EPA
United States
Environmental Protection
Agency

EPA/600/R-08/146 | January 2010 | www.epa.gov/ncea

Inferring Causes of Biological Impairment in the Clear Fork Watershed, West Virginia

National Center for Environmental Assessment
Office of Research and Development, Washington, DC 20460

EPA/600/R-08/146
January 2010

Inferring Causes of Biological Impairment in the Clear Fork Watershed, West Virginia

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

NOTICE

The U.S. Environmental Protection Agency through its Office of Research and Development prepared this report in consultation with the West Virgina Department of Environmental Protection. It has been subjected to the Agency's peer and administrative review and has been approved for publication as an U.S. EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

ABSTRACT

Human activities such as mining, logging, agriculture, and residential development have degraded biological conditions in many West Virginia (USA) streams. Using benthic macroinvertebrates as biological indicators of stream condition, the West Virginia Department of Environmental Protection (WVDEP) identified streams across the State that do not meet aquatic life use designations; these streams are considered to be biologically impaired. Total Maximum Daily Loads (TMDLs) are required for all streams classified as biologically impaired, and the TMDL process mandates that stressors to the biological community are identified so that pollutants resulting from human activities can be controlled within each watershed. We used the U.S. EPA's Stressor Identification (SI) guidance (U.S. EPA, 2000) to identify and rank the probable physical, chemical, and biological stressors that have impaired the aquatic community in the Clear Fork of Coal River, West Virginia. We developed a comprehensive conceptual model to establish the causal pathways for each stressor. The conceptual model illustrates linkages between candidate causes and their biological effects based on general ecological knowledge. Stressor-response (S-R) threshold values were based on statistical analyses of statewide data. We used these analyses and thresholds to infer whether the stressor occurred at a sufficient intensity to cause biological impairments in specific portions of the watershed. We plotted and analyzed quantitative data spatially using a "geo-order" format. Through this method, we were able to assign relative positions of sampling locations (from downstream to upstream), along each impaired stream and its tributaries, within a subwatershed. We included watershed characteristics such as land use and soils, point-source inventories, site observations, and other evidence in these analyses to help identify stressor sources.

Candidate causes were screened to eliminate those that did not co-occur with effects. Remaining candidate causes were ranked according to the strength of evidence (strongest to weakest) of occurrence within each watershed. Types of evidence included co-occurrence of stressors with observed biological impairment, S-R threshold values from the statewide data analyses, and the predictive models to rank multiple stressors. We obtained the strongest inferences where the models agreed with on-site observations of stressors.

Probable causes were different throughout the watershed, and the combination of all these causes was evident in the mainstem, which exhibited some resiliency due to dilution and different geophysical attributes. In particular, causes included metal contamination and acidification from mine draining, aluminum toxicity in association with low pH, sediment deposition, organic enrichment from direct releases and from algal productivity enhanced by nutrients, and low dissolved oxygen.

- Lick Run—the principal cause of biological impairment of Lick Run appears to be sediment deposition and erosion most likely from abandoned minelands, and riparian disturbance along the stream corridor, both of which also contribute to degraded aquatic habitat. In addition, ionic stress, likely from abandoned minelands and current mining activity is also apparent. There is no residential land use and no livestock.
- Toney Fork and Buffalo Creek—the principal cause of impairment appears to be excess sulfate/conductivity, or an unmeasured substance that occurs with the sulfate. Mining activities and mine effluent ponds are present within these watersheds and known sources of sulfate.
- White Oak Creek—the principal cause is most likely organic and nutrient enrichment and appears to be from inadequately treated domestic sewage.
- Stonecoal Branch is impaired by acid mine drainage and likely sediment too from abandoned minelands and dirt roads.
- Clear Fork—a receiving stream of all of the above, is apparently biologically impaired by multiple causes, such as: organic/nutrient enrichment from untreated domestic wastewater, excess sedimentation, and the residual metals and conductivity effects of mining.

Preferred citation:
Gerritsen, J., L. Zheng, J. Burton, C. Boschen, S. Wilkes, J. Ludwig, and S. Cormier. 2010. Inferring Causes of Biological Impairment in the Clear Fork Watershed, West Virginia. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-08/146.

Cover photo:
Jeffery Bailey, West Virginia Department of Environmental Protection.

TABLE OF CONTENTS

Page

LIST OF TABLES vi
LIST OF FIGURES vi
LIST OF ABBREVIATIONS vii
PREFACE ix
AUTHORS, CONTRIBUTORS, AND REVIEWERS xi

1. DEFINE THE CASE 1

    1.1. REGULATORY CONTEXT FOR THE CASE 1
    1.2. DESCRIPTION OF THE WATERSHED 2
    1.3. POTENTIAL SOURCES OF STRESSORS 2
    1.4. SPECIFIC BIOLOGICAL IMPAIRMENTS 5

2. CANDIDATE CAUSES 9

    2.1. DATA SOURCES FOR CAUSAL ANALYSIS 9

        2.1.1. West Virginia Water Quality Monitoring 9
        2.1.2. Pollutant Source Report and Source Tracking 9

    2.2. CONCEPTUAL MODEL 11

        2.2.1. Enrichment 13
        2.2.2. Acidity 15
        2.2.3. Sulfate, Conductivity, and Other Metals 16
        2.2.4. Sediment 17
        2.2.5. Habitat 18

3. EVALUATE DATA FROM THE CASE 19

    3.1. SPATIAL CO-OCCURRENCE 19
    3.2. STRESSOR-RESPONSE (S-R) RELATIONSHIPS IN THE FIELD 19
    3.3. CAUSAL PATHWAY 19

4. EVALUATE DATA FROM ELSEWHERE 21

    4.1. STRESSOR-RESPONSE (S-R) RELATIONSHIPS FROM STATEWIDE DATA 21

        4.1.1. Thresholds 27

TABLE OF CONTENTS cont.

Page

4.2. EMPIRICAL MODELS TO RANK MULTIPLE STRESSORS 32

5. IDENTIFY PROBABLE CAUSES 35

5.1. LICK RUN 36
5.2. TONEY FORK AND BUFFALO FORK 37
5.3. WHITE OAK CREEK 38
5.4. STONECOAL BRANCH 39
5.5. CLEAR FORK 41

6. DISCUSSION 43

7. REFERENCES 44

APPENDIX A: STRESSOR-RESPONSE (S-R) RELATIONSHIPS IN WEST VIRGINIA 49
APPENDIX B: CLEAR FORK 80
APPENDIX C: LICK RUN 83
APPENDIX D: STONECOAL BRANCH 86
APPENDIX E: TONEY FORK AND BUFFALO FORK 89
APPENDIX F: WHITE OAK CREEK 92

LIST OF TABLES

No.	Title	Page
1	Thresholds for Evaluating Stressor-Response Information, Quantitative and Semi-Quantitative Stressor Data, Ecoregion 69	28
2	Clear Fork Mainstem Sites Showing Tributaries With Measured Stressors Above Stressor-Response Thresholds	41

LIST OF FIGURES

No.	Title	Page
1	Confluence of Clear Fork and Sycamore Creek Near Ameagle, WV	3
2	Clear Fork Watershed Showing Numbered Sampling Sites and Several Tributaries	4
3	West Virginia Stream Condition Index Scores for Clear Fork and its Tributaries	8
4	Clear Fork Watershed Showing Current and Abandoned Mining Areas, Oil and Gas Wells, and Burned Areas	10
5	Conceptual Model of Sources and Stressors in the Coal River Watershed, WV	12
6	Geographic-Order Scatter Plot for pH	20
7	Diagram Showing Interpretation of Stressor-Response Relationship	22
8a	Scatter Plots of the Full Data Set of Biological Responses with a Candidate Stressor, Conductivity	23
8b	Scatter Plots of Constrained Data Set of Biological Responses with a Candidate Stressor, Conductivity.	24
8c	Scatter Plots of More Sensitive Biological Responses with a Candidate Stressor, Conductivity	25
9	“Yellow boy” (Fe(III)hydroxide) Deposition in Fickey Run, WV	40

LIST OF ABBREVIATIONS

AMD	acid mine drainage
AML	abandoned minelands
BOD	biological oxygen demand
CADDIS	Causal Analysis/Diagnosis Decision Information System
DO	dissolved oxygen
EPT	Ephemeropteran, Plecopteran, and Trichopteran
FeS_2	pyrite
GIS	geographic information system
HBI	Hilsenhoff's Biotic Index
LOWESS	locally weighted estimation
NH_3	un-ionized ammonia
NH_4^+	ammonium ions
NHD	National Hydrographic Dataset
NPDES	National Pollutant Discharge Elimination System
RBP	Rapid Bioassessment Protocol
SI	stressor-identification
S-R	stressor-response
TMDL	Total Maximum Daily Load
TP	total phosphorus
TSS	total suspended solids
USGS	U.S. Geological Survey

LIST OF ABBREVIATIONS cont.

WQC	water quality criterion
WVDEP	West Virginia Department of Environmental Protection
WVSCI	West Virginia Stream Condition Index

PREFACE

Biological impairments were identified in portions of the Clear Fork Watershed in West Virginia. The Clear Fork and some tributaries were listed on the West Virginia's 303(d) list of impaired waterbodies, thus triggering the need for a determination of a TMDL that can allow the waters to support aquatic life uses. In West Virginia, Total Maximum Daily Loads (TMDLs) are developed together with the state's geographically based Watershed Management Framework. This framework partitions the state's 32 major watersheds into five U.S. Geological Survey-designated, 8-digit Hydrologic Unit Code or hydrologic groups (A−E), and operates as a 5-year, five-step process. In the first year of the cycle, the TMDL process begins with stream selection, informational public meetings in the watersheds of the selected streams, and pre-TMDL sampling. In the second year of the process, data compilation starts, and TMDL development begins, including the Stressor Identification (SI) process.

The results of the SI process are reported here. The text was reorganized and formatted for U.S. EPA publication during and subsequent to a workshop at Canaan Valley, WV in May of 2005; however, the sampling, analysis, and conclusions are those of researchers of the authors. Only comments indicating alternative approaches and suggestions were prepared by the U.S. EPA Office of Research and Development of NCEA-Cincinnati. NCEA provided editorial and formatting assistance to make the original WVDEP report similar to four other case studies that were solicited as examples for other practitioners of causal assessment.

The Clear Fork Watershed case study is one of five causal assessments that were completed prior to 2005 by states. These cases, as all cases, could be improved with more resources, but represent the state of the capability and analysis that was available in 2005. Since then, additional analytical tools and databases continue to reduce the uncertainty of the analysis. All of these case studies use a biological index to define the impairment. To demonstrate causal relationships, most of the case studies, including the Clear Fork Watershed case study, used biological indices or metrics. This practice diminishes the ability to detect associations because summing the metrics dampens the overall signal from individual metrics and species that are responding differently to environmental conditions or stressors. However, WVDEP did use metrics in the analysis to good effect, and this area of research continues to be very active in West Virginia.

To address these and other issues, text boxes have been inserted throughout the Clear Fork Watershed case study to supply commentary or to suggest other approaches that could strengthen the case. The analyses in the cases cannot be modified as they are already a part of West Virginia's public record. Throughout the case study, you will find links to relevant tools and guidance on the U.S. EPA Web site: www.epa.gov/caddis.

The Clear Fork case study illustrates what can be done with a comprehensive statewide database, which enables quantitative analysis. No doubt, the potential to better understand causal relationships can be further investigated and improved by adding paired measurements for other causes. Nevertheless, this Clear Fork case study demonstrates that a watershed-wide causal assessment has several advantages for making analysis practical, defensible, and showing the relationships among interconnected waterbodies.

This case used the SI process to eliminate candidate causes that did not co-occur with effects. Remaining candidate causes were ranked according to the strength of evidence (strongest to weakest) of occurrence within each watershed. Types of evidence included co-occurrence of stressors with observed biological impairment; Stressor-Response (S-R) threshold values from the statewide data analysis, and the predictive models to rank multiple stressors. We obtained the strongest inferences where the models agreed with on-site observations of stressors.

Probable causes were different throughout the watershed, and all these diverse causes combined to impair the biological condition of the mainstem, which exhibited some resiliency due to dilution and different geophysical attributes. In particular, causes included metal contamination and acidification from mine draining, aluminum toxicity in association with low pH, sediment deposition, organic enrichment from direct releases and from algal productivity enhanced by nutrients, and low dissolved oxygen.

The Clear Fork Watershed case study is a good example of several strategic techniques:

1. Assessment of an Appalachian watershed.
2. Differential comparisons of tributaries and sections of the mainstem.
3. Development of state-wide S-R associations, thresholds, and predictive models.
4. Inclusion of source identification whenever reasonable and practical.
5. Surrogate indicators contributing to SI when primary indicators have not been monitored.
6. Candidate causes: excess sulfate/conductivity, organic and nutrient enrichment, AMD, residual metals with special attention to Al and moderately acidic pH, excess sediment, and multiple stressors.
7. Types of evidence: co-occurrence, causal pathway, S-R relationships from other field studies, and predictive performance based on biological alterations.
8. Analytical methods: geographic tools, scatter plots with Spearman correlation, locally weighted estimation regression, local weighted averaging regression, nonmetric multidimensional scaling, ordinary linear regression, local weighted polynomial regression, deviance reduction changing point analysis (regression tree), and conditional probability.

Editor: Susan M. Cormier January 2010

AUTHORS, CONTRIBUTORS, AND REVIEWERS

AUTHORS

Clint Bosche
Tetra Tech, Inc.
Fairfax, VA 22030

June Burton
Tetra Tech, Inc.
Fairfax, VA 22030

Jeroen Gerritsen
Tetra Tech, Inc.
Owings Mills, MD 21117

Lei Zheng
Tetra Tech, Inc.
Owings Mills, MD 21117

CONTRIBUTING AUTHORS

Jon Ludwig
Tetra Tech, Inc.
Charleston, WV 25301

Sam P. Wilkes
Tetra Tech, Inc.
Charleston, WV 25301

EDITOR

Susan M. Cormier, Ph.D.
U.S. EPA, Office of Research and Development, National Center for Environmental Assessment
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS cont.

INTERNAL REVIEWERS

Jeff Hollister
U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory
Narragansett, RI 02882

Jan Kurtz
U.S. EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory
Gulf Breeze, FL 32561

Paul Wagner
U.S. EPA, Office of Research and Development, National Center for Environmental Assessment
Research Triangle Park, NC 27711

EXTERNAL REVIEWERS

Peter V. Caldwell, Ph.D.
North Carolina Department of Environment and Natural Resources
Division of Water Quality
Raleigh, NC 27699

Peter L. deFur, Ph.D.
Virginia Commonwealth University
Center for Environmental Studies
Richmond, VA 23284

Mark T. Southerland, Ph.D.
Versar, ESM Operations
Columbia, MD 21045

AUTHORS, CONTRIBUTORS, AND REVIEWERS cont.

ACKNOWLEDGMENTS

We would like to thank Jeff Bailey, Ben Lowman, Dave Montali, and John Wirts of the West Virginia Department of Environmental Protection for insightful contributions while consulting with U.S. EPA staff and contractors during the development of this case study. Without their guidance this case study would not have been possible. Futhermore, we recognize these scientists for designing a high quality monitoring program in West Virginia and populating a rich database of biological, physical, and chemical data without which analyses such as these would not be possible and good examples would not be available to advance the practice of causal assessment.

We would also like to thank Cris Broyles of IntelliTech Systems, Inc and Heidi Glick of ECFlex, Inc. for their editorial services, the TSS staff of ECFlex, Inc. and IntelliTech Systems, Inc. for their formatting services, and Bette Zwayer and Ruth Durham of the U.S. EPA for shepherding this document.

1. DEFINE THE CASE

Much of the State of West Virginia lies within the Appalachian Mountains of the eastern United States. As a largely rural state with relatively rich natural resources, the economy of West Virginia historically has relied on extracting natural resources, such as timber, coal, oil, and gas. Urban and industrial centers in West Virginia are located along the major rivers (the Kanawha River and the Ohio River, in particular). Agriculture is restricted to smaller areas but is most concentrated in the Potomac River Watershed in the eastern part of the state.

Comment 1. What are these Comment Boxes?
At various points in this document, the U.S. EPA editor and reviewers provide comments. These are not meant to indicate that the causal analysis is in error. The Stressor Identification (SI) process does not address every possible option, nor does it provide details on implementation, so there are many opportunities for interpretation (U.S. EPA, 2000). The U.S. EPA encourages states and tribes to improve and interpret the methodology in ways that are appropriate to their circumstances. Hence, the inserted comments are meant to help other SI users by indicating alternative approaches that they might apply to their cases.

1.1. REGULATORY CONTEXT FOR THE CASE

West Virginia's narrative water quality criterion (WQC) (47 CSR 2-3.2.i) "prohibits the presence of wastes in state waters that cause or contribute to significant adverse affects on the chemical, physical, hydrologic, and biological components of aquatic ecosystems." Various human activities such as mining, logging, agriculture, and residential development have altered the chemical, physical, hydrologic, and biological composition of streams, thus degrading biological conditions in many West Virginia streams. The West Virginia Department of Environmental Protection (WVDEP), through its benthic macroinvertebrate monitoring program, has identified streams across the State that do not meet aquatic life use designations. These streams are considered to be biologically impaired. Under the Clean Water Act, waters that are impaired must be restored to an unimpaired state through the Total Maximum Daily Load (TMDL) process (see Comment 2).

Comment 2. U.S. EPA Regulations and Programs.
To learn more, see a synopsis in the CADDIS Step by Step guide on The Role of Stressor Identification in Various Water Management Programs.

TMDLs currently are being developed for all biologically impaired streams in West Virginia. TMDL development requires that the causes of impairment (i.e., stressors to the biological community) be identified so that pollutants can be controlled in each watershed. We used U.S. EPA's Stressor Identification (SI) guidance to identify and rank physical, chemical, and biological stressors that may have impaired aquatic biological communities (U.S. EPA, 2000). The SI process involves the analysis of all available water quality, habitat, physical, biological, historical, anecdotal, and observational data, and it allows inferences about the likely causes of impairment for each stream.

In West Virginia, TMDLs are developed together with the state's geographically based Watershed Management Framework. This framework partitions the state's

32 major watersheds into five U.S. Geological Survey (USGS)-designated, 8-digit Hydrologic Unit Code, or hydrologic groups (A−E), and operates as a 5-year, five-step process. In the first year of the cycle, the TMDL process begins with stream selection, informational public meetings in the watersheds of the selected streams, and pre-TMDL sampling. In the second year of the process, data compilation starts, and TMDL development begins, including the SI process. These two activities continue through the third year, as draft TMDLs are calculated. Also during the third year, modeled baseline and TMDL conditions of predraft TMDLs are evaluated by participating agencies. This allows subwatersheds to be prioritized for future restoration. In the fourth year of the process, the TMDLs are finalized. Implementation occurs in the fifth year, through the National Pollutant Discharge Elimination System (NPDES) permitting process. If remediation is required (typically aimed at nonpoint source pollution abatement), these too are started in the fifth year. Public participation and stakeholder involvement is encouraged throughout TMDL development.

1.2. DESCRIPTION OF THE WATERSHED

To illustrate the SI process in West Virginia, we describe a single TMDL watershed, the Clear Fork Subwatershed of the Coal River (see Figures 1 and 2). The Coal River Watershed (about 571,000 acres) is in central-southwestern West Virginia and joins the Kanawha River at Saint Albans, WV. The Coal River and its tributaries generally flow northwesterly and are divided into two major subwatersheds—the Little Coal River and Big Coal River. These two subwatersheds are similar in size and are further comprised of smaller sub-basins or subwatersheds; Spruce Fork and Pond Fork converge at Madison, WV, to form the Little Coal River, while the Big Coal River originates from the confluence of Marsh Fork and Clear Fork, south of Whitesville, WV.

Most of the Coal River basin is in the Central Appalachians (Level III, Ecoregion 69) and is an elevated, dissected, and rugged plateau comprised largely of sandstone, shale, conglomerate, and coal (Woods et al., 1996). The soils resulting from the rugged terrain and cool climate are mostly shallow and relatively infertile. The potential natural vegetation for this area is mixed mesophytic forest types, including Appalachian oak, northern hardwood, and northeastern spruce/fir forests. The ecoregion is further subclassified into (Level IV) Ecoregions 69a, Forested Hills and Mountains, and 69d, Cumberland Mountains, with the former comprising a small portion of the upper Watershed (Woods et al., 1996). The other (Level III) ecoregion found within the Watershed (Ecoregion 70) consists of wooded, hilly, unglaciated terrain near the mouth of the Coal River. Further classified (Level IV) to Ecoregion 70b, Monongahela Transition Zone, the hills and ridges of this area are lower in elevation and support primarily mixed mesophytic forests, with white oak (*Quercus alba*) and red oak (*Q. rubra*) being dominant.

1.3. POTENTIAL SOURCES OF STRESSORS

Coal, oil, and natural gas extraction has contributed to the degradation of streams in the Clear Fork Watershed. Coal mining, primarily in the form of mountaintop

FIGURE 1

Confluence of Clear Fork and Sycamore Creek (on right) Near Ameagle, WV. Pile of dark material to right (left bank of Clear Fork) is old mining spoil (pre-1970). Lower Clear Fork substrate is influenced by historic mine spoil piles, such as in the photo. Photo by Jeffrey Bailey, WVDEP.

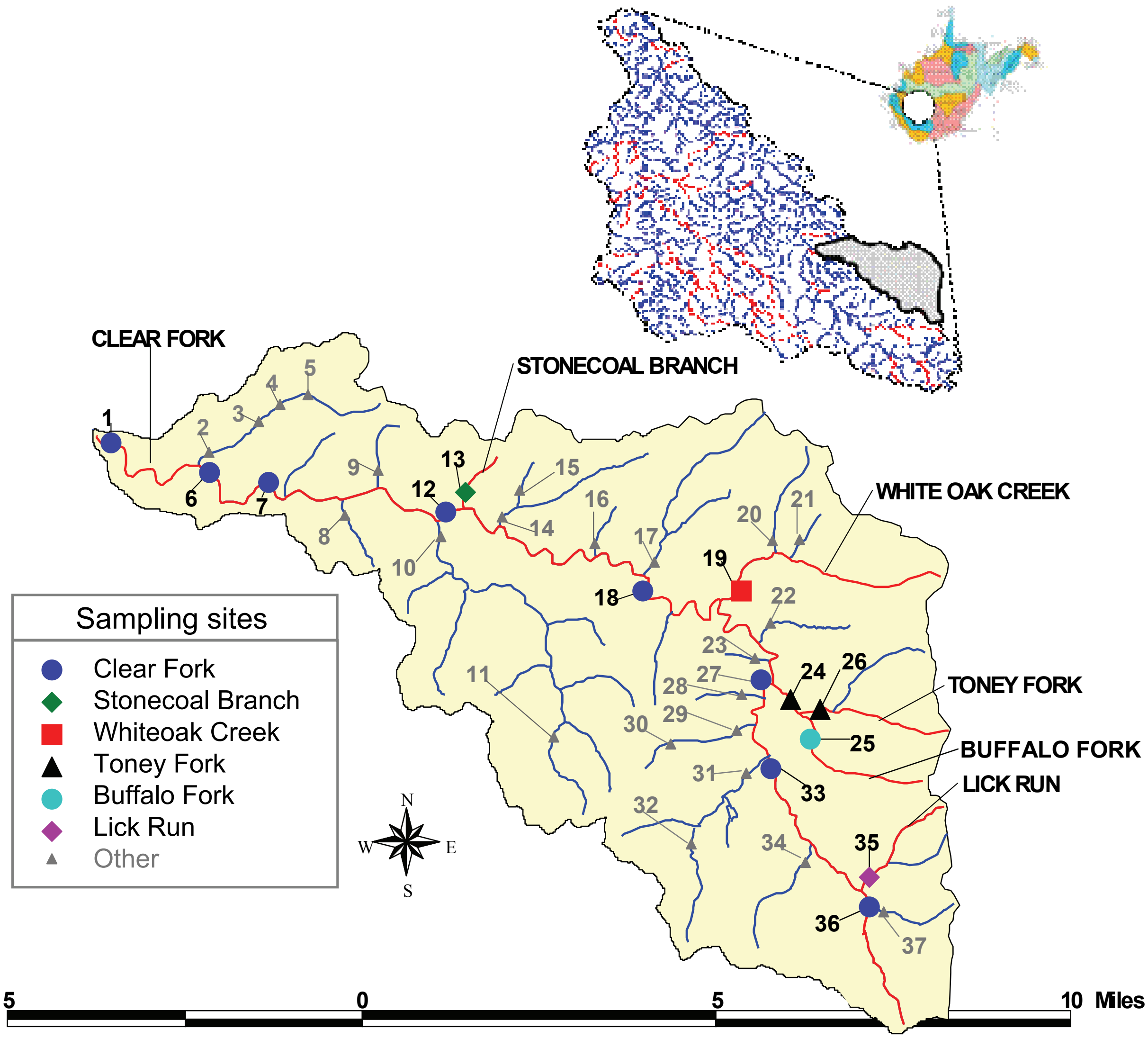


FIGURE 2

Clear Fork Watershed Showing Numbered Sampling Sites and Several Tributaries removal, is the major industry in this region. This method of surface mining is commonly used to unearth coal seams once considered too thin to be mined. As a byproduct of this process, excess rock (overburden) is pushed into headwater valleys, creating valley fills. These large landscape alterations significantly affect stream hydrology and morphology, and otherwise stress receiving streams (Pond et al., 2008). Residential land use also affects streams in the Clear Fork Watershed: residential inputs include organic and nutrient enrichments via discharges from improperly sewered homes and failed septic systems.

The Clear Fork sub-basin is about 20% of the Big Coal River's drainage area, and its land use practices are representative of the larger Coal River Watershed. The Clear Fork Watershed is forested, primarily with deciduous hardwoods, except for areas with logging, mining, or residential uses. Agriculture also occurs throughout the Watershed but has declined since 1950. The Clear Fork Watershed is entirely within the Cumberland Mountains (Level IV, Ecoregion 69d). This ecoregion is strongly dissected with steep slopes, narrow ridge tops, and extensive forests.

1.4. SPECIFIC BIOLOGICAL IMPAIRMENTS

Biological condition was assessed using the West Virginia Stream Condition Index (WVSCI), a biotic index calculated from the species composition and relative abundances of benthic macroinvertebrates (see Comment 3). The WVSCI was developed using WVDEP's watershed assessment data and U.S. EPA's Environmental Monitoring and Assessment Program data collected from riffle/run habitats in wadeable streams. The WVSCI is based on family level taxonomic identification and consists of six metrics (Tetra Tech, Inc., 2000):

- total Taxa Richness (the number of distinct taxa);
- total Ephemeropteran, Plecopteran, and Trichopteran (EPT) Taxa (number of taxa within the orders Ephemeroptera [mayflies], Plecoptera [stoneflies], or Trichoptera [caddisflies]);
- percent EPT Taxa (percent of individuals that are in the Orders Ephemeroptera, Plecoptera or Trichoptera);
- percent Chironomidae (percent of individuals in the family that includes true midges);

Comment 3. Specific Impairments.
The causal assessment in the Coal River was done because streams within the watershed did not attain a WVSCI score $\geq$60.6, the threshold for 303(d) listing for aquatic life impairment in West Virginia. The section entitled "specific biological impairment" describes how the WVSCI and threshold for attainment were derived. The specific metrics that cause the score to vary are listed but are not used to indicate how each one differed at each impaired site. This could lead stakeholders to think that he same biological timpairment and the same cause occurs at all impaired locations. To avoid this potential misconception, the U.S. EPA guidance recommends that a specific impairment be identified for each site. The guidance further indicates when sites may be considered as a group with similar effects.
See the Long Creek, Little Scioto, Willimantic, and Bogue Homo Rivers' case studies for examples of specific impairments. See Define the Case-Specific Impairment for the advantages of using a specific impairment as the assessment endpoint for the causal analysis.

- percent 2 Dominant Taxa (the cumulative percent of individuals within the two numerically dominant taxa); and
- the Hilsenhoff Family Biotic Index (Plafkin et al., 1989).

For a particular location, the WVSCI score is determined by calculating the average of the six standardized scores. The standardized score for each metric is determined by comparing an individual metric value (from a site of interest) to the "best standard value." The best standard value represents either the 95th or 5th percentile (depending on whether the metric increases or decreases with increasing perturbation) of all sites sampled throughout the state within a similar classification. All metrics values are converted to a standardized scale from 0 to 100 (worst to best), so the WVSCI score for each site ranges from 0 (worst) to 100 (best). See Tetra Tech, Inc. (2000) for details.

Determination of biological impairment using the WVSCI is based on dissimilarity from the population of reference sites. Accordingly, the 5th percentile of the range of WVSCI scores of 107 original reference sites was selected as the impairment threshold (WVSCI threshold = 68). The 5th percentile was selected because the West Virginia reference sites were determined to meet the "minimally stressed" criterion (Stoddard et al., 2006), yet allowing for anomalous outliers and unknown anthropogenic stress at the reference sites. A site's suitability as a reference site was established by comparing the site's habitat and physicochemical data to a list of minimum degradation criteria or "reference site" criteria. Sites that met all of the minimum criteria were designated reference sites. WVDEP developed the minimum degradation criteria with the assumption that sites meeting the following criteria would provide a reasonable approximation of least-disturbed conditions:

Comment 4. Different Information Content from Different Measurements of the Same Stressor.

Most causal assessments do not have optimal data. For example, single measurements do not capture episodic events or daily fluctuations. This is particularly important for DO, which is lowest in the early morning and can become supersaturated due to photosynthesis in the afternoon. See the Ways to Measure sections for many Candidate Causes.

One reviewer also offered this commentary: "The case study refers to oxygen concentrations in water in units of mg/L. While regulatory standards are written in this fashion, oxygen concentration is not … a toxicological and physiological point of view. The condition that matters is oxygen partial pressure. Raw concentrations of oxygen are influenced by ambient temperature, barometric pressure, and salt content. An accurate metric when discussing the level of oxygen in water is percent oxygen saturation, which takes into account the above factors."

<u>Primary WQC (must be met)</u> (see Comment 4)

1. dissolved oxygen (DO) > 5.0 mg/L;
2. pH between 6.0 and 9.0;

<u>Secondary WQC (flag values – exceedances may require further investigation)</u>

3. conductivity < 500 μS/cm;

4. fecal coliform < 800 colonies/100 mL (mean of 2 or more measurements);

Rapid Bioassessment Habitat Criteria (must be met)

5. epifaunal substrate/available cover score > 10;
6. channel alteration score > 10;
7. sediment deposition score > 10;
8. bank vegetative protection score of lowest side > 5 (right and left banks);
9. undisturbed vegetation zone width score of lowest side > 5 (right and left banks);
10. total habitat score ≥ 130 points;

Other

11. evaluation of anthropogenic activities and disturbances at site using field data;
12. evaluation of landuse and landcover upstream of the site using geographic information system (GIS) coverages;
13. no known violations of State water quality standards;
14. no obvious sources of nonpoint pollution; and
15. no known point source discharges upstream.

Based on the initial data analysis (Tetra Tech, Inc., 2000), the 5th percentile of the WVSCI score in reference sites was 68. WVDEP further sampled 26 sites in duplicate to determine the precision of the scoring methodology. Using the duplicate data, the 90% 1-tailed confidence interval above a WVSCI score from a single sample was estimated as 7.4. Therefore, the effective 303(d) listing value is a WVSCI score of 60.6, such that a single sample having a score of <60.6 is considered impaired by WVDEP, with 90% confidence.

While monitoring for TMDL development (see below), the WVSCI score for Clear Fork of the Coal River ranged from about 55 to about 75 (see Figure 3). Although both the headwaters and the mouth of Clear Fork scored above 68.0, mid-reach sections of the stream scored below 60.6. Additionally, several tributaries to Clear Fork (e.g., Lick Run, Toney Fork, Buffalo Fork, Whiteoak Creek, and Stonecoal Branch) scored below 60.6. Several sites on the mainstem and several tributary streams had WVSCI scores in the “fair” range (60.6−68.0).

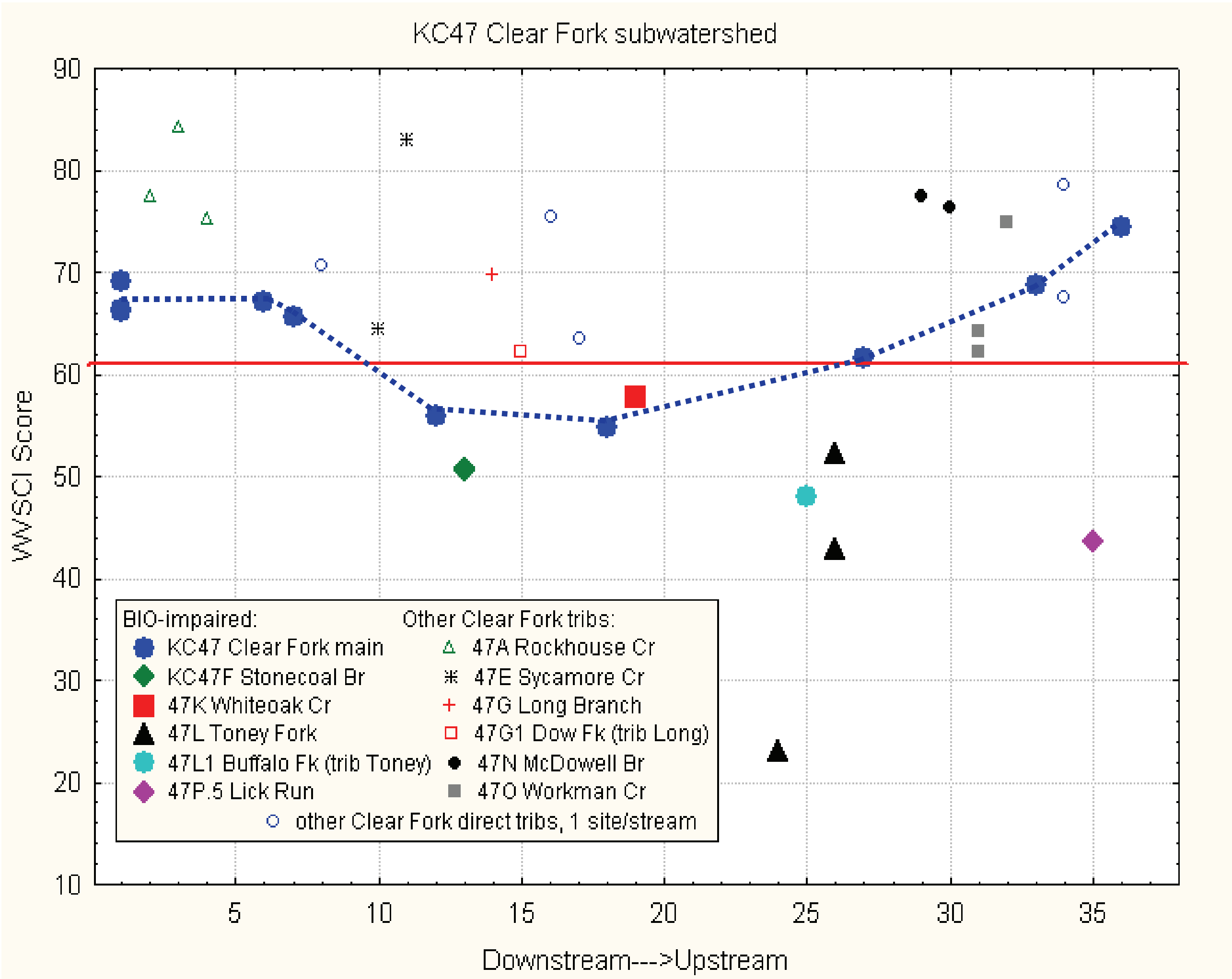


FIGURE 3

West Virginia Stream Condition Index (WVSCI) Scores for Clear Fork and its Tributaries. Solid red line is the WVSCI biocriterion for single sample determination of impairment. Symbols indicate sampled stream. Large symbols are assessed in this report; small samples are not because they scored greater than the WVSCI biocriterion. Site locations relative to the Clear Fork Mainstem are shown in kms from the River mouth (left) to furthest upstream location at River km 35 on the x-axis (right). Dotted line connects Clear Fork sites for easier viewing. See also Figure 2.

2. CANDIDATE CAUSES

2.1. DATA SOURCES FOR CAUSAL ANALYSIS

2.1.1. West Virginia Water Quality Monitoring

To support TMDL development, the WVDEP stream-assessment strategy uses a tiered process for monitoring streams and rivers. Water quality data are collected from long-term monitoring stations, targeted stations (within watersheds, on a rotating schedule), randomly selected stations, and stations strategically located to provide additional information on impaired stream segments (WVDEP, 2005).

Pre-TMDL monitoring is used to characterize existing water quality, biological condition, and to refine impairment listings. West Virginia's 303(d) list is used as the basis for initial site selection and additional sites are used to identify suspected sources of impairment. Pre-TMDL monitoring consists of monthly chemical sampling conducted over a 1-year period, plus a single biological sampling event during West Virginia's index period. The monthly sampling is intended to capture various weather conditions and flow regimes that could indicate specific nonpoint and point sources of impairment (WVDEP, 2005). Although the WVSCI is based on family level data, benthic macroinvertebrates are now identified to genus when possible (WVDEP, 2005).

2.1.2. Pollutant Source Report and Source Tracking

As part of the TMDL program, WVDEP developed a GIS-based report of potential pollutant sources (see Figure 4). This report is used both for SI and in TMDL modeling. The report consists of shapefiles representing the geographical features of the Watershed and possible pollutant sources, including

- NPDES outlets for mining operations;
- NPDES outlets for other (nonmining) operations;
- permitted mining areas;
- valley fills;
- abandoned minelands (AML) portals;
- AML highwalls;
- total AML area;
- other AML disturbances;
- oil and gas wells;
- harvest and burn history of managed forest land;
- water quality sampling locations;

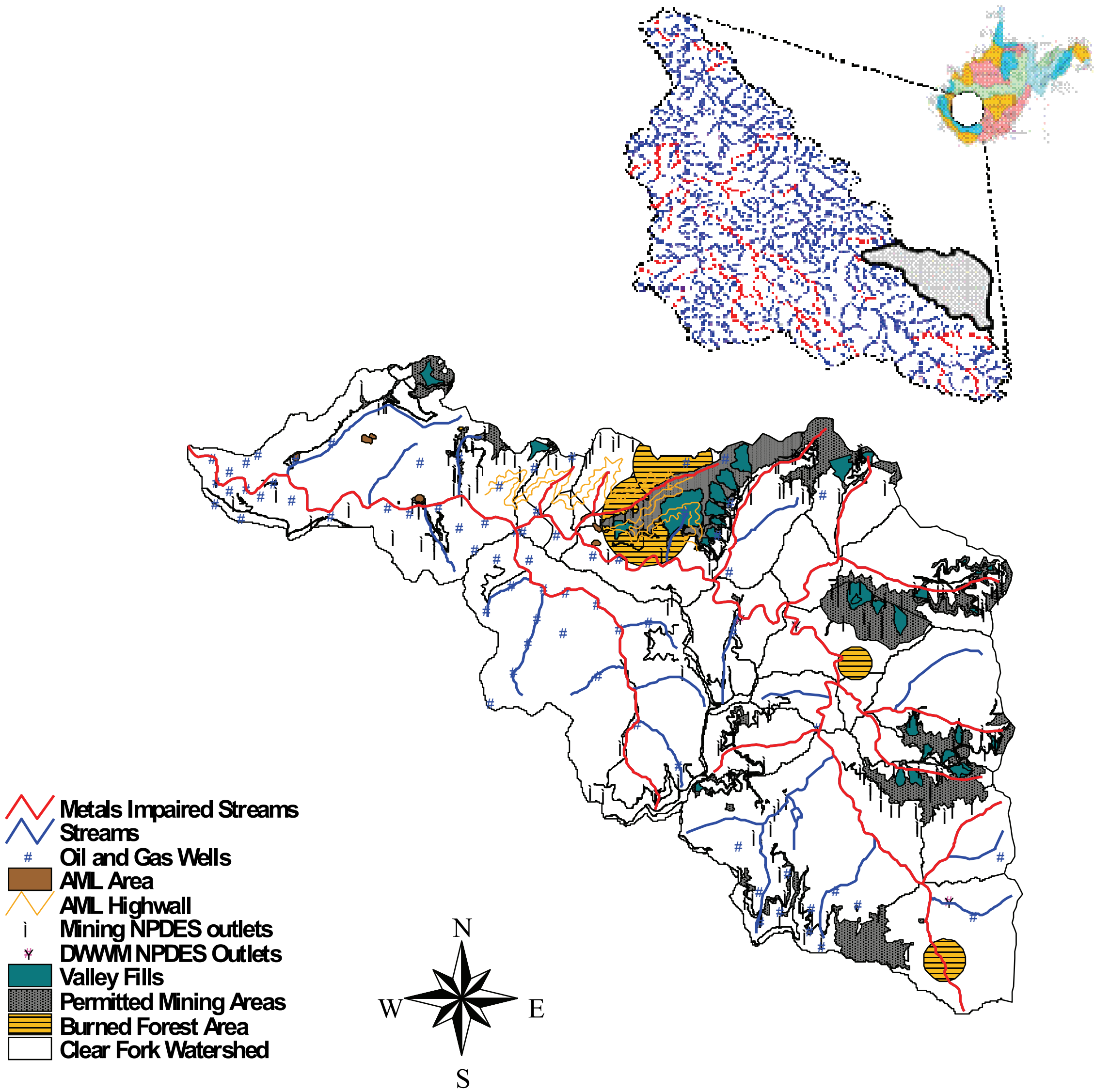


FIGURE 4

Clear Fork Watershed Showing Current and Abandoned Mining Areas, Oil and Gas Wells, and Burned Areas. AML = Abandoned Minelands; DWWM = Division of Water and Waste Management; NPDES = permitted discharges (National Pollutant Discharge Elimination System).

- source tracking photos (WVDEP);
- roads, including unmapped roads discovered in source tracking;
- weather stations;
- USGS gauging stations;
- towns;
- streams defined in the National Hydrographic Dataset (NHD), as well as unmapped streams discovered in source tracking;
- subwatershed delineation; and
- land use.

Potential sources of stressors in subwatersheds were validated or refuted by ground-truthing. The ground-truth source tracking process consisted of walking all NHD stream length identified as impaired in the 303(d) list, and documenting and photographing potential sources of pollution (point sources, nonpoint sources, and general riparian condition and activities). The process located undocumented AML drains, discharges of untreated sewage, livestock, and unmapped roads.

2.2. CONCEPTUAL MODEL

We developed a comprehensive conceptual model to show the linkages between potential causes of impairment, the sources of these stressors, and the pathways by which the stressors could affect the benthic macroinvertebrate community. This model was based on initial data analyses, knowledge of the watersheds, and experience in defining impairment causes in similar watersheds (see Comment 5). Sources of stressors, the pathways leading to stressors, and resulting effects to the biological community depend on the stream or watershed in question. In some cases, impairments were linked to a single stressor; in other cases, multiple stressors were responsible for the impairments. Our conceptual model (see Figure 5) includes all reasonable potential causes and their likely sources.

Comment 5. Dealing with Data Limitations. When data are insufficient or a candidate cause is unlikely based on professional judgment, then assessment of a candidate cause may be deferred until a later time. This is commonly the case when analyses are expensive and additional data are not collected. For example, in this case study, pesticide and organic chemicals were not evaluated as candidate causes. This does not deny these as potential causes if such information later came to light. However, as a general principle, a lack of information on some candidate causes need not impede incremental progress in addressing others in a causal assessment.

The candidate causes of an altered benthic macroinvertebrate assemblage depicted in the conceptual model are summarized below (see Comment 6):

1. elevated concentrations of metals (including metals contributed through soil erosion) may be toxic;
2. high pH (>9) may be harmful;

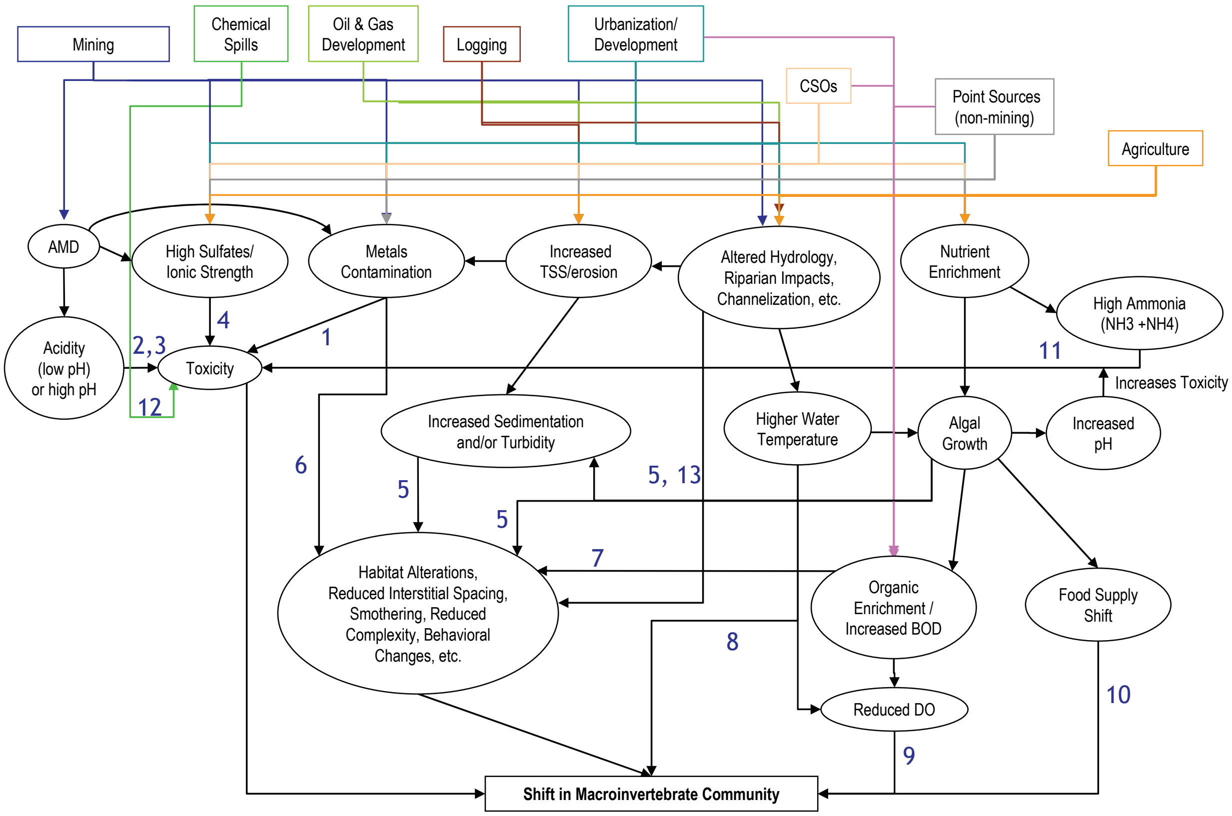


FIGURE 5

Conceptual Model of Sources and Stressors in the Coal River Watershed, WV. Potential sources are listed in top-most rectangles. Potential stressors and interactions are in ovals. Candidate causes are numbered (1) through (13) (see numbered list in Section 2.2). Note that some causes have more than one stressor or more than one associated step.

3. high pH (>9) may be harmful;
4. high sulfates/increased ionic strength causes toxicity;
5. increased total suspended solids (TSS)/erosion, altered hydrology, and algal growth causes sedimentation and other habitat alterations;
6. elevated levels of some metals in water, such as iron, aluminum, or manganese, can result in the formation of flocs, which can increase embeddedness;
7. nutrient and organic enrichment (e.g., sewage discharges, agricultural runoff) promote growth of filamentous algae and fungi, causing habitat alterations;
8. altered hydrology causes higher water temperatures, resulting in direct impacts;
9. altered hydrology, nutrient enrichment, and increased biological oxygen demand (BOD) cause reduced DO in water;
10. algal growth causes food supply shift;
11. high ammonia levels cause toxicity (including increased toxicity due to algal growth);
12. chemical spills cause toxicity; and
13. altered hydrology can cause flow permanence that can influence insect life cycles.

Comment 6. Value of Very Specific Candidate Causes.
Candidate causes are described very specifically in this case study. The candidate cause includes an agent, a mode of action, and in some cases, a source. This is valuable because it reduces ambiguity when communicating the assessment to others. When the assessment is complete, the specificity of the identified cause narrows the scope of subsequent assessments that will identify the source and the interventions that are needed to reduce exposure and restore biological condition.
One disadvantage is that the list of candidate causes is long. This requires that there is special care in managing the analysis and in presenting the findings to stakeholders and resource managers.

The remainder of this section provides a brief description about what is generally known from the literature about these candidate causes in streams and in some cases for sources in the Clear Fork Watershed.

2.2.1. Enrichment

2.2.1.1. *Enrichment Pathways—Low DO.*

Low concentrations of DO can stress many aquatic species, and low DO in streams often results from organic or nutrient enrichment (see Comment 7). Sources of nutrients to Clear Fork included sewage discharges, animal wastes, runoff from fertilized fields and

Comment 7. Nutrient Enrichment.
Nutrient enrichment of a waterbody often results in high levels of primary production and may lead to depletion of dissolved oxygen. For more information and detailed conceptual models, see common candidate causes: nutrients and dissolved oxygen at the Causal Analysis/Diagnosis Decision Information System (CADDIS) Web site.

lawns (WVDEP, 2005), and atmospheric deposition of nitrogen. Untreated sewage and animal wastes also contain organic matter. In these cases, fecal coliform was used with caution as a surrogate for organic enrichment, although both nutrients and organics can be unrelated to fecal waste. Some homes in the Clear Fork Watershed still lack satisfactory sewer systems, and even properly designed septic systems may fail, releasing organic wastes.

Direct organic enrichment (usually measured as BOD or chemical oxygen demand) can be decomposed by aerobic microbes, which can lower the concentrations of DO. If sufficient light is available, nutrient enrichment can allow rapid algal growth. Greater levels of algae result in greater production of oxygen during periods of photosynthesis and greater consumption of oxygen during periods dominated by respiration. Thus, in highly enriched streams, which have high levels of algal biomass, photosynthesis is accelerated, and oxygen is often supersaturating on sunny days, but DO can decline to levels that are stressful to biota at night, when photosynthesis ceases but respiration continues (e.g., Hynes, 1960, 1970).

Elevated levels of ammonia, such as those originating from inadequately treated animal or human wastes, also can contribute to low levels of DO. The ammonium is nitrified by bacteria that consume oxygen in the process (e.g., Rysgaard et al., 1994). In addition, un-ionized ammonia (NH_3) is toxic to many aquatic organisms (see below).

Nutrient concentrations were not sampled regularly in the Clear Fork streams, and phosphorus detection limits (most common: 0.10 mg/L) were higher than needed for determining background or reference phosphorus concentration. BOD was only rarely measured. Fecal coliform was sampled regularly throughout the Watershed, and we used it rather than nutrient concentration as a surrogate measure for both nutrient and organic enrichment. We recognize that nutrient enrichment and organic enrichment are not the same, nor do they always co-occur, but the existing data did not permit their separation.

2.2.1.2. *Enrichment Pathways—Altered Food Supply.*

The food base of undisturbed high gradient forested streams primarily comes from plant litter (or allochthonous inputs), with only a small amount of autochthonous production (Cummins and Klug, 1979; Minshall et al., 1983; Vannote et al., 1980). The native benthic macroinvertebrates are adapted to this food source, with a large group of shredders that process the leaves and other organic matter. Autochthonous food is mostly algae (diatoms) and is consumed primarily by organisms with scraping mouthparts and by filter-feeders. Changes in the relative abundance of plant litter and algae can cause subsequent changes in the benthic macroinvertebrate assemblage. Nutrient enrichment or increased light can cause increased abundance of algae in a stream and may be accompanied by increased abundance of snails (scrapers), black fly larvae (suspension feeders), and hydropsychid caddisfly larvae (suspension feeders) (see Comment 8). In oligotrophic, forested mountain streams, both increased light penetration and nutrient enrichment are required for large increases in algae abundance

(Carpenter et al., 1998; Hill et al., 1995). Trees are removed with most human activities.

2.2.1.3. *Enrichment Pathways—Ammonia Toxicity.*

> **Comment 8. Specific Biological Responses is a Type of Evidence.**
> Specific biological responses that are often seen associated with certain situations are listed in the last paragraph for each candidate cause described in this case. This is important information. If these changes are not observed, then this weakens the case for that candidate cause. If they are observed, then this strengthens the case for that candidate cause. This information was not explicitly used in the analysis but could have been used as a form of assemblage symptomology. (See Touchet River case study for an example.)

Ammonium ions (NH_4^+) exist in equilibrium with NH_3, and the equilibrium is pH-dependent (Stumm and Morgan, 1996): higher pH values drive the reaction towards greater concentrations of the un-ionized (more toxic) form. U.S. EPA's toxicity criterion for ammonia is a function of total ammonia concentration, pH and water temperature (U.S. EPA, 2002). In eutrophic waters, relatively low concentrations of ammonia increase episodically, sometimes to toxic levels, as a result of elevated water temperatures and photosynthesis-driven increases in pH. During warm and sunny periods, photosynthesis can reduce CO_2 and bicarbonate ion concentrations, which increases pH (Wetzel, 2001). Alkaline pH contributes to the formation of unionized ammonia. When this occurs, toxicity can increase, at least until levels of dissolved CO_2 are re-established as photosynthesis declines near sunset.

2.2.2. Acidity

Most headwater streams in the Central Appalachian ecoregion are poorly buffered (low alkalinity) and have naturally low nutrient concentrations due to the underlying sandstone and shale formations and thin soils. The Central Appalachians and the Western Appalachian Plateau also contain North America's largest group of coal formations (the coal-rich region includes seven states, from Pennsylvania to Alabama; USGS, 1999). Some of this coal is sulfur rich, as are some of the marine-derived shales associated with the coal deposits (USGS, 1999). Mining of high-sulfur coal exposes pyrite (FeS_2) to oxidation by chemosynthetic bacteria, resulting in the formation of sulfuric acid, and potentially leading to acid mine drainage (AMD). In addition, the region is exposed to atmospheric acidic deposition from combustion sources. Consequently, the poor buffering capacity and sources of acid from both atmospheric acidic deposition and AMD often leads to acidification of streams (DeNicola and Stapleton, 2002).

Acidic deposition includes wet and dry deposition of sulfates and nitrates; these originate largely as emissions of sulfur dioxide and nitrous oxides from fossil fuel combustion, primarily coal. The northern Central Appalachians (southwest PA and northern WV) have particularly high rates of atmospheric sulfate deposition, mostly from the burning of coal in electric generating plants in the Ohio River valley (NAPAP, 1991). Sulfate deposition rates peaked in the mid 1990s and have declined since enactment of the Clean Air Act amendments of 1992 (Driscoll et al., 2001). Acidic deposition can exert a strong influence on small, poorly buffered headwater streams by decreasing pH and increasing the solubility of aluminum (e.g., Driscoll et al., 2001; Stumm and Morgan, 1981) which is toxic to many aquatic organisms in its ionic form (e.g., Baker and Schofield, 1982; Baldigo and Murdoch, 1997; McDonald et al., 1989). Acidity alone is also very damaging to some types of organisms (e.g., cyprinid fish; see McDonald et al., 1989).

In many Central Appalachian streams, pH is affected more by AMD than by atmospheric deposition. Large-scale land disturbances, such as mining, can increase inputs of sulfates and metals to streams. Mining operations (surface and underground) and commercial land development both increase rock fracturing and movement; this exposes unweathered bedrock to oxidation and weathering and allows leaching of rock constituents to surface waters. Oxidation of pyrite in high-sulfur marine shales associated with coal deposits results in the formation of sulfuric acid and ferric ions. The increase in acidity causes the dissolution of aluminum from clays and aluminosilicates. In addition to acidity and dissolution of aluminum, AMD is characterized by high conductivity, elevated concentrations of iron and sulfate, and often other metals such as manganese (e.g., DeNicola and Stapleton, 2002). These other substances, acting in combination with acidity, can create conditions that are especially stressful to the stream biota, due to synergistic toxicity (Grippo and Dunson, 1996; McDonald et al., 1989). These are discussed below under "Sulfate, Conductivity, and Other Metals."

Some aquatic organisms are adapted to surviving in low-pH conditions. For example, some stoneflies (Plecoptera: *Leuctra* and *Amphinemura*) persist in nutrient-limited, dilute waters of headwater streams, where buffering potential is low due to the underlying geology. These organisms can be exposed to episodic acidification (Lepori et al., 2003), and may dominate benthic assemblages in some streams.

2.2.3. Sulfate, Conductivity, and Other Metals

Present-day coal mine operations are required to treat AMD by adding alkaline materials or anhydrous ammonia. The treatment neutralizes acidity, causes potentially toxic metals to precipitate from solution, but leaves soluble salts in solution. The salts generally are less toxic than the metals that are precipitated but can be present at concentrations high enough to adversely affect aquatic biota. Organisms that are adapted to low-conductivity waters may be particularly vulnerable to increased levels of dissolved solids (Koel and Peterka, 1995). For example, certain mayflies, such as *Drunella* sp., have respiratory structures (tracheal gills) that are efficient at taking up oxygen but vulnerable to metal ions. The sensitivity to metals may be due to a relatively large number of ionoregulatory cells (chloride cells) on the gill surfaces (e.g., Buchwalter and Luoma, 2005). In contrast, other organisms have integuments and gill surfaces with relatively few chloride cells (e.g., Chironomidae, pollution-tolerant species indicative of poor water quality) that may provide a selective advantage in streams where concentrations of ions are high. Treated and untreated mine drainage are typically very high in sulfate, calcium, and magnesium ions. Salts are deleterious to freshwater organisms when present in high concentrations. However, test organisms are typically highly tolerant species adapted to high-conductivity hard water or lake taxa with little relevance to streams (e.g., *Ceriodaphnia, amphipods, and fathead minnow).* Except for the Greenbrier Karst subregion, the Central Appalachians consist primarily of shale and sandstone with little calcium carbonate. Consequently, streams are poorly buffered, with natural background conductivity in the range 30-180 μS/cm (median 46). Native aquatic insects are adapted to these conditions and may be sensitive to increased ionic strength, which affects osmotic balance, gas exchange, and uptake of potentially toxic substances through the gills.

Manganese is frequently, but not always, found in mine drainage. Manganese causes bad taste in drinking water, and gray staining of laundry in wash water (U.S. EPA, 2002). High concentrations can cause a fine black floc of manganese and iron hydroxides to form on substrates (Diz, 1997), which can smother invertebrates. It is moderately toxic to amphipods and *Ceriodaphnia* (Lasier et al., 2000).

2.2.4. Sediment

Fine sediment is frequently a stream pollutant and often results from activities associated with agriculture, logging, mining, road construction, and urbanization (Henley et al., 2000; Walsh et al., 2005; Waters, 1995). Adverse effects of increased sediment on aquatic life in streams are well documented (e.g., summaries in Waters, 1995). Several studies have shown that aquatic invertebrates decrease in abundance, and that benthic macroinvertebrate communities change taxonomically and functionally in response to increased sediment (Wood and Armitage, 1997). Suspended sediments reduce water transparency and thereby can lower rates of primary production by aquatic plants (Relyea et al., 2000; Vannote et al., 1980). Reduced primary production can affect other organisms in the aquatic food web by reducing the supply rates of food, reducing the availability of appropriate refugia, and altering the habitat for aquatic invertebrates and fish. Suspended particles can damage or clog the delicate gill structures of aquatic organisms resulting in decreased abundance and diversity of filter-feeding invertebrates whose filter-feeding structures have become clogged with suspended sediment (Wood and Armitage, 1997). When sediment deposits are present in excess, some aquatic invertebrates may emigrate (via drift) and be subject to increased predation, encounter physiological challenges, and are lost from the system (Shaw and Richardson, 2001). Deposited sediments reduce the amount of habitat available to benthic invertebrates by filling interstitial spaces between boulder, cobble, and gravel substrates. Many stream-dwelling aquatic animals deposit their eggs in gravel or on cobble substrates. When substrates are buried under fine sediment, egg mortality can increase due to reduced availability of DO. Organisms with an affinity for cobble and gravel bottoms may be replaced by ones that are more tolerant of increased sedimentation. Therefore, the presence of many sediment-tolerant organisms can be used as an indicator of increased sedimentation (Wood and Armitage, 1997).

2.2.5. Habitat

Disturbances to streamside features can threaten the resident biota in numerous ways (Allan, 2004). Among the more important habitat components indicative of degraded biological potential are standard measures of bank stability, vegetative bank protection, riparian vegetation, and hydrologic stability. These parameters, which are quantified as part of stream assessments, often mirror in-stream living conditions, and thereby reflect benthic community health (Barbour et al., 1999).

Bank stability, one of the habitat assessment measures used by WV DEP, is a measure of the erosion potential of a streambank (Barbour et al., 1999). It is affected by armoring, bank vegetation cover, excessive stream energy, and the long-term stability of the stream valley. Increased bank erosion may lead to extensive habitat degradation, including embeddedness, scour, habitat instability, and reduced habitat availability for both fish and macroinvertebrates (Allan, 1995; Cummins, 1974; Hynes, 1970).

Measurements of vegetative bank protection primarily indicate whether vegetation cover is sufficient to stabilize the stream bank, as well as to provide information on stream shading and available streamside habitat. Vegetated banks may be undercut by erosion caused by increases in stream energy, or may be devegetated by logging, agriculture, construction, recreational overuse, etc. Such disturbances may initiate other processes, such as stream channel shifts (changes in channel morphology), that may further degrade biological communities (Allan, 2004). Some macroinvertebrate functional feeding groups, such as shredders, depend strongly on riparian vegetation inputs (woody debris as substrate; allochthonous organic matter as an energy supply; Allan, 2004; Cummins and Klug, 1979; Cummins et al., 1989). Therefore, disturbing or removing stream bank vegetation can substantially reduce the abundance of these taxa.

The condition of riparian vegetation typically reflects local landuse, which affects biological condition (Allan, 2004). Activities that reduce riparian vegetation may disrupt food-web dynamics, alter temperature regimes, and reduce the system’s ability to tolerate toxicants entering the stream from the surrounding landscape (e.g., Allan, 1995; Hynes, 1970). In addition, some species of aquatic insects have adult forms that require certain types or amounts of riparian vegetation to complete their lifecycle.

In Central Appalachia, industrial activities and residential land use can affect the hydrologic stability of streams, negatively affecting the benthic community. Disturbances such as valley fill construction (resulting from surface mining) and pumped discharges (from underground sources, including subsided streams) threaten benthic communities by changing the annual flow regimes from highly variable to fairly stable. Although such a flow regime augments stream flow during low-flow conditions and provides additional habitat for some species, the loss of natural flow variability may be more detrimental. Flow stabilization may be particularly harmful to organisms that are well adapted to variable thermal regimes.

3. EVALUATE DATA FROM THE CASE

3.1. SPATIAL CO-OCCURRENCE

Available quantitative data were plotted and analyzed spatially from upstream to downstream in the mainstem, as well as in tributaries, by assigning relative positions to the sampling sites (from downstream to upstream) (see Figure 6). Each tributary stream is plotted at its confluence with the mainstem, so that tributary sites are adjacent in the plot, but mainstem sites are dispersed over the plot. For example, Station 1 is located nearest to the mouth of Clear Fork (see Figure 6); just upstream of this sampling site is a tributary that had four sampling sites (Stations 2, 3, 4, and 5, going progressively upstream; see Figure 6). The next mainstem (upstream) sampling location in Clear Fork is Station 6. When plotted in numerical order with unique symbols for mainstem sites and for each tributary (see Figure 6), the serial arrangement of all sampled sites is preserved. This allows an estimation of the degree of influence of a tributary on conditions in the mainstem stream. We prepared scatter plots for each numeric parameter to spatially represent all data collected in the Watershed. This process allowed us to evaluate spatial co-occurrence of stressors and biological responses.

3.2. STRESSOR-RESPONSE (S-R) RELATIONSHIPS IN THE FIELD

Owing to the rich West Virginia database, we examined S-R relationships throughout the Central Appalachians ecoregion and throughout the state. S-R relationships within the Clear Fork Watershed were similar to the statewide associations and used the same sampling protocols. As such, we did not examine within-Clear Fork S-R relationships separately from the statewide analysis, except to identify Clear Fork data in statewide data plots (see Chapter 4).

3.3. CAUSAL PATHWAY

The Causal Pathway was evaluated for complex candidate causes using information about intermediate stressors that are known to increase the intensity of the proximate stressors that affect the biological community (see Comment 9). This consideration was important for all of the nutrient enrichment-related candidate causes including algal growth, change in food supply, low DO, and ammonia toxicity. Qualitative field observations of sources and stressors were also used to determine the strength of the causal pathway.

> **Comment 9. More on Causal Pathways.**
> Evidence of a causal pathway may relate to any part of the causal pathway. Evidence may include measures of: stressor sources, intermediate causes, or factors influencing the proximate cause, or associations between these and the proximate or specific biological effect. See these links for more information on causal pathway as a type of evidence at Evaluate Data from the Case: Causal Pathway and analyzing data at Organizing Data along Causal Pathways.

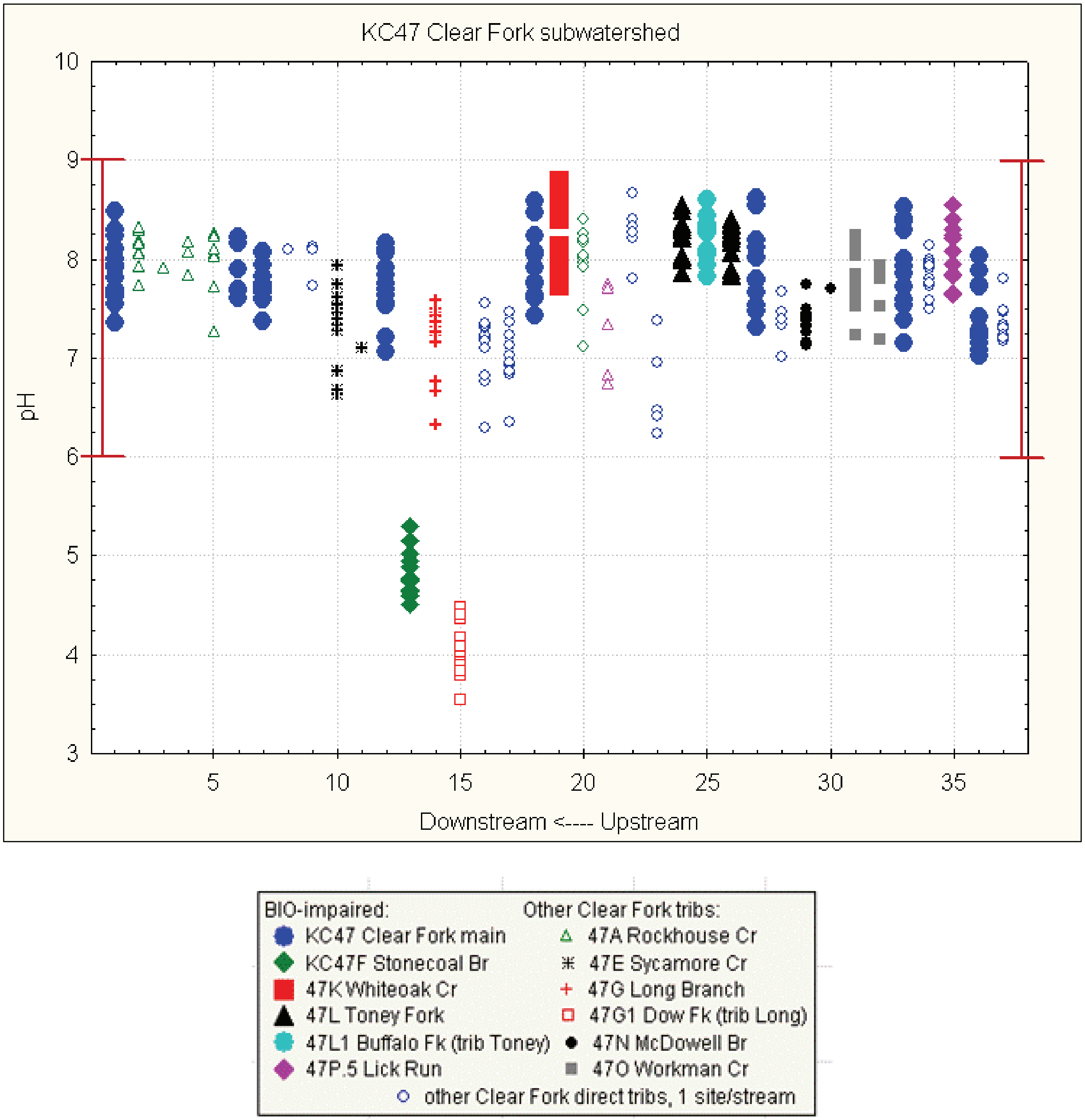


FIGURE 6

Geographic-Order Scatterplot for pH. Graph illustrates one example of how observations for mainstem and tributaries were depicted for pre-TMDL monthly sampling in 2003. The mainstem of Clear Fork is indicated by solid circles; data from Clear Fork tributaries are represented by open circles. Bars at either end of graph indicate pH range that was considered acceptable.

4. EVALUATE DATA FROM ELSEWHERE

4.1. STRESSOR-RESPONSE (S-R) RELATIONSHIPS FROM STATEWIDE DATA

The large amount of data collected by WVDEP throughout the state for the TMDL process allowed us to characterize S-R (dose-response) relationships for each quantitatively measured stressor, the WVSCI score, and critical response metrics that illustrate specific responses. It also allowed us to extract information specifically from individual metrics that contribute to the total WVSCI score. We estimated thresholds of response and nonresponse by (1) graphical analyses of scatter plots of biological indicator values and measured stressors values (see Figure 7) (see Comment 10); and (2) several statistical techniques for deriving thresholds of response (see Appendix A).

These scatter plots (e.g., see Figures 8a, b, c) often show a "wedge-shaped" scatter of points. At low levels of the stressor, there are both high and low values of the biological indicator, but at high values of the stressor, there are typically only low values of the biological indicator.

The objective of the scatter plots and their statistical analysis is to define thresholds of responses:

1. a threshold of the stressor at which no response is observed but a response is seen beyond that stressor value threshold; and
2. a threshold where the response has become strong enough to have biological relevance to the community or assemblage.

Comment 10. Characterizing Associations.
Scatter plots were used to depict associations between measures of different candidate causes and measures of biological response. Curves were drawn by LOWESS (Locally Weighted Estimation), a statistical technique that draws a curve through the scatter plot of points. Examples of other, repeatable options are ordinary least square regression and quantile regression.
Although not used in this instance, the assessors of this case later used this data set to examine stressor-response relationships using several different statistical methods. The conclusions from the study remained unchanged by these analyses, but they now provide West Virginia with methods that can be reproduced by all assessors. These are presented in Appendix A.
Causal assessment is a form of inductive inference; it is not hypothesis testing. A null hypothesis is not rejected, and therefore, use of *p*-values is inappropriate and in fact was not used to assess the cause in Clear Fork. Rather, general causal relationships are used to support or weaken a case for specific causality. See CADDIS for Using Statistics Responsibly for a more complete discussion.

In these stressor plots, we defined three regions of "plausibility" of a biological response (see Figure 7):

1. if the concentration or intensity of the stressor is similar to that found in regional reference sites, then it is implausible or unlikely to cause impairment and weakens the case for that candidate cause (below response threshold);
2. at intermediate concentrations of the stressor, the stressor may have effects on the biota (above a response threshold), but alone, the level of the candidate

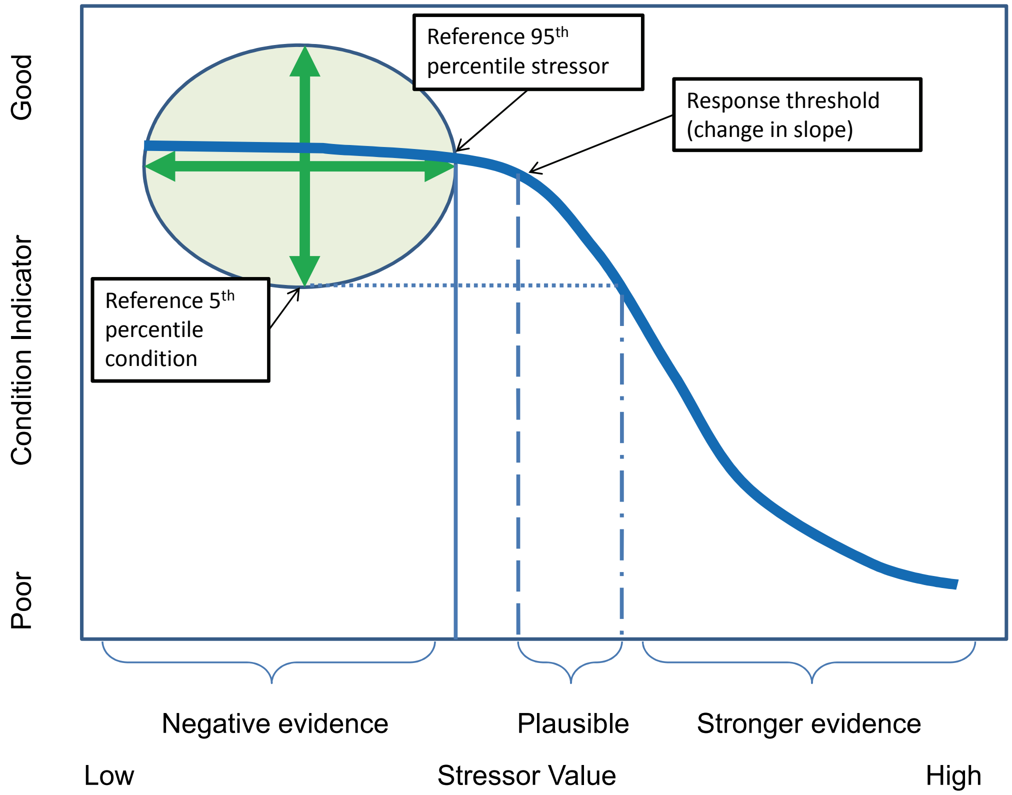


FIGURE 7

Diagram Showing Interpretation of Stressor-Response Relationship. The data are plotted, and a locally weighted estimation (LOWESS) regression reveals an S-shaped association between the stressor and the response or condition indicator. The ellipse with broad-crossed arrows indicates the 90% distribution of both stressor and response indicator values in regional reference sites. The solid vertical line shows the 95^{th} percentile of stressor value in reference sites, and the horizontal dotted line shows the 5^{th} percentile of biological indicator or response values in the reference sites. The curve reveals a response threshold (vertical dashed line), where the mean response value begins to decline as stressor increases (breakpoint). The dot-dash line indicates the point where mean-predicted response value (from LOWESS or linear regression) is equal to the 5^{th} percentile of biological condition, i.e., where the mean condition is substantially less than the reference condition. Relative strength of evidence is indicated by the brackets below the x-axis, negative evidence discounts, plausible evidence supports, and stronger evidence strongly supports.

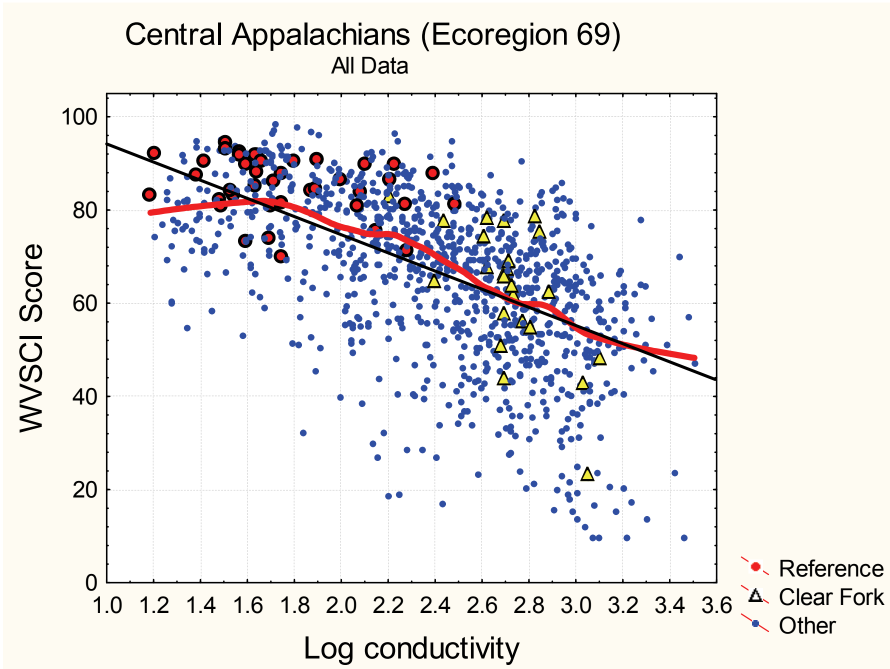


FIGURE 8a

Scatterplots of the Full Data Set of Biological Responses (WVSCI) with a Candidate Stressor, Conductivity (on log scale, µS/cm). Central Appalachian (Ecoregion 69) reference sites and Clear Fork sites are identified. Heavy red curve is LOWESS, and straight black line shows linear regressions. Note threshold in LOWESS estimate at conductivity near 60 µS/cm (log conductivity 1.8), and crossing WVSCI = 71 at conductivity near 250 µS/cm (log conductivity 2.4).

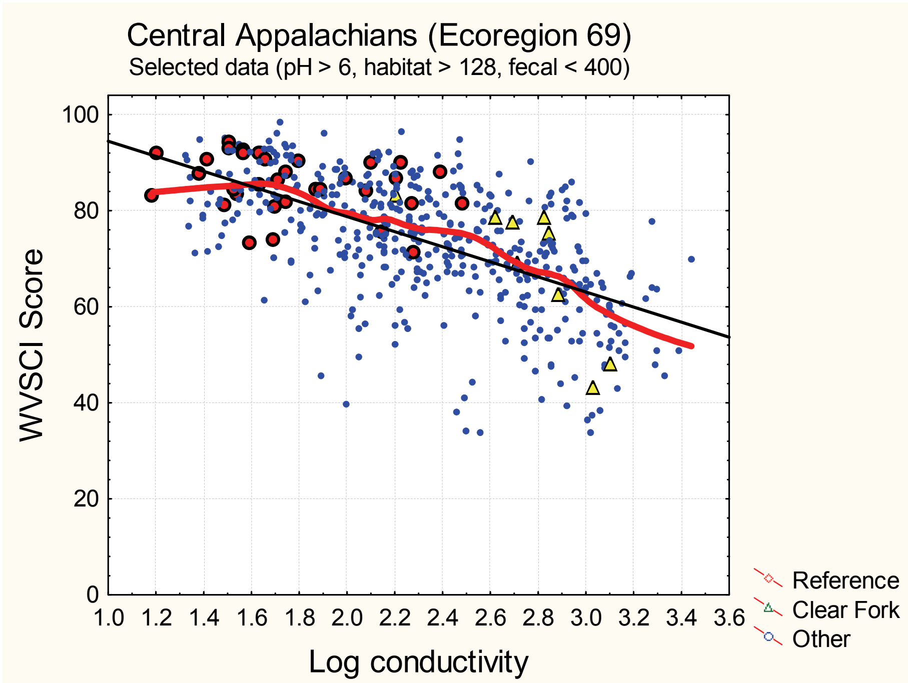


FIGURE 8b

Scatter Plots of a Constrained Data Set of Biological Responses (WVSCI) with a Candidate Stressor, Conductivity (on log scale, µS/cm). Samples removed: pH > 6, habitat score > 128, and fecal coliform < 400 colonies. LOWESS estimated threshold is the same as Figure 8a, but conductivity at WVSCI = 71 increased to approximately 400 µS/cm (log conductivity 2.6).

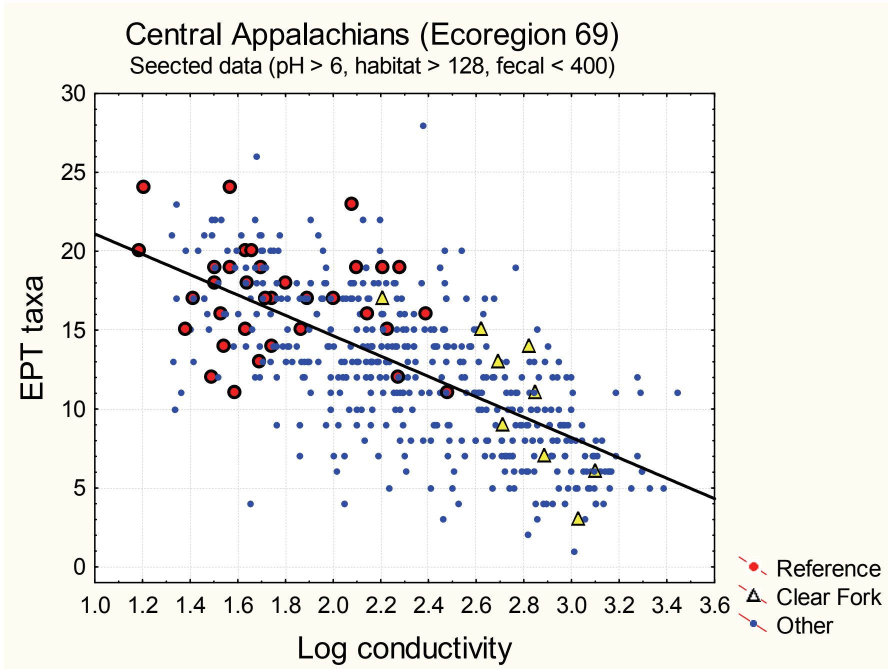


FIGURE 8c

Scatter Plots of More Sensitive Biological Responses (EPT taxa) with a Candidate Stressor, Conductivity (on log scale, µS/cm). EPT is a component of WVSCI, the number of taxa that are Mayflies, Stoneflies, or Caddisflies (EPT taxa). Linear regression only is shown because LOWESS showed no improvement over linear; hence, there is no threshold that can be assigned. Note that this is not a wedge-shaped scatter plot. Reference 95th percentile of conductivity is 180 µS/cm (log conductivity 2.25), and regression line crosses the 5th percentile of EPT taxa at 250 µS/cm.

cause may not be strong enough to result in severe biological change on a regular basis (response detectable, but below substantial change threshold); and

3. at high concentrations of the stressor, the biota are clearly different from reference in the regional data set, and the concentration is deemed sufficient to cause strong change in other cases, thus strengthening the case for that candidate cause (above strong change threshold).

Stressor values in the first region are similar to reference values, or are below an observed threshold, and are almost never associated with a decline in condition. Stressor levels in the second region, above the initial response threshold, are not associated with the best biological condition (highest WVSCI scores), but neither are they substantially degraded. The assumption here is that the stressor is only causing slight to moderate degradation. Evidence of stressor levels in the second region was considered plausible but not strong enough to have contributed to degradation. Stressor values in the third region are nearly always associated with substantial biological degradation, and this was considered strong evidence that the candidate stressor could be a cause of biological degradation (see Figure 7).

We estimated these regions and the thresholds from two data sources: (1) the distribution of the stressors in regional reference sites, to estimate the range of the stressor with no effect, or almost no effect, on biological response; and (2) an S-shaped response curve based on the selected stressor gradient, showing an initial decline in condition (the response threshold) at the shoulder of the curve, and also showing the point where the mean response declines below the 5th percentile of reference condition (see Figure 7). Not all responses show an S-shaped curve with a shoulder—some are more nearly a straight line. For example, Figures 8a, b and c show the response to increasing conductivity, which can be as readily interpreted as a straight-line response as a curve.

We examined other statistical methods (see Appendix A) that can be used to estimate the initial response threshold. Throughout, we made a concerted effort to examine the effects of single stressors, with the effects of confounding and collinear multiple stressors removed as far as possible. The other analyses included conditional probability analysis (Paul and McDonald, 2005), which estimates the probability of impairment for cumulative increases in levels of stressors in a regional sample, and change-point analysis (Qian et al., 2003), which partitions the response distribution (WVSCI) into two groups based on minimizing within-group variance. We found that the locally weighted estimation (LOWESS) estimation often represented the overall response, when the response is a nonlinear S-shaped response curve. Exceptions where the response was adequately explained by linear regression included the responses to aluminum and to conductivity (see Figure 8).

4.1.1. Thresholds

From the statewide data, we attempted to estimate the thresholds shown in Figure 7 (see Comment 11):

- Weakening threshold—stressor values below the reference site 95th percentile were considered to weaken the case for that candidate cause. If reference site data were insufficient, we used the WVDEP- or in its absence the U.S. EPA-recommended criteria (presumed, but not demonstrated, to be protective against that potential cause).
- Plausible threshold—stressor values above an empirical weakening threshold, and above an observable "shoulder" in the stress-response were considered plausible to induce a biological response. The shoulder was estimated visually from the LOWESS regressions of the response data on the stressor values (see Appendix A), as the midpoint where the LOWESS slope changed from shallow to steep on the S-shaped response curve. If the response appeared to be a straight line, then there is no shoulder, and the reference 95th percentile is assumed to capture natural variability in undisturbed sites. Because of unknown anthropogenic sources of stressors, we recognize that the reference 95th percentile may also include some anthropogenic stress.
- Substantial Effects threshold—For S-shaped responses, we used the upper 95th confidence interval of change-point analysis to identify substantial effects (Qian et al., 2003). A change-point identifies a midpoint between two clusters of data. The upper confidence interval is conservative and often coincides closely to the point where the LOWESS line crosses the WVSCI = 71 point (see Appendix A). For straight-line responses, we used conditional probability analysis on the probability that WVSCI score would be less than 71, followed by change-point analysis to find the median point where the expectation is that more than half of sites would have WVSCI < 71 for a given stressor level.

Comment 11. Analytical Transparency. Describing how and why evidence is judged is essential for causal analysis. Table 1 and its accompanying text clearly describe how thresholds were set and used as evidence that either weakened or strengthened the case for each candidate cause in the Clear Fork River Watershed case study. These thresholds were developed for streams in West Virginia and are not recommended for use elsewhere. Analyses should always be appropriate to the geophysical and biogeographical characteristics of the case.

Thresholds used are listed in Table 1, and the analysis results are shown in Appendix A. Several stressors showed a response "shoulder" within the reference 95% envelope (ionic stressors and sedimentation). Given the extent of historic human activity in the coal region, these could be in part due to undetected AML or other historic disturbances.

TABLE 1

Thresholds for Evaluating Stressor-Response Information, Quantitative and Semi-Quantitative Stressor Data, Ecoregion 69. For stressors with sufficient data, Stressor-response thresholds are derived in Appendix A. “Weakening evidence” means that stressor values in this range weaken the case for the particular stressor, “Plausible” indicates potential for effects (U.S. EPA CADDIS Web site 2007: http://cfpub.epa.gov/caddis/step.cfm?section=91&step=4&parent_section=12). Note that thresholds derived from reference sites may not agree with those derived from stress-response. Values in this table were revised by WVDEP after completion of this case study and do not reflect current WVDEP practice.

Candidate Cause	Stressor Indicator Measures	Weakening Evidence from Reference Sites or Other Data		Supporting Evidence from Stress-Response		Comments
		Reference Threshold	Data Source	Plausible S-R Threshold (LOWESS line declines)	Substantial Effects Threshold	
1. Metals Toxicity	Al (dissolved)	max < 0.18 mg/L	95%ile reference	Al(dis) > 0.2 mg/L (see Figure A-4a,b); mayfly threshold lower (see Figure A-5b)	Al(dis) > 0.4 mg/L	Effects from conditional probability median (see Figure A-4b,e)
	Fe (total)	max < 0.8 mg/L	95%ile reference	No observed effect	No observed effect	Detailed analysis (see Appendix A) suggests that dissolved Fe and Mn alone (in absence of other stressors) have weak effects on stream invertebrates
	Mn(total)	max < 0.05 mg/L	95%ile reference	>0.05 mg/L	NA	
2. Acid pH	pH	min ≥ 6.5	Break in pH-aluminum relation	No observed effect of pH alone to pH > 4	No observed effect of pH alone to pH > 4	Detailed analysis (see Appendix A and Figure A-3) suggests that pH < 6 and >4, when dissolved Al < 0.1 mg/L, is not harmful to macroinvertebrate condition
3. High pH	pH	max ≤ 9	WV WQC	>9.0	Insufficient data	

TABLE 1 cont.

Candidate Cause	Stressor Indicator Measures	Weakening Evidence from Reference Sites or Other Data		Supporting Evidence from Stressor-Response		Comments
		Reference Threshold	Data Source	Plausible S-R Threshold (LOWESS line declines)	Substantial Effects Threshold	
4. Ionic Strength	Conductivity	max < 180 µS	95%ile reference	>180 Used reference because nearly straight line (see Figure A-11a)	>300	Nearly straight line relationship between WVSCI and conductivity (see Figure A-12a,f, Table A-1). Conditional probability median for substantial effects
	Sulfate	max < 43 mg/L	95%ile reference	>43	>43	LOWESS and conditional probability analysis indicated threshold near 30−33 (see Figure A-12b,e, Table A-1), but note that reference 95% is higher, at 43 mg/L
	Chloride	max < 10 mg/L	95%ile reference	>10	>17	Conditional probability analysis indicated threshold around 17 (see Figure A-12c,f, Table A-1)
5. Sedimentation	TSS	max < 7 mg/L	95%ile reference	No S-R impairment	No S-R impairment	See Appendix A and Figure A-17b
	% Fines (sand + silt + clay)	max $\leq$ 30%	95%ile reference	>30%	>30%	LOWESS suggested threshold around 20% (see Figure A-15a); change point of raw data suggested substantial effects above 24%
	RBP: Embedded-ness	min $\geq$ 13	5%ile reference	<13	<9	Change Point Analysis; (see Appendix A and Figure A-13b,f; Table A-1)

TABLE 1 cont.						
Candidate Cause	Stressor Indicator Measures	Weakening Evidence from Reference Sites or Other Data		Supporting Evidence from Stressor-Response		Comments
		Reference Threshold	Data Source	Plausible S-R Threshold (LOWESS line declines)	Substantial Effects Threshold	
5. cont.	RBP: Sediment	min ≥ 11	5%ile reference	<11	<8	Change Point Analysis; (see Appendix A and Figure A-13c,g; Table A-1)
	RBP: Total (adjusted to post-1998 RBP)	min ≥ 147	5%ile reference	<140	<130	Change Point Analysis; (see Appendix A and Figure A-13a,e; Table A-1)
	RBP: bank stability	min ≥ 13	5%ile reference	<13	<12	Change Point Analysis; (see Appendix A and Figure A-13d,h); Table A-1
6. Other Habitat	RBP: channel alteration	min ≥ 16	5%ile reference	<10	No severe S-R impairment	From RBP “marginal” threshold (Barbour et al., 1999)
	RBP: cover	min ≥ 15	5%ile reference	<10	No severe S-R impairment	From RBP “marginal” threshold (Barbour et al., 1999)
	RBP: riparian vegetation	min ≥ 14	5%ile reference	<10	No severe S-R impairment	From RBP “marginal” threshold (Barbour et al., 1999)
7. Nutrient Enrichment Leading to Excess Algae	NO_3	max < 0.6 mg/L	90%ile reference			Insufficient data
	TKN	max < 1.7 mg/L	90%ile reference			Insufficient data
	TP	max < 0.04 mg/L	90%ile reference			Insufficient data

TABLE 1 cont.

Candidate Cause	Stressor Indicator Measures	Weakening Evidence from Reference Sites or Other Data		Supporting Evidence from Stressor-Response		Comments
		Reference Threshold	Data Source	Plausible S-R Threshold (LOWESS line declines)	Substantial Effects Threshold	
7. cont.	Field observations of algae			≥ "moderate"	Soft algae "high"	
8. Altered Food/ Energy Source	Fecal coliform	max < 250 colonies/ 100 mL	95%ile reference	>250	>500	Used response shown for all data (see Appendix A and Figure A-16a,c) because no high values found in partitioned data (strongly collinear with other stressors)
	Excess algae	see 7				
9. Low DO	DO	min ≥ 5.0 mg/L	WV WQC	min < 5 mg/L (WVC)	min < 4 mg/L	
10. Temperature (direct)	Temperature	max < 30.6^{o}C May through November; or max < 22.8^{o}C December through April	WV WQC	Exceeds WQC	Insufficient data	
11. Ammonia Toxicity	NH_3	max ≤ 0.5 mg/L	100%ile reference	Exceeds U.S. EPA chronic criteria		Insufficient data from WAP
12. Chemical Spills	NA					No data

NA = not applicable; RBP = Rapid Bioassessment Protocol; TKN = total Kjeldahl nitrogen; TP = total phosphorus; WAP = Watershed Action Plan.

4.2. EMPIRICAL MODELS TO RANK MULTIPLE STRESSORS

Identifying the causes of impairment is essential to the development of environmental regulations and the ability of water resource managers to restore aquatic ecosystems. Ideally, based on the biological information found in a stream and the relationships between organisms and environmental variables, aquatic ecologists can predict environmental variables, as well as diagnose stressors that impair water quality (Cairns and Pratt, 1993) (see Comment 12). Field data were used to develop empirical models using both bottom-up and top-down approaches, and both approaches were used to identify probable causes:

1. by developing models from individual taxa response to stressor (bottom-up); and
2. from specific stressor to biological response (top-down).

Comment 12. Predictive Performance and Diagnosis.

The results using the top-down approach (dirty model) is an example of the type of evidence termed "predictive performance." This is a strong form of evidence because it makes a prediction and then checks for agreement.

In this example, a top-down model was developed and used to predict site concurrence or lack thereof with a candidate cause. An underlying premise is that different species are present or absent when certain stressors are present or absent. If we consider, benthic invertebrates as traits or symptoms of an ecosystem, and if those "symptoms" are specific for a "disease" of the ecosystem, or the lack of the "disease," then this would be a very strong model and would be diagnostic of the cause. The "dirty" model uses these assumptions, but the diagnostic proof is suggestive but not definitive and thus does not meet the very tough standards of a diagnostic tool.

We developed both bottom-up and top-down empirical models to predict the stressors most likely to have caused an observed impairment among multiple stressors (Zheng and Gerritsen, 2007). The description below summarizes those results. Because the WVDEP data set is very large, we were able to partition the data to examine the macroinvertebrate community response to single stressors. Four types of environmental stressors shown to affect species composition were identified: conductivity/sulfate, habitat/sediment, acidic/nonacidic metals, and organic/nutrient enrichment. We did not examine stressor interactions because there are many interaction terms, and although the data set is large, even moderate colinearity among the stressors severely reduces the ability to detect interactions. Also, WVDEP typically acts on stressors singly and independently, except where there is demonstrated chemical interaction (e.g., pH and aluminum or ammonia toxicity). The exception was pH and aluminum toxicity, where we did examine the interaction because the effect could be confidently characterized (see Figure A-2).

The bottom-up approach used weighted averaging regression models to develop response-based taxonomic indicators of environmental stress. Weighted averaging regression is a statistical procedure used to estimate the optimal environmental conditions for the occurrence of a taxon (ter Braak and Barendregt, 1986; ter Braak and Looman, 1986). Optimal values of stressors and the breadth of response (whether the taxa are narrowly [stenotopic] or widely tolerant [eurytopic] of departures from optimal) were determined for individual taxa based on available literature and professional judgment. Weighted averaging regression models were then calibrated following the methods of Birks et al. (1990) and used to predict the environmental variables for each site based on these tolerance values and individual abundance. The goodness of fit for the weighted averaging regression inference models were measured by calculating coefficients of determination (R^2) among derived and observed environmental variables. Eight weighted averaging regression models were developed and tested using four groups of candidate stressors based on generic-level abundance. The strongest predictive models were for acidic metals (dissolved Al) and conductivity, $R^2 = 0.76$ and $R^2 = 0.54$, respectively. Benthic macroinvertebrates also responded to environmental variables with good predictive power. Habitat, sediment, sulfate, and fecal coliform R^2 values ranged from 0.38−0.41. Macroinvertebrate taxa had weaker responses and predictive power to total phosphorus (TP) ($R^2 = 0.25$) and nonacidic Al models ($R^2 = 0.29$).

The top-down approach was based on the hypothesis that exposure to various stressors leads to specific changes in macroinvertebrate assemblages and taxonomic composition. A "dirty" reference approach was used to define groups of sites affected by single stressors. Four "dirty" reference groups were identified and consisted of sites primarily affected by one of the following stressor categories: dissolved metals (Al and Fe), excessive sedimentation, high nutrients and organic enrichment (using fecal coliform as a surrogate measure of wastewater and livestock runoff), and increased ionic strength (using sulfate concentration as a surrogate measure). In addition, a "clean" reference group of sites was identified based on low levels of stressors. Nonmetric multidimensional scaling and multiple responses of permutation procedures were used to examine the separation of the "dirty" reference groups from the "clean" reference groups based on the biological communities observed in the two groups. The results indicated that the centroids of the "dirty" reference groups were significantly different from the "clean" reference group ($p < 0.0001$). Note that in this instance a prediction is made and therefore the use of p-values are appropriate. Of the "dirty" reference groups, the dissolved metals group was significantly different from the other three "dirty" reference groups ($p < 0.001$). The other three "dirty" reference groups, though overlapping in ordination space to some extent, were also significantly different from one another ($p < 0.05$). Overall, each of the five "dirty" reference models were significantly different from one another ($p < 0.001$), indicating that differences among stressors may have led to unique macroinvertebrate assemblages. Thus, independent biological samples known to be impaired by a single stressor were used to test the performance of these diagnostic models. The Bray-Curtis similarity index was used to measure the similarity of test sites to each of the reference groups. Multiple stressors were then ranked according to the measured similarity to each reference group. The relative similarity and the variation explained by each model were accounted for in the final ranking of the predicted stressors for each impaired site. The majority of test results indicated that the model agreed with the stressor conclusions based on the physical and chemical data collected at each site. Most of the "clean" test sites (80%) were correctly identified as unimpaired, with 10% considered unclassified. None of the "dirty" test sites was classified as "clean." In addition, the sites in the metal test group were either correctly classified as impaired by metals (87.5%) or were not classified (12.5%). The majority of the sulfate test sites (75%) were correctly identified as sulfate affected. The "dirty" reference models also identified most of the fecal test group (78%)

as fecal impaired, although 22% of the fecal test sites were misclassified as sediment affected. Some of the sediment test sites (37.5%) were also misclassified as fecal affected.

The weighted averaging regression indicator approach (based on taxa tolerance values) and the “dirty” reference approach provide valid and useful tools for identifying evidence of environmental stressors in multiple stressor environments. The applications of these biologically based diagnostic models were used to help identify stressors. Model predictions for each sample were incorporated into the strength-of-evidence analysis for final stressor determinations. Discrepancies between the model predictions from the “dirty” reference models and the stressor-response models from field observations discounted the candidate cause, but in some cases had no effect on the weight of evidence if there was evidence of episodic exposures, or if the model was unable to discriminate certain stressors, such as nutrients and sedimentation.

5. IDENTIFY PROBABLE CAUSES

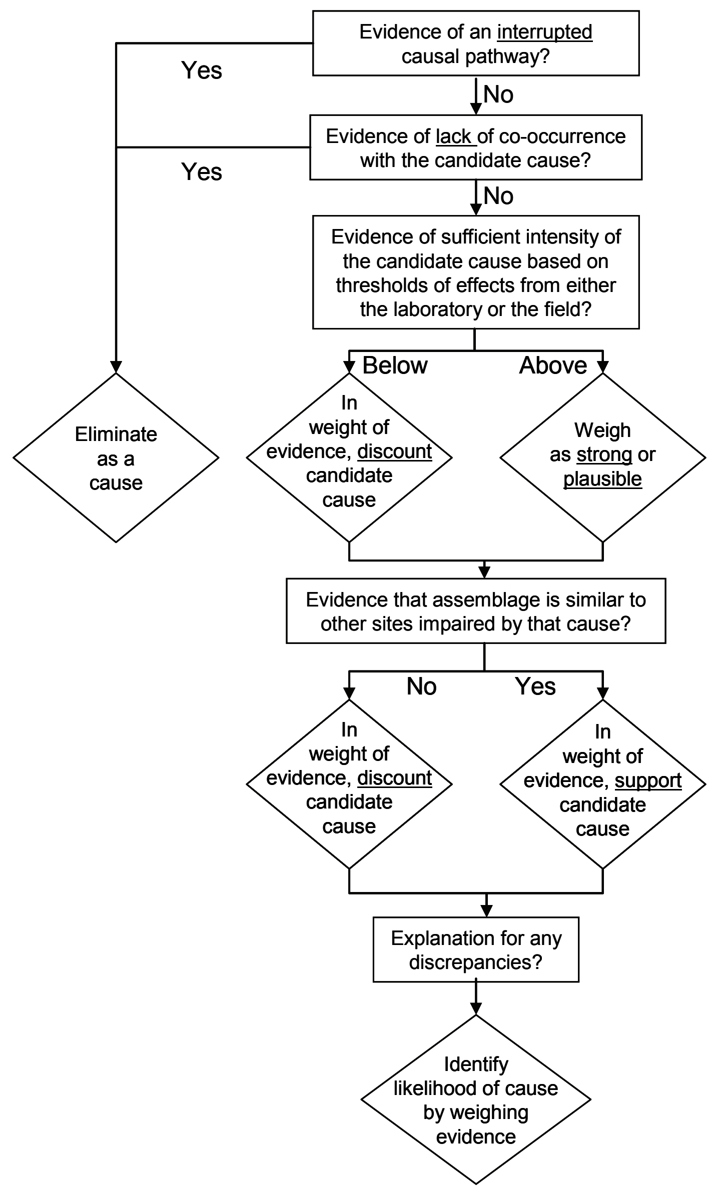
The final step in WVDEP SI required the integration of watershed-based conceptual models of impairment, field biological and chemical monitoring databases (including field notes from pre-TMDL monitoring and TMDL source tracking efforts), empirical models of biological impairment, and ecotoxicological principles in a strength-of-evidence approach to infer causes of impairment (see Comment 13). Primary candidate causes included known toxic contaminants (metals), conventional pollutants (organic and nutrient enrichment), sedimentation, habitat degradation, and ionic concentration (conductivity).

Candidate causes were screened to eliminate exposures that were too low in concentration or intensity to be seriously considered as a potential cause, or if all possible sources of a cause were demonstrated to be absent. For example, AMD can be eliminated as a cause

1. if there are no coal mines in a watershed, now or in the past; or
2. if both conductivity is low and pH is near neutral. Note that high conductivity and neutral pH <u>can</u> occur with AMD that has been treated; therefore, when conductivity is high, AMD cannot be eliminated as a candidate cause.

Remaining candidate causes were ranked according to considerations

Comment 13. Depicting the Process.
The U.S. EPA SI process was intended as guidance. If a very clear process is used to logically determine causes, then it may be helpful to prepare a diagram. This diagram illustrates the process used to identify the candidate causes in this case study.



of evidence within each watershed, from statewide empirical models, and from other published sources. Appendices B-F contain the values used to evaluate if the amount of the stressor was sufficient to cause the effect. Strongest inferences were obtained where the independent predictive model agreed with within-watershed observations of stressor measures. Final stressor determinations for each biologically impaired stream were used to identify specific pollutants for TMDL development. This method will continually evolve and improve as it is applied to current and future TMDL development efforts throughout West Virginia.

Probable causes were identified for each biologically impaired tributary, followed by the Clear Fork mainstem.

5.1. LICK RUN

Lick Run is a 2nd order tributary near the headwaters of Clear Fork (Site 35; see Figure 2). It is severely impaired biologically (WVSCI score = 44). Lick Run has several permitted mining discharges and a current mining area on its northern watershed ridge, and consists mostly of reclaimed mine land throughout. There is logging in the Watershed (clearcutting in advance of surface mining) but no residential land use and no livestock.

The S-R evidence derived from the statewide data analysis suggested the following:

- strong evidence for sedimentation causing impairment as indicated by sediment deposition and embeddedness metrics and, consequently, in reduced total habitat score;
- plausible evidence for ionic stress (conductivity/sulfate) impairment;
- S-R results suggested that iron is not toxic to benthic macroinvertebrates (see Table A-1, Appendix A);
- plausible, but weak evidence for manganese toxicity (statewide data indicated only weak evidence for Mn toxicity at high concentrations);
- plausible but weak evidence for nutrient enrichment as measured by fecal coliform;
- discounting evidence against high temperature as a cause;
- discounting evidence against AMD as a cause; and
- acidic deposition eliminated.

The “dirty” reference model for the Lick Run sample indicated nutrient/organic enrichment as the strongest stressor, followed by conductivity/sulfate stress, and finally sedimentation stress. Model calibration indicated that the model does not distinguish well between nutrient enrichment and sedimentation, and sedimentation stress alone can result in the model identifying both nutrients and sedimentation. Both nutrients and sedimentation appeared as a prediction for Lick Run.

Field observations indicated severe sediment deposition due to the reclaimed mining areas, poor riparian vegetation throughout the Watershed, and moderate algal growth.

Evidence supporting excess sediment deposition as the principal stressor of Lick Run was strong. Secondary stressors in Lick Run include moderate conductivity or sulfate effects and moderate algal growth causing a benthic macroinvertebrate food source shift, but the effects of these are masked to some extent by the sedimentation. The source of the conductivity is reclaimed mine lands or current mining activity. Algal growth is most likely stimulated because the lack of riparian vegetation allows increased light penetration into this headwater stream. There are no known anthropogenic nutrient sources in the Lick Run Watershed.

5.2. TONEY FORK AND BUFFALO FORK

Toney Fork and its tributary, Buffalo Fork, are two of the most impaired tributaries to Clear Fork (see Figure 3). The southern half of the Toney Fork Watershed, which includes Buffalo Fork, is an active mining area with numerous NPDES mining discharges. The West Virginia pollutant source database recorded three permitted valley fills in upper Toney Fork and five in Buffalo Fork. Field observations indicated a moderate amount of houses and lawns, and some cattle and poultry.

The S-R evidence derived from the statewide data analysis suggested the following:

- strong evidence for ionic stress (measured as sulfate/conductivity) causing impairment;
- moderate evidence for enrichment in Toney Fork, but weak in Buffalo Fork;
- weak evidence for iron and manganese;
- discounting evidence against excess sediment and high temperature;
- discounting evidence against AMD; and
- acidic deposition eliminated.

The community similarity from the “dirty” reference model causal consideration indicated that the macroinvertebrate community was most similar to communities strongly affected by sulfate/conductivity, and that sediment was a secondary stressor in Toney Fork, and organic/nutrient enrichment was secondary in Buffalo Fork.

Field observations suggested intermittent sediment deposition and removal, including “fine black sludge” of small coal particles. These observations were made monthly at each of the three sites in Toney and Buffalo and covered the entire length of the reach sampled by the field crews. Quantitative sediment measurements made at the time of macroinvertebrate sampling were confined to the 100-m sampling reach at the time of sampling only. We therefore considered the numerous qualitative observations a more reliable indicator of the potential for sediment impairment than the one-time quantitative sediment measurements, because deposited sediment shifts with changing hydrology. Field observations also indicated a moderate level of algal abundance, no sewage odors, and no observations of domestic sewage pipes.

The conclusions for these tributaries were that the principal stressor causing the impairment is excess sulfate/conductivity, or an unmeasured substance that occurs with the sulfate. The source of the sulfate is mining and the mine effluent treatment ponds. The second vital stressor is sedimentation, which may be variable and intermittent. The sources of the sediments include current mining operations, AML and tailings piles, valley fills, roads and tracks, and residential activities and construction. The third key stressor is moderate nutrient enrichment from septic systems, lawns, and livestock.

5.3. WHITE OAK CREEK

White Oak Creek has two tributaries, Left Fork and Road Fork. The Pollutant Source database recorded mining in the Watershed, including several small valley fills in the headwaters of White Oak Creek and two larger valley fills in the headwaters of Left Fork. There are more than 75 dwellings in the creek valley, near the stream channel.

The S-R evidence derived from the statewide data analysis suggested the following:

- moderate to strong evidence for enrichment as indicated by episodically high fecal coliform concentrations during baseflow periods;
- plausible evidence for sulfate/conductivity causing impairment;
- plausible but weak evidence for manganese;
- plausible but weak evidence for habitat degradation causing biological effects;
- discounting evidence against excess sediment and high temperature;
- discounting evidence against AMD; and
- acidic deposition eliminated.

The “dirty reference” model did not identify any stressor as being stronger or more likely than others. This result indicates that the biological community, while impaired, was not more similar to any one of the single-stress communities than to any other, suggesting that the stream was subject to multiple, cumulative causes.

Observational data collected at the White Oak Creek also indicated that organic enrichment was a major stressor. Evidence included periphyton ratings of moderate to high sewage odors, fecal coliform concentrations exceeding the established significant impairment threshold during baseflow, and evidence of some agricultural runoff. Visual source-tracking by walking the entire stream, also indicated sedimentation stress in parts of the Watershed. Source-tracking consists of observations on the entire length of a stream and is more reliable at capturing excessive erosion and sedimentation than observations at a single point.

The most important cause of impairment was concluded to most likely be organic and nutrient enrichment and appeared to be from inadequately treated domestic sewage. Ionic stress (measured as conductivity/sulfate) was identified as a secondary stressor in White Oak Creek. Surface mining in the headwater reaches is the most likely source. Sedimentation and moderately degraded habitat were identified as a tertiary stressor.

5.4. STONECOAL BRANCH

Stonecoal Branch is a small tributary of Clear Fork that is mostly forested with small dirt roads along the drainage and a mountain top mine/valley fill permit in the headwaters. In addition to the permitted mining activity, there are extensive AML (not restored) throughout the drainage. There are no residences or agriculture within its drainage. In terms of biological measures, the WVSCI score in Stonecoal Branch (50.7) was well below the impairment threshold, and the "dirty" reference model indicated a strong AMD signature.

The S-R evidence derived from the statewide data analysis suggested the following:

- strong evidence for AMD impairment (mean pH = 4.8; mean dissolved aluminum = 3.7 mg/L). In the pH range 4−6, more than 80% of sites were negatively affected (WVSCI < 71) when Al concentration exceeded 1 mg/L (see Appendix A; Figure A-4c);
- substantial evidence for ionic stress (mean conductivity = 499 μS/cm); however, AMD is always associated with high conductivity and sulfate; and
- plausible evidence for manganese impairment.

Similarly, field notes indicated that AMD was a primary stressor in Stonecoal Branch. Indicators of severe AMD noted in the field included cementing of substrate particles by iron hydroxides (colloquially known as "yellow boy"; see Figure 9), and presence of aluminum hydroxide floc. There is no doubt that Stonecoal Branch is impaired by AMD (see Comment 14).

Comment 14. Visual Inspection at the Site.

Acid mine drainage is an extreme case of metals contamination that results in visible evidence. When acid mine drainage mixes with the higher pH water of a receiving stream, the metal hydroxides precipitate (ferric hydroxide, aluminum hydroxide, manganese oxide) from the water column as flocs that coat the streambed (see Figure CC.1-3).

Photographic documentation can be an effective type of evidence illustrating co-occurrence. For other images that illustrate co-occurrence, visit the sections for each Candidate Cause section of CADDIS in the subsections entitled Site Evidence that Suggests Listing as a Candidate Cause.

FIGURE 9

“Yellow boy” (Fe(III)hydroxide) Deposition in Fickey Run, WV. The floc coats the entire stream bottom, fills interstitial spaces, and coats the exposed rocks. In severe cases, the floc can form a hard cement matrix holding all substrate (photo: Joe Cochran, WVDEP).

5.5. CLEAR FORK

The mainstem of Clear Fork receives waters from its biologically impaired tributaries described above, as well as several other tributaries that exceed West Virginia water quality standards, but were not listed as biologically impaired. Clear Fork's biological condition is good in the headwaters (Site 36; see Figure 2), then declines below Lick Run and other tributaries to become impaired from Site 27 to Site 12. Below Site 12, the stream condition recovers to "marginal" status to the mouth. Influent tributaries carrying stressors above S-R thresholds are shown in Table 2.

TABLE 2

Clear Fork Mainstem Sites Showing Tributaries With Measured Stressors Above Stressor-Response Thresholds

Clear Fork Site	Upstream Tributary (sites)	Measured Stressors in Tributary
33	Lick Run (35)	sediment, iron, manganese
27*	Workman Creek (31, 32)	manganese, excess conductivity/sulfate
	McDowell Branch (29, 30)	fecal coliform (nutrients, enrichment)
18*	Toney, Buffalo Forks (24−26)	sulfate/conductivity
	White Oak Creek (19)	fecal coliform (nutrients, enrichment), some iron
12*	Long Branch	some dissolved aluminum from AMD in Dow Fork (trib. to Long Branch), conductivity/sulfate
	Stonecoal Branch	AMD, iron, manganese
7 to mouth	Sycamore Creek	slight iron, fecal coliform (nutrients, enrichment)

* = impaired.

In addition to the tributaries, there are a few mining areas with direct drainage to Clear Fork (no named tributaries), as well as numerous oil and gas wells along the mainstem and along tributaries to the south. There are residences and roads throughout the stream valley and floodplain, mostly near the stream.

The S-R evidence derived from the statewide data analysis suggested the following:

- moderate (plausible) evidence for organic and nutrient enrichment as measured by fecal coliform;
- plausible evidence for sulfate/conductivity causing impairment;
- plausible evidence for excess sediment in upper and lower thirds of mainstem;
- weak evidence for manganese;
- weak evidence for AMD: dissolved aluminum weak, but low pH is not present; and
- weak evidence for iron toxicity in the lower mainstem.

The “dirty” reference model did not identify any single stressor as the greatest potential cause of impairment.

- Field personnel (J. Bailey) observed sedimentation in the upper and lower thirds of the mainstem. The middle third has too high a gradient for sediments to deposit and remain (high hydraulic power). Moderate levels of algae, both periphytic diatoms and soft (filamentous) forms, were observed in the lower portion of the mainstem. Sewage was observed, and sewage odor was noted several times during sampling in the lower mainstem.

Clear Fork (as a receiving stream of Lick Run, Toney Fork and Buffalo Fork, White Oak Creek, and Stonecoal Branch) is an example of a stream affected by multiple stressors and multiple, cumulative causes (organic/nutrient enrichment from untreated domestic wastewater, excess sedimentation, and residual metals and conductivity effects of mining). No single stressor is overwhelming by itself, and the condition ranges from unimpaired in the upper third to moderately impaired in the middle third, and recovering to marginally impaired in the lower third. The upper third of Clear Fork is affected by scour and suspended sediment, but nevertheless remains above WV’s biological threshold. In the middle third, organic enrichment from sewage most likely has the strongest effect, although suspended sediment during high flows and residual metals toxicity from tributaries may be contributing factors. The lower third is most likely affected by sedimentation, poor habitat, moderately elevated conductivity, and moderate enrichment causing algal growth. The biota in the lower third of Clear Fork is in fair condition, but the apparent multiple, cumulative or combined stressors in this receiving stream have prevented recovery to good condition.

6. DISCUSSION

The Clear Fork case study illustrates what can be done with a comprehensive statewide database that enables quantitative analysis. No doubt, the potential to better understand causal relationships can be further investigated and improved by adding paired measurements for other causes. Nevertheless, this Clear Fork case study demonstrates that a watershed-wide causal assessment has several advantages for making analysis practical, defensible, and establishing the relationships among interconnected waterbodies.

This case used the SI process to eliminate candidate causes that did not co-occur with effects. Remaining candidate causes were ranked according to the strength of evidence (strongest to weakest) of occurrence within each watershed. Types of evidence included co-occurrence of stressors with observed biological impairment, S-R threshold values from the statewide data analysis, and the predictive models to rank multiple stressors. We obtained the strongest inferences where the models agreed with on-site observations of stressors.

Probable causes were different throughout the Watershed, and the combination of all these causes was evident in the mainstem, which exhibited some resiliency due to dilution and different geophysical attributes. In particular, causes included metal contamination and acidification from mine draining, aluminum toxicity in association with low pH, sediment deposition, organic enrichment from direct releases and from algal productivity enhanced by nutrients, and low DO.

- Lick Run—the principal cause of biological impairment of Lick Run appears to be sediment deposition and erosion most likely from abandoned minelands, and riparian disturbance along the stream corridor, both of which also contribute to degraded aquatic habitat. In addition, ionic stress, likely from abandoned minelands and current mining activity is also apparent. There is no residential land use and no livestock.
- Toney Fork and Buffalo Creek—the principal cause of impairment appears to be excess sulfate/conductivity, or an unmeasured substance that occurs with the sulfate. Mining activities and mine effluent ponds are present within these watersheds and known sources of sulfate.
- White Oak Creek—the principal cause is most likely organic and nutrient enrichment and appears to be from inadequately treated domestic sewage.
- Stonecoal Branch is impaired by acid mine drainage and likely sediment too from abandoned minelands and dirt roads.
- Clear Fork— a receiving stream of all of the above, is apparently biologically impaired by multiple causes, such as: organic/nutrient enrichment from untreated domestic wastewater, excess sedimentation, and the residual metals and conductivity effects of mining.

7. REFERENCES

Allan, J.D. 1995. Stream Ecology: Structure and Function of Running Waters. Chapman and Hall, New York, NY.

Allan, J.D. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Ann. Rev. Ecol. Evolut. Systematics. 35:257−284.

Baker, J.P. and C.L. Schofield. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Poll. 18(1−3):289−309.

Baldigo, B.P. and P.S. Murdoch. 1997. Effect of stream acidification and inorganic aluminum on mortality of brook trout (*Salvelinus fontinalis*) in the Catskill Mountains, New York. Can. J. Fish. Aquat. Sci. 54:603−615.

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/841/B-99/002.

Birks, H.J.B., J.M. Line, S. Juggins, A.C. Stevenson and C.J.F. ter Braak. 1990. Diatoms and pH reconstruction. Phil. Trans. Roy. Soc. Lond. B. Ser. Biol. Sci. 327:263−278.

Buchwalter, D.B. and S.M. Luoma. 2005. Differences in dissolved cadmium and zinc uptake among stream insects: Mechanistic explanations. Environ. Toxicol. Chem. 39(2):498−504.

Cairns, J., Jr. and J.R. Pratt. 1993. A history of biological monitoring using benthic macroinvertebrates. In: Freshwater Biomonitoring and Benthic Macroinvertebrates, D.M. Rosenberg and V.H. Resh, Eds. Chapman and Hall, New York, NY. p. 10−27.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley and V.H. Smith. 1998. Non-point pollution of surface waters with phosphorus and nitrogen. Ecol. Appl. 8:559−568.

Cummins, K.W. 1974. Structure and function of stream ecosystems. BioScience 24(1):631−641.

Cummins, K.W. and M.J. Klug. 1979. Feeding ecology of stream invertebrates. Ann. Rev. Ecol. Syst. 10:147−172.

Cummins, K.W., M.A. Wilzbach, D.M. Gates, J.B. Perry and W.B. Taliaferro. 1989. Shredders and riparian vegetation. BioScience. 39:24−30.

DeNicola, D.M. and M.G. Stapleton. 2002. Impact of acid mine drainage on benthic communities in streams: The relative roles of substratum vs. aqueous effects. Environ. Poll. 119(3):303−315.

Diz, H.R. 1997. Chemical and biological treatment of acid mine drainage for the removal of heavy metals and acidity. Ph.D. dissertation, VPI, Blacksburg, VA. Available at http://scholar.lib.vt.edu/theses/available/etd-81697-135443/unrestricted/etd.pdf.

Driscoll, C.T., G.B. Lawrence, A.J. Bulger et al. 2001. Acidic deposition in the northeastern United States: Sources and inputs, ecosystem effects, and management strategies. BioScience. 51(3):180−198. Available at http://www.esf.edu/efb/mitchell/Class%20Readings%5CBioSci.51.180.198.pdf.

Grippo, R.S. and W.A. Dunson. 1996. The body ion loss biomarker. 1. Interactions between trace metals and low pH in reconstituted coal mine-polluted water. Environ. Toxicol. Chem. 15(11):1955−1963.

Henley, W.F., M.A. Patterson, R.J. Neves and A.D. Lemly. 2000. Effects of sedimentation and turbidity on lotic food webs: A concise review for natural resource managers. Rev. Fisheries Sci. 8(2):125−139.

Hill, W.R., M.G. Ryon and E.M. Schilling. 1995. Light limitation in a stream ecosystem – Responses by primary producers and consumers. Ecology. 76(4):1297−1309.

Hynes, H.B.N. 1960. The Biology of Polluted Waters. Liverpool University Press, Liverpool England. 202 pp.

Hynes, H.B.N. 1970. The Ecology of Running Waters. University of Toronto Press, Toronto. 555 pp.

Koel, T.M. and J.J. Peterka. 1995. Survival to hatching of fishes in sulfate-saline waters, Devils Lake, North Dakota. Can. J. Fish. Aquat. Sci. 52:464−469.

Lasier, P.J., P.V. Winger and K.J. Bogenrieder. 2000. Toxicity of Manganese to *Ceriodaphnia dubia* and *Hyalella azteca.* Arch. Environ. Contam. Toxicol. 38(3):298−304.

Lepori, F., A. Barbieri and S.J. Ormerod. 2003. Effects of episodic acidification on macroinvertebrate assemblages in Swiss Alpine streams. Freshwater Biol. 48:1873−1885.

McDonald, D.G., J.P. Reader and T.R.K. Dalziel. 1989. The combined effects of pH and trace metals on fish ionoregulation. In: Acid Toxicity and Aquatic Animals, R. Morris, E.W. Taylor, D.J.A. Brown and J.A. Brown, Eds. Cambridge University Press, Port Chester, NY, USA. p. 221−242.

Minshall, G.W., R.C. Petersen, K.W. Cummins et al. 1983. Interbiome comparison of stream ecosystem dynamics. Ecol. Monogr. 53:1−25.

NAPAP (National Acid Precipitation Assessment Program). 1991. 1990 Integrated Assessment Report. National Acid Precipitation Assessment Program, Washington, DC.

Paul, J.F. and M.E. McDonald. 2005. Development of empirical, geographically specific water quality criteria: A conditional probability analysis approach. J. Am. Water Resourc. Assoc. 41(5):1211−1223.

Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. U.S. Environmental Protection Agency, Office of Water, Washington, DC. EPA/440/4-89/001.

Pond, G.J., M.E. Passmore, F.A. Borsuk, L. Reynolds, and C.J. Rose. 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. J.N. Am. Benthol. Soc. 27(3):717−737.

Qian, S.S., R.S. King, and C.J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. Ecological Modelling. 166:87−97.

Relyea, C.D., G.W. Minshall and R.J. Danehy. 2000. Stream insects as bio-indicators of fine sediment. In: Watershed Management 2000 Conference Proceedings, Vancouver, B.C., July 9-12, 2000. Sponsored by the Water Environment Federation and the British Columbia Water and Wastes Association. Water Environment Federation, Alexandria, VA.

Rysgaard, S., N. Risgaard-Petersen, N.P. Sloth, K. Jensen and L.P. Nielsen. 1994. Oxygen regulation of nitrification and denitrification in sediments. Limnol. Oceanogr. 39:1643−1652.

Shaw, E.A. and J.S. Richardson. 2001. Direct and indirect effects of sediment pulse duration on stream invertebrate assemblages and rainbow trout (*Oncorhynchus mykiss*) growth and survival. Can. J. Fish. Aquat. Sci. 58(11):2213−2221.

Soucek, D.J. 2004. Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. Final Report to Illinois Environmental Protection Agency and Illinois Coal Association. January 14, 2004.

Stoddard, J.L., D.P. Larsen, C.P. Hawkins, R.K. Johnson and R.H. Norris. 2006. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecol. Appl. 16(4):1267−1276.

Stumm, W. and J.J. Morgan. 1981. Aquatic Chemistry. Wiley and Sons, New York, NY.

Stumm, W. and J.J. Morgan. 1996. Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters, 3rd ed. John Wiley & Sons, Inc., New York, NY. 1022 pp.

ter Braak, C.J.F. and L.G. Barendregt. 1986. Weighted averaging of species indicator values: its efficiency in environmental calibration. Math. Biosci. 78:57−72.

ter Braak, C.J.F. and C.W.N. Looman. 1986. Weighted averaging, logistic regression and the Gaussian response model. Vegetatio. 65:3−11.

Tetra Tech, Inc. 2000. A Stream Condition Index for West Virginia Wadeable Streams. Report Prepared for U.S. EPA Region 3 Environmental Services Division, and U.S. EPA Office of Science and Technology, Office of Water. Prepared by Tetra Tech, Inc., Owings Mills, MD. Available at http://www.littlekanawha.com/536_WV-Index.pdf.

U.S. EPA. 2000. Stressor Identification Guidance Document. Office of Water, Office of Research and Development, Washington, DC. EPA/822/B-00/025. Available at http://www.epa.gov/waterscience/biocriteria/stressors/stressorid.html.

U.S. EPA. 2002. National Recommended Water Quality Criteria: 2002. Office of Water, Washington, DC. EPA/822/R-02/047. Available at http://env1.kangwon.ac.kr/sdwr%202003/literature%20survey/international%20web%20sites/epa-ost/pc/revcom.pdf.

USGS (U.S. Geological Survey). 1999. Coal resource assessments in the northern and central Appalachian coal region. USGS Fact Sheet FS-115-99. Available at http://pubs.usgs.gov/fs/fs115-99/.

Vannote R.L., G.W. Minshall, K.W. Cummings, J.R. Sedell and C.E. Cushing. 1980. The river continuum concept. Can. J. Fish. Aquat. Sci. 37:130−137.

Walsh, C.J., A.H. Roy, J.W. Feminella, P.D. Cottingham, P.M. Groffman and R.P. Morgan. 2005. The urban stream syndrome: current knowledge and the search for a cure. J. N. Am. Benthol. Soc. 24:706−723.

Waters, T.F. 1995. Sediment in Streams. American Fisheries Society, Bethesda, MD. 251 pp.

Wetzel, R.G. 2001. Limnology: Lake and River Ecosystems, 3rd ed. Academic Press, New York, NY.

Wood, P.J. and P.D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environ. Manage. 21(2):203−217.

Woods, A.J., J.M. Omernik, D.D. Brown and C.W. Kiilsgaard. 1996. Level III and IV Ecoregions of Pennsylvania and the Blue Ridge Mountains, the Ridge and Valley, and Central Appalachians of Virginia, West Virginia, and Maryland. U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Corvallis, OR. EPA/600/R-96/077. 50 pp.

WVDEP (West Virginia Department of Environmental Protection). 2005. Surface water monitoring and assessment. In: 2004 Integrated Water Quality Monitoring and Assessment Report. p. 11−13. Available at http://www.wvdep.org/Docs/7742_EPA%20Approved%20Integrated%20Report%20AND%20303(d)%20List%20AND%20Supplements%20A-F%20Redused%20for%20web.pdf.

Zheng, L. and J. Gerritsen. 2007. Development of diagnostic indicators for Appalachian streams. To be submitted to Ecol. Appl. (In Preparation)

APPENDIX A:

STRESSOR-RESPONSE (S-R) RELATIONSHIPS IN WEST VIRGINIA

A.1. INTRODUCTION

The objective of this appendix is to describe analyses that explore the associations between candidate stressors and biological metrics, and to infer thresholds of biological impairment for each stressor. The West Virginia Benthic Stream Condition Index (WVSCI) has been developed (Tetra Tech, Inc., 2000) for West Virginia streams. This index is composed of six macroinvertebrate community metrics, including total taxa score, Ephemeropteran, Plecopteran, and Trichopteran (EPT) Index, percent contribution of two dominant taxa score, percent EPT score, percent Chironomidae, and Hilsenhoff's Biotic Index (HBI) score. WVSCI was determined by calculating the average of the standardized score of each metric. A reference data set (189 samples) was selected based on physical-chemical and geological characteristics of the streams statewide. To infer thresholds of biological response to stressors, analyses were performed to reveal associations between biological metrics and candidate causes that were used in the Clear Fork Causal Assessment (see Table A-1).

A.2. STATISTICAL APPROACHES

A total of 3766 macroinvertebrate samples were collected in West Virginia from 1999 to 2003. These samples were considered during the TMDL project to assess the ecological status of West Virginia wadeable streams. Sites were fairly equally distributed among all basins, and they included reference and non-reference sites. The large data set enabled us to examine the biological patterns along gradients of interest.

In order to prevent multiple stressor effects from confounding the analyses, we partitioned the data set to separate out gradients of single stressors, to the extent allowed by the data. The procedure here was to identify sites with high values of stressors (high metals concentrations, acidic pH, high conductivity, high fecal coliform, high sedimentation, etc.). To analyze a single stressor, sites with high values of all other stressors were removed, leaving a gradient of the stressor of interest.

Environmental variables were transformed to normalize distributions as necessary. Most analyses were done using either Systat® version 10, or the open-source language software R (R Development Core Team, 2005). Spearman correlation was used to examine the relationship among environmental variables and biological metrics. Locally weighted estimation (LOWESS) was used to explore the biological response to environmental gradients. This technique is designed to address nonlinear relationships where linear methods do not perform well. LOWESS combines much of the simplicity of linear least squares regression with the flexibility of nonlinear regression. It achieves this by fitting simple models to localized subsets of the data to build a function that describes the deterministic part of the variation in the data, point by

TABLE A-1

Change Points and Their 95th Percentile Confidence Limits for All Stressors Based on a Deviance Reduction Analysis. Both biological metrics and the conditional probabilities of these metrics exceeding biological benchmarks were used as response variables to delineate stressor change points.

Figure	Stressor	Response Variable	Raw Biological Metric			Conditional Probability		
			Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit	Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit
Figure A-3	Low pH (pH $\leq$ 6)	WCSCI	3.43	3.55	4.61	4.0495	4.205	4.300
Figure A-4	Dissolved Al (mg/L)	WVSCI	0.457	3.635	7.775	0.1035	0.115	0.270
	Dissolved Al (mg/L) When pH < 6	WVSCI	0.907	5.935	8.765	0.350	0.385	0.455
	Dissolved Al (mg/L) When pH < 6 & pH > 4	WVSCI	0.32	1.2	5.775	0.265	0.385	3.225
Figure A-5	Dissolved Al	Genus-level HBI	1.63	8.765	11.5	0.754	1.240	1.813
	Dissolved Al	Percent Ephemeroptera	0.040	0.135	0.452	0.375	3.115	15.400
	Dissolved Al	No. Emphemeroptera genera	0.043	0.137	0.310	0.265	0.290	0.320
	Dissolved Al	Percent EPT	0.505	5.415	9.825	1.18	1.36	2.29
	Dissolved Al	Total no. genera	0.135	0.295	0.535	0.095	33.360	66.245
	Dissolved Al	No. EPT genera	0.0622	0.265	0.545	0.095	1.360	3.635
Figure A-6	Dissolved Al excluding all stressors	Genus-level HBI	0.505	6.245	9.925	0.795	1.215	1.870
	Dissolved Al excluding all stressors	Percent Ephemeroptera	0.051	0.385	6.31	0.3600	0.475	3.708
	Dissolved Al excluding all stressors	No. Emphemeroptera genera	0.061	0.11	3.775	0.2550	0.295	1.545

TABLE A-1 cont.								
Figure	Stressor	Response Variable	Raw Biological Metric			Conditional Probability		
			Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit	Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit
Figure A-6 cont.	Dissolved Al excluding all stressors	Percent EPT	0.5	6	9.925	1.045	1.360	2.207
	Dissolved Al excluding all stressors	Total no. genera	0.11	0.29	9.251	0.100	3.360	3.935
	Dissolved Al (mg/L)	No. EPT genera	0.095	0.38	6.31	0.095	1.315	3.360
Figure A-8	Fe all data	WVSCI	1.355	2.43	2.6	0.780	0.801	0.823
	Dissolved Fe all data	WVSCI	0.335	0.4	2.34	0.137	0.145	0.235
	Mn all data	WVSCI	0.069	0.1385	0.288	0.069	0.071	0.121
Figure A-9	Fe excluding other stressors	WVSCI	0.121	0.152	1.095	0.117	0.12	11.259
	Dissolved Fe excluding other stressors	WVSCI	0.034	0.037	0.105	0.385	0.409	0.424
	Mn excluding other stressors	WVSCI	0.026	0.071	0.269	0.042	0.047	0.055
Figure A-11	Conductivity excluding other stressors	WVSCI	92.8	267.8	508.3	280	292.5	298.8
	Sulfate excluding others stressors	WVSCI	11.75	47.25	260	32.95	34.45	43.2
	Chloride excluding other stressors	WVSCI	2.46	3.28	4.06	16.	16.55	18.8
Figure A-12	Conductivity	WVSCI	185	288	495	243.7	251	262
	Sulfate	WVSCI	42.9	186	250.5	30.65	30.95	32.85
	Cl	WVSCI	3.28	4.05	5.51	14.15	15.72	16.50

TABLE A-1 cont.								
Figure	Stressor	Response Variable	Raw Biological Metric			Conditional Probability		
			Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit	Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit
Figure A-13	RBP habitat all	WVSCI	134	141.5	147.5	138	141	144
	Embeddedness score	WVSCI	9.5	12.5	13.5	9.23	13	15.
	Sedimentation score	WVSCI	8.5	13.5	14.5	8.5	13.0	15
	Bank stability score	WVSCI	13.5	14.5	16.5	8.5	13.0	16.5
Figure A-14	RBP score excluding all stressors	WVSCI	131	133	139.5	131.0	133.5	139
	Embeddedness score excluding all stressors	WVSCI	9.5	10.5	13.5	9	12.5	16.5
	Sedimentation score excluding all stressors	WVSCI	8.5	9.5	13.5	9.2	11.5	14
	Bank stability score excluding all stressors	WVSCI	12.5	14.5	15.5	NA	NA	NA
Figure A-15	% Fine all	WVSCI	13.5	20.25	24	13	15.5	21.0
	% Sand all	WVSCI	7.5	15.5	19	10.5	15.5	71.5
	% Silt all	WVSCI	6.5	9.5	12.5	5	30	55
	% Clay all	WVSCI	3.5	4	7.5	NA		

TABLE A-1 cont.								
Figure	Stressor	Response Variable	Raw Biological Metric			Conditional Probability		
			Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit	Lower 95th Conf. Limit	Median Change Point	Upper 95th Conf. Limit
Figure A-16	% Fine excluding other stressors	WVSCI	13.5	24	37.5	16	20.5	22.5
	% Sand excluding other stressors	WVSCI	8.5	16	28	15.5	25.0	45
	% Silt excluding other stressors	WVSCI	1.5	6	13.5	NA		
	% Clay excluding other stressors	WVSCI	1	7.5	7.5	NA		
Figure A-17	Fecal coliform count	WVSCI	187	305	472	324	397	412
	TSS	WVSCI	2.5	8.4	29.2	19.8	69.0	90.5
	TP	WVSCI	0.015	0.015	1.279	0.34	0.383	1.14
	NO_{2+3}	WVSCI	0.051	0.098	1.305	0.641	0.65	0.667
Figure A-18	Fecal coliform excluding other stressors	WVSCI	14.5	68.5	241	54.5	346	355
	TSS excluding other stressors	WVSCI	3.17	3.8	19	8.7	12.5	57
	TP excluding other stressors	WVSCI	0.015	0.055	0.302	0.225	0.265	0.314
	NO_{2+3} excluding other stressors	WVSCI	0.086	0.093	0.266	0.102	0.655	1.315

NH_3 = un-ionized ammonia.

point. This method does not require specification of a global function of any form to fit a model to the data, rather it fits segments of the data. We used K-nearest neighbor smoothing, which applies kernel-weighted robust binomial regression. The 95^{th} percentile of confidence interval of the LOWESS line based on bootstrapping resampling was calculated and plotted.

We used logistic regression to examine the probability of macroinvertebrate impairment at different level of pollutants. Logistic regression is part of a category of generalized linear models. Logistic regression allows one to predict a discrete outcome, such as group membership, from a set of variables that may be continuous, discrete, dichotomous, or a mix of any of these. Generally, the dependent or response variable is dichotomous, such as presence/absence or success/failure. The independent or predictor variables in logistic regression can take any form. That is, logistic regression makes no assumption about the distribution of the independent variables. The relationship between the predictor and response variables is a logistic regression function instead of linear.

We also used a conditional probability approach (Paul and McDonald, 2005) to examine change of biological community along multiple stressor gradients. In this case, conditional probability is the probability of an event (exceeding the biocriterion) when it is known that some other event ($\geq$ a stressor value) has occurred or has been exceeded. For use in developing a numeric WQC, a conditional probability statement provides the likelihood (probability) of observing an impairment from the cumulative population of sites from zero to the selected value of the stressor or from the maximum to the value of stressor. The method tested does not provide the probability of observing an impairment at a specific value of the stressor or range of the stressor.

Finally, we used nonparametric deviance reduction to identify ecological thresholds, or change points (Qian et al., 2003) in response variables to increasing stressor levels for both raw biological metrics and conditional probabilities. This technique is similar to regression tree models, which are used to generate predictive models of response variables for one or more predictors. The change-point, in our application, was the first split of a tree model with a single predictor variable (total phosphorus [TP] concentration). After generating change points, we used a bootstrapping resampling technique to calculate confidence estimates for the change points. All these statistical approaches were performed in R, an open source statistical program.

A.3. RESULTS AND DISCUSSION

As discussed above, a number of potential stressors could contribute to biological degradation in streams. Along with lethal effects of metal toxicity (at low pH and high pH), ionic strength, habitat degradation, organic and nutrient enrichment, and thermal pollution are all potential candidate causes of biological impairments in streams. We examined each of these candidate causes against macroinvertebrate metrics and partitioned covariates of each stressor variable to estimate thresholds for biological effects in WV streams.

A.3.1. Reference Conditions and Biological Criteria

West Virginia Department of Environmental Protection sampled a total of 187 reference stations, which had been selected based on a set of strict criteria (Bailey, 2006). The cumulative frequency distributions of several metrics are shown in Figure A-1. For the stressor-response development, we selected a threshold indicating a departure from reference condition, i.e., where a site begins to exhibit characteristics that would lead one to conclude that it is no longer similar to other reference sites. This does not necessarily mean that a site is impaired in the sense of WQC, but only that it is no longer similar to reference, for that particular metric or index.

We chose the lower 5^{th} percentile or upper 95^{th} percentile of the frequency distributions for metric scores in the reference sites, as the criterion of departure from reference. According to this criterion, stations with WVSCI score less than or equal to 71, Ephemeropteran genera $\leq$ 3, Ephemeropteran individuals $\leq$ 8%, the number of EPT genera $\leq$ 11, total number of genera $\leq$ 23, and HBI score $\geq$ 5 were considered to be biologically different from reference streams (see Figure A-1).

A.3.2. Metal Toxicity: Aluminum

Acidity of water increases the solubility of common metals that are associated with coal mining (Al, Fe, Zn, Mn). Among these, dissolved aluminum is known to be highly toxic to aquatic organisms. At pH values less than 6.5, water quality is dominated by a nearly linear relationship between pH and the concentration of dissolved Al (see Figure A-2), indicating that low pH streams are mostly impacted by acid mine drainage, though a small number of acidic streams may be potentially affected by acid precipitation. At pH values above 6.5, only two samples had dissolved Al above 0.5 mg/L, which could be outliers due to sampling error.

The relationship between WVSCI index score and pH is relatively weak (see Figure A-3). The weak association could be mostly driven by increased dissolved Al concentrations with declined pH level (see Figure A-3b). When pH is within the 4~6 range and when dissolved Al concentration is below 0.1 mg/L (see Figure A-3c), WVSCI scores are mostly above the 5% reference criterion. We applied the conditional probability approach to examine macroinvertebrate decline along the pH gradient at different dissolved Al levels (see Figure A-3c, f). The risk of WVSCI scores falling below the 5% reference criterion remains relatively stable at each of the dissolved Al levels along the pH gradient, indicating that pH itself is less of a factor affecting macroinvertebrate WVSCI index scores.

On the other hand, the WVSCI index score for macroinvertebrates is strongly associated with dissolved aluminum (see Figure A-4a), and the association is much

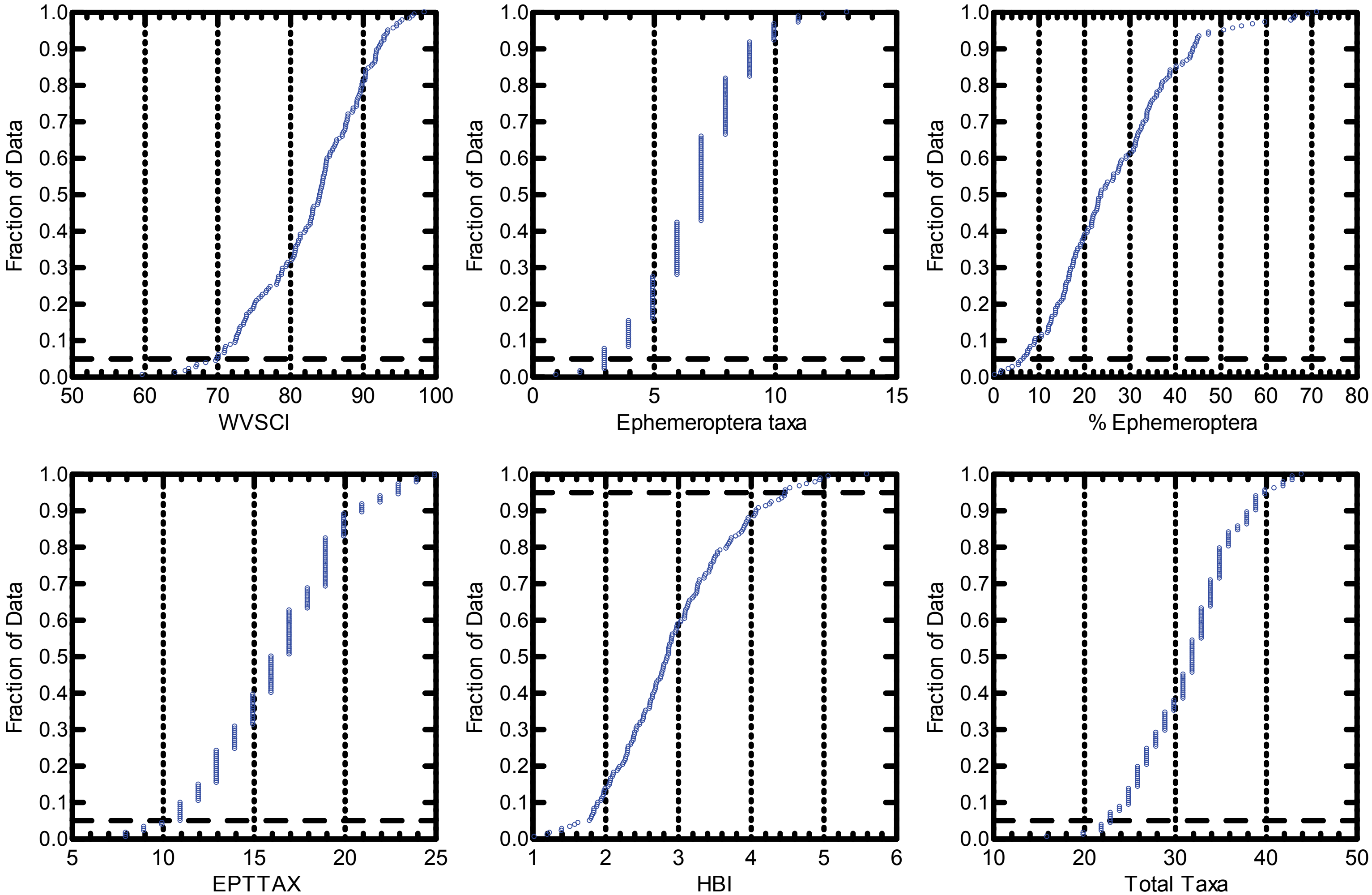


FIGURE A-1

Cumulative Frequency Distributions of Biological Metrics in Reference Streams in West Virginia

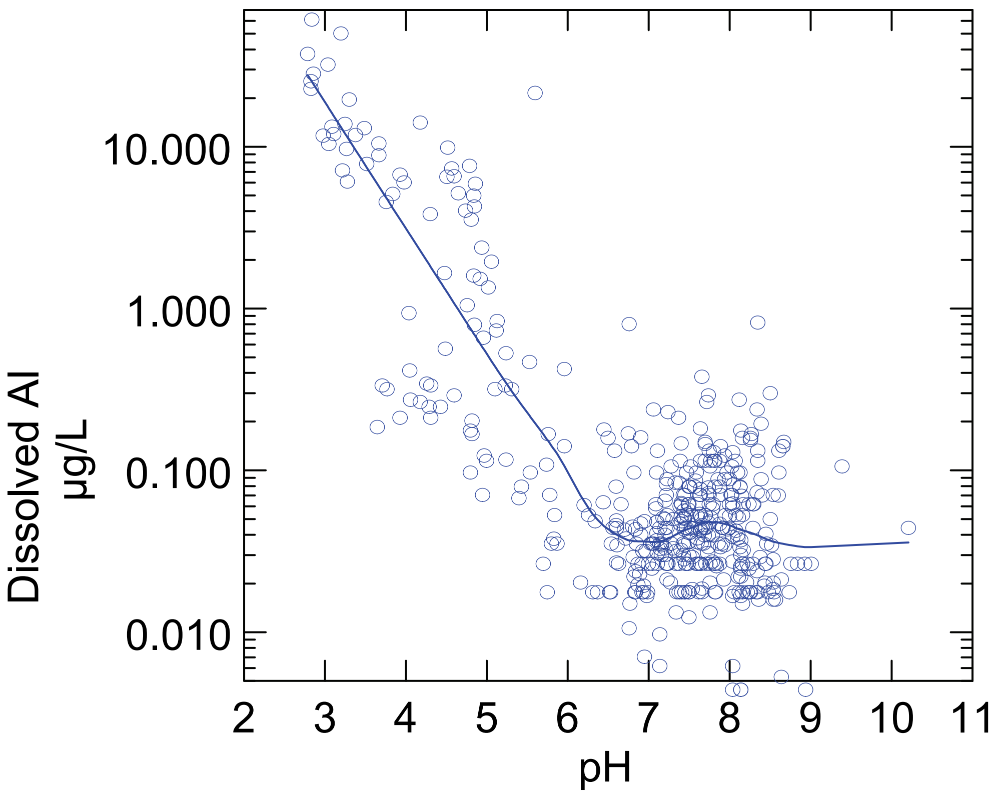


FIGURE A-2

Relationship Between pH and Dissolved Aluminum Concentrations. Data reported here only include those above detection limits. The smooth line shows locally weighted smoothing. Dissolved Al levels off at 0.040 µg/L.

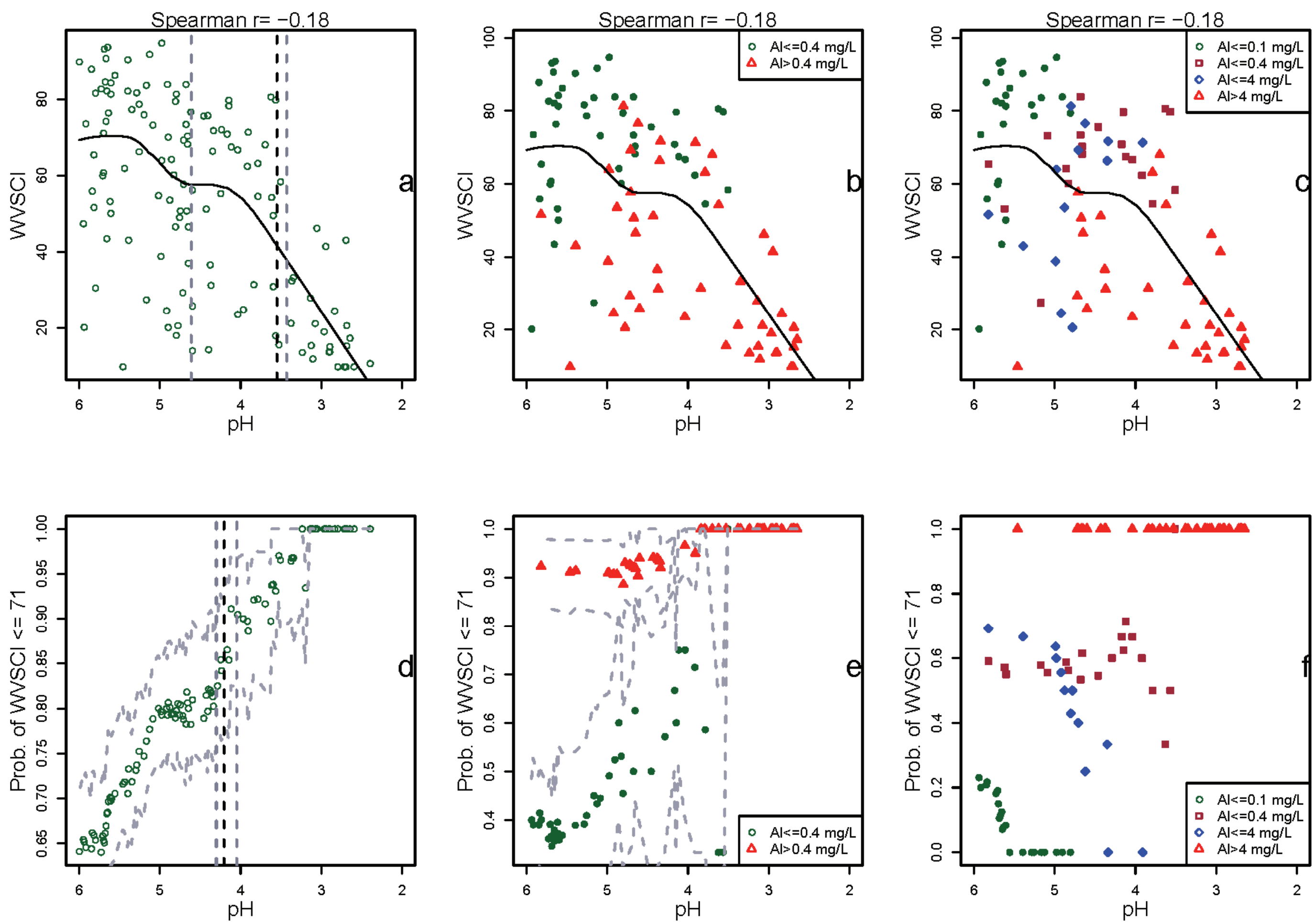


FIGURE A-3

Relationship Between WVSCI Score and pH (pH $\leq$ 6) When Dissolved Aluminum Concentrations are in Different Ranges, and Their Conditional Probabilities of Impairment (WVSCI $\leq$ 71) Along pH Gradients. (a and d). WVSCI response to pH when all dissolved Al concentrations are included; (b and e). WVSCI response to pH when dissolved Al is below or above 0.40 mg/L; (c and f). WVSCI response to pH when dissolved Al is < 0.1; >0.1 to 0.4; >0.4 to 4; and >4 mg/L. The vertical dashed lines show the change point and 95th confidence limits.

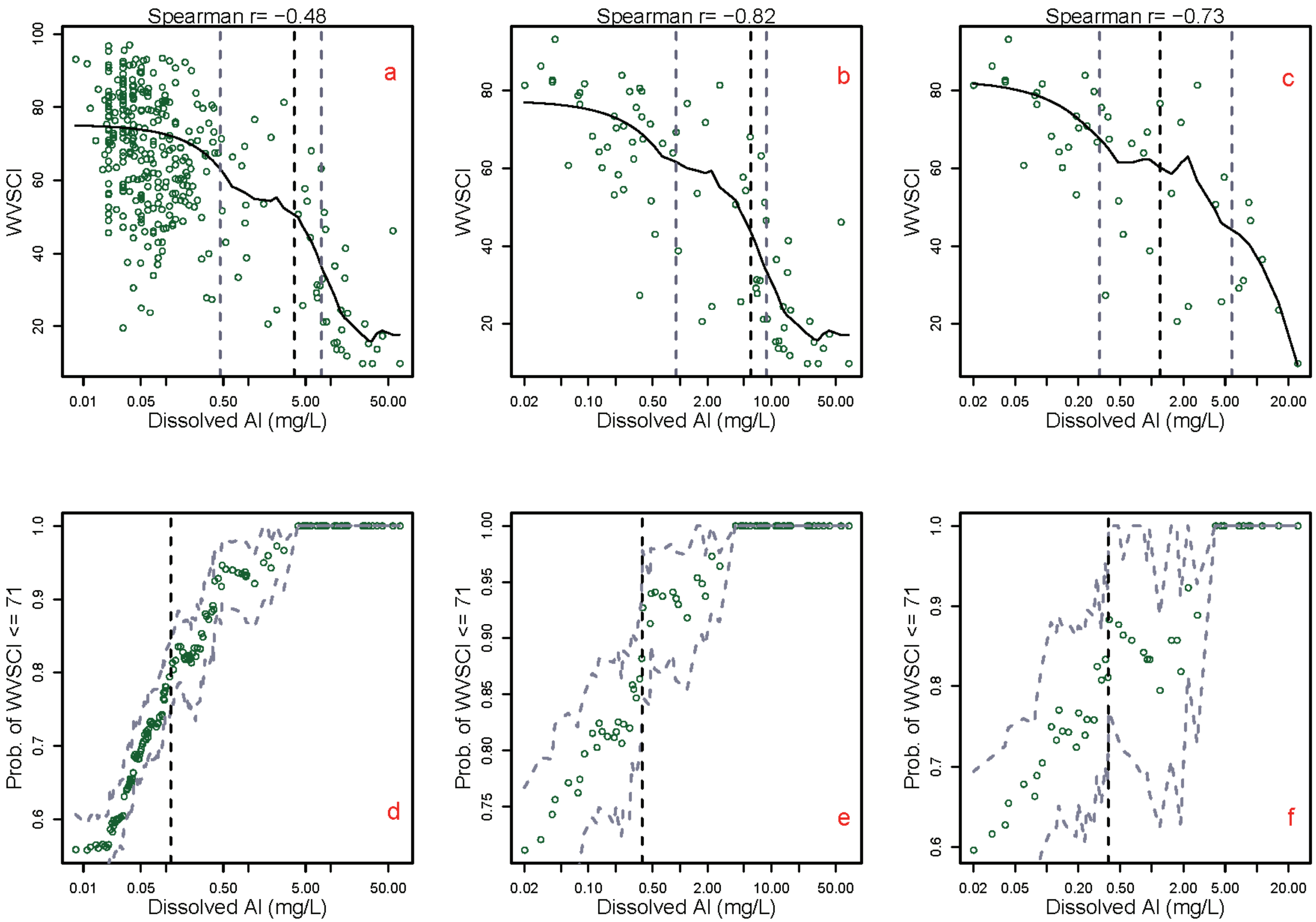


FIGURE A-4

Relationship Between WVSCI Score and Dissolved Al Concentrations in Different pH Ranges (a-c), and Ttheir Conditional Probabilities of Impairment (WVSCI $\leq$ 71) Along Dissolved Al Concentrations (d-f). (a and d). Entire pH range; (b and e). pH $\leq$ 6 only; (c and f). WVSCI response to dissolved Al when pH is within the range of 4−6. The vertical dashed lines show the change point and 95^{th} confidence limits.

stronger at a pH below 6 (see Figure A-4b). At pH levels from 6 to as low as 4, there are many sites with high WVSCI scores (see Figure A-4d), but the WVSCI score within the range pH 4−6 is associated most closely with aluminum (see Figure A-4c). The decline of WVSCI score at pH < 4 could be due to rising dissolved Al concentrations since the relationship between Al and WVSCI is consistently linear along the Al gradient within the pH $\leq$ 6 range (see Figure A-4b). These results suggest that Al has stronger and more consistent influence on WVSCI scores than does pH.

Individual metrics (see Figure A-5) along the entire pH range mostly show wedge-shaped relationships with dissolved Al. The Ephemeroptera (mayflies) appear to be the most sensitive to Al, starting to decline if Al is above 30 µg/L. Total taxa and EPT taxa also decline when dissolved Al is around 100 µg/L. HBI score seems less sensitive to Al concentrations and starts to increase after Al is above 200 µg/L. Percent EPT taxa was not considered a good indicator of Al and pH because some stoneflies are very tolerant to Al and low pH. We applied the conditional probability approach to examine macroinvertebrate decline along the dissolved Al gradient using the full data set (see Figure A-5g-l). The probabilities of HBI score above the 95% criterion, and other five metrics falling below the 5% criterion increase with the elevated Al concentrations. Changing point analysis indicates that the thresholds of change vary widely among response variables. The most stringent metric, Ephemeroptera (mayflies), shows a change point around 0.043~0.320 mg/L according to different methods applied.

All metrics still show a response to increased Al concentration (see Figure A-6a-f) when pH $\leq$ 6, though total taxa, EPT taxa, and HBI score have tighter relationships between metric scores and Al concentrations. The conditional probability analyses also show similar increase of probabilities exceeding the biological criterion along the dissolved Al gradient as the full data set (see Figure A-6g-l). Ephemeroptera (mayflies) percent and richness start to decline around 0.05 mg/L and show a change point around 0.250~0.385 mg/L according to the change point analyses.

Another way of looking at the relationship between probability of impairment and stressor is through logistic regression analyses (see Figure A-7). The Al concentrations are log-transformed and categorized into equal proportions to predict the probability of macroinvertebrate impairment. The results indicate that probabilities of biological impairment, represented by WVSCI score and % Emphemeroptera in the samples, also increase as a logistic function. The % Emphemeroptera, as shown in Figures A-5 and A-6, are sensitive to Al concentration, and a changing point could be seen at dissolved Al between 52 and 79 µg/L.

A.3.3. Metal Toxicity: Fe and Mn

Correlations among Al, Fe, and Mn concentrations in the water column are strong (*r* values between 0.5 and 0.6). Overall, WVSCI scores declined with elevated total Fe, dissolved Fe, and total Mn concentrations (see Figure A-8a-c). However, causes may be other factors, such as pH, conductivity, organic enrichment, or habitat

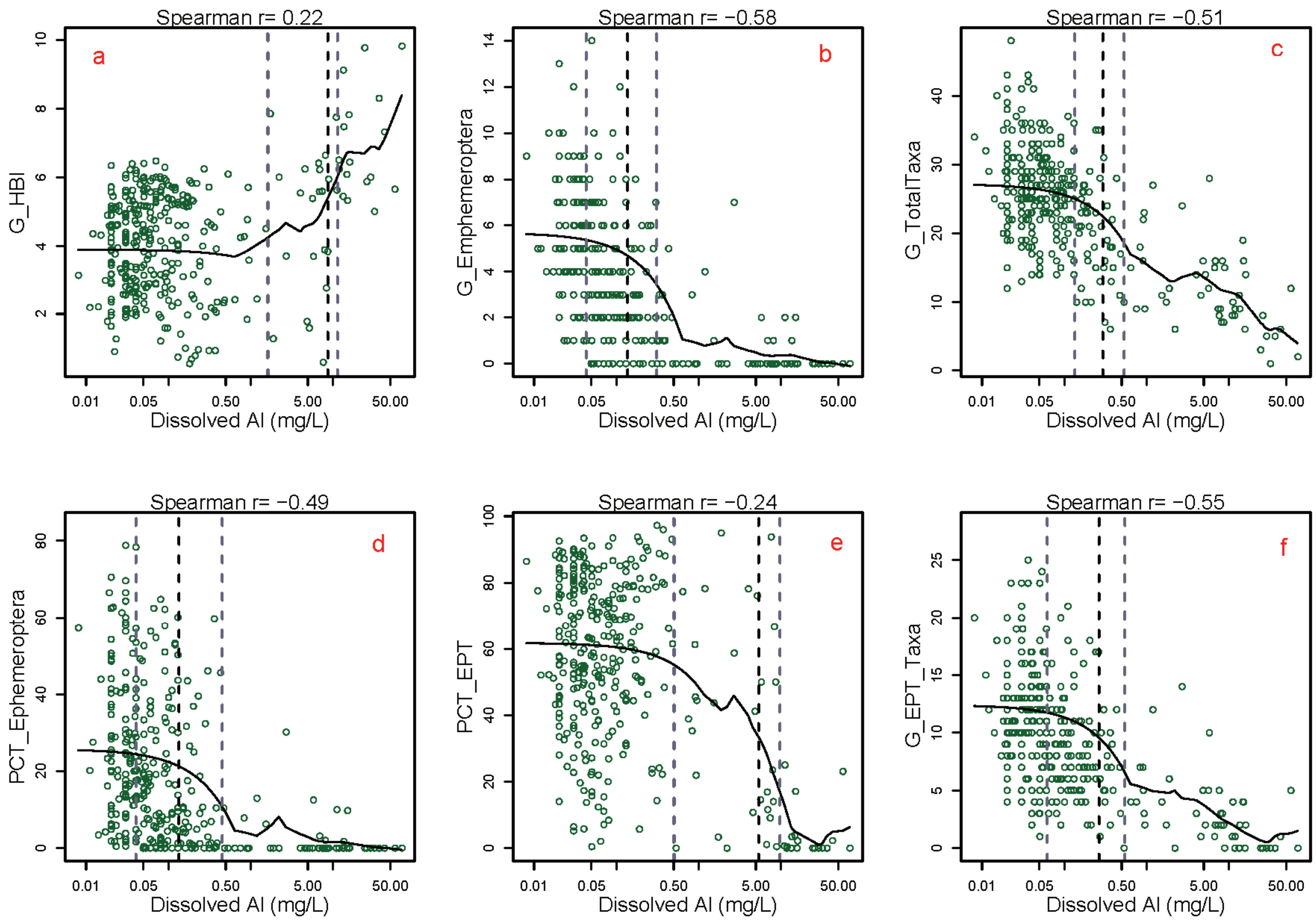


FIGURE A-5

Relationship Between Six Genus-Level Macroinvertebrate Metrics and Dissolved Aluminum Concentrations Within Entire pH Range. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against dissolved Al concentrations. A deviance reduction technique was used to calculate the change points, or Al threshold above which the probability of exceeding the 5% biological criterion is much higher. The vertical dashed lines are the potential Al thresholds and 95th confidence limits (gray).

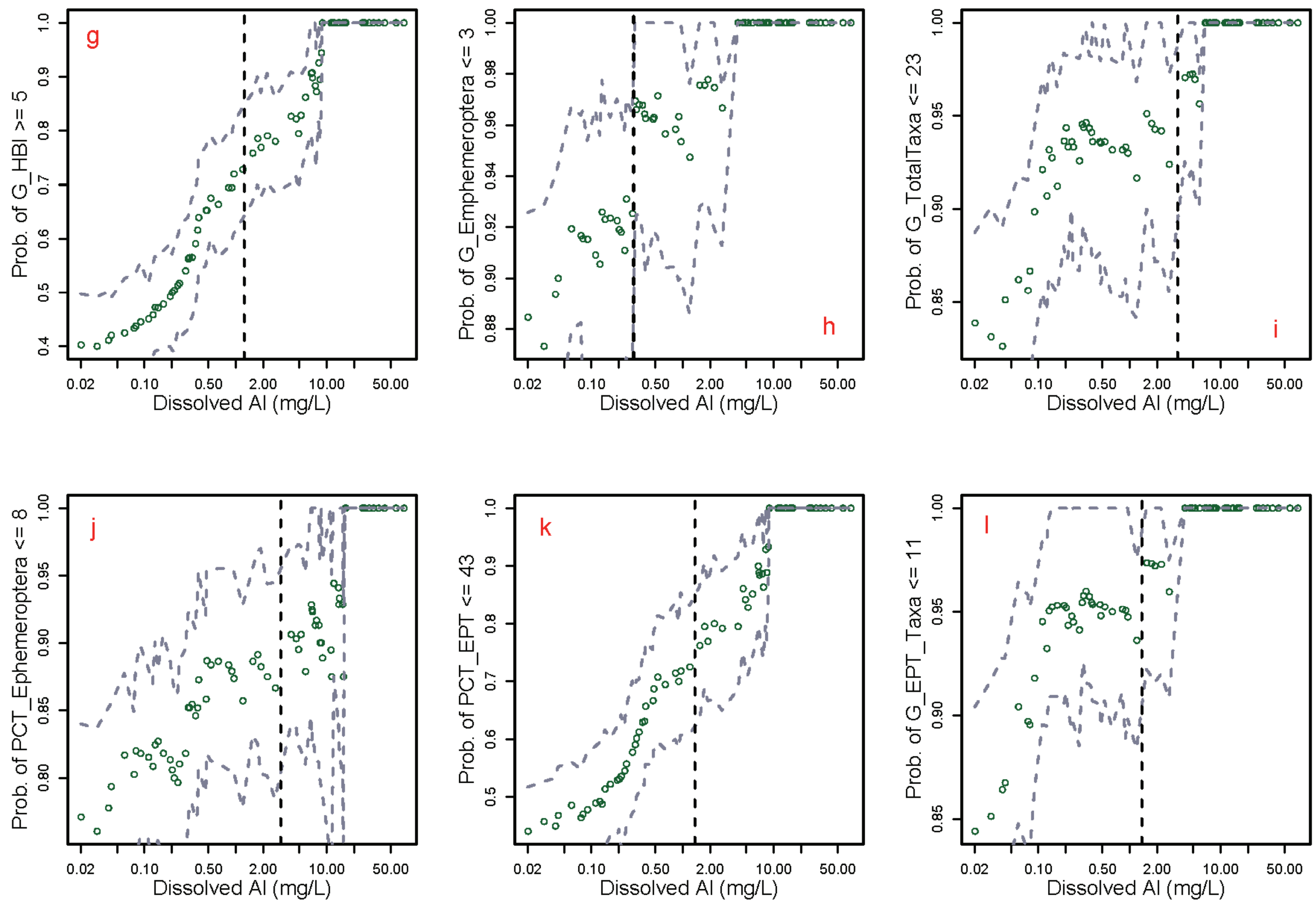


FIGURE A-5 cont.

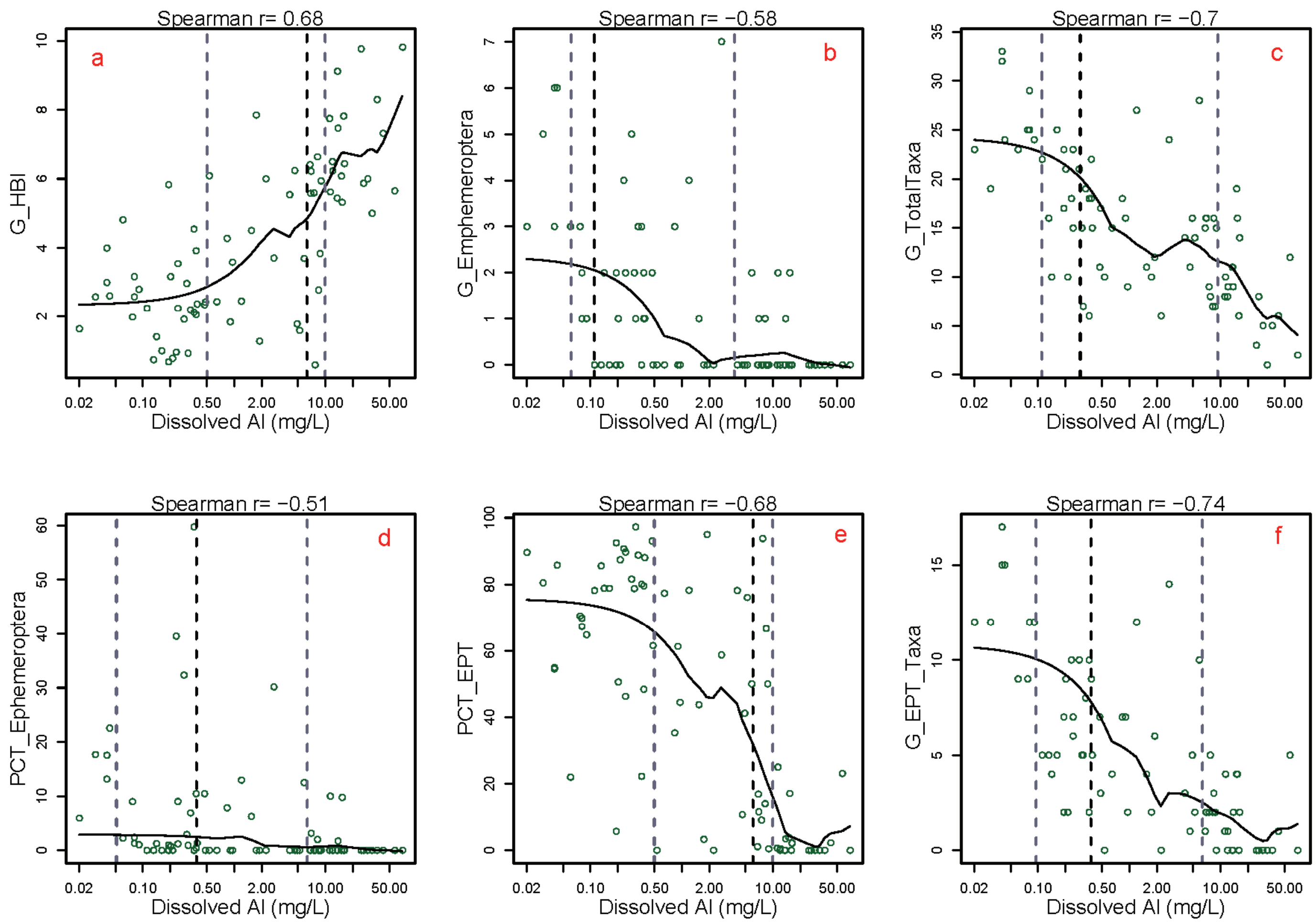


FIGURE A-6

Relationship Between Six Genus-Level Macroinvertebrate Metrics and Dissolved Aluminum Concentrations When pH $\leq$ 6. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against dissolved Al concentrations. A deviance reduction technique was used to calculate the change points, or Al threshold above which the probability of exceeding the 5% biological criterion is much higher. The vertical dashed lines are the potential Al thresholds and 95th confidence limits (gray).

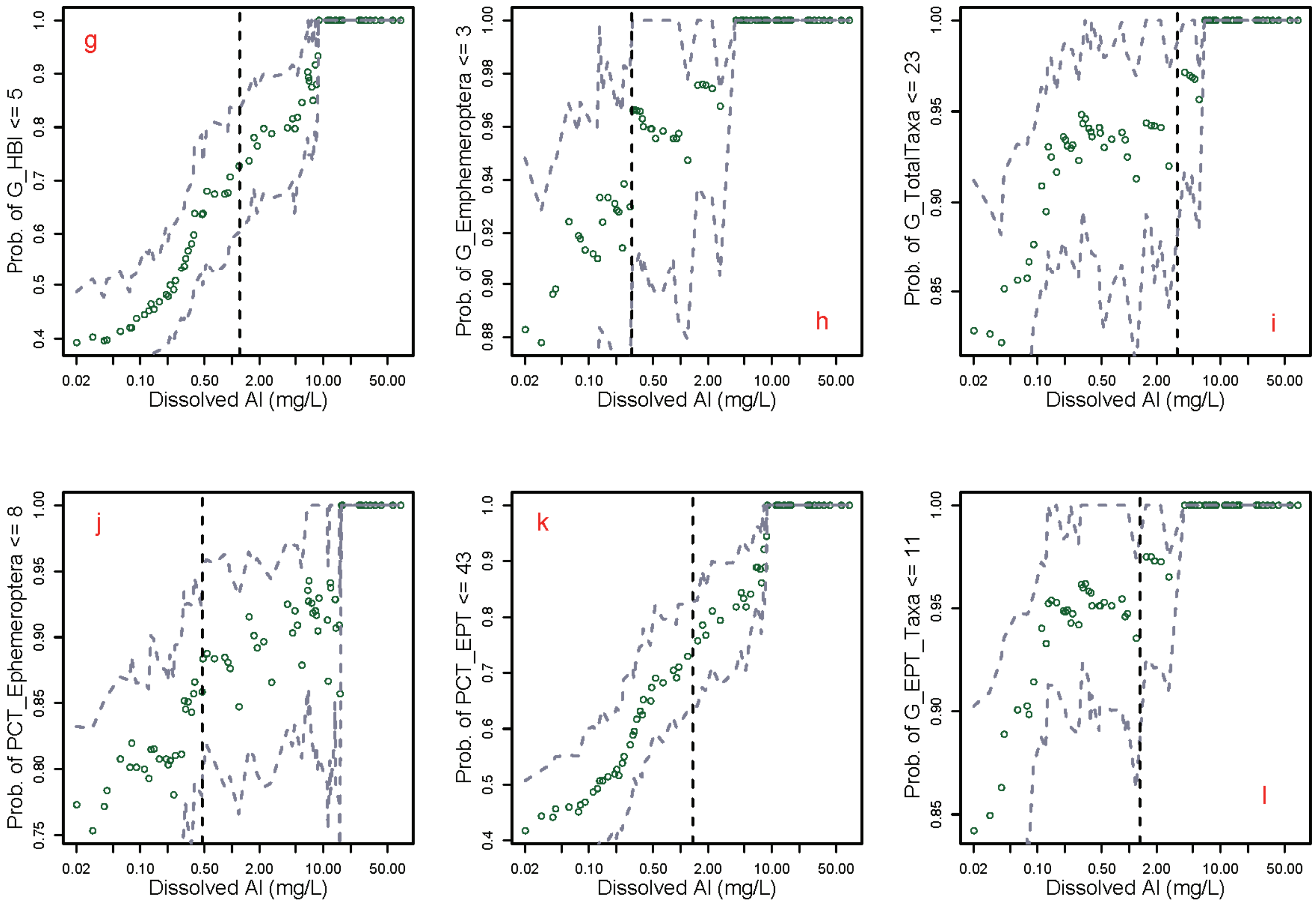
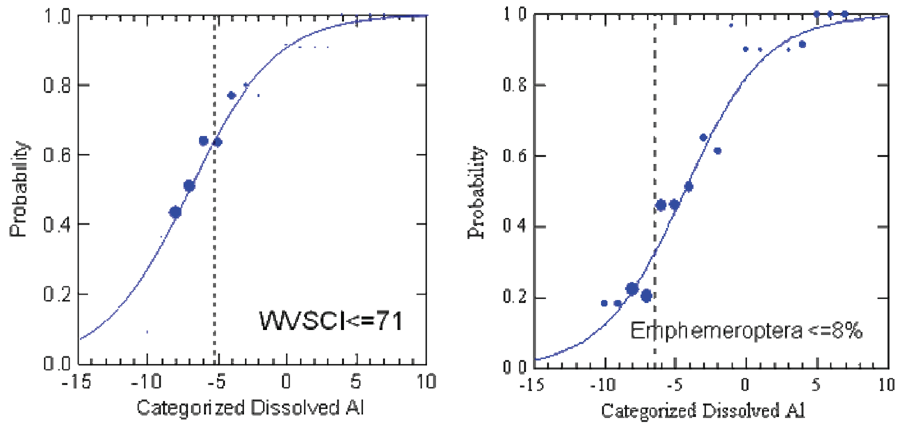


FIGURE A-6 cont.



-10	0.008-0.012 mg/L
-9	0.014-0.019mg/L
-8	0.020-0.031mg/L
-7	**0.032-0.050mg/L**
-6	**0.052-0.079mg/L**
-5	**0.080-0.123mg/L**
-4	0.130-0.192mg/L
-3	0.200-0.310mg/L
-2	0.318-0.480mg/L
-1	0.530-0.750mg/L
0	0.830-1.190mg/L
1	1.53-1.88mg/L
2	2.21-2.7mg/L
3	3.836-4.85mg/L
4	5.17-7.63mg/L
5	8.1-11.9 mg/L
6	13.3-19.6 mg/L
7	24.4-69.3 mg/L

FIGURE A-7

Logistic Regression Analyses Showing the Probability of WVSCI and % Emphemeropteran Changes With Increasing Aluminum Concentrations. Vertical dashed line represents threshold used in weight-of-evidence analysis.

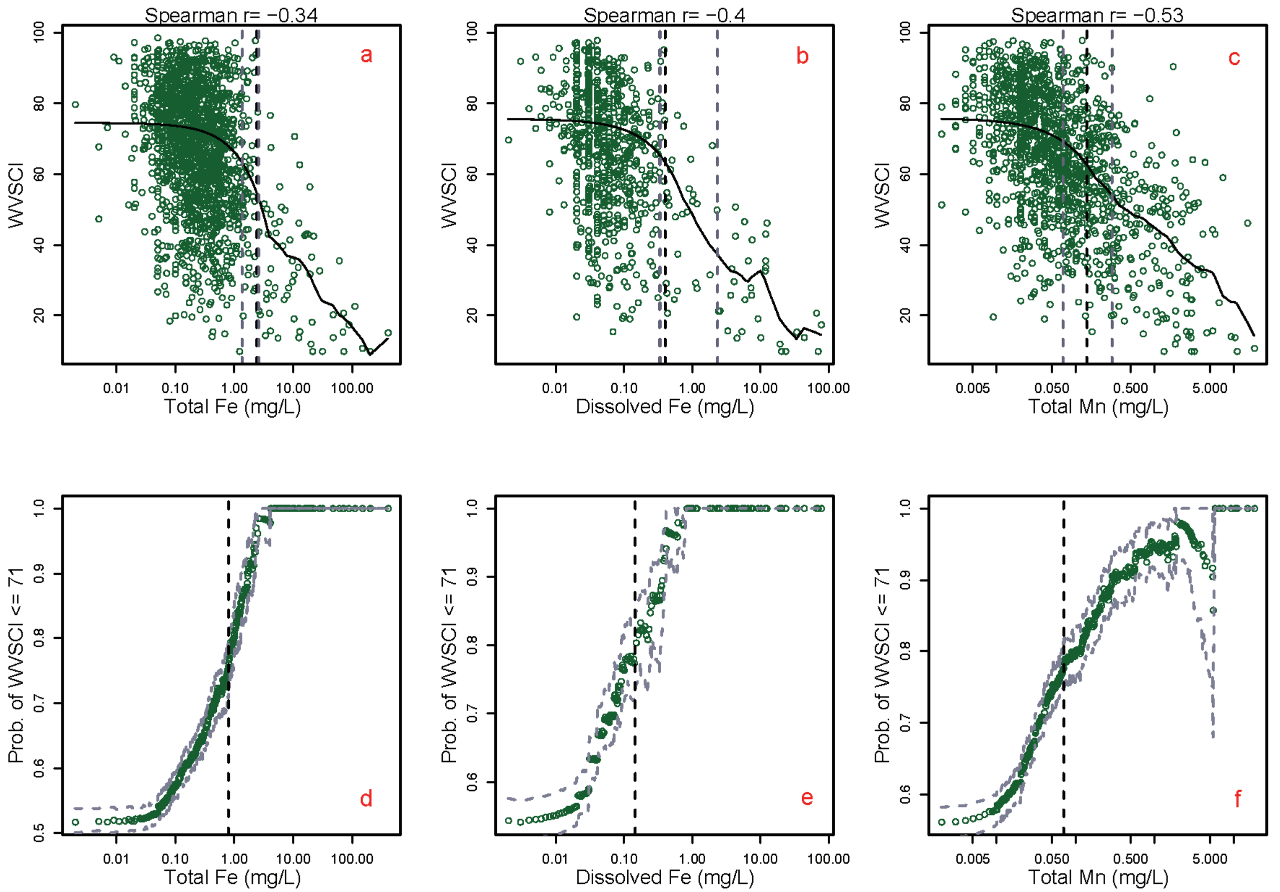


FIGURE A-8

Relationships Between Metal (Mn and Fe) Concentrations and WVSCI Scores When All Samples are Included. The smooth line in the scatter plots shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against metal concentrations. A deviance reduction technique was used to calculate the change points, or metal threshold above which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential metal thresholds and 95th confidence limits.

impairment. When other candidate causes are excluded from the analysis (pH > 6, fecal < 400, Rapid Bioassessment Protocol (RBP) habitat score > 128, conductivity < 300), there was barely any association between WVSCI and total Fe, dissolved Fe, and total Mn (see Figure A-9a-c). The very weak correlations between WVSCI and dissolved Fe ($r = -0.151$) and total Mn ($r = -0.158$) could be due to associations between WVSCI and other factors such as sulfate ($r = -0.268$) or conductivity increases along the same gradient. These results suggest that manganese and iron at neutral pH are only marginally toxic, or that the toxic effects are confounded with other stressors.

A.3.4. Ionic Strength

Conductivity is generally an excellent predictor of biological condition of streams. It measures total solutes in the water column and includes both cations (Ca and Mg) and anions (Cl, SO_4, CO_3, HCO_3). Both Cl and SO_4 have been reported to be deleterious or toxic at high concentrations and to cause biological impairment (Goodfellow et al., 2000), though these effects are not as strong as dissolved toxic metals. Meanwhile, both sulfate and chloride are correlated with conductivity in the water column (see Figure A-10a,b) (Spearman $r = 0.92$ and 0.67, respectively) as well as correlated with each other, though the correlation is not very strong ($r = 0.49$). In West Virginia streams, the proportion of sulfate (as molar milliequivalent, meq/L) to total anion tends to increase along with increasing sulfate concentration ($r = 0.50$), while the Cl proportion is more unpredictable along increasing Cl concentrations ($r = -0.14$). Sulfate is the major anion in high conductivity streams, while bicarbonates and chloride may also compose a large proportion of anions in low conductivity streams.

WVSCI scores tend to decline with increasing conductivity, sulfate, and chloride gradients no matter whether other potential stressors are excluded (pH > 6.5, fecal < 200, and RBP score > 128) (see Figure A-11) or not (see Figure A-12). Conductivity had the strongest correlation with WVSCI score ($r = -0.61$ and -0.56 for full data set and selected set), while the two single anions had less strong correlations with WVSCI ($r = -0.58$ and -0.49 with sulfate and $r = -0.48$ and -0.48 with chloride in full data set and the selected set, respectively). We also used a multiple linear regression approach to examine the combined effect of sulfate and chloride concentrations on WVSCI. It is interesting that the total variance explained by the two anions ($R^2 = 0.31$) is almost equivalent to the variance explained ($R^2 = 0.31$) by conductivity in the simple linear regression of WVSCI vs. conductivity. Although the proportion of sulfate to total anion tends to increase along with increasing sulfate concentration, the proportion of sulfate is only weakly associated with WVSCI score ($r = -0.16$). These results suggest that declines of the macroinvertebrate community are not caused by increasing sulfate concentration alone, but that the combined effect of sulfate and other ions has a much stronger effect than single parameters.

LOWESS smoothing and change point analysis were applied to the regressions between ionic variables and WVSCI scores when other stressors were excluded (see Figure A-11a-c). WVSCI scores show linear decline with increased ionic strength, and

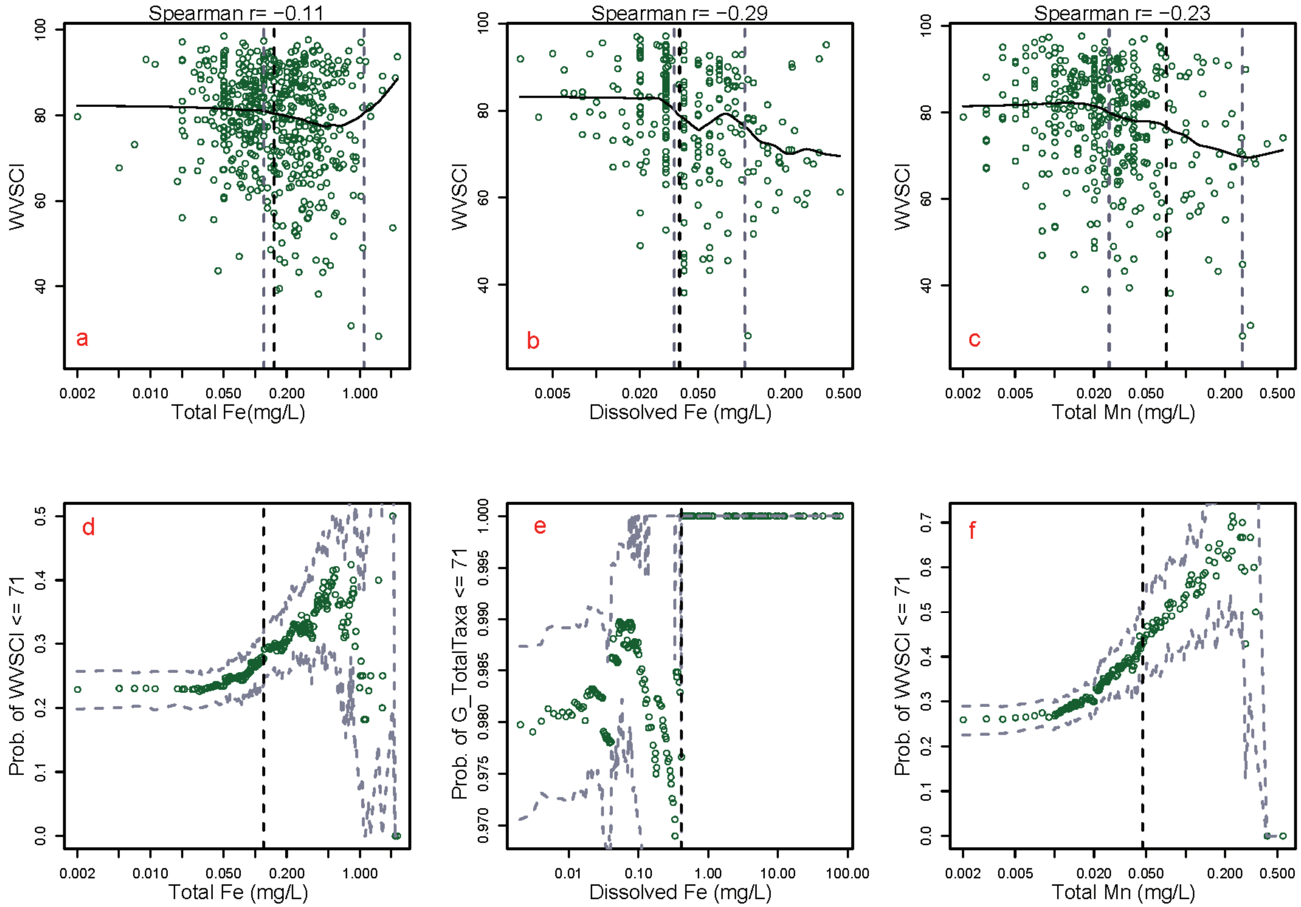


FIGURE A-9

Relationships Between Metal (Mn and Fe) Concentrations and WVSCI Scores When Other Stressors are Excluded (pH > 6, fecal < 400, habitat > 128, conductivity < 300). The smooth line in the scatter plots shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against dissolved Al concentrations. A deviance reduction technique was used to calculate the change points, or metal threshold above which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential metal thresholds and 95th confidence limits.

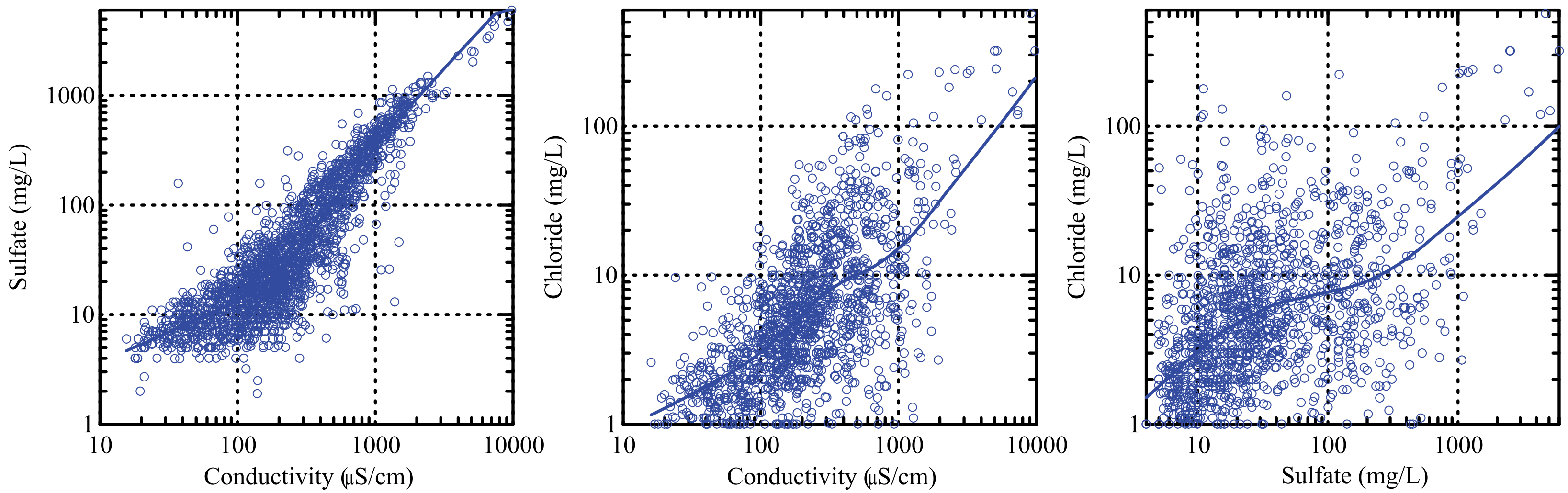


FIGURE A-10

Relationships Among Conductivity, Sulfate, and Chloride Concentrations. The smooth line shows locally weighted smoothing.

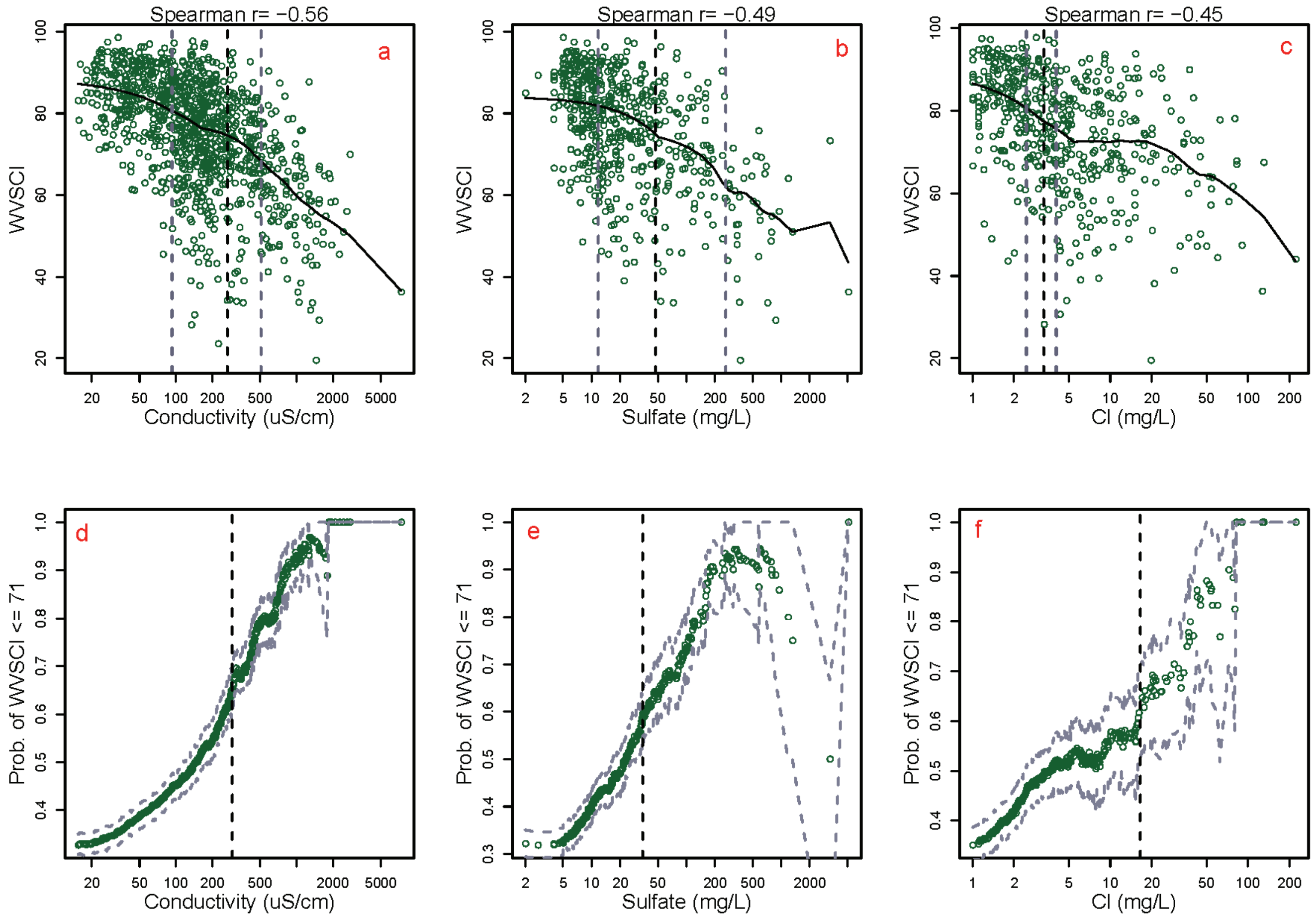


FIGURE A-11

Relationships Between WVSCI Scores and Potential Stressors Representing Ionic Strength (conductivity, sulfate, and chloride) When Other Stressors are Partitioned Out (pH > 6, habitat > 128, and fecal coliform counts < 400). The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against ionic variables. A deviance reduction technique was used to calculate the change points, or ionic strength threshold above which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential ionic strength thresholds and 95th confidence limits.

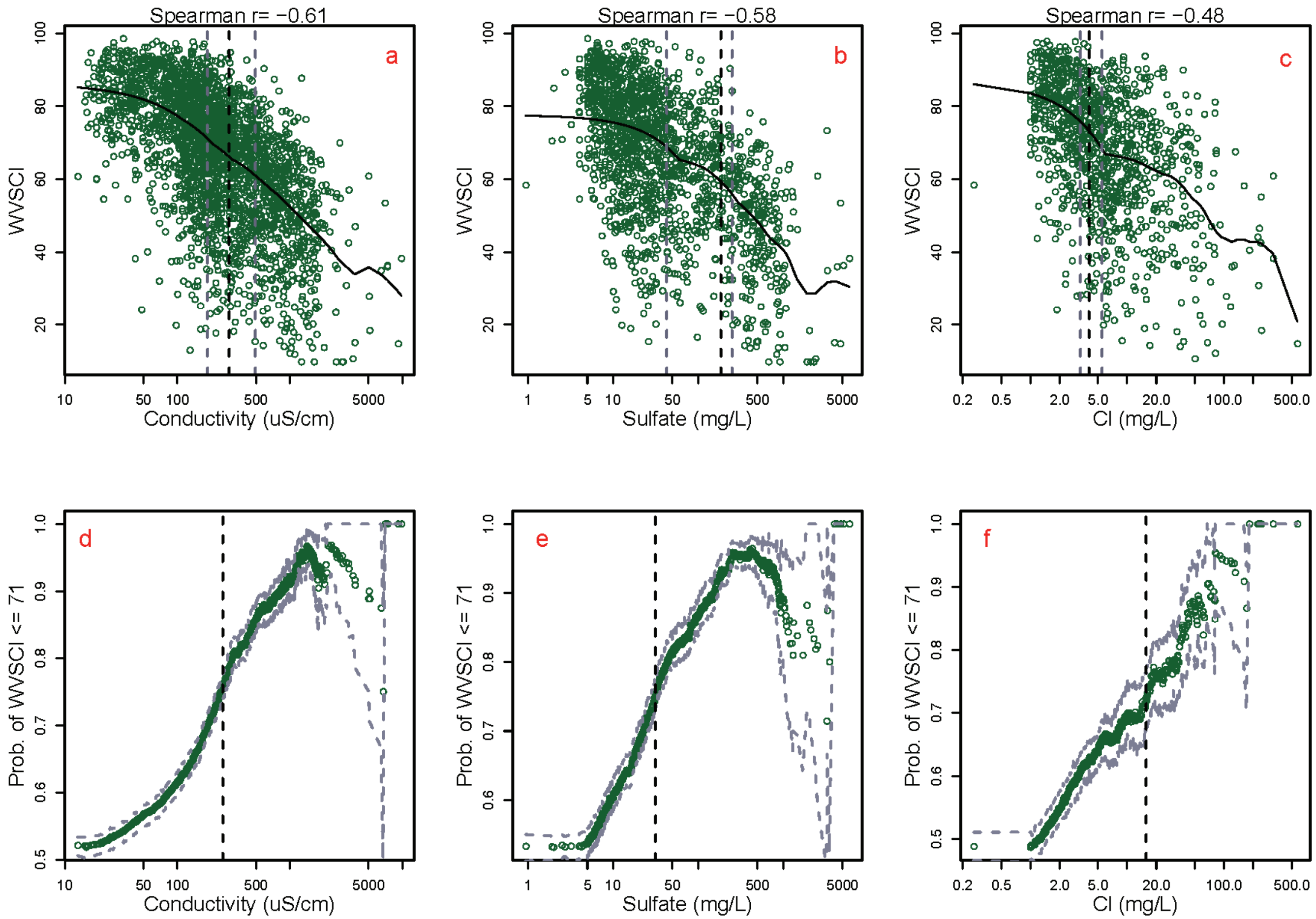


FIGURE A-12

Relationships Between WVSCI Scores and Potential Stressors Representing Ionic Strength (conductivity, sulfate, and chloride) in Full Data Set. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against ionic variables. A deviance reduction technique was used to calculate the change points, or ionic strength threshold above which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential ionic strength thresholds and 95th confidence limits.

the confidence interval for change points for all three variables is wide. Conditional probability analysis was also applied to the probability of decline (WVSCI < 71) with these three ionic variables (see Figure A-11d-f). The probability increased almost linearly for all three variables. There is no actual threshold or change point, but deviance reduction identifies a midpoint at about 300 µS/cm conductivity, 35~50 mg/L sulfate, and 3~16 mg/L chloride.

A.3.5. Habitat Degradation and Sedimentation

We examined effects of habitat and particularly of sedimentation-related parameters on macroinvertebrate community. These parameters were first examined without partitioning out other stressors (see Figure A-13). When all sites were included in the analyses, the total RBP score and the three parameters are correlated with WVSCI scores (r = 0.48 with total score, 0.37 with embeddedness, 0.30 with sedimentation, 0.26 with bank stability score). When a subset of sampling sites excluding other potential stressors (pH > 6, conductivity < 300, and fecal < 400) was used for the analysis, the correlations were weaker (r = 0.41 for total score, 0.31 for embeddedness, 0.27 for sedimentation, 0.28 for bank stability score) (see Figure A-14). The potential threshold for total habitat score is around 130~138 based on deviance reduction analysis from the raw data. The conditional probability analyses seem less useful for this type of semiquantitative predictor variables (see Figure A-13e-h and Figure A-14e-h).

Pebble counts identify benthic substrates into different categories and percent cover. Percent fine (<2 mm) is the sum of % sand, % silt, and % clay in a catchment. Fine sediments were negatively correlated with WVSCI scores (r = −0.33 with % fine, −0.27 with % sand, −0.24 with silt, −0.07 with % clay) (see Figure A-15). After data partition, the correlations still existed (r = −0.24 with % fine, r = −0.20 with % sand, r = −0.17 with % silt, r = −0.07 with % clay) (see Figure A-16), suggesting that substrate deposition and sedimentation can contribute to decline of WVSCI score. According to LOWESS regression and change point analysis, WVSCI starts to decline when % fine (see Figure A-16a) is at 16% and reaches the change point at 20% fine.

A.3.6. Organic and Nutrient Enrichment

Several candidate stressors representing nutrient and organic enrichment were plotted against WVSCI (see Figure A-17). Because of lack of biological oxygen demand and chemical oxygen demand measurement, fecal coliform bacteria in water column and total suspended solids (TSS), along with nutrient parameters, were used as surrogate parameters to represent organic pollution. Fecal coliform count is significantly correlated with WVSCI score (r = −0.34). TSS, TP, and NO_{2+3} were not strongly correlated with WVSCI score in the total data set (see Figure A-17). When other potential stressors (RBP > 128, conductivity < 300, and pH > 6) were excluded from the analyses, fecal coliform was still the strongest variable in this category (r = −0.26) (see Figure A-18). TSS and TP were not associated with WVSCI scores.

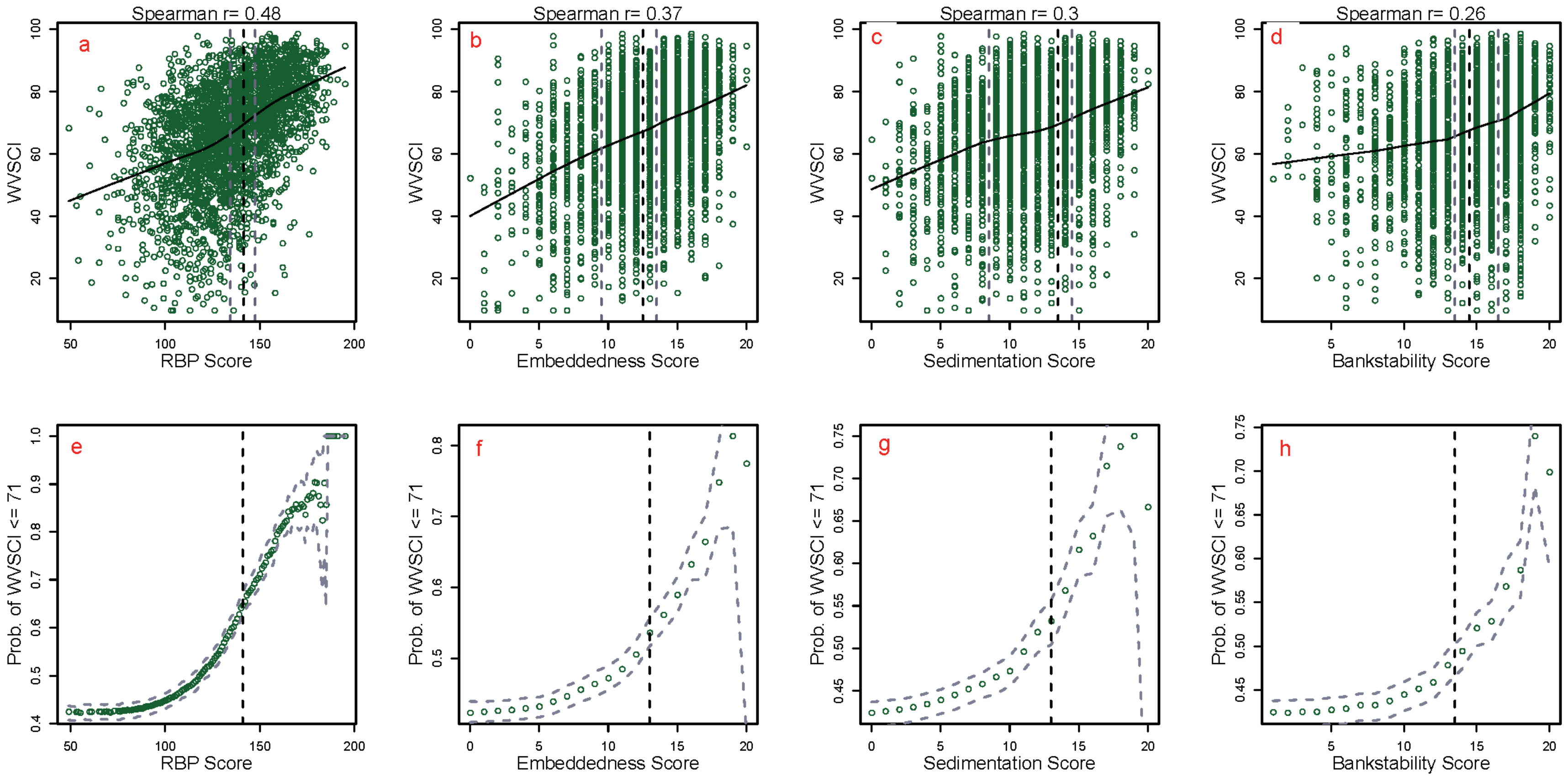


FIGURE A-13

Relationships Between RBP Habitat Scores, Sedimentation Scores, Embeddedness Scores, Bank Stability Scores, and WVSCI Scores When All Samples are Included. The smooth line shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against ionic variables. A deviance reduction technique was used to calculate the change points, or habitat threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential ionic strength thresholds and 95th confidence limits.

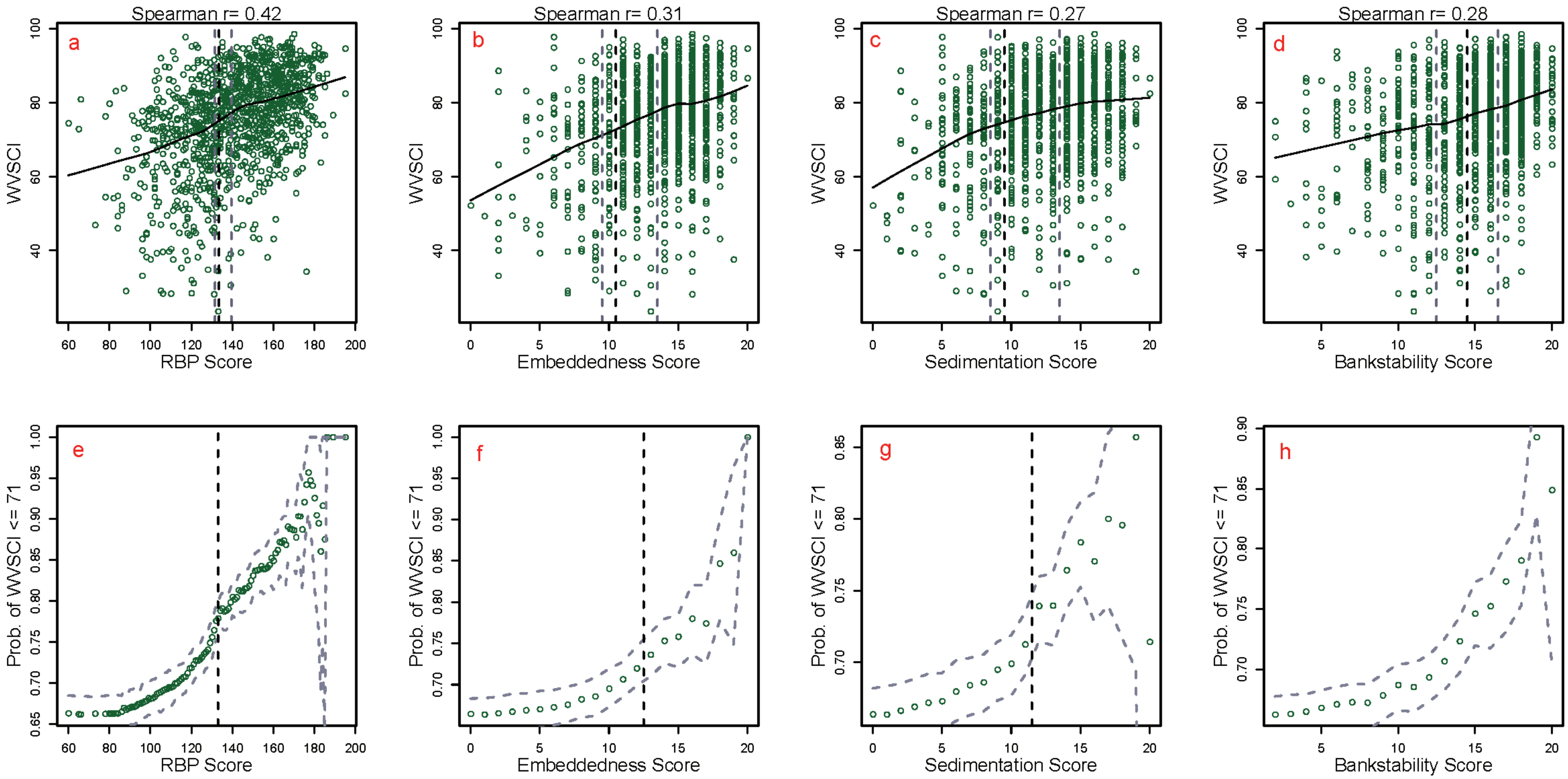


FIGURE A-14

Relationships Between RBP Habitat Scores, Sedimentation Scores, Embeddedness Scores, Bank Stability Scores, and WVSCI Scores When Other Stressors are Partitioned Out (pH > 6, conductivity < 300, and fecal coliform counts < 400). The smooth line shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against habitat variables. A deviance reduction technique was used to calculate the change points, or habitat threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential ionic strength thresholds and 95th confidence limits.

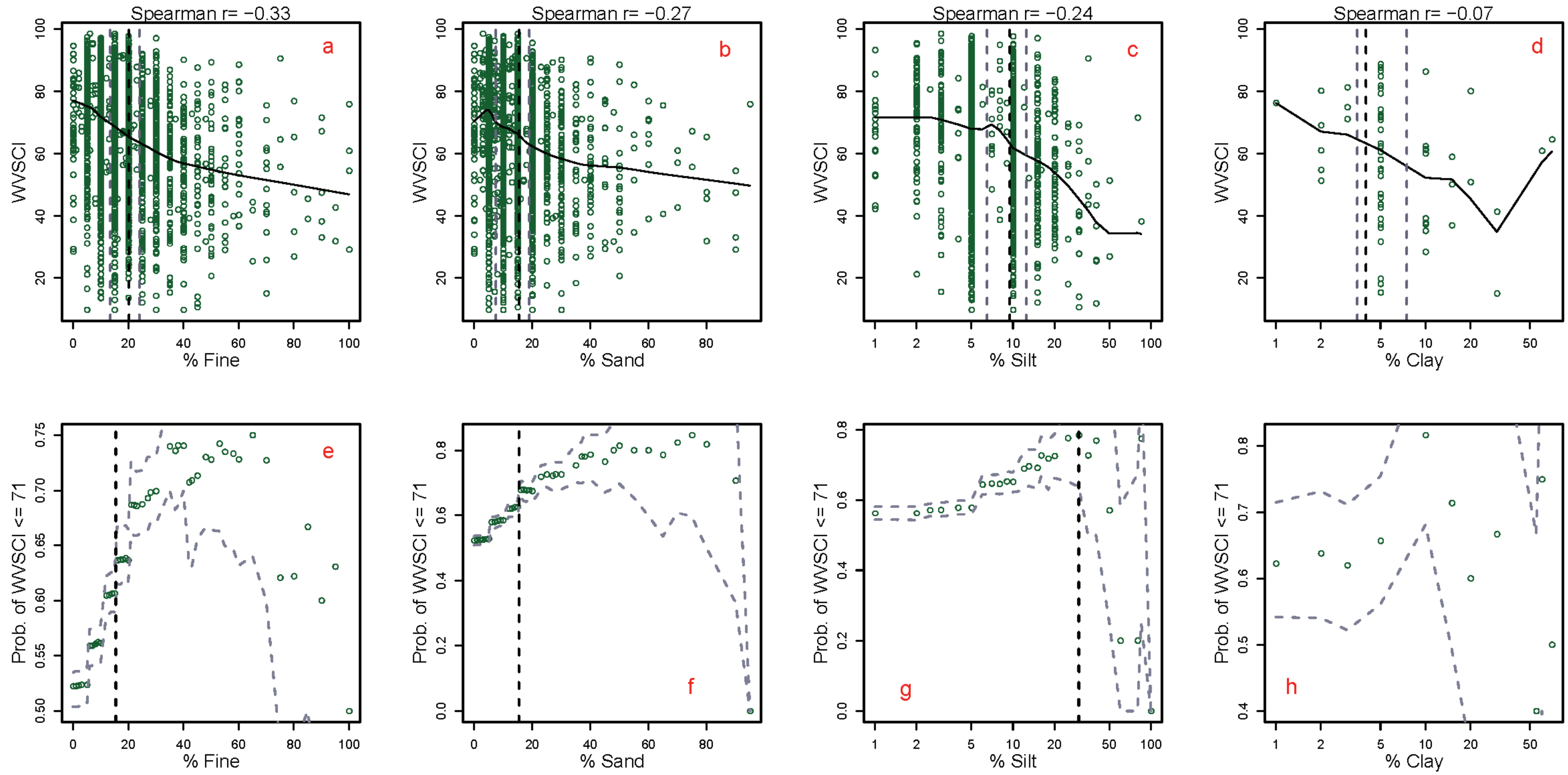


FIGURE A-15

Relationships Between % Fine and Its Components in Substrate Size Classes and WVSCI Scores. The smooth line shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against sedimentation variables. A deviance reduction technique was used to calculate the change points, or sedimentation threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential sedimentation thresholds and 95th confidence limits.

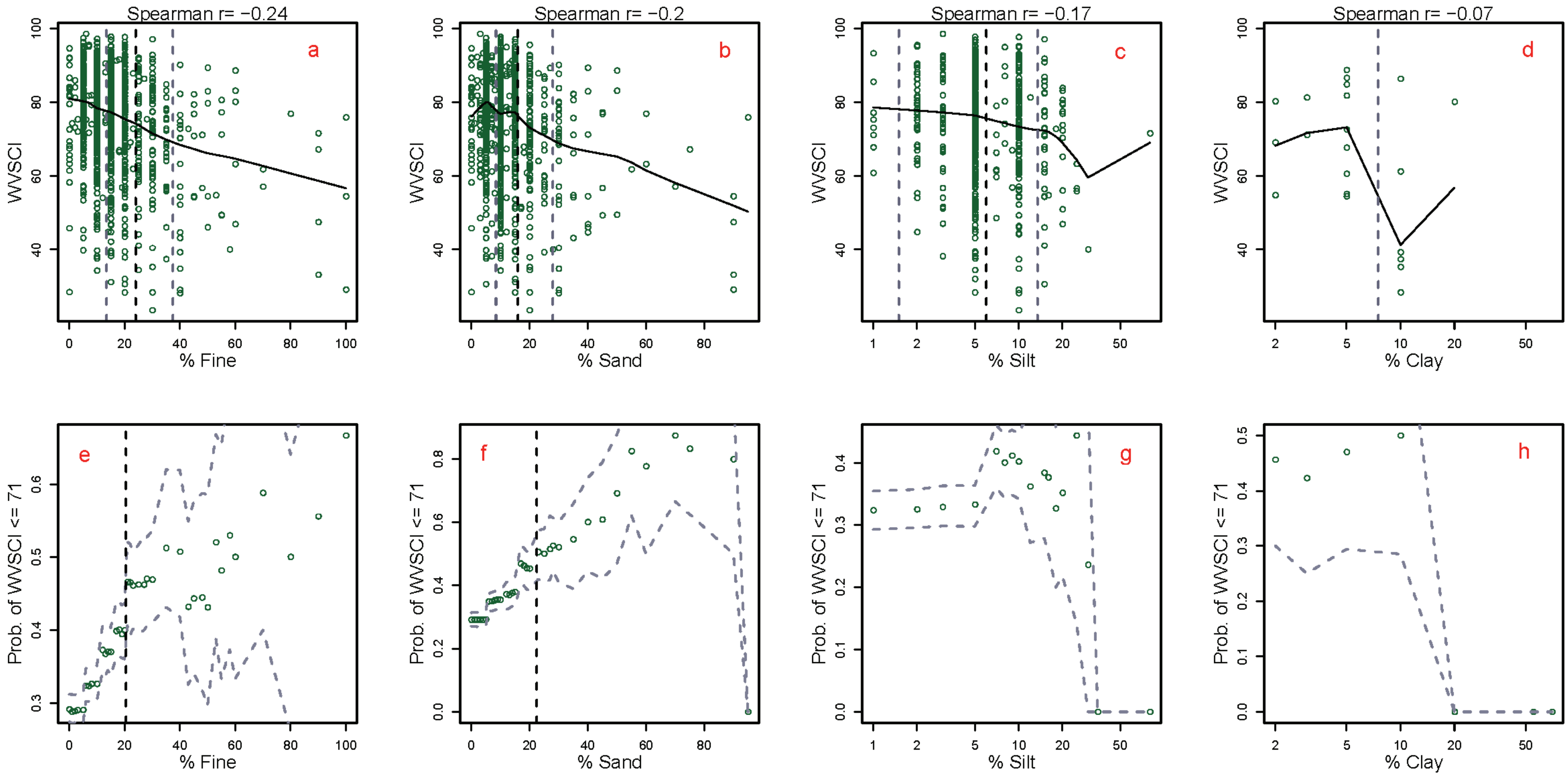


FIGURE A-16

Relationships Between % Fine and Its Components in Substrate Size Classes and WVSCI Scores When Other Stressors are Partitioned Out (pH > 6, conductivity < 300, and fecal coliform counts < 400). The smooth line shows locally weighted smoothing. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against sedimentation variables. A deviance reduction technique was used to calculate the change points, or sedimentation threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential sedimentation thresholds and 95th confidence limits.

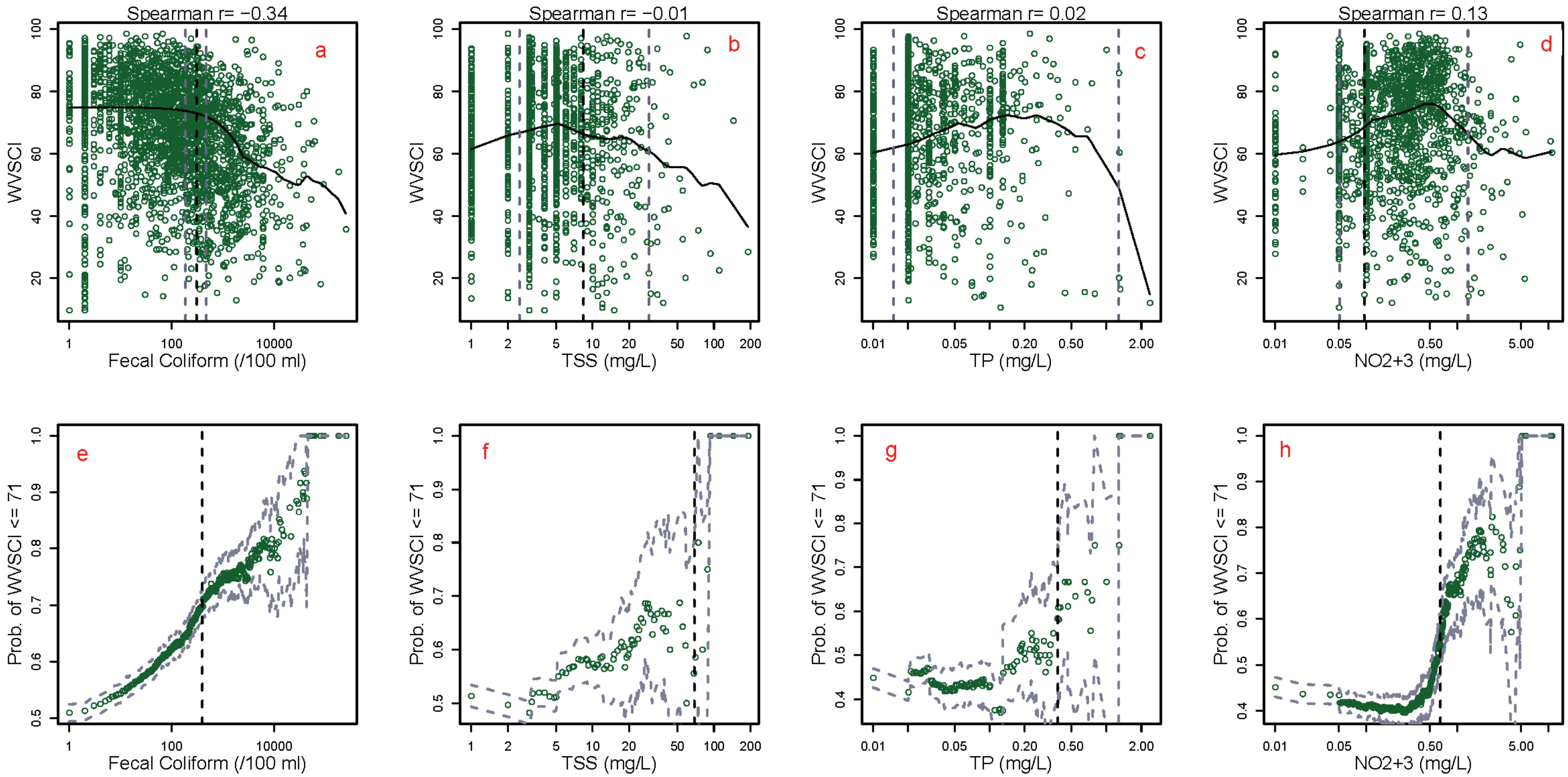


FIGURE A-17

Relationships Between Nutrient- and Organic-Related Environmental Variables (fecal coliform counts, total suspended solids, total phosphorus, and nitrate and nitrite concentrations) and WVSCI Scores. The conditional probabilities of exceeding biological endpoints and their 95th confidence limits of the conditional probabilities were calculated and regressed against sedimentation variables. A deviance reduction technique was used to calculate the change points, or nutrient stressor threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential thresholds and 95th confidence limits.

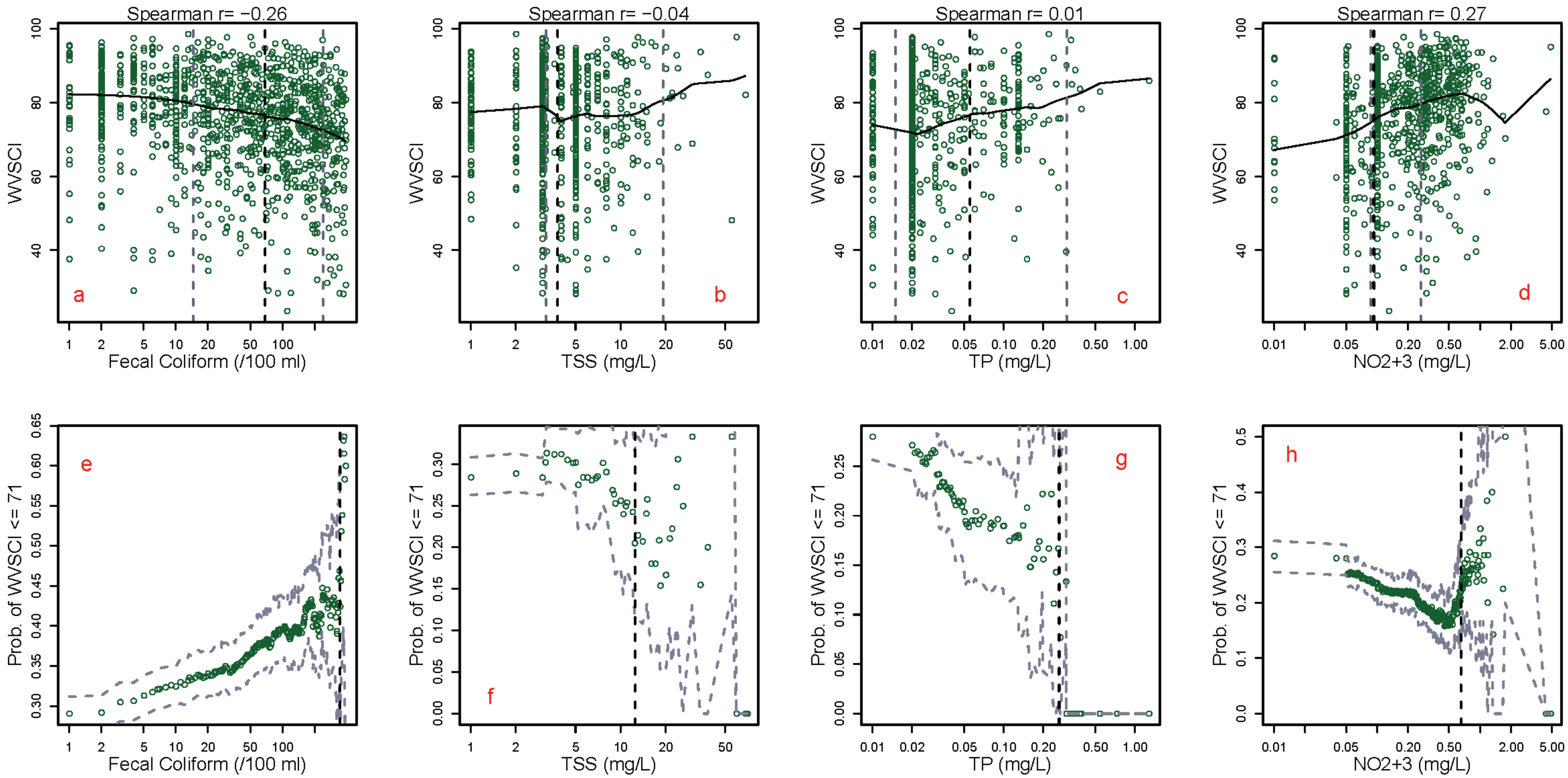


FIGURE A-18

Relationships Between Nutrient- and Organic-Related Environmental Variables (fecal coliform counts, total suspended solids, total phosphorus, and nitrate and nitrite concentrations) and WVSCI Scores When Other Potential Stressors are Excluded (pH > 6.0, conductivity < 300, RBP habitat score > 128). The conditional probabilities of exceeding biological endpoints and their 95th confidence limits were calculated and regressed against sedimentation variables. A deviance reduction technique was used to calculate the change points, or nutrient threshold below which the probability of exceeding the biological criterion is much higher. The vertical dashed lines are the potential thresholds and 95th confidence limits.

A.4. CONCLUSIONS

Of the candidate stressors, metal toxicity is of most concern. Al is highly toxic when pH is below 6. Fe and Mn appear to have relatively weak effects on macroinvertebrate assemblages and are mostly masked by co-occurrence of Al toxicity. Acidity (low pH) as a potential stressor is less strong than Al toxicity, and low pH leads to increased Al toxicity. Ionic strength, indicated by conductivity, is more strongly associated with macroinvertebrate WVSCI scores than any single component ions, including Cl and SO_4^- concentrations. Habitat degradation, measured by RBP scores in a reach, showed stronger effects on macroinvertebrate metrics than any single sedimentation-related parameters. More than 20% percent fines in a reach was associated with decline of WVSCI scores. As a surrogate measure of nutrient and organic enrichment, fecal coliform count also was associated with declines of WVSCI scores.

A.5. REFERENCES

Bailey, J. 2006. Watershed Assessment Section's 2006 Standard Operating Procedures. West Virginia Department of Environmental Protection, Division of Water and Waste Management, Watershed Branch.

Goodfellow, W.L., L.W. Ausley, D.T. Burton et al. 2000. Major ion toxicity in effluents: A review with permitting recommendations. Environ. Toxicol. Chem. 19:175−182.

Paul, J.F. and M.E. McDonald. 2005. Development of empirical, geographically specific water quality criteria: A conditional probability analysis approach. J. Am. Water Resourc. Assoc. 41(5):1211−1223.

R Development Core Team. 2005. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. Available at http://www.R-project.org.

Tetra Tech, Inc. 2000. A Stream Condition Index for West Virginia Wadeable Streams. Report Prepared for U.S. EPA Region 3 Environmental Services Division, and U.S. EPA Office of Science and Technology, Office of Water. Prepared by Tetra Tech, Inc., Owings Mills, MD. Available at http://www.littlekanawha.com/536_WV-Index.pdf.

Qian, S.S., R.S. King and C.J. Richardson. 2003. Two statistical methods for the detection of environmental thresholds. Ecol. Model. 166:87−97.

APPENDIX B

Clear Fork
(Highlighted cells indicate condition for Clear Fork)

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
1. Metals Toxicity	Al (diss)	0.020	0.050	0.260	max < 0.18	max > 0.2	med > 0.2	med > 0.4	min > 0.4
	Fe (tot)	0.050	0.255	28.100	max < 0.8	indeterminate (no S-R)			
	Mn (tot)	0.020	0.095	1.470	max < 0.05	min > 0.05	med > 0.05		min > 0.5
2. Acid pH	pH	7.02	7.76	8.61	min > 6.5	min < 6.5	med ≤ 4		max < 4
3. High pH	pH	7.02	7.76	8.61	max < 9	max > 9	med > 9		min > 9
4. Ionic Strength	Conductivity	129	464	974	max < 180	max > 180	med > 180	med > 300	min > 300
	SO_4	29	173	433	max < 43	max > 43	med > 43		
	Chloride		ND		max < 10	min > 10	med > 10	med > 17	min > 17
5. Sedimentation	TSS	3	5	934	max < 7	indeterminate (no S-R)			
	% fines (SSC)	10	17.5	45	max ≤ 30%	max > 30%	med > 30%		min > 30%
	RBP embeddedness	5	14	18	≥13		<13	<9	
	RBP sediment	5	14	16	≥11		<11	<8	
	RBP bank stability				≥13		<13	<12	

APPENDIX B cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
6. Other Habitat	RBP total score	103	134.5	155	≥147	<147	<140	<130	
	RBP: channel alteration				≥16		<10		
	RBP: cover	10	16	18	≥15		<10	no substantial effects	
	RBP: riparian Vegetation	5	7.5	13	≥14		<10	no substantial effects	
7. Nutrient Enrichment Leading to Excess Algae	NO_3		ND		max < 0.6	indeterminate (no S-R)			
	TKN		ND		max < 1.7				
	TP		ND		max < 0.04	indeterminate (no S-R)			
	Algae obs.	observed in upper reaches			few observed		moderate	high	

APPENDIX B cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
8. Altered Food/ Energy Source	Fecal coliform	2	80	5000	max < 250	max > 250	med > 250	med > 500	min > 500
	Excess algae	See 7							
9. Low DO	DO	8.20	10.60	15.60	min ≥ 5.0		min < 5.0	min < 4.0	
10. Temperature (direct)	Temperature	0.06	12.41	28.80	max < 30.6				
11. Ammonia Toxicity	NH_3		ND		max ≤ 0.5		max > 0.5		

Min = Minimum; Med = Median; Max = Maximum.

APPENDIX C

Lick Run
(Highlighted cells indicate condition for Lick Run)

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > Thresh
1. Metals Toxicity	Al (diss)	0.020	0.050	0.200	max < 0.18	max > 0.18	med > 0.2	med > 0.4	min > 0.4
	Fe (tot)	0.090	0.470	19.700	max < 0.8	indeterminate (no S-R)			
	Mn (tot)	0.070	0.320	2.110	max < 0.05	min > 0.05	med > 0.05		min > 0.05
2. Acid pH	Ph	7.63	8.01	8.54	min > 6.5	min < 6.5	med ≤ 4		max < 4
3. High pH	pH	7.63	8.01	8.54	max < 9	max > 9	med > 9		min > 9
4. Ionic Strength	Conductivity	263	475	804	max < 180	max > 180	med > 180	med > 300	min > 300
	SO_4	70	106	223	max < 43	max > 43	med > 43		
	Chloride		ND		max < 10	min > 10	med > 10	med > 17	min > 17
5. Sedimentation	TSS	3	7	528	max < 7	indeterminate (no S-R)			
	% fines (SSC)	35	35	35	max ≤ 30%	max > 30%	med > 30%		min > 30%
	RBP embeddedness	5	5	5	≥13		<13	<9	
	RBP sediment	2	2	2	≥11		<11	<8	
	RBP bank stability				≥13		<13	<12	

APPENDIX C cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > Thresh
6. Other Habitat	RBP total score	85	85	85	≥147	<147	<140	<130	
	RBP: channel alteration				≥16		<10		
	RBP: cover	10	10	10	≥15		<10	no substantial effects	
	RBP: riparian Vegetation	6	6	6	≥14		<10	no substantial effects	
7. Nutrient Enrichment Leading to Excess Algae	NO_3		ND		max < 0.6	indeterminate (no S-R)			
	TKN		ND		max < 1.7				
	TP		ND		max < 0.04	indeterminate (no S-R)			
	Algae obs.	algae low to high			few observed		moderate	high	
8. Altered Food/ Energy Source	Fecal coliform	2	42	950	max < 250	max > 250	med > 250	med > 500	min > 500
	Excess algae	See 7							
9. Low DO	DO	8.53	10.93	13.20	min ≥ 5.0		min < 5.0	min < 4.0	

APPENDIX C cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > Thresh
10. Temperature (direct)	Temperature	3.95	13.03	24.94	max < 30.6				
11. Ammonia Toxicity			ND		max ≤ 0.5		max > 0.5		

Min = Minimum; Med = Median; Max = Maximum.

APPENDIX D

Stone Coal Branch
(Highlighted cells indicate condition for Coal Branch)

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
1. Metals Toxicity	Al (diss)	0.340	3.340	6.600	max < 0.18	max > 0.2	med > 0.2	med > 0.4	min > 0.4
	Fe (tot)	0.050	0.355	12.100	max < 0.8	indeterminate (no S-R)			
	Mn (tot)	0.920	1.350	1.700	max < 0.05	min > 0.05	med > 0.05		min > 0.5
2. Acid pH	pH	4.50	4.77	5.30	min > 6.5	min < 6.5	med ≤ 4		max < 4
3. High pH	pH	4.50	4.77	5.30	max < 9	max > 9	med > 9		min > 9
4. Ionic Strength	Conductivity	432	497	623	max < 180	max > 180	med > 180	med > 300	min > 300
	SO_4	208	262	330	max < 43	max > 43	med > 43		
	Chloride		ND		max < 10	min > 10	med > 10	med > 17	min > 17
5. Sedimentation	TSS (mg/L)	3	10	382	max < 7	indeterminate (no S-R)			
	% fines (SSC)	25	30	35	max ≤ 30%	max > 30%	med > 30%		min > 30%
	RBP embeddedness	3	7	11	≥13		<13	<9	
	RBP sediment	2	6.5	11	≥11		<11	<8	
	RBP bank stability				≥13		<13	<12	

APPENDIX D cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
6. Other Habitat	RBP total score	102	104	106	≥147	<147	<140	<130	
	RBP: channel alteration				≥16		<10		
	RBP: cover	11	11.5	12	≥15		<10	no substantial effects	
	RBP: riparian Vegetation	1	4.5	8	≥14		<10	no substantial effects	
7. Nutrient Enrichment Leading to Excess Algae	NO3		ND		max < 0.6	indeterminate (no S-R)			
	TKN		ND		max < 1.7				
	TP		ND		max < 0.04	indeterminate (no S-R)			
	Algae obs.	observed once			few observed		moderate	high	
8. Altered Food/ Energy Source	Fecal coliform	2	3.5	5600	max < 250	max > 250	med > 250	med > 500	min > 500
	Excess algae	See 7							
9. Low DO	DO	7.70	10.52	13.02	min ≥ 5.0		min < 5.0	min < 4.0	

APPENDIX D cont.									
Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
10. Temperature (direct)	Temperature	3.85	12.53	28.73	max < 30.6				
11. Ammonia Toxicity	NH_3		ND		max ≤ 0.5		max > 0.5		

Min = Minimum; Med = Median; Max = Maximum.

APPENDIX E

Toney and Buffalo
(Highlighted cells indicate condition for Toney and Buffalo)

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > thresh
1. Metals Toxicity	Al (diss)	0.020	0.050	0.050	max < 0.18	max > 0.2	med > 0.2	med > 0.4	min > 0.4
	Fe (tot)	0.030	0.300	2.890	max < 0.8	indeterminate (no S-R)			
	Mn (tot)	0.006	0.020	0.290	max < 0.05	max > 0.05	med > 0.05		min > 0.5
2. Acid pH	pH	7.83	8.24	8.54	min > 6.5	min < 6.5	med ≤ 4		max < 4
3. High pH	pH	7.83	8.24	8.54	max < 9	max > 9	med > 9		min > 9
4. Ionic Strength	Conductivity	8.44	1206	1650	max < 180	max > 180	med > 180	med > 300	min > 300
	SO_4	349	602	871	max < 43	max > 43	med > 43		
	Chloride	3	3	3	max < 10	min > 10	med > 10	med > 17	min > 17
5. Sedimentation	TSS	3	6	63	max < 7	indeterminate (no S-R)			
	% fines (SSC)	5	5	15	max ≤ 30%	max > 30%	med > 30%		min > 30%
	RBP embeddedness	13	14	18	≥13		<13	<9	
	RBP sediment	16	16	16	≥11		<11	<8	
	RBP bank stability				≥13		<13	<12	

APPENDIX E cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > thresh
6. Other Habitat	RBP total score	124	129	137	≥147	<147	<140	<130	
	RBP: channel alteration				≥16		<10		
	RBP: cover	15	16	18	≥15		<10	no substantial effects	
	RBP: riparian Vegetation	1	3	5	≥14		<10	no substantial effects	
7. Nutrient Enrichment Leading to Excess Algae	NO_3	0.22	0.22	0.22	max < 0.6	indeterminate (no S-R)			
	TKN		ND		max < 1.7				
	TP	0.02	0.02	0.02	max < 0.04	indeterminate (no S-R)			
	Algae obs.	Algae moderate			few observed		moderate	high	
8. Altered Food/ Energy Source	Fecal coliform	2	102	6600	max < 250	max > 250	med > 250	med > 500	min > 500
	Excess algae	See 7							
9. Low DO	DO	8.41	10.91	13.23	min ≥ 5.0		min < 5.0	min < 4.0	

APPENDIX E cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > thresh	Min > thresh
10. Temperature (direct)	Temperature	3.67	12.42	22.80	max < 30.6				
11. Ammonia Toxicity	NH_3	0.5	0.5	0.5	max ≤ 0.5		max > 0.5		

Min = Minimum; Med = Median; Max = Maximum.

APPENDIX F

White Oak
(Highlighted cells indicate condition for White Oak)

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
1. Metals Toxicity	Al (diss)	0.020	0.050	0.050	max < 0.18	max > 0.2	med > 0.2	med > 0.4	min > 0.4
	Fe (tot)	0.060	0.190	1.740	max < 0.8	indeterminate (no S-R)			
	Mn (tot)	0.007	0.030	0.880	max < 0.05	min > 0.05	med > 0.05		min > 0.5
2. Acid pH	pH	7.73	8.13	8.80	min > 6.5	min < 6.5	med ≤ 4		max < 4
3. High pH	pH	7.73	8.13	8.80	max < 9	max > 9	med > 9		min > 9
4. Ionic Strength	Conductivity	249	468.5	750	max < 180	max > 180	med > 180	med > 300	min > 300
	SO_4	71	137	275	max < 43	max > 43	med > 43		
	Chloride		ND		max < 10	min > 10	med > 10	med > 17	min > 17
5. Sedimentation	TSS	3	5.2	85	max < 7	indeterminate (no S-R)			
	% fines (SSC)	10	10	10	max ≤ 30%	max > 30%	med > 30%		min > 30%
	RBP embeddedness	16	16	16	≥13		<13	<9	
	RBP sediment	14	14	14	≥11		<11	<8	
	RBP bank stability				≥13		<13	<12	

APPENDIX F cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
6. Other Habitat	RBP total score	137	137	137	≥147	<147	<140	<130	
	RBP: channel alteration				≥16		<10		
	RBP: cover	15	15	15	≥15		<10	no substantial effects	
	RBP: riparian Vegetation	9	9	9	≥14		<10	no substantial effects	
7. Nutrient Enrichment Leading to Excess Algae	NO_3		ND		max < 0.6	indeterminate (no S-R)			
	TKN		ND		max < 1.7				
	TP		ND		max < 0.04	indeterminate (no S-R)			
	Algae obs.	moderate to high			few observed		moderate	high	
8. Altered Food/ Energy Source	Fecal coliform	2	320	12000	max < 250	max > 250	med > 250	med > 500	min > 500
	Excess algae	See 7							
9. Low DO	DO	7.69	10.32	12.91	min ≥ 5.0		min < 5.0	min < 4.0	

APPENDIX F cont.

Candidate Cause	Stressor Measures	Min	Median	Max	Weakening Evidence	Weakly Plausible	Plausible S-R Threshold	Substantial Effects	Sustained Effects
					Min < Ref	Min > Thresh, Med < Thresh	Med > Thresh	Med > Thresh	Min > Thresh
10. Temperature (direct)	Temperature	4.48	12.44	25.54	max < 30.6				
11. Ammonia Toxicity	NH_3				max ≤ 0.5		max > 0.5		

Min = Minimum; Med = Median; Max = Maximum.