE	NAT_INTLPTAX	% Native intolerant taxa (BPJ-based tolerance assignments)
TOLERANCE	NAT_TOLRNTAX	No. Native tolerant species(BPJ-based tolerance assignments)
TOLERANCE	NAT_TOLRPIND	% Native tolerant individuals(BPJ-based tolerance assignments)
TOLERANCE	NAT_TOLRPTAX	% Native tolerant taxa (BPJ-based tolerance assignments)
TOLERANCE	TOLRNTAX	No. Tolerant species (BPJ-based tolerance assignments)
TOLERANCE	TOLRPIND	% Tolerant individuals (BPJ-based tolerance assignments)
TOLERANCE	TOLRPTAX	% Tolerant taxa (BPJ-based tolerance assignments)
TROPHIC	CARNNTAX	No. Carnivore species
TROPHIC	CARNPIND	% Carnivore individuals
TROPHIC	CARNPTAX	% Carnivore taxa
TROPHIC	NAT_CARNNTAX	No. Native carnivore species
TROPHIC	NAT_CARNPIND	% Native carnivore individuals
TROPHIC	NAT_CARNPTAX	% Native carnivore taxa
TROPHIC	NTOLCARNNTAX	No. Not tolerant carnivore species
TROPHIC	NTOLCARNPIND	% Not tolerant carnivore individuals
TROPHIC	NTOLCARNPTAX	% Not tolerant carnivore taxa
TROPHIC	NAT_NTOLCARNNTAX	No. Native not tolerant carnivore species
TROPHIC	NAT_NTOLCARNPIND	% Native not tolerant carnivore individuals
TROPHIC	NAT_NTOLCARNPTAX	% Native not tolerant carnivore taxa
TROPHIC	HERBNTAX	No. Herbivore species
TROPHIC	HERBPIND	% Herbivore individuals
TROPHIC	HERBPTAX	% Herbivore taxa

METRIC CLASS	METRIC VARIABLE NAME	DESCRIPTION
TROPHIC	NAT_HERBNTAX	No. Native herbivore species
TROPHIC	NAT_HERBPIND	% Native herbivore individuals
TROPHIC	NAT_HERBPTAX	% Native herbivore taxa
TROPHIC	INVNTAX	No. Invertivore species
TROPHIC	INVPIND	% Invertivore individuals
TROPHIC	INVPTAX	% Invertivore taxa
TROPHIC	NAT_INVNTAX	No. Native invertivore species
TROPHIC	NAT_INVPIND	% Native invertivore individuals
TROPHIC	NAT_INVPTAX	% Native invertivore taxa
TROPHIC	NTOLINVNTAX	No. Not tolerant invertivore species
TROPHIC	NTOLINVPIND	% Not tolerant invertivore individuals
TROPHIC	NTOLINVPTAX	% Not tolerant invertivore taxa
TROPHIC	NAT_NTOLINVNTAX	No. Native not tolerant invertivore species
TROPHIC	NAT_NTOLINVPIND	% Native not tolerant invertivore individuals
TROPHIC	NAT_NTOLINVPTAX	% Native not tolerant invertivore taxa
TROPHIC	OMNINTAX	No. Omnivore species
TROPHIC	OMNIPIND	% Omnivore individuals
TROPHIC	OMNIPTAX	% Omnivore taxa
TROPHIC	NAT_OMNINTAX	No. Native omnivore species
TROPHIC	NAT_OMNIPIND	% Native omnivore individuals
TROPHIC	NAT_OMNIPTAX	% Native omnivore taxa
TROPHIC (HABITAT)	BENTINVNTAX	No. Benthic invertivore species
TROPHIC (HABITAT)	BENTINVPIND	% Benthic invertivore individuals
TROPHIC (HABITAT)	BENTINVPTAX	% benthic invertivore taxa
TROPHIC (HABITAT)	NAT_BENTINVNTAX	No. Native benthic invertivore species
TROPHIC (HABITAT)	NAT_BENTINVPIND	% Native benthic invertivore individuals
TROPHIC (HABITAT)	NAT_BENTINVPTAX	% Native benthic invertivore taxa
TROPHIC (HABITAT)	INTLINVNTAX	No. Intolerant invertivore species
TROPHIC (HABITAT)	INTLINVPIND	% Intolerant invertivore species
TROPHIC (HABITAT)	INTLINVPTAX	% Intolerant invertivore taxa
TROPHIC (HABITAT)	NAT_INTLINVNTAX	No. Native intolerant invertivore species
TROPHIC (HABITAT)	NAT_INTLINVPIND	% Native intolerant invertivore species
TROPHIC (HABITAT)	NAT_INTLINVPTAX	% Native intolerant invertivore taxa

7 WATER CHEMISTRY ANALYSES

Water samples were collected as a grab sample either at the midpoint of the reach in wadeable systems or at the upper most point of the sample reach in boatable systems (see NRSA 2018-19 Field Operations Manual and Laboratory Operations Manual for additional details). The main report presents assessments for four chemical stressors: total nitrogen (TN), total phosphorus (TP), acidity, and salinity. These benchmark values and class definitions were identical to those used in the NRSA 2008-09. Water chemistry data, including additional parameters not assessed in the report, are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

7.1 ACIDITY AND SALINITY BENCHMARKS

For acidity, criteria values were determined based on values derived during the National Acid Precipitation Assessment Program (Baker et al. ,1990; Kaufmann et al., 1991). Sites with acid neutralizing capacity (ANC) less than zero were considered acidic. Acidic sites with dissolved organic carbon (DOC) greater than 10 mg/L were classified as organically acidic (natural). Acidic sites with DOC less than 10 and sulfate less than 300 µeq/L were classified as acidic deposition impacted, while those with sulfate above 300 µeq/L were considered acid mine drainage impacted. Sites with ANC between 0 and 25 µeq/L and DOC less than 10 mg/L were considered acidic-deposition-influenced but not currently acidic. These low ANC sites typically become acidic during high flow events (episodic acidity).

Salinity data values were divided into good, fair, or poor classes. Salinity classes were defined by specific conductance using ecoregional specific values (**Table 7-1**).

7.2 TOTAL PHOSPHORUS AND TOTAL NITROGEN BENCHMARKS

The process for setting the good/fair/poor benchmark for nutrients (TP, TN) in NRSA was derived from EPA's approach for the development of regional nutrient criteria (USEPA 1998). Implicit in this approach is the recognition that excess nutrients are a major cause of water quality impairment in the United States. The approach also acknowledges that because of diverse geology, climate, and geomorphology, a single national nutrient criteria level for all types of water bodies is inappropriate.

7.2.1 SELECTING AN APPROACH

Various approaches have been used to develop nutrient benchmarks such as reference sites, best professional judgment (BPJ), paleolimnological analysis, use of historical data, and dose-response modeling. In evaluating possible approaches for NRSA, analysts determined that three of these approaches (BPJ, historical data from undisturbed sites, and paleolimnological approaches) did not work for NRSA. Best professional judgment was considered too subjective, there is little if any of the needed historical data available from rivers and streams prior to human disturbance, and paleolimnological approaches were not deemed applicable for NRSA due to the erosional/depositional nature of rivers and streams.

NRSA analysts also investigated other approaches to determine their efficacy for developing nutrient benchmarks. For example, dose-response models, both *watershed disturbance-nutrient* (see Herlihy and Sifneos, 2008; Herlihy et al., 2010) and *nutrient-chlorophyl*, were examined and deemed insufficient due to the nation-wide extent of the NRSA sampling effort for rivers and streams; and the fact that many other factors besides nutrients (e.g., light, substrate) control biological responses in rivers and streams. Additionally, dose-response modeling does not eliminate the need to define "good" or undisturbed condition for the response variable to implement the model. Finally, breakpoint analysis was evaluated. The analysis did not show clear breakpoints at the ecoregional scales assessed and reported on by NRSA. As a result, this approach was not considered appropriate for NRSA. Analysts determined that the reference approach lends itself to the scale of NRSA data and provides a means of establishing least-disturbed conditions which can be used for setting benchmarks.

As a result of the evaluation of multiple approaches and the pros/cons identified, analysts selected the reference site approach as the process to set good/fair/poor condition for the NRSA nutrient analyses. Following the same basic approach as that used for biological data, regionally relevant nutrient benchmarks were calculated from conditions observed at the population of least-disturbed NRSA reference, except for nutrients not being used in the reference site screening criteria.

7.2.2 APPLYING THE REFERENCE-BASED APPROACH TO NRSA

Total nitrogen and phosphorus concentrations were classified as "good", "fair," or "poor" using a method similar to that described in Chapter 4. The benchmarks have been updated since the Herlihy and Sifenos 2008 paper as a result of adding additional sites to the reference (least-disturbed) set of sites, categorizing them based on the 9 aggregated ecoregions used in NRSA rather than nutrient ecoregions, and the expansion of the analysis to include non-wadeable systems in addition to wadeable ones.

For nutrients, the value at (and below) the 75th percentile of the reference distribution was used for each ecoregion to define the least-disturbed condition class (good–fair boundary). The 95th percentile (and above) of the reference distribution in each ecoregion defines the most disturbed condition class (fair-poor boundary) **Table 7-1**.

A set of "nutrient reference sites" was defined for this analysis using both WSA and NRSA data. All available WSA and NRSA sample sites were screened for water chemical and physical habitat disturbances using the process described in **Chapter 4** with the exception that total phosphorus and total nitrogen values were not used as screens to avoid circularity in defining nutrient benchmarks. Sites with screening values exceeding criteria for the remaining parameters in **Table 4-2** were excluded as nutrient reference sites.

To adjust the process after the removal of the nutrient screens, we incorporated screens for land cover disturbance. A single national criterion was used to exclude sites that had watershed %Urban LULC (Land Use Land Cover) >10%, watershed road density > 3 km/km2, and watershed population density >100 people/km2. For watershed %Agriculture LULC screening, ecoregional specific criteria were used as screens; NAP, WMT, XER (>10%), CPL, NPL, SAP, SPL, UMW (>25%), TPL (>50%). Before calculating ecoregional nutrient reference site percentiles, outliers (values outside 1.5 times the interquartile range above and below the quartiles) were removed.

Ecoregion	Salinity as Conductivity (µS/cm) Good-Fair	Salinity as Conductivity (µS/cm) Fair-Poor	Total N (µg/L) Good-Fair	Total N (µg/L) Fair-Poor	Total P (µg/L) Good-Fair	Total P (µg/L) Fair-Poor
CPL	500	1000	624	1081	55.9	103
NAP	500	1000	345	482	17.1	32.6
SAP	500	1000	240	456	14.8	24.4
UMW	500	1000	583	1024	36.3	49.9
TPL	1000	2000	700	1274	88.6	143
NPL	1000	2000	575	937	64.0	107
SPL	1000	2000	581	1069	55.8	127
WMT	500	1000	139	249	17.7	41.0
XER	500	1000	285	529	52.0	95.9

Table 7-1. Nutrient and Salinity Category Benchmarks for NRSA Assessment.

7.3 SIGNAL TO NOISE

To examine within-year variability of water chemistry data, analysts used the revisit sites from the Wadeable Streams Assessment, NRSA 2008-09, and NRSA 2013-14 to calculate S:N estimates for the national dataset. The results were a S:N ratio of 12.3 for total nitrogen, 10.2 for total phosphorus, 31.2 for conductivity, and 39.2 for ANC.

7.4 LITERATURE CITED

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8 PHYSICAL HABITAT ASSESSMENT

An assessment of river and stream (fluvial) physical habitat (PHab) condition is a major component of the National Rivers and Streams Assessment (NRSA). Of many possible general and specific fluvial habitat indicators measured in the NRSA surveys, the assessment team chose streambed stability and excess fine sediments, instream habitat cover complexity, riparian vegetation, and riparian human disturbances for the 2013-14 assessment. These four indicators have been used in earlier U.S. Environmental Protection Agency (USEPA) national assessments, are important nationwide, can be reliably and economically measured, and their reference conditions and degree of anthropogenic alteration can be interpreted with reasonable confidence (Paulsen et al., 2008).

In the broadest sense, fluvial habitat includes all physical, chemical, and biological attributes that influence or sustain organisms within streams or rivers. We use the term *physical habitat* to refer to the structural attributes of habitat. NRSA made field measurements aimed at quantifying eight general attributes of physical habitat condition, including direct measures of human disturbance.

- Habitat Volume/Stream Size
- Habitat Complexity and Cover for Aquatic Biota
- Streambed Particle Size
- Bed Stability and Hydraulic Conditions
- Channel-Riparian and Floodplain Interaction
- Hydrologic Regime
- Riparian Vegetation Cover and Structure
- Riparian Disturbance

These attributes were previously identified during EPA's 1992 national stream monitoring workshop (Kaufmann 1993) as those essential for evaluating physical habitat in regional monitoring and assessments. They are typically incorporated in some fashion in regional habitat survey protocols (Platts et al., 1983, Fitzpatrick et al., 1998, Lazorchak et al., 1998, Peck et al. 2006, USEPA 2004) and were applied in the NRSA 2008-09 assessment (USEPA 2016), the National Wadeable Streams Assessment (WSA: USEPA 2006) and the Western Rivers and Streams Pilot (EMAP-W) surveys conducted between 2000 and 2005 (Stoddard et al., 2005a, b).

The major habitat metrics used in those past assessments and considered in NRSA are listed and defined in **Table 8-1**. Some measures of these attributes are useful measures of habitat condition in their own right (e.g., channel incision as a measure of channel-riparian interaction); others are important controls on ecological processes and biota (e.g., bed substrate size), still others are important in the computation of more complex habitat condition metrics (e.g., bankfull depth is used to calculate Relative Bed Stability [*RBS*]). Like biological characteristics, most habitat attributes vary according to their geomorphic and ecological setting. Even direct measures of riparian human activities and disturbances are strongly influenced by their geomorphic setting. And even within a region, differences in precipitation and stream drainage area channel gradient (slope) lead to variation in many aspects of stream habitat. Those geoclimatic factors influence discharge, flood stage, stream power (the product of discharge times gradient), bed shear stress (proportional to the

product of depth and slope), and riparian vegetation. However, all eight of the major habitat attributes can be directly or indirectly altered by anthropogenic activities.

NRSA follows the precedent of EMAP-W and WSA in reporting the condition of fluvial physical habitat condition on the basis of four habitat indicators that are important nationwide, can be reliably and economically measured, and their reference condition under minimal anthropogenic disturbance can be interpreted with reasonable confidence. These are: relative bed stability (*RBS*) as an indicator of bed sedimentation or hydrologic alteration, the areal cover and variety of fish concealment features as a measure of in-stream habitat complexity, riparian vegetation cover and structure as an indicator of riparian vegetation condition, and a proximity-weighted tally of streamside human activities as an indicator of riparian human disturbances (Paulsen et al., 2008).

In this document, we describe the approach taken by NRSA in 2013-14 (which applied to the NRSA 18-19 data) for assessing physical habitat condition in rivers and streams based on the four above-mentioned indicators. We revisited the screening of reference sites, consistently defining a set of reference sites from the combined 2013-14 and 2008-09 NRSA surveys, thereby increasing the number of sample sites available for modeling expected condition, and for evaluating precision and responsiveness. We recalculated PHab condition assignments in all previous surveys using the current NRSA 2013-14 assessment procedures described here for our estimates of change or trends in PHab. We also examined the rationale, importance, and measurement precision of each of the four indicators, including the analytical approach for estimating reference conditions for each. Reference conditions for each indicator were interpreted as their expected value in sites having the least amount of anthropogenic disturbance within appropriately stratified regions. In most cases, we also refine the expected values as a function of geoclimatic controlling factors within regions. Finally, we examine patterns of association between physical habitat indicators and anthropogenic disturbance by contrasting habitat indicator values in least-, moderately-, and most-disturbed sites nationally and within regions.

Physical habitat metrics and condition assessment data from NRSA are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys

8.1 METHODS

8.1.1 PHYSICAL HABITAT SAMPLING AND DATA PROCESSING

Sample sites visited in NRSA are shown in **Figure 8-1.** In the wadeable streams sampled in NRSA, field crews took measurements while wading the length of each sample reach (Peck et al., 2006); in non-wadeable rivers, these measurements were made from boats (Hughes and Peck 2008). Physical habitat data were collected from longitudinal profiles and from 11 cross-sectional transects and streamside riparian plots evenly spaced along each sampled stream reach (USEPA 2007, 2013a, b). The length of each sampling reach was defined proportional to the wetted channel width, and measurements were placed systematically along that length to represent the entire reach. Sample reach lengths were 40 times the wetted channel-width (ChW) long in wadeable streams and rivers, with a minimum reach length of 150 m for channels less than 3.5 m wide. In non-wadeable (boatable) rivers, reach lengths were also set to 40 ChW with a maximum length of 2,000 m.

Thalweg (maximum) depth measurements (in the deepest part of channel), habitat classification, and mid-channel substrate observations were made at tightly spaced intervals; whereas channel cross-sections and shoreline-riparian stations for measuring or observing substrate, fish cover (concealment features), large woody debris, bank characteristics and riparian vegetation structure were spaced further apart. Thalweg depth was measured at points evenly spaced every 0.4 ChW along these reaches to give profiles consisting of 100 measurements (150 in streams <2.5 m wide). The tightly spaced depth measures allow calculation of indices of channel structural complexity, objective classification of channel units such as pools, and quantification of residual pool depth, pool volume, and total stream volume. Channel slope and sinuosity on non-wadeable rivers were estimated from 1:24,000-scale digital topographic maps.

In wadeable streams and rivers, wetted width was measured, and substrate size and embeddedness were evaluated using a modified Wolman pebble count of 105 particles spaced systematically along 21 equally spaced cross-sections (Faustini and Kaufmann 2007), in which individual particles were classified visually into seven size-classes plus bedrock, hardpan and other (e.g., organic material).

The numbers of pieces of large woody debris in the bankfull channel were tallied in 12 size classes (3 length by 4 width classes) along the entire length of sample reaches. Channel incision and the dimensions of the wetted and bankfull stream channel were measured at 11 equally-spaced transects. Bank characteristics and areal cover of fish concealment features were visually assessed in 10 m long instream plots centered on transects, while riparian vegetation structure, presence of large (legacy) riparian trees, non-native (alien) riparian plants, and evidence of human disturbances (presence/absence and proximity) in 11 categories were visually assessed on adjacent 10 m x 10 m riparian plots on both banks. In addition, channel gradient (slope) in wadeable streams was measured to provide information necessary for calculating residual pool depth and RBS. In wadeable streams, crews used laser or hydrostatic levels, but if necessary, were allowed to use hand-held clinometers in channels with slopes >2.5%. Compass bearings between stations were obtained for calculating channel sinuosity. Channel constraint and evidence of debris torrents and major floods were assessed over the whole reach after the other components were completed. Discharge was measured by the velocity-area method at the time of sampling, or by other approximations if that method was not practicable (Peck et al., 2006; USEPA 2007, 2013a, b).

In boatable rivers, NRSA field crews measured the longitudinal thalweg depth profile (approximated at mid-channel) using 7.5 m telescoping survey rods or SONAR. At the same time, crews tallied snags and off-channel habitats, classified main channel habitat types, and characterized mid-channel substrate by probing the bottom. At 11 littoral/riparian plots (each 10 m wide x 20 m long) spaced systematically and alternating sides along the river sample reach, field crews measured channel wetted width, bankfull channel dimensions, incision, channel constraint. They assessed near-shore, shoreline, and riparian physical habitat characteristics by measuring or observing littoral depths, riparian canopy cover, substrate, large woody debris, fish cover, bank characteristics, riparian vegetation structure, presence of large ("legacy") riparian trees, non-native riparian plants, and evidence of human activities. As was the case for wadeable streams, NRSA 2013-14 did not assess presence of large (legacy) trees and non-native (alien) riparian plants in boatable rivers, as had been done in previous surveys. After all the thalweg and littoral/riparian measurements and observations were completed, the crews estimated the extent and type of channel constraint (see USEPA 2007, 2013a, b). Channel slope and sinuosity on all boatable rivers were estimated from 1:24,000-scale digital topographic maps.

See Kaufmann et al. (1999) for calculations of reach-scale summary metrics from field data, including mean channel dimensions, residual pool depth, bed particle size distribution, wood volume, riparian vegetation cover and complexity, and proximity-weighted indices of riparian human disturbances. See Faustini and Kaufmann (2007) for details on the calculation of geometric mean streambed particle diameter, Kaufmann et al. (2008, 2009) for calculation of bed shear stress and relative bed stability (modified since published by Kaufmann et al., 1999), and Kaufmann and Faustini (2012) for demonstrating the utility of EMAP and NRSA channel morphologic data to estimate transient storage and hydraulic retention in wadeable streams.

8.1.2 QUANTIFYING THE PRECISION OF PHYSICAL HABITAT INDICATORS

The absolute and relative precision of the physical habitat condition metrics used in NRSA are shown in **Table 8-2**, based on data from 4,193 sites (2,113 from NRSA 2008-09 and 2,080 from NRSA 2013-14) and repeat visits to a random subset of 388 of those sites (197 and 191 revisits in the two surveys). The RMSrep expresses the precision or replicability of field measurements, quantifying the average variation in a measured value between same-season site revisits, pooled across all sites where measurements were repeated. We calculated RMSrep as the root-mean-square error of repeat visits during the same year, equivalent to the pooled standard deviation of repeat visits relative to their site means, as discussed Kaufmann et al. (1999) and Stoddard et al. (2005a). S:N is the ratio of variance among streams ("signal") to that for repeat visits to the same stream ("noise") as described by Kaufmann et al. (1999).

The ability of a monitoring program to detect trends is sensitive to the spatial and temporal variation in the target indicators as well as the design choices for the network of sites and the timing and frequency of sampling. Sufficient temporal sampling of sites was not available to estimate all relevant components of variance for the entire U.S. However, Larsen et al. (2004) examined the survey sampling variance components for a number of the EMAP-NARS physical habitat variables, including some of interest in this chapter (residual depth, canopy cover, fine sediment, and in-channel large wood). Their analysis was based on evaluation on six Pacific Northwest surveys that included 392 stream reaches and 200 repeat visits. These surveys were conducted in Oregon and Washington from 1993 to 1999. Most were from one to three years in duration, but one survey lasted six years. They modeled the likelihood of detecting a 1–2% per year trend in the selected physical habitat characteristics, if such a trend occurs, as a function of the duration of a survey. To calculate the number of years required to detect the defined trends in a monitoring network with a set number of sites, they set the detection probability at >80% with <5% probability of incorrectly asserting a trend if one is not present. We used the same survey data sets to duplicate their analysis for several variables not included in the Larsen et al. (2004) publication, including log transformed relative bed stability (*LRBS_BW5*) and riparian vegetation cover complexity (*XCMGW*, the combined cover of three layers of riparian woody vegetation); the results of that trend detection potential is summarized in **Table 8-3**.

8.2 PHYSICAL HABITAT CONDITION INDICATORS

8.2.1 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., 1983, 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 1982, Dietrich et al., 1989).

"Unscaled" measures of surficial streambed particle size, such as percent fines or *D50*, can be useful descriptors of streambed conditions. In a given stream, increases in percent fines or decreases in *D50* may result from anthropogenic increases in bank and hillslope erosion. However, a great deal of the variation in bed particle size among streams is natural: the result of differences in stream or river size, slope, and basin lithology. The power of streams to transport progressively larger sediment particles increases in direct proportion to the product of flow depth and slope. All else being equal, steep streams tend to have coarser beds than similar size streams on gentle slopes. Similarly, the larger of two streams flowing at the same slope will tend to have coarser bed material, because its deeper flow has more power to scour and transport fine particles downstream (Leopold et al., 1964, Morisawa 1968). For these reasons, we "scale" bed particle size metrics, expressing bed particle size in each stream as a deviation from that expected as a result of its size, power, and landscape setting (Kaufmann et al., 1999, 2008, 2009).

The scaled median streambed particle size is expressed as *RBS*, calculated as the ratio of the geometric mean diameter, *Dgm*, divided by *Dcbf*, the critical diameter (maximum mobile diameter) at bankfull flow (Gordon et al., 1992), where *Dgm* is based on systematic streambed particle sampling ("pebble counts") and *Dcbf* is based on the estimated streambed shear stress calculated from slope, channel dimensions, and hydraulic roughness during bankfull flow conditions.

RBS is a measure of habitat stability for aquatic organisms as well as an indication of the potential for economic risk to streamside property and structures from stream channel movement. In many regions of the U.S., we may also be able to use *RBS* to infer whether sediment supply is augmented by upslope or bank erosion from anthropogenic or other disturbances, because it can indicate the degree of departure from a balance between sediment supply and transport. In interpreting *RBS* on a regional scale, Kaufmann et al. (1999, 2009) argued that, over time, streams and rivers adjust sediment transport to match supply from natural weathering and delivery mechanisms driven by the natural disturbance regime, so that *RBS* in appropriately stratified regional reference sites should tend towards a range characteristic of the climate, lithology, and natural disturbance regime. Values of the *RBS* index either substantially lower (finer, more unstable streambeds) or higher (coarser, more stable streambeds) than those expected based on the range found in least-disturbed reference sites within an ecoregion are considered to be indicators of ecological stress.

Excess fine sediments can destabilize streambeds when the supply of sediments from the landscape exceeds the ability of the stream to move them downstream. This imbalance can result from numerous human uses of the landscape, including agriculture, road building, construction, and grazing. Lower-than-expected streambed stability may result either from high inputs of fine sediments (from erosion) or increases in flood magnitude or frequency (hydrologic alteration). When low *RBS* results from fine sediment inputs, stressful ecological conditions result from fine sediments filling in the habitat spaces between stream cobbles and boulders (Bryce et al., 2008, 2010). Instability (low *RBS*) resulting from hydrologic alteration can be a precursor to channel incision and arroyo formation (Kaufmann et al., 2009). Perhaps less well recognized, streams that have higher than expected streambed stability can also be considered stressed—very high bed stability is typified by hard, armored streambeds, such as those often found below dams where fine sediment flows are interrupted, or within channels where banks are highly altered. Values of *RBS* higher than reference expectations can indicate anthropogenic coarsening or armoring of streambeds, but streams containing substantial amounts of bedrock may also have very high *RBS*, and at this time it is difficult to determine the role of human alteration in stream coarsening on a national scale. For this reason, NRSA reported only on the "low end" of *RBS* relative to reference conditions, generally indicating streambed excess fine sediments or augmented stormflows associated with human disturbance of stream drainages and riparian zones.

8.2.1.1 PRECISION OF SEDIMENT AND BED STABILITY MEASUREMENTS

The geometric mean bed particle diameter (*Dgm*) and *RBS*, respectively, varied over 6 and 9 orders of magnitude in the NRSA surveys. Because of this wide variation and the fact that both exhibit repeat-visit variation that is proportional to their magnitude at individual streams, it is useful and necessary to log transform these variables (*LSUB_DMM* and *LRBS_g08*). The RMSrep of *LSUB_DMM* in the two combined NRSA surveys was 0.39, but the wadeable stream "pebble count" procedure was more precise (RMSrep=0.25) than the bottom-probing procedure applied in boatable rivers (RMSrep=0.51). For NRSA's wadeable streams, this precision for *LSUB-DMM* was similar to that reported by Faustini and Kaufmann (2007) for EMAP-W (0.21). For a *Dgm* = "*y*" mm, the log-based RMSrep of 0.25 translates to an asymmetrical 1SD error bound of 0.56y to 1.78*y* mm, and for a log-based RMSrep of 0.51, a 1SD error bound of 0.31*y* to 3.24*y* mm.

The RMSrep of *LRBS_g08* in NRSA wadeable and boatable sites was 0.52, approximately 6% of its observed range, but less precise (surprisingly) than that for EMAP-W (RMSrep = 0.365). The log-based RMSrep of 0.52 for NRSA *LRBS_g08* translates to an asymmetrical error bound of 0.30*y* to 3.3*y* around an untransformed RBS value of "*y*" (**Table 8-2**). Compared with the high S:N ratio for LSUB_DMM in NRSA wadeable+boatable waters (S:N=10.9), relative precision for *LRBS_g08* was lower (S:N=4.2), reflecting the reduction in total variance that results from "modeled out" a large component of natural variability by scaling for channel gradient, water depth, and channel roughness. Nevertheless, the moderate relative precision of *LRBS_g08* is easily adequate to make it a useful variable in regional and national assessments (Kaufmann et al., 1999, 2008, Faustini and Kaufmann 2007). The transformation of the unscaled geometric mean bed particle diameter *Dgm* to the ratio *RBS* by dividing by the critical diameter reduced the within-region variation by accounting for some natural controlling factors. As a result, we feel that the scaled variable helps to reveal alteration of bed particle size and mobility from anthropogenic erosion and sedimentation (Kaufmann et al., 2008, 2009).

We have examined the components of variability of *LRBS* based on earlier surveys and modeled its potential utility in trend detection in the Pacific Northwest region of the U.S. with the same data and procedures as used by Larsen et al. (2004), in which all methods were the same as used in EMAP-W and WSA except that bed substrate mean diameter data used by Larsen et al. was determined based on 55, rather than 105 particles. (NRSA data differed from data used in that analysis by using laser levels rather than hand-held clinometers to measure wadeable stream slopes <2.5%) That analysis showed that a 50-site monitoring program could detect a subtle trend in *LRBS_BW5* of 2% per year within 8 years, if sites were visited every year (**Table 8-3**).

8.2.2 RIPARIAN VEGETATION

8.2.2.1 QUANTIFYING RIPARIAN VEGETATION COVER COMPLEXITY

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.

The presence of a complex, multi-layered vegetation corridor along streams and rivers is an indicator of how well the stream network is buffered against sources of stress in the watershed. Intact riparian areas can help reduce nutrient and sediment runoff from the surrounding landscape, prevent streambank erosion, provide shade to reduce water temperature, and provide leaf litter and large wood that serve as food and habitat for stream organisms (Gregory et al., 1991). The presence of large, mature canopy trees in the riparian corridor reflects its longevity, whereas the presence of smaller woody vegetation typically indicates that riparian vegetation is reproducing and suggests the potential for future sustainability of the riparian corridor (Kaufmann and Hughes 2006).

NRSA evaluated 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 (<0.5 m), mid-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 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, Kaufmann and Hughes 2006).

8.2.2.2 PRECISION OF RIPARIAN VEGETATION INDEX

XCMGW ranged from 0 to 2.6 (260% cover), with RMS_{rep} of Log(0.01+*XCMGW*) = 0.148 (**Table 8-2**), meaning that an *XCMGW* value of 10% at a single stream site has a $\pm$1.0 RMS_{rep} error bound of 7% to 14%. Its S:N ratio was 8.45, indicating very good potential for discerning differences among sites. We examined the components of variability of *XCMGW* and modeled its potential

utility in trend detection in the Pacific Northwest region of the U.S. with the same data and procedures used by Larsen et al. (2004). Based on that analysis, a 50-site monitoring program could detect a trend in *XCMGW* of 2% per year within 8 years if sites were visited every year (**Table 8-3**).

8.2.3 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. (see 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, we use 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 were estimated visually by NRSA field crews.

8.2.3.1 QUANTIFYING INSTREAM HABITAT COMPLEXITY

Habitat complexity is difficult to quantify; it could be quantified or approximated by a wide variety of measures. The NRSA Physical Habitat protocols provide estimates for nearly all the following components of complexity identified during EPA's 1992 stream monitoring workshop (Kaufmann 1993):

- Habitat type and distribution (e.g., Bisson et al., 1982, O'Neill and Abrahams 1984, Frissell et al., 1986, Hankin and Reeves 1988, Hawkins et al., 1993, Montgomery and Buffington 1993, 1997, 1998).
- Large wood count and size (e.g., Harmon et al., 1986, Robison and Beschta 1989, Peck et al. 2006).
- In-channel cover: percentage areal cover of fish concealment features, including undercut banks, overhanging vegetation, large wood, boulders (Hankin and Reeves 1988, Kaufmann and Whittier 1997, Peck et al., 2006).
- Residual pools, channel complexity, hydraulic roughness (e.g., Kaufmann 1987a, b, Lisle 1987, Stack and Beschta, 1989; Lisle and Hilton 1992, Robison and Kaufmann 1994, Kaufmann et al., 1999, Kaufmann et al., 2008, Kiem et al., 2002; Kaufmann et al., 2011).
- Width and depth variance, bank sinuosity (Kaufmann 1987a, Moore and Gregory 1988, Kaufmann et al., 1999, Madej 1999, 2001, Kaufmann et al., 2008, Mossop and Bradford 2006, Pearsons and Temple 2007, 2010, Kaufmann and Faustini 2012).

Residual depth is a measure of habitat volume, but also serves as one of the indicators of channel habitat complexity, particularly when expressed as a deviation from reference expectations, including the influences of basin size. A stream with more complex bottom profile will have greater residual depth than one with similar drainage area, discharge and slope, but lacking that complexity (Kaufmann 1987a). Conversely, between two streams of equal discharge and slope, the one with greater residual depth (i.e., larger, more abundant residual pools) will have greater variation in cross-sectional area, slope, and substrate size. A related measure of the complexity of channel morphology

is the coefficient of variation in thalweg depth, calculated entirely from the thalweg depth profile (*SDDEPTH / XDEPTH*). The thalweg profile is a systematic survey of depth in the stream channel along the path of maximum depth (i.e., the thalweg). In addition to measures of channel morphometric complexity, NRSA physical habitat protocols measure in-channel large wood (sometimes called "large woody debris" or simply "LWD"), and several estimates of the areal cover of various types of fish and macroinvertebrate "cover" or concealment features. The large wood metrics include counts of wood pieces per 100 m of bankfull channel and estimates of large wood volume in the sample reach expressed in cubic meters of wood per square meter of bankfull channel. The "fish cover" variables are visual estimates of the areal cover of single or combined types of habitat features.

NRSA required a general summary metric as a holistic indicator of many aspects of habitat complexity, so NRSA used the metric *XFC_NAT*, summing the areal cover from large wood, brush, overhanging vegetation, live trees and roots, boulders, rock ledges, and undercut banks in the wetted stream channel. Habitat complexity and the abundance of particular types of habitat features differ naturally with stream size, slope, lithology, flow regime, and potential natural vegetation. For example, boulder cover will not occur naturally in streams draining deep deposits of loess or alluvium that do not contain large rocks. Similarly, large wood will not be found naturally in streams located in regions where riparian or upland trees do not grow naturally. Though the index *XFC_NAT* partially overcomes these differences by summing divergent types of cover, we set stream-specific expectations for habitat complexity metrics in NRSA based on region-specific reference sites and further refined them as a function of geoclimatic controls.

8.2.3.2 PRECISION OF HABITAT COMPLEXITY MEASURES

The instream habitat complexity index *XFC_NAT* ranged from 0 to 2.3, or 0% to 230% in NRSA (2008-09 and 2013-14 combined), expressing the combined areal cover of the five cover elements contributing to its sum. The RMS_{rep} of Log(0.01+*XFC_NAT)* was 0.21, meaning that an *XFC_NAT* value of 10% cover at a single stream site has a $\pm$1.0 RMS_{rep} error bound of 6% to 16% (**Table 8-2**). S:N was relatively low for this indicator (2.27), though higher in wadeable streams (2.76) than in boatable rivers (1.66). Despite its relatively low S:N, the RMS_{rep} for *LXFC_NAT* was 9% of its observed range. It was retained as a habitat complexity indicator because it contains biologically relevant information not available in other metrics, shows moderate responsiveness to human disturbances, and has precision adequate to discern relatively large differences in habitat complexity.

8.2.4 RIPARIAN HUMAN DISTURBANCES

Agriculture, roads, buildings, and other evidence of human activities in or near stream and river channels 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 follows 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., 1999). Observations within the stream or on its banks are weighted by 1.5, those within the 10 x 10 m 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.

8.2.4.1 PRECISION OF RIPARIAN DISTURBANCE INDICATORS

The proximity-weighted human disturbance indicator *W1_HALL* ranged from 0 to 8.3 in NRSA, and its precision was proportional to the level of disturbance. The RMSrep of *log*(0.1+*W1_HALL*) was 0.178 (Table 8-2), meaning that a *W1_HALL* value of 1.0 at a single stream site has a +1.0 RMSrep error bound of 0.66 to 1.51. The relative precision of *Log*(0.1+*W1_HALL*) was moderate (S:N=5.46), indicating good potential for discerning differences among sites.

8.3 ESTIMATING REFERENCE CONDITION FOR PHYSICAL HABITAT

8.3.1 REFERENCE SITE SCREENING AND ANTHROPOGENIC DISTURBANCE CLASSIFICATIONS

As part of the routine application of its field and GIS protocols, NRSA (2008-09 and 2013-14 combined) obtained various measures of human disturbance associated with each site and its catchment. Following a similar approach as described in Chapter 4 and Herlihy et al. (2008), indicators of local scale human disturbance and water chemistry (Chloride, Total Phosphorus, Total Nitrogen, Sulfate, and Turbidity) were used to screen probability and hand-picked sites and designate them as least- moderately-, and most-disturbed, relative to other sites within each of the nine aggregate ecoregions used in NRSA. To avoid circularity, we did not use any field measures of sediment, in-channel habitat complexity, or riparian vegetation to screen least-disturbed sites used to estimate reference condition for excess streambed fining, instream fish cover, and riparian vegetation. Nor did we use such measures in defining levels of disturbance to use in examining the associations of these habitat metrics with human disturbances. We did, however, use field observations of the level and proximity of streamside human activities (*W1-HALL, W1_HAG, W1H_CROP,* and *W1H_WALL*) in screening reference sites and defining levels of disturbance for evaluating indicator responsiveness (**Table 8-4**). In this chapter, the designation "**R**" refers to least-disturbed ("reference") sites; "**S**" to moderately-disturbed sites, and "**T**" to the most-disturbed sites within each of the nine aggregate ecoregions discussed herein. We defined these site disturbance categories independent of the habitat indicators we evaluate in this report (other than riparian human disturbances), allowing an assessment of fluvial habitat response to a gradient of human activities and disturbances. We also used sub-basin row crop and urban land use percentages, and the density of dams and impoundments to reject potential reference sites.

Screening the NRSA 2008-09 and 2013-14 survey sites by the disturbance variables in **Table 8-4** yielded 708 reference sites, 349 from the first survey and 359 from the second (**Table 8-5**). Fewer reference sites were identified for boatable (281) than for wadeable (427) streams and rivers; except in several regions, reference sites were approximately evenly distributed between the two surveys. Notably, only 2 boatable reference sites were identified in the SPL (both in the 2008-09 survey), and only 7 boatable and 7 wadeable reference sites were identified in the SAP. Interestingly, more reference sites were identified in the 2013-14 survey of the CENPL and Western regions than in the 2008-09 survey of those regions. The opposite was true of the Appalachians.

8.3.2 MODELING EXPECTED REFERENCE VALUES OF THE INDICATORS

8.3.2.1 MODELING APPROACHES

In the following paragraphs, we describe the conceptual basis for modeling the expected range of values for the each of the physical habitat indicators under least-disturbed (reference) condition. The details of these models are presented in **Table 8-6**–**Table 8-8**, and with more detail in Appendix 8.A. For riparian human activities, we applied uniform criteria based on professional judgement and literature to assign high, medium and low disturbance to individual sample sites across the entire U.S. For the other three PHab indicators, we assigned habitat condition based on the distribution of PHab metric values within the combined set of NRSA reference sites, employing several types of modeling:

NULL MODELS based expected least-disturbance values and their distribution on the mean and SD of the indicator metric (e.g., *LRBS_g08*, *XCMGW*, or *XFC_Nat*) in the set of reference sites representing least-disturbed condition within resource types (e.g., wadeable and boatable) in their respective regions (*ECOwsa9*) or aggregations of those regions (e.g., Central Plains = CENPL = NPL+SPL+TPL). For example, in NAP boatable sites, *LRBS_g08* null model condition classes were defined based on normal approximations of the 5th and 25th percentiles of the actual reference distributions. The definition of "Poor" condition was set for those sites with *LRBS_g08* < the reference mean *LRBS_g08* minus 1.65(SD_{ref}). Sites in "Good" condition with respect to this indicator were those with *LRBS_g08* > the reference mean *LRBS_g08* minus 0.67(SD_{ref}). As for RBS_g08, we log-transformed *XCMGW* and *XFC_Nat* to approximate statistical normality in distributions (e.g., *LRBS_g08* = Log_{10}[*RBS_g08*], *LPt01_XCMGW* = Log_{10}[0.01+*XCMGW*], and *LPt01_XFC_Nat* = Log_{10}[0.01+*XFC_Nat*]).

REFERENCE-SITE OBSERVED/EXPECTED (O/E) MODELS: In cases where reference sites were sufficiently numerous and spanned a representative range of the natural controlling variables, we applied Multiple Linear Regression (MLR) to regional reference sites (only) in order to factor out the influence of natural controlling factors on habitat separate from the influences of anthropogenic disturbances. These MLR models estimate site-specific expected values of habitat metrics under least-disturbed conditions, given their geoclimatic and geomorphic setting (e.g., ecoregion, latitude, longitude, drainage area, channel width, slope, elevation, and soil erodibility). If there were less than 22 reference sites in a region, or we determined that reference sites may not fully encompass the geoclimatic variables controlling a habitat metric, we combined regions with similar controlling factors in the modelling. The variables made available to MLR were *LAT_DD83, LON_DD83, L_AreaWSkm2_use, ELEV_PT_use, LXSlope_use, LXWidth_use*, and *KFCT_WS_use*. We then calculated observed/expected (O/E) values of the habitat metrics for every site within the

modelled region, including non-reference sites. We set expectations of the O/E values based on the mean and SD of the O/E values in the regional reference distribution, and set Good, Fair, and Poor condition determinations based on normal approximation of log-transformed O/E values as described for the *LRBS* null model in the previous paragraph.

8.3.3 REFERENCE-SITE O/E MODELS WITH DISTURBANCE ADJUSTMENT

In cases where reference sites were sufficiently numerous and spanned a representative range of the natural controlling variables, but had substantial anthropogenic disturbances that influenced the habitat metric response variable, we included riparian and basin disturbance variable(s) as predictors in the Reference Site MLRs. As with the Reference Site models with no adjustment, we combined regions with similar controlling factors in the modelling, where the number and representativeness of reference sites were inadequate in a given region. Besides the geoclimatic and geomorphic variables listed in the previous paragraph, we considered the following disturbance variables in these MLRs: *W1_Hall, W1_HNOAG, W1_HAG, W1H_Crop, DAM_dii, AG_1KMCircle, URB_1KMCircle, RDDEN_WS_use, PCT_AG_WS_use*, and *AGws_X_KFct* (interaction of basin % crop agriculture with soil erodibility factor). Site-specific expected ("E") values of the habitat metric were then calculated by setting the anthropogenic disturbance metric values to the lowest value observed ("O") among reference sites in the modelled region. Because we had already modeled-out disturbance to some extent in our calculation of E values, the distributions of O/E in reference sites did not necessarily have a mean of 1/1 (Log=0), although means were very close to 1/1. We then calculated O/E values of the habitat metrics for every site within the modelled region, including non-reference sites. We set expectations of the O/E values based on the mean and SD ofthe distributions of Log_{10}(O/E) values in regional reference sites, analogous to that described for reference site regressions in the previous paragraph, and set Good, Fair, and Poor condition determinations based on normal approximation of log-transformed O/E values analogous to that described for the *LRBS* null model above.

"ALL-SITES" O/E MODELS: In cases where reference sites were generally disturbed and where the number and distribution of minimally-disturbed reference sites were insufficient to accurately quantify geoclimatic influences on a given habitat metric, we employed "All-Sites" O/E models. We used two steps to calculate reference expected values. The first step was to calculate expected values from MLRs that employed all sites (not just reference sites) in the model region; and considered both geoclimatic and anthropogenic predictors. Site-specific expected ("E") values of the habitat metric were then calculated using the MLR equation with the anthropogenic disturbance metric values set to their lowest value observed ("O") in the modelled region. We then calculated O/E values of the habitat metrics for every site within the modelled region. In the second step, we examined the distribution of O/E values in reference sites and their association with anthropogenic disturbance within the region. In cases where reference site O/E values showed no association with disturbance, we based reference expectations on the mean and SD of the distributions of Log_{10}(O/E) values in these regional reference sites, analogous to that described for unadjusted regression site regressions in the previous paragraph. We then set Good, Fair, and Poor condition determinations based on normal approximation of log-transformed O/E values analogous to that described for the LRBS null model in the previous paragraph. In cases where reference site O/E values were still associated with anthropogenic disturbance, our second step included regressing the Log_{10}(O/E) values against anthropogenic disturbance variables to determine expected O/E values under least-disturbed conditions. We then set the anthropogenic disturbance variables in the MLR to

their regional minimum values, effectively choosing the y-intercept of these equations as the central tendency for expected reference condition. We set expectations of the O/E values based on the y-intercept and regression RMSE of Log_{10}(O/E) values in regional reference sites, analogous to that described for unadjusted reference site regressions in the previous paragraph, and set Good, Fair, and Poor condition determinations based on normal approximation of log-transformed O/E values analogous to that described for the *LRBS* null model above.

8.3.3.1 BED SEDIMENT CONDITION MODELING

We used reference site null models to estimate expected reference values of Log_{10} Relative Bed Stability (*LRBS_g08*) in boatable rivers and streams in 5 of the 9 ecoregions (NAP, SAP, CPL, WMT, and XER). RMSE's for these null models ranged from 0.365 in the WMT to 1.539 in the NAP. Modeling for boatable sites in the 4 remaining regions were MLR models with R^2 ranging from 18% to 56%, RMSE from 0.365 to 1.539, and included one to three predictors. Predictors were primarily drainage area (*LAws*), channel width (*LXWidth*), and extent of agricultural land use in the contributing drainages, or within a 1 km radius of the sample sites on these rivers (**Table 8-6**, and more detail in **Appendix 8.A**). For boatable rivers in the NPL, SPL, and TPL, we employed All-Sites MLR models that incorporated similar predictors as those used in the reference site MLRs.

For wadeable streams in all except the Central Plains regions (NPL, SPL, and TPL), we used reference site MLRs to estimate Log10 Relative Bed Stability (*LRBS_g08*) in least-disturbed sites. These MLRs most commonly included a basin or stream size variable (*LAws* or *LXWidth*), slope (*LXSlope*), and usually a site-scale or basin measure of human land use intensity. In the NPL, SPL, and TPL, we employed All-Sites MLR models typically incorporating *Lat* and/or *Lon* with *LAws*, Elevation or Slope and one or more variables representing the intensity of human land use activityin the drainage basin, vicinity, or near the banks of the sample reaches. MLR model R^2 values ranged from 20% to 41%, and RMSE ranged from 0.430 to 0.990. The reference site models had 1to 3 predictors and the All-Sites models had 4 to 5 predictors.

8.3.3.2 RIPARIAN VEGETATION COVER & STRUCTURE CONDITION MODELING

Reference site null models were employed for estimating expected reference condition for Riparian Vegetation Cover & Structure (*LPt01_XCMGW*) only for boatable rivers in the TPL and WMT (**Table 8-7** with greater detail in **Appendix 8.A**). All-Sites MLR models were used for boatable rivers in the combined NPL and SPL and for wadeable streams in the NPL. The boatable All-Sites MLR incorporated *Lat*, *Lon*, site-level agriculture (*W1_HAG*), basin road density (*RDDEN_ws*), and % of agricultural land use in the drainage basin (*PCT_AG_ws*). The NPL wadeable stream All-Sites model was similar, incorporating *Lat, Lon*, *LXSlope*, *LXWidth*, site-level agriculture (*W1_HAG*), basin road density (*RDDEN_ws*), and *PCT_AG_ws*. Expected condition models for boatable or wadeable streams in all the remaining ecoregions were reference site regression modelswith 1 to 4 geoclimatic predictors including *Lat* or *Lon*, along with *LAws, LXWidth, LXSlope*, or *Elev*. Most of these MLRs also included one or more variables representing the intensity of human land use activity in the drainage basin, vicinity, or near the banks of the sample reaches. Model R^2 was 1% for CPL wadeable streams, and 14% to 40% elsewhere. The precision of these reference site MLRs and All-Sites models (RMSE 0.119 to 0.487) was generally greater (smaller RMSE) for these riparian vegetation models than for the *LRBS* models.

8.3.3.3 INSTREAM HABITAT COVER & COMPLEXITY CONDITION MODELING

Reference site null models were employed for estimating expected reference condition for Instream Habitat Cover Complexity (*LPt01_XFC_Nat*) only in the CPL, where we used separate null models for wadeable and boatable sites (**Table 8-8** with greater detail in **Appendix 8.A**). All the remaining expected condition models were reference site regression models incorporating 1 to 5 predictors, with R^2 ranging from 7% to 53% and RMSE's from 0.175-0.335, somewhat less precise (larger) than those for riparian vegetation condition. These expected condition MLRs typically included 1 to 3 predictors from the set of geoclimatic variables including *Lat, Lon, LAws, LXWidth, LXSlope*, or *Elev*. Except for NAP and UMW wadeable stream MLRs and the XER boatable river model, all the other instream habitat condition MLRs also included one or more variables representing the intensity of human land use activity in the drainage basin, vicinity, or near the banks of the sample reaches.

8.3.3.4 RIPARIAN HUMAN DISTURBANCE INDICATOR CONDITION DETERMINATION

For the riparian human disturbance indicator, we did not base condition benchmarks on the reference distributions or expected condition MLRs, as was done for bed sediments, riparian vegetation condition and habitat complexity. Instead, we set these classes using uniform judgement-based criteria for all regions. *W1_Hall*, the database variable name for this indicator, is a direct measure of human disturbance "pressure," unlike the other habitat indicators, which are actually measures of habitat response to human disturbance pressures. It is very difficult to define reference sites without screening sites based on *W1_Hall*. For this reason, we took this different approach for setting riparian disturbance benchmarks, defining low disturbance sites as those with *W1_Hall* <0.33 and high riparian disturbance sites as those with W1_*Hall* ≥1.5; we applied these same benchmarks in all ecoregions. A value of 1.5 for a stream means, for example, that at 22 locations along the stream the field crews found an average of one of 11 types of human disturbance within the stream or its immediate banks. A value of 0.33 means that, on average, one type of human disturbance was observed at one-third of the 22 riparian plots along a sample stream or river.

8.4 RESPONSE OF THE PHYSICAL HABITAT INDICATORS TO HUMAN DISTURBANCE

Riparian human disturbance (*W1_Hall*) values between 0 and 3 were found in all regions and in both boatable and wadeable sites (**Figure 8-2**). Among regional reference sites, UMW boatable and wadeable reference sites and WMT wadeable reference sites had the lowest riparian disturbance (**Figure 8-3**). Very high values of W1_Hall were found in all regions with the exception of wadeable streams in the UMW (note tradition of riparian buffer protection that is visible from the air), and steep gradients of *W1_Hall* were found across the three disturbance classes in all regions (**Figure 8-3**). Because the field-obtained measures of riparian disturbance used in the NRSA are themselves direct indicators of human disturbance, and were used to screen reference sites, we did not do t-tests to quantify the strength of relationship between *W1_Hall* and general disturbance class in **Table 8-9**. However, we do illustrate the relationship of *W1_Hall* to the human disturbance gradient in **Figure 8-3** to compare the relative magnitudes of *W1_Hall* among least-, moderately-, and most-disturbed streams in the various regions of the U.S.

We quantified the responsiveness of NRSA physical habitat condition metrics to levels of human disturbance by the t-values (t_{rt}) of the difference between mean of the indicator Log_{10}(O/E) values in least-disturbed reference sites (*prk3RRT_NRSA1314*=R) minus the mean for the most-disturbed sites (those screened as *prk*RRT_*NRSA1314*=T). Throughout the text, figures, and tables, we indicate the order of magnitude of p-values of these comparisons by the number of asterisks following the t value. For example, t_{rt} = +2.34* indicates that the mean Log_{10}(O/E) in reference sites exceeds that in the most-disturbed sites by 2.34 log units, and = p~ 0.1. Multiple asterisks denote the magnitude of p values (* = p~ 0.1; ** = p~ 0.01; *** = p~ 0.001; and **** = p~0.0001).

Regional differences in bed substrate texture do not necessarily indicate anthropogenic sedimentation. In other words, there are streams and rivers that are naturally fine-bedded. Examination of the distribution of the Log_{10} of geometric mean bed surface substrate diameter (*LSUB_dmm*) shows that the wadeable streams clearly separate into fine-bedded and coarse-bedded regions (**Figure 8-4**). Wadeable streams in CPL, UMW, and Central Plains (NPL, SPL, TPL) are largely low gradient streams, and median bed sediments with *LSUB_dmm* ≤0 (≤1 mm) which is sand or finer (**Figure 8-4b**). A similar, but less distinct pattern is seen in boatable rivers, but NPL and XER rivers are relatively more coarse-bedded than expected from the pattern in wadeable streams. These patterns are driven largely by the slope and lithology of these sites. Patterns in the distribution of *LRBS_g08* (=*LRBS_use*) show less difference among regions, and a number of the fine-bedded regions have similar bed stability as those found in coarser regions (**Figure 8-4a** and **Figure 8-4b**). Once scaled as an O/E variable (*LOE_LRBS_use*) to adjust for natural controls on bed material size and more clearly reflect anthropogenic influences, *LRBS* showed modest to strong negative response to human disturbance for combined boatable and wadeable sites in most regions and aggregations of regions, as illustrated by t_{rt} values ranging from +3.38*** to +12.84****, showing substantial and statistically significant differences between means of least-disturbed minus most-disturbed sites (**Table 8-9**). The strength of associations of instream sediments with human disturbance (**Table 8-9** and **Figure 8-5**) tended to be similar and relatively strong for both boatable and wadeable rivers and streams (t_{rt} = +2.24** to +11.32****). We observed moderate to strong declines *LRBS* with disturbance in all regions, the strongest associations were in the UMW boatable sites (t_{rt} = 6.59 ****), the Western Rivers (t_{rt} = 3.96***), and in EHIGH, CENPL, and WEST wadeable sites (t_{rt} = 4.12**** to 8.25****).

Riparian vegetation cover (*LPt01_XCMGW*) adjacent to both wadeable and boatable rivers and streams was markedly lower in the NPL than in any other region (**Figure 8-6**). By contrast, riparian vegetation cover for both types of waters was consistently higher in the CPL, NAP, and SAP, with the other regions having moderately high median values of riparian cover. Once scaled as an O/E variable to adjust for natural geoclimatic controls (*LOE_XCMGW_use*), riparian vegetation cover complexity showed modest to strong negative response to human disturbance for combined boatable and wadeable sites in most regions and aggregations of regions, as illustrated by t_{rt} values from +2.95*** to +14.17****), showing substantial and statistically significant differences between means of least-disturbed minus most-disturbed sites (**Table 8-9**). Compared with the similar response of sediment to disturbance in boatable and wadeable sites, the association between riparian vegetation and disturbance was much stronger for wadeable sites (t_{rt} = +4.06**** to +13.46****) than for boatable sites (t_{rt} = -0.13 to +3.44***) sites (**Table 8-9** and **Figure 8-7**). Among boatable rivers, riparian vegetation cover complexity was moderately correlated with the disturbance levels only in the Coastal Plain (t_{rt} = 2.99***) and West (t_{rt} = 2.24**), and relatively weakly associated

elsewhere (t_{rt} = -0.13$^{n.s.}$ to 1.73*). Among wadeable streams, however, riparian vegetation was strongly correlated with disturbance in all regions (t_{rt} = 4.06**** in the UMW to 7.35**** in CENPL). Note of course that expected riparian vegetation cover complexity is much higher in the CPL and EHIGH, for example, than in the CENPL.

Instream habitat cover complexity (*LPt01_XFC_NAT*) in boatable and wadeable rivers and streams was markedly lower in the NPL than in any other region (**Figure 8-8)**. In wadeable streams, the Central Plains ecoregions (NPL, SPL, and TPL) had markedly lower instream cover complexity than the other regions. Boatable and wadeable rivers and streams in the SAP, CPL, and NAP, and wadeable rivers and streams in the WMT had generally higher instream habitat cover complexity than the other regions (**Figure 8-8**). We scaled instream cover complexity as an O/E variable (*LOE_XFC_NAT_use*) to adjust for geoclimatic influences on instream cover, we examined the associations between instream cover and anthropogenic influences (**Table 8-9** and **Figure 8-9**). Except for the weak response in the Upper Midwest (t_{rt} = +1.08*), the instream habitat complexity indicator showed moderate response to human disturbance, with t_{rt} values ranging from +2.30** to +6.62**** for combined boatable and wadeable sites (**Table 8-9**).

However, as was the case for the riparian vegetation indicators, associations were in most cases much stronger for wadeable (t_{rt} = +1.73* to 8.16****), than for boatable sites (**Figure 8-9**), where most regional associations of instream habitat complexity to human disturbance levels were non-significant, with low or negative t values (-1.78* to +0.91*). Among wadeable sites, however, the associations of instream habitat complexity with disturbance ranged from weak in the EHIGH and UMW (t_{rt} = 1.73* and 2.02**) to very strong in the WEST (t_{rt} = 8.16 and $p < 0.0001$). Note that expected instream habitat complexity is generally higher in the CPL and upland regions (EHIGH and WEST) than for the CENPL and UMW.

8.5 LITERATURE CITED

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Table 8-1. Metrics used to characterize the general attributes of stream/river physical habitat.

Habitat Volume:

- *LRP100* = *log(RP100)* = Log of Mean Residual Depth (cm)

Scaled Habitat Volume:

- *LDVRP100* = *log(RP100)* – *log(Predicted RP100)* = Deviation in Mean Residual Depth from expected value

Habitat Complexity:

- *CVDPTH* = *SDDEPTH* / *XDEPTH* = Coefficient of Thalweg Depth Variation
- *C1WM100* = Number of Large Woody Debris pieces/100m of channel.
- *LV1W_MSQ* = log[Volume of Large Woody Debris per m^2 of bankfull channel area (m^3/m^2)].
- *XFC_NAT* = Areal Cover of Woody Debris, Brush, Undercut Banks, Overhanging Vegetation, plus Boulders andRock Ledges.
- *XFC_NORK* = Areal Cover of Woody Debris, Brush, Undercut Banks, Overhanging Veg.
- *XFC_AQM* = Areal Cover of Aquatic Macrophytes
- *XFC_ALG* = Areal Cover of Filamentous Algae detectable by the unaided eye.

Streambed Particle Size:

- *LSUB_dmm* = log[Streambed surface particle D_{gm} – mm] = log of geometric mean diameter of bed surfacesediments in millimeters.
- *PCT_FN* = % Streambed Silt & Finer
- *PCT_SAFN* = % Streambed Sand & Finer
- *XEMBED* = % Substrate Embedded by Sand and Fines

Scaled Streambed Particle Size:

- *DPCT_FN* = Deviation of *PCT_FN* from expected value ("excess Fines")
- *DPCT_SF* = Deviation of *PCT_SAFN* from expected value ("excess Sand+Fines")
- *DEVLSUB* = Deviation of *LSUB_DMM* from expected value (Streambed Fining Index)

Relative Bed Stability:

- *LRBS*= $\log_{10}$ of diameter ratio: Geometric mean bed particle diameter / Critical (mobile) diameter at bankfull flowstage. (LRBS_bw5: see Kaufmann et al. 1999; LRBS_g08: see Kaufmann et al. 2008, 2009).

Floodplain Interaction:

- *LSINU* = *Log(SINU)* = Log(Channel Sinuosity).
- *LINCIS_H* = *log(XINC_H - XBKF_H + 0.1)* = Log of Incision from terrace to bankfull ht (m).
- *LBFWDRAT* = *log{BKF_W / BKF_H+(XDEPTH/100)}* = log (Bankfull Width/Depth Ratio)
- *LBFXWRAT* = *log(BKF_W / XWIDTH)*= log (Bankfull Width / Wetted Width) (an index of streamside floodinundation potential)

Hydrologic Regime:

- *LQSLTR_RAT* = *log{(Qsp+0.0000001)/LTROFF_M}*=log{low flow /annual mean runoff} (~ an inverse index of "droughtiness",
  where: *Qsp = Flow_mps/WSAREAKM= (flow_cfs/35.315)/WSAREAKM*
- *LBFXDRAT =log{(XBKF_H+(XDEPTH/100) / (XDEPTH/100)}*= log(ratio of bankfull depth / wetted depth), a morphometric index of "flashiness".

Riparian Vegetation:

- *XCDENMID*: % Canopy Density measured midstream.
- *XCMG* = Riparian Canopy+Mid-+Ground Layer Vegetation (areal cover proportion)
- *XCMGW* = Riparian Canopy+Mid+Ground Layer Woody Veg.(areal cover proportion)

Riparian Habitat Alteration:

- *QR1=(QRVEG1*QRVEG2*QRDIST1)$^{0.3333}$;* where:
  if *XCMGW* <=2.00 then *QRVeg1*=.1+(0.9(*XCMGW* / 2.00));
  if *XCMGW* >2.00 then *QRVeg1*=1*;*
- *QRVeg2*=.1+(0.9(*XCDENBK* / *100*)); and *QRDIST1*=1/(1+*W1_HALL*)

Riparian Human Disturbances:

- *W1_HAG* = Riparian & near-Stream Agriculture – all types (proximity-weighted tally)
- *W1H_ROAD* = Riparian & near-Stream Roads (proximity-weighted tally)
- *W1H_CROP* = Riparian & near-Stream Row Crop Agriculture (proximity-weighted tally)
- *W1H_WALL* = Riparian & near-Stream Walls, Dikes, Revetment (proximity-weighted tally)
- *W1_HALL* = Proximity-weighted Index of Human Disturbances of All Types
- *QRDIST1* = 1/(1+*W1_HALL*) = Proximity-weighted Inverse Index of Human Disturbances of All Types

Table 8-2. Sampling revisit precision (repeatability) of the four physical habitat condition indicators. Repeat visits within the summer sampling season were used to calculate RMS_{rep}, which is essentially the standard deviation of repeat sampling pairs to the same stream or river reach. Dividing the square of the RMS_{rep} into the variance among sites gives the S:N variance ratio. (*See* Kaufmann et al., 1999 for ANOVA methods to calculate RMS_{rep} and S:N, where RMS_{rep} is equal to their RMSE.)

Metric	Group	Sites (n)	mean	Repeat pairs (n)	RMS_{rep}	S:N
LRBS_g08	**All Sites**	**4058**	**-0.938**	**375**	**0.519**	**4.17**
	All (0809 / 1314)	(2032 / 2025)	(-0.942 / -0.933)	(191 / 184)	(0.482 / 0.556)	(5.13 / 3.42)
	Boatable	1484	-0.661	178	0.479	6.70
	Wadeable	2573	-1.104	197	0.553	2.58
	EHIGH	1075	-0.541	134	0.539	3.65
	PLNLOW	2060	-1.242	164	0.514	3.89
	WMTNS	921	-0.740	77	0.493	4.07
L_xcmgw	**All Sites**	**4193**	**-0.252**	**388**	**0.148**	**8.45**
	All (0809 / 1314)	(2112 / 2080)	(-0.286 / -0.218)	(197 / 191)	(0.146 / 0.150)	(9.38 / 7.46)
	Boatable	1599	-0.154	187	0.144	4.70
	Wadeable	2593	-0.315	201	0.151	10.08
	EHIGH	1100	-0.051	138	0.083	8.05
	PLNLOW	2158	-0.341	173	0.188	6.72
	WMTNS	933	-0.293	77	0.135	7.79
L_xfc_nat	**All Sites**	**4193**	**-0.603**	**388**	**0.214**	**2.27**
	All (0809 / 1314)	(2112 / 2080)	(-0.590 / -0.617)	(197 / 191)	(0.240 / 0.184)	(1.87 / 2.99)
	Boatable	1599	-0.626	187	0.220	1.66
	Wadeable	2593	-0.589	201	0.209	2.76
	EHIGH	1100	-0.494	138	0.200	1.57
	PLNLOW	2158	-0.670	173	0.227	2.24
	WMTNS	933	-0.584	77	0.211	2.28
L_W1_Hall	All Sites	**4193**	**-0.129**	**388**	**0.178**	**5.46**
	All (0809 / 1314)	(2112 / 2080)	(-0.152 / -0.106)	(197 / 191)	(0.186 / 0.170)	(5.18 / 5.76)
	Boatable	1599	-0.091	187	0.137	9.03
	Wadeable	2593	-0.154	201	0.210	3.89
	EHIGH	1100	-0.078	138	0.181	5.15
	PLNLOW	2158	-0.151	173	0.168	5.85
	WMTNS	933	-0.142	77	0.196	5.10

Table 8-3. Estimated number of years to detect trends in habitat attributes. Number of years required for a 50-site monitoring network to detect 1% and 2% per year trends in habitat attributes with 80% likelihood (beta, or power) and alpha = 0.05, if specified trends occur, and sites are visited each year. Data were taken from Larsen et al. (2004),[a] or calculated using the same data and analytical procedures used in that publication.[b]

Variable	Description	1% trend	2% trend
SDDEPTH[b]	(Std. Deviation of Thalweg Depth)	13 years	8 years
LRP100[a]	(log[Mean Residual Depth])	20	12
PCT_SAFN[a]	(% Sand + Silt)	21	13
XEMBED[b]	(% Embeddedness)	20	12
LRBS_BW5[b]	(log[Rel. Bed Stability])	12	8
LV1W_MSQ[a]	(log[Large Wood Volume/m^2])	27	17
XCMGW[b]	(3-Layer Riparian Woody Veg Areal Cover)	12	8
XCDENMID[a]	(Canopy Density measured midstream)	13	8

Table 8-4. Anthropogenic disturbance screening criteria.

Criteria used to characterize least-disturbed reference (R), moderately-disturbed (S), and most-disturbed (T) sample reaches for developing physical habitat condition criteria. In addition to the tabulated criteria, potential reference sites were rejected if DAM_DII > 1, or URB_1KMCIRCLE > 5%, or AG_1KMCIRCLE > 15%.

- Values > than those before the slash (/) are EXCLUSION criteria for least-disturbed reference sites.
- Values ≥ those after slash are INCLUSION criteria for most-disturbed sites.
- W, B, and G refer to Wadeable, Boatable, and Great River sites.

Region	PTL	NTL	Cl	SO4	Turb	W1_HALL	W1_HAG Wadeable	W1H_CROP Wadeable	W1H_WALL Wadeable
NAP	20/100	750/3500	250/10000	250/1000	5/10	2.0/4.0	0.1/0.4	0.05/0.10	0.2/0.4
SAP	20/100	750/3500	200/1000	400/1000	5/20	2.0/4.0	0.1/0.4	0.05/0.10	0.2/0.4
UMW	50/150	1000/5000	300/2000	400/2000	5/30	2.0/4.0	0.15/1.4	0.1/0.4	0.2/0.4
CPL	75/250	2500/8000	999999/ 999999	600/4000	10/50	2.0/4.0	0.15/1.4	0.05/ 0.4	0.2/0.4
TPL	100/500	3000/15000	2000/5000	999999/ 999999	50/100	2.0/4.0	0.67/1.4	0.25/0.48	0.4/0.6
NPL & SPL	150/500	4500/10000	1000/5000	999999/ 999999	50/100	2.0/3.0	1.0/1.4	0.15/ 0.25	0.2/0.4
WMT:									
Southwest	50/100	750/1500	300/1000	99999/ 99999	5/10	W:0.5/3.0 B,G:1.5/3.0	0.25/1.4	0.10/0.25	0.2/0.4
S.Rockies	25/100	750/1500	200/1000	200/1000	5/10	W:1.0/3.0 B,G:1.5/3.0	0.3/1.4	0.1/0.25	0.2/0.4
N.Rockies & Pacific	25/100	750/1500	200/1000	200/1000	5/10	W:0.5/3.0 B,G:1.5/3.0	0.3/1.4	0.10/0.25	0.2/0.4
XER	50/150	1500/5000	1000/5000	999999/ 999999	25/75	1.5/3.0	0.6/1.4	0.15/0.25	0.2/0.4

Table 8-5. NRSA boatable and wadeable least-disturbed reference sites from combined 2008-09 & 2013-14 surveys, selected using consistent criteria listed in Table 8-4. Numbers of reference sites identified from the 2008-09 and 2013-14 surveys are parenthesized and separated by a slash (/).

ECO9	ECOp5	Total	Boatable	Wadeable
NAP	APPAL	88 (45/43)	47 (24/23)	41 (21/20)
SAP	APPAL	54 (40/14)	22 (15/7)	32 (25/7)
CPL	CPL	103 (55/48)	52 (25/27)	51 (30/21)
UMW	UMW	79 (40/39)	36 (18/18)	43 (22/21)
TPL	CENPL	83 (44/39)	22 (12/10)	61 (32/29)
NPL	CENPL	85 (29/56)	33 (11/22)	52 (18/34)
SPL	CENPL	44 (23/21)	2 (2/0)	42 (21/21)
WMT	WEST	112 (47/65)	43 (16/27)	69 (31/38)
XER	WEST	60 (26/34)	24 (6/18)	36 (20/16)
Totals for lower 48 states		**708 (349/359)**	**281 (129/152)**	**427 (220/207)**

Table 8-6. Summary of regression models used in estimating site-specific expected values of Log10 Relative Bed Stability (*LRBS_g08)* under least-disturbed reference conditions. See Appendix 8.A for model details.

REGION/Realm

NAP/Boatable
ExpRef LRBS_g08 = (mean)$_{REF\text{-}47}$ (R^2=0%, RMSE=1.539)
NAP/Wadeable
ExpRef LRBS_g08 = f(LAws, W1_HAG)$_{REF\text{-}41}$, *where W1_HAG=0* (R^2=22%, RMSE=0.525)

SAP/Boatable
ExpRef LRBS_g08 = (mean)$_{REF\text{-}22}$ (R^2=0%, RMSE=0.704)
SAP/Wadeable
ExpRef LRBS_g08 = f(LAws, W1_Hall)$_{REF\text{-}32}$ *where W1_Hall=0* (R^2=28%, RMSE=0.691)

CPL/Boatable
ExpRef LRBS_g08 = (mean)$_{REF\text{-}52}$ (R^2=0%, RMSE=1.331)
CPL/Wadeable
ExpRef LRBS_g08 = f(LSlope, LWidth, W1_Hall)$_{REF\text{-}51}$ *where W1_Hall=0* (R^2=35%, RMSE=0 .736)

UMW/Boatable
ExpRef LRBS_g08 = (Lat, W1_Hall)$_{REF\text{-}36}$ *where W1_Hall=0* (R^2=18%, RMSE=1.259)
UMW/Wadeable
ExpRef LRBS_g08 = (LSlope, W1_Hall)$_{REF\text{-}43}$ *where W1_Hall=0* (R^2=41%, RMSE=0.925)

NPL/Boatable
Exp LRBS_g08 = f(LAws, LSlope, [AGws-x-KFct])$_{ALL\text{-}51}$, *where AGws-x-KFct =0* (R^2=56%, RMSE=0.610)
ExpRef (LRBS_g08/Exp LRBS_g08) = f(PCT_AG_WS)$_{REF\text{-}28}$ *where PCT_AG_WS =0* (R^2=23%, RMSE=0.512)
NPL/Wadeable
Exp LRBS_g08 = f(Elev, LSlope, LWidth, W1_Hall, W1_Crop)$_{ALL\text{-}314}$ (R^2=39%, RMSE=0.837)
where W1_Hall, W1_Crop [AGws-x-KFct]) = 0
ExpRef (LRBS_g08/Exp LRBS_g08) = f(W1_Hall)$_{REF\text{-}51}$ (R^2=3%, RMSE=0.839)
where W1_Hall=0

SPL+TPL/Boatable
Exp LRBS_g08 = f(LAws, AG_1KMCircle)$_{REF\text{-}47}$ *(SPL+TPL+NPL)* *where AG_1KMCirle =0* (R^2=18%, RMSE=1.139)
SPL/Wadeable
Exp LRBS_g08 = f(Lat, LAws, LSlope, W1_HAG, AG_1KMCircle)$_{ALL\text{-}297}$ (R^2=35%, RMSE=0.952)
where *W1_HAG, AG_1KMCircle = 0*
ExpRef (LRBS_g08/Exp LRBS_g08) = f(W1H_NOAG, Dam_dii, RdDen_ws, PCT_AG_ws)$_{REF\text{-}42}$ (R^2=26%, RMSE=0.990)
where *W1H_NOAG, Dam_dii, RdDen_ws, PCT_AG_ws = 0*
TPL/Wadeable
Exp LRBS_g08 = f(Lat, Lon, LSlope)$_{ALL\text{-}342}$ (R^2=20%, RMSE=0.976)
ExpRef (LRBS_g08/Exp LRBS_g08) =
f(W1H_NOAG,W1H_Crop, AG_1KMCircle, PCT_AG_WS, AgWS-x-KFct)$_{REF\text{-}58}$ (*R*^{2}=26%, RMSE=0.990)
where *W1H_NOAG,W1H_Crop, AG_1KMCircle, PCT_AG_WS, AgWS-x-KFct = 0*

WMT/Boatable
ExpRef LRBS_g08 = (mean)$_{REF\text{-}43}$ (R^2=0%, RMSE=0.365)
WMT/Wadeable
ExpRef LRBS_g08 = f(LSlope, LWidth)$_{REF\text{-}69}$, (R^2=27%, RMSE=0.430)

XER/Boatable
ExpRef LRBS_g08 = (mean)$_{REF\text{-}24}$ (R^2=0%, RMSE=0.985)
XER/Wadeable
ExpRef LRBS_g08 = f(LWidth)$_{REF\text{-}36}$, (R^2=23%, RMSE=0.794)

Table 8-7. Summary of regression models used in estimating site-specific expected values of Riparian Vegetation Cover and Structure (Log10[0.01+XCMGW]) under least-disturbed reference conditions. See Appendix 8.A for model details.

REGION/Realm

NAP/Boatable
ExpRef L_XCMGW = f(Lat, , AG_1KMCircle, PCT_AG_WS, AgWS-x-KFct)$_{REF-47}$ (R^2=40%, RMSE=0.156)
where AG_1KMCircle, PCT_AG_WS, AgWS-x-KFct = 0
NAP/Wadeable
ExpRef L_XCMGW = f(LAws, LWidth, W1_Hall)$_{REF-41}$, *where W1_Hall=0* (R^2=24%, RMSE=0.121)

SAP/Boatable
ExpRef L_XCMGW = f(W1_HAG)$_{REF-22}$, *where W1_HAG=0* (R^2=17%, RMSE=0.141)
SAP/Wadeable
ExpRef L_XCMGW = f(LAws, ELEV, W1_Hall)$_{REF-32}$ *,where W1_Hall=0* (R^2=32%, RMSE=0.141)

CPL/Boatable
ExpRef L_XCMGW = f(Lon, LAws, W1_HAG)$_{REF-52}$, *where W1_HAG=0* (R^2=26%, RMSE=0.119)
CPL/Wadeable
ExpRef L_XCMGW = f(Lon)$_{REF-51}$ (R^2=1%, RMSE=0 .152)

UMW/Boatable
ExpRef L_XCMGW = f(Lat, LAws, LSlope, LWidth)$_{REF-55\ (SPL+TPL+UMW)}$ (R^2=34%, RMSE=0.373)
UMW/Wadeable
ExpRef L_XCMGW = f(LSlope, LWidth, W1_Hall)$_{REF-43}$, *where W1_Hall=0* (R^2=33%, RMSE=0.130)

NPL+SPL /Boatable
Exp L_XCMGW = f(Lat, Lon, W1_HAG, RDDEN_ws, PCT_AG_ws)$_{ALL-249\ (NPL+SPL+TPL)}$ (R^2=25%, RMSE=0.362)
where *W1_HAG, RDDEN_ws, PCT_AG_ws = 0*
ExpRef (L_XCMGW/Exp L_XCMGW) = f(PCT_AG_WS)$_{REF-28}$, *where PCT_AG_WS =0* (R^2=31%, RMSE=0.324)
TPL/Boatable
ExpRef L_XCMGW = (mean)$_{REF-22}$ (R^2= 0%, RMSE=0.159)

NPL/Wadeable
Exp L_XCMGW = f(Lat, Lon, LSlope, LWidth, W1_HAG, PCT_AG_ws)$_{ALL-922\ (NPL+SPL+TPL)}$ (R^2=31%, RMSE=0.487)
where W1_HAG, PCT_AG_ws = 0
ExpRef (L_XCMGW/Exp L_XCMGW) = f(Damm_dii, PCT_AG_ws, AgWs-x-KFct)$_{REF-152\ (NPL+SPL+TPL)}$ (R^2=14%, RMSE=0.386)
where *Damm_dii, PCT_AG_ws, AgWs-x-KFct = 0*
SPL+TPL/Wadeable
ExpRef L_XCMGW = f(Lon, ELEV, AG_1KMCircle, PCT_AG_ws, AGws-x-KFct)$_{REF-143\ (SPL+TPL+UMW)}$ R^2=40%, RMSE=0.267)
where *AG_1KMCircle, PCT_AG_ws, AGws-x-KFct = 0*

WMT/Boatable
ExpRef L_XCMGW = (mean)$_{REF-43}$ (R^2= 0%, RMSE=0.262)
WMT/Wadeable
ExpRef L_XCMGW = f(LAws, ELEV, LSlope,)$_{REF-68}$, (R^2=20%, RMSE=0.153)

XER/Boatable
ExpRef L_XCMGW = f(W1_HNOAG, W1_HAG)$_{REF-24}$, where *W1_HNOAG, W1_HAG = 0* (R^2=29%, RMSE=0.153)
XER/Wadeable
ExpRef L_XCMGW = f(LAws, LSlope, LWidth)$_{REF-36}$, (R^2=23%, RMSE=0.253)

Table 8-8. Summary of regression models used in estimating site-specific expected values of Instream Habitat Cover Complexity (Log10[0.01+XFC_NAT]) under least-disturbed reference conditions. See Appendix 8.A for model details.

REGION/Realm

NAP/Boatable
ExpRef L_XFC_NAT = f(Lon, LAws, LWidth, W1H_Crop)$_{REF\text{-}47}$*, where W1H_Crop = 0* (R^2=34%, RMSE=0.319)
NAP/Wadeable
ExpRef L_XFC_NAT = f(LWidth)$_{REF\text{-}41}$ (R^2= 7%, RMSE=0.285)

SAP/Boatable
ExpRef L_XFC_NAT = f(Lat, W1_Hall)$_{REF\text{-}22}$*, where W1_Hall = 0* (R^2=53%, RMSE=0.175)
SAP/Wadeable
ExpRef L_XFC_NAT = f(Lat, ELEV, W1_HAG)$_{REF\text{-}32}$*, where W1_HAG =0* (R^2=42%, RMSE=0.310)

CPL/Boatable
ExpRef L_XFC_NAT = (mean)$_{REF\text{-}52}$ (R^2= 0%, RMSE=0.235)
CPL/Wadeable
ExpRef L_XFC_NAT = (mean)$_{REF\text{-}51}$ (R^2= 0%, RMSE=0.298)

UMW/Boatable
ExpRef L_XFC_NAT = f(Lon, W1_HAG)$_{REF\text{-}36}$*, where W1_HAG =0* (R^2=23%, RMSE=0.316)

UMW/Wadeable
ExpRef L_XFC_NAT = f(LAws, LWidth)$_{REF\text{-}43}$, (R^2= 7%, RMSE=0.290)

NPL+SPL+TPL/Boatable
Exp L_XFC_NAT = f(Lat, Lon, LAws, ELEV, AG_1KMCircle)$_{REF\text{-}47\ (NPL+SPL+TPL)}$ (R^2=34%, RMSE=0.323)
where AG_1KMCircle = 0

NPL+SPL+TPL/Wadeable
Exp L_XFC_NAT = f(Lon, LAws, ELEV, AG_1KMCircle, URB_1KMCircle)$_{REF\text{-}152\ (NPL+SPL+TPL)}$ (R^2=17%, RMSE=0.335)
where AG_1KMCircle, URB_1KMCircle = 0

WMT/Boatable
ExpRef L_XFC_NAT = f(LWidth, W1H_Crop, RDDEN_ws)$_{REF\text{-}43}$, (R^2= 24%, RMSE=0.230)
where W1H_Crop, RDDEN_ws = 0
WMT/Wadeable
ExpRef L_XFC_NAT = f(Lat, Lon, LAws, W1_HAG, RDDEN_ws)$_{REF\text{-}68}$, (R^2=35%, RMSE=0.217)
where W1_HAG, RDDEN_ws = 0

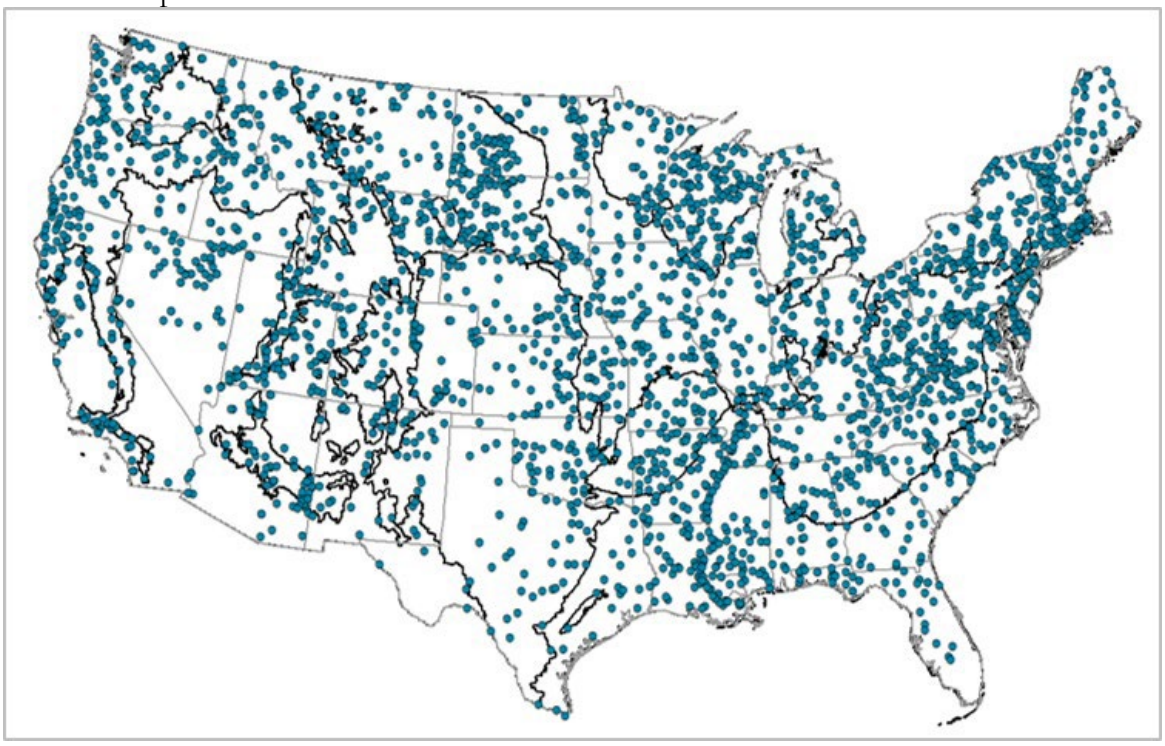
XER/Boatable
ExpRef L_XFC_NAT = f(ELEV, LWidth)$_{REF\text{-}23}$, (R^2=13%, RMSE=0.310)
XER/Wadeable
ExpRef L_XFC_NAT = f(Lon, LSlope, W1H_Crop)$_{REF\text{-}36}$*, where W1H_Crop = 0* (R^2=27%, RMSE=0.242)

Table 8-9. Responsiveness to levels of human disturbance.

Responsiveness of NRSA physical habitat condition metrics to levels of human disturbance, as quantified by t-values of the difference between means of least-disturbed reference sites (prk3RRT_NRSA1314=R) minus most-disturbed sites (those screened as prkRRT_NRSA1314=T). Values shown in red have a sign contrary to expectations. Order of magnitude of p-values shown by number of asterisks (e.g., * = p≃ 0.1; **** = p≃ 0.0001)

Metric	Region	t-value R-T (Boatable)	t-value R-T (Wadeable)	t-value R-T (All sites)
LOE_RBS_g08	USA-48	+11.32****	+7.26****	+12.84****
	CPL	+2.47**	+2.68**	+3.38***
	EHIGH (NAP+SAP)	+3.22***	+4.12****	+4.79****
	UMW	+6.59****	+2.24**	+5.32****
	CENPL (TPL+NPL+SPL)	+2.64**	+6.39****	+6.93****
	West (WMT+XER)	+3.96****	+8.25****	+8.98****
LOE_XCMGW	USA-48	+3.44***	+13.46****	+14.17****
	CPL	+2.99***	+5.69****	+6.32****
	EHIGH (NAP+SAP)	+1.73*	+5.61****	+5.25****
	UMW	-0.13	+4.06****	+2.95***
	CENPL (TPL+NPL+SPL)	+1.43	+7.35****	+7.95****
	West (WMT+XER)	+2.24**	+7.16****	+7.35****
LOE_XFC_Nat	USA-48	-0.64	+7.84****	+6.62****
	CPL	+0.59	+3.64****	+3.52***
	EHIGH (NAP+SAP)	+0.91*	+1.73*	+2.30**
	UMW	-0.85	+2.02**	+1.08*
	CENPL (TPL+NPL+SPL)	+0.56	+3.68****	+2.82**
	West (WMT+XER)	-1.78*	+8.16****	+5.37****

A. NRSA 2008-09 sample sites



B. NRSA 2013-14 sample sites

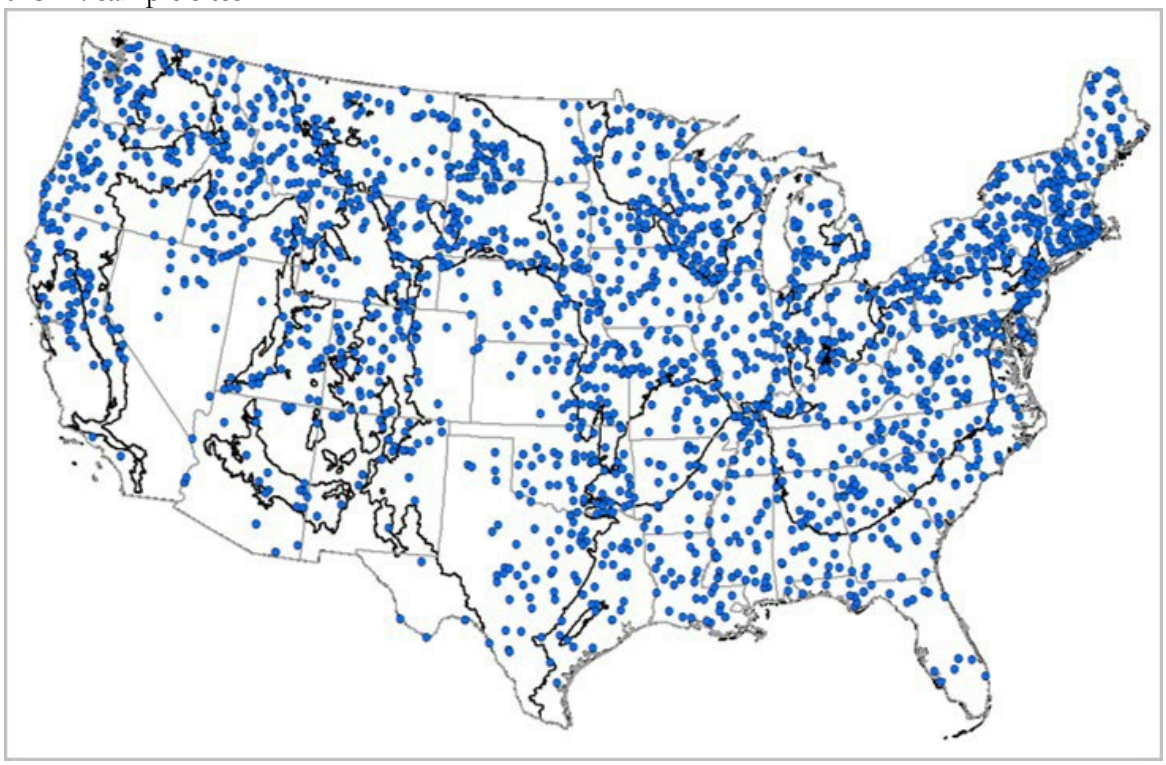
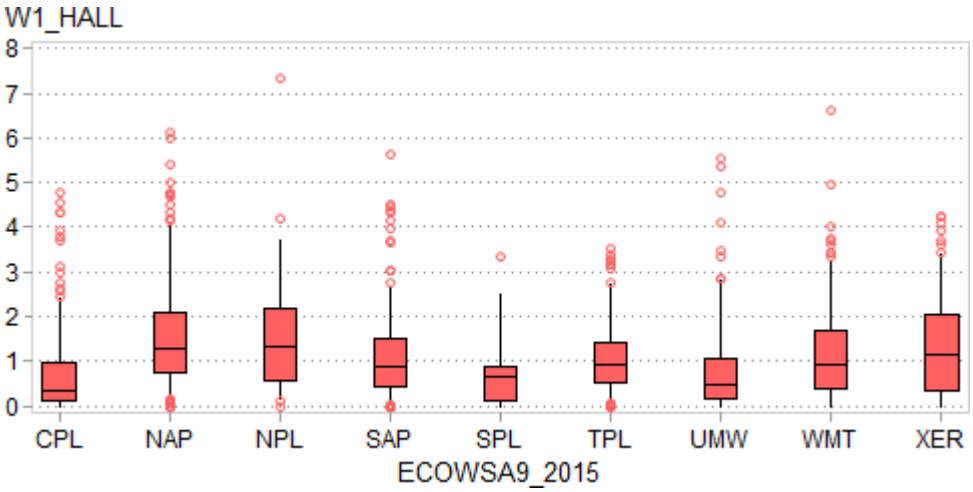


Figure 8-1. Sample sites for NRSA 2008-09 and NRSA 2013-14.

A. **Boatable**



B. **Wadeable**

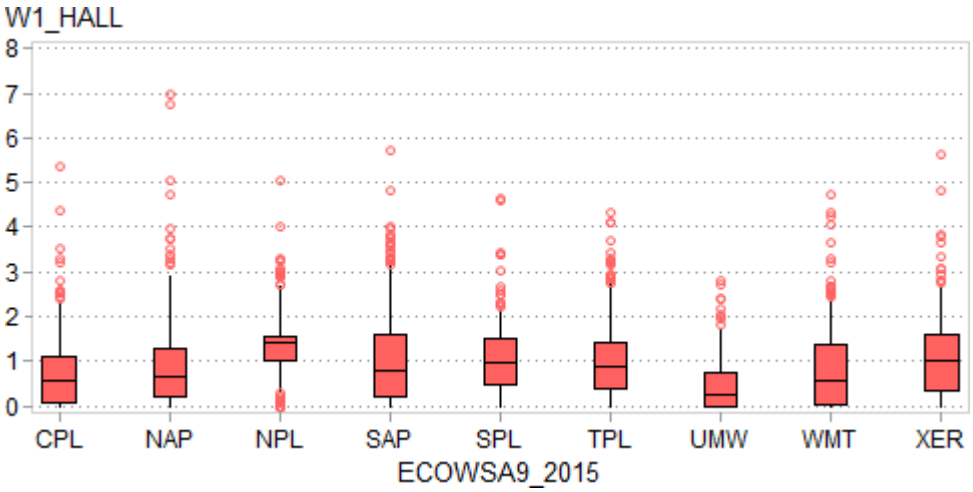
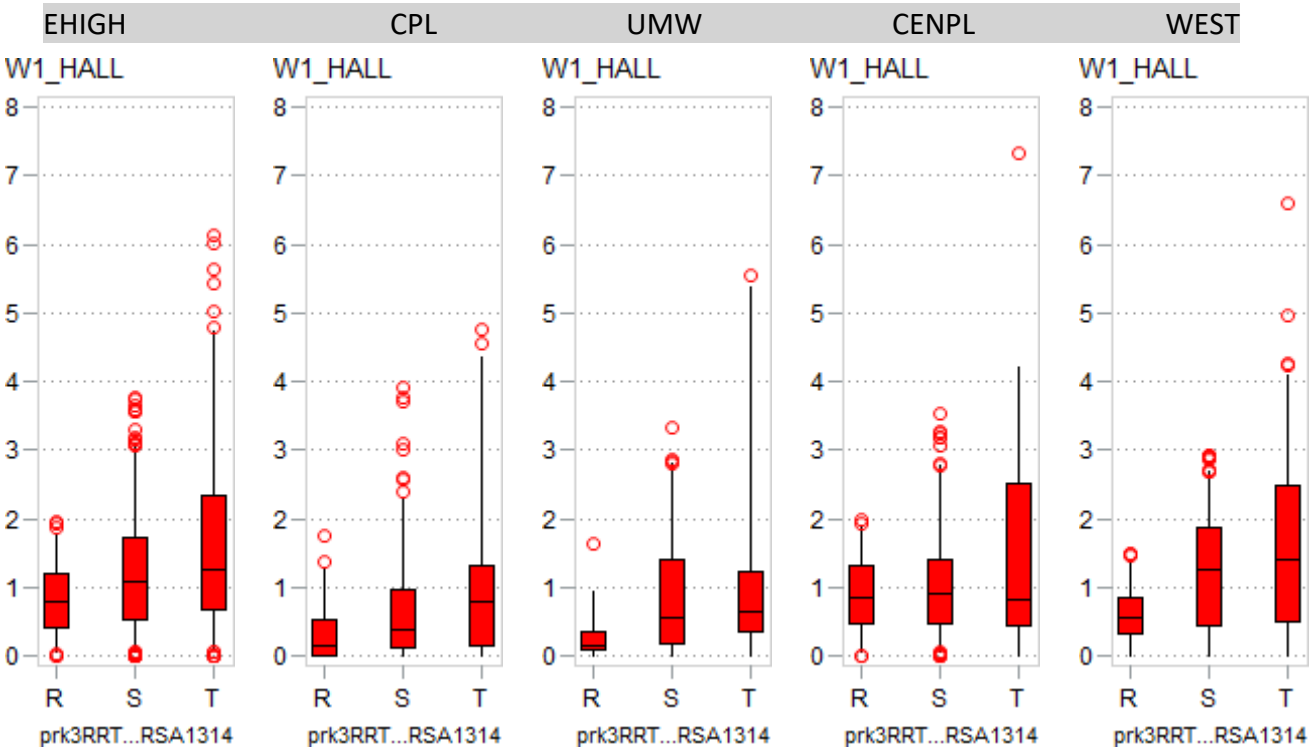


Figure 8-2. Riparian Disturbance (W1_Hall) in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S. Boxplots show 5th, 25th, median, 75th, and 95th percentiles ofthe unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A.Boatable



B. Wadeable

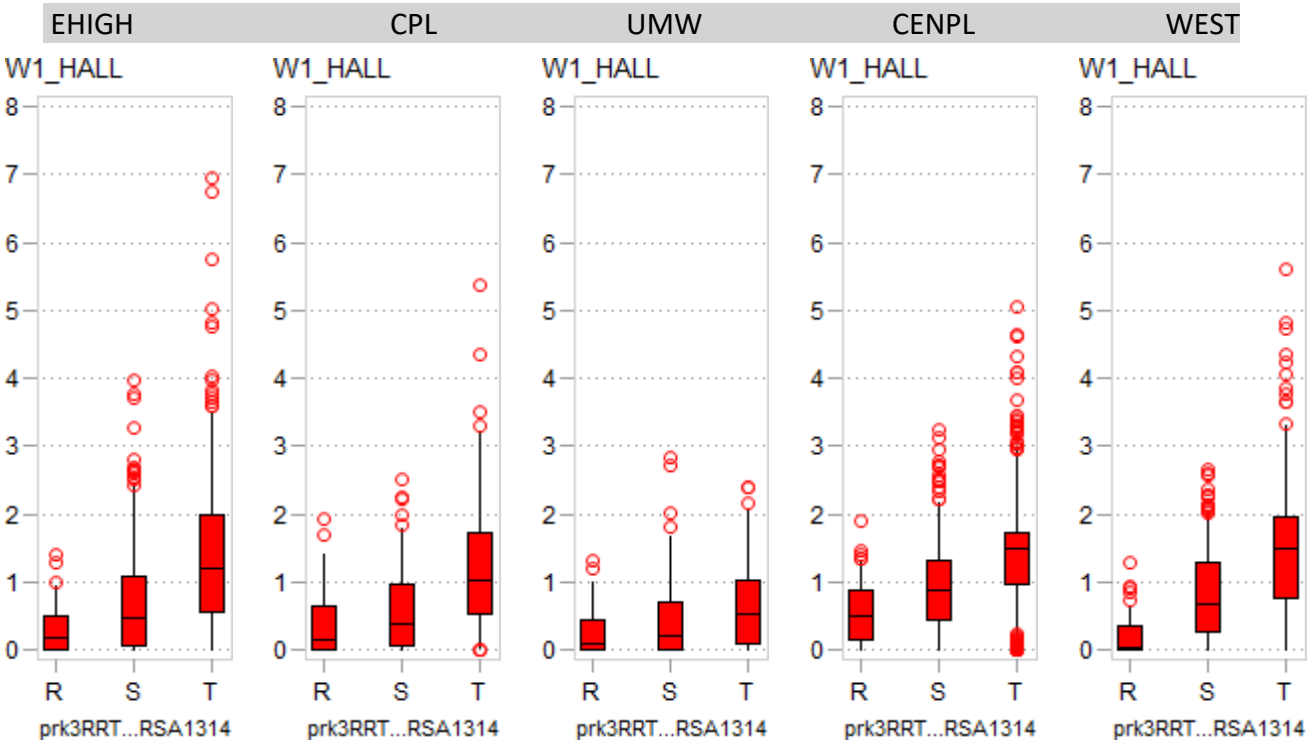
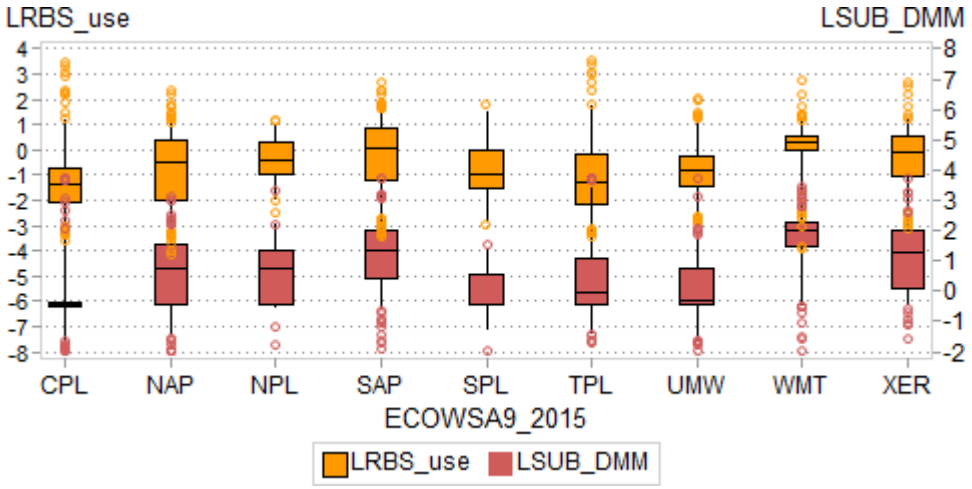


Figure 8-3. Riparian Disturbance (W1_Hall) in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S., contrasting distributions in least-, moderately-, and most-disturbed sites within each aggregated ecoregion. Boxplots show 5th, 25th, median, 75th, and 95th percentiles of the unweighted \sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A. Boatable



B. Wadeable

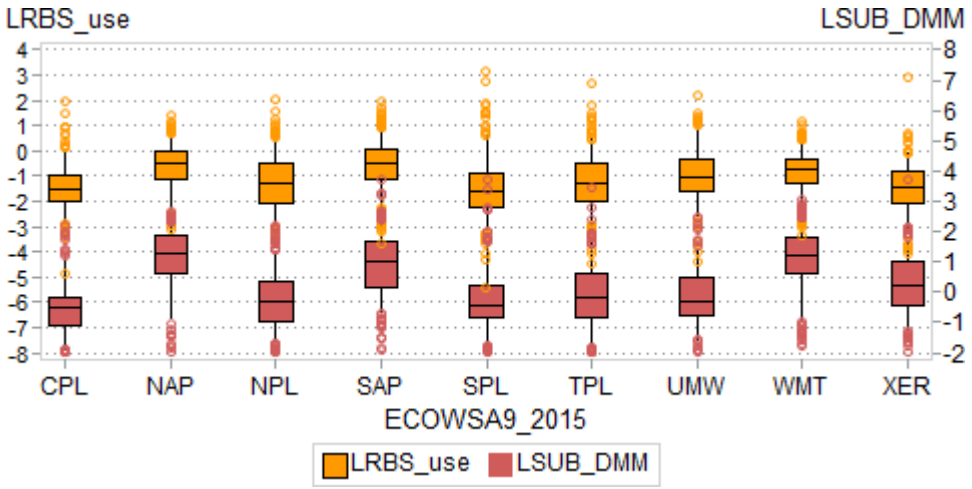
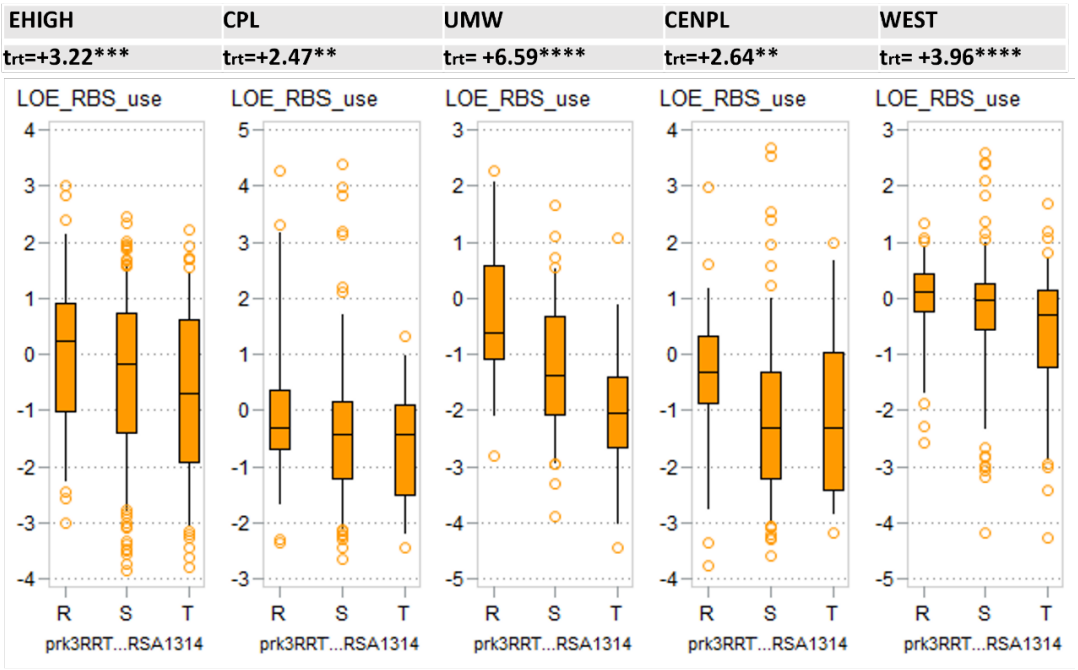


Figure 8-4. Log Relative Bed Stability (LRBS_use) and Log10 geometric mean bed surface substrate diameter (LSUB_dmm) in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions ofthe conterminous U.S. Boxplots show 5th, 25th, median, 75th, and 95th percentiles of the unweighted sampledistributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A. Boatable



B. Wadeable

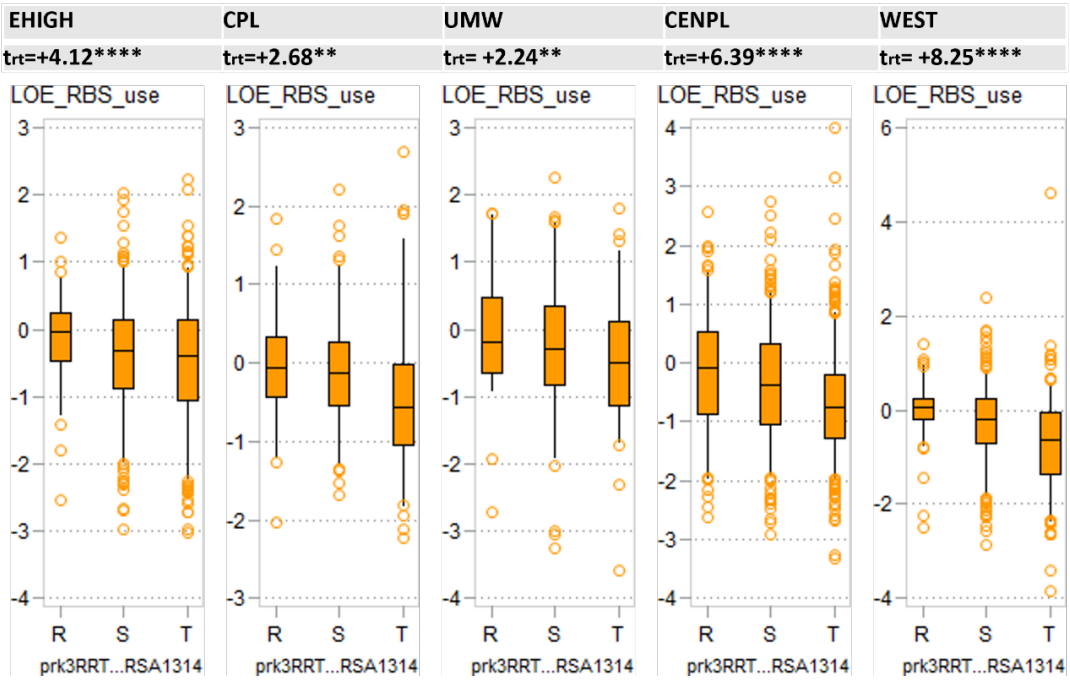
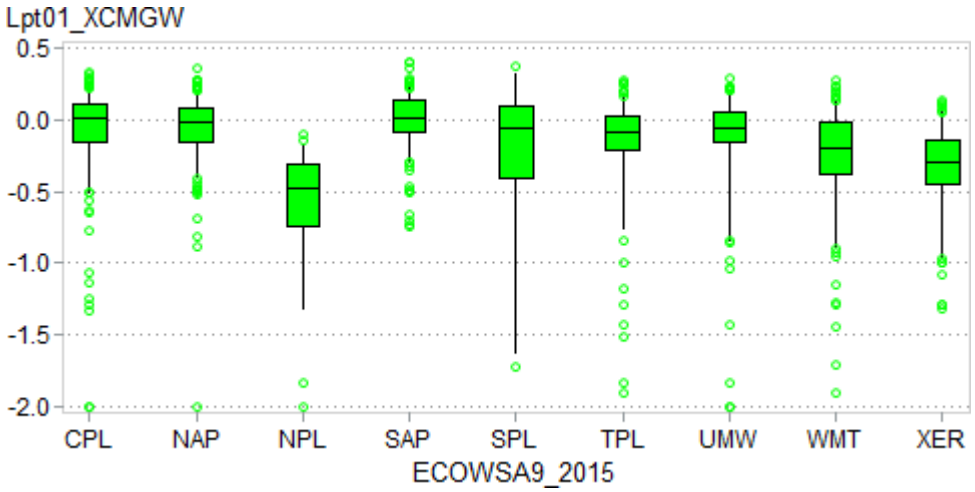


Figure 8-5. Observed/Expected Relative Bed Stability (LOE_LRBS_use) in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S., contrasting distributions in least-, moderately-, and most-disturbed sites within each aggregated ecoregion. Boxplots show 5th, 25th, median,75th, and 95th percentiles of the unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A. **Boatable**



B. **Wadeable**

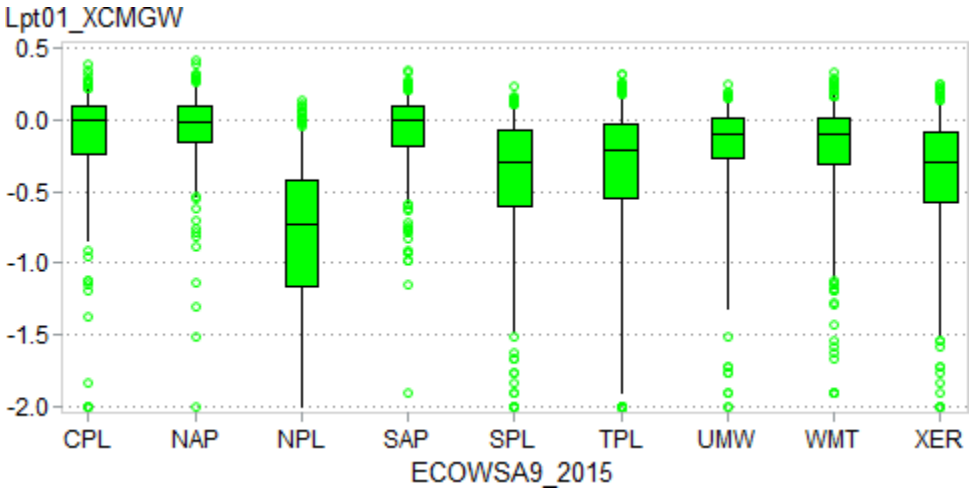
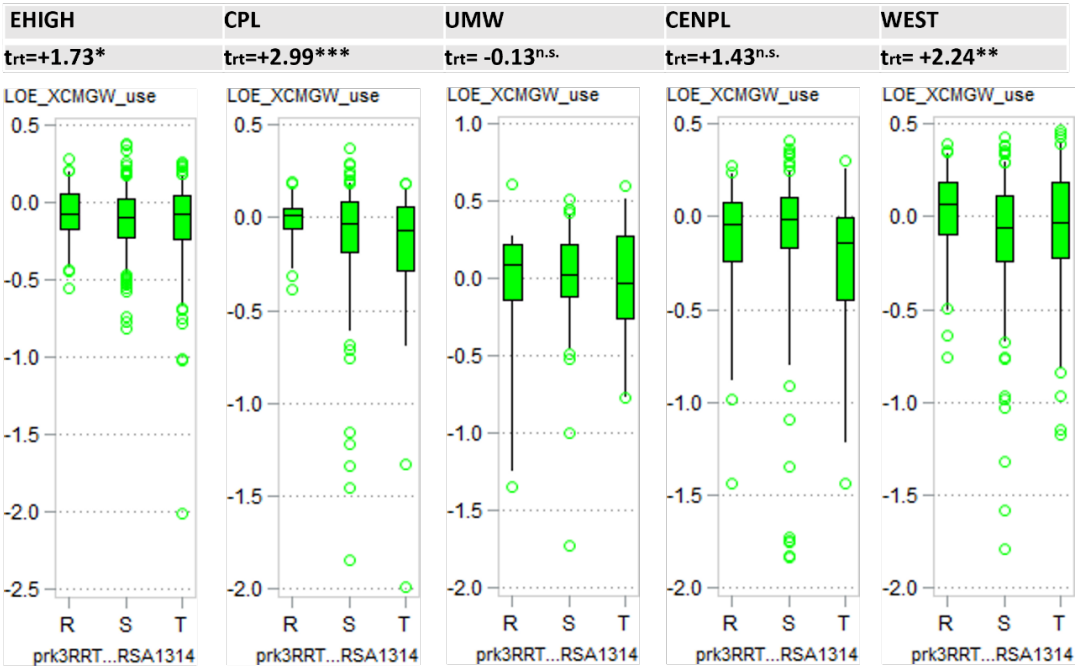


Figure 8-6. Riparian Vegetation Cover Complexity (*LPt01_XCMGW*) in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S. Boxplots show 5th, 25th, median, 75th, and 95th percentiles of the unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A. Boatable



B. Wadeable

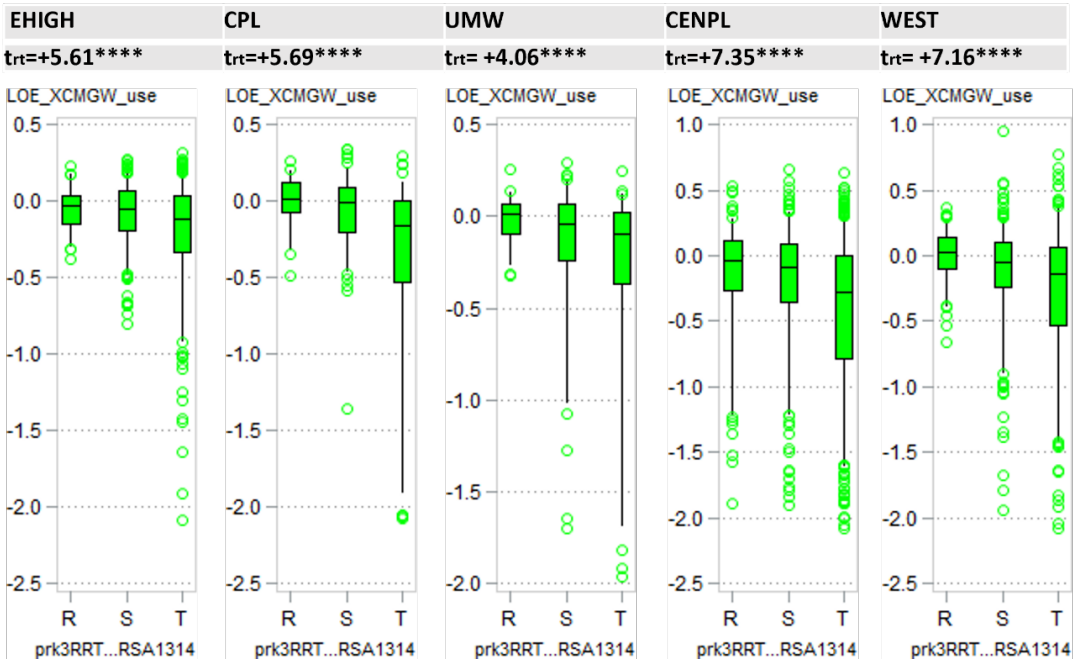
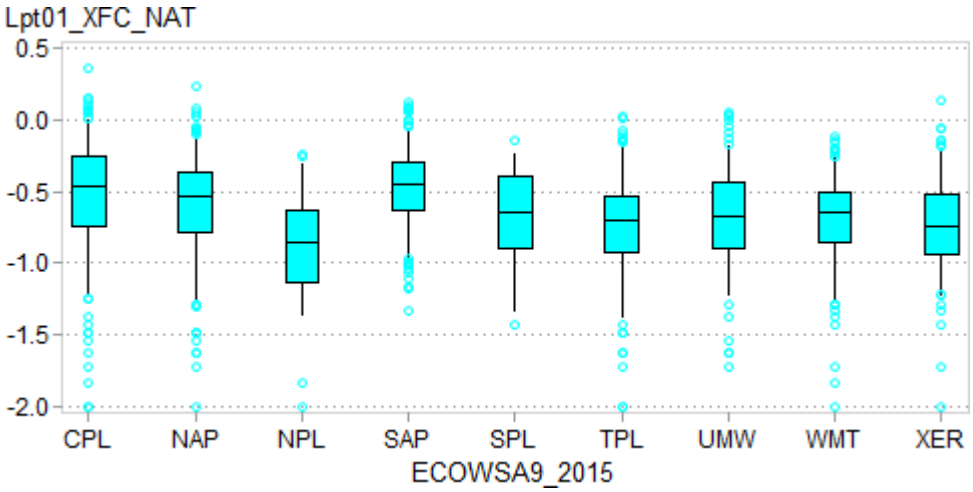


Figure 8-7. Observed/Expected Riparian Vegetation Cover Complexity *(LOE_XCMGW_use)* in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S., contrasting distributions in least-, moderately-, and most-disturbed sites within each aggregated ecoregion. Boxplots show 5th, 25th, median, 75th, and 95th percentiles of the unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

A. Boatable



B. Wadeable

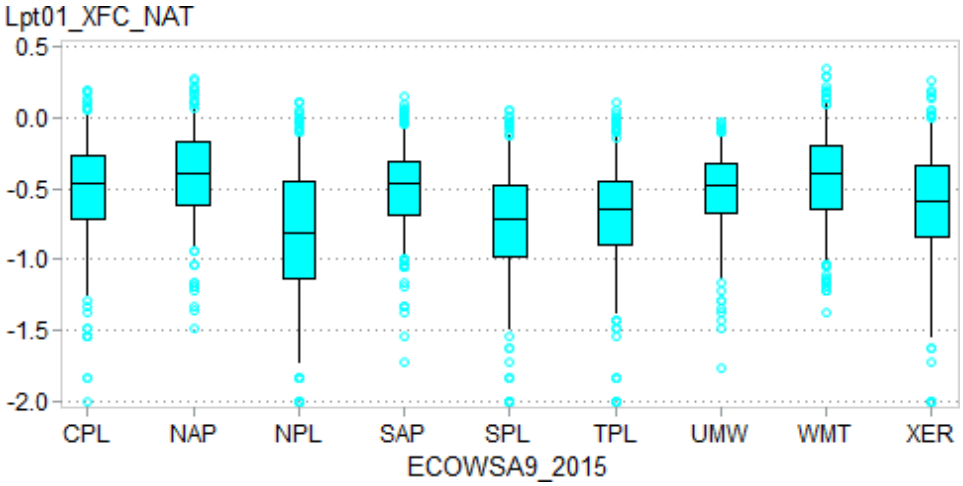


Figure 8-8. Instream Habitat Complexity *(LPt01_XFC_NAT)* in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S. Boxplots show 5th, 25th, median, 75th, and95th percentiles of the unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

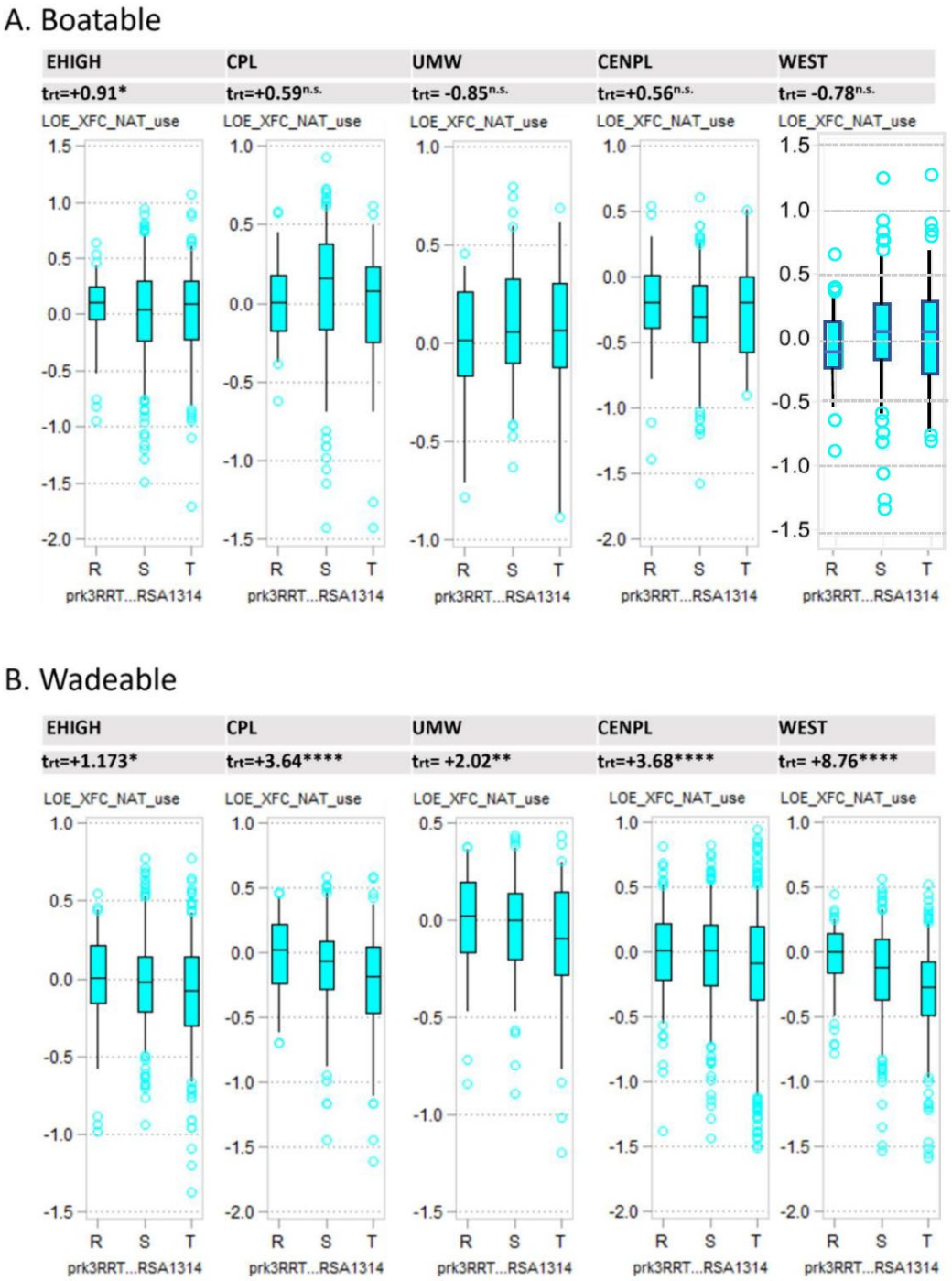


Figure 8-9. Observed/Expected Instream Habitat Complexity *(LOE_XFC_NAT_use)* in combined NRSA 2008-09 and 2013-14 sample sites in 9 aggregate ecoregions of the conterminous U.S., contrasting distributions in least-, moderately-, and most-disturbed sites within each aggregated ecoregion. Boxplots show 5th, 25th, median, 75th, and 95th percentiles of the unweighted sample distributions (not population estimates). A. Boatable sites; B. Wadeable sites.

APPENDIX 8.A

NRSA 2008-09 & 2013-14 Expected Condition Models and Condition Criteria

NOTES:

- Uni-Fixed Models are fixed values of a metric that are uniform across all ecoregions andboth Boatable and Wadeable "Realms."
- NULL MODELS are based on mean & SD for reference sites (prk3RRT_NRSA1314=R)from NRSA0809 and NRSA1314.
- Cond_1 and Cond_1b MODELS are MLRs using reference sites (prk3RRT_NRSA1314=R)from NRSA0809 and NRSA1314. Cond_1 MLRs may have disturbance variable(s) as predictors in cases where reference sites have anthropogenic disturbance that influences response variable.
- Cond_1D MODELS are "All-Sites" MLRs using all sites (except Great Rivers) and incorporate disturbance variables as predictors. We use 2-steps to calculate reference expected values. First step is to calculate All-Sites Model Expected values then calculate O/E values by setting disturbance to empirical minimum values for the ecoregion/realm. Second value is to examine distribution of All-Sites Model O/E values within the ReferenceSites of the appropriate ecoregion/realm.
- The expected reference value of the All-Sites Model OE is calculated from the reference sitedistribution of All-Sites model O/E values (refOE mean & refSD) or a regression factoring out disturbance in the reference sites (refOE y-intercept and refRMSE from disturbance regression) *** note that there is no requirement that the disturbance variable be the same asin the All-Sites model regression --- in fact it is likely to be a different variable because the influence of the disturbance variable used in the "All-Sites Model" has already been accounted for.

Condition Benchmarks for Riparian Human Disturbances(RDist_COND) based on W1_HALL

We applied uniform condition benchmarks nationwide. The Low (1 Low), Medium (2 Medi), and High (3 High) disturbance levels are analogous to the Good, Fair, Poor condition classification usedfor the other indicators.

All Ecoregions and both Boatable and Wadeable sites

If W1_Hall<0.33 then RDIST_COND= '1 Low';
If W1_Hall>=0.33 and W1_Hall<1.5 then
RDIST_COND='2 Medi';If
W1_Hall>=1.5 then RDIST_COND='3
High';

Reference Condition Models for Channel Bed Sedimentationbased on Relative Bed Stability (LRBS_use = LRBS_g08)

Coastal Plain (CPL) Boatable Sites Cond_Null
(eco9-B n=52)
RfNullM_LRBS=
–0.92405
RfNullSD_LRBS= 1.33124

Coastal Plain (CPL) Wadeable Sites
Cond_1 (eco9-W n=51):
LRBS_use= –1.67044 –0.77290(LXSlope_use)–0.49218(LXWidth_use)
–0.12031(W1_Hall) R^2=0.3497; AdjR^2=0.3054; RMSE=0.73642;
n=48/51; p=0.0003;p1<0.0001; p2=0.2637; p3=0.5799
---- Set W1_Hall= 0 = minimum in ref sites:

RfE1_LRBS=-1.67044 –
0.77290(LXSlope_use) –
0.49218(LXWidth_use)
RfE1_RMSE_LRBS=0.73642

Northern Appalachian (NAP) Boatable Sites
Cond_Null (eco9-B n=47):
RfNullM_LRBS= –0.63226
RfNullSD_LRBS= 1.53888

Northern Appalachian (NAP) Wadeable Sites
Cond_1 (eco9-W n=41):
LRBS_use= –0.64678 +0.32478(L_AreaWSkm2_use) –8.04380(W1_HAG)
R^2= 0.2250; AdjR^2=0.1842; RMSE=0.52529; n=41/41; p=0.0079;p1=0.0097;p2=0.1123
---- Set W1_HAG=0 = minimum in ref sites:

RfE1_LRBS= –0.64678
+0.32478(L_AreaWSkm2_u
se)
RfE1_RMSE_LRBS=0.5252

9

Southern Appalachian (SAP) Boatable Sites

Cond_Null (eco9-B n=22):
RfNullM_LRBS= 0.44138;RfNullSD_LRBS=0.70357;

Southern Appalachian (SAP) Wadeable Sites

Cond_1 (eco9-W n=32):
LRBS_use= –0.74349 +0.48842(L_AreaWSkm2_use) –0.75562(W1_Hall)
R^2= 0.2835; AdjR^2=0.2341; RMSE=0.69081; n=32/32; p=0.0079;p1=0.0026;p2=0.0472
---- Set W1_Hall=0 = minimum in ref sites:

RfE1_LRBS= –0.74349
+0.48842(L_AreaWSkm2_u
se)
RfE1_RMSE_LRBS=0.6908
1

Northern Plains (NPL) Boatable Sites

Cond_1D (eco9-B n= 51)
"All-Sites Model" Regression on all 51 NPL boatable sites:
LRBS_use= –0.42002
+0.44371(L_AreaWSkm2_use)+1.26686(LXSlope_use) –
0.08698(AGws_X_KFct)
--- Set AGws_X_KFct = 0 = minimum for the ecoregion:
RfE1D_LRBS= –0.42002
+(0.44371*L_AreaWSkm2_use)
+(1.26686*LXSlope_use)R^2=0.5598; AdjR^2=0.5311;
RMSE All-Sites model= 0.61027; n=50/51;
p<0.0001;p1=0.0089;p2<0.0001;p3=0.0011
If don't have KFactor the following is very equivalent, as KFactors are close to 0.35:LRBS_use= –0.50236 +0.44371(L_AreaWSkm2_use)+1.29164(LXSlope_use) – 0.02628(PCT_AG_WS_use)

RfOE1D_LRBS=LRBS_use – RfE1D_LRBS

Regression using only NPL boatable Reference sites (n=28):
RfOE1D_LRBS = 0.15939 –0.02276(PCT_AG_WS_use)
R^2=0.2322; AdjR^2=0.2026; RMSE=0.51215; n=28/28; p=0.0094; p1=0.0094
-- Set PCT_AG_WS_use=0 = minimum in ref sites.

RfE_OE1D_LRBS
= 0.15939
RfE_OE1D_RMSE
_LRBS=0.51215;

Northern Plains (NPL) Wadeable Sites

Cond_1D (eco9-W n=314)
"All-Sites Model" Regression on all 314 NPL wadeable sites:
LRBS_use= –2.80718 +0.00084015(ELEV_PT_use) –0.70092(LXSlope_use)
+0.64948(LXWidth_use)
–0.20932(W1_HALL) –0.49739(W1H_Crop)
--- Set W1_HALL and W1_Crop = 0, the minima for
the ecoregion: RfE1D_LRBS= –2.80718
+0.00084015(ELEV_PT_use) –0.70092(LXSlope_use)
+0.64948(LXWidth_use) ;
R^2 = 0.3854; AdjR^2=0.3754; RMSE All-
Sites model =0.83720; n=314;
p<0.0001;p1-
3<0.0001;p4=0.0048;p5=0.0553;

RfOE1D_LRBS=LRBS_use – RfE1D_LRBS;
Regression using only NPL
Wadeable Reference sites (n=52):
RfOE1D_LRBS = +0.19752 –
0.31987(W1_Hall);
R^2=0.0280; AdjR^2=0.0086; RMSE=0.83941; n=51/52; p=0.2356;p1=0.2356

RfE_OE1D_LRBS= 0.19752
RfE_OE1D_RMSE_LRBS= 0.83941

Southern Plains & Temperate Plains (SPL + TPL) Boatable Sites
Cond_1 (cenpl-B) -------- n=47 ref sites from TPL, SPL, and NPL
LRBS_use= 1.44046 -0.32356*L_AreaWSkm2_use –
0.02377*AG_1KMCIRCLE R^2=0.1789; Adj R^2=0.1416;
RMSE=1.13936; n=47/47; p=0.0131;p1=0.0852;p2=0.0084
---- Set AG_1KMCircle=0 = minimum in reference sites:

RfE1_LRBS=1.44046 –
0.32356(L_AreaWSkm2_
use)
RfE1_RMSE_LRBS=1.14
939

Southern Plains (SPL) Wadeable Sites
Cond_1D (eco9-W) n= 301
"All-Sites Model" Regression on all SPL wadeable sites:
LRBS_use= 0.89319 –0.06565(LAT_DD83) –0.09181(L_AreaWSkm2_use) –
0.86897(LXSlope_use)
–0.24209(W1_HAG) –0.00308(AG_1KMCIRCLE)
–0.02727(AGws_X_KFct)R^2=0.3525; Adj
R^2=0.3391; RMSE All-Sites=0.95158; n=297/301;
p<0.0001;p1=0.0002;p2=0.0519;p3<0.0001;p4=0.0
155;p5=0.2490;p6=0.0049

--- Set W1_HAG, AG_1KMCircle, AGws_x_KFct = 0 =
minima for SPL wadeable sites:RfE1D_LRBS= +0.89319 –
0.06565(LAT_DD83) –0.09181(L_AreaWSkm2_use) –
0.86897(LXSlope_use)

RfOE1D_LRBS= LRBS_use – RfE1D_LRBS
Regression on SPL wadeable ref sites:
RfE1D_LRBS=
–0.00983 –0.83096(W1_HNOAG) –3.3658(Dam_dii) +0.6857(RdDen_ws_use) –
0.02242(PCT_Ag_ws_use)
R^2 = 0.2616; Adj R^2=0.1817;
RMSE=0.99030; n=42/42;
p=0.0214;p1=0.0618;p2=0.2
056;p3=0.0364;p4=0.0338
---- Set W1_HNOAG, Dam_dii, PCT_Ag_ws_use = 0 = minima for ref sites;
-----Set RdDen_ws_use = 0 (Ref site mimimum = 0.19) ---- zero leads to more lenient expected condition.

RfE_OE1D_LRBS= –0.00983
RfE_OE1D_RMSE_LRBS= 0.99030

Temperate Plains (TPL) Wadeable Sites
Cond_1D (eco9-W) -- All-Sites Model Regression on all 344 TPL
wadeable sites: LRBS_use= 0.22205 +0.04387(LAT_DD83)
+0.03596(LON_DD83) –0.49057(LXSlope_use)
–0.08247(W1_HAG) –0.01116(AG_1KMCIRCLE);
--- Set W1_HAG and AG_1KMCIRCLE = 0 = minima for region:
RfE1D_LRBS=0.22205 +0.04387(LAT_DD83)
+0.03596(LON_DD83) –0.49057(LXSlope_use) ;R^2=0.1974; Adj
R^2=0.1854; RMSE- All-Sites =0.97639; n=342/344;
p<0.0001;p1=0.0556;p2=0.0074;p3<0.0001;p4=0.4971;p5<0.0001

RfOE1D_LRBS= LRBS_use – RfE1D_LRBS
Regression on TPL ref sites:
RfE1D_LRBS= +0.21704 –0.83169(W1_HNOAG) +6.55336(W1H_Crop) –
0.0228(Ag_1KmCircle)
–0.05988(PCT_Ag_ws_use)
+0.19465(AgWS_x_KFct)R^2 =
0.3279; Adj R^2=0.2633;
RMSE=0.93335; n=58
/61 ; p=0.0007;p1=0.0608;p2=0.0295;p3=0.0065;p4=0.0107;p5=0.0036
--- Set W1_HNOAG, W1H_Crop, Ag_1KmCircle , PCT_Ag_ws_use, and
AgWS_x_KFct = 0 = minimafor ref sites;

RfE_OE1D_LRBS=0.217
04 = y-intercept from
above
RfE_OE1D_RMSE_LRB
S=0.93335

Upper Midwest (UMW) Boatable Sites

Cond_1 (eco9-B n=36):
LRBS_use= 22.86206 –0.50298(LAT_DD83) –0.92704(W1_HALL)
R^2=0.1820; Adj R^2=0.1324; RMSE=1.25933; n=36/36; p=0.0363;p1=0.0113;p2=0.2028
--- Set W1_HALL= 0 = minimum for regional ref sites

RfE1_LRBS=22.86
206 –
0.50298(LAT_DD8
3)
RfE1_RMSE_LRB
S=1.25933;

Upper Midwest (UMW) Wadeable Sites

Cond_1 (eco9-W n=43):
LRBS_use = –1.38974 –0.69289(LXSlope_use) –0.26824(W1_HALL)
R^2=0.4103; Adj R^2=0.3808; RMSE=0.92535; n=43/43; p<0.0001;p1<0.0001;p2=0.5347
--- Set W1_HALL=0 = minimum for regional ref sites:

RfE1_LRBS= –
1.38974 –
0.69289(LXSlope_us
e)
RfE1_RMSE_LRBS
=0.92535

Western Mountain (WMT) Boatable Sites

Cond_N (eco9-B n=43):
RfNullM_LRBS= 0.36550RfNullSD_LRBS=0.48996

Western Mountain (WMT) Wadeable Sites

Cond_1 (eco9-B n=69):
LRBS_use = –0.77810 –0.31541(LXSlope_use) +0.48616(LXWidth_use)
R^2 = 0.2739; Adj R^2=0.2516; RMSE=0.42995; n=68/69; p<0.0001;p1=0.0382;p2=0.022333

RfE1_LRBS= –0.77810 –0.31541(LXSlope_use)
+0.48616(LXWidth_use)RfE1_RMSE_LRBS=0.42995

Xeric (XER) Boatable Sites

Cond_N (eco9-B n=24):
RfNullM_LRBS= 0.08641RfNullSD_LRBS=0.98518

Xeric (XER) Wadeable Sites

Cond_1 (eco9-W n=36):
LRBS_use = –2.01510 +1.33328(LXWidth_use)
R^2=0.2333; Adj R^2=0.2107; RMSE=0.79439; n=36/36 ; p=0.0028;p1=0.0028

```
RfE1_LRBS= –
2.01510
+1.33328(LXWidth_u
se)
RfE1_RMSE_LRBS=
0.79439
```

CONDITION ASSIGNMENTS FOR LRBS_use NULL MODELS:

```
RfNull25_LRBS=RfNullM_LRB
S-(0.67*RfNullSD_LRBS);
RfNull05_LRBS=RfNullM_LRB
S-(1.65*RfNullSD_LRBS);

RfOENull_LRBS=LRBS_us

e-RfNullM_LRBS;

LRBS_Cond_N='XXXX';
if LRBS_use<=RfNull05_LRBS then LRBS_Cond_N='Poor';
if LRBS_use>RfNull05_LRBS and
LRBS_use<=RfNull25_LRBSthen
LRBS_Cond_N='Medi';
if LRBS_use>RfNull25_LRBS then LRBS_Cond_N='Good';

If RfOENull_LRBS=. then
LRBS_COND_N='XXXX';
If LRBS_use=. then
LRBS_COND_N='XXXX';
```

CONDITION ASSIGNMENTS FOR LRBS_use COND_1 O/E MODELS:

```
RfOE1_LRBS=LRBS_use-RfE1_LRBS;

RfE1_25_LRBS=RfE1_LRBS-
(0.67*RfE1_RMSE_LRBS);
RfE1_05_LRBS=RfE1_LRBS-
(1.65*RfE1_RMSE_LRBS);

LRBS_Cond_1='XXXX';
if LRBS_use<=RfE1_05_LRBS then LRBS_Cond_1='Poor';
if LRBS_use>RfE1_05_LRBS and
 LRBS_use<=RfE1_25_LRBSthen
 LRBS_Cond_1='Medi';
if LRBS_use>RfE1_25_LRBS then LRBS_Cond_1='Good';

If RfE1_LRBS=. then LRBS_COND_1='XXXX';
If LRBS_use=. then LRBS_COND_1='XXXX';
```

CONDITION ASSIGNMENTS FOR LRBS_use COND_1D ("All-Sites") O/E MODELS:

*** NOTE RfOE1D_LRBS=LRBS_use-RfE1D_LRBS;
*** We base expectations on the distribution of OE in ref sites;

RfE_OE1D_25_LRBS=RfE_OE1D_LRBS-
(0.67*RfE_OE1D_RMSE_LRBS);
RfE_OE1D_05_LRBS=RfE_OE1D_LRBS-
(1.65*RfE_OE1D_RMSE_LRBS);

if RfOE1D_LRBS<=RfE_OE1D_05_LRBS then LRBS_Cond_1D='Poor';
if RfOE1D_LRBS> RfE_OE1D_05_LRBS and RfOE1D_LRBS<=RfE_OE1D_25_LRBS
then LRBS_Cond_1D='Medi';
if RfOE1D_LRBS> RfE_OE1D_25_LRBS then LRBS_Cond_1D='Good';

If RfOE1D_LRBS=. then LRBS_COND_1D='XXXX';
If LRBS_use=. then LRBS_COND_1D='XXXX';

Reference Condition Models for Riparian Vegetation CoverCondition based on Log_{10}(0.01+XCMGW)

Coastal Plain (CPL) Boatable Sites

Cond_1 (eco9-B n=52):
LPt01_XCMGW= 0.83657 +0.00658(LON_DD83) –
0.06020(L_AreaWSkm2_use) –0.57160(W1_HAG);
R^2=0.2583; AdjR^2=0.2121; RMSE=0.11862; n=52/52 ; p=0.0023; p1=0.0570; p2=0.0461;
p3=0.0166
--- Set W1_HAG = 0 = minimum for ref sites in region:

RfE1_LXCMGW=0.83657 +0.00658(LON_DD83) –
0.06020(L_AreaWSkm2_use);
RfE1_RMSE_LXCMGW=0.11862;

Coastal Plain (CPL) Wadeable Sites

Cond_1 (eco9-W n=51):
LPt01_XCMGW= –0.58185 –0.00700(LON_DD83)
R^2= 0.0551; AdjR^2= 0.0358; RMSE=0.15238; n=51/51 p=0.0972 p1=0.0972

RfE1_LXCMGW= –
0.58185 –
0.00700(LON_DD83)
RfE1_RMSE_LXCMGW
=0.15238

Northern Appalachian (NAP) Boatable Sites

Cond_1-eco9-B:
Lpt01_XCMGW= 2.51398 –0.05498(LAT_DD83)
-0.00786(AG_1KMCIRCLE) –
0.79370(PCT_AG_WS_use) +2.68820(Agws_X_KFct)R^2
= 0.4025; AdjR^2=0.3456; RMSE=0.15628; n=47/47
p=0.0002; p1=0.0056; p2=0.0107;p3=0.0005;p4=0.0007
--- Set AG_1KMCIRCLE, PCT_AG_WS_use and AGws_X_KFct = 0 = minima for reference sites:

RfE1_LXCMGW=2.51
398 –
0.05498(LAT_DD83)
RfE1_RMSE_LXCMG
W=0.15628

Northern Appalachian (NAP) Wadeable Sites

Cond_1 (eco9-W n=41):
LPt01_XCMGW=0.21141+0.09026(L_AreaWSkm2_use)
–0.30883(LXWidth_use) –0.14456(W1_HALL)
R^2 = 0.2411; AdjR^2=0.1795; RMSE=0.12059; n= 41/41
p=0.0159;p1=0.0894;p2=0.0130;p3=0.0293
--- Set W1_HALL = 0 = minimum for reference sites:

RfE1_LXCMGW=0.21141
+0.09026(L_AreaWSkm2_use) –0.30883(LXWidth_use);
RfE1_RMSE_LXCMGW=0.12059

Southern Appalachian (SAP) Boatable Sites

Cond_1 (eco9-B n=22):
LPt01_XCMGW= 0.02698 –0.44778(W1_HAG)
R^2 = 0.1689; AdjR^2=0.1274; RMSE= 0.14138; n= 22/22 p=0.0574; p1=0.0574
--- Set W1_HAG = 0 = minimum for reference sites:

RfE1_LXCMGW=
0.02698
RfE1_RMSE_LXC
MGW=0.14138;

Southern Appalachian (SAP) Wadeable Sites

Cond_1 (eco9-W n=32):
LPt01_XCMGW= –0.14633+0.04120(L_AreaWSkm2_use) +0.00051106(ELEV_PT_use)
–0.16089(W1_HALL);
R2=0.3232; AdjR^2=0.2507; RMSE= 0.14090; n= 32/32 ; p=0.0111; p1=0.2142;
p2=0.0028; p3=0.0429
Set W1_HALL = 0 = minimum for reference sites:

RfE1_LXCMGW= –0.14633 +0.04120(L_AreaWSkm2_use)
+0.00051106(ELEV_PT_use);
RfE1_RMSE_LXCMGW=0.14090;

Northern Plains (NPL) & Southern Plains (SPL) Boatable Sites

Cond_1D (CENPL-B n=249): -- All-Sites Regression on All CENPL Boatable sites (NPL, SPL, & TPL) LPt01_XCMGW= 1.80926 –0.02245(LAT_DD83)
+0.01036(LON_DD83)
–0.24323(W1_HAG) –
0.11970(RDDEN_WS_use)+0.00306(PCT_AG_WS_
use)R^2 = 0.2485; AdjR^2=0.2331; RMSE All-Sites=
0.36204; n= 249/249
p<0.0001;p1=0.0685;p2<0.0001;p3=0.0283;p4=0.01
13.
--- Set W1_HAG and PCT_AG_WS_use = 0 = minimum in NPL+SPL ref sites
--- Set RDDEN_WS_use, = 0 --------(minimum for NPL+SPL ref sites = 0.043):
RfE1D_LXCMGW= 1.80926 –0.02245*LAT_DD83)+(0.01036*LON_DD83)

Regression on TPL CENPL ref sites:
RfOE1D_LXCMGW=LPt01_XCMG
W – RfE1D_LXCMGW;
RfE_OE1D_LXCMGW = –0.08047 –
0.01773(PCT_AG_WS_use)
R^2 = 0.3141; AdjR^2=0.2877; RMSE = 0.32423; n= 28/28; p=0.0019;p1=0.0019
--- Set PCT_AG_WS_use = 0 = min in ref sites

RfE_OE1D_LXCMGW = –0.08047 ;
RfE_OE1D_RMSE_LXCMGW=0.32423;

Northern Plains (NPL) Wadeable Sites

Cond_1D (CENPL-W n=959) All-Sites Regression on All CENPL
Wadeable sites (NPL, SPL, & TPL) :LPt01_XCMGW= 2.43249 –
0.02325(LAT_DD83) +0.01579(LON_DD83)+0.16417(LXSlope_use)
–0.32696(W1_HAG) –0.00256(PCT_AG_WS_use)
R^2 = 0.3126; AdjR^2=0.3088; RMSE- All-Sites =0.48720; n=922/959; p<0.0001;p1-p4<0.0001;p5=0.0002
--- Set W1_HAG and PCT_AG_WS_use = 0 = minima in ref sites of NPL (also SPL & TPL):

RfE1D_LXCMGW= 2.43249 –0.02325*LAT_DD83) +(0.01579*LON_DD83)
+(0.16417*LXSlope_use)

RfOE1D_LXCMGW=LPt01_XCMGW – RfE1D_LXCMGW;
---- Regression using only CENPL Wadeable Ref sites (155)
RfOE1D_LXCMGW = –0.13159 –2.01216(Dam_dii) –0.02708(PCT_AG_WS_use) +
0.08125(AgWs_x_KFct);
R^2 =0.1443; AdjR^2=0.1270; RMSE=0.38555; n=152/155;

p<0.0001;p1=0.0015;p2=0.0006;p3=0.0006
--- Set Dam_dii, PCT_AG_WS_use & AgWs_x_KFct = 0 = minima in CENPL and NPL alone:

RfE_OE1D_LXCMGW= –0.13159;
RfE_OE1D_RMSE_LXCMGW=0.38555;

Southern Plains (SPL) Boatable Sites

----- see combined NPL & SPL Boatable Sites above

Southern Plains (SPL) & Temperate Plains (TPL) Wadeable Sites

Cond_1b (spl-tpl-umw-W -- ref sites only n=146):
LPt01_XCMGW= 1.25746 +0.01355(LON_DD83) –0.00024404(ELEV_PT_use)
–0.00636(AG_1KMCIRCLE) –0.02587(PCT_AG_WS_use)
+0.08055(AGws_X_KFct) R^2=0.3956; AdjR^2=0.3735; RMSE=0.26692;
n=143/146 p<0.0001;p1=0.0088;p2=0.0048;p3=0.0013;p4-5<0.0001
--- Set AG_1KMCIRCLE, PCT_AG_WS_use & AGws_X_KFct = 0 = minima in both SPL and TPL refsites:

RfE1b_LXCMGW= 1.25746 +0.01355(LON_DD83) –
0.00024404(ELEV_PT_use);
RfE1b_RMSE_LXCMGW=0.26692;

Temperate Plains (TPL) Boatable Sites

Cond_N (eco9-B
n=22):
RfNullM_LXCM
GW= –0.08249 ;
RfNullSD_LXCMGW= 0.15980 ;

Temperate Plains (TPL) Wadeable Sites

----- see combined SPL & TPL Wadeable sites above

Upper Midwest (UMW) Boatable Sites

Cond_1b (SPL + TPL+ UMW Boatable Ref sites n=55):

LPt01_XCMGW=1.52755 –0.03762(LAT_DD83) –0.33101(L_AreaWSkm2_use)
+0.17072(LXSlope_use)
+0.82145(LXWidth_use) R^2-
Square=0.3354 AdjR^2=0.2822;
RMSE=0.37273 n=55/55;
p=0.0003;p1=0.0057;p2=0.0019;
p3=0.0284;p4=0.0003

Expected Ref condition model applied only to UMW

Boatable sites: RfE1b_LXCMGW= 1.52755 –

0.03762*LAT_DD83) –0.33101*L_AreaWSkm2_use)
+(0.17072*LXSlope_use
) +(0.82145*LXWidth_use)
RfE1b_RMSE_LXCMGW=0.37
273

Upper Midwest (UMW) Wadeable Sites

Cond_1 (eco9-W n=43):
LPt01_XCMGW= –0.13511 +0.05069(LXSlope_use)+0.17937(LXWidth_use) –0.06747(W1_HALL)
R^2=0.3303 AdjR^2=0.2465; RMSE=0.12999 n=43/43 ;
p=0.0028;p1=0.0115;p2=0.0025;p3=0.2867
--- Set W1_HALL = 0 = minimum for ref sites:

RfE1_LXCMGW= –0.13511
+(0.05069*LXSlope_use) +(0.17937*LXWidth_use)
RfE1_RMSE_LXCMGW= 0.12999

Western Mountains (WMT) Boatable Sites

Cond_N (eco9-B n=43):
RfNullM_LXCMGW= –0.12272
RfNullSD_LXCMGW= 0.26191

Western Mountains (WMT) Wadeable Sites

Cond_1 (eco9-W n=69):
LPt01_XCMGW=0.24290 –0.09638(L_AreaWSkm2_use) –0.00007192(ELEV_PT_use) –.11520(LXSlope_use)
R^2= 0.2037; AdjR^2=0.1669; RMSE= 0.15289; n=68/69;
p=0.0019;p1=0.0063;p2=0.0024;p3=0.0425

RfE1_LXCMGW= 0.24290 –0.09638(L_AreaWSkm2_use) –0.00007192(ELEV_PT_use)
–
0.11520
*LXSlo
pe_use)
RfE1_R
MSE_L
XCMG
W=0.15
289

Xeric (XER) Boatable Sites

Cond_1 (eco9-B n=24):
LPt01_XCMGW= –0.32820 +0.24638(W1_HNOAG) –0.15614(W1_HAG)
R^2=0.2896; AdjR^2=0.2220; RMSE=0.15263; n=24/24 ; p=0.0276;p1=0.0273;p2=0.1633;

Set W1_HNOAG (positive beta) and W1_HAG (negative beta) = 0 = minima for ref sites; Note this results in lower ref mean, smaller RMSE, but lower (more lenient) percentile values than NULL

RfE1_L
XCMG
W= –
0.32820
RfE1_R
MSE_L
XCMG
W=0.15
263

Xeric (XER) Wadeable Sites

Cond_1 (eco9-W n=36):
LPt01_XCMGW= –0.21113 –0.19122(L_AreaWSkm2_use) +0.19148(LXSlope_use) +0.65498(LXWidth_use)
R^2=0.2294; AdjR^2=0.1571; RMSE=0.25328; n=36/36;
p=0.0374;p1=0.0695;p2=0.0730;p3=0.0086

RfE1_LXCMGW= –0.21113 –0.19122*L_AreaWSkm2_use) +(0.19148*LXSlope_use) +(0.654
98*LX
Width_
use);
RfE1_R
MSE_L
XCMG
W=0.25
328

CONDITION ASSIGNMENTS FOR RIPARIAN VEGETATION COVER NULL MODELS:

```
RfNull25_LXCMGW=RfNullM_LXCMGW-
(0.67*RfNullSD_LXCMGW);
RfNull05_LXCMGW=RfNullM_LXCMGW-
(1.65*RfNullSD_LXCMGW);
RfOENull_LXCMGW=LPt01_XCMGW-
RfNullM_LXCMGW;

LXCMGW_Cond_N='XXXX';
if LPt01_XCMGW<=RfNull05_LXCMGW then LXCMGW_Cond_N='Poor';
if LPt01_XCMGW>RfNull05_LXCMGW and
 LPt01_XCMGW<=RfNull25_LXCMGWthen
 LXCMGW_Cond_N='Medi';
if LPt01_XCMGW>RfNull25_LXCMGW then
LXCMGW_Cond_N='Good';if LPt01_XCMGW
```

=. then LXCMGW_Cond_N='XXXX';

CONDITION ASSIGNMENTS FOR RIPARIAN VEGETATION COVER COND_1 O/E MODELS:

```
RfOE1_LXCMGW=LPt01_XCMGW-RfE1_LXCMGW;

RfE1_25_LXCMGW=RfE1_LXCMGW-
(0.67*RfE1_RMSE_LXCMGW);
RfE1_05_LXCMGW=RfE1_LXCMGW-
(1.65*RfE1_RMSE_LXCMGW);

LXCMGW_Cond_1='XXXX';
if LPt01_XCMGW<=RfE1_05_LXCMGW then LXCMGW_Cond_1='Poor';
if LPt01_XCMGW>RfE1_05_LXCMGW and LPt01_XCMGW<=RfE1_25_LXCMGW
then LXCMGW_Cond_1='Medi';
if LPt01_XCMGW>RfE1_25_LXCMGW then
LXCMGW_Cond_1='Good';If
RfE1_LXCMGW=. then
LXCMGW_Cond_1='XXXX';
if LPt01_XCMGW =. then LXCMGW_Cond_1='XXXX';
```

CONDITION ASSIGNMENTS FOR RIPARIAN VEGETATION COVER COND_1b O/EMODELS:

```
RfE1b_25_LXCMGW=RfE1b_LXCMGW-
(0.67*RfE1b_RMSE_LXCMGW);
RfE1b_05_LXCMGW=RfE1b_LXCMGW-
(1.65*RfE1b_RMSE_LXCMGW);

LXCMGW_Cond_1b='XXXX';
if LPt01_XCMGW<=RfE1b_05_LXCMGW then LXCMGW_Cond_1b='Poor';
if LPt01_XCMGW>RfE1b_05_LXCMGW and LPt01_XCMGW<=RfE1b_25_LXCMGW
then LXCMGW_Cond_1b='Medi';

if LPt01_XCMGW>RfE1b_25_LXCMGW then
LXCMGW_Cond_1b='Good';If
RfE1b_LXCMGW=. then
LXCMGW_Cond_1b='XXXX';
if LPt01_XCMGW =. then LXCMGW_Cond_1b='XXXX';
```

CONDITION ASSIGNMENTS FOR RIPARIAN VEGETATION COVER COND_1D ("All-Sites") O/E MODELS:

```
RfE_OE1D_25_LXCMGW=RfE_OE1D_LXCMGW-
(0.67*RfE_OE1D_RMSE_LXCMGW);
RfE_OE1D_05_LXCMGW=RfE_OE1D_LXCMGW-
(1.65*RfE_OE1D_RMSE_LXCMGW);
```

LXCMGW_Cond_1D='XXXX';
if RfOE1D_LXCMGW<=RfE_OE1D_05_LXCMGW then
LXCMGW_Cond_1D='Poor';if RfOE1D_LXCMGW>
RfE_OE1D_05_LXCMGW and
RfOE1D_LXCMGW<=RfE_OE1D_25_LXCMGW
then LXCMGW_Cond_1D='Medi';
if RfOE1D_LXCMGW> RfE_OE1D_25_LXCMGW then LXCMGW_Cond_1D='Good';

If RfE_OE1D_LXCMGW=. then LXCMGW_Cond_1D='XXXX';
if RfOE1D_LXCMGW =. then LXCMGW_Cond_1D='XXXX';

Reference Condition Models for Instream Fish Coverbased on $Log_{10}(0.01+XFC\_NAT)$

Coastal Plain (CPL) Boatable Sites

Cond_N (eco9-B n=52):
RfNullM_LXFC_NAT= –0.57048 ;
RfNullSD_LXFC_NAT= 0.23527 ;

Coastal Plain (CPL) Wadeable Sites

Cond_N (eco9-B n=51):
RfNull
M_LXF
C_NAT
= –
0.39218
;
RfNullS
D_LXF
C_NAT
=0.2982
0 ;

Northern Appalachian (NAP) Boatable Sites

Cond_1 (eco9-B n=47):
LPt01_XFC_NAT= –5.46962 –0.06654(LON_DD83) –0.46088(L_AreaWSkm2_use)
+0.92383(LXWidth_use)
–1.05887(W1H_Crop);
R^2= 0.3404; AdjR^2=0.2776; RMSE=0.31921; n=47;
p=0.0013;p1=0.0047;p2=0.0897;p3=0.0483;p4=0.0082
--- Set W1H_Crop = 0 = minimum in ref sites:

RfE1_LXFC_NAT= –5.46962 –0.06654(LON_DD83) –0.46088(L_AreaWSkm2_use)
+(0.92383(LXWidt

h_use) ;
RfE1_RMSE_LXF
C_NAT=0.31921;

Northern Appalachian (NAP) Wadeable Sites

Cond_1 (eco9-W n=41):
LPt01_XFC_NAT= –0.08246 –0.26338(LXWidth_use) ;
R^2=0.0736; AdjR^2=0.0499; RMSE=0.28459; n=41; p=0.0862;p1=0.0862

RfE1_LXFC_NAT= –
0.08246 –
0.26338(LXWidth_use);
RfE1_RMSE_LXFC_NAT=
0.28459;

Southern Appalachian (SAP) Boatable Sites

Cond_1 (eco9-W n=22) :
LPt01_XFC_NAT= -3.54570+0.07646(LAT_DD83) +0.22940(W1_HALL);
R^2= 0.5343; AdjR^2=0.4852; RMSE=0.17528; n=22/22; p=0.0007;p1=0.0089;p2=0.0065
--- Set W1_HALL= 0 -- note it is a positive association (mimimum in ref sites=0.03; in all sites=0):

RfE1_LXFC_NAT= –
3.54570+(0.07646*LAT_D
D83);
RfE1_RMSE_LXFC_NAT=
0.17528;

Southern Appalachian (SAP) Wadeable Sites

Cond_1 (eco9-W n=32):
LPt01_XFC_NAT= –2.89088 +0.06090(LAT_DD83)
+0.00062631(ELEV_PT_use) –7.37514(W1_HAG) ;
R^2=0.4169; AdjR^2=0.3544; RMSE=0.31006; n=32/32;
p=0.0015;p1=0.0896;p2=0.0785;p3=0.0041
--- Set W1_HAG = 0 = minimum for ref sites:

RfE1_LXFC_NAT= –2.89088 +(0.06090*LAT_DD83)
+(0.00062631*ELEV_PT_use) ;RfE1_RMSE_LXFC_NAT=
0.31006;

CENPL (NPL, SPL, TPL) Northern, Southern & Temperate Plains -- Boatable Sites

Cond_1 (CENPL-B n=47):
LPt01_XFC_NAT= 2.42961 –0.02335(LAT_DD83) +0.01564(LON_DD83) –
0.11096(L_AreaWSkm2_use)
–0.00934(AG_1KMCIRCLE) ;
R^2=0.3446; AdjR^2=0.2822; RMSE=0.32257; n=47/47;

p=0.0012;p1=0.1204;p2=0.1228;p3=0.0400;p4=0.0070
--- Set AG_1KMCIRCLE = 0 = min for CENPL {Minima are 0%,
3.6%, 0.06% for NPL(n=33),SPL(n=2), TPL(n=22)}:

RfE1_LXFC_NAT= 2.42961 –0.02335*LAT_DD83) +(0.01564*LON_DD83) –
0.11096*
L_AreaW
Skm2_use
);
RfE1_RM
SE_LXFC
_NAT=
0.32279;

CENPL (NPL, SPL, TPL) Northern, Southern & Temperate Plains -- Wadeable Sites

Cond_1 (CENPL-W n=155):
LPt01_XFC_NAT= –0.20615 +0.00409(LON_DD83) –
0.08735(L_AREAWSkm2_use)+0.00025270(ELEV_PT_use)
–0.00258(AG_1KMCIRCLE)
+0.04332(URB_1KMCIRCLE);R^2
=0.1740; AdjR^2=0.1457;
RMSE=0.33531; n=152/155;
p<0.0001;p1=0.5890;p2=0.0039;p3=0.0154;p4=0.1134;p5=0.0032
---- Set AG_1KMCIRCLE and URB_1KMCIRCLE =0 = minima for ref sites each of the 3 regions:

RfE1_LXFC_NAT= –0.20615 +(0.00409*LON_DD83) –
0.08735*L_AREAWSkm2_use)+(0.00025270*ELEV_PT_use);
RfE1_RMSE_LXFC_NAT=0.33531;

Upper Midwest (UMW) Boatable Sites

Cond_1 (eco9-B n=36)
LPt01_XFC_NAT= 3.97716 +0.05232(LON_DD83) –0.20032(W1_HAG);
R^2=0.2349 AdjR^2=0.1885; RMSE=0.31606; n=36/36 ; p=0.0121;p1=0.0049;p2=0.8532
--- Set W1_HAG = 0 = minimum in ref sites:

RfE1_LXFC_NAT= 3.97716 +(0.05232*LON_DD83);
RfE1_RMSE_LXFC_NAT= 0.31606;

Upper Midwest (UMW) Wadeable Sites

Cond_1 (eco9-W n=43):
LPt01_XFC_NAT= –0.48451
+0.17605(L_AreaWSkm2_use) –0.35844(LXWidth_use) ;R^2
=0.0740; AdjR^2=0.0277; RMSE=0.29010;
n=43/43;p=0.2151;p1=0.0818;p2=0.1406

RfE1_LXFC_NAT= –0.48451

+(0.17605*L_AreaWSkm2_use) –0.35844*LXWidth_use);
RfE1_RMSE_LXFC_NAT= 0.29010;

Western Mountain (WMT) Boatable Sites

Cond_1 (eco9-B n=43):
LPt01_XFC_NAT= –1.40552 +0.48649(LXWidth_use)
–5.67454(W1H_Crop) –0.11975(RDDEN_WS_use) ;
R^2=0.2408; AdjR^2=0.1824; RMSE=0.23044; n=43/43;
p=0.0124;p1=0.0175;p2=0.0077;p3=0.0654
--- Set W1H_Crop and RDDEN_WS_use = 0 = minima for ref sites:

RfE1_LXFC_NAT= –1.40552 +(0.48649*LXWidth_use)
RfE1_RMSE_LXFC_NAT= 0.23044;

Western Mountain (WMT) Wadeable Sites

Cond_1 (eco9-W n=69):
LPt01_XFC_NAT= 1.57993 +0.01058(LAT_DD83) +0.01895(LON_DD83) –
0.08287(L_AreaWSkm2_use)
–11.24156(W1_HAG) –
0.05374(RDDEN_WS_use
) ; R^2 =0.3466;
AdjR^2=0.2939;
RMSE=0.21669 n=68/69;
p<0.0001;p1=0.1652;p2=0.0013;p3=0.0414;p4=0.0054;p5=0.1064
--- Set W1_HAG and RDDEN_WS_use = 0 = minima for ref sites:

RfE1_LXFC_NAT= 1.57993 +(0.01058*LAT_DD83) +(0.01895LON_DD83) –
0.08287(L_AreaW
Skm2_use);
RfE1_RMSE_LXF
C_NAT=0.21669;

Xeric (XER) Boatable Sites

Cond_1 (eco9-B n=24):
LPt01_XFC_NAT= –0.03292 –0.00013276(ELEV_PT_use) –
0.42159(LXWidth_use) ; R^2 =0.1266; AdjR^2=0.1266;
RMSE=0.31024; n=23/24; p=0.2582;p1=0.1323;p2=0.1973

RfE1_LXFC_NAT= –0.03292 –0.00013276*ELEV_PT_use)
–0.42159*LXWidth_use) ;RfE1_RMSE_LXFC_NAT=
0.31024;

Xeric (XER) Wadeable Sites

Cond_1 (eco9-W n=36):
LPt01_XFC_NAT= 0.96284
+0.01132(LON_DD83)+0.18104(LXSlope_use) –19.86518(W1H_Crop);
R^2=0.2738; AdjR^2=0.2057; RMSE=0.24231 n=36/36;
p=0.0155;p1=0.1628;p2=0.0431;p3=0.0353

--- note LXSlope distribution is similar across the range of the other model variables in all sites;
--- Set W1H_Crop = 0 = minimum in ref sites:

```
RfE1_LXFC_NAT= 0.96284 +(0.01132*LON_DD83)
+(0.18104*LXSlope_use);
RfE1_RMSE_LXFC_NAT=0.24231;
```

CONDITION ASSIGNMENTS FOR INSTREAM FISH COVER NULL MODELS:

```
RfNull25_LXFC_NAT=RfNullM_LXFC_NAT-
(0.67*RfNullSD_LXFC_NAT);
RfNull05_LXFC_NAT=RfNullM_LXFC_NAT-
(1.65*RfNullSD_LXFC_NAT);
RfOENull_LXFC_NAT=LPt01_XFC_NAT-
RfNullM_LXFC_NAT;

LXFC_NAT_Cond_N='XXXX';
if LPt01_XFC_NAT<=RfNull05_LXFC_NAT then LXFC_NAT_Cond_N='Poor';
if LPt01_XFC_NAT>RfNull05_LXFC_NAT and
 LPt01_XFC_NAT<=RfNull25_LXFC_NATthen
 LXFC_NAT_Cond_N='Medi';
if LPt01_XFC_NAT>RfNull25_LXFC_NAT then
LXFC_NAT_Cond_N='Good';If LPt01_XFC_NAT =.
then LXFC_NAT_Cond_N='XXXX';
```

CONDITION ASSIGNMENTS FOR INSTREAM FISH COVER COND_1 O/E MODELS:

```
RfE1_25_LXFC_NAT=RfE1_LXFC_NAT-
(0.67*RfE1_RMSE_LXFC_NAT);
RfE1_05_LXFC_NAT=RfE1_LXFC_NAT-
(1.65*RfE1_RMSE_LXFC_NAT);
RfOE1_LXFC_NAT=LPt01_XFC_NAT-
RfE1_LXFC_NAT;

if LPt01_XFC_NAT<=RfE1_05_LXFC_NAT then LXFC_NAT_Cond_1='Poor';
if LPt01_XFC_NAT>RfE1_05_LXFC_NAT and
LPt01_XFC_NAT<=RfE1_25_LXFC_NAT
then LXFC_NAT_Cond_1='Medi';
if LPt01_XFC_NAT>RfE1_25_LXFC_NAT then
LXFC_NAT_Cond_1='Good';If
RfE1_LXFC_NAT=. then
LXFC_NAT_COND_1='XXXX';
If LPt01_XFC_NAT =. then LXFC_NAT_Cond_1='XXXX';
```

9 HUMAN HEALTH FISH TISSUE INDICATOR

Fish are time-integrating indicators of persistent pollutants, and the bioaccumulation of contaminants in fish tissue has important human health implications. Contaminants in fish pose various health risks to human consumers (e.g., cancer risks, and noncancer risks such as reproductive effects or impacts to neurological development). The NRSA 2018-19 human health fish tissue indicator consists of collection and analysis of two types of fish composite samples, including whole fish samples for homogenized fillet analyses and fish fillet plug samples. Collectively, these samples provide information on the national distribution of selected persistent, bioaccumulative, and toxic (PBT) chemical residues (specifically, mercury, polychlorinated biphenyls or PCBs, and per- and polyfluoroalkyl substances or PFAS) in fish species that people might catch and eat. The whole fish samples for homogenized fillet analyses were collected from a subset of 290 rivers 5th order and greater in size in the conterminous United States (because these rivers are more likely to contain predator fish commonly consumed by humans) whereas the fish fillet plug samples were collected from all river and stream sites regardless of river or stream size. Results of analyses of mercury, PCB, and PFAS fillet tissue concentrations are presented for this indicator.

9.1 FIELD FISH COLLECTION

9.1.1 WHOLE FISH SAMPLES FOR HOMOGENIZED FILLET ANALYSIS

The human health fish tissue indicator field and analysis procedures described below were based on EPA's National Study of Chemical Residues in Lake Fish Tissue (EPA 2009) and EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*, Volumes 1-2 (third edition) (EPA 2000).

The NRSA crews collected whole fish samples for the fillet tissue indicator from a subset of rivers 5th order and greater in size. The fish samples collected for fillet tissue analysis consisted of a composite of fish specimens (i.e., typically five similarly sized individuals of one target species)[5] from each site. The fish had to be large enough to provide sufficient tissue for analysis and for archiving, when possible. Additional criteria for each fish composite sample included fish that were:

- All of the same species (for each site);
- Harvestable size per legal requirements or of consumable size if there were no harvest limits; and
- At least 190 mm in length and of similar size so that the smallest individual in the composite was no less than 75% of the total length of the largest individual in the composite.

Crews were provided with a recommended list of target and alternate fish species (**Table 9-1**), but they could choose an appropriate substitute if none of the recommended fish species were available. Fish collection data were screened to exclude individual fish specimens with lengths less than 190 mm or composite samples where field crews collected non-target species.

[5] Use of composite sampling for screening studies is a cost-effective way to estimate average contaminant concentrations while also ensuring that there is sufficient fish tissue to analyze for all contaminants of concern.

To prepare fillet composite samples for chemical analysis, fish composite samples from each site were scaled and filleted in the laboratory. In filleting individual fish, muscle tissue was removed from both sides of each fish leaving the skin on and the belly flap attached. Fillets from the individual specimens that comprised a composite sample were homogenized together before being analyzed for contaminants.

9.1.2 FISH TISSUE PLUGS

NRSA crews attempted to collect fish for the tissue plug analysis from all river and stream sites regardless of river or stream size. Two fish tissue plugs for mercury analysis were removed from two fish of the same species (one plug per fish) from the target list. These fish were collected during the fish assemblage sample collection effort. A plug tissue sample was collected by inserting a biopsy punch into a descaled thicker area of dorsal muscle section of a live fish. After collection, antibiotic salve was placed over the wound and the fish was released.

Crews were provided with a recommended list of target and alternate fish species (**Table 9-1**), but they could choose an appropriate substitute if none of the recommended fish species were available. Suitable alternate species include species that are typically consumed by humans and meet the minimum size requirements (i.e., at least 190 mm in length).

Table 9-1. Recommended Target Species and Alternate Species for Fish Tissue Indicator Sample Collection.

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

9.2 MERCURY ANALYSIS AND FISH TISSUE CRITERION FOR HUMAN HEALTH

All fish tissue samples (both homogenized fillet composite tissue and fillet tissue plug samples) were analyzed for total mercury. The samples were prepared using EPA Method 1631B, Appendix A (EPA 2001a) and analyzed using EPA Method 1631E (EPA 2002), which utilizes approximately 1 g of fillet tissue for analysis. In screening-level studies of fish contamination, EPA guidance recommends monitoring for total mercury rather than methylmercury (an organic form of mercury) since most mercury in adult fish is in the toxic form of methylmercury, which will be captured during an analysis for total mercury. Applying the assumption that all mercury is present in fish tissue as methylmercury is also protective of human health. The fish tissue criterion used to interpret mercury concentrations in fillet tissue for human health protection is 0.3 milligrams (mg) of

methylmercury per kilogram (kg) of tissue (wet weight), or 300 parts per billion (ppb), which is EPA's fish tissue-based CWA Section 304(a) water quality criterion recommendation for methylmercury (EPA 2001b).[6] For more information on the fish tissue criterion or screening levels for human health protection, see Section **9.5**. This criterion represents the concentration of mercury that, if exceeded, may adversely impact human health.

Application of this criterion to the fish tissue composite data from this study identifies the number and percentage of river miles in the sampled population containing fish with mercury tissue concentrations that are above the recommended mercury fish tissue-based water quality criterion. Results for the fish fillet composite data are presented for the sampled population of miles of rivers, which are defined as 5th order or larger, and for the percentage of miles containing fish with mercury fillet concentrations that are above the criterion. Mercury concentration data from analysis of homogenized fish fillet composite samples are available to download from the NRSA Fish Tissue Studies webpage, https://www.epa.gov/fish-tech/national-rivers-and-streams-assessment-fish-tissue-studies. In addition, summary statistics, including the number of detections, are reported in Table 9-2.

Results for the fish tissue plugs are presented for all rivers and streams in the NRSA target population including the unassessed portion where fish tissue plugs could not be collected. To examine within-year variability, analysts used the revisit sites to calculate a signal: noise (S:N) estimate for the national mercury in fish tissue plug dataset. For NRSA 2013-14 the result was a S:N value of 6.35. Mercury concentration data from fish tissue plugs are available to download from the NARS data webpage, https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

9.3 PCB ANALYSIS AND FISH TISSUE SCREENING LEVELS TO PROTECT HUMAN HEALTH

Fillet tissue samples from 290 whole fish composite samples collected at river sites were analyzed for PCBs. EPA Method 1668C (EPA 2010) was used to analyze homogenized fillet tissue samples from each fish composite sample. This method uses approximately 10 g of fillet tissue for analysis and provides results for the full suite of 209 PCB congeners. The total PCB concentration for each sample was determined by summing the concentration results for any of the 209 congeners that were detected, using zero for any congeners that were not detected in the sample.

In the NRSA 2018-19 report, EPA included total PCB results for general fish consumers and for high-frequency fish consumers, such as subsistence fishers or those who eat several meals of locally caught river fish per week, which include some recreational fishers or some individuals in underserved communities. EPA used fish tissue screening levels, expressed as wet-weight concentrations of total PCBs, to characterize cancer human health risks for general fish consumers and high-frequency fish consumers. EPA applied a total PCB fish tissue screening level of 12 ppb (wet weight) for cancer effects among general fish consumers, which is based on a fish consumption

[6] Because EPA relies on the CWA Section 304(a) water quality criterion for methylmercury to interpret the mercury results, EPA is only reporting mercury results for the general population and is not including an additional analysis and interpretation for general fish consumers or high-frequency consumers.

rate of 32 grams per day (or one 8-ounce meal of locally caught river fish per week). EPA also applied a total PCB fish tissue screening level of 2.8 ppb (wet weight) for cancer effects among high-frequency fish consumers, which is based on a fish consumption rate of 142 grams per day (or four to five 8-ounce meals of locally caught river fish per week). For more information on the fish tissue screening levels for human health protection, see Section **9.5**.

Application of these screening levels to the PCB fillet tissue composite data identifies the number and percentage of river miles in the sampled population containing fish with total PCB fillet concentrations that are above each total PCB fish tissue screening level. Results are presented for sampled population of the miles of rivers (defined as 5th order or larger) and for the percentage of river miles containing fish with total PCB fillet concentrations that are above each total PCB fish tissue screening level to protect human health. PCB concentration data from analysis of homogenized fish fillet composite samples are available to download from the NRSA Fish Tissue Studies webpage, https://www.epa.gov/fish-tech/national-rivers-and-streams-assessment-fish-tissue-studies.

9.4 PFAS ANALYSIS AND AND FISH TISSUE SCREENING LEVELS TO PROTECT HUMAN HEALTH

Fillet tissue samples prepared from 290 whole fish composite samples collected at river sites were analyzed for 33 per- and polyfluoroalkyl substances (PFAS). At the time when the composite samples were analyzed for PFAS, there were no standard EPA methods for PFAS analysis of tissue, so the samples were analyzed by SGS AXYS Analytical Services using a proprietary procedure developed by their laboratory in Sidney, British Columbia. That procedure, which utilizes approximately 1 g of fillet tissue for analysis, uses high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) and applies the technique known as isotope dilution to determine the concentration of each of the 33 PFAS.

For PFOS, the EPA applied a 0.25 ppb noncancer screening level for general fish consumers to interpret PFOS concentrations in each fillet tissue composite sample.[7] Note that although the EPA classifies PFOS as likely to be carcinogenic to humans, the agency applied a noncancer screening level for this analysis. Noncancer health effects from PFOS exposure can occur at lower PFOS levels than cancer does, so applying a lower screening level to reduce the risk of noncancer health effects from dietary exposure to PFOS *also* reduces risks of cancer.

In April 2024, EPA released the National Primary Drinking Water Regulation for six PFAS, including PFOS, and issued a final Human Health Toxicity Assessment for Perfluorooctane Sulfonic Acid (PFOS) and Related Salts (EPA 2024). EPA developed an overall RfD for PFOS of $1x10^{-7}$ mg/kg/day. This RfD value was used to derive the PFOS fish tissue screening level mentioned above. For more information on the fish tissue screening levels for human health, see Section **9.5**.

Application of the PFOS noncancer screening level for general fish consumers to the PFOS fillet

[7] The calculated screening level represents a fillet tissue concentration slightly below the method detection limit (MDL) for PFOS for the NRSA 2018-19 (MDL = 0.35 ppb), making it difficult to determine the full extent of exceedances due to the possibility of some samples containing PFOS concentrations above 0.25 but below 0.35 ppb.

tissue data identifies the number and percentage of river miles in the sampled population containing fish with PFOS fillet concentrations that are above the PFOS fish tissue screening level for human health. Results are presented for the miles of rivers (defined as 5th order or larger) that could be sampled and for the percentage of river miles containing fish with PFOS fillet concentrations that are above the PFOS fish tissue screening level to protect human health. PFAS concentration data from fish fillet tissue composite samples are available to download from the NRSA Fish Tissue Studies webpage, https://www.epa.gov/fish-tech/national-rivers-and-streams-assessment-fish-tissue-studies.

Summary statistics, including the number of detections for mercury, total PCBs, and each of the 33 PFAS are provided in **Table 9-2**.

Table 9-2. NRSA 2018-19 Fish Fillet Tissue Composite Sample Summary Data.

Chemical	Number of Detections	Detection Frequency (%)	Method Detection Limit (MDL) (ppb)	Measured Minimum Concentration (ppb) *	Weighted Median Concentration (ppb) *	Measured Maximum Concentration (ppb) *
Mercury	290	100	0.090	9.44	180	1340.00
Total PCBs	290	100	0.00022-0.00561**	0.171	9.04	1212.00
Perfluorobutanoic acid (PFBA)	18	6	0.551	0.517	<MDL	0.806
Perfluoropentanoic acid (PFPeA)	0	0	0.192	0.00	<MDL	0.00
Perfluorohexanoic acid (PFHxA)	0	0	0.203	0.00	<MDL	0.00
Perfluoroheptanoic acid (PFHpA)	0	0	0.170	0.00	<MDL	0.00
Perfluorooctanoic acid (PFOA)	6	2	0.162	0.161	<MDL	0.354
Perfluorononanoic acid (PFNA)	120	41	0.129	0.122	<MDL	1.44
Perfluorodecanoic acid (PFDA)	256	88	0.116	0.115	0.332	29.80
Perfluoroundecanoic acid (PFUnA)	246	85	0.151	0.143	0.516	105
Perfluorododecanoic acid (PFDoA)	201	69	0.156	0.155	0.277	140
Perfluorotridecanoic acid (PFTrDA)	161	56	0.398	0.386	0.421	140
Perfluorotetradecanoic acid (PFTeDA)	105	36	0.309	0.310	<MDL	62.8
Perfluorobutanesulfonic acid (PFBS)	0	0	0.097	0.00	<MDL	0.00
Perfluoropentansulfonic acid (PFPeS)	0	0	0.129	0.00	<MDL	0.00
Perfluorohexanesulfonic acid (PFHxS)	6	2	0.153	0.194	<MDL	0.611

Chemical	Number of Detections	Detection Frequency (%)	Method Detection Limit (MDL) (ppb)	Measured Minimum Concentration (ppb) *	Weighted Median Concentration (ppb) *	Measured Maximum Concentration (ppb) *
Perfluoroheptanesulfonic acid (PFHpS)	1	<1	0.154	0.162	<MDL	0.162
Perfluorooctanesulfonic acid (PFOS)	265	91	0.354	0.353	3.07	131
Perfluorononanesulfonic acid (PFNS)	1	<1	0.155	0.224	<MDL	0.224
Perfluorodecanesulfonic acid (PFDS)	88	30	0.207	0.201	<MDL	4.97
Perfluorododecanesulfonic acid (PFDoS)	0	0	0.291	0.00	<MDL	0.00
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid (4:2FTS)	0	0	0.234	0.00	<MDL	0.00
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid (6:2FTS)	14	5	0.404	0.436	<MDL	12.1
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid (8:2FTS)	0	0	0.670	0.00	<MDL	0.00
Perfluorooctanesulfonamide (PFOSA)	69	24	0.152	0.149	<MDL	2.87
N-methyl perfluorooctanesulfonamide (N-MeFOSA)	0	0	0.288	0.00	<MDL	0.00
N-ethyl perfluorooctanesulfonamide (N-EtFOSA)	0	0	0.248	0.00	<MDL	0.00
N-methyl perfluorooctanesulfonamidoacetic acid (N-MeFOSAA)	7	2	0.304	0.303	<MDL	0.756
N-ethyl perfluorooctanesulfonamidoacetic acid (N-EtFOSAA)	10	3	0.143	0.144	<MDL	1.38
N-methyl perfluorooctanesulfonamidoethanol (N-MeFOSE)	0	0	3.36	0.00	<MDL	0.00
N-ethyl perfluorooctanesulfonamidoethanol (N-EtFOSE)	16	6	1.45	1.41	<MDL	3.62
Hexafluoropropylene oxide dimer acid (HFPO-DA)	0	0	0.460	0.00	<MDL	0.00
4,8-Dioxa-3H-perfluorononanoic acid (ADONA)	0	0	0.884	0.00	<MDL	0.00
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (9Cl-PF3ONS)	0	0	0.708	0.00	<MDL	0.00

Chemical	Number of Detections	Detection Frequency (%)	Method Detection Limit (MDL) (ppb)	Measured Minimum Concentration (ppb) *	Weighted Median Concentration (ppb) *	Measured Maximum Concentration (ppb) *
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (11Cl-PF3OUdS)	0	0	0.889	0.00	<MDL	0.00

*Detection frequencies and concentrations are for fish fillet tissue composite samples.
**PCB MDLs presented as a range because there are 209 PCB congeners with associated MDLs.

9.5 CALCULATION OF FISH TISSUE SCREENING LEVELS FOR HUMAN HEALTH PROTECTION

For the 2018-19 NRSA, EPA analyzed fish fillet composite samples[8] for three contaminants or groups of contaminants: methylmercury (measured as total mercury), PCBs (reported as total PCBs for all 209 congeners), and PFAS (reported as individual concentrations for 33 PFAS). For methylmercury, EPA used the Agency's recommended CWA Section 304(a) fish tissue-based ambient water quality criterion for protection of human health as a benchmark. For PCBs and PFOS, because EPA does not currently have a fish tissue-based water quality criterion for these contaminants, EPA generally followed the approach in its *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories* (EPA 2000a) to develop fish tissue screening levels for human health protection.

Methylmercury: EPA used the Agency's recommended CWA Section 304(a) fish tissue-based ambient water quality criterion for methylmercury (EPA 2001b) as the benchmark for human health protection to evaluate mercury fish fillet tissue and fish tissue plug results. (Note: EPA applies the conservative assumption that all mercury in fish is methylmercury and therefore measures total mercury in fillet tissue to be most protective of human health.)

PCBs: EPA analyzed fish fillet tissue samples for the full set of 209 PCB congeners. There is no EPA recommended CWA Section 304(a) fish-tissue based ambient water quality criterion for protection of human health for PCBs, so EPA derived fish tissue screening levels using the equations found in EPA's Fish Advisories Guidance (EPA 2000a). Inputs to these equations consist of the Agency's oral noncancer and cancer toxicity values for PCBs (EPA 1994), updated human body weights in EPA's Exposure Factors Handbook (USEPA 2011) and different fish consumption rates for general fish consumers and high-frequency fish consumers. An oral noncancer toxicity value (or reference dose, RfD) of 0.00002 mg/kg day was used to derive a screening level for noncancer health effects, and an oral cancer slope factor of 2 $(mg/kg/d)^{-1}$ and a cancer risk level of 10^{-5} were used to derive a screening level for cancer health effects. A human adult body weight default value of 80 kg was used to derive the PCB screening levels for each group of fish consumers. For the screening level for general consumers, EPA used a fish consumption rate of 32 grams per

[8] For the NRSA survey, a composite sample was formed by combining fillet tissue from up to five adult fish of the same species and similar size from the same site. Use of composite sampling for screening studies is a cost-effective way to estimate average contaminant concentrations while also ensuring that there is sufficient fish tissue to analyze for all contaminants of concern.

day (or one 8-ounce meal of locally caught river fish per week), consistent with the U.S. Department of Agriculture and Department of Health and Human Services' *Dietary Guidelines for Americans, 2020-2025* (USDA and HHS 2020). For the screening level for high-frequency fish consumers (such as subsistence or recreational fishers or individuals from underserved populations), EPA used a fish consumption rate of 142 grams per day (or four to five 8-ounce meals of locally caught river fish per week) which is described in the EPA 2000 Human Health Methodology (USEPA 2000b). Because the total PCBs screening levels associated with cancer effects were lower than the screening levels associated with noncancer effects, EPA only evaluated the PCB concentrations in fish samples against the screening levels associated with cancer effects. This conservative approach is also likely to be protective against noncancer effects, which may occur at higher levels of total PCB contamination.

PFAS: For the NRSA 2018-19 report, EPA analyzed fish fillet tissue samples for 33 PFAS chemicals. PFOS was the most commonly detected of the PFAS – in 91 percent of the fish fillet composite samples – so EPA derived PFOS fish tissue screening levels for cancer and non-cancer effects using the equations found in EPA's *Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories*. The screening levels represent the concentration of PFOS in fish tissue that should not be exceeded based on a total consumption-weighted rate of 0.032 kg of fish/day for general fish consumers. The PFOS screening levels were based on an average adult human body weight default value of 80 kg[9], an RfD of $1*10^{-7}$ mg/kg day (for non-cancer effects), a cancer slope factor of 39.5 $(mg/kg/d)^{-1}$ and a cancer risk level of 10^{-5} (for cancer effects). Because the PFOS screening level associated with noncancer effects was lower than the screening level associated with cancer effects, EPA intended to apply only the screening level associated with noncancer effects. This conservative approach is also likely to be protective against cancer effects, which may occur at higher levels of PFOS contamination.

9.6 LITERATURE CITED

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10 ENTERCOCCI INDICATOR

The EPA has developed and validated a molecular testing method employing quantitative polymerase chain reaction (qPCR) as a rapid approach for the detection of enterococci in recreational water. NRSA used this method to estimate the presence and quantity of these fecal indicator bacteria in the nation's rivers and streams. The statistical threshold value of 1,280 calibrator cell equivalents (CCE)/100 mL from EPA's 2012 *Recreational Water Quality Criteria* document (RWQC) was then applied to the enterococci data to assess the recreational condition ofrivers and streams.

10.1 FIELD COLLECTION

To collect enterococci samples, field crews took a water sample for the fecal indicator at the last transect after all other sampling was completed. Using a pre-sterilized 250 mL bottle, they collected the sample approximately 1 m off the bank at about 0.3 m (12 inches) below the water. Following collection, crews placed the sample in a cooler and kept it on ice prior to filtration of two 50 mL volumes. Samples were filtered and frozen on dry ice within 6 hours of collection. In addition to collecting the sample, crews looked for signs of disturbance throughout the reach that would contribute to the presence of fecal contamination to the waterbody.

10.2 LAB METHODS

The sample collections and the laboratory method followed EPA's Enterococcus qPCR Method 1609.1 (USEPA 2015; available on-line at https://www.epa.gov/cwa-methods/other-clean-water-act-test-methods-microbiological). As with EPA Draft Method A, used in the NRSA 2008-09 study, Method 1609.1 describes a quantitative polymerase chain reaction (qPCR) procedure for the detection of DNA from enterococci bacteria in ambient water matrices based on the amplification and detection of a specific region of the large subunit ribosomal RNA gene (lsrRNA, 23S rRNA) from these organisms. Both methods use an arithmetic formula (the comparative cycle threshold (CT) method; Applied Biosystems, 1997) to calculate the ratio of enterococcus lsrRNA gene target sequence copies (TSC) recovered in total DNA extracts from the water samples relative to those recovered from similarly prepared extracts of calibrator samples containing a consistent, pre-determined quantity of Enterococcus cells. Mean estimates of the absolute quantities of TSC recovered from the calibrator sample extracts were then used to determine the quantities of TSC in the water samples and then converted to CCE values as described in the section below. To normalize results for potential differences in DNA recovery, monitor signal inhibition or fluorescence quenching of the PCR analysis caused by a sample matrix component, or detect possible technical error, CT measurements of sample processing control (SPC) and internal amplification control (IAC) target sequences were performed as described in Method 1609.1.

The primary differences between EPA Draft Method A (subsequently published as EPA Method 1611,USEPA 2012a) and Method 1609.1 are that Method 1609.1 includes the IAC assay, an improved polymerase reagent with greater resistance to inhibitory compounds and allows direct analyses of undiluted sample DNA extracts. Analyses of diverse river water samples have indicated no significant difference in the quantitative estimates obtained by the two methods (Sivaganesan et al., 2014).

10.3 APPLICATION OF BENCHMARKS

10.3.1 CALIBRATION

Estimates of absolute TSC recoveries from the calibrator samples were determined from standard curves using EPA-developed plasmid DNA standards of known TSC concentrations as described in Method 1609.1. Estimates of TSC recovered from the test samples were determined by the comparative cycle threshold (C_T) method, as also described in Method 1609.1. Before applying the EPA benchmark to the qPCR data, it was necessary to convert the TSC estimates to CCE values.

The standardized approach developed for this conversion is to assume 15 TSC/CCE (USEPA 2015). This approach allows the CCE values to be directly compared to the EPA RWQC values (Haugland et al., 2014). A slightly modified approach was employed in the earlier NRSA 2008-09 study toobtain the same conversions of TSC to standardized CCE units.

10.3.2 BENCHMARKS

For the data analysis of the enterococci measurements determined by Method 1609.1, analysts used a benchmark as defined and outlined in EPA's recommended recreational criteria document for protecting human health in ambient waters designated for swimming (USEPA 2012b). Enterococci CCE/100 mL values were compared to the EPA benchmark of 1280 CCE/100 mL[10] (USEPA 2012b). Enterococci concentration data are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic- resource-surveys/data-national-aquatic-resource-surveys. To examine within-year variability, analysts used the revisit sites to analyze signal to noise of enterococci concentrations and to analyze condition classes in a 2x2 contingency table. Condition classes were defined as "above benchmark" and "at or below benchmark" based on the EPA benchmark value of 1,280 CCE/100 mL. The S:N ratio for concentration values was 0.36. However, results from the contingency table analysis show that 78% of sites had the same condition class during both visits (i.e., 64.7% of the 184 revisits that were assessed were at or below benchmark in both visits and 13.6% were above benchmark in both visits) and 21.7% had mixed classes between visits.

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11 ALGAL TOXINS

Cyanobacteria are one-celled photosynthetic organisms that normally occur at low levels. Under eutrophic conditions, cyanobacteria can multiply rapidly. Not all cyanobacterial blooms are toxic, but some may release toxins, such as microcystins and cylindrospermopsin. Recreational exposure is typically a result of inhalation, skin contact, or accidental ingestion. When people are exposed to cyanotoxins, adverse health effects may range from a mild skin rash to serious illness or in rare circumstances, death. Acute illnesses caused by short-term exposure to cyanobacteria and cyanotoxins during recreational activities include hay fever-like symptoms, skin rashes, respiratory and gastrointestinal distress.

Microcystins refers to an entire group of toxins (all of the different congeners, rather than just one congener). Cyanobacteria can produce one or many different congeners at any one time, including Microcystin-LR (used in the kit's calibration standards), Microcystin-LA, and Microcystin-RR. The different letters on the end signify the chemical structure (each one is slightly different) which makes each congener different.

For the NRSA, both microcystins and cylindrospermopsin were analyzed.

11.1 FIELD METHODS

The algal toxin sample was collected as a grab sample from Transect A (non-wadeable) or the X-site[11] (wadeable) in a flowing portion near the middle of the channel. Water was collected in a 3 L beaker and then transferred to a 500 mL bottle. The bottle was kept on ice and then stored frozen until analysis. Both microcystins and cylindrospermopsin were analyzed from the 500 mL bottle.

11.2 ALGAL TOXIN ANALYSIS AND APPLICATION OF BENCHMARKS

The microcystins sample was measured using an enzyme-linked immunosorbent assay (ELISA) procedure with an Abraxis Microcystins-ADDA Test Kit. For freshwater samples, the procedure's reporting range is 0.15 µg/L to 5.0 µg/L, although, theoretically, the procedure can detect, but not quantify, microcystins concentrations as low as 0.10 µg/L. Microcystin concentrations were evaluated against the EPA recommended criterion and swimming advisory level of 8 µg/L (USEPA 2019). Microcystin concentration data are available to download from the NARS data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

The cylindrospermopsin sample was measured using an enzyme-linked immunosorbent assay (ELISA) procedure with an Abraxis Cylindrospermopsin Test Kit. For freshwater samples, the procedure's reporting range is 0.02 µg/L to 2.0 µg/L, although, theoretically, the procedure can detect, but not quantify, concentrations as low as 0.04 µg/L. Cylindrospermopsin concentrations were evaluated against the EPA recommended criterion and swimming advisory level of 15 µg/L (USEPA 2019). Cylindrospermopsin concentration data are available to download from the NARS

[11] The "X-site" is the mid-point of the sampling reach, and it determines the location and extent for the rest of the sampling reach.

data webpage - https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys.

To examine within-year variability, analysts used the revisit sites to calculate a S:N ratio estimate for the national microcystin dataset. The result was a S:N value of 4.8. For this calculation, non-detect values were excluded due to the fact that no variance between repeat sites when both were non-detect may overestimate the S:N.

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12 FROM ANALYSIS TO RESULTS

12.1 CONDITION CLASSES

The NRSA database contained the field and laboratory data for all sampled sites, whether selected as potential reference sites (i.e., hand-selected sites) or from the statistical design. NRSA analysts reviewed the raw data for each indicator independently and assigned the values in each dataset to categories (for example, "above criterion" or "at or below criterion"; good, fair, or poor). To assign the appropriate condition category, EPA used two broad types of assessment benchmarks for NRSA 2018-19.

The first type consisted of fixed benchmarks applied nationally based on values in the peer-reviewed scientific literature, EPA published values, or EPA-derived screening levels. For example, EPA's recommended water quality criteria were used nationally to classify rivers and streams as above or below a criterion or benchmark for microcystins, cylindrospermopsin, enterococci, and mercury. Similarly, EPA fish tissue screening levels, developed using information on human health risk and fish consumption rates for PCBs, were applied for human health fish fillet tissue indicators. See Chapters 9, 10, and 11 for additional information.

The second type consisted of NRSA-specific ecoregional benchmarks based on the distribution of indicator values from a set of river and stream least disturbed (reference) sites. Within each region, least-disturbed sites (i.e., reference sites described in Chapter 4) provided a benchmark against which all other sites were compared and classified. The condition classes for each stressor and biological response were determined from data and observations from the least-disturbed sites in each ecoregion and the continuous gradient of observed values at all sites.
The resulting condition classes were defined as follows:

- Good: Not different from the reference sites
- Fair: Somewhat different from the reference sites
- Poor: Markedly different from the reference sites
- Not Assessed: indicator not available for the site

While the "Not Assessed" category was included in the assessment (for instance, if fish were not caught at a site or a sample was damaged) for stressor and response extent analyses, these sites were not utilized in the relative risk or attributable risk analysis.

12.2 STRESSOR EXTENT, RELATIVE RISK, AND ATTRIBUTABLE RISK

A major goal of the National Aquatic Resource Surveys is to assess the relative importance of stressors that impact aquatic biota on a national basis. EPA assesses the influence of stressors in three ways: stressor extent, relative risk, and population attributable risk. In NRSA, each targeted and sampled river and stream reach was classified as being in either *Good*, *Fair*, or *Poor* condition, separately for each stressor variable and for each biological response variable. From this data, we estimated the stressor extent (prevalence) of rivers and streams in *Poor* condition for a specified stressor variable. We also estimated the relative risk of each stressor for a biological response.

Relative risk is the ratio of the probability of a poor biological condition when the stressor is poor to the probability of a poor biological condition when the stressor is not poor (Van Sickle et al. (2006)). Finally, we estimated the population attributable risk (AR) of each stressor for a biological response. AR combines RR and stressor extent into a single measure of the overall impact of a stressor on a biological response, over the entire population of rivers and streams (Van Sickle and Paulsen (2008)).

12.2.1 STRESSOR EXTENT

For each particular stressor, the stressor extent (SE) may be reported as the number of miles, the proportion of miles, or the percent of miles in *Good*, *Fair*, *Poor*, or *Not Assessed* condition. If the SE is reported as the proportion of miles, then it can be interpreted as the probability that a stream chosen at random from the population will be in *Poor* condition for the stressor.

Stressor extent in *Poor* condition is estimated as

(1) SE_p, the sum of the sampling weights for sites that are assessed in *Poor* condition

$$SE_p = \sum_{i=1}^{n_p} w_{pi}$$

,

(2) SEP_p, as the ratio of the sums of the sampling weights for the probability selected sites that are assessed in *Poor* condition divided by the sum of the sampling weights of all the selected sites regardless of condition, i.e.,

$$SEP_p = \frac{\sum_{i=1}^{n_p} w_{pi}}{\sum_{i=1}^{n} w_i}$$

, or

(3) SER_p, the percent of stressor extent in *Poor* condition (i.e., stressor relative extent)

$$SER_p == 100 * SEP_p = 100 * \frac{\sum_{i=1}^{n_p} w_{pi}}{\sum_{i=1}^{n} w_i}$$

where w_{pi} is the weight for the *i*th selected site in the *Poor* condition category, w_i is the weight for the *i*th selected site regardless of condition category, n_p is the number of selected sites that are in *Poor* condition, and n is the total number of sites regardless of their condition category. A stressor condition category may use other terminology to identify if a site is in poor condition but generically, we use the term *Poor*. Note that the extent for a response variable is defined similarly.

12.2.2 RELATIVE RISK AND ATTRIBUTABLE RISK

To estimate relative risk and attributable risk, we restrict the sites to those that both the stressor and response variable assessed as *Good*, *Fair*, or *Poor* (or their equivalents). That is, if a site is *Not Assessed* for either the stressor or response variable, it is dropped. Next, for these sites the condition classes are combined to be either *Poor* or *Not Poor* for the stressor and response variables. For example, *Not*

Poor combines the *Good* and *Fair* condition classes. Thus, each sampled river or stream was designated as being in either *Poor* (P) or *Not Poor* (NP) condition for each stressor and response variable separately.

To estimate the relative risk and attributable risk for one stressor (S) and one response (B) variable, we compiled a 2x2 table (**Table 12-1**), based on data from all river and stream sites that were included in the probability sample and that had both the stressor and response variable measured. A separate table must be compiled for each pair of stressor and response variables.

Table 12-1. Extent estimates for response and stressor categories

Response (B)	**Stressor (S)**	
	Not Poor (NP)	Poor (P)
Not Poor (NP)	$a = \sum_{i=1}^{n_{nn}} w_{nni}$	$b = \sum_{i=1}^{n_{np}} w_{npi}$
Poor (P)	$c = \sum_{i=1}^{n_{pn}} w_{pni}$	$d = \sum_{i=1}^{n_{pp}} w_{ppi}$

Table entries (a, b, c, d) are the sums of the sampling weights of all sampled rivers and streams that were found to have each combination of *Poor* or *Not Poor* condition for stressor and response. For example, $d = \sum_{i=1}^{n_{pp}} w_{ppi}$ where n_{pp} is the number of sites with both the stressor and response in poor condition and w_{ppi} is the weight for the *i*th site. Note that the estimates in Table 12.1 may differ from the stressor extent estimates since both the stressor and response variables must be measured at each site.

12.2.3 RELATIVE RISK

Relative risk (RR) is the ratio of the probability of a *Poor* biological condition when the stressor is *Poor* to the probability of a *Poor* biological condition when the stressor is *Not Poor*. That is,

$$RR = \frac{Pr(B = P|S = P)}{Pr(B = P|S = NP)}$$

Using the simplified notation in Table 12.1, relative risk (RR) is estimated as:

$$RR_{est} = \frac{d/(b+d)}{c/(a+c)}$$

A RR = 1.0 indicates there is no association between the stressor and response. That is, a *Poor* response condition in a river or stream is equally likely to occur whether or not the stressor condition is *Poor*. A RR > 1.0 indicates that a *Poor* response condition is more likely to occur when the stressor is *Poor*. For example, when the RR is 2.0, the chance that a stream is in *Poor* biological (response) condition is twice as likely when the stressor is *Poor* than when the stressor is *Not Poor*.

Further details of RR and its interpretation, including estimation of a confidence interval for RR_{est}, can be found in Van Sickle et al. (2006).

12.2.4 ATTRIBUTABLE RISK

Population attributable risk (AR) measures what percent of the extent in *Poor* condition for a biological response variable can be attributed causally to the *Poor* condition of a specific stressor. AR is based on a scenario in which the stressor in *Poor* would be entirely eliminated from the population of river and streams, e.g., by means of restoration activities. That is, all rivers and streams in *Poor* condition for the stressor are restored to the *Not Poor* condition. AR is defined as the proportional decrease in the extent of *Poor* biological response condition that would occur if the stressor were eliminated from the population of rivers and streams. Mathematically, AR is defined as (Van Sickle and Paulsen (2008))

$$AR = \frac{Pr(B = P) - Pr(B = P|S = NP)}{Pr(B = P)}$$

We estimated AR as

$$AR_{est} = \frac{BEP_p - c/(a + c)}{BEP_p}$$

where

$$BEP_p = \frac{(c + d)}{(a + b + c + d)}$$

and is the estimated proportion of the biological response that is in *Poor* condition. We calculated a confidence interval for AR_{est} following Van Sickle and Paulsen (2008).

An AR can take a value between 0 and 1. A value of 0 indicates either "No association" between stressor and response, or else a stressor has a zero extent, i.e., is not present in the population. A strict interpretation of AR in terms of stressor elimination, as described above, requires one to assume that the stressor-response relation is strongly causal and that stressor effects are reversible. Van Sickle and Paulsen (2008) discuss the reality of these assumptions, along with other issues such as interpreting them when multiple, correlated stressors are present, and using them to express the joint effects of multiple stressors.

However, AR can also be interpreted more informally, as a measure that combines RR and SE into a single index of the overall, population-level impact of a stressor on a response. Van Sickle and Paulsen (2008) show that the population attributable risk can be written as

$$AR = \frac{SEP_p(RR - 1)}{1 + SEP_p(RR - 1)}$$

This shows that the numerator of AR is the product of the SE of *Poor* stressor condition and the "excess" RR, i.e., RR-1, of that stressor. The denominator standardizes this product to yield AR values between 0 and 1. Thus, a high AR for a stressor indicates that the stressor is widely prevalent

(has a high SE of *Poor* condition), and the stressor also has a large effect (high RR) in those river and stream reaches where it does have Poor condition.

12.3 CHANGE ANAYLSES

One of the objectives of the NRSA is to track changes and trends over time. Previously, EPA and partners reported on the condition of all rivers and streams for NRSA 2008-09 and 2013-14, and on the condition of wadeable streams in the Wadeable Streams Assessment (WSA) 2004. The 2018-19 report presents the difference in percentage points of river and stream miles in various condition categories between NRSA 2013-14 and 2018-19. Additional change comparisons back to 2008-09 can be found in the NRSA data dashboard.

12.3.1 DATA PREPARATION

The survey frame inclusion variables were used to identify sites for change estimation. Only sites that were included in the survey frame for all surveys were used to calculate change estimates. The same set of benchmarks and analyses were applied to all applicable datasets (e.g., NRSA 2008-09, 2013-14, 2018-19) in order for results to be directly comparable. Change analysis was not conducted for cylindrospermopsin because this indicator was not included in earlier surveys.

12.3.2 ANALYSIS

Change analysis was conducted through the use of the spsurvey 5.4.0 package in R (Dumelle et al., 2022). Within the GRTS (Generalized Random Tessellation Stratified) survey design, change analysis can be conducted on continuous or categorical variables. When using categorical variables, change is estimated by the difference in category estimates from the two surveys. Category estimates were defined as the estimated proportion of values in each category (i.e., good, fair, and poor categories). Change between the two years was statistically significant when the resulting error bars around the change estimate did not cross zero.

Early trend information, calculated using a linear regression, are also available in the data dashboard by hovering over the bar graphs in the "Change" section of the dashboard.

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