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Stressor Identification Guidance Document

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STRESSOR IDENTIFICATION
GUIDANCE DOCUMENT

U.S. Environmental Protection Agency

Office of Water
Washington, DC 20460

Office of Research and Development
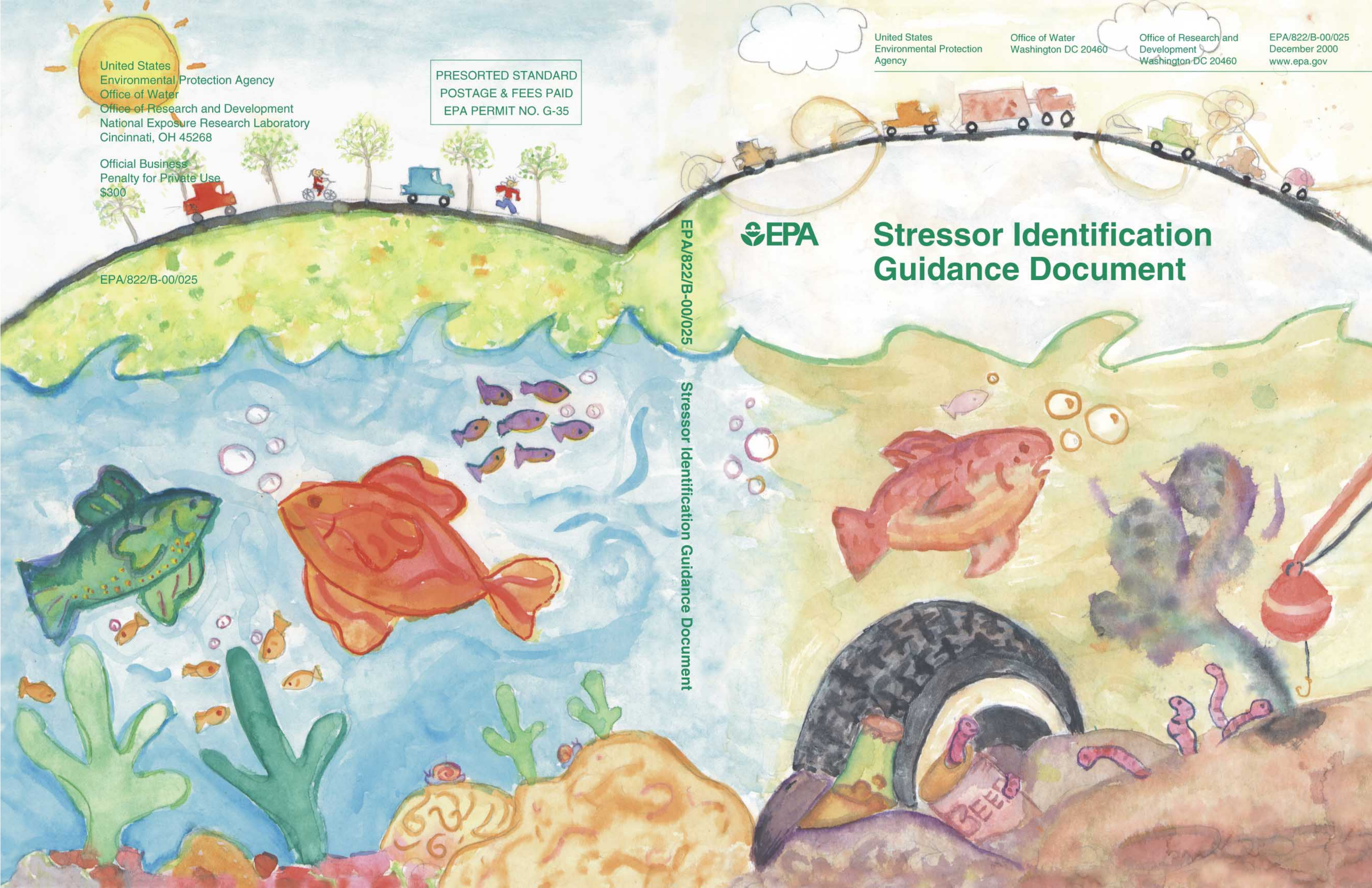Washington, DC 20460

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Disclaimer

This Stressor Identification Guidance Document provides guidance to assist EPA Regions, States, and Tribes in their efforts to protect the biological integrity of the Nation's waters, one of the primary objectives of the Clean Water Act (CWA). It also provides guidance to the public and the regulated community on identifying stressors that cause biological impairment. While this document constitutes the U.S. Environmental Protection Agency's (EPA's) scientific recommendations regarding stressor identification, this document does not substitute for the CWA or EPA's regulations, nor is it a regulation itself. Thus, it cannot impose legally binding requirements on EPA, States, Tribes, or the regulated community, and may not apply to a particular situation based upon the circumstances. When appropriate, State and Tribal decisionmakers retain the discretion to adopt approaches on a case-by-case basis that differ from this guidance. EPA may change this guidance in the future.

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The cover illustration was provided by a fifth grade student at Ursula Villa Elementary School, Mount Lookout, OH. According to the illustrator, the front cover is the river when you first pick up this book, and the back cover is the river after you've followed the instructions.

Table of Contents

Table of Contents (continued)

List of Figures

List of Figures (continued)

Figure **Page**

List of Tables

Table **Page**

List of Tables (continued)

Acronym List

303(d) The section of the Clean Water Act that requires a listing by states, territories, and authorized tribes of impaired waters, which do not meet the water quality standards that states, territories, and authorized tribes have set for them, even after point sources of pollution have installed the minimum required levels of pollution control technology.

305(b) The section of the Clean Water Act that requires EPA to assemble and submit a report to Congress on the condition of all water bodies across the Country as determined by a biennial collection of data and other information by States and Tribes.

7Q10 Lowest average 7 consecutive days flow with average recurrence frequency of once every 10 years

BAP Benzo[a]pyrene

BOD Biological Oxygen Demand

CERCLA Comprehensive Environmental Response, Compensation, and Liability Act

COD Chemical Oxygen Demand

CSOs Combined Sewer Outfalls

CWA Clean Water Act

DELTA Deformities, Erosions, Lesions, Tumors, and Anomalies

DDT Dichlorodiphenyltrichloroethane

DNR Department of Natural Resources

DO Dissolved Oxygen

DQA Data Quality Assessment

DQO Data Quality Objectives

ECBP Eastern Cornbelt Plains

EMAP Environmental Monitoring and Assessment Program

EPA U.S. Environmental Protection Agency

EPT Ephemeroptera-Plecoptera-Tricoptera

EROD Ethoxy Resorufin[o]deethylase

FACA	Federal Advisory Committee Act
GIS	Geographic Information System
IBI	Index of Biotic Integrity
ICI	Invertebrate Community Index
IFIM	Instream Flow and Incremental Methodology
KBI	Kansas Biotic Index
KDHE	Kansas Department of Environmental Protection
MBI	Macroinvertebrate Biotic Index
MIWB	Modified Index of Well-Being
MWH	Modified Warmwater Habitat
NA	Not Applicable/Available
NAPH	Naphthalene
NE	No Evidence
ND	Not Detected
NEP	National Estuaries Program
NIH	National Institute of Health
NO$_x$	Nitrites
NPDES	National Pollution Discharge Elimination Act
NPS	Non-point Source
NRC	National Research Council
OEPA	Ohio Environmental Protection Agency
PAHs	Polycyclic Aromatic Hydrocarbons
PEL	Probable Effect Level
PO$_4$	Ortho-phosphate
POTWs	Publicly Owned Treatment Works
QHEI	Qualitative Habitat Evaluation Index

RM	River Mile
SECs	Sediment Effect Concentrations
SEP	Supplemental Environmental Protection
SI	Stressor Identification
TEL	Threshold Effect Level
TKN	Total Kjeldahl Nitrogen
TMDL	Total Maximum Daily Load
TP	Total Phosphorus
TIE	Toxicity Identification Evaluation
TRE	Toxicity Reduction Evaluation
TSS	Total Suspended Solids
USEPA	U.S. Environmental Protection Agency
WET	Whole Effluent Toxicity
WWH	Warm Water Habitat
WWTP	Waste Water Treatment Plant

Executive Summary

ES.1   The Clean Water Act, Biological Integrity, and Stressor Identification

Since the inception of the Clean Water Act (CWA) in 1972, the rivers, lakes, estuaries, and wetlands of the United States have indeed become cleaner. The standard for measuring these improvements are both chemical and biological. Yet, we know that many waterbodies still fail to meet the goal of the Clean Water Act – to maintain the chemical, physical and biological integrity of the nation's waters.

Biological assessments have become increasingly important tools for managing water quality to meet the goals of the CWA. These methods, which use measurements of aquatic biological communities, are particularly important for evaluating the impacts of chemicals for which there are no water quality standards, and for non-chemical stressors such as flow alteration, siltation, and invasive species. However, although biological assessments are critical tools for detecting impairment, they do not identify the cause or causes of the impairment.

The ability to accurately identify stressors and defend the evidence supporting those findings is a critical step in developing strategies that will improve the quality of aquatic resources.

The Office of Water and Office of Research and Development of the US EPA have developed a process for identifying any type of stressor or combination of stressors that cause biological impairment. The Stressor Identification (SI) Guidance is intended to lead water resource managers through a formal and rigorous process that

▸   identifies stressors causing biological impairment in aquatic ecosystems, and

▸   provides a structure for organizing the scientific evidence supporting the conclusions.

The ability to accurately identify stressors and defend the evidence supporting those findings is a critical step in developing strategies that will improve the quality of aquatic resources.

The Stressor Identification process (SI) is prompted by biological assessment data indicating that a biological impairment has occurred. The general SI process entails critically reviewing available information, forming possible stressor scenarios that might explain the impairment, analyzing those scenarios, and producing conclusions about which stressor or stressors are causing the impairment. The SI process is iterative, usually beginning with a retrospective analysis of available data. The accuracy of the identification depends on the quality of data and other information used in the SI process. In some cases, additional data collection may be necessary to accurately identify the stressor(s). The conclusions can be translated into management actions and the effectiveness of those management actions can be monitored.

ES.2 Intended Audience

This guidance should prove useful to anyone involved in managing impaired aquatic ecosystems. The results of Stressor Identification investigations are valuable to many types of environmental managers— including land-use planners, industrial and municipal dischargers, reclamation companies, and any individuals or organizations involved in activities that directly or indirectly affect water quality or aquatic habitats.

Although the Stressor Identification process is scientifically rigorous, it is flexible enough to support various water management requirements.

The process of stressor identification draws upon a broad variety of disciplines and is most effective when the SI investigator has input from professionals in a number of environmental areas such as aquatic ecology, biology, geology, geomorphology, statistics, chemistry, environmental risk assessment, and toxicology. Sophisticated knowledge in certain fields may increase the tools available to investigators (e.g., physiological responses to certain stressors), but the SI process also can be used by investigators with very general tools (e.g., fish population estimates). Results of general measures, however, may not be as precise as when more specialized measures are used (e.g., stomach-lining histological evaluations).

ES.3 Applications of the SI Process

Although the Stressor Identification process is scientifically rigorous, it is flexible enough to support various water management requirements. Some potential applications of the SI process include the following:

- ▶ **Characterizing the Quality of the Nation's Waters:** Stressor Identification procedures can assist states in more accurately identifying the causes of biological impairment in 305(b) reporting.

- ▶ **Identifying Waterbodies and Wetlands that Exceed Water Quality Standards:** Accurate, reliable stressor identification procedures are necessary for EPA and the states/tribes to accurately identify the cause(s) of water quality standards violations for 303(d) listing and Total Maximum Daily Load (TMDL) calculations. The SI process can help achieve higher degrees of accuracy and reliability in identifying pollutants causing impacts. The SI process is not designed, however, to allocate the amount of responsibility for an impact to a particular source, especially when multiple sources of a stressor are present.

- ▶ **Regulatory and Non-Regulatory Pollution Management Programs:** Stressor identification procedures can help identify different types of stressors within a watershed that are contributing to biological impairment. Stressors can then be prioritized and controlled through a combination of voluntary and mandatory programs.

Other types of programs in which the SI process is useful include: State/Local Watershed Management Programs, National Pollutant Discharge Elimination System (NPDES) Permitting Programs, Dredge and Fill Permitting, Compliance and Enforcement Actions, Risk Assessments, Preservation and Restoration Programs, and Control Effectiveness Assessments.

If a legal challenge to the conclusions drawn is possible, or if costly remediation efforts are indicated as the means to control a stressor, it is essential to have a high level of confidence in the accuracy of the identification. However, because requirements for confidence levels and stressor precision can vary with the intended use of the findings, managers also require flexibility in evaluation systems. Table ES.1 summarizes various levels of rigor required in eight water quality management programs where the SI process can be applied.

Table ES.1. Summary of the use of Stressor Identification (SI) in water quality management programs.

Water Program	Type of Program			Level of Rigor Needed for SI			
	Advisory	Regulatory	Enforcement	Low	Medium	High	ID Source
305(b) Water Quality Reports	✔			✔	✔		✔
303(d) Impaired Waterbody Lists		✔				✔	✔
319 Non-point Source Control	✔			✔	✔		✔
402 Point Source Permitting		✔	✔			✔	✔
316(b) Cooling Water Intake Permitting		✔	✔		✔		✔
401 Water Quality Certifications		✔			✔		
404 Wetlands Permitting		✔	✔		✔		✔
Water Enforcement			✔			✔	✔

ES.4   Document Overview

The SI guidance document describes the organization and analysis of available evidence to determine the cause of biological impairment. The document does not directly address biological assessment, impairment detection, source allocation, management actions, or data collection, although these activities interact with SI in significant ways. This document is intended to guide water resource managers through the Stressor Identification process.

Section One: *The Stressor Identification Process*
Introduces SI process and provides detailed guidance on implementing a stressor identification program. The guidance applies principles of ecoepidemiology to evaluating causes of biological impairment at specific locations.

Chapter 1: *Introduction to the SI Process*
Provides the background and justification for the SI process.

Chapter 2: *Listing Candidate Causes*
Provides an overview of and guidance on the first step of the SI process, listing candidate causes for the impairment.

Chapter 3: *Analyzing Evidence*
Provides an overview of and guidance on the second step of the SI process, analyzing new and previously existing data to generate evidence.

Chapter 4: *Characterizing Causes*
Provides an overview of and guidance on the third step of the SI process, using the evidence from Step 2 to draw conclusions about the stressors that are most likely to have caused the impairment.

Chapter 5: *Iteration Options*
Provides options for stressor identification if no clear cause is found in the first iteration.

Section Two: *Case Studies*
Provides two case studies illustrating the SI process.

Chapter 6: *Presumpscot River, Maine*

Chapter 7: *Little Scioto River, Ohio*

Appendix A: *Overview of Water Management Programs Supported by the SI Process*

Appendix B: *Worksheet Model*

Appendix C: *Glossary of Terms*

Appendix D: *Literature Cited*

Chapter 1:

Introduction to the Stressor Identification (SI) Process

1.1   Introduction

The use of biological assessments and biocriteria in state and tribal water quality standards programs is a top priority of the U.S. Environmental Protection Agency (EPA). As such, one of the agency's objectives is to ensure that all States and Tribes develop water quality standards and programs that

- ▸ use bioassessment information to evaluate the condition of aquatic life in all waterbodies,

- ▸ establish biologically-based aquatic life use designations,

- ▸ protect aquatic life use standards with narrative or numeric biocriteria (see box below),

- ▸ regulate pollution sources,

- ▸ assess the effectiveness of water quality management efforts, and

- ▸ communicate the condition of their waters.

SI is an invaluable component of any bioassessment/biocriteria program concerned with protecting the biological integrity of aquatic ecosystems.

Although bioassessments are useful for identifying biological impairments, they do not identify the causes of impairments. Linking biological effects with their causes is particularly complex when multiple stressors impact a waterbody. Investigation procedures are needed that can successfully identify the stressor(s) and lead to appropriate corrective measures through habitat restoration, point and non-point source controls, or invasive species control. Water management programs have historically shown that aquatic life protection is best accomplished using integrated information from various sources. For example, the whole effluent toxicity program has utilized methods for more than a decade that help resource managers understand and control the toxicity

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Defining Terms– *Aquatic Life Use* and *Biocriteria*

Aquatic Life Use is a beneficial use designation, identified by a state, in which a waterbody provides suitable habitat for the survival and reproduction of desirable fish, shellfish, and other aquatic organisms. ***Beneficial Use Designation*** is a management objective defining desirable uses that water quality should support. Examples include drinking water supply, primary contact recreation (swimming), and aquatic life use.

Biocriteria are narrative expressions (qualitative) or numeric values (quantitative) describing the biological characteristics of aquatic communities based on appropriate reference conditions.

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of complex effluents. Similarly, the Stressor Identification process will enable water resource managers to better understand and control stressors affecting aquatic biota. SI is an invaluable component of any bioassessment/biocriteria program concerned with protecting the biological integrity of aquatic ecosystems.

1.2   Scope of this Guidance

The SI guidance covers the organization and analysis of available evidence to determine the cause of biological impairment. It does not directly address biological assessment, reference condition, impairment detection, source allocation, management actions, data collection, or stakeholder involvement– although these activities interact with SI in significant ways. After stressors are identified, the appropriate management actions depend on the nature of those stressors, and on other factors– including economics. Identifying appropriate management actions is beyond the scope of this document, but examples of management actions are included in the case studies described in Chapters 6-7 of this document.

The SI process may be applied to any level of biological organization (e.g., individuals, populations, communities) and to any type of waterbody (e.g., freshwater streams, estuaries, wetlands, etc.).

Many methods exist for measuring impacts, exposure, land-use, habitat changes and other parameters that are important pieces of evidence in an SI investigation. Descriptions of those methods are beyond the scope of this guidance. The SI guidance, however, relies on the proper use of many tools to collect evidence. EPA recognizes the need for a tools compendium as well as software to help organize evidence, to make use of available databases and technical publications and to prompt proper collection of additional data when needed. The SI process should be viewed as a "logic backbone" in determining the cause of impacts to aquatic biota.

1.3   Data Quality Issues

The SI process is a procedure for analyzing available evidence and determining if the available evidence is adequate to draw a conclusion about the causes of impairment. Since evidence may be collected from a variety of sources using a variety of tools, proper documentation of the data is critical. Each technique for collecting data has associated quality control measures. The higher the quality of data analyzed, the better the chances will be of correctly identifying stressors. Guidance on assessing data quality and making use of various types of data may be found in the Comprehensive State Water Quality Assessment (305b) guidelines (USEPA 1997) and Ecological Risk Assessment guidelines (USEPA 1998a, also Chapter 3). Data of unknown or poor quality can sometimes be used for very rough estimates if the goals of the study allow, but, in general, the quality of all data should be acceptable and well documented. If the available data are not adequate, the SI process can show where data are missing or deficient, but it does not address designing new data collection efforts. Chapter 2, however, does provide advice on quality control when new data are collected.

After stressors are identified, the appropriate management actions depend on the nature of those stressors and on other factors, including economics. Evaluating whether stressor controls have allowed biological recovery is critically important in verifying that the stressors were accurately identified.

1.4 Overview of the SI Process

The SI process may be applied to any level of biological organization (e.g., individuals, populations, communities) and to any type of waterbody (e.g., freshwater streams, estuaries, wetlands, etc.). Some of the criteria presented for evaluating evidence may be specific, however, to a waterbody type (e.g., references to upstream/downstream associations). Similarly, the logic of the SI process may be applied in straightforward, single stressor situations or in complex situations with multiple stressors and cumulative impacts. Complex situations may require investigators to refine the definition of the study area, gather new data, or do multiple iterations of SI to identify all the important stressors. The Little Scioto Case Study (Chapter 7) is given as an example of a complex stressor situation where river segments were analyzed separately because impacts and stressors differed at each location.

1.4.1 The SI Process

Figure 1-1 provides an overview of the Stressor Identification process within the context of water quality management and data collection. The SI process is initiated by the observation of a biological impairment (shown in the topmost box). Decision-maker and stakeholder involvement is shown along the left-hand side; their involvement is particularly important in defining the scope of the investigation and listing candidate causes. At any point in the process of identifying stressors, a need for additional data may be identified; the acquisition of this data is shown by the box on the right-hand side of the diagram. The accurate characterization of the probable cause allows managers to identify appropriate management action to restore or protect biological condition. Once stressors are identified and management actions are in place to control them, the effectiveness of the SI process (as demonstrated by improved conditions) can be monitored using appropriate monitoring tools and designs.

The core of the SI process is shown within the bold line of Figure 1-1 and consists of three main steps:

1. listing candidate causes of impairment (Chapter 2),

2. analyzing new and previously existing data to generate evidence for each candidate cause (Chapter 3), and

3. producing a causal characterization using the evidence generated in Step 2 to draw conclusions about the stressors that are most likely to have caused the impairment (Chapter 4).

The first step in the SI process is to develop a list of candidate causes, or stressors, that will be evaluated. This is accomplished by carefully describing the effect that is prompting the analysis (e.g., unexplained absence of brook trout) and gathering available information on the situation and potential causes. Evidence may come from the case at hand, other similar situations, or knowledge of biological processes or mechanisms. The outputs of this initial step are a list of candidate causes and a conceptual model that shows cause and effect relationships.

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Detect or Suspect Biological Impairment

Stressor Identification

LIST CANDIDATE CAUSES

ANALYZE EVIDENCE

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause

Identify/ Apportion Sources

MANAGEMENT ACTION: Eliminate or Control Causes; Monitor Results

Biological Condition Restored or Protected

Decision-maker and Stakeholder involvement

As Necessary: Acquire Data, Iterate Process

Figure 1-1. The management context of the SI process. (The SI process is shown in the center box with bold line. SI is initiated with the detection of a biological impairment. Decision-maker and stakeholder involvement is particularly important in defining the scope of the investigation and listing candidate causes. Data can be acquired at any time during the process. The accurate characterization of the probable cause allows managers to identify appropriate management action to restore or protect biological condition.)

The second step, analyzing evidence, involves analyzing the information related to each of the potential causes. Virtually everything that is known about an impaired aquatic ecosystem is potentially useful in this step. For example, useful data may come from chemical analysis of effluents, organisms, ambient waters, and sediments; toxicity tests of effluents, waters, and sediments; necropsies; biotic surveys; habitat analyses; hydrologic records; and biomarker analyses. These data do not in themselves, however, constitute evidence of causation. The investigator performing the analysis must organize the data in terms of associations that could support or refute proposed causal scenarios. Chapter 3 discusses several levels of associations between:

▸ measurements of the candidate causes and responses,

▸ measures of exposure at the site and measures of effects from laboratory studies

▸ site measurements and intermediate steps in a chain of causal processes, and

▸ cause and effect in deliberate manipulations of field situations or media.

These associations comprise the body of evidence used to characterize the cause.

In the third step, characterize causes, the investigator uses the evidence to eliminate, to diagnose, and to compare the strength of evidence in order to identify a probable cause. The input information includes a description of the effects to be explained, the set of potential causes, and the evidence relevant to the characterization. Evidence is brought in and analyzed as needed until sufficient confidence in the causal characterization is reached. In straightforward cases, the process may be completed in linear fashion. In more complex cases, the causal characterization may require additional data or analyses, and the investigator may iterate the process.

1.4.2 SI Process Iterations

The SI process may be iterative, beginning with retrospective analysis of available data. If the stressor is not adequately identified in the first attempt, the SI process continues using better data or testing other suspected stressors. The process repeats until the stressor is successfully identified. The certainty of the identification depends on the quality of information used in the SI process. In some cases, additional data collection may be necessary to confidently identify the stressor(s). Although the SI process cannot accurately identify stressors without adequate data, completing the SI process is helpful even without adequate data because the exercise can help target future data collection efforts.

Although the SI process cannot accurately identify stressors without adequate data, completing the SI process is helpful even without adequate data because the exercise can help target future data collection efforts.

1.4.3 Using the Results of Stressor Identification

Stressor Identification is only one of several activities required to improve and protect biological condition (Figure 1-1). In some cases, the most effective management action will be obvious after the probable cause has been identified. In many cases, however, the investigation must identify sources and apportion responsibility among them. This can be even more difficult than identifying the stress in the first place (e.g., quantifying the sources of sediment in a

large watershed), and may require environmental process models. The identification and implementation of management alternatives can also be a complex process that requires additional analyses (e.g., economic comparisons, engineering feasibility) and stakeholder involvement. Once a management alternative is selected and implemented, monitoring its effectiveness can ensure that biological goals are attained, and provides valuable feedback to the SI process. All of these important activities are outside the scope of the current document. However, accurate and defensible identification of the cause through the SI process is the key component that directs management efforts towards solutions that have the best chance of improving biological condition.

1.5   Use of the SI Process in Water Quality Management Programs

Identifying the cause of biological impairments is an essential element of many water quality management programs. Table 1-1 summarizes the stressor identification needs of several water management programs. An extended discussion of some major regulatory programs and their requirements is presented in Appendix A.

Table 1-1. The role of SI in various water management programs.

Program Type/Name	Purpose	Role of SI
305(b) Characterizing the Quality of the Nation's Waters	Under section 305(b) of the Clean Water Act (CWA), states and tribes are required to assess the general status of their waterbodies and identify, in general terms, known or suspected causes of water quality impairments, including biological impairments.	Stressor identification procedures will assist states and tribes to accurately identify the causes of biological impairment. This is a non-regulatory, information reporting effort. A high degree of certainty in identifying the causes of impairment is not always needed for 305(b) reports.
303(d) Listings and TMDLs Identifying Waterbodies and Wetlands that Exceed Water Quality Standards	Under section 303(d) of the CWA, states and tribes are required to prepare and submit to EPA lists of specific waterbodies that currently violate, or have the potential to violate water quality standards, including designated uses and numeric or narrative criteria such as biocriteria. Wetlands assessment programs are also being developed and wetlands may be listed on 303(d) lists.	Accurate, reliable stressor identification procedures are necessary for EPA and the states/tribes to accurately identify the cause(s) of water quality standards violations. A high degree of accuracy and reliability in the stressor identification process is necessary and sources will need to be identified.
State/Local Watershed Management Programs	Managing water resources on a watershed basis involves examining the quality of a waterbody relative to all the stressors within its watershed. Stressors, once identified, are prioritized and controlled through a combination of voluntary and mandatory programs, possibly employing the CWA 402, 319, 404, 401, and other programs.	Stressor identification procedures will help to identify the different types of stressors within a watershed that may be contributing to biological impairment. A high degree of certainty in identifying the causes of impairment is needed.

Table 1-1 (continued). The role of SI in various water management programs.

Program Type/Name	Purpose	Role of SI
319 Non-point Source Control Program	The 319 Program is a voluntary, advisory program under which the states develop plans for controlling the impacts of non-point source runoff using guidance and information about different types of non-point source pollution.	Stressor identification procedures will help to identify the different types of non-point sources within a watershed that may be contributing to biological impairment. A high degree of certainty in identifying the causes of impairment is not always needed.
NPDES Permit Program	Under Section 402 of the CWA, it is illegal to discharge pollutants to waters of the United States from any "point source" (a discrete conveyance) unless authorized by a National Pollutant Discharge Elimination System permit issued by either the states or EPA. NPDES permits are required whenever a discharge is found to be causing a violation of water quality, including biological impairment.	Accurate stressor identification can be very critical in NPDES permitting cases, both for fairness and success in stressor control. The SI process can help to determine if the discharge is the cause of biological impairment. This is especially important when site-specific modifications of state standards or national criteria are used. A high degree of accuracy and reliability in the stressor identification process is necessary and sources will need to be identified. The SI process is not designed to allocate the amount of responsibility for an impact when multiple sources for a stressor are present.
316(b) Cooling Water Intake Program	Under Section 316(b) of the CWA, any NPDES permitted discharger which also intakes cooling water must not cause an adverse environmental impact to the waterbody.	To determine if a cooling water intake structure is causing adverse environmental impacts to the waterbody, the overall health of the waterbody should be known. Where biological impairments are found, stressor identification procedures should be used to identify the different stressors causing the waterbody to be impaired, including the intake structure. A high degree of certainty is needed.
401 Water Quality Certifications	Under Section 401 of the CWA, different types of federal permitting activities (such as wetlands dredge and fill permitting) require a certification that there will be no adverse impact on water quality as a result of the activity. This certification process is the 401 Water Quality Certification.	Stressor identification procedures will help to identify the different types of stress an activity may place on water quality that can then be addressed through conditions in the 401 Certification.

Table 1-1 (continued). The role of SI in various water management programs.

Program Type/Name	Purpose	Role of SI
Wetlands Permitting	Under Section 404 of the CWA, the discharge of dredge and fill materials into a wetland is illegal unless authorized by a 404 Permit. The 404 Permit must receive a 401 Water Quality Certification.	Stressor identification procedures may help to identify unanticipated stress from a dredge and fill activity on water quality or the biological community after the activity is underway. Stressor identification procedures will also help in pre-permitting evaluations of the potential impacts of 404 permitting by assessing different potential stressors on the wetland in advance.
Compliance and Enforcement	Whenever an enforcement action is taken by a regulatory authority, the type of pollution, the source, and other stressors that play a role in causing the violation need to be clearly identified and related to the violating source.	Stressor identification procedures must be able to clearly identify the different types of pollution causing the violation with a high degree of confidence. Legal defensibility is required. Identifying the source with a high degree of confidence is also needed, though the current SI process does not provide that guidance.
Risk Assessments	Results of bioassessment studies can be used in watershed ecological risk assessments to predict risk from specific stressors and anticipate the success of management actions.	Accurate stressor identification is an integral part of this process and can help ensure that management actions are properly targeted and efficient in producing the desired results.
Wetlands Assessments	States are beginning to develop wetlands assessment procedures. In the future, wetlands protection is expected to be increasingly incorporated into state water quality standards.	Stressor identification procedures, as well as future tools specific to wetland investigations, are very much needed by wetlands managers. The biological assessment methods will allow resource managers to evaluate the condition of wetlands and may provide some indication of the type of stressor damaging a wetland. Once bioassessment methods are completed and incorporated into monitoring programs, wetlands may be listed on 305(b) lists as impaired due to biological impairment. The SI process should help identify stressors causing biological impairment so resource managers can better remedy the problems.

Table 1-1 (continued). The role of SI in various water management programs.

Program Type/Name	Purpose	Role of SI
Preservation Programs	The National Estuary Program (NEP) was established in 1987 by amendments to the Clean Water Act to identify, restore, and protect nationally significant estuaries of the United States. The program focuses on improving water quality in an estuary, and on maintaining the integrity of the whole system --its chemical, physical, and biological properties--as well as its economic, recreational, and aesthetic values.	Stressor identification procedures should be useful to the NEP, and other preservation programs, by helping stakeholders identify causes of impairments. This information would feed into the development of a management plan.
Restoration Programs	The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund, was enacted in 1980 (and amended in 1986) for hazardous waste cleanup.	As in enforcement and compliance programs, stressor identification procedures must be able to clearly identify the different types of pollution causing the impairment with a high degree of confidence. Legal defensibility is required. Identifying the source with a high degree of confidence is also needed, though the current SI process does not provide that guidance.
Pollution Control Effectiveness	A key component of any pollution control program or watershed management effort is the ability to ascertain (or predict) the likely effectiveness of pollution control measures or management strategies.	Stressor identification procedures will help to identify the different types of pollution a control measure needs to reduce and the different types of stressors a management strategy needs to address.

Chapter 2

Listing Candidate Causes

2.1 Introduction

The first step in the stressor identification process is to develop the list of candidate causes, or stressors. This is accomplished by carefully describing the effect that is prompting the analysis, and gathering available information on the situation and potential causes (see the box below for definitions of some key terms). Potential causes are evaluated and those that are sufficiently credible are retained as candidate causes used in the analysis stage. The outputs of this initial step are a list of candidate causes and a conceptual model that shows the relationship between the causes and the effect.

Stressor Identification

LIST CANDIDATE CAUSES

ANALYZE EVIDENCE

CHARACTERIZE CAUSES

| Eliminate | Diagnose | Strength of Evidence |

Identify Probable Cause
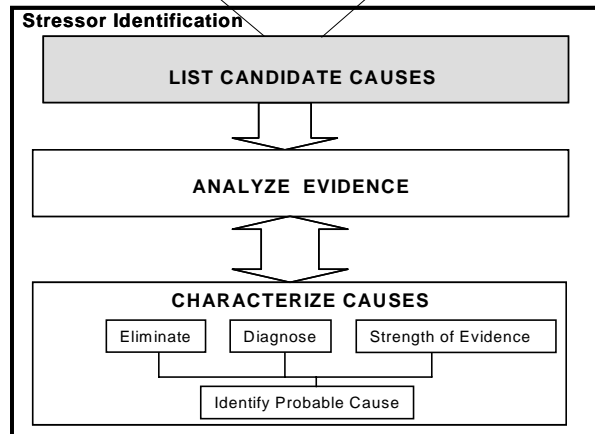
Defining Terms – *Exposures, Effects, Causes, Sources*

An **effect** is a biological change traceable to a cause.

Exposure is the co-occurrence or contact of a stressor with the biological resource.

A **cause** is defined as a stressor that occurs at an intensity, duration, and frequency of exposure that results in a change in the ecological condition.

A **source** is the origin of a stressor. It is an entity or action that releases or imposes a stressor into the waterbody.

note: the processes of detecting impairment and identifying sources are beyond the scope of this document

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2.2 Describe the Impairment

The first important piece of information to be documented is a careful description of the effect that prompted the evaluation. Whenever possible, the impairment should be described in terms of its nature, magnitude, and spatial and temporal extent (see worksheet in Appendix B, Unit I, page B-4). Making inferences about causes is easier when the impairment is defined in terms of a specific effect, or response. The response should be quantified as a count (abundance of darter species) or continuous variable (mean length of darters). If multiple effects with different causes are described as a single impairment, it may be mistakenly assumed that there is only a single cause.

The importance of biological entities as resources and as sentinels of the overall integrity of ecosystems is recognized in the Clean Water Act as well as in subsequent legislation and regulations (See Chapter 1). Observations made in streams and rivers can alert

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environmental managers or the public to a potential problem. If the biological or ecological impairment is of sufficient magnitude, it may necessitate identifying the cause and the potential management controls needed to prevent further damage or to restore the ecosystem. Observations that might prompt the initiation of a stressor identification investigation include:

- ▸ kills of fish, invertebrates, plants, domestic animals, or wildlife,

- ▸ anomalies in any life form, such as tumors, lesions, parasites, disease,

- ▸ altered community structure such as the absence, reduction, or dominance of a particular taxon–this can include increased algal blooms, loss of mussels, increase of tolerant species, etc.,

- ▸ loss of species or shifts in abundance,

- ▸ response of indicators designed to monitor or detect biological, community, or ecological condition, such as the Index of Biotic Integrity (IBI) or the Invertebrate Community Index (ICI),

- ▸ changes in the reproductive cycle, population structure, or genetic similarity,

- ▸ alteration of ecosystem function, such as nutrient cycles, respiration, and photosynthetic rates, and

- ▸ alteration of the aerial extent and pattern of different ecosystems: for example, shrinking wetlands, change in the mosaic of open water, wet meadows, sandbars and riparian shrubs and trees.

It can be important to describe how the observed condition makes the waterbody unfit for its intended use. This makes the purpose and relative importance of the assessment clear. For instance, if the fish are covered with lesions, no one wants to fish for them.

In addition to describing the impairment, it is useful to prepare a background statement articulating the steps taken that revealed the biological impairment. For example, it might be appropriate to refer to a numerical or narrative biocriterion, or a reference condition that has been created for this type of waterbody, including the documentation for its derivation.

If conditions are below expectations, it is important to discuss how the quality or condition of the stream compares to other streams, or to the same stream in other places or times. Photographs of the water body provide visual evidence of a lost resource and can later be used in describing potential pathways that may have lead to the impairment. Equally important are photographs of what the resource could be like (e.g., taken from other locations), what it used to be like, or what valued attributes are still retained.

Maps or other geographical representations that show the location and severity of impairments are essential for orienting the investigators, examining spatial relationships, and eliciting information from stakeholders (see worksheet in Appendix B, Unit I, page

The scope of the investigation determines the extent of the data sets that will be analyzed. It defines the geographic area and time frame under consideration and the types of data that will be examined.

B-5). Maps can range from simple hand-drawn to computer-generated versions. Useful geographic information includes location of the impairment and known point sources, cities, roads, dams, tributaries, and land use. Examples of maps are included in the case studies presented in Section 2 of this document (Chapters 6 and 7). The depiction of this geographic information is also used to determine the scope of the evaluation; that is, the overall spatial and temporal extent of the study.

2.3 Define the Scope of the Investigation

The scope of the investigation influences the selection of candidate causes, and has ramifications for the final outcome and the practical use of the entire stressor identification effort. In a sense, the scope reflects perceptions about the ecosystem and beliefs about the level of restoration, or change, that is possible.

The scope of the investigation determines the extent of the data sets that will be analyzed (see worksheet in Appendix B, Unit I, page B-4). It defines the geographic area and time frame under consideration as well as the types of data that will be examined. The scope of the investigation may be limited or broad. An example of a limited scope is an evaluation of whether a particular stressor is responsible for an impairment. A broader objective would be to evaluate which, among several candidate causes, could be responsible for the observed effect. This broader approach might be appropriate for waters that are not attaining their designated use, and for which TMDLs (Total Maximum Daily Loads; see glossary) must be developed.

Early communication with the stakeholders will help ensure that relevant information has been identified and that potential causes are considered.

Several factors influence the overall scope of the investigation, including:

- ▸ the regulatory context,
- ▸ the purpose of the investigation,
- ▸ the relative importance of stressors emanating from outside the watershed,
- ▸ stakeholder expectations and interests,
- ▸ logistical constraints,
- ▸ cost,
- ▸ personnel, and
- ▸ available data.

Other factors to consider are the geographic extent of the impairment, and the extent of knowledge about the impairment. Early communication with the stakeholders will help ensure that relevant information has been identified, and that potential causes are considered. After these factors are carefully reviewed, a definition of the geographical area should be clearly stated. The regulatory context sometimes limits the scope of the study. For acid rain regulation, the geographical area is very large, whereas an NPDES violation may involve less than a kilometer of stream reach. The investigators should

document any regulatory authorities involved and discuss the regulatory requirement for making a causal determination of the impairment.

The depth of the study may be limited by a paucity of data. In this case, it may still be appropriate to attempt a causal determination with the available data, and then indicate what additional information is needed to more confidently ascribe the cause.

2.4   Make the List

In developing a list of candidate causes, investigators should consider available evidence from the case at hand, other similar situations, and knowledge of biological processes or mechanisms (see text box entitled "Using Existing Programs to List Candidate Causes" and worksheet in Appendix B, Unit I, page B-6). The causes of ecological condition usually involve multiple spatial and temporal scales; both of which must be considered in defining the scope of the study and in listing candidate causes. Recent environmental events are overlaid on historical events, even those spanning geological time. Global and regional influences form the backdrop for local factors.

Where multiple stressors contribute to cause an effect, the stressor that makes the largest contribution is the principal cause. Usually a principal cause is so dominant that removing other causes has no effect on the condition of the resource. For example, if benthic habitat is both physically altered and chemically contaminated, restoring the physical habitat may have no effect until the chemical

In some cases, two or more stressors must be present for the effect to occur.

contamination is removed. In this situation the chemical contamination is the principal cause. The habitat alteration is still a cause of impairment, but it is ancillary and masked by the toxic chemical impact. Nevertheless, pervasive ancillary causes like habitat alteration, nutrient enrichment, and sediment loading can lower the potential improvement to the waterbody even after the controlling or principal cause is removed.

Finding and Using Existing Lists of Stressors

Monitoring programs conducted by government agencies and non-governmental organizations may identify types and levels of stressors. For example, EPA's Environmental Monitoring and Assessment Program (EMAP) has monitored common stressors found in estuarine systems.[1] Among those listed are elevated nutrient concentrations, prolonged phytoplankton blooms, low dissolved oxygen, and sediment contamination.

State agencies and volunteer monitoring programs may also be good sources of information on stressors. Maryland's Department of Natural Resources (DNR), for example, maintains a website on which are links to maps indicating long term trends in total nitrogen, total phosphorus, and total suspended solids for 3rd order and larger streams in the state of Maryland.[2]

[1] See EPA "Condition of the Mid-Atlantic Estuaries." Office of Research and Development, Washington, D.C. #600-R-98-147. November, 1998.

[2] See Maryland DNR website, http://www.dnr.state.md.us/streams/status_trend/index.html

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In some cases, two or more stressors must be present for the effect to occur. For example, a moderate level of nutrients poses no toxicological threat, but if sparse riparian cover permits sufficient sunlight to allow algal growth, then eutrophication can occur, with a subsequent cascade of effects. Another example is when a combination of reduced stream flow and lack of shading cause an elevation of temperature beyond the limit that native species can tolerate. Stressors acting together to cause an effect should be listed as a single scenario.

There are some ways to simplify the process of identifying and listing candidate causes. In the beginning, it helps to make a relatively long list and then pare the list down to the most likely causes. For the initial long list, it is a good idea to include all stressors known to occur in a waterbody. Even if these stressors have not previously been shown to cause this type of impairment, someone is likely to want proof that they were not causal agents. Include stressors that stakeholders have good reason to believe may be important. Consult other ecologists for potential causes of the impairment.

Knowledge about pollution sources near the waterbody can also suggest potential stressors. Point sources, such as drainage pipes, outfalls, and ditches are easily identified as sources. Constituents of the effluent can be listed as candidate causes. Other sources may be located some distance from the resource, such as motor vehicles and smoke stacks that generate candidate causes such as acid rain or nitrogen enrichment. Particular land uses often generate a consistent suite of stressors. For example, siltation and pesticides are commonly associated with agriculture. Locations of sources and stressors should be added to the impairment maps developed in Section 2.2.

Once an exhaustive list of candidate causes is developed, the next step is to pare the list down. Including very unlikely causes can make the identification process unwieldy and will distract stakeholders and managers from the more likely candidates. Unlikely stressors are those that are believed to be mechanistically implausible or absent from the watershed. Although they need not be evaluated, we recommend that you document the rationale for not including the less likely causes.

2.5   Develop Conceptual Models

The final part of this initial step is to develop conceptual models for the candidate causes, linking the cause with the effect (see worksheet in Appendix B, Unit I, page B-6). This part of the process documents a likely explanation of how the stressor could have caused the impairment. Conceptual models provide a good way to communicate hypotheses and assumptions about how and why effects are occurring. Models can also show where different causes may interact and where additional data collection may provide useful information.

The conceptual model can help the investigator see the pathway between the candidate cause and the eventual impact.

Conceptual models will vary in complexity, depending on the mechanisms and ecological processes involved. A generalized conceptual model might show land uses in the watershed that generate in-stream stressors impacting valued resources. For instance, if fish communities are impacted by moderate levels of nutrients in a sunlit stream, it is important to show that the effect could have occurred via several possible pathways, or a combination of pathways, such as:

▸ decaying algal blooms that result in low dissolved oxygen,

▸ the dominance of prey, causing a change in abundance of species,

▸ conditions favorable for opportunistic pathogens,

▸ diatom-rich water that is so turbid that sight-feeding fish cannot find prey and starve, and

▸ embedded substrates smothered with decaying and overgrown algal mats that reduce habitat for foraging, refugia, and reproduction.

The primary causes in this example are nutrients and incident sunlight. The secondary cause in the pathway could be any of the stressors that are formed from the initial cause. It is usually a good idea to consult with ecologists experienced with similar streams when developing conceptual models, especially when complex pathways and ecological process are involved.

Using a pictorial, poster-style conceptual model is useful to introduce the ecological relationships. Then a box and arrow diagram can be used to show details of the relationships among stressors, receptors, and intermediate processes. Some models get too complicated to be helpful. The diagram should show only the pathways and causes considered in the study. Separate diagrams for each stressor or pathway can keep the focus on the analysis steps that will follow. Figure 2-1 is an example of a box and arrow conceptual model illustrating the impacts of logging on salmon production in a forest stream. Additional examples and advice on conceptual model development can be found in Jorgensen (1994), Suter (1999), Cormier et al. (2000c), USEPA (1998a) (especially Appendix C), and in the case studies shown in Chapters 6 and 7.

In addition to helping the investigators to elucidate the relationships among multiple cause and multiple effects, conceptual models are also powerful tools for communicating among the investigative team and obtaining additional insights from stakeholders and managers.
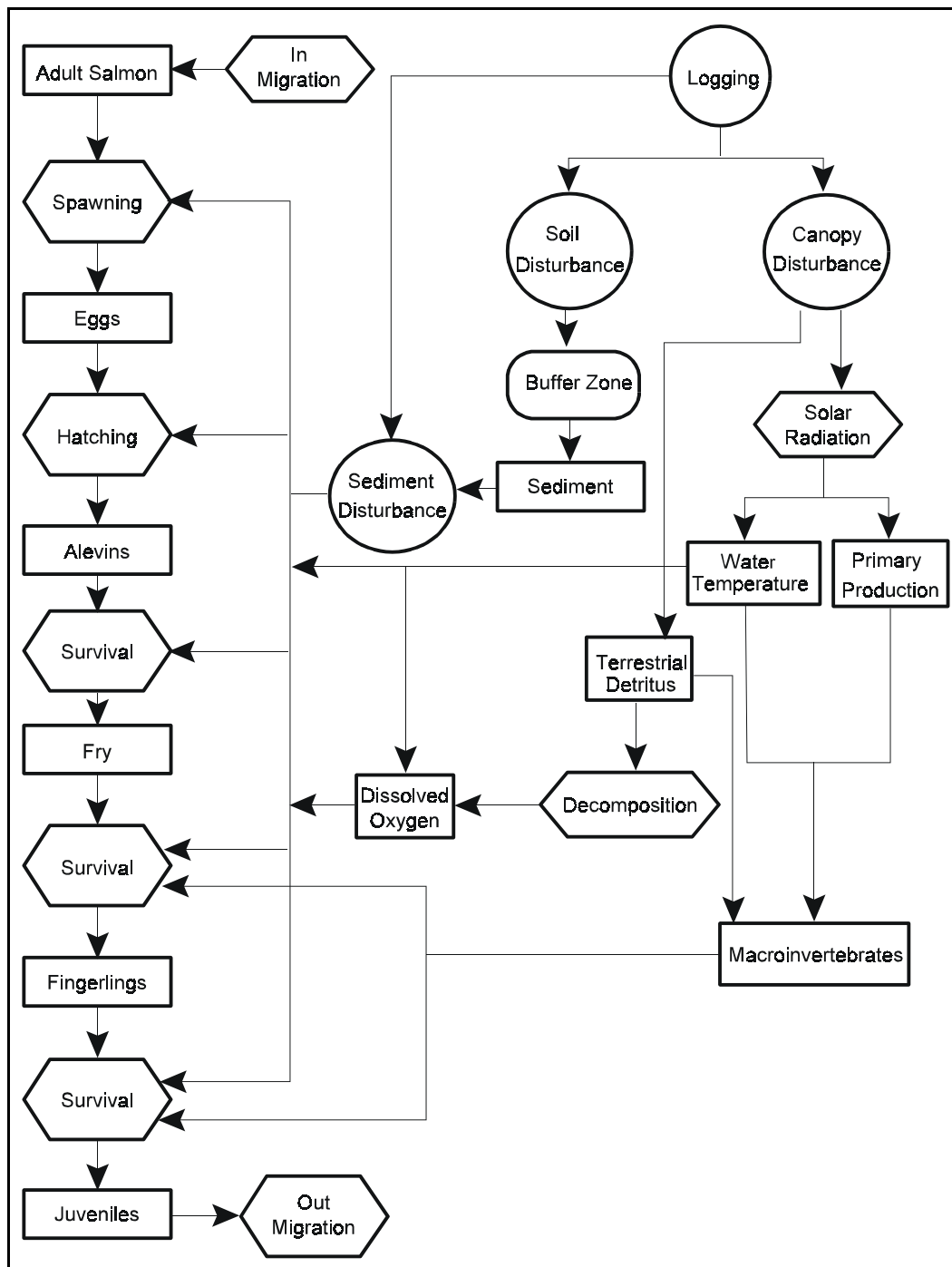
Figure 2-1. A conceptual model for ecological risk assessment illustrating the effect of logging in salmon production in a forest stream. (The assessment includes a series of exposures and responses. In the diagram, the circles are stressors, the rectangles are states of receptors, and the hexagons are processes of receptors. The rectangle with rounded corners is an intervention, establishment of buffer zones, that is being considered (Suter et al. 1994).)

Chapter 3

Analyzing the Evidence

3.1 Introduction

The second step in the SI process is to analyze the information that is related to each of the candidate causes identified in Chapter 2. Virtually everything that is known about an impaired aquatic ecosystem and about the candidate causes of the impairment may be useful for inferring causality. Potentially useful data that may come from studies of the site include chemical analysis of effluents, organisms, ambient waters, and sediments; toxicity tests of effluents, waters, and sediments; necropsies; biotic surveys; habitat analyses; hydrologic records; and biomarker analyses. A similar array of data may be obtained from other sites and from laboratory studies (performed *ad hoc* or reported in the literature). However, these data do not in themselves constitute evidence of causation.

Stressor Identification

LIST CANDIDATE CAUSES

ANALYZE EVIDENCE

CHARACTERIZE CAUSES

Eliminate | Diagnose | Strength of Evidence

Identify Probable Cause

The investigators performing the causal analysis must organize and analyze the data in terms of associations that might support or refute proposed causal scenarios.

Existing data are often sufficient to determine the cause of impairment.

The SI process does not require a minimum data set, and existing data are often sufficient to determine the cause of impairment. However, the investigator has the responsibility of evaluating whether the data used are sufficient to support the SI process. If the investigator decides to generate additional data, its quality must be assured (see text box entitled "Data Quality Objectives").

The primary inputs to the analysis step are the list of candidate causes and the associated conceptual models that link the causes with the observed effects (developed in Chapter 2). Other inputs include data and information that come from the case at hand, other similar cases, the laboratory, and the literature that synthesizes biological and ecological knowledge (Figure 3-1). In the analysis step, this information is converted into causal evidence that falls into four general categories of relationships:

1. associations between measurements of the candidate causes and effects (Section 3.2),

2. associations between measures of exposure at the site and measures of effects from laboratory studies (Section 3.3),

3. associations of site measurements with intermediate steps in a chain of causal processes (Section 3.4), and
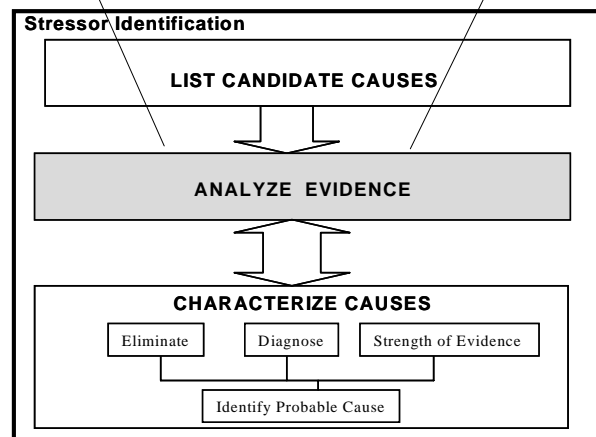
4. associations of cause and effect in deliberate manipulations of field situations or media (Section 3.5).

The evidence produced in the analysis step is used to characterize the cause or causes of the observed effect (see Chapter 4). The analysis and characterization of causes is usually done iteratively and interactively, as illustrated by the two-way arrows between the analysis and characterization boxes in Figures 1-1 and 3-1. Evidence is brought in and analyzed as needed until there is sufficient confidence in the causal characterization. In straightforward cases, the process may be completed in linear fashion. In more complex cases, the causal characterization may require additional data or analyses, and the investigator may repeat the process.

Data Quality Objectives

If new data will be generated for an SI investigation, consider following U.S. EPA's Data Quality Objectives (DQO) process. The DQO process combines a problem formulation exercise with conventional sampling statistics to determine the type, quantity, and quality of data needed to make an environmental decision with a desired probability of error (Quality Management Staff 1994). The DQO process is not directly applicable to SI since it is designed to determine the probability of exceeding a threshold. However, using a formal process to define the problem, examine information needs, and determine study boundaries is important in planning any sampling and analysis program. The criteria for defining an optimum design for an SI study will vary depending on the circumstances. Following sampling and analysis, a Data Quality Assessment (DQA) should be performed to determine whether the goals of the DQO process have been achieved (Quality Assurance Division 1998). The EPA's Quality System, including requirements for non-EPA organizations, can be found at www.epa.gov/quality/index.html.

Quality Assurance Division. 1998. Guidance for Data Quality Assessment. EPA QA/G-9, QA97 Version, or EPA/600/R-96/084. U.S. EPA, Washington, D.C.

Quality Management Staff. 1994. Guidance for the Data Quality Objectives Process. EPA QA/G-4, or EPA/600/R-96/055. U.S. EPA, Washington D.C.

3.2 Associations Between Measurements of Candidate Causes and Effects

The first type of evidence of causation is associations among measurements of candidate causes and effects (Table 3-1). The objective of this analysis is to provide evidence that:

▸ the candidate cause and the effect are observed at the same time or place,

▸ when the candidate cause is not observed, the effect is also not observed, or

▸ the intensity of the causal factor is related to the magnitude of the effect.

Causal evaluations often begin by examining associations from the case at hand. For example, effects are observed downstream, but not upstream of a candidate cause. These associations provide the core of information used for characterizing causes (see worksheet in Appendix B, Unit II, page B-7). Associations may be revealed by plotting data on common axes, as shown in Figure 3-2. In this figure, the spatial pattern of a toxicity bioassay results are clearly associated with the spatial pattern of a community metric. Causal inference is easier when the stressors and effects are located together (co-

located) in time and space. Inference becomes more difficult as stressors are dispersed over larger scales, occur intermittently, or cannot be measured. Inference is also more difficult when there is a time lag between exposures. For example, if a stressor, such as a diversion of water flow prevents salmon from reaching the sea on their out-migration, the effect (i.e., destruction of the salmon run) may not be observed until three years later. In some cases, models may be useful for extrapolating inferences from available measurements.

Causal evaluations often begin by examining associations from the case at hand.
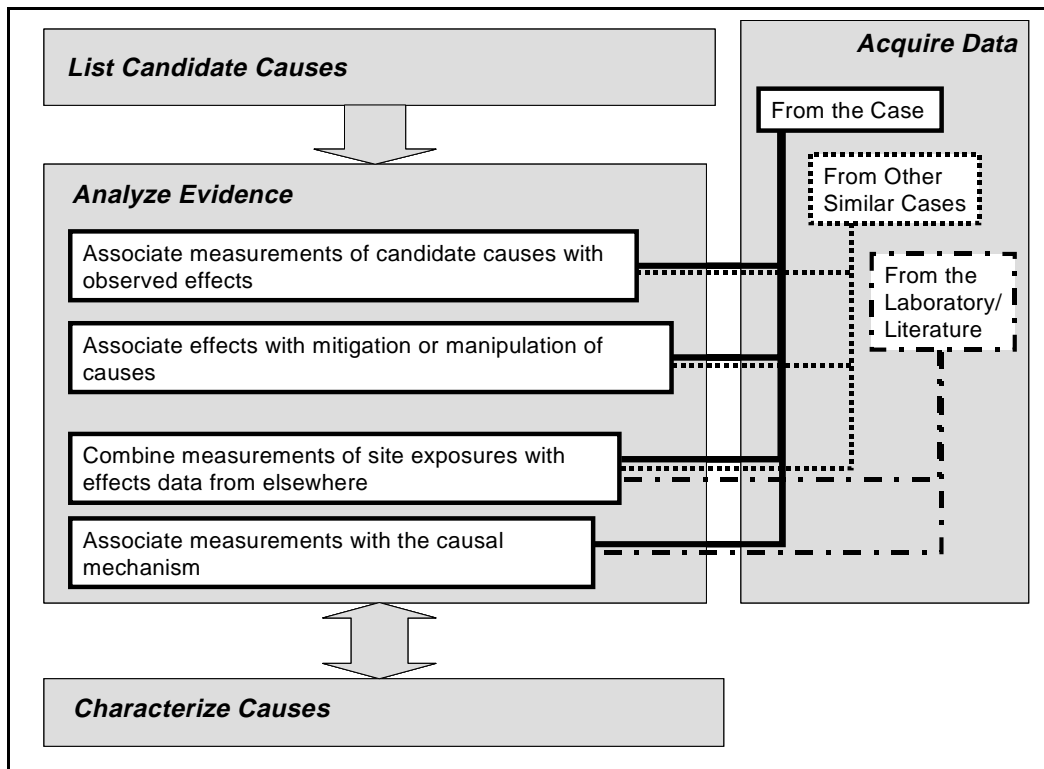
Figure 3-1. The flow of information from data acquisition to the analysis phase of the SI process.

Table 3-1. Types of associations between measurements of causes and effects among site data and the evidence that may be derived from each.

Type of Association	Example Evidence

Spatial co-location	Effects are occurring at same place as exposure
	Effects do not occur where there is no exposure
	For candidates with discrete sources on streams and rivers:
	    Effects occur downstream of a source
	    Effects do not occur upstream of a source
	For candidates with dispersed sources:
	    Effects occur where there is exposure, but not at carefully matched reference sites where exposure does not occur

Spatial gradient	Effects decline as exposure declines over space
Temporal relationship	Exposure precedes effects in time Effects are occurring simultaneously with exposure (allowing for response and recovery rates) Intermittent sources are associated with intermittent exposure and effects
Temporal gradient	Effects increase or decline as exposure increases or declines over time
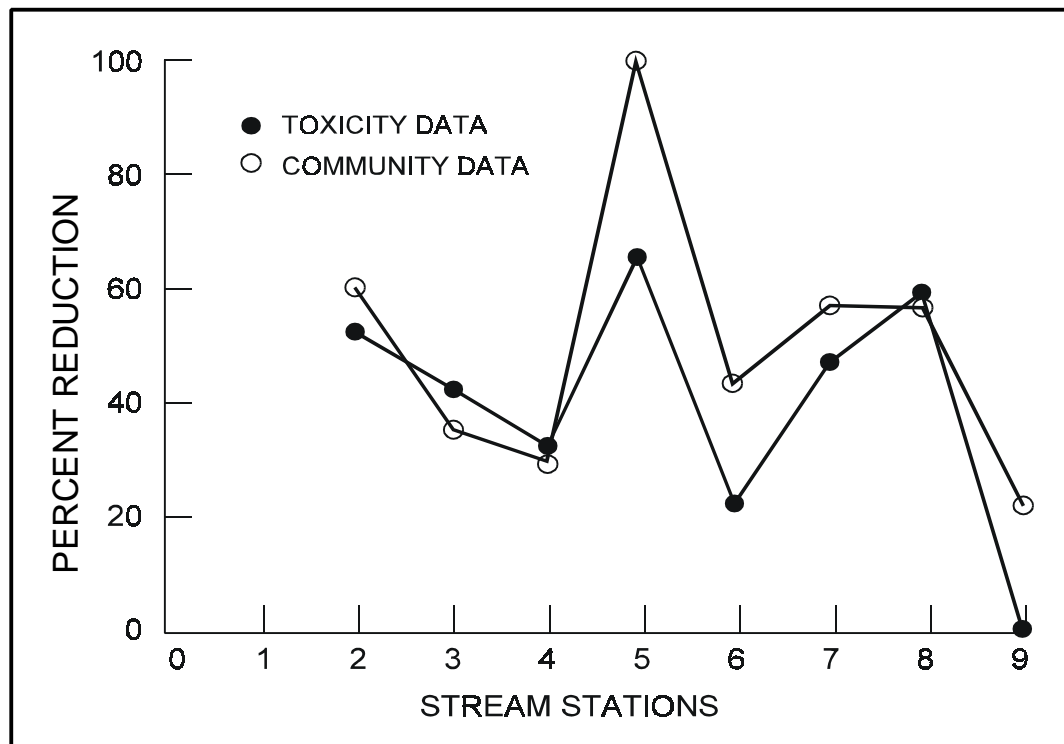
Figure 3-2. Plot of toxicity data from a 7-day subchronic test of ambient waters and a community metric obtained on a common stream gradient (Norberg-King and Mount 1986).

The evaluation of associations must consider whether potentially affected organisms may have moved since exposure. It is helpful to consider the mobility of organisms relative to the extent of the observed exposed and unexposed reaches or areas. Clearly, fish are capable of swimming long distances and invertebrates may drift downstream or fly upstream. However, extensive experience with bioassessment of fish and invertebrate communities has demonstrated that the movements of these organisms are usually not so great as to prevent the observation of spatial associations. The movement of a few individual organisms from contaminated reaches to upstream reaches will diminish, but generally not eliminate, the contrast or gradient among reaches. However, salmon and other species that regularly move long distances require special consideration when analyzing spatial associations. In such cases, consider the logic of the situation and possibly use a GIS as a platform for modeling spatial relationships.

Obtaining measurements of the stressor that can be associated with the effect can be challenging. In the most straightforward cases, the measurements of the stressor itself are available; for example, nutrient concentrations, degree of siltation, dissolved oxygen concentrations, or chemical concentrations. In some cases, the candidate cause is the lack of a required resource, such as nesting habitat. In these cases, measurements can establish that the resource is indeed missing at the place and time it would be required by an organism. When measurements of the stressor are not available, surrogates can be used, although the uncertainty in the analysis will increase. Information on the location and attributes of sources can be useful surrogates.

> *In some cases, the candidate cause is the lack of a required resource, such as nesting habitat.*

This information can be particularly important for stressors that are intermittent in nature (e.g., high flow events), or degrade quickly (e.g., some pesticides). In these cases, source information may be used as a surrogate for the stressors. As sources become larger in scale and more diffuse, information on the sources becomes more difficult to use in site-specific causal evaluation.

Similarly, measuring the immediate or direct response to a stressor increases the confidence in a causal evaluation. For example, a fish kill may be associated with nutrient enrichment, acting through algal growth, decomposition, and oxygen depletion. Measurements of the initial algal growth and oxygen depletion would increase an investigator's confidence that nutrient enrichment was the cause of the fish kill. Conceptual models are very useful for illustrating linkages between complex pathways of cause and effects, and for illustrating where measurements are (and are not) available.

Whenever possible, associations should be quantified. For categorical data, calculate the frequencies of associations. For count or continuous data use, linear or nonlinear models. For example, the abundance of Ephemeroptera at a site may be regressed against concentration of total sediment PAHs. Similarly, the community data plotted in Figure 3-2 might be regressed against the toxicity data. If effects data are categorical or heterogeneous and exposure data are continuous, categorical regression may be used (Dourson et al. 1997). Select the analysis technique that best illuminates the association, based on the amounts and types of data available. Some statistical descriptions of the associations include correlation coefficients, confidence intervals, and p-values. However, avoid statistical hypothesis testing of the associations (see text box entitled "Using Statistics and Statistical Hypothesis Testing for Analyzing Observational Data in Stressor Identification"). Because groups are not randomly assigned in a way that minimizes the influence of confounding variables, a significant outcome in a hypothesis test may be falsely attributed to a candidate cause, when in fact it is due to another

factor. On the other hand, the small sample sizes that are usually seen in these studies decrease the ability to statistically discriminate groups, and may lead to mistakenly eliminating a true cause.

Often associations between candidate causes and effects can be improved by identifying and isolating confounding factors in either the receptors or the environment. For example, the frequency of hepatic neoplasms in fish is associated both with the age structure of the fish population and the concentration of PAHs in sediment (Baumann et al.1996). Correction for age of fish would increase the consistency and, potentially, the biological gradient in the relationship between hepatic neoplasm frequency and industrial contaminants. Similarly, a decline in fish species richness is a common measure of impairment, but the number of species present generally increases with increasing stream size (e.g., OEPA 1988a). Therefore, including a correction for stream size could strengthen the association between the degradation and species loss.

Associations observed from other studies can provide useful supporting information, particularly when the specific type or constellation of effects is consistently observed in association with a candidate stressor. Keep in mind that, as evidence, associations observed from other sites is not as strong as those observed from the study site. Therefore, if associations of effects and potential causes are analyzed at other sites, they should be evaluated separately from those at the site of concern.

3.3   Using Effects Data from Elsewhere

Measures of exposure from the case at hand can also be matched with measures of effect from other situations. The objective of this analysis is to provide evidence showing that the stressor is present at the study site in sufficient quantity or frequency that the investigator would expect to see a particular effect based on effect information from laboratory tests, field tests, or exposure-response relationships developed at other sites (see worksheet in Appendix B, Unit II, page B-12). This type of evidence is familiar to ecotoxicologists who combine measures of exposure from the study site with measures of effect from laboratory tests. For example, concentrations of chemicals measured in water may be compared to concentrations that are thresholds for effects in toxicity tests, or they may be used in concentration-response models to estimate the frequency or magnitude of effects. When doing these comparisons, the investigator should keep in mind that laboratory conditions or organisms may not accurately represent field conditions or organisms.

Equivalent measures of exposure and effects are available for non-chemical stressors (Table 3-2). As in toxicological assessments, it is important to choose the most applicable high-quality effect measurements. It is also important to ensure that the measures of exposure and effects are consistent. For example, long-term field exposures are most appropriately compared with chronic test data. In some cases, exposure-response information will not be available for a candidate cause, but will be available for an analogous agent, such as an effluent with a structurally similar chemical or an introduced species with similar feeding behavior.

**Using Statistics and Statistical Hypothesis Testing
for Analyzing Observational Data in Stressor Identification**

Statistical techniques are essential tools for summarizing and analyzing environmental measurements for SI. Good SI uses a variety of techniques, including descriptive statistics (e.g., means, ranges, variances), exploratory statistics (e.g., multivariate correlations), statistical modeling (e.g, exposure-response relationships), quality assurance statistics (e.g., accuracy and precision of analyses of duplicates and standard reference materials) and comparison of alternative models of candidate causes (e.g., goodness-of-fit or maximum likelihood). However, the use of statistical hypothesis tests is problematic. Statistical hypothesis testing was designed for analyzing data from experiments, where treatments are replicated and randomly assigned to experimental units that are isolated from one another. The application of these tests to data from observational studies can result in erroneous conclusions. In observational studies, treatments are very seldom replicated and are never randomly assigned to experimental units.

If experimental units are replicated at all, they are replicated within the same water body and hence are likely to influence one another. As a result, samples are replicated rather than treatments. This is known as pseudoreplication (Hurlbert 1984). Finally, the location of a candidate cause is a given, rather than being randomly placed, so it is likely that candidate causes will co-vary with each other and with important natural attributes of the system (e.g., salinity, depth). The following table summarizes several common analytical techniques and discusses their use in SI.

Activity	Application to observational data in SI	Comments
Using summary statistics (e.g., mean water concentrations, 7Q10 flow rates) to summarize measurements	Encouraged	Pay attention to the biological or physical relevance of the summary statistic used. For example, the mean of chemical concentrations over time is often the most relevant (USEPA 1998a). As another example, the bankfull flow event is considered to be an important determinant of stream morphology (Rosgen 1996).
Using statistics to determine the probability that two sets or samples are drawn from the same distribution, or that they differ by a prescribed amount	Use Caution	Note that this use is not hypothesis testing in that it does not test a null hypothesis about a treatment (cause). It simply tells you the likelihood that differences are due to sampling variance. Also, the conventional criteria for statistically significant differences are not relevant; the differences must be shown to be biologically significant and the probabilities must be shown to affect the overall strength of evidence. Because the sample sizes are often small relative to variance, the power to detect real differences may be small.
Using the results of statistical hypothesis tests to conclude that a candidate is (or is not) the cause	Wrong	The assumptions of statistical hypothesis testing are violated. In observational studies, replicate treatments cannot be randomly assigned in a way that minimizes the influence of confounding variables. For this reason, a significant outcome in a hypothesis test may be falsely attributed to a candidate cause when in fact it is due to another factor.
Using correlations or regression techniques to quantify relationships between variables.	Encouraged	The type of data (continuous, ordinal, or categorical) and the type of relationship (e.g., linear, non linear) will determine the best technique to use.
Using statistics to determine the probability that a relationship is nonrandom, or that the slope of a regression differs from zero.	Use Caution	Note that this analysis indicates only the probability that an apparent relationship is due to sampling variance. It does not test the hypothesis that the relationship is causal. Also, the number of samples is likely to be low, so even correlations or models that are not statistically significant can be biologically significant and contribute to the strength of evidence.
Concluding that statistically significantly correlated variables have a causal relationship	Wrong	Correlation does not indicate causation, and a highly improbable regression model does not indicate that the independent variable caused the relationship. Because stressors often covary with each other and with natural environmental attributes, a strong relationship between a candidate cause and a biological variable may be due to a factor other than the candidate cause.

Table 3-2. Example associations between site-derived measures of exposure and measures of effects from controlled studies for different types of stressors.

Stressor	Characterization of Exposure: Intensity, Time, and Space	Characterization of Exposure-Response
Chemical	External concentration in medium Internal concentration in organism Biomarker	Concentration-response or time-response relationships from laboratory or other field studies
Effluent	Dilution of effluent	Effluent dilution - response in the laboratory (WET)
Contaminated Ambient Media	Location and time of collection Analysis of medium	Lab or *in situ* tests using the medium: Medium dilution - response Medium gradient - response
Habitat	Structural attributes	Empirical models (e.g., Habitat suitability models)
Water withdrawal/ drought	Hydrograph and associated summary statistics (e.g., 7Q10)	In-stream flow models (e.g., IFIM)
Thermal energy	Temperature	Thermal tolerances
Siltation (suspended)	Suspended concentration (e.g., TSS)	Concentration-Response relationships from laboratory or other field studies
Dissolved oxygen and oxygen-demanding contaminants (e.g. BOD, COD)	Dissolved Oxygen	Oxygen concentration-response relationships from laboratory or other field studies.
Siltation (bed load)	Degree of embeddedness, texture	Empirical siltation-response relationships from laboratory or other field studies.
Excess mineral nutrients	Dissolved concentration	Empirical concentration-response relationships from laboratory or other field studies. Eutrophication models
Pathogen	Presence or abundance of pathogen	Disease, Symptoms
Non-indigenous invasive species	Presence or abundance of the species	Ecological models (food web, energetics, predator-prey, etc.)

In developing mechanistic conceptual models depicting the induction of effects, it is often apparent that there are intermediate steps in the causal process that may be observed or measured.

Laboratory toxicity tests and other controlled studies provide the bases for models depicting the induction of effects by particular causes. For example, an acute lethality test of a chemical provides a concentration-response model which may be used to determine whether fish kills might be attributable to observed or estimated ambient concentrations. More complex causal mechanisms, particularly those involving indirect causation, require more complex mechanistic models. As models of causal processes become more complex, it becomes more difficult to judge whether an individual model provides an acceptable representation of the causes of ecological degradation at a site. In such cases, the best strategy is to generate mechanistic models of each proposed causal scenario and determine which model best explains the site data (Hilborn and Mangel 1997).

3.4   Measurements Associated with the Causal Mechanism

In developing mechanistic conceptual models depicting the induction of effects, it is often apparent that there are intermediate steps in the causal process that may be observed or measured. Documenting those intermediate steps increases confidence in the proposed causal mechanism (see worksheet in Appendix B, Unit II, page B-8). This type of evidence is particularly useful when the ultimate effects of multiple candidate causes are similar, but act through different mechanistic pathways. Types and examples of intermediate steps are presented in Table 3-3. In some cases it is sufficient to document the occurrence of the intermediate step, but in many cases, the level of the metric must be shown to be adequate. For example, if competition for prey by an introduced species is the proposed mechanism by which an endpoint species has been lost, then the investigator should show that the number of prey are reduced sufficiently.

Table 3-3. Example associations between site data and the processes by which stressors induce effects.

Type of Measurement	Example Mechanistic Association
Symptoms (i.e., responses specific to, or characteristic of, a type of stressor and causing the overt impairment)	Fish have lesions characteristic of a bacterium
Biomarkers	Metalothionine induction is an intermediate step in the glomerular toxicity of cadmium
Intermediate product of an ecological process	Algal abundance and DO are measures of intermediate steps in the induction of fish kill by nutrient additions
Changes in abundance of predators, prey, or competitors	Abundance of prey decreases upon introduction of a new predator

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Table 3-3 (continued). Example associations between site data and processes by which stressors induce effects.

Type of Measurement	Example Mechanistic Association
Effects on other receptors	If impairment is defined in terms of effects on fish, then the responses of invertebrates or plants may suggest what causes are operating
Distributions of stressors and receptors coincide	For a stressor to cause an effect, it must contact or co-occur with the receptor organisms. For causes that act through the deprivation of a resource, the deprivation must actually occur

3.5   Associations of Effects with Mitigation or Manipulation of Causes

Strong causal evidence can be provided by deliberately eliminating or reducing a candidate cause and noting whether the effects disappear or remain (see worksheet in Appendix B, Unit II, page B-10). Causes can be eliminated as a part of a field experiment or by bringing site media into the laboratory (Table 3-4). Field experiments may also be performed by manipulating the source (see text box entitled "Associating Effects with Mitigation or Manipulation of a Cause"). For example, cattle may be fenced away from some locations where they usually have access to a stream channel, or an effluent may be eliminated for a time due to plant shut-down. These experiments may be conducted at the site being assessed, or may be conducted at other sites where the same type of source operates. Occasionally, a regulatory or remedial action may be treated as an experimental manipulation. Alternatively, experiments may be conducted that control the exposure of organisms or communities to potential causes. Examples include caging previously unexposed organisms at contaminated locations, placing containers of uncontaminated sediments in locations with contaminated water. These field experiments typically cannot be replicated, so their results are potentially subject to confounding (see text box "Using Statistics and Statistical Hypothesis Testing for Analyzing Observational Data in Stressor Identification"). Finally, site media can be brought into the laboratory and manipulated to eliminate different candidate causes. Then the results of the manipulation can be tested using laboratory organisms. These methods have been most extensively developed for the purpose of attributing causality among different chemicals in effluents.

Table 3-4. Types of field experiments and the evidence that may be derived from each.

Example Experiment	Example Evidence Derived from the Experiment
Manipulation of a source in the field	Elimination of a source reduces or eliminates the effect.
Manipulation of exposure in the field	Introduction of previously unexposed organisms results in effects. Isolation of organisms from one cause reveals the effects of others.
Laboratory manipulation and testing of media from the case	Extracting site media into fractions containing different chemical classes results in toxicity being associated with only one fraction.

Associating Effects with Mitigation or Manipulation of a Cause

Biological data collected by the Kansas Department of Health and Environment (KDHE) have played an increasingly important role in the state's efforts to document water quality impairments. KDHE historically has applied a modification of Davenport and Kelly's (1983) macroinvertebrate biotic index (MBI) to identify impairments resulting from nutrient loading and organic enrichment. Recently, a genus- and species-level indicator known as the Kansas Biotic Index (KBI) was developed to specifically respond to different stressor categories, including nutrients and oxygen demanding substances (KBI_{org}). Data collected by KDHE have shown that declines in the MBI and KBI_{org} have been consistently associated with increased organic enrichment, nutrient loading, and ammonia contamination.

The MBI and KBI_{org} were used to document the ***association between effects and the mitigation or manipulation of causes***. After a nitrification process was installed at the city of Wichita's municipal wastewater treatment facility, median concentrations of total ammonia-nitrogen in the Arkansas River decreased from 1.1 mg/L (1982-91) to 0.06 mg/L (1992-99). Concomitant decreases in the upper quartile MBI and KBI_{org} values were sufficiently large to justify a formal change in the Arkansas River's 305(b) impairment status. Moreover, city officials documented the recolonization of this river by several rare or previously extirpated fish species. Comparable improvements in MBI and KBI_{org} scores were documented in the Smoky Hill River below the city of Salina sewage treatment plant after ammonia levels were reduced by implementing wastewater nitrification and an industrial pretreatment initiative.

Outcome
In the 2000 KDHE 305(b) assessment, the Smoky Hill River was upgraded from non-supporting to fully supporting of aquatic life.

References
Davenport, E. and H. Kelly. (1983); Huggins, G. and F. Moffett. (1988); KDHE. (1993, 1998, 2000).

Chapter 4

Characterizing Causes

4.1   Introduction

Characterizing causes involves using the evidence analyzed in Chapter 3 to reach a conclusion and to state the levels of confidence in that conclusion. The input information in this process includes a description of the effects to be explained, the set of candidate causes developed in Chapter 2, and the causal evidence analyzed in Chapter 3.

Stressor Identification

LIST CANDIDATE CAUSES

ANALYZE EVIDENCE

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause

4.2   Methods for Causal Characterization

After available evidence has been compiled and analyzed, the cause(s) may be obvious. In other cases, a more systematic method for reaching a conclusion may be needed. The use of clearly documented inferential logic increases the defensibility of causal attribution. This chapter describes three methods for using the evidence developed in Chapter 3 to characterize the cause: (1) eliminating alternatives, (2) using diagnostic protocols, and (3) weighing the strength of evidence supporting each candidate cause. Figure 4-1 depicts a procedure that combines these multiple methods to reach a conclusion of causality. Although this approach uses a combination of methods for characterizing causes, each method may also be used independently.

This integrated approach does not include all possible methods of causal analysis, particularly the use of expert judgment. When evidence is ambiguous, the process of developing consensus among a panel of experts may be more acceptable to stakeholders than any systematic evaluation of evidence. Utilizing expert judgment is certainly a more flexible approach in that it does not require any particular data set or type of model. In addition, experts can reach conclusions on the basis of experience and pattern recognition. For example, an experienced extension agent may visit a farm pond that is not producing bass and, without taking any measurements, know that the pond is too small or receives too much manure runoff from surrounding pastures to support bass reproduction. However, when the issue of causation is contentious, the attempt to develop consensus may be complicated by experts who represent the interests of the contending parties. Even when the experts are neutral, expert consensus may not be acceptable to some parties due to subjectivity. Finally, the process of developing expert consensus may not be practical. An NIH consensus development conference or an NRC panel may be practical for large-scale issues, such as the carcinogenicity of electromagnetic fields. It may not be practical to convene an expert panel for each outfall causing ecological injuries.

> *Although this approach uses a combination of methods for characterizing causes, each method may also be used independently.*
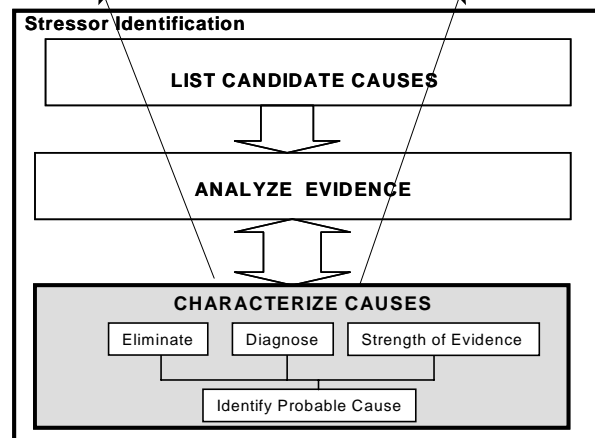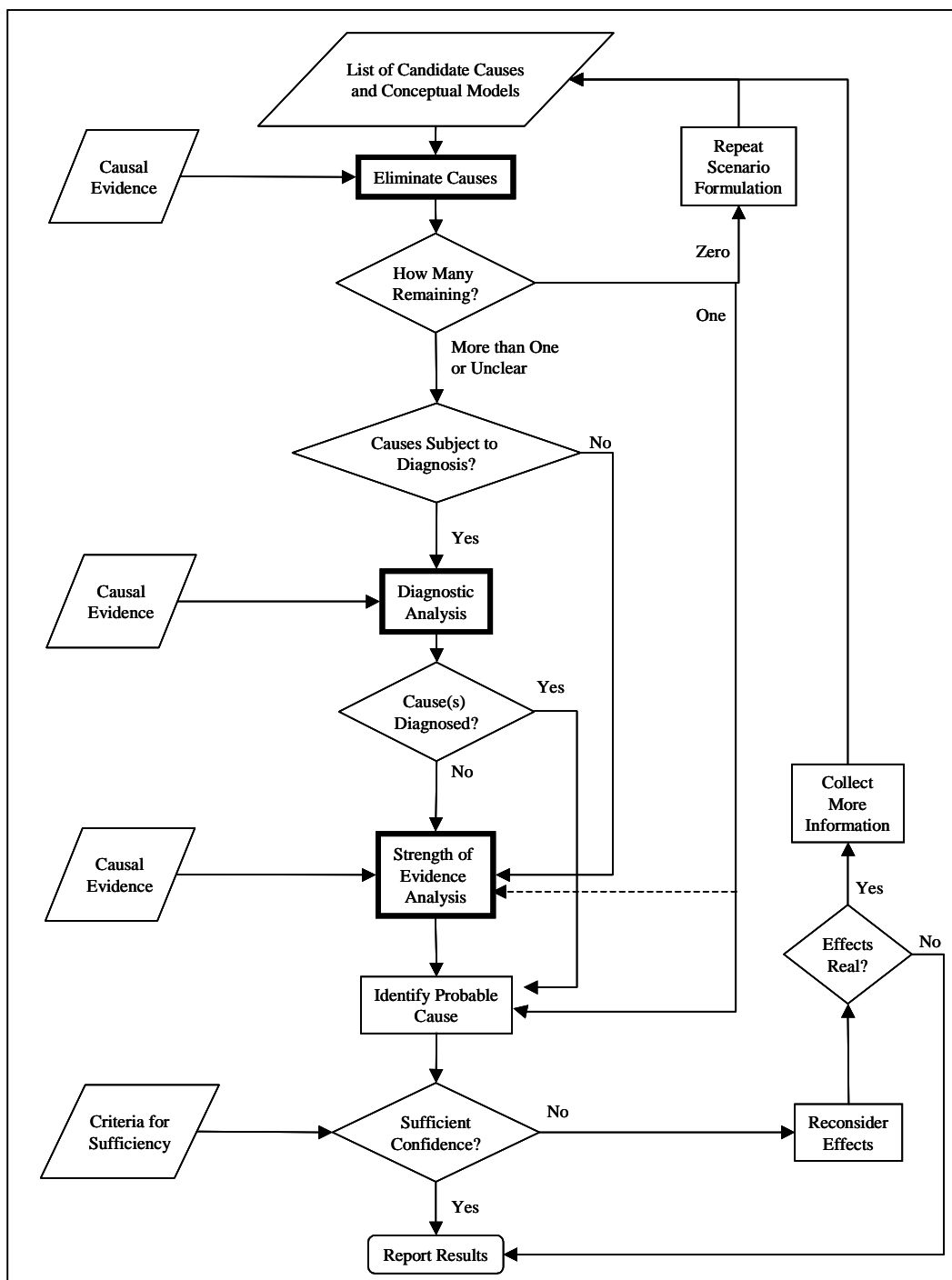
Figure 4-1. A logic for characterizing the causes of ecological injuries at specific sites. (Processes are rectangles, and the three inferential methods have heavy borders. Decisions are diamonds, and inputs are parallelograms.)

Inputs to the characterization process (the parallelogram at the top of Figure 4-1) include a description of the effects to be explained, the list of candidate causes, and the associated conceptual models (Chapter 2). The set of candidate causes should include stressors that consist of multiple factors that act together and are not individually sufficient to cause the effect (i.e., causal scenarios). Other inputs to characterization include the causal evidence produced in the analysis step (the three parallelograms on the left side of Figure 4-1). As discussed above, analyses are usually conducted in combination, as needed, throughout the characterization process. For example, the evidence necessary for eliminating candidate causes is analyzed first, then evidence for diagnosis, and, finally if necessary, the strength of evidence for each candidate cause is analyzed.

4.2.1   Eliminating Alternatives

The causal characterization methods shown in Figure 4-1 are presented in order, from the most conclusive to the least conclusive. The first method, eliminating alternatives, is a powerful approach to evaluating information. The ability to eliminate all but one alternative is a strong standard of proof for causality, and it is easily understood and widely practiced. It is the basic technique of literature's most famous master of inference, Sherlock Holmes:

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause
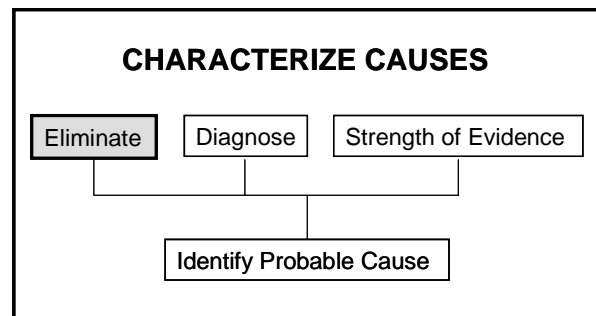
> "When you have eliminated the impossible, whatever remains, however improbable, must be the truth."
>   -- (Sir Arthur Conan Doyle, *Sign of Four*, 1890).

Elimination is also an effective way of reducing the numbers of alternatives to be considered before using another method (e.g., strength of evidence, Section 4.1.3, and see worksheet in Appendix B, Unit III, page B-15). Eliminating evidence is a particularly good option for SI when the set of alternatives is limited, and when disproof does not rely on statistics (see text box in Chapter 3 entitled "Using Statistics and Statistical Hypothesis Testing for Analyzing Observational Data in Stressor Identification"). Specifically, if the SI is conducted to support a permitting action, logical elimination of the permitted source as a potential cause of the observed injury is a sufficient causal analysis. Because of the complexity associated with ecological systems and multiple stressors, many SI investigations will not have the evidence necessary to confidently eliminate causes. These evaluations will rely on a strength of evidence analysis (Section 4.1.3).

Elimination as a method for establishing causality has strong roots in the philosophy of science. Popper, Platt, and other conventional philosophers of science have argued that it is logically impossible to prove a hypothesized relationship, but it is possible to disprove hypotheses (Platt 1964, Popper 1968). If a set of possible causes has been identified, once all but one alternative has been eliminated, the remaining hypothesis must be true. For example, if a body of water is found to be acidic, it is possible to establish the cause as acid deposition by eliminating acid mine drainage, geologic sulphate, and biogenic acids as causes (Thornton et al. 1994).

The elimination of alternatives has three major limitations:

▸ Due to limited knowledge, it may not be possible to identify a complete set of candidate. Also, the array of possible causes is potentially infinite, as there is no clear boundary between plausible and absurd hypothetical causes (Susser 1986b, Susser 1988).

▸ The process of elimination is limited by the ability to perform reliable tests and obtain unambiguous results. Such tests are often difficult in ecology. One may fail to reject a hypothesis but be uncertain of that result due to sampling variance, biases, and temporal variance. If all but one cause is rejected on uncertain grounds, it is difficult to accept the remaining candidate cause with confidence.

▸ Elimination of causes should be done with particular care when multiple sufficient causes may be operating. The evidence for one cause may be so strong that it masks the effects of another sufficient cause and appears to be the sole cause. In addition, beware that the temporal sequence of cause and effect may appear to be wrong when one sufficient cause precedes another. For example, an industrial effluent may impair a biological community. If the stream is subsequently channelized, the effects would be obscured by the industrial effluent. The channelization would have been sufficient to degrade biological communities within a pristine stream and therefore should be retained as a candidate cause. As shown in Table 4-1, similar issues are also relevant to spatial sequences such as those occurring in streams or rivers.

Most often the objective of SI is to identify all sufficient causes (for example, when the goal is to remediate or restore a water body). In these cases, the elimination step should be performed iteratively. That is, each cause eliminated during the first round should be reevaluated to determine if its effects may have been masked by another cause. If so, the candidate cause should be retained. In extreme cases, the masked secondary causes will remain unidentified, because the primary causes are so conspicuous. For example, if channelization has eliminated nearly all fish, it may not be apparent that episodic pesticide runoff would affect sensitive species. Such occult secondary causes will become apparent only after the primary causes have been remediated.

Some types of evidence can be used to eliminate candidate causes, and when those causes might be retained because of masking. Only associations derived from measurements taken from the case under evaluation are strong enough to eliminate an alternative. Associations derived from similar cases cannot be used to eliminate alternatives, but are useful in strength of evidence analyses which allow for uncertain or indecisive evidence (Section 4.1.3).

Only associations derived from measurements taken from the case under evaluation are strong enough to eliminate an alternative.

A stressor can be confidently eliminated if case-specific measurements clearly show that a necessary step in the causal chain of events has not occurred. For example, if a chemical must be taken up by an organism in order to cause an effect, and it can be demonstrated that uptake has not occurred (e.g., though biomarkers or body burdens), the chemical can be eliminated as a cause. Similarly, if sedimentation causes

effects by silting-in riffles, and riffles can be demonstrated to be free of silt, sedimentation can be eliminated as a cause.

Although another potential way to eliminate a candidate cause is through experimental manipulations, the results of field experiments are seldom sufficiently conclusive to eliminate a cause. Uncertainties exist in field experiments due to a lack of thorough knowledge of recovery and recolonization rates following exposure. As a result, reduction or elimination of exposure may not appear to eliminate the effects. Field experiment data can, however, be used in the strength of evidence analysis discussed in Section 4.1.3. In addition, removal of one sufficient cause may unmask the effects of another. The protocols associated with the Toxicity Identification and Evaluation (TIE) program can be applied here, but not all effects of concern occur in these tests (e.g., tumors). Further, there may be questions concerning the sensitivity of the 7-day tests and test species relative to field durations and species (USEPA 1993a,b). TIE, therefore, is considered as part of the strength of evidence analysis.

Table 4-1. Application of common types of evidence in eliminating alternatives.

Type of Evidence (See Chapter 3)	Reason for Rejection	Masking Considerations	Causal Consideration [1] (See Section 4.1.3)
Associations between measurements of candidate causes and effects: Did the stressor precede the effect in time?	If the effects preceded a candidate cause in time, it cannot be the primary cause.	If the candidate cause is preceded by both the effect and another sufficient cause, its effects may be masked, and it should be retained.	Temporality
Associations between measurements of candidate causes and effects: Is there an upstream/ downstream conjunction of candidate cause and effect?	If the effect occurs upstream of the candidate cause's source or does not occur regularly downstream (e.g., is distributed spatially independently of a plume, sediment deposition areas, etc.), it cannot be the primary cause.	If the candidate cause is downstream of another sufficient cause, its effects may be masked and it should be retained.	Co-occurrence
Associations between measurements of candidate causes and effects: Is there a reference site/test site conjunction of candidate cause and effect?	If a candidate cause occurs at reference sites and occurs at equal or greater levels, it can be eliminated.		Co-occurrence

Table 4-1 (continued). Application of common types of evidence in eliminating Alternatives.

Type of Evidence (See Chapter 3)	Reason for Rejection	Masking Considerations	Causal Consideration [1] (See Section 4.1.3)
Associations between measurements of candidate causes and effects: Is a decrease in the magnitude or proportion of an effect seen along a decreasing gradient of the stressor?	A constant or increasing level of effect with significantly decreasing exposure would eliminate a cause.	If a decreasing gradient of one sufficient cause coincides with an increasing gradient of second, recovery from the first cause may be obscured.	Biological Gradient
Measurements associated with the causal mechanism: Has the stressor co-occurred with, contacted, or entered the receptor(s) showing the effect?	If the candidate cause never contacted or co-occurred with the receptor organisms, the cause may be eliminated. For appropriate stressors, if tissue burdens or other measures of exposure are found not to occur in affected organisms, the cause may be eliminated. For stressors that act through a known chain of events, if a link in the chain can be shown to be missing, the candidate cause can be eliminated.		Complete Exposure Pathway
Association of effects with mitigation or manipulation of causes: Did effects continue when a source or stressor was removed?	If the effect continues even after the stressor is removed, then the candidate cause can be eliminated. This assumes that there is no impediment to recolonization.	The effect may also continue if another sufficient cause is present.	Experiment, Temporality

[1] Many of the same types of evidence can also be used in the strength of evidence analysis (see Section 4.1.3). This column denotes the corresponding causal consideration used there.

In some cases all causes but one will be eliminated, and the part of the process is to describe the level of confidence in the characterization. It is often desirable to perform a strength of evidence analysis of that cause to demonstrate that it is probable, given all available evidence. If the true cause was not identified as a candidate, it may be possible to eliminate all candidate causes. In that case, one must repeat the process of identifying candidate causes (Chapter 2). In most cases, the elimination of causes will simply narrow the set of candidates, which is always helpful. Then the process continues to the next step, which is the use of diagnostic protocols or keys.

4.2.2   Diagnostic Protocols or Keys

If more than one cause remains after the elimination step, the next step is to consider whether any of the causes are subject to a diagnostic analysis. Whereas the elimination step relies on negative evidence (e.g., an exposure pathway is not present), diagnostic protocols rely on positive evidence (e.g., a particular symptom *is* present). Diagnostic symptoms are also used in the strength of evidence analysis (under consistency of association and specificity; see Section 4.1.3). The diagnostic protocols referred to here have been used and tested sufficiently to be considered authoritative and some have been formalized into a set of rules or a key (e.g., Meyer and Barclay 1990).

CHARACTERIZE CAUSES

| Eliminate | Diagnose | Strength of Evidence |

Identify Probable Cause
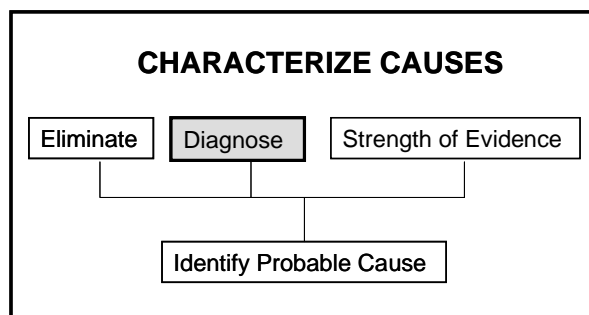
In medicine, diagnostic protocols identify a disease by examining its signs and symptoms. The diagnostic process requires an understanding of mechanism, so most of the evidence comes from measurements associated with the causal mechanism (see Section 3.4 and worksheet in Appendix B, Unit III, page B-19). As in medical practice, diagnostic information in the SI process comes from the exposed organisms and includes symptomatology (i.e., signs of the action of the causal agent on the organisms), measures of internal exposure (e.g., isolation of pathogens or analysis of chemicals in organisms), or measurements of intermediate processes (e.g., a depressed pre-dawn dissolved oxygen level).

The diagnostic approach is a good alternative for SI when organisms are available for examination, when the candidate causes are familiar enough that they have made it into the protocols, and when there is a high degree of specificity in the cause, the effect, or both.

The diagnostic approach is a good alternative for SI when organisms are available for examination, when the candidate causes are familiar enough that they have made it into the protocols, and when there is a high degree of specificity in the cause, the effect, or both. As an example, protocols for the investigation of fish kills are particularly well established (e.g., Meyer and Barclay 1990) and consist of collection of site data concerning candidate causes (e.g., oxygen, pH, temperature, contaminant levels, and presence of toxic algae), site data concerning effects (e.g., taxa killed, duration of event, behavior of live fish), and necropsy results (e.g., lesions, pathogens, tissue contamination, or clinical signs such as blue stomach which indicates molybdenum toxicity). Meyer and Barclay (1990) even provide a dichotomous key for determining the causes of fish kills. Since an SI investigation is more likely to examine current biological

community compositions that might reflect past chronic exposures rather than the effects of acute lethality, the methods for fish kill investigations often are not directly applicable. However, a diagnostic approach can potentially be employed.

Diagnostic tools are well developed for pathogens and to a slightly lesser extent for chemicals (e.g., certain bill deformities are diagnostic of exposure to dioxin-like compounds) (Gilbertson et al. 1991). Diagnostics are also well developed for a few other agents such as low dissolved oxygen (low blood oxygen, gasping at the surface, etc.). For many other stressors and for most non-vertebrate aquatic organisms, reliable diagnostics are seldom available. Expert judgment has been used to assign tolerance values to taxonomic groups for nutrients and this concept has been extended to other stressor types (Hilsenhoff 1987, Huggins and Moffet 1988). The utility of using these tolerance values in multimetric indices along with some recent statistical analyses indicate that the structure of fish and invertebrate communities may prove valuable for diagnosis (Yoder and Rankin 1995b, Norton et al. 2000). Although the use of multimetric information for diagnosing cause and effect is not yet widely accepted or validated, this information can be brought into the strength of evidence analysis discussed in the next section.

4.2.3   Strength of Evidence Analysis

In many SI cases, the candidate causes are not identified by elimination or diagnosis, and an analysis of the strength of evidence for each of the candidate causes is required (see worksheet in Appendix B, Unit III, page B-20). This analysis organizes information so that the evidence that supports, or doesn't support, each candidate cause can be easily compared and communicated. When there are many candidate causes or when evidence is ambiguous, strength of evidence analysis is more useful than elimination of alternatives because it identifies the alternative that is best supported by the evidence. Even when a cause has been identified by a process of elimination or diagnosis, it is often desirable to complete the strength of evidence analysis in order to organize all of the evidence for the decision makers and stakeholders.
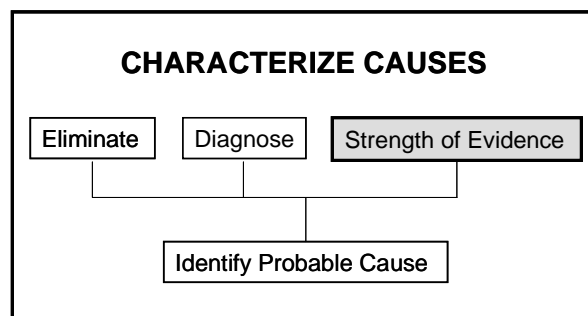
The strength of evidence analysis discussed in the remainder of this section defines a group of causal considerations used to organize the information concerning each alternative. Causal considerations are logical categories of evidence that are consistently applied to support or refute a hypothesized cause. They are defined in Section 4.2.3.1. Section 4.2.3.2 discusses how the types of evidence described in Chapter 3 provide information relevant to each consideration. Finally, Section 4.2.3.3 shows how to evaluate the strength of each piece of evidence in supporting or refuting a candidate cause.

For the purposes of this approach, we treat Koch's postulates (see text box entitled "Koch's Postulates") as a special case of analysis of the strength of evidence. That is, for pathogens or chemical contaminants, if Koch's postulates are satisfied, the strength of evidence is particularly high.

4.2.3.1 Causal Considerations for Strength of Evidence Analysis

This section describes various causal considerations used for strength of evidence analyses. These considerations draw on the work of epidemiologists and ecologists over the last 30 years (Fox 1991, Hill 1965, Susser 1986a).

Koch's Postulates

Koch's postulates combine different lines of evidence in a formal way to provide compelling evidence for causation. The approach was originally developed for pathogen-induced diseases. It has been adapted for demonstrating that particular toxicants cause human diseases (Yerushalmy and Palmer 1959, Hackney and Kinn 1979) or ecological effects (Adams 1963, Woodman and Cowling 1987, Suter 1990, Suter 1993), and has been recommended for ecological risk assessment (EPA 1998a). The following is an adaptation of Koch's postulates for causal inference in ecological epidemiology for effects of pathogens or chemicals.

1. The injury, dysfunction, or other potential effect of the pathogen or toxicant must be regularly associated with exposure to the pathogen or toxicant in association with any contributory causal factors.

2. The pathogen, toxicant, or a specific indicator of exposure must be found in the affected organisms.

3. The effects must be seen when healthy organisms are exposed to the pathogen or toxicant under controlled conditions, and any contributory factors should contribute in the same way during the controlled exposures.

4. The pathogen, toxicant, or a specific indicator of exposure must be found in the experimentally affected organisms.

The power of Koch's postulates arises from the way the four types of evidence are combined. The requirement of regular association in the field ensures that the association is relevant to the field, but, because field observations are uncontrolled, one cannot determine whether the association is, in fact, caused by an another agent that happens to be correlated with the proposed cause. In addition, associations in field data fail to demonstrate the temporal sequence between the candidate cause and effect. The requirement that the candidate causal agent induce the effect under controlled conditions eliminated the potential for confounding and demonstrates that the cause precedes the effect. However, the artificial conditions of toxicity tests and other experimental studies means that the demonstrated causal association may not be relevant to the field. The second and fourth postulates provide the ties that bind the two lines of evidence together. That is, evidence of exposure must be obtained in the field and must correspond to the experimental exposure. This correspondence of the exposure metrics makes it highly unlikely that the correspondence of effects in the field and the experiment are coincidental.

Koch's four postulates were derived for addressing the general issue of whether a stressor could be a cause at all (i.e., could DDT cause reproductive failure in birds). SI investigations typically choose among causal scenarios that have already been established as having the ability to produce impairment. For this reason, the emphasis is placed on postulate 2, identifying the pathogen, toxicant, or specific indicator of exposure in the affected organisms. This case-specific information is then combined with previously established information discussed in postulates 1, 3, and 4. This approach works best for simple causal agents that have a known indicator of exposure. When causal scenarios have multiple insufficient causes, the requirements of regular association and experimental evidence can rarely be met for the specific mixture that is encountered in the field situation. In cases where multiple sufficient causes can be assumed to be acting independently, the evidence for each cause can be evaluated separately.

The first four considerations, *co-occurrence, temporality, biological gradient,* and *complete exposure pathway* draw primarily on associations that are derived from the case itself. These considerations form the strongest basis for causal inference. The next two considerations, *consistency of association* and *experiment* can be based either on data from the case at hand or may draw from similar situations. The next four *considerations, plausibility, specificity, analogy, and predictive performance*, combine information from the case at hand with experiences from other cases or test situations, or from knowledge of biological, physical, and chemical mechanisms. These considerations provide corroborative information that can be used to supplement the basic observations of association of observed effects and potential causes from the case. The last two considerations, *consistency* and *coherency of evidence*, evaluate the relationships among all of the available lines of evidence.

Each of these causal considerations is discussed below:

  Co-occurrence – The spatial co-location of the candidate cause and effect. In SI, this consideration is case-specific; for example, effects may be occurring downstream but not upstream of an identified source (see text box entitled "Arkansas River Case Study"). This consideration should be interpreted with caution when several sufficient causes may be present and when the objective of the analysis is to identify all potential and contributing causes. In this situation, the causes occurring the furthest upstream may mask the effects of causes occurring later in the downstream sequence.

  Temporality – A cause must always precede its effects. For example, a baseline monitoring study showing a productive trout population before a dam was built provides some evidence that the dam caused the subsequent population decline. As with co-occurrence, this criterion should be applied with caution when several sufficient causes may be present and when the objective of the analysis is to identify all potential and contributing causes. In this situation, the causes occurring early in the time sequence may mask the effects of causes occurring later.

  Biological Gradient – The effect should increase with increasing exposure. This is the classic toxicological requirements that effects must be shown to increase with dose. Biological gradient is also applicable to other types of causes (see text box entitled "Arkansas River Case Study"). For example, if fine substrate texture is believed to cause reduced diversity of benthic invertebrates, then diversity should decline along a gradient of texture. In SI, evidence for biological gradient is case-specific. Examples include demonstrating recovery of a community downstream of an outfall, or evidence that an effect decreases with decreasing concentration of an effluent or with increasing mean flow. Investigators should be aware that some stressors elicit non-linear response. For example, community diversity can increase at low levels of nutrient enrichment, then decline again as enrichment increases. Regression and correlation analyses are common tools used to quantify biological gradient; both high slopes and large correlation coefficients increase the strength of evidence.

  Complete Exposure Pathway – The physical course a stressor takes from the source to the receptors (e.g., organisms or community) of interest. If the exposure pathway is incomplete, the stressor does not reach the receptor, and cannot cause an effect. Evidence for a complete exposure pathway is case-specific and may include measurements such as body burdens of chemicals, presence of parasites or pathogens, or biomarkers of exposure (see text box entitled "Arkansas River Case Study"). For stressors that do not leave internal evidence (e.g., siltation), measurements that show the

stressor co-occurring in space and time with the receptor may be useful. For causes that induce effects indirectly, observations or measurements of the intermediate products or conditions are evidence of a complete exposure pathway (see Chapter 7, Little Scioto case study).

Consistency of Association – Refers to the repeated observation of the effect and candidate cause in different places or times (see text box entitled "Lake Washington Case Study"). A consistent association of an effect with a candidate cause is likely to indicate true causation. The case for causation is stronger if the number of instances of consistency is greater, if the systems in which consistency is observed are diverse, and if the methods of measurement are diverse. Consistency can be demonstrated using evidence from the case at hand, or may draw on evidence from many cases. For example, if fish kills repeatedly occur below a particular outfall, there is a consistent association over time of those incidents with a candidate cause. Less commonly, a particular case may have multiple instances of exposure to an agent spread over space rather than time. Consistent association can also be demonstrated across multiple sites or cases. For example, a decrease in benthic arthropod diversity may be consistently observed at many different sites having low dissolved oxygen levels. Consistency of association across many sites is seldom demonstrated because the same causal agent seldom occurs at multiple sites that are sufficiently similar to demonstrate a consistent response. However, when it is demonstrated, consistency across sites is stronger evidence for causation than the simple co-occurrence or temporal association of the agent with the response in a single case.

Arkansas River Case Study:
Using Strength of Evidence Analysis

This example highlights strength of evidence evaluations used in the SI process. Specifically, the example presents several lines of evidence used to support the hypothesis that heavy metal exposure impairs benthic macroinvertebrate communities.

Several sites in the Arkansas River (CO) were monitored over a 10-year span to examine the effects of cadmium (Cd), zinc (Zn), and copper (Cu) on benthic macroinvertebrates. More specifically, metal contamination was related to the abundance of heptageniid mayflies. It was found that heptageniid mayflies were abundant upstream of known metal inputs, and sparse downstream of these inputs, an example of **spatial co-occurrence**. In addition, a **complete exposure pathway** was evident: concentrations of Cd, Cu and Zn were elevated in benthic invertebrates collected at stations downstream of the source. Evidence of a **biological gradient** was observed using multiple regression analysis; the abundance of heptageniid mayflies decreased with increasing zinc concentrations.

Evidence from other studies was also available and demonstrated that effects from metals would be **plausible** based on **stressor-response** relationships observed in the laboratory. Chronic toxicity tests of water collected from the Arkansas using *Ceriodaphnia dubia* and microcosm tests using mayflies established that effects would be expected at the concentrations of Zn, Cu, and Cd measured in the Arkansas.

Evidence from other studies also supported the hypothesis that heavy metal exposures reduce abundance of mayflies. Regional Environmental Monitoring and Assessment Program (R-EMAP) data from other locations in the Rocky Mountains showed a **consistent association** between metal exposures and reduced abundance of heptageniid mayflies.

Finally, efforts were undertaken by several agencies to reduce ambient metal concentrations, an example of a remedial **experiment**. Increases in the abundance of heptageniid mayflies were observed at the sites with greatest metal reduction. Further, little biological improvement was observed where metal levels have remained elevated.

References: Clements and Kiffney 1994, Kiffney and Clements 1994a, Kiffney and Clements 1994b, Clements 1994, Clements et al. 2000, Nelson and Roline 1996.

Experiment – Refers to the manipulation of a cause by eliminating a source or altering exposure (Hill 1965) (see text boxes entitled "Lake Washington Case Study" and "Arkansas River Case Study"). Experiments of greatest relevance to SI (see Section 3.3) include manipulating and testing site media in the laboratory (e.g., using TIE), and conducting field experiments by controlling a source (e.g., fencing cattle) (USEPA 1991b, 1993a, 1993b). The strongest evidence is case-specific. If evidence from experiments conducted on a similar situation is used, the relevance to the case at hand should be described.

Plausibility – Refers to the degree to which a cause and effect relationship would be expected given known facts. Two types of plausibility are discussed below:

Mechanism: Given what is known about the biology, physics, and chemistry of the candidate cause, the receiving environment, and the affected organisms, is it plausible that the effect resulted from the cause? It is important to distinguish a lack of information concerning a mechanism (e.g., the ability of chemical x to induce tumors is unknown) from evidence that a mechanism is implausible (e.g., chemical x is not tumorogenic). It is also important to carefully consider whether some indirect mechanism may be responsible. For example, increased nutrient levels cause algal blooms that decompose and reduce epibenthic oxygen concentrations, which in turn decrease invertebrate diversity. If a mechanism is known and there is evidence that the mechanism is operating in a specific case, the positive evidence is particularly strong.

Stressor-Response: Given a known relationship between the candidate cause and the effect, would effects be expected at the level of stressor seen in the environment? The comparison of environmental concentrations to laboratory-derived concentration-response relationships is a common approach used in chemical risk assessments. It provides strong evidence of causality if concentrations are higher than a level that causes a relevant effect (see Table 3-2) (see text box entitled "Arkansas River Case Study"). Note that exceedence of water quality criteria or standards does not necessarily imply causation because regulatory values are intended to be set at safe levels. Whole effluent toxicity tests may be used with dilution models. Although used mostly for chemical stressors, a similar approach could also be used for other types of stressors, such as siltation.

Analogy – Examines whether the hypothesized relationship between cause and effect is similar to any well-established cases. Hill (1965) used the criterion of analogy to refer specifically to similar causes. For example, a new pesticide with a similar structure to another one may induce similar effects. The idea can be extended to other types of stressors. For example, an introduced species that has similar natural history characteristics to one that had been previously introduced may have similar impacts on the ecological system.

Lake Washington Case Study[1]

Lake Washington, located in Seattle and draining into Puget Sound, first began receiving street runoff and raw sewage input from Seattle at the turn of the 20th century. Although the sewer outlets were eventually replaced by wastewater treatment plant effluents, the growing human population in the surrounding area put increasing demands on the lake. By 1953, 10 wastewater treatment plants discharged into Lake Washington. Shortly thereafter, the first report describing nutrient loadings in the lake was issued by researchers at the University of Washington.

While the problems associated with eutrophication were not widely recognized by the public at the time, a University of Washington professor, W.T. Edmondson, used the concept of **consistency of association** to make an important observation: the recent discovery of a blue-green alga (*Oscillatoria rubescens*) in Lake Washington coincided with other documented cases where water quality had declined in response to nutrient input. The lakes described in these reports ranged geographically from Wisconsin to western Europe, yet the **highly specific** occurrence of *Oscillatoria* was identified in each case as an early response to water enrichment. Thus, Edmondson asserted that the water quality in Lake Washington was declining in response to nutrient input, and would continue to decline in predictable ways.

Edmondson developed a model based on principles of mass balance and stoichiometry to define the quantitative relationships between nutrient levels and algal biomass. He used the model to forecast that water quality in Lake Washington would continue to decline in predictable ways. This is an example of **predictive performance**, since continued monitoring confirmed his assertions.

Outcome
Edmondson's letters and popular science articles describing the problems of the lake successfully brought about public and political support for the eventual clean-up of Lake Washington. Between 1963 and 1968, all 10 wastewater treatment plant discharges were diverted out of Lake Washington and sent to a common collection system that ultimately discharged deep within Puget Sound.

Until the diversions were constructed, water quality had continued to decline as predicted by Edmondson, with water transparency at less than 1 m in 1962. However, in the years following the improvements, nutrient levels decreased substantially. By the 1970s, visibility had reached 12 m, and the presence of the blue-green alga *O. rubescens* was undetectable. The swift recovery of Lake Washington following the removal of nutrient inputs in this field **experiment** left little uncertainty about the true cause of its water quality decline.

[1] Summarized from J. T. Lehman (1986).

Specificity of Cause – Applicable only if the proposed cause is plausible or if it has been consistently associated with the effect. Specific cause-effect relationships are more likely to be demonstrated to be causal (see text box "Lake Washington Case Study"). If an effect (e.g., hepatic tumors in fish) observed at the site has only one or a few known causes (e.g., PAHs), then the occurrence of one of those causes in association with the effect is strong evidence of causation. In the extreme, causation is clear when both effects and causes are specific (*x* causes specific effect *y*, and *y* is caused only by *x*). One implication of this consideration is that both effects and causes should be defined as specifically as possible in order to increase the specificity of the association. For example, a specific cause such as highly embedded substrate can be more clearly associated with identified effects than a general cause like overall poor habitat quality.

Predictive Performance – Refers to whether the candidate cause has any initially unobserved properties that were predicted to occur. Was that prediction confirmed at the site? The ability to make and confirm predictions is one of the hallmarks of a good

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scientific process. For example, if the proposed cause of a fish kill is drift of an organophosphate insecticide into a stream, one could make the specific prediction that cholinesterase levels would be reduced, or the more general prediction that insects and crustaceans would also be killed. If these predicted conditions are then observed at the site, it increases confidence in the causal relationship (see text box entitled "Lake Washington Case Study"). Multiple predictions in both the positive and negative direction would strengthen this criterion (e.g., plants and protozoa would not be harmed, but arthropods would be).

Consistency of Evidence – Refers to whether the hypothesized relationship between cause and effect is consistent with all available evidences. The strength of this consideration increases with the number of lines of evidence (Yerushalmy and Palmer 1959).

Coherence of Evidence – Examines whether a conceptual or mathematical model can explain any apparent inconsistencies among the lines of evidence. For example metal concentrations at the site may be sufficient to impair reproduction in fish, and yet both juvenile and adult fish occur at the site. This evidence may be coherent if reproduction is not occurring at the site, but juvenile fish re-colonize the site from unexposed locations. Another explanation may be that the measured total metal concentration is not 100% bioavailable. The strength of these explanations depend on the expertise and judgment of the assessors. It is a weak line of evidence, because of the possibility that *post hoc* explanations are wrong. However, the hypotheses may lead to experiments or predictions in future iterations of the causal assessment (e.g., testing the bioavailability of the metals), which could support stronger inferences.

4.2.3.2 Matching Evidence with Causal Considerations

Table 4-2 illustrates the different types of evidence discussed in Chapter 3 with the causal considerations they support. The relationship between types of evidence and causal considerations is not one-to-one. Each type of evidence may be relevant to several causal considerations, and a causal consideration may be evaluated using several different types of evidence. In any specific application of SI, evidence will probably exist for only some of the causal considerations, and the evidence will be uneven across the candidate causes. After the evidence relevant to each consideration is identified, it is evaluated as discussed in the next section.

4.2.3.3 Weighing Causal Considerations

Epidemiologists and ecoepidemiologists have attempted to develop guidance for weighing the causal considerations described below (Fox 1991, Hill 1965, Susser 1986a). Table 4-3 presents the possible outcomes for each consideration and provides symbols to represent the influence of each outcome on the inference.

Table 4-3 illustrates a format that can be applied to specific SI cases. In this table, the causal considerations are listed in the left-hand column. Each of the other columns presents results for a candidate cause. The rows show the appropriate number of +, -, or 0 symbols associated with the strength of evidence for each consideration evaluated for each candidate cause. Supporting narratives should describe how the scores were obtained from the evidence. We do not recommend adding up the scores for each candidate cause. Adding the scores erroneously implies that each consideration is of equal importance and is equitable only if the same types of evidence are available across

all candidates. In difficult cases, it may be valuable to compare the evidence for each individual consideration across the candidate causes. Particular attention should be paid to negative results, which are more likely to be decisive.

Table 4-2. Types of evidence (columns) that contribute to each causal consideration (rows).

Causal Considerations	Associations of Measurements of Cause and Effect				Measurements Related to Causal Mechanisms					Case Exposure/ Other Exposure Response			Experiments		
	Spatial Co-location	Spatial Gradient	Temporal Co-occurrence	Temporal Gradient	Symptoms	Biomarkers	Indirect Effects (Abundances of other Species)	Effects on Other Receptors	Cause and Receptor are Co-located	Laboratory Exposure Response	Field-derived Exposure-response	Mechanistic model of Exposure-response	Manipulation of Sources	Manipulation of Exposure	Lab Manipulation and Testing of Media from Site
Co-occurrence	x														
Temporality			x										x		
Biological Gradient		x		x											
Consistency of Association	x		x												
Complete Exposure Pathway					x	x	x	x	x						
Specificity of Cause					x	x									
Plausibility: Mechanism					x	x	x	x	x			x			
Plausibility: Stressor-Response										x	x	x			
Experiment													x	x	x
Analogy	x	x	x	x						x	x	x			
Predictive Performance					x	x	x	x					x	x	

Table 4-3. Format for a table used to summarize results of an inference concerning causation in case-specific ecoepidemiology. (Table adapted from Susser (1986a), Fox (1991), Suter (1998), Beyers (1998).)

Consideration	Results	Score[1]
Case-Specific Considerations		
Co-occurrence	Compatible, Uncertain, Incompatible	+, 0, − − −
Temporality	Compatible, Uncertain, Incompatible	+, 0, − − −
Consistency of Association	Invariant, In many places and times, At background frequencies or many exceptions to the association	++, +, −
Biological Gradient	Strong and monotonic, Weak or other than monotonic, None, Clear association but wrong sign	+++, +, −, − − −
Complete Exposure Pathway	Evidence for all steps, Incomplete evidence, Ambiguous, Some steps missing or implausible	++, +, 0, −
Experiment	Experimental studies: Concordant, Ambiguous, Inconcordant	+++, 0, − − −
Considerations Based on Other Situations or Biological Knowledge		
Plausibility		
Mechanism	Actual Evidence, Plausible, Not known, Implausible	++, +, 0, −
Stressor-Response[2]	Quantitatively consistent, Concordant, Ambiguous, Inconcordant	+++, +, 0, −
Consistency of Association	Invariant, In most places, In some places, At background frequency or many exceptions to the association	+++, ++, +, −
Specificity of cause[3]	Only possible cause, One of a few, One of many	+++, ++, 0
Analogy Positive Negative	Analogous cases: Many or few but clear, Few or unclear	++, + − −, −
Experiment	Experimental studies: Concordant, Ambiguous, Inconcordant	+++, 0, − − −
Predictive Performance	Prediction: Confirmed specific or multiple, Confirmed general, Ambiguous, Failed	+++, ++, 0, − − −
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	Evidence: All consistent, Most consistent, Multiple inconsistencies	+++, +, − − −
Coherence of Evidence	Evidence: Inconsistency explained by a credible mechanism, No known explanation	+, 0

[1] In addition to the scores noted, there ay be No Evidence (NE) available relevant to the consideration, or the consideration may be Not Applicable (NA) for the particular case (see especially stressor-response and specificity).
[2] Stressor-response is not applicable (NA) if the mechanism is clearly implausible.
[3] Specificity of cause is not applicable (NA) if either the mechanism is clearly implausible, or if there are many exceptions to the association.

Other methods for combining different lines of evidence include expert systems based on the logic of abduction and Bayesian statistical approaches (Josephson and Josephson 1996, Clemens 1986). As of this writing, these more quantitative approaches have not yet been developed for combining evidence for SI.

4.3   Identify Probable Cause and Evaluate Confidence

Whichever method is used to infer causation, the results of the characterization must be summarized. That is, the cause must be described, the logical basis for its determination summarized, and the uncertainties concerning that determination presented. As discussed above, there may be multiple sufficient causes, all of which should be characterized. In extreme cases, the effects of the primary causes are so severe that other potential causes will remain unidentified.

CHARACTERIZE CAUSES

| Eliminate | Diagnose | Strength of Evidence |

Identify Probable Cause
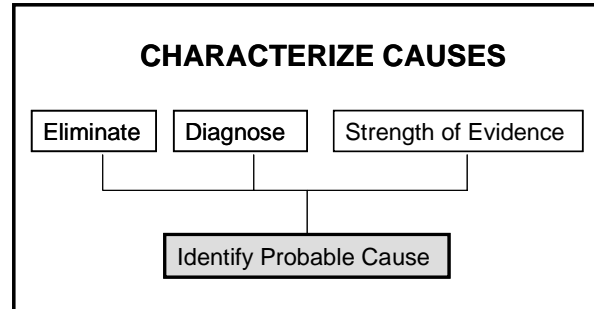
The level of confidence in causal identification may be assessed in quantitative or qualitative terms. Confidence is determined in part by uncertainty concerning the data, the models, and the observations that contribute to the inference. The uncertainty associated with the data may be partially estimated by conventional statistical analysis (see text box in Chapter 3 entitled "Data Quality Objectives," and "Using Statistics and Statistical Hypothesis Testing"), but also includes uncertainty concerning the applicability of the data. If data must be extrapolated between species or life stages, if old data are used to estimate current conditions, or if, for some other reason, data are not directly applicable, the associated uncertainty should be estimated. The uncertainty in statistical models, such as regressions of biological properties against levels of potential causes, may be estimated using goodness-of-fit statistics or confidence bounds. The uncertainty due to the parameters in mathematical models, such as models of dissolved oxygen depression due to nutrient input, may be estimated analytically or by Monte Carlo simulation (USEPA 1996a, 1999). If a causal inference is logically clear and is based predominantly on the results of a statistical or mathematical model, the uncertainties concerning the results may serve to estimate the uncertainties concerning the inference.

 In most cases, unquantified uncertainties will dominate. These include lack of data concerning the presence or levels of particular stressors, incomplete biological data, uncertainty concerning the time when the impairment began, and many more. In addition, most causal inferences are based on the strength of evidence, so that no single source of uncertainty characterizes the uncertainty concerning the conclusion. Therefore, the uncertainty concerning most identifications of causes must be characterized qualitatively. That qualitative judgement should be accompanied by a list of major sources of uncertainty and their possible influence on the results.

In some cases, investigators will be able to clearly demonstrate that a particular cause is responsible for the ecological injuries of concern. However, in many if not most cases, there will be significant uncertainty concerning the relative contributions of alternative causal factors. In such cases, it is necessary to determine whether the evidence is sufficient to justify a management action. Standards and criteria for establishing epidemiological causation are not generally agreed upon. In particular, there is no

consistent standard for adequacy of proof. While conventional science sets a high standard to prove causation, the precautionary principle begins by assuming that an agent is harmful and requires disproof of causation (Botti et al. 1996). Such decisions are made by risk managers, rather than risk assessors, and may be based on considerations such as the cost of remediation and the nature and magnitude of the ecological injury. Ideally, that judgment would be made on the basis of *a priori* criteria. That is, each program that uses SI should specify a standard basis for deciding whether the characterization of the cause is sufficient for the management purpose. For example, for the permitting of POTW effluents, a particular state might develop standards for proof that those effluents cause particular types of injuries. However, standards and criteria for establishing causation are not generally agreed upon, and many decisions are made *ad hoc*. That is, the evidence concerning causation may be presented to the risk manager as a best estimate of causation along with an accompanying analysis of uncertainties. The risk manager may use that result to help reach a decision.

As discussed in Chapter 1, the SI process may be conducted iteratively until sufficient confidence in the causal characterization is reached. In the most uncertain and complex cases, the SI process may best serve to guide further data collection, modeling, or analysis efforts. Options for iterating the process are discussed further in Chapter 5, below. If the cause is confidently identified, then the investigation may proceed to identifying sources, developing and implementing management options, and monitoring their effectiveness (Figure 1-1).

Chapter 5

Iteration Options

This chapter describes iterations if no clear cause can be identified. If the SI process has yielded no clear cause for the biological impairment, it may be because (1) there is actually no effect (Section 5.1), or (2) there is insufficient information concerning the identified causes or the true cause was not among the list of candidate causes (Section 5.2). These alternatives, all leading to a reiteration of the investigation (Figure 4-1), are discussed in this section.

5.1 Reconsider the Impairment

When no cause was identified, it may be that there is actually no effect, or the actual effect may be different from the identified impairment (see worksheet in Appendix B, Unit V, page B-35). This situation is known as a false positive, or in statistical terms, a Type I error. It should be noted that both false positive and false negative errors (failure to detect an effect that exists) are inherent to any detection system, whether it is medical diagnostics, aircraft radar, or environmental monitoring.

A false positive might result from errors in a biological survey or in the analysis of data. The samples may have been collected improperly; therefore, the biotic community appears to be less abundant or species rich than it truly is. The individuals performing the identifications may have misidentified organisms. There may have been errors in data recording or analysis. Any of these errors may artificially obscure the responses. A quality assurance program can minimize, but not entirely eliminate these errors. If the causal analysis reveals weaknesses in the evidence for the occurrence of a real effect, a careful audit of the biological survey may be appropriate.

Other reasons for a false positive result include sampling error and the natural variability of the biological indicators. In any monitoring program, sampling is stratified among perceived natural classes and subdivisions of systems (e.g., habitat type, salinity, sediment, elevation, biogeographic region), and often by season (sampling index period in defined season). A sample may have been taken outside of an index period. A site may belong to a poorly characterized system type or may have been incorrectly classified (e.g., cold water system evaluated using warm water criteria). Any unrecognized misclassification can result in either a false positive or false negative. Intensive monitoring and characterization of natural systems, combined with quality assurance and peer review of results, can reduce both types of errors.

In other cases, the impairment may have been defined too broadly or investigators may have made wrong assumptions about mechanisms when developing their conceptual model. For example, the first investigations into bird population declines and DDT focused on mortality rather than egg-shell thinning, and failed to find a connection with DDT (see text box entitled "Revisiting the Impairment in the Case of DDT"). Careful reconsideration of the nature of the impairment can put the investigation back on the right track.

Finally, natural variability of the indicators, not due to any measurement or analytical errors, can result in both false positives and false negatives. Environmental criteria may

be defined by exceedence of a percentile or extreme value of some statistical distribution. This means that natural, or unimpaired conditions, may also exceed the criteria at some frequency. Ideally, acceptable error rates should be specified for decisions resulting from the biological assessment system. If confidence in a finding of biological impairment is low (that is, if the indicator just exceeds the threshold value), then increased sampling may reduce uncertainty and increase confidence (see next section).

5.2 Collect More Information on Previous and Additional Scenarios

If a causal scenario has not been established with sufficient confidence and the effect appears to be real, management should be consulted to discover if knowing the cause is still required for decision-making. If so, then more information must be collected (see worksheet in Appendix B, Unit VI, page B-36). Because the cost of field data collection and data analysis increases with each iteration, it is important to carefully plan what additional information is needed to determine the cause of impairment. This information may include previously considered scenarios for which information was inadequate, or candidate causal scenarios that were not previously considered.

Revisiting the Impairment in the Case of DDT[1]

The fact that DDT played a role in the decline of bald eagle and other bird-of-prey populations (e.g., osprey, brown pelicans) is now commonly appreciated among most biologists. However, the link between DDT and the eggshell thinning that caused reproductive failure in these birds was not initially recognized. Ultimately, the connection was made by re-examining the description of the impairment.

The first link between DDT and diminishing bald eagle and other bird-of-prey populations was the consistent observation of high body burdens of DDT metabolites. In other words, there was **co-occurrence** of the declining bird populations and the candidate cause, DDT. There was also evidence of **a complete exposure pathway** to birds based on body burden of DDT. However, extensive toxicity testing of DDT on adult bird mortality revealed no relationship. This suggested that the proposed mechanism, toxicity, was implausible. However, lethality was <u>not</u> the impairment; decline of birds-of prey was the impairment. A new **conceptual model** was required that considered other mechanisms that could result in declines in bird populations. In re-examination of the overall analysis, it became apparent that the species chosen for testing had been relatively tolerant of DDT exposure compared to those that were affected in the wild, and that the endpoint observed in these tests (lethality) would not reflect reproductive success or failure resulting from DDT exposure.

Field observations eventually revealed a potential **plausible mechanism** of reproductive failure due to eggshell thinning among bald eagles and other birds-of-prey. Laboratory **experiments** showed that DDE could cause eggshell thinning. Field studies showed that field exposures to DDE, a metabolite of DDT, were sufficient to cause effects in many species of birds based on the **stressor-response** relationship. Together these findings provided lines of evidence by which DDT might cause eggshell thinning and reduce reproductive success, a more specific impairment than declines in bird population.

Outcome
In 1972, DDT was banned from most uses in the United States. In the years following the ban, bald eagle and other bird-of-prey populations slowly recovered. The recovery of bird populations after banning the use of DDT, is an example of mitigation of the effect following manipulation of the cause, and is very strong evidence that the use of DDT was, in fact, the true cause of bald eagle and other bird-of-prey population declines.

References
Grier, J.W. 1982; Blus, L.J., and C.J. Henny, 1997

Even when the characterization of causes has not determined the cause with sufficient confidence, the set of candidate causes should have been reduced, and the critical evidence should be apparent. In particular, it should be possible to design experiments or observations that will potentially eliminate certain causes (Chapter 4.1.1). However, such experiments are not always feasible. Alternatively, one may identify critical pieces of positive evidence that would strongly support one scenario and none of the others. In most cases, it will be appropriate and prudent to plan a sampling and testing program that will generate a set of potentially decisive positive and negative evidence.

If all of the most common causes have been eliminated or have been determined to be unlikely, then additional causal scenarios need to be identified. The process is similar to that described in Chapter 2. New data may have come to light during the first iteration of the SI process. These data should be carefully reviewed to determine if there are any clues to suggest additional causal scenarios. Details of the available data should be considered, such as weather patterns, new construction, or land use information. If the descriptions of the effect or the scope were too broad, they may need to be refined or more clearly defined. Additional potential causal scenarios may include new stressors or combinations of stressors that occur simultaneously or in a specific sequence. After the additional candidate causal scenarios are developed, key evidence should be identified that is likely to allow identification of the cause.

The most important tools to bring to the SI process are experience in multiple disciplines (especially ecology), careful, deliberate critical thinking, and a strong desire to find the true cause of biological impairment.

Chapter 6

Presumpscot River, Maine

6.1   Executive Summary

The Presumpscot River is located in southern Maine and forms the outlet of one of Maine's largest lakes, Sebago Lake. From 1984 to 1996, biological monitoring downstream of a pulp and paper mill discharge consistently revealed non-attainment of Maine's Class C aquatic life standards. The river is impounded above and below the discharge. The discharge releases high concentrations of TSS and total phosphorus, and on occasion releases metals above the chronic criteria but below acute criteria. Upstream samples consistently indicated attainment of Class C or better standards.

Description of the Impairment

Biological impairment was characterized by a shift in the benthic macroinvertebrate community from 90% insects upstream of a pulp and paper mill discharge to about 50% insects downstream. This shift included a 15-35% loss of taxonomic richness, and 40-60% loss of Ephemeroptera-Plecoptera-Trichoptera (EPT) taxa. Moreover, many insect taxa found upstream of the discharge were pollution-sensitive, while those found downstream were primarily pollution-tolerant species, such as snails and worms.

List Candidate Causes

Eight candidate causes for non-attainment were considered in the Stressor Identification process:

1. Excess toxic chemicals from the discharge;

2. High TSS combined with floc causes high BOD and reduced DO;

3. High TSS combined with floc causes smothering;

4. Excess nutrients (from POTWs, nonpoint sources, and the mill) cause excess algal growth;

5. Impoundment increases sedimentation that smothers biota;

6. Impoundment decreases flow velocity and causes algal growth, leading to reduced DO;

7. Impoundment causes low DO; and

8. Impoundment causes loss of suitable habitat.

Characterizing Causes: Eliminate

Four of the eight candidate causes were logically eliminated from examination of the evidence. Reduced DO sufficient to cause the impairment was not observed in the Presumpscot River, and bottom-water DO concentrations were stable throughout the

river, above and below the discharge. Therefore, causal scenarios #2, #6 and #7 could be eliminated. Although elevated concentrations of total phosphorus (TP) were observed below the discharge, the increase in chlorophyll *a* concentration was negligible. Water column chlorophyll *a* is a surrogate measure for algal biomass. Because excess algal growth was necessary for causal pathway #4, and there was none, it was also eliminated from further consideration.

Characterizing Causes: Diagnose

No evidence strong enough to support diagnosis was available for any of the candidate causes.

Characterizing Causes: Strength of Evidence

A strength of evidence approach was then used to examine the remaining four candidate causes. The four remaining causes were toxic chemicals, flocculent TSS causing smothering, impoundment increasing sedimentation, and impoundment causing loss of suitable habitat. There was no strong evidence for or against the toxic chemical hypothesis (#1). Several lines of strong evidence favored the TSS hypothesis (#3):

- ▸ The exposure pathway from discharge to biological impairment was complete and plausible.

- ▸ Other rivers with similar elevated flocculent TSS also had impaired biological assemblage.

- ▸ Removal of flocculent TSS from a nearby river resulted in recovery of the biological assemblage.

Two lines of evidence disfavored the two impoundment hypotheses (#5 and #8):

- ▸ Other impoundments with similar potential sediment loadings (not from mill discharge) and similar habitat support diverse invertebrate assemblages that meet aquatic life use criteria; and

- ▸ a site upstream of the mill effluent, and within the same impoundment, met aquatic life use criteria.

Characterizing Causes: Identify Probable Cause

The evidence supporting scenario #3, that non-attainment was due to high loads of flocculent TSS from the discharge, was consistent throughout the lines of evidence. Strength of association, spatial co-occurrence, and experimental lines of evidence strongly supported this scenario. Evidence for the toxicity scenario (#1) was extremely weak. Evidence for the two impoundment scenarios (#5 and #8) was negative. The State of Maine concluded that high TSS was sufficient for causing the biological impairment. Quality of the data were adequate, and confidence in the conclusion was high. Subsequently, the State took management action to reduce loadings of TSS through a TMDL that was approved by EPA. This was the first time in New England that bioassessment findings had served as the quantitative response variable for development of a TMDL and resulting pollutant discharge limits, including the pulp and paper mill.

Moreover, it provided a means for Maine to control a pollutant (TSS) for which it had no specific criterion in its water quality standards.

6.2   Background

This case study is presented as an example of how the stressor identification (SI) process could have been used to determine the cause of non-attainment of aquatic life use in a small river in Maine. The case study begins with the presentation of background information on the regulations in the State of Maine and the geographical location of the case study. This is followed by a brief discussion of the evidence found at the site and in other situations. Several causal scenarios are then presented and analyzed separately to illustrate how the SI process could be used to eliminate four of the eight candidate causes. A strength of evidence analysis is then used to identify the most likely cause. The case study concludes with a brief discussion of the management actions taken to remedy the situation. One of the most significant results of this effort was that the State of Maine, Department of Environmental Protection, used bioassessment findings to control a stressor for which the State has no standards.

Impairment Trigger: Biological monitoring in the Presumpscot River in Westbrook, below a pulp and paper mill discharge, has consistently revealed non-attainment of Class C aquatic life standards (1984, 1994, 1995, 1996) using standard Maine Department of Environmental Protection methods (invertebrate) (Davies and Tsomides 1997).

Regulatory Authority

The Maine Department of Environmental Protection (MDEP) issues wastewater discharge licenses that set the allowable amounts of pollutants that industries may discharge to waters of the State. These limits are scientifically determined in order to preserve water quality sufficient to maintain all designated uses and criteria established, by law, for the river. In recent years USEPA has required that a Total Maximum Daily Load (TMDL) be established for impaired river systems, such as the Presumpscot, for which existing, required pollution controls are inadequate to attain applicable water quality standards.

The State of Maine established minimum standards for three water quality classifications, Class A, Class B, and Class C. These classes specify designated aquatic life uses from Class C, the minimum state standard, to the most protected waters with the Class A/AA designation. Class C requires that the structure and function of the biological community be maintained and provides for the support of all indigenous fish species.

Under this system, attainment of the aquatic life classification standards for a given water body is evaluated using numeric biological criteria. The MDEP numeric aquatic life criteria are based on statewide data collections over a 14-year period with analysis of over 400 sampling events. Artificial substrates (rock baskets) are incubated on the bottom at stream sites, retrieved, and benthic macroinvertebrates that have colonized the substrates are identified and enumerated (Davies and Tsomides 1997). Aquatic life classification standards for a given water body are evaluated using numeric biological criteria that were statistically derived from the statewide database. The criteria are in the form of a statistical model (linear discriminant model) which yields the probability that a test sample belongs to one of the 3 water quality classes, or non-attainment of the lowest

class (Davies et al. 1995). The model uses a set of metrics derived from the species composition and abundance enumerated from the substrates.

Geography

The Presumpscot River is the outlet of Sebago Lake. The river flows through the most densely-populated county in the State of Maine, crossing the towns of Gorham, Windham, Westbrook, Portland, and Falmouth. The Presumpscot then empties into Casco Bay at the Martins Point Bridge (Figure 6-1).
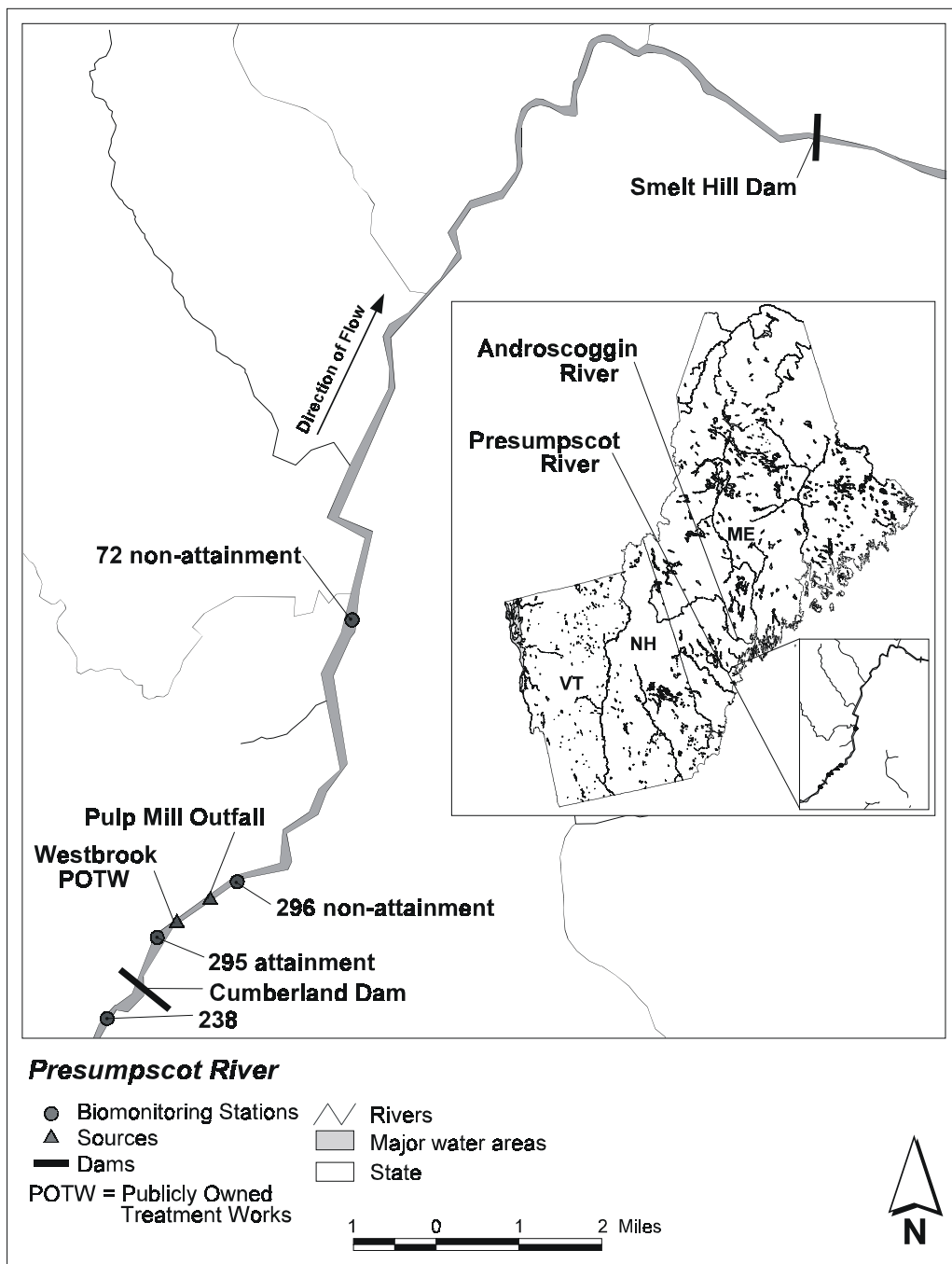

Figure 6-1. Map of the Presumpscot River showing biomonitoring stations, potential sources of impairment, and their location relative to the Androscoggin River (inset).

Compared to industrial receiving waters in the State of Maine, the Presumpscot is a relatively small river, having a drainage area of only 647 square miles. These circumstances contribute to a low dilution ratio in the lower Presumpscot River.

The river has six impoundments and four industrial and municipal waste discharges. This study comprises an area immediately downstream of a pulp and paper mill effluent discharge. Approximately 3.2 km, downstream of the discharge is an impoundment; upstream is a municipal discharge and (further upstream) two impoundments.

Evidence of Impairment

Biological Evidence: Biological monitoring in the Presumpscot River in Westbrook, below a pulp and paper mill discharge, consistently revealed non-attainment of Class C aquatic life standards (1984, 1994, 1995, 1996) using the standard Maine Department of Environmental Protection methods (Davies et al. 1995, Davies and Tsomides 1997).

Biological evidence indicating impairment on the lower Presumpscot River is summarized in Table 6-1 and Figure 6-2. Upstream samples consistently indicated attainment of Class C or better aquatic life standards (Davies et al. 1999). Three kilometers downstream the Presumpscot within the impounded area did not attain Class C aquatic life standards.

Table 6-1. Evidence of biological impairment in the Presumpscot River upstream and downstream of a pulp and paper mill effluent discharge.

Evidence	Upstream of Effluent	Downstream of Effluent
Aquatic Life Standard	Class C	Non-Attainment
Benthic Macroinvertebrate Community	90% insects	50% insects
Taxonomic Richness	--	15%-35% decrease relative to upstream
Sensitive Species (EPT)	--	46%-60% decrease relative to upstream
Snails and Worms	Low	High

The Presumpscot River biological monitoring samples reveal a shift in the benthic macroinvertebrate community from 90% insects above the mill to about 50% insects below the mill, with 15%-35% loss of taxonomic richness and 46%-60% loss of the sensitive Ephemeroptera-Plecoptera-Trichoptera (EPT) groups (Mitnik 1998). Pollution-sensitive insect taxa found in the upstream samples were replaced by a predominance of snails and worms, which are more tolerant of pollution, in the downstream samples.

6.3   List Candidate Causes

Eight candidate causes for the non-attainment of biological standards were considered. The candidate causes for the biological impairment of the Presumpscot River are shown in terms of a conceptual model (Figure 6-3), wherein the candidate causes are ordered from left to right. Each scenario is explained below:

1. Excess Toxic Chemicals - Potentially toxic compounds may be discharged from the paper mill and these compounds adversely affect aquatic life.

Biological Indicators of Non-Attainment
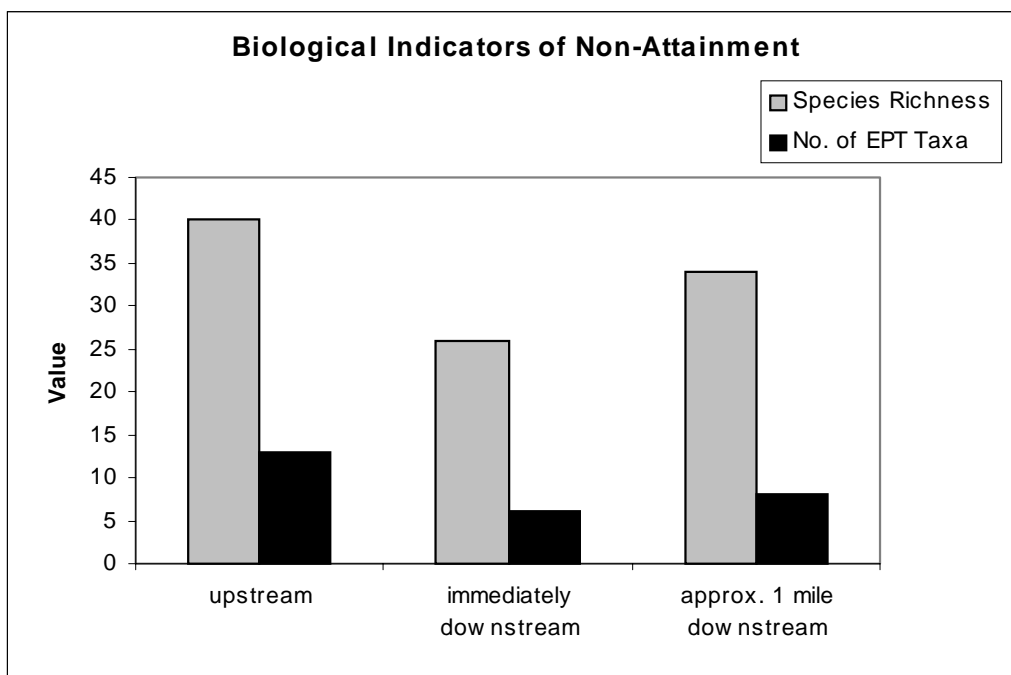
Figure 6-2. Species richness and number of EPT taxa in the Presumpscot River upstream and downstream of a pulp and paper mill effluent discharge.

2. BOD (produced by high TSS with floc) reduces DO - Excess total suspended solids (TSS with floc) may be released by the paper mill effluent, and these solids create biological oxygen demand (BOD), reducing dissolved oxygen (DO) levels in the river. Consequently, the river has insufficient oxygen to support sensitive species of benthic invertebrates.

3. TSS with floc - The increased levels of TSS discharged to the river could impact the benthic communities by accumulating as (non-biodegradable) sediment, resulting in fewer interstitial spaces in which animals can live, and possibly smothering benthic biota.

4. Excess Nutrients - Excess nutrients, deriving from either upstream, non-point sources or from the paper mill effluent, may affect water quality by promoting algal blooms. In this scenario, an overabundance of plant nutrients such as phosphorus is delivered to the stream, and over-stimulates algal growth (a process known as *eutrophication).* An increase of algae in the river may affect benthic macroinvertebrates in two ways. If the algal growth is severe, the resulting detritus becomes a source of BOD, reducing dissolved oxygen levels in the river. If the growth is modest, the algae may still affect the benthic macroinvertebrate community by providing an increased food supply for opportunistic invertebrates that use algae as a food source. Consequently, the community would shift in such a way that the opportunistic species would thrive and outcompete other, less opportunistic species.
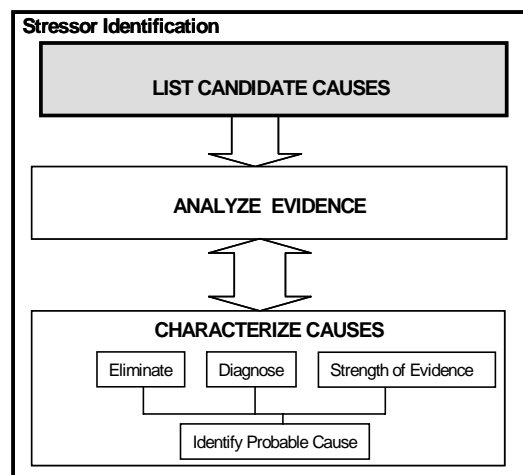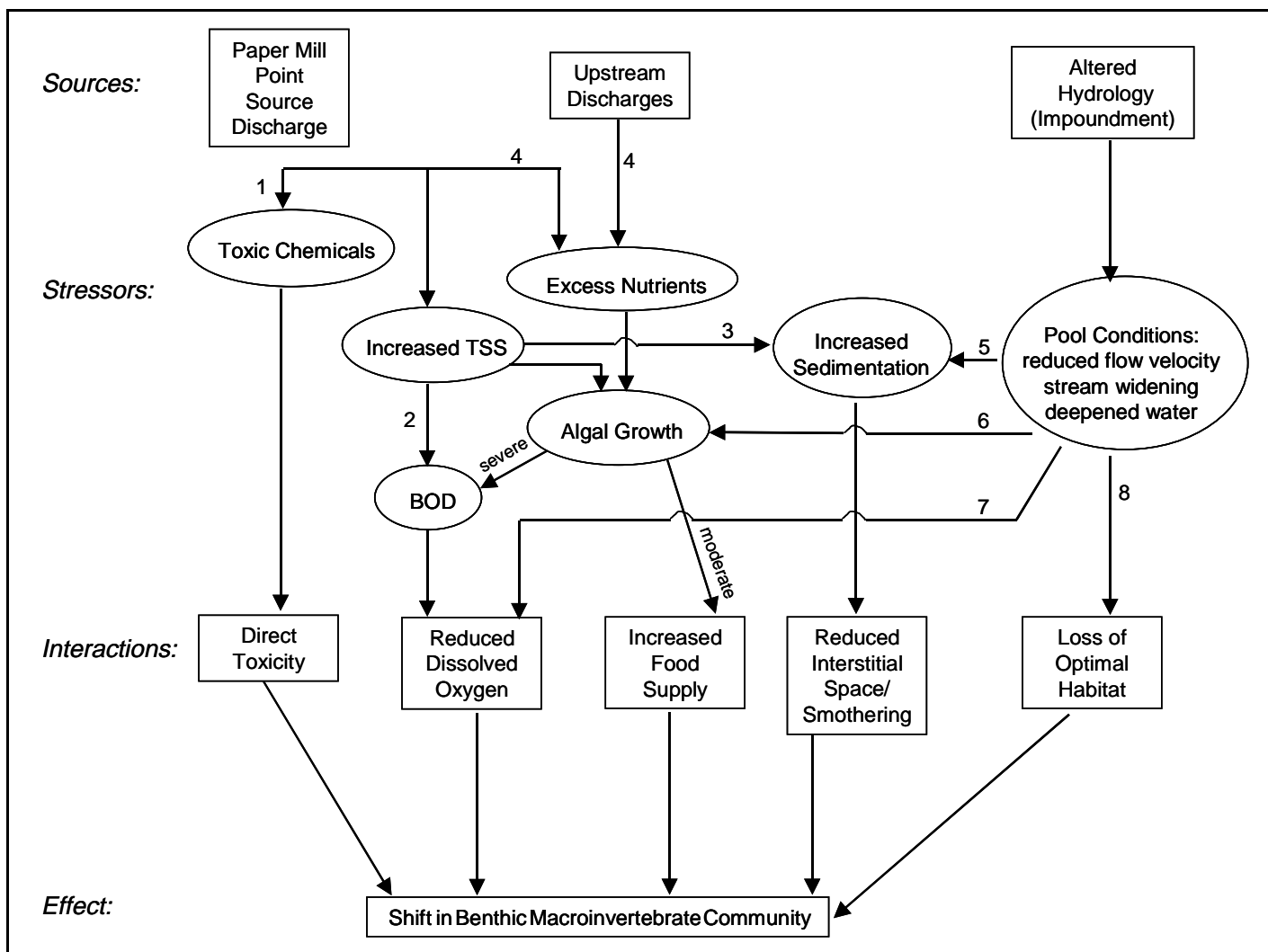
Figure 6-3. Conceptual model showing the potential impact of stressors on the benthic community of the Presumpscot River. (Arrow with minus sign (-) indicates inhibition.)

The fifth through eighth candidate causes are based on impoundment of the river just downstream of the paper mill effluent. Each cause begins with the idea that the impoundment is causing adverse changes in the physical nature of the Presumpscot. Impoundments generally widen and deepen a stream corridor, reducing flow velocity and creating pool-like conditions. Such alterations can have several effects:

5. Impoundment Increases Sedimentation - One effect of impoundment is increased sedimentation due to reduced flow velocity, which leads to fewer interstitial spaces in which animals can live, and potentially smothers benthic ones.

6. Impoundment Promotes Algal Growth - The pool-like conditions created by the impoundment become a better habitat for algal growth, and algal blooms occur. Subsequently, benthic communities shift as a result of oxygen depletion or the dominance of algae-consuming invertebrates, as described previously.

7. DO Reduction in Impoundment - An impounded river is deeper and slower, which results in less potential for mixing and more potential for stratification, particularly in warmer months. As a result, underlying water may not be sufficiently aerated, and benthic diversity decreases in response to low dissolved oxygen levels.

8. Habitat Degradation caused by Impoundment - Changes in physical conditions of the river caused by impoundment reduce optimal habitat for benthic organisms. The effect is a direct one: native benthic macroinvertebrates are unable to thrive under the altered conditions. Dissolved oxygen levels and other water quality parameters are not a factor.

6.4   Analyze Evidence and Characterize Causes: Eliminate

<u>Physical and Chemical Evidence</u>: Physical and chemical evidence indicating impairment on the lower Presumpscot River is summarized in Table 6-2. Upstream of the pulp and paper mill outfall, it was possible to see samplers on the river bottom at 2.5 meters of depth, whereas in the effluent plume, just 600 m downstream, visibility was less than 0.5 meter. Visibility at a sampling station 3.2 km downstream of the outfall remained significantly impaired. This evidence was used to eliminate candidate causes.

1. Toxic Chemicals - No in-stream or sediment chemistry data were available. Therefore, toxic chemicals cannot be eliminated as a candidate cause.
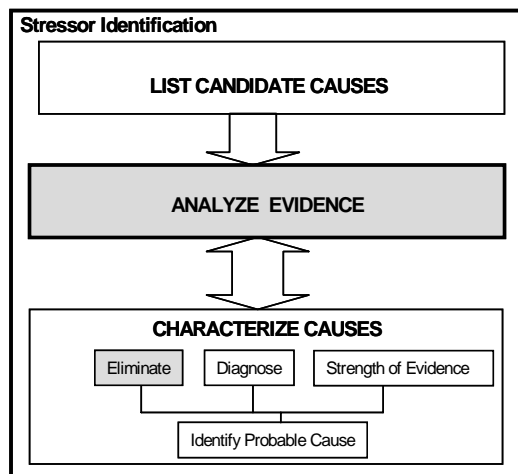
Table 6-2. Physical and chemical parameters measured in the Presumpscot River upstream and downstream of a pulp and paper mill effluent discharge.

Observation	Source	Upstream of Mill	Downstream of Mill
Visibility	Mitnik 1998	2.5 m	<0.5 m (600 m below outfall) and visibility remained "significantly impaired" 3.2 km downstream
Observations on Sampling Equipment (e.g., ropes, nets)	Mitnik 1998	Free of brown floc	Coated with brown floc
Mean TSS (ppm)[1]	Courtemanch et al. 1997	3 ppm	5.9 ppm
Mean BOD (ppm)[2]	Mitnik 1994	3.96	6.19
DO range (ppm) [3]	Mitnik 1994	5.9 - 8.4	5.8 - 8.0
Mean nitrate - nitrite (ppm)[2]	Mitnik 1994	0.03	0.05
Mean ammonia (ppm)[2]	Mitnik 1994	0.03	0.12
Mean Total phosphorus (ppb)[2]	Mitnik 1994	12.8	61.2
Mean Orthophosphate (ppb)[2]	Mitnik 1994	3.5	44.3
Mean Chlorophyll *a* (ppb)[2]	Mitnik 1994	2.1	2.3

Notes
1 Observations from 1995-96; number unknown
2 4 sites above mill and 5 sites below on 3 consecutive days
3 Bottom water; 9 sites above mill and 8 sites below on 6 non-consecutive days

2. BOD (produced by high TSS with floc) reduces DO - Elevated BOD was associated with the biological impairment in the Presumpscot River. In this candidate scenario, reduced DO is the actual stressor that acts on the organisms to cause impairment. Monitoring in the Presumpscot River above and below the mill discharge indicated that DO concentrations did not decrease upstream to downstream (Table 6-2 and Figure 6-4). The reported DO measurements were taken at stations indicated on the map (Figure 1). Most of the sites shown in Figure 1 were impounded water; only 7.7, and 6.3 were free-flowing. The results reported in Tables 6-2 and 6-3 were all sampled between 0640 and 0850 hours, within 1m of the bottom, in July and August, 1993 (Mitnik 1994). This is the time, depth, and season at which minimum DO is found in lakes and impoundments, because of the diurnal cycle of photosynthesis and respiration, and because photosynthesis (but not respiration) is inhibited in deeper and darker waters. This analysis demonstrated that low DO does not occur in the Presumpscot River under any of the candidate causes involving reduced dissolved oxygen. **Therefore, candidate causal scenarios # 2** (High TSS with floc causes high BOD and reduced DO) **# 6** (Impoundment promotes algal growth that in turn reduces dissolved oxygen), **and # 7** (Impoundment causes low DO through decreased water flow rate) **could be eliminated without further analysis.** The elimination of scenario #6 is reinforced by the evidence described in scenario #4, below.
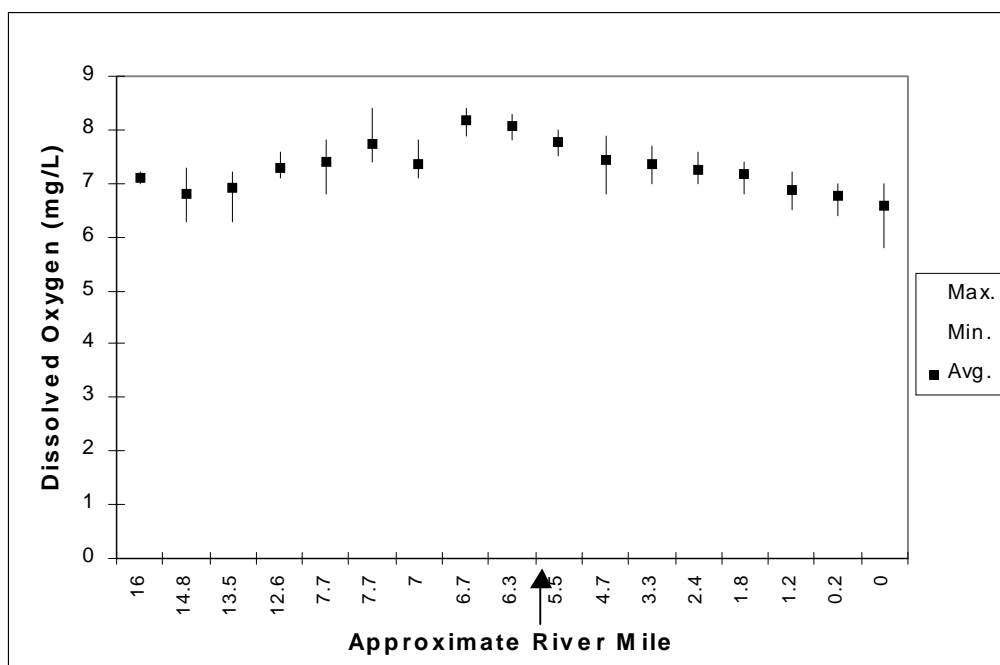
Figure 6-4. Bottom dissolved oxygen concentration in the Presumpscot River. (Means of 6 observations on 6 days in July and August, 1993, for each site. All observations within 1m of bottom. Whiskers extend to minimum and maximum measurements. All measurements were taken between 06:40 and 08:50 am. Arrow indicates location of the pulp and paper mill discharge: sites to the left of arrow are incrementally upstream of the discharge, and all sites to the right are incrementally downstream. The darkened square represents the average DO measurement. The lines above and below the square represent the maximum and minimum measurements, respectively.)

3. TSS with floc - In the Presumpscot River, TSS and floc are elevated at the impaired site (Tables 6-2 and 6-3). TSS with floc cannot be eliminated as a candidate cause.

4. Nutrients and Algal Growth - The nitrogen to phosphorus ratio ((TKN + NO_3 + NO_2)/TP) upstream of the paper mill discharge was approximately 25, indicating phosphorus limitation, as is typical of New England fresh waters. Below the paper mill discharge, elevated phosphorus concentrations were associated with biological impairment (Table 6-2). Specifically, total phosphorus (TP) and ortho-phosphate (PO_4) increase 5- to 10-fold downstream of the discharge (Table 6-2). Moreover, the discharge alone contained an average of 723 mg/L TP. A five-fold increase in TP (ten-fold in PO_4) would normally result in increased algal growth (measured as chlorophyll *a* concentration). However, the observed increase in chlorophyll *a* with the phosphorus enrichment below the discharge was negligible, increasing by just 0.2 ppb. Because excess algal growth is necessary for the causal pathway to be complete, **causal scenario #4 was eliminated from further consideration.**

5. Impoundment Increases Sedimentation - Biological impairment downstream of the paper mill discharge coincided with the presence of an impoundment (Table 6-3). However, no measurements of sediment loadings were available to determine if the biological impairment was the result of increased sedimentation caused by the impoundment. Therefore, scenario # 5 could not be eliminated.

8. Habitat Degradation caused by Impoundment - Again, the biological impairment found downstream of the paper mill discharge coincided with the presence of an impoundment (Table 6-3). However, no measurements of habitat quality were available to determine if the biological impairment was the result of habitat loss caused by the impoundment. Therefore, scenario #8 could not be eliminated.

Following the process of elimination, 4 candidate causes remained:

1. Excess toxic chemicals.

3. High TSS with floc causes smothering.

5. Impoundment increases sedimentation that smothers biota (with or without discharge of TSS and floc).

8. Impoundment causes loss of suitable habitat.

6.5   Analyze Evidence and Characterize Causes: Strength of Evidence

Direct observations in the Presumpscot River during macroinvertebrate and fish tissue sampling revealed a heavy suspended and settled solids load. Samplers and gill nets were coated with flocculent fibers and water clarity was dramatically reduced. In comparison to other paper mills in the State, the pulp and paper mill effluent released to the Presumpscot was considered high strength for solids. However, the conditions faced on the Presumpscot were similar to those found below the discharge from another paper mill on the Androscoggin River in Jay, Maine. Because of this, observations in the vicinity of the paper mill on the Androscoggin River were used to support the evidence found for this case study.

Stressor Identification

LIST CANDIDATE CAUSES

ANALYZE EVIDENCE

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause
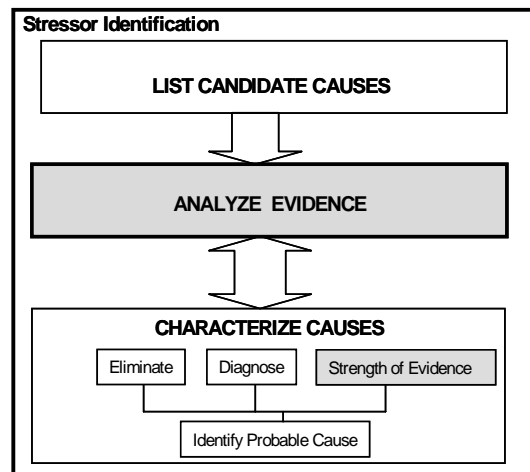
A comparison of the two rivers and discharge loadings to each is given in Table 6-4. Paper mill discharges on both rivers were subject to impoundments with similar hydraulic properties (e.g., velocity and depth) and background TSS concentrations (about 3 ppm). Two or more dams impounded both rivers upstream of the discharges.

Table 6-3. Considerations for eliminating candidate causes.

Candidate Cause	Impairments occur same place as exposure?	Exposure increased over closest upstream location?	Gradient of recovery at reduced exposure?	Exposure pathway complete?	Candidate Causes Remaining
Toxic Chemicals	NE	NE	NE	NE	X
BOD (produced by TSS) reduces DO	BOD Yes; TSS Yes; DO No	BOD Yes; TSS Yes; DO No	NE	No	
TSS with floc	Yes	Yes	NE	Yes	X
Nutrients and algal growth	Nutrients Yes; Algal Yes	Nutrients Yes; Algal No	NE	No	
Impoundment increases sediment	Yes	NE	NA	NE	X
Impoundment promotes algal growth	Algal Yes; DO No	Algal No; DO No	NA	No	
DO reduction in Impoundment	Imp. Yes; DO No	Imp. Yes; DO No	NA	No	
Habitat degradation caused by impoundment	Yes	NE	NA	NE	X

Table 6-4. Comparison of TSS loadings in the Presumpscot and Androscoggin Rivers. (Sample points were located below a pulp and paper mill effluent discharge.)

	Presumpscot		Androscoggin		
Mill & Year Sampled	1995	1996	1995	1996	1997
Aquatic Life Status	Non-Attainment	Non-Attainment	Non-Attainment	Attainment	Attainment
TSS treatment	none	none	none	TSS removal	TSS removal
Sampling Months	June-Aug	Aug-Sept	June-Aug	Aug-Sept	June-Aug
Flow, cubic feet/second (cfs)	418	463	2114	2982	4116
TSS Discharged, pounds/day	7454	8795	19804	5750	13495
TSS discharged/flow	3.31	3.52	1.74	0.36	0.61

Moreover, the upstream impoundments on both rivers attained at least Class C aquatic life standards. However, both rivers were found to be in non-attainment of aquatic life standards downstream of the paper mill discharges in 1995. Calculated mean ambient concentrations of TSS in the Presumpscot downstream of the mill were 32% to 39% greater than ambient levels downstream of the mill on the Androscoggin River. For the most part, the incremental TSS increase on the Androscoggin River, due to paper mill discharges, was within 1 ppm of background, while on the Presumpscot, the mill discharge was about 3 ppm greater than background.

In 1996, efforts were made by another paper mill on the Androscoggin River to reduce TSS discharge into the Androscoggin River. Following these efforts, the site's biological score improved and the river met Class C aquatic life standards. This recovery of biological conditions following TSS reduction provided experimental evidence that TSS could also be the cause of ecological stress in the Presumpscot River. Table 6-6 summarizes the types of evidence weighed in the analysis of potential stressors in the Presumpscot River.

Other evidence used in the strength of evidence comparison is shown in Table 6-5. Some metals exceeded chronic criteria when the maximum concentration in the effluent was evaluated with a low flow scenario (Table 6-5). Although low DO was eliminated in the previous step of this case study. Maine DEP performed an extensive modeling effort to investigate the potential for low DO below the mill outfall. The modeling results supported the conclusion that the DO concentrations did not fall below minimum levels for Class C aquatic life uses (Mitnik 1998). Furthermore, during the same time period as the biological monitoring, there were not violations of criteria for DO.

Table 6-5. 1996 - 1999 metal concentrations in the pulp and paper mill effluent.

Metals	Range µg/L in Effluent Grab Samples 1996-1999	Maximum Receiving Water Concentration (µg/L) at Low Flow[1] (7Q10[2])	Chronic Criteria (µg/L)	Acute Criteria (µg/L)
Aluminum	108 - 1920	207.9	87	750
Lead	3 - 14	1.52	0.41	10.52
Mercury	0.0001 - 0.9	0.097	0.012	2.4
Silver	10	1.083	0.12	0.92

Notes
1 The receiving water concentration is calculated from the maximum effluent concentration divided by a dilution factor of 9.
2 7Q10 + 7-day low flow over a ten year period.

Table 6-6: Strength of evidence of non-attainment in the Presumpscot River.

	Consideration	TSS with Floc		Toxic Compound		Impoundment increases Sedimentation		Impoundment causes Loss of Habitat	
		Results	Score	Results	Score		Score		Score
Case-Specific Evidence:	**Spatial Co-occurrence**	Compatible: Non-attainment observed in area of high TSS and floc loading. Attainment observed in upstream areas without TSS loading.	+	Evidence unavailable.	NE	Uncertain: Non-attainment observed in area of impoundment, but no measurements of sedimentation were available.	0	Uncertain: Non-attainment observed in area of impoundment, but no observations of habitat quality were available.	0
	Temporality	No observations prior to paper mill discharge.	NE	No observations prior to paper mill discharge.	NE	No observations prior to impounding	NE	No observations prior to impounding	NE
	Consistency of Association	No evidence	NE	No evidence	NE	A site within the same impoundment, upstream of the mill met aquatic life uses.	_	A site within the same impoundment, upstream of the mill met aquatic life uses.	_
	Biological Gradient	No evidence	NE	No evidence	NE	Not Applicable	NA	Not Applicable	NA
	Complete Exposure Pathway	Evidence for all steps: High TSS and floc discharge into river well-documented.	++	No evidence	NE	No evidence	NE	No evidence	NE
	Experiment	No evidence	NE	No evidence	NE	No evidence	NE	No evidence	NE

Table 6-6 (continued): Strength of evidence for causes of non-attainment in the Presumpscot River.

	Consideration	TSS with Floc		Toxic Compound		Impoundment increases Sedimentation		Impoundment causes Loss of Habitat	
		Results	Score	Results	Score		Score		Score
Information from Other Situations or Biological Knowledge:	**Plausibility - Mechanism**	Plausible: Snails and worms are adapted to utilization of settled solids.	+	Plausible: Toxic compounds could alter community composition.	+	Plausible: Sediment could alter habitat and community composition.	+	Plausible: Altered habitat could change community composition.	+
	Plausibility - Stressor-Response	TSS response from Androscoggin study and modeling sufficient to cause impairment.	++	Ambiguous: Assuming low flow conditions and at the highest concentrations reported for effluent from the mill, chronic aquatic life criteria might be exceeded for aluminum, lead, mercury and silver. However, if we assume high flows at the time of sampling then neither acute nor chronic aquatic life criteria are likely to be exceeded.	0	Other impoundments with similar potential sediment loadings support diverse invertebrate communities.	_	Other impoundments with similar habitat support diverse invertebrate communities.	_
	Consistency of Association	Invariant: Other sites on other rivers with TSS have impaired biological communities.	+++	In some places: Possibly could cause effects if at maximum values most of the time, but unlikely	0	Other impoundments on other rivers are not impaired.	_	Other impoundments on other rivers are not impaired.	_
	Specificity of Cause	Low: Other causes elicit similar responses.	0	Low: Other causes elicit similar responses.	0	Low: Other causes elicit similar responses.	0	Low: Other causes elicit similar responses.	0
	Analogy	No evidence	NE	No evidence	NE	No evidence	NE	No evidence	NE
	Experiment	Concordant: Removal of TSS in the Androscoggin river improved invertebrate assembleges.	+++	No evidence	NE	No evidence	NE	No evidence	NE

**Table 6-6 (continued):** Strength of evidence for causes of non-attainment in the Presumpscot River.

	Consideration	TSS		Toxic Compound		Impoundment increases Sedimentation		Impoundment causes Loss of Habitat	
		Results	Score	Results	Score		Score		Score
	Predictive Performance	No evidence	NE	No evidence	NE	No evidence	NE	No evidence	NE
Considerations Based on Multiple Lines of Evidence:	**Consistency of Evidence**	All Consistent.	**+++**	Not consistent: data collected during the same time period as the biological monitoring indicated that there were no violations of criteria for toxic materials (Mitnik 1998).	0	Not consistent: Other sites with impoundments maintained diverse communities.	0	Not consistent: Other sites with impoundments maintained diverse communities.	0
	Coherence of Evidence			Could be due to unmeasured chemical or episodic exposure.	0	No known explanation.	0	No known explanation.	0

6.6   Characterize Causes: Identify Probable Cause

Following the process of elimination, four causal scenarios remained to compare for strength of analysis (Table 6-6). These scenarios were: #1 (excess toxic chemicals), #3 (high TSS with floc causing smothering), #5 (impoundment increasing sedimentation that smothers biota, with or without discharge of TSS and floc), and #8 (impoundment causing loss of suitable habitat).

CHARACTERIZE CAUSES

| Eliminate | Diagnose | Strength of Evidence |

Identify Probable Cause
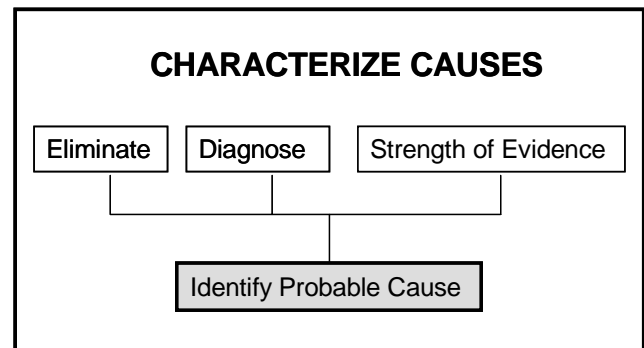
The evidence supporting scenario #3, that non-attainment was due to high TSS loads combined with floc, was consistent throughout the lines of evidence. Moreover, the strength of association, spatial co-occurrence, plausible stressor-response and experiment lines of evidence strongly supported this scenario. Therefore, high TSS with floc was sufficient for causing the biological impairment. The quality of the data are adequate for this conclusion, and our confidence is high.

In contrast, evidence for the toxicity scenario was weak, because the stressor-response association was unlikely based on levels of chemicals in the effluent and the likely dilution provided by the river at the time of discharge. If greater certainty was required, ambient receiving water toxicity tests could be used.

Likewise, evidence for the candidate causes involving impoundments lacked field measurements of sedimentation and habitat quality. However, our confidence in rejecting these scenarios as the primary cause of impairment is strengthened by the fact that several upstream sites along the Presumpscot River were impounded with no associated biological impairment (Mitnik 1998, Davies et al. 1999), and within the same impoundment upstream from the mill, the Presumpscot met aquatic life uses. Furthermore, several other impounded rivers of the state are able to meet Class B and C biological criteria (Davies et al. 1999).

Nutrient levels were elevated; however, the algal concentration was not different from the nearest upstream sampling location. As a result, candidate cause # 4, excess nutrients, was eliminated; however, it is possible that the growth of algae was inhibited by other factors, such as shading from floc. If floc were removed, then effects due to eutrophication might become evident.

Low dissolved oxygen was also eliminated based on spatial patterns of DO along the river. Other data is also available that increases the confidence that could have been presented in a strength of evidence analysis. At the site, DO was not below 6 ppm. The minimum DO level for Class C waters is 5 ppm. Maine DEP also performed an extensive modeling effort to investigate the potential for low DO below the mill outfall. The modeling results supported the conclusion that the DO concentrations did not fall below minimum levels for Class C aquatic life uses (Mitnik 1998).

6.7   Significance and Use of Results

In December 1998, the U.S. Environmental Protection Agency approved a Total Maximum Daily Load (TMDL) finding, prepared by Maine Department of Environmental Protection, for the Presumpscot River. This approval was significant for several reasons:

1. It was the first TMDL that addressed a listed 303(d) water to be approved in Region 1 USEPA (the New England States);

2. It was the first time in New England that bioassessment findings had served as the quantitative response variable from which a pollutant discharge limit was developed.

The wastewater discharge license that has resulted from this effort requires an initial 30% reduction in the TSS discharge from a pulp and paper mill in Westbrook. Provisions are included in the license for further reductions (up to 61%) if the initial levels still fail to provide for attainment of aquatic life standards.

Main Factors Influencing Success

The Department was able to apply this innovative approach to improving water quality and aquatic life conditions in the Presumpscot River because of the convergence of several factors:

▸ The State has a sound legal basis for use of biological monitoring findings to force action. Clearly defined aquatic life standards exist in the Water Quality Classification law and technically-defensible numeric criteria have been established by the Department;

▸ Data essential to the modeling of the recommended total suspended solids load reductions on the Presumpscot River had been collected to assess aquatic life issues on the Androscoggin River (under State requirements for a 401 Water Quality certification for a hydropower license renewal);

▸ Teamwork and collaboration between DEP, water quality modelers, and aquatic biologists resulted in an approach that integrated technical information and expertise from both disciplines. It also provided a means for the Department to control a stressor (TSS) for which the State has no standards.

6.8   References

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Chapter 7

Little Scioto River, Ohio

7.1   Executive Summary

This case study of the Little Scioto River represents an application of the SI process to a complicated system. Impairment of the Little Scioto River reflected several impacts caused by different stressors. Originally, the data on the Little Scioto were collected and analyzed as part of the Ohio Environmental Protection Agency (OEPA) state monitoring program during 1987, 1991, 1992 and 1998 (OEPA 1988b, 1992, 1994, unpublished data from 1998) and as research for a USEPA methods development program. The monitoring data were subsequently analyzed for this SI case study to demonstrate how data collected from monitoring programs could be used to identify probable causes of biological impairment.

The SI investigation was initiated because criteria in the state of Ohio's water quality standards were violated in parts of the Little Scioto, a small river in north-central Ohio (Yoder and Rankin 1995b). The SI investigation involved a 9-mile stretch of the Little Scioto River near Marion, Ohio, where there was evidence of biological impairment.

The State of Ohio has a "tiered" set of aquatic life use designations based on narrative definitions of specific aquatic uses that are protected by a set of numeric biocriteria, chemical criteria, and habitat criteria. Ohio EPA determines biological impairment of stream segments by comparing study sites to the numeric biocriteria in their water quality standards. OEPA uses standard multimetric indices, including the Index of Biotic Integrity (IBI), the Invertebrate Community Index (ICI) (OEPA 1989a), and the Qualitative Habitat Evaluation Index (QHEI) (OEPA 1989c). Little Scioto River data collected in 1987 and 1992 showed a condition of "fair" to "severe impairment" in the stretch from river mile (RM) 9.2 to where the Little Scioto joins the Scioto River, just downstream of RM 0.4.

Describe the Impairment

Three distinctive impairments (A, B, and C) were identified for the causal evaluation (at RM 7.9, 6.5, and 5.7, respectively). Impairment A was characterized by a loss of fish and benthic invertebrate species, a decrease in the number of individual fish, and an increase in the relative weight of fish. Impairment B was characterized by a decrease in the relative weight of fish and a large increase in deformities, fin erosion, lesions, tumors and anomalies (DELTA). Impairment C was characterized as having a further increase in DELTA and extirpation of a Tribe of midges, the Tanytarsini.

List Candidate Causes

Stressors impacting the upper portion of the river were identified as mostly non-point nutrient and sediment loadings associated with agriculture. Beginning at river mile 9.0 and continuing to the mouth, the river is channelized. The Little Scioto River at and below Marion, Ohio, however, has been notably contaminated with elevated levels of polycyclic aromatic hydrocarbons (PAH). Creosote and metals in sediment samples and

ammonia, phosphorous (P), total nitrogen (N) were detected in water samples (OEPA 1994).

Based on the knowledge about the site and effects, six candidate causes were hypothesized to account for the three major biological impairments observed in the Little Scioto study area:

1. Habitat alteration: embedded stream and deepened channel

2. Exposure to PAHs

3. Metal contamination

4. Ammonia Toxicity

5. Low Dissolved Oxygen/High Biological Oxygen Demand

6. Nutrient Enrichment

Characterize Causes: Eliminate

Candidate causes were eliminated because the level of exposure to the candidate cause did not increase compared to the nearest upstream location. Candidate causes that remained after the elimination step are listed below:

▸ Impairment A (RM 7.9) — habitat alteration, metal contamination, and nutrient enrichment remained as probable causes.

▸ Impairment B (RM 6.5) — PAH contamination, metal contamination, ammonia toxicity, low dissolved oxygen/high biological oxygen demand, and nutrient enrichment remained as probable causes.

▸ Impairment C (RM 5.7) — metal contamination, ammonia, and nutrient enrichment remained as probable causes.

Characterize Causes: Diagnose

No evidence strong enough to support diagnosis was available for any of the candidate stressors.

Characterize Causes: Strength of Evidence

A strength of evidence approach was used to examine the remaining causes with regard to each impairment. Evidence based on other situations and biological knowledge were especially important including consistency of association and plausibility of mechanism and stressor-response.

Characterize Causes: Identify Probable Causes

Impairment A
At Impairment A, the increased relative weight is probably caused by the artificial deepening of the channel that allows larger fish to live there. The mechanisms were

probable, and consistency of association and experiments from other sites in Ohio and elsewhere supported this finding for the specific impairments. The extirpation of fish and benthic invertebrates seems to be most likely due to embedded substrates. Although low DO could also be a cause, upstream locations had even lower DO levels and yet had a greater variety of fish and invertebrate species.

Although metals were present, the likelihood of response at these concentrations is low. Furthermore, the types of changes in the community, especially an increase in the relative weight of fish, is very unlikely with the candidate cause of metals. Although P levels are slightly higher, effects are not associated with these phosphorous concentrations elsewhere, and they do not exceed Ohio proposed criteria values for effects. PAH and ammonia had already been eliminated because levels were the same or lower than upstream. Low DO /BOD was also eliminated as an overall pathway; however, low DO associated with channelization may still play a role, especially with respect to the slight increase in the percentage of DELTA.

Impairment B
A single probable cause, toxic levels of PAH-contaminated sediments, is likely for the three manifestations of Impairment B: decreased relative weight, increased DELTA, and decreased species. All of the evidence support PAH contamination as the cause. There is a complete exposure pathway at the location, and a clear mechanism of action for each of the effects. The single most convincing piece of evidence is that the cumulative toxic units of PAH were more than 300 times the probable effects level.

Metals are at sufficient concentrations to cause effects; however, they are at levels close to upstream concentrations, and are less than 2% as toxic as the lowest cumulative toxic units of PAH. Metal concentrations are high enough that they should be considered a potentially masked cause. Reduced DO resulting from increased BOD is unlikely because, downstream, even greater levels of BOD did not cause reduction of dissolved oxygen. Ammonia and nutrient enrichment are unlikely given that state criteria levels were met and given the much stronger evidence for PAH. Habitat alteration continues to impair the site, but it is not the cause of the increased DELTA, decreased relative weight, or the additional decline in the number of species, because the level of embeddedness was similar to upstream.

Impairment C
At Impairment C, increased % DELTA and % Tanytarsini may have different causes. Increased DELTA in fish is probably caused by increased P and N. Nutrients, especially P, have been associated with increased fin erosion and lesions, but some uncertainty exists since P acts indirectly. Another candidate cause is also probable, namely, ammonia. Ammonia is slightly higher at Impairment C than at Impairment B, and exceeded ammonia criterion values. Biological gradients were absent for ammonia; however, this may have been a statistical artifact given the number of sites available to perform the analysis, and the potential interference from other stressors downstream.

Metals are considered unlikely, because very specific surface lesions are only occasionally noted as effects from long-term exposure, and only some metal concentrations were slightly greater than at Impairment B. Metal concentrations are high enough that they should be considered a potentially masked cause.

The probable cause of extirpation of Tanytarsini at Impairment C is more uncertain because less is known about the natural history and stressor-response relationships of

these benthic invertebrates. Nutrient enrichment still seems to be the most likely cause since all of the strength of evidence considerations were consistent.

PAH contamination and habitat alteration continue to impair the site, but they are not the cause of the increased percent DELTA or extirpation of Tanytarsini.

Identify Probable Cause

The most probable causes were:

- ▸ Impairment A (RM 7.9) — Siltation and deepened channel are consistent with impairment A. The magnitude of the alteration and clear difference from upstream locations strongly support this cause.

- ▸ Impairment B (RM 6.5) — PAH-contaminated sediments are likely causes for the three manifestations of Impairment B.

- ▸ Impairment C (RM 5.7) — The causal characterization at Impairment C is less certain, but the strength of evidence favors increased nutrient enrichment as the cause.

The Little Scioto case study is a good example of a complex system requiring a detailed analysis. Although it was possible to identify the dominant causes of specific impairments, other causes were present that had the potential to cause impairments if the dominant cause was removed. For instance, habitat alteration associated with channelization would still impair the entire river below RM 9.0.

7.2 Introduction

The Little Scioto case study involves a nine-mile stretch of a river suffering from several impairments with different causes. Typical of similar stressor investigations, the data examined for this case study were not collected or originally analyzed specifically for the Stressor Identification Technical Guidance Document. Rather, they were collected as a part of the Ohio EPA state monitoring program during 1987, 1991, 1992 and 1998 (OEPA 1988b, 1992, 1994, unpublished data from 1998), and as research for a USEPA methods development program. These monitoring data were subsequently analyzed in this study to demonstrate how data collected from existing monitoring programs could be used to identify probable causes of biological impairment.

Various types of data were used in this case study, including chemical analyses (sediment, water, and fish tissue) and biological assessment (biological community and physical habitat). Methods for the collection and analysis of chemical data are described in Ohio EPA (1989c). In 1992, one grab sample was taken, whereas in 1987, multiple grab samples were taken. Other Ohio EPA data sets included biological assessment data on fish and invertebrate assemblages and physical habitat measurements. In Ohio, impairment of stream aquatic life uses are defined by standard multimetric indices including the Index of Biotic Integrity (IBI) and the Invertebrate Community Index (ICI) (OEPA 1989a). These indices have been promulgated as numeric biocriteria in the State's water quality standards. The quality of the habitat is characterized using the Qualitative Habitat Evaluation Index (QHEI) (OEPA 1989c). These methods are described in detail by Ohio EPA (1989c). Biochemical measurements of impairment included bile metabolites measured according to Lin et al. (1996) and ethoxy

resorufin[O]deethylase (EROD) activity measured according to Cormier et al. (2000b). Although the attempt was made to use biological and chemical data from the same locations, in some cases, chemical measurements were recorded at a location that did not exactly coincide with the location of biological assessment (e.g., RM 5.8 and RM 5.7, respectively). However, the distance between the chemical and biological sample sites was negligible or overlapped, and the data were able to be used to analyze associations between candidate causes and the biological impairment.

The Little Scioto River is a small river in north-central Ohio that empties into the Scioto River (Figure 7-1). It drains primarily farmland in the northeastern quadrant of the Eastern Corn Belt Plains ecoregion. The soils in this area are glacial till overlying limestone, dolomite, and shale bedrock. The water table has been lowered in much of the watershed by extensive use of tile drainage in crop fields. Near Marion, Ohio, the Little Scioto is biologically impaired.

This causal investigation was initiated because the State of Ohio water quality standards related to biological criteria were violated (Yoder and Rankin 1995a). The State of Ohio has a "tiered" set of aquatic life use designations based on narrative definitions of specific aquatic uses, which are protected by numeric criteria.

The majority of Ohio rivers and streams are designated as *Warmwater Habitat* (WWH) (Yoder and Rankin 1995a). This designation is narratively defined as *supporting a balanced, reproducing aquatic community*. Quantitatively, the minimum criteria required to be in attainment of WWH standards are defined as the 25th percentile values of reference condition scores for a given index, site type, and ecoregion. The choice of the 25th percentile is considered to be conservative and will likely be influenced by the inclusion of marginal sites as well as reference quality sites.

The Little Scioto River is considered Warmwater Habitat above RM 7.9 and a Modified Warmwater Habitat at and below RM 7.9 (see Figure 7-1). The *Modified Warmwater Habitat* (MWH) criteria are based on comparisons to a different reference condition than are used for the WWH criteria (Yoder and Rankin 1995a). The MWH designation is a *non-fishable* aquatic life use, and is designed to protect streams that have been too impacted, or modified, to meet WWH standards. MWH streams are unlikely to recover sufficiently to meet WWH designation. Consequently, MWH criteria are typically lower than WWH criteria. In spite of poorer water quality conditions (such as low dissolved oxygen, high ammonia concentration, and increased nutrient input), MWH streams are nonetheless able to support permanent assemblages of tolerant species.

7.3   Evidence of Impairment

In 1987 and 1992, sampling and measurements for community and habitat indices (IBI, ICI, QHEI) were conducted by OEPA along the Little Scioto River. Standardized field, laboratory and data processing methods followed OEPA procedural guidelines (OEPA 1988a, OEPA 1989a,b,c, Rankin 1989). Fish and macroinvertebrates were sampled at seven sites along the river, from river mile (RM) 9.5 to 0.4 (Figure 7-1). Index and metric scores for IBI, ICI, and QHEI used in this study were obtained from data sets that were generated and made available by OEPA as well as various OEPA reports (1988b, 1992, and 1994).
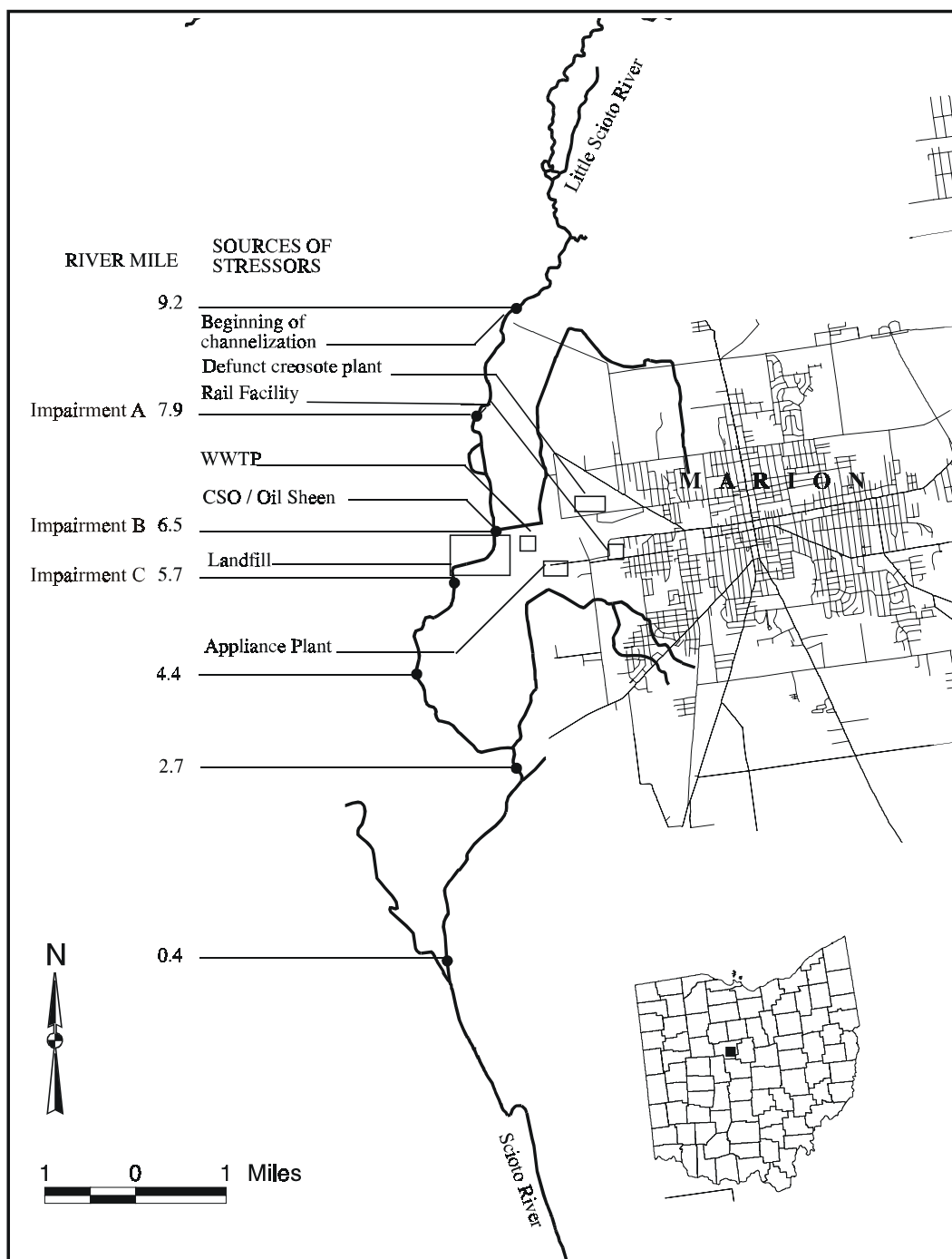
Figure 7-1. Map of the Little Scioto River, Ohio, showing sites where fish were sampled. (Approximate locations of significant physical features, tributaries and point source inputs are noted. The small inset shows the location of the study area in the state of Ohio. Locations of Impairments A, B and C are also shown.)

Of the seven sites sampled in 1987, the highest IBI score was 34 (out of a possible score of 60), which occurred at RM 9.2. This score translates to a *fair* ranking according to WWH standards. The remainder of sites were described as *severely impaired*, with IBI scores between 25 and 12 (the lowest possible IBI score) (OEPA 1994, Yoder and Rankin 1995a). In 1992, the IBI score at RM 9.2 decreased by one point to 33. However, in 1992, the IBI score dropped 9 points to a score of 24 between RM 9.2 and

RM 7.9. Another 5 point drop occurred at RM 6.5 and scores stayed between 19 and 20 through RM 2.7. At RM 0.4, the IBI score climbed back to 25, greater than the adjacent upstream site's score, but still indicating impairment. Figure 7-2A illustrates the fluctuation of the IBI at the seven sites during the two sampling years (1987 and 1992).

Figure 7-2B traces a similar pattern of impairment for the invertebrate index during the 1987 and 1992 sampling years. The ICI met WWH aquatic life use standards in 1987 and 1992 at RM 9.2, with scores of 40 and 38, respectively (Figure 7-2). In 1992, the ICI score declined 22 points at RM 7.9 with a score of 16, considered fair, but below MWH aquatic life use standards. Scores further declined 12 or more points at RM 6.5, 5.7 and 4.4, with scores ranging between 6 and 10. These scores are indicative of highly impaired conditions (OEPA 1994, Yoder and Rankin 1995a). ICI scores increased to a value of 18 downstream at RM 2.7 and RM 0.4. In 1987, both IBI and ICI scores were greater at RM 6.5 and then declined at RM 5.7, and remained very low to the mouth of the Little Scioto.
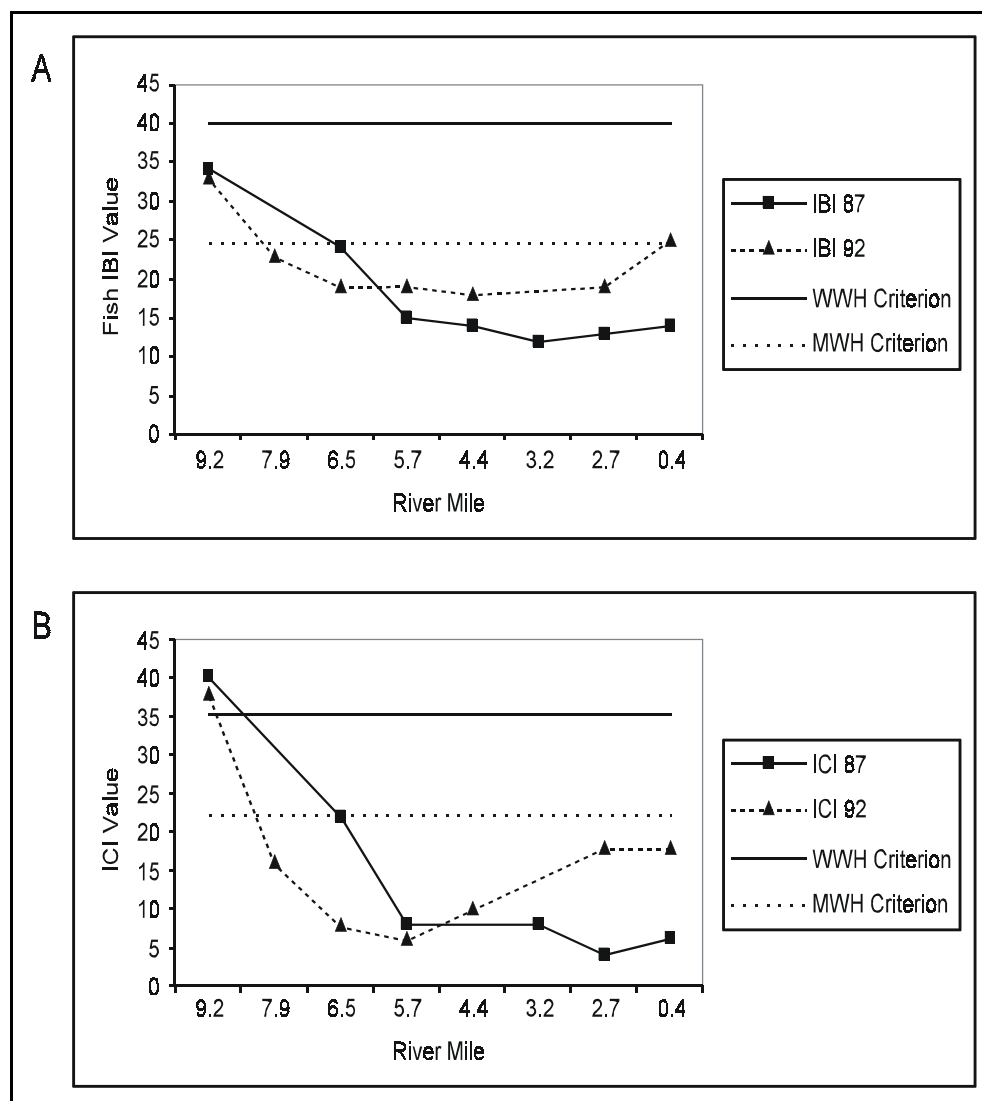

Figure 7-2. Spatial changes in fish IBI (A) and benthic macroinvertebrate ICI (B) values in the Little Scioto River in 1987 (OEPA 1988) and 1992 (OEPA 1994).

The impairments seen below RM 9.2 were more specifically described by examining the metrics that make up the IBI and the ICI. This information was combined with the changes seen in the overall IBI and ICI scores to determine whether distinctive patterns of impairment could be identified. Each distinctive impairment required a separate causal evaluation.

A subset of the fish and macroinvertebrate metrics, selected to highlight differences in community patterns, is shown in Figures 7-3A (fish) and 7-3B (macroinvertebrates). The complete list of values for the metrics is shown in Tables 7-13 and Table 7-14 (Please note that Tables 7-13 through 7-20 are located in Section 7.13, "Additional Data Tables"). One of the metrics, relative weight of fish, is not a component of the IBI but a component of another index, the Modified Index of Well-being (MIWB).
Examination of the spatial distribution of the IBI, ICI, and metric patterns in 1992 indicates that at least three distinct impairments occurred:

▸ Impairment A was seen at RM 7.9 where a marked drop in both the IBI and ICI occurred relative to the upstream location at RM 9.2. Specific fish metrics that appeared to correspond to this drop included decreases in the number of individuals minus tolerant fish, decreased total number of species, and increased relative weight. In addition, the percentage of mayfly species decreased.

▸ Impairment B occurred at RM 6.5 and corresponded with an additional decrease in both the IBI and the ICI. Relative the upstream location at RM 7.9, fish relative weight decreased, the number of deformities, erosions, lesions, tumors and anomalies (DELTA) increased, and the percentages of mayflies and Tanytarsini midges also decreased while the percentage of tolerant organisms increased.

▸ Impairment C occurred at RM 5.7. There was no change in the IBI relative to RM 6.5, although relative weight of fish decreased and DELTA increased. The invertebrates had variable changes depending on the sampling year. In 1987 and 1992, the % Tanytarsini midges decreased or disappeared entirely. Changes in the metrics at these three locations are summarized in Table 7-1.

The biological assessment data for the remaining locations showed a pattern similar to Impairment C, with the possibility of intensification at RM 4.4 and some improvement in metric scores occurring at RM 2.7 and 0.4. A fourth impairment was not hypothesized for RM 4.4 because the pattern of fish and invertebrate metrics were fairly similar to those seen at RM 5.7.
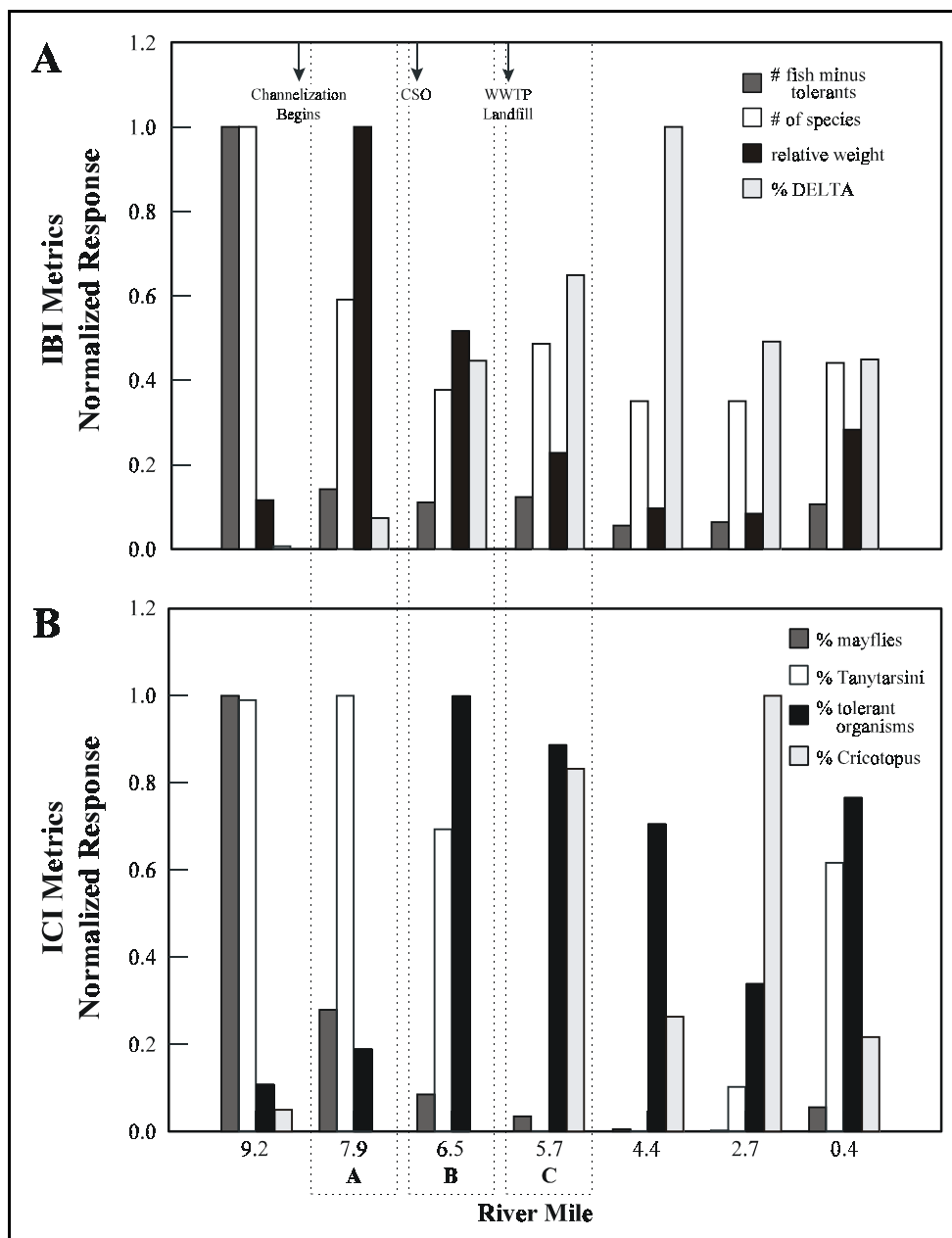
Figure 7-3. Changes in the IBI and ICI scores over distance in the Little Scioto River, 1992. ((A) Changes in the relative scores for the total number of individual fish minus tolerant fish (# fish minus tolerant), the number of species (# species), the relative weight of fish (relative weight) and the percentage of DELTA. (B) Changes in the relative abundances of percent *Ephemeroptera, Tanytarsini*, tolerant organisms, and *Cricotopus*, in the Little Scioto River. Normalized values were calculated by dividing the value at the individual site by the highest value for all sites.)

Table 7-1. Summary of the three impairments that were considered in the Little Scioto River. (Each location is scored relative to the location immediately upstream, based on 1992 data.)

Response	Impairment A RM 7.9	Impairment B RM 6.5	Impairment C RM 5.7
Fish			
# of individuals minus tolerant individuals	-	+	-
# Species	-	-	0
Relative Weight	7.9	-	-
DELTA	0	0	5.7
Invertebrates			
% Mayflies	-	-	-
% Tanytarsini midges	0	-	-
% Tolerant taxa	0	0	-
% *Cricotopus* sp.	-	0	0

(+) indicates an increase in the metric relative to the next upstream location
(-) indicates a decrease
(0) indicates no change.

7.4 List Candidate Causes

Evidence Used to Develop Candidate Causes

Many point and non-point sources of pollutants are associated with the Little Scioto River. Stressors impacting the upper portion of the river are mostly non-point nutrient and sediment loadings associated with agriculture. However, the Little Scioto River, at and below Marion, Ohio, has been notably contaminated with elevated levels of polycyclic aromatic hydrocarbons (PAH). Creosote and metals were found in sediment samples, and ammonia was detected in water samples (OEPA 1994). The OEPA has, in fact, recently requested Superfund support in the clean-up of an abandoned wood creosote plant suspected of polluting the river since the 1860's (Edwards and Riepenhoff 1998). An oily

sheen was noted on the river between river miles 6.5 and 5.8 during a site visit in 1992 (Cormier, pers. observ.). In-stream habitat quality was also degraded by channelization that took place in the early 1900's (OEPA 1994). Locations of the potential sources and stressors, including a landfill and wastewater treatment plant (WWTP), are shown in Figure 7-1.
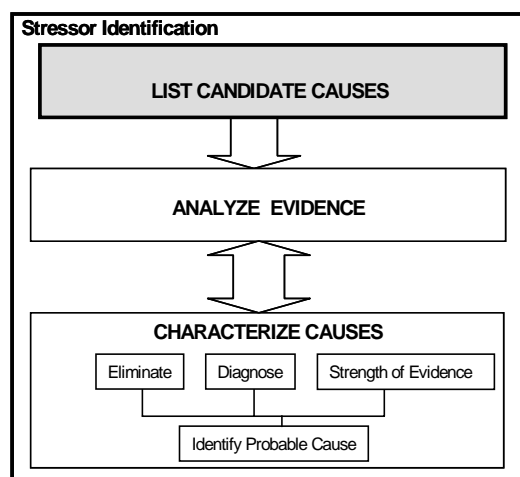
List of Candidate Causes and Scenarios

As noted previously, three distinctive impairments were identified for the causal evaluation. Based on the knowledge of the sources and effects, six candidate causes were formulated to account for the impairment observed at each site. A conceptual model of these candidates is provided in Figure 7-4.

1. Habitat Alteration - Habitat alteration, resulting from channelization, combines a complex interaction of several stressors. These stressors are evident at RM 7.9 and continue to the mouth of the river. Channelization can alter biological communities by changing the physical structure of the stream and the flow characteristics of the water, ultimately lowering dissolved oxygen, increasing siltation, and reducing substrate complexity. This complex suite of stressors also includes: decreased woody debris, which reduces available substrate and changes the energy source; decreased sinuosity, which changes flow characteristics; erosional patterns and substrates; increased channel depth that favors larger species of fish; loss of pools that act as refugia; and loss of riffles that oxygenate water and transport sediment (Tarplee et al. 1971, Karr and Schlosser 1977, Yount and Niemi 1990, Allan 1995).

2. PAH and 3. Metals - Biological impairment could also have been caused by toxic stress. Historically, the river has provided a means of waste disposal for various industries, whose effluents have contained metals, PAH, and creosote. Waste materials may have also been buried in the landfill below RM 6.5 (OEPA 1994). All are potentially toxic to aquatic life, and some have the ability to bioaccumulate through the food web (Eisler 2000a,b). Thus, two candidate causes emerge: candidate cause #2 is that biological impairment has occurred due to PAH exposure (with PAH emanating from creosote deposits), and candidate cause #3 attributes impairment to metal contamination.

4. Ammonia Toxicity - Ammonia is directly discharged into streams by point sources (Russo 1985, Miltner and Rankin 1998). Ammonia can also be formed as the result of nutrient enrichment. When dissolved oxygen levels are low, nitrates are reduced to ammonium ion. If pH is high, some of the ammonium ion is converted to un-ionized ammonia, which is toxic to aquatic organisms (Russo 1985). Moreover, pH may rise during periods of high photosynthetic rates from bicarbonate depletion. High amounts of nutrients often lead to increased algal growth rates, and the conversion of ammonium to un-ionized ammonia is expedited (Dodds and Welsh 2000).

5. Low Dissolved Oxygen/ High Biological Oxygen Demand - Depletion of DO commonly occurs from organic enrichment (Smith et al. 1999). Organic enrichment is the most common cause of increased biological oxygen demand (BOD) (Allan 1995). Potential sources of excess organic matter within the study area include a waste water treatment plant (WWTP) and several combined sewer outfalls (CSOs), as well as upstream, non-point sources. Organic matter is also produced by excess algal growth from nutrient enrichment (Dodds and Welsh 2000). Algal blooms themselves result in increased organic matter regardless of DO depletion. The algal bloom may suffice to raise BOD so that DO is depleted. Because no chlorophyll *a* or algal biomass data were collected in this study, the cause of BOD to the river can only be estimated from BOD, measured at several points, and COD (chemical oxygen demand) measured at point sources such as the WWTP above RM 5.4 in 1998.

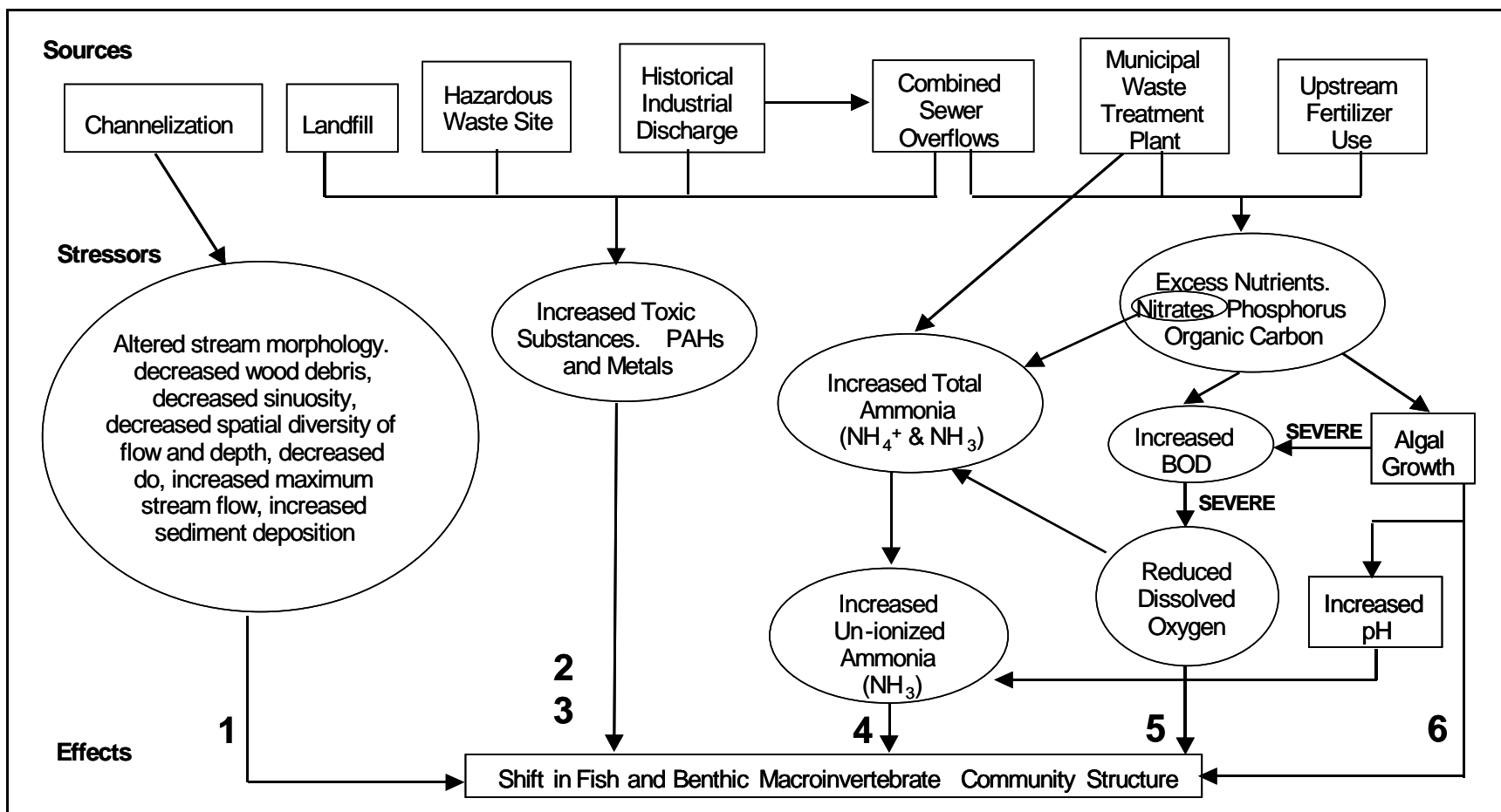**Figure 7-4.** A conceptual model of the six candidate causes for the Little Scioto stressor identification. (Potential sources are listed in top most rectangles. Potential stressors and interactions are located in ovals. Candidate causes are numbered 1 through 6. Note that some causes have more than one stressor or more than one step associated with it. The impairments are located in the lower rectangle.)

6. Nutrient Enrichment - The sixth and final candidate cause is a less extreme form of nutrient enrichment. Primary production and organic matter loading to the sediments are increased, but not enough to reduce DO. This can cause changes in fish and benthic macroinvertebrate assemblages, including changes in dominant species, and greatly increased abundance and biomass (Carpenter et al. 1988, Rankin et al.1999, Smith et al. 1999, Dodds and Welsh 2000, Edwards et al. 2000). This form of nutrient enrichment is also associated with fin erosion (Rankin et al. 1999).

7.5   Analyze Evidence to Eliminate Alternatives

7.5.1   Data Analyzed

Habitat alteration-related data
Data on the spatial location of habitat alteration was obtained by using the Qualitative Habitat Evaluation Index (QHEI). The QHEI incorporates measures of habitat condition and has been correlated with the IBI. This index uses eight interrelated metrics, which assess substrate type and quality; in-stream cover type and amount; channel morphology; riparian width and quality and bank erosion; pool / riffle characteristics including depth, current, pool morphology, substrate stability and riffle embeddedness; and finally gradient (Rankin 1989). Based on these metrics, a total score is assigned to a stream reach out of a possible 100 points, with greater scores indicating higher quality. The channel morphology and substrate metrics are particularly relevant for this case because of the channelization (Figure 7-5). Values for the QHEI and its component metrics are given in Table 7-15 (see Section 7.13).
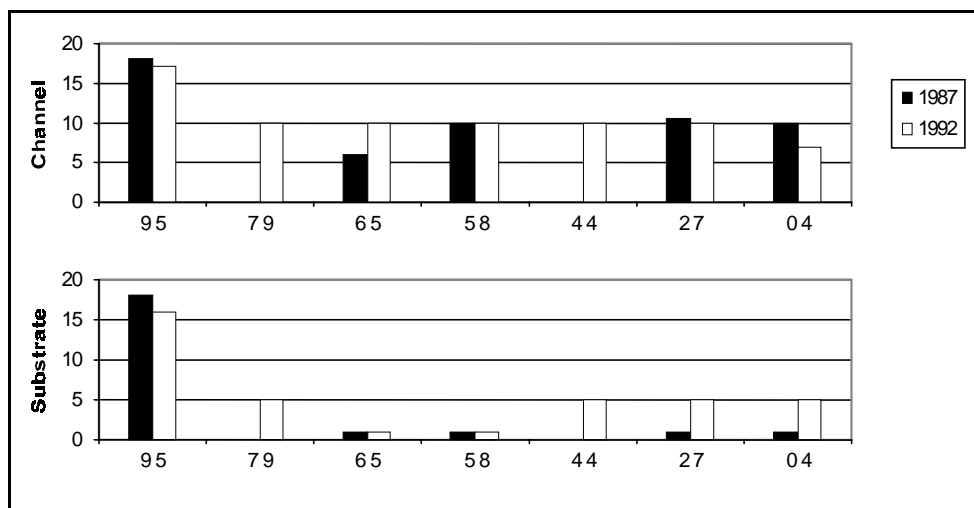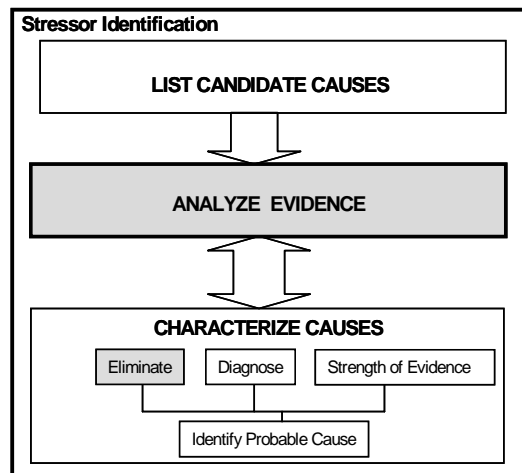

Figure 7-5. Selected QHEI metrics for 1987 and 1992. (Scores are qualitative ranks.)

<u>Chemical Data</u>
Data on sediment and in-stream chemistry were used to evaluate the spatial location of
the remaining candidate causes (#2-6). Nutrient concentrations measured in water
included ammonia, nitrates and nitrites (NO_x), phosphorus (P), and BOD. Ambient
levels of potential toxic chemicals were determined for sediment and water. Results of
chemical analyses are presented in Tables 7-16, 7-17 and 7-18 (See Section 7.13), and
Figures 7-6, 7-7, and 7-8.

While PAHs were not detectable at the upstream sites (RM 9.5 and 7.9), many PAHs
were detected between RM 6.5 and 0.4 (Table 7-16) (Figure 7-6). Spearman Rank
Correlations between chemical and biological data from 1992 at RM 5.7 to 0.4 are
shown in Table 7-2 through 7-5.

Metals were found in sediments at relatively high concentrations at RM 6.5 and
downstream (Table 7-17; see Section 7.13) (Figure 7-7). These included lead, cadmium,
copper, chromium, zinc, and mercury. Arsenic was relatively high at upstream reference
and study sites. Spearman rank correlations between metals and biological data from
1992 at RM 5.8 to 0.4 are shown in Table 7-3. Strong correlations having the sign that is
consistent with the hypothesis were noted for copper and mercury.

The water quality parameters ammonia, nitrates and nitrites (NO_x), and BOD increased
substantially at RM 5.8, and remained elevated. Dissolved oxygen declined at 7.9 and
remained low to RM 0.4 (Table 7-18; see Section 7.13) (Figure 7-8). Spearman rank
correlations of water chemistry and biological endpoints are presented in Table 7-4.
Percent Tanytarsini are significantly correlated with DO, BOD, NO_x and P, and the
negative direction of the slope was consistent with ecological theory. Percent DELTA
was correlated with the same parameters (DO, BOD, NO_x and P) but at the 0.8 level,
whereas percent Cricotopus was associated with ammonia and the QHEI.

7.5.2   Associations between Candidate Causes and Effects

The associations between candidate causes and effects were analyzed by combining data
on the location of the three impairments with data on habitat quality and chemical
concentrations in water and sediments. The analyses evaluated whether the candidate
causes and each of the three impairments were spatially co-located, and whether a
gradient in recovery corresponded with a decrease in the candidate cause. These
associations are organized in table format (Table 7-5).

The first objective of the analysis was to determine if there was evidence that the
candidate cause occurred at the same place as the impairment but not where that
particular impairment was absent. Plots of the channel quality and substrate metrics
from the QHEI are shown in Figure 7-5. The chemistry values relevant to each of the
causal scenarios are shown in Figures 7-6, 7-7 and 7-8. Each graph shows the level or
concentration of the parameter. The presence or absence of candidate causes at the
locations of Impairments A, B, and C are summarized in Table 7-5.
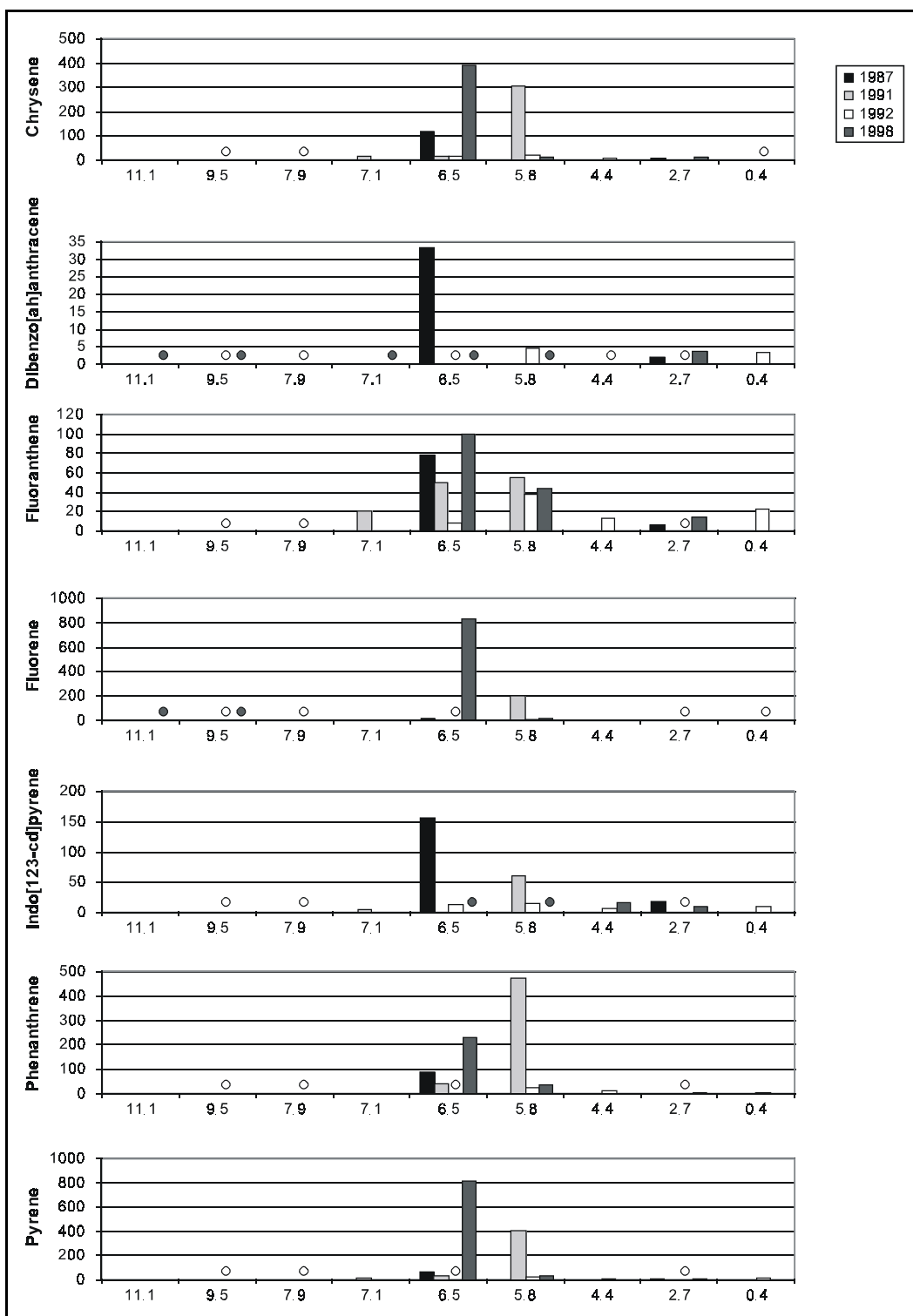
Figure 7-6. Mean PAH concentrations from the sediment (mg/kg) in the Little Scioto River 1987-1998. ((o) indicates below detection limit. Absence of bar indicates no data available.)
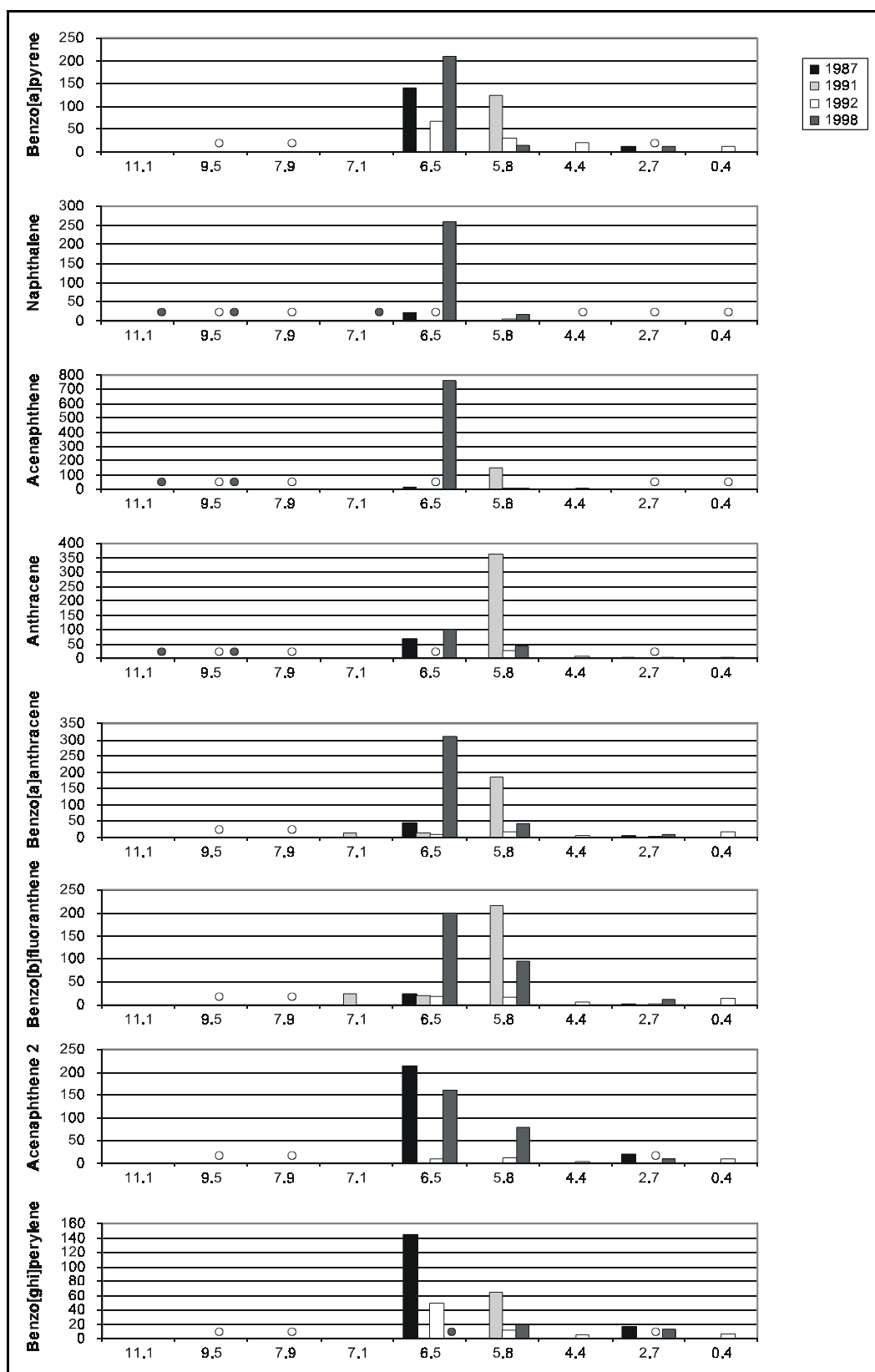
Figure 7-6 (continued). Mean PAH concentrations from the sediment (mg/kg) in the Little Scioto River 1987-1998. ((o) indicates below detection limit. Absence of bar indicates no data available.)
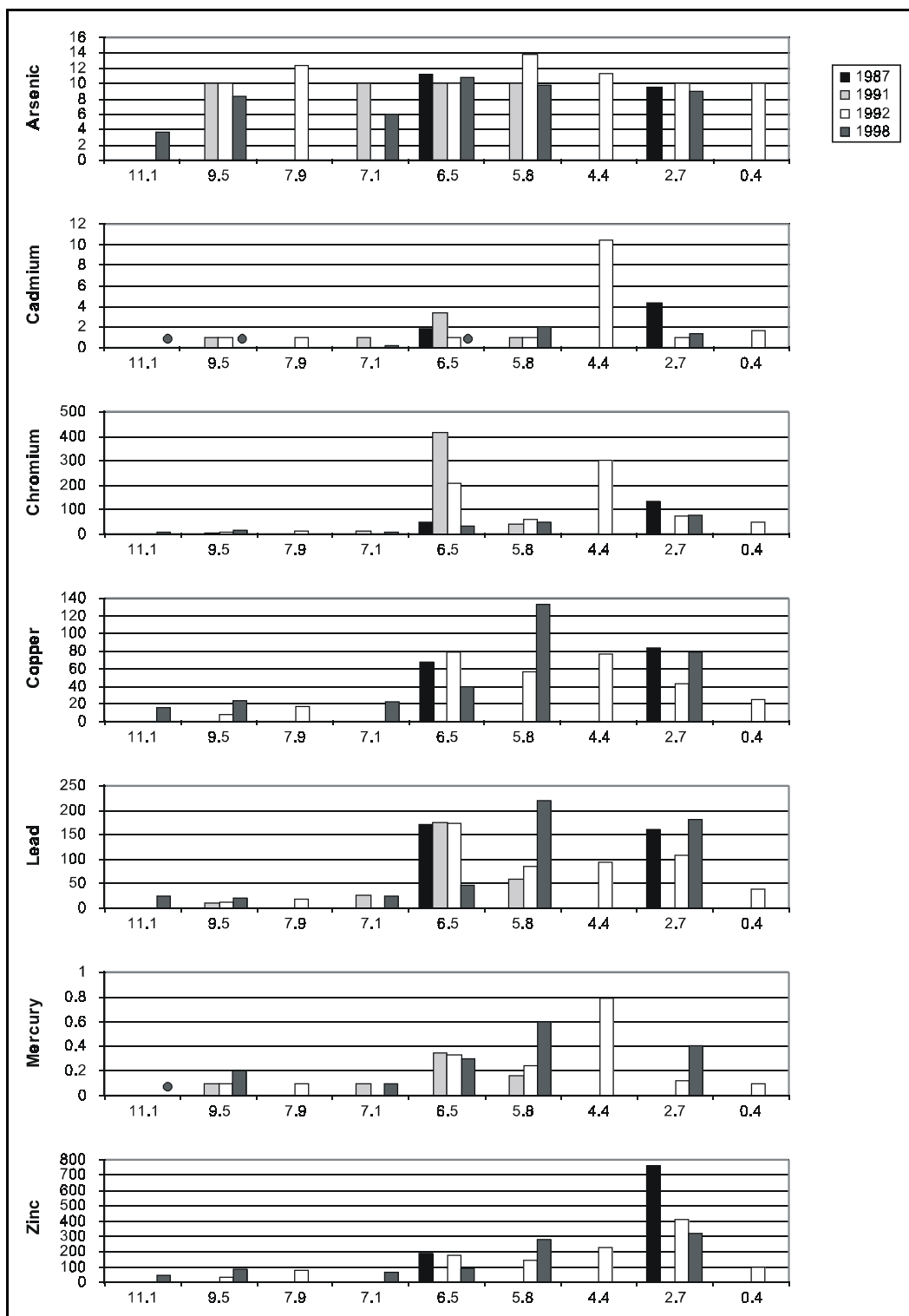
Figure 7-7. Mean metal concentrations from the sediment (mg/kg) in the Little Scioto River from 1987-1998. (Absence of bar indicates no data available.)
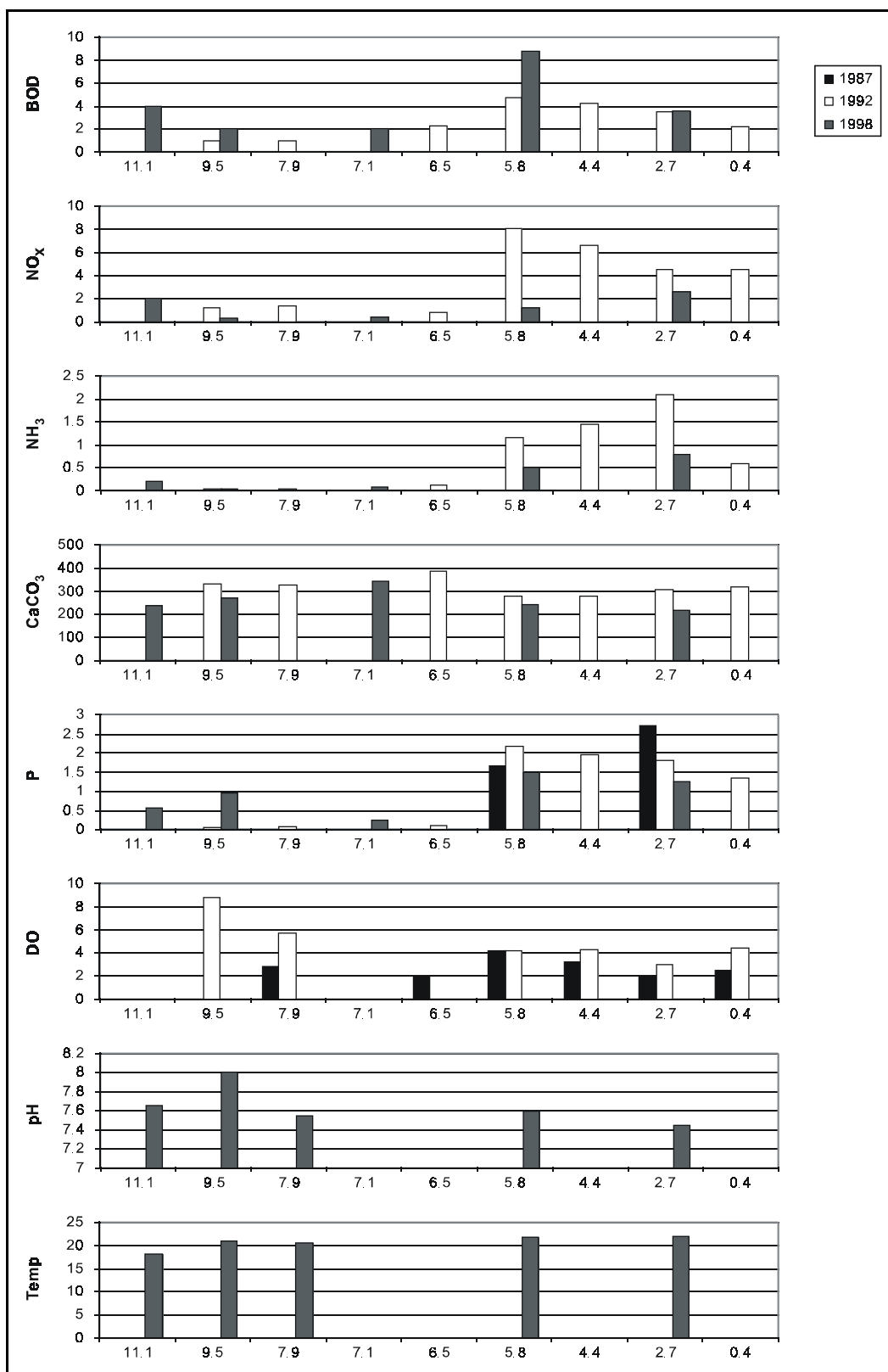
Figure 7-8. Mean water chemistry values from the Little Scioto River from 1987-1998. (BOD, NO_x, Ammonia, $CaCO_3$, PO_4, are all mg/L, Temperature (^0C). DO is also mg/L and is the minimum value obtained from grab samples for each year. Absence of a bar indicates no data for that year.)

Table 7-2. Spearman rank correlations with selected metrics and the IBI and ICI from 1992 and selected PAHs. (Reflects only values from RM 5.8 to 0.4. Correlations N=4).

Parameter	DELTA	% Tanytarsini Midges	% Cricotopus
Anthracene (#2)	0.60	-0.74	-0.20
Benzo[a]anthracene (#2)	0.00	-0.21	-0.40
Benzo[ghi]perylene(#2)	0.00	-0.21	-0.40
Benzo[a]pyrene (#2)	0.00	-0.21	-0.40
Chrysene (#2)	0.80*	-0.95*	0.40
Dibenzo[a,h]anthracene (#2)	-0.21	-0.06	-0.21
Fluoranthene (#2)	0.00	-0.21	-0.40
Fluorene (#2)	0.74	-0.89*	0.11
Naphthalene (#2)	0.26	-0.54	0.26
Phenanthrene (#2)	0.60	-0.74	-0.20
Pyrene (#2)	0.00	-0.21	-0.40

* Correlations above 0.8

Table 7-3. Spearman rank correlations with selected metrics and the IBI and ICI from 1992 and selected metals. (Reflects only values from RM 5.8 to 0.4. Correlations N=4).

Parameter (Candidate Cause)	DELTA	% Tanytarsini Midges	% Cricotopus
Arsenic (#3)	0.74	-0.89*	0.11
Cadmium (#3)	0.20	0.11	-0.60
Chromium (#3)	0.80*	-0.63	0.40
Copper (#3)	1.00*	-0.95*	0.20
Lead (#3)	0.40	-0.32	0.80*
Mercury (#3)	1.00*	-0.95*	0.20
Zinc (#3)	0.40	-0.32	0.80*

* Correlations above 0.8

Table 7-4. Spearman rank correlations with selected metrics and the IBI and ICI from 1992 and selected water quality and habitat quality measurements. (Reflects only values from RM 5.8 to 0.4. Correlations N=4).

Parameter (Candidate Cause)	DELTA	% Tanytarsini Midges	% Cricotopus
Channel Metric (#1)	0.77	-0.82*	0.77
QHEI (#1)	0.20	-0.32	1.00*
Ammonia, N (#4)	0.40	-0.32	0.80*
Dissolved oxygen maximum (#5)	0.80*	-0.95*	0.40
Dissolved oxygen minimum (#5)	0.60	-0.74	-0.20
BOD (#5)	0.80*	-0.95*	0.40
Nitrate-nitrite, N (#4,5,6)	0.80*	-0.95*	0.40
Phosphorus, total P (#5,6)	0.80*	-0.95*	0.40

* Correlations above 0.8

The second objective was to determine if the cause increased compared to the nearest upstream location. Statistical analyses were not used to determine an increase because the power would be very weak due to small sample sizes. Even a small increase was accepted since it might represent a threshold for the effect (Table 7-5).

The third objective of the analyses was to evaluate whether a gradient in the intensity of the potential cause corresponded to a gradient of recovery in impairment. The gradient analysis was conducted only for Impairment C, which was observed at four contiguous locations (i.e., RM 5.8 to 0.4). The recovery of Impairment B could not be analyzed since it would be masked by Impairment C. Similarly, any recovery of Impairment A would be masked by both B and C. The gradients in environmental parameters and the IBI and ICI were examined visually by comparing Figures 7-2 and 7-3 with Figures 7-5 through 7-8. The IBI and ICI metrics for 1987 and 1992 data are shown in Table 7-13 and Table 7-14, respectively. In addition, Spearman's rank correlations were calculated using the 1992 data set to relate the biological metrics (shown in Figure 7-3) with each of the parameters related to the candidate causes. The results of this analysis are shown in Tables 7-2 through 7-4.

Two metrics are more severe at Impairment C: % DELTA and % Tanytarsini midges decrease and % *Cricotopus* increases. Percent DELTA were significantly correlated with copper and mercury, and moderately correlated with chrysene, chromium, BOD, nitrate, phosphorous, and maximum DO. The change in tanytarsini midges was negatively and strongly correlated with chrysene, copper, mercury, BOD, nitrate, phosphorous, maximum dissolved oxygen, and moderately correlated with fluorene, arsenic, and the channel metric. The change in % *Cricotopus* was strongly positively correlated with QHEI and moderately correlated with lead, zinc, and ammonia.

Table 7-5. Evidence for eliminating candidates causes at Impairments A, B, and C.

	Impairment A	Impairment B	Impairment C
Habitat Alteration (Candidate Cause 1)			
Is there exposure at the same location as the impairment?	Yes	Yes	Yes
Is exposure increased over the closest upstream location?	Yes	No	No
Is there a gradient of recovery as exposure decreases?	NA* (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	No (Correlation coefficients have the wrong signs, with % DELTA and % Tanytarsini)
Is the exposure pathway complete?	Yes	Yes	Yes
PAH Contamination (Candidate Cause 2)			
Is there exposure at the same location as the impairment?	No	Yes	Yes
Is exposure increased over the closest upstream location?	No	Yes	No (based on metabolite values in fish)
Is there a gradient of recovery as exposure decreases?	NA (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	Inconclusive (Mixed results)
Is the exposure pathway complete?	No	Yes	Yes

Table 7-5 (continued). Evidence for eliminating candidates causes at Impairments A, B, and C.

	Impairment A	Impairment B	Impairment C
Metal Contamination (Candidate Cause 3)			
Is there exposure at the same location as the impairment?	Yes	Yes	Yes
Is exposure increased over the closest upstream location?	Yes (all metals greater in some years)	Yes (all metals greater)	Yes (copper and zinc increased)
Is there a gradient of recovery as exposure decreases?	NA (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	Yes (Tanytarsini midges and % DELTA are strongly correlated with copper and mercury)
Is the exposure pathway complete?	Yes	Yes	Yes
Ammonia (Candidate Cause 4)			
Is there exposure at the same location as the impairment?	Yes	Yes	Yes
Is exposure increased over the closest upstream location?	No	Yes	Yes
Is there a gradient of recovery as exposure decreases?	NA (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	NA (ammonia increases below RM 5.8)
Is the exposure pathway complete?	No	Yes	Yes

Table 7-5 (continued). Evidence for eliminating candidates causes at Impairments A, B, and C.

	Impairment A	Impairment B	Impairment C
Low Dissolved Oxygen/High BOD (Candidate Cause 5)			
Is there exposure at the same location as the impairment?	Yes	Yes	Yes
Is exposure increased over the closest upstream location?	No (DO is depressed, BOD unchanged)	Yes (BOD is two times greater in 1992, DO slightly less)	No (BOD is elevated, but DO is greater than either RM 7.9 or RM 6.5)
Is there a gradient of recovery as exposure decreases?	NA (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	NA (ammonia increases below RM 5.8)
Is the exposure pathway complete?	Yes	Yes	No
Nutrient Enrichment (Candidate Cause 6)			
Is there exposure at the same location as the impairment?	Yes	Yes	Yes
Is exposure increased over the closest upstream location?	Yes	Yes	Yes
Is there a gradient of recovery as exposure decreases?	NA (Gradient in impairment is masked by B and C)	NA (Gradient in impairment is masked by C)	Yes (% Tanytarsini and % DELTA are strongly correlated with NO_x and Total P)
Is the exposure pathway complete?	Yes	Yes	Yes

NA* = not applicable

7.5.3   Measurements Associated with the Causal Mechanism: Exposure Pathways

The exposure pathways are shown in Figure 7-4. Lines of evidence for each exposure pathway are discussed below and are summarized in Table 7-11. To refute an hypothesis, a step in the pathway must be absent.

Habitat Alteration (#1) - Channelization results in a constellation of stressors, including loss of riffles with increased sediment deposition, and decreased DO. The QHEI metrics can yield insights into specific changes: for example, riffle scores are zero throughout the channelized portion of the stream (Table 7-15; see Section 7.13), substrate quality and embeddedness due to fine sediment drops at RM 7.9, and DO also drops at RM 7.9. The co-occurrence of macroinvertebrates with changes in physical structure may be somewhat lessened, because Hester-Dendy samplers create an artificial solid substrate for colonization. The ICI score does include a qualitative kick net sample that is independent of the artificial substrates. The exposure pathway for habitat alteration is complete for Impairments A, B, and C.

PAHs (#2) - Exposure to PAHs involves two steps: direct contact with external tissues and uptake into the organism. Because the PAH information in this case is from the sediments, we assume that fish and benthic invertebrates between river miles 6.5 and 0.4 will contact this contamination. Concentrations of PAH in the sediment were used only from samples collected in 1992, as it was the only year in which we were confident that the samples were collected from the top six inches. It is unlikely that fish or invertebrates would be exposed to deeper sediments.

The exposure pathway for PAHs could be interrupted if there was no sign of internal exposure. Aquatic contaminants such as PAHs have been monitored by measuring the metabolites of xenobiotics in fish bile (Roubal et al. 1977, Gmur and Varanasi 1982, Varanasi et al. 1983). Samples from white suckers (*Catostomus commersoni*) taken in 1992 from the Little Scioto River were analyzed for concentrations of benzo[a]pyrene (BAP) and naphthalene (NAPH)-type metabolites. Results of the analysis of PAH bile metabolites in white suckers from the Little Scioto River are shown in Figure 7-9. Biomarkers of NAPH and BAP are elevated from RM 6.5 to the mouth of the river, providing evidence that the exposure pathway is complete at these locations. Exposure criteria, concentrations considered to be above background, were exceeded at RMs 6.5 through 0.4. PAHs are also known to cause induction of detoxifying enzymes such as EROD. EROD activity was elevated at RM 6.5 - 0.4. Based on the absence or presence of bile metabolites, the exposure pathway for PAHs is incomplete at Impairment A, and complete at Impairments B and C.

Metals (#3) - Metals must be taken into organisms to cause adverse effects. Data from fish tissue sampled in 1992 confirm uptake of lead and zinc. For common carp (*Cyprinus carpio)* at RM 9.2, zinc concentrations were 79.6 mg/kg, at RM 6.5, zinc concentrations were 68.3 mg/kg. For white suckers at RM 6.5, zinc concentrations were 17.8 mg/kg, and lead concentrations were 81.4 mg/kg. At RM 2.7, fish tissues levels were 15.8 mg/kg for zinc and 0.34 mg/kg for lead. For the other metals, we have conservatively assumed that external exposure will represent internal exposure for fish. Making this assumption, increased exposure to at least one of the metals occurs at all sampling locations in the reach RM 7.9 to 0.4. Concentrations of metals in sediment were from samples taken in 1992 from the top six inches of sediment. For 1987 and 1998 data, the depth of samples is unknown.
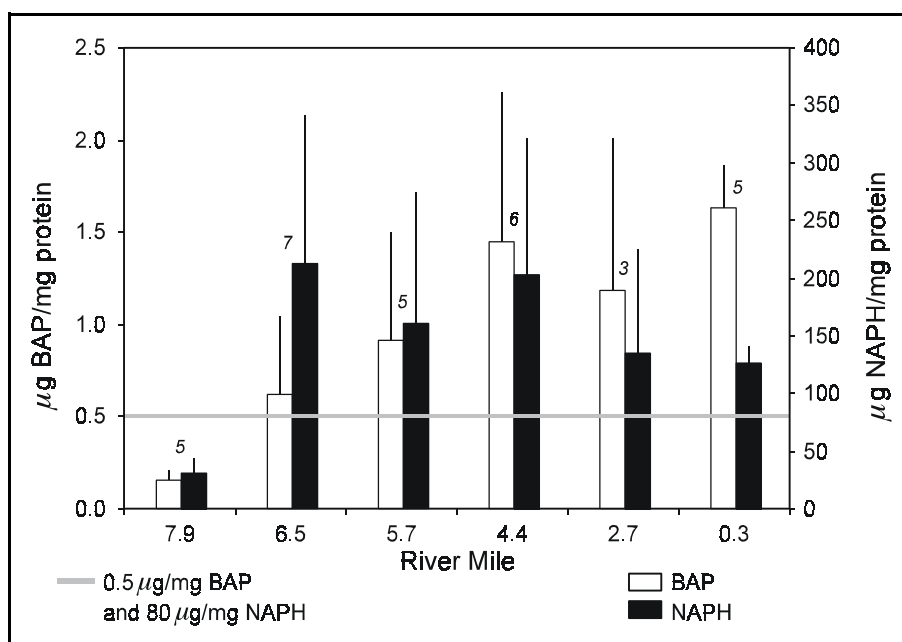
Figure. 7-9. Bile metabolites (µg/mg protein) measured in white suckers from the Little Scioto River in 1992. (Median levels of PAH metabolites below RM 7.9 were up as much as 4 times the Exposure Criteria, (dashed horizontal line) which are upper limits of background for the state of Ohio. The numbers above the bars equal number of fish sampled. Vertical lines are standard errors.)

Ammonia (#4) - There are several interweaving pathways by which ammonia can be produced in the river and cause effects. We have evidence for two of these steps: total ammonia, and nitrate and nitrite concentrations that are converted to ammonia when DO is low. Toxic unionized ammonia is formed at high pH. Hard water streams of the Eastern Corn Belt Plains typically have pH from 7.5-8; pH may rise even above 9.0 in the summer during maximum photosynthesis in nutrient-enriched waters. Data on pH are not available in 1992, however, in 1998 grab samples, pH ranged between 7.4 to 8.0. The Little Scioto is highly enriched, and it is highly likely that there are periods when pH is greater than indicated by grab samples. Thus, we assume that the exposure pathway is complete in the Little Scioto when total ammonia is present. This occurs from RM 11.1 to 0.4. Because ammonia concentrations are measured in the water column, both fish and macroinvertebrates are exposed.

Low Dissolved Oxygen/High Biological Oxygen Demand (#5) - Dissolved oxygen can be depleted by high BOD due to the bacterial respiration associated with allochthonous organic matter or decaying algal mats. We have measurements of several relevant parameters: NO_x, total P, BOD and DO concentrations. This exposure pathway is considered complete under two scenarios: (1) BOD is elevated and DO is reduced compared with the most upstream location, or (2) if BOD data is unavailable, NO_x and P are elevated and assumed to cause algal growth, and DO is reduced as compared with the most upstream location. At RM 7.9, DO is reduced, but BOD is unchanged, so that the exposure pathway is considered incomplete. RM 6.5 is more difficult to evaluate because data are scanty and are used from different years. In 1987, DO data were low at

RM 6.5, and in 1992, the BOD was slightly elevated; thus, the pathway is complete. At RM 5.8 to 0.4, because BOD is elevated but DO is similar or greater than at 7.9, the exposure pathway is considered incomplete.

Nutrient Enrichment (#6) - We have evidence for the presence of elevated levels of both NO$_x$ and total P concentrations. This exposure pathway appears to be complete at RM 5.8 to 0.4, and at RM 7.9.

7.5.4   *Summary of Analyses for Elimination*

The results of the analysis of spatial associations are summarized in Table 7-5 (pages 7-21 to 7-23). The table addresses four questions for each combination of impairment and candidate cause. If any of the answers are no, then the candidate cause can be eliminated:

▸ The first question is whether a candidate cause and impairment are spatially co-located. Regardless of concentration, the answer is yes if the stressor is present. If the stressor is not present, the answer is no and the impairment could not have been caused by exposure to that stressor.

▸ The second question asks whether the exposure is elevated compared to the closest upstream location where the impairment does not occur. The candidate cause could have been responsible for the impairment only if exposure increased. The candidate cause can be eliminated if the answer to the second question is no.

▸ The third question asks whether there is a decrease in exposure that corresponds with recovery of the impairment. As discussed above, this question is relevant only to Impairment C. If the answer is no with results clearly showing a lessening of impairment with consistent exposure, then the candidate cause can be eliminated.

▸ The last question asks if the exposure pathway is complete. If it is interrupted or clearly incomplete so that exposure could not have taken place, then it can be eliminated as a potential cause.

7.6   Characterize Causes: Eliminate

Potential causes may be eliminated if the evidence indicates that they do not co-occur with effects, if effects decrease with increasing influence of the cause, or if the exposure pathway is incomplete. Each of the three Impairments (A, B, and C) are discussed below in relation to the elimination of specific causes. Conclusions about which candidate causes remain for each impairment are also listed.

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause
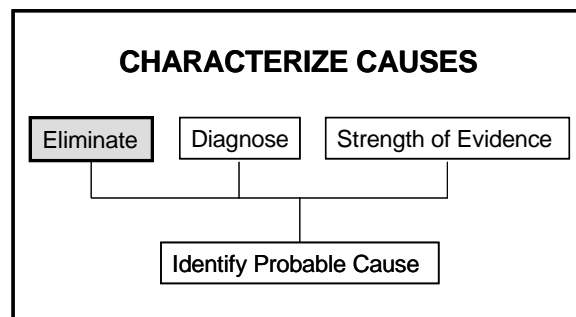
Impairment A: RM 7.9
Habitat alteration and metal contamination are the only candidate causes known to co-occur at RM 7.9 and to increase compared to upstream locations. All metals were slightly greater at RM 7.9 compared to RM 9.2. PAHs and ammonia were not elevated at RM 7.9 relative to the upstream reference, thus candidate causes #2 and

#4 are eliminated. DO concentrations were about 30% lower than upstream, but BOD concentrations were not different from the upstream reference location (RM 9.2), thus candidate cause #5 is eliminated. NO_x increased from 1.2 mg/L to 1.4 mg/L. The shift is small, but precludes elimination of candidate cause #6.

Conclusion: Habitat Alteration (#1), Metal Contamination (#3), and Nutrient Enrichment (#6) remain.

Impairment B: RM 6.5.

At this site, only candidate cause #1 can be eliminated because the degree of habitat alteration is not elevated compared with those at RM 7.9. The decline in QHEI score is associated with the obvious presence of organic chemical contamination rather than physical stream characteristics. Organic chemicals, including benzo[a]pyrene and naphthalene, were present and were elevated above concentrations at RM 7.9. Exposure to these organic chemicals was demonstrated by internal concentrations of metabolites. The metals chromium, copper, lead, and mercury were elevated compared to upstream concentrations in all years for which there is data, including 1988, 1991, 1992 and 1998. Dissolved oxygen levels were among the lowest in the river in 1987, and BOD levels were slightly greater than upstream locations. Ammonia concentrations were also slightly greater, and total P concentrations were 0.02 mg/L greater.

Conclusion: PAH Contamination (#2) Metal Contamination (#3), Ammonia Toxicity (#4), Low Dissolved Oxygen/High Biological Oxygen Demand (#5), and Nutrient Enrichment (#6) remain.

Impairment C: RM 5.7.

In this reach of the river, the degree of habitat alteration and PAH levels were similar or lower than at RM 6.5, thus candidates #1 and #2 are eliminated. Candidate cause #5, low DO/high BOD, can be eliminated, even though BOD, P and NO_x are elevated because the subsequent event in the pathway, decreased DO, did not occur. DO is unchanged from RM 7.9 in 1992, and RM 6.5 in 1987. The metals (copper and zinc) increased slightly, and the copper gradient was significantly correlated with % Tanytarsini midges and % DELTA, thus candidate cause #3 remains. NO_x and P were elevated in the reach compared to upstream locations and were significantly correlated with % Tanytarsini midges. Candidate cause #6 remains. Candidate cause #4 could be eliminated since ammonia was not correlated with the specific impairments. However, the increase in ammonia was 10 times greater than upstream, and because the data available for correlations were very limited, a conservative decision could be made to retain this cause for further evaluation by the strength of evidence approach.

Conclusion: Metal Contamination (#3), Ammonia (#4), and Nutrient Enrichment (#6) remain.

A summary of the candidate causes that remain after the elimination process are listed in Table 7-6. Only those causes remaining need to be evaluated by diagnostic or strength of evidence analyses.

Table 7-6. Candidate causes remaining after elimination.

	Impairment A	Impairment B	Impairment C
#1 Habitat alteration	X		
#2 PAH Contamination		X	
#3 Metal Contamination	X	X	X
#4 Ammonia		X	X
#5 Low DO/BOD		X	
#6 Nutrient Enrichment	X	X	X

7.7   Analyze Evidence for Diagnosis

Diagnosis is the identification of causes based on characteristic signs or symptoms (see 4.2.2). No evidence strong enough to support diagnosis was available for any of the candidate stressors. However, the pattern of community change is considered to be suggestive, and is used in the strength of evidence analysis below.

The deformities, fin erosion, tumors, physical lesions and anomalies on fish that constitute the DELTA are pathologies that are also potentially subject to diagnosis. Some DELTA are strongly associated with known toxic substances and others with increased nutrients (Yoder and Rankin 1995b). However, no pathologist has examined the fish in question. DELTA cannot be used to distinguish among toxic substances unless specific anomalies are identified, and even these may be too non-specific to diagnose without additional information (e.g., histopathology).
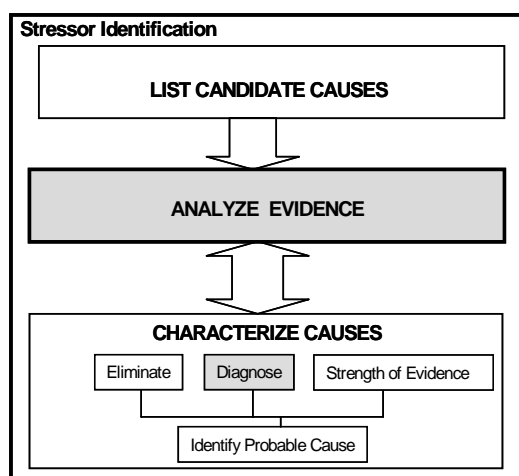

7.8   Analyze Evidence to Compare Strength of Evidence

All of the remaining candidate causes are subjected to a strength of evidence analysis to verify the elimination step and to identify the most likely cause from the multiple hypothesized causes that remained after the elimination process. The strength of evidence analysis examined case specific evidence as well as evidence from other situation and biological knowledge.

Case Specific Evidence

The evidence presented earlier for the elimination step is useful here as well. In addition, some data on loadings are available from the Waste Water Treatment Plant (WWTP), which discharges at RM
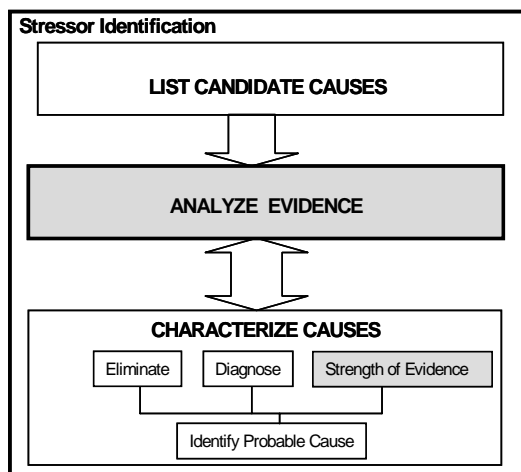
6.2 and also has combined sewer overflows that discharge during wet weather periods. No clear trends were evident in the loadings of total non-filterable residue or biological oxygen demand between 1977 and 1992. Ammonia values were generally low, with fifty percent of loading below 10 kg/day between 1977 and 1991. The highest ammonia loading occurred during 1992, with a median of 12.6 kg/day and a maximum of 130 kg/day (OEPA 1994).

Evidence from Other Situations or Biological Knowledge

This section presents evidence that uses information from other studies that are related to either exposures or effects found in segments of the Little Scioto River. In particular, associations are made between the exposures known at the site and reports of effects caused by similar exposures. This section also uses levels of effects seen at the site and effects seen at other sites where the same candidate cause occurred. It also considers special experimental evidence; that is, reports about places with similar stressors and effects that improved when the stressor was removed, and laboratory studies of candidate cause-effect relationships.

Exposure-response data are available for PAHs and metals, although not for the community parameters of greatest interest for this study. Sediment effect concentrations (SECs) developed for *Hyalella azteca* and *Chironomus riparius* were considered, but only *Hyalella azteca* was used since *Chironomus riparius* values were always less sensitive. Sediment effect concentrations for *Hyalella azteca* are expressed as threshold effect level (TEL) and probable effect level (PEL) (Table 7-19; see Section 7.13) (USEPA 1996b).

The TEL and PEL are sediment concentrations associated with toxicity in laboratory tests. The interpretation is that toxicity rarely occurs below the TEL and frequently occurs above the PEL (USEPA 1996b). Values were derived from a data set consisting of many similar studies, and they consider both effect and no-effect data for field-contaminated sediments. The TEL and PEL values used in this study are listed in Tables 7-19 and 7-20 (see Section 7.13). Since many metals and PAHs were present at sites, partial toxicity contributed by individual chemicals were calculated and summed to estimate the overall toxicity of metals and PAH at each site. TELs and PELs are used with caution because they are based on sediments with multiple contaminants.

The TEL and PEL values were compared with the concentrations seen at the locations of impairment in Table 7-19. As shown in Table 7-19, the most striking result is that no PAH exceeded any criterion level at Impairment A for 1992. For metals only, the TEL for arsenic was exceeded at Impairment A in 1992. At Impairment B and C, the *Hyalella azteca* PEL and TEL were exceeded for all PAH that were measured and in every year except 1992, when there were more samples below the detection limit. *Hyalella azteca* TEL values were exceeded for most metals, but only a few PEL values were exceeded, including those for lead, copper, and chromium.

For PAHs, the cumulative toxic units were exceeded at Impairments B and C in every year (Table 7-7). Exceedances ranged from 339 to 18,820 times the value that would probably kill *Hyalella azteca*. For metals, the cumulative toxic units were also exceeded at Impairments B and C in every year. However, exceedances were never more than six times the cumulative probable effect level.

Table 7-7. Cumulative toxic units for PAHs and metals based on the PEL values. (Values greater than 1.0 exceed PEL*).

Chemical	Cumulative Toxic Units			
	Nearest Upstream Location	Impairment A	Impairment B	Impairment C
PAH	/0\ (0) [1.2]*	/0\ (0) [2.5]*	/604.5* (339.4)* [18819.9]*	/9697.8* (821)* [1633.4]*
Metals	/0.4\ (0.6) [0.9]	/0.7\ (1.1)* [0.9]	/4.3* (5.1)* [1.6]*	/1.5* (2.8)* [5.8]*

* Exceeds PEL and TEL. / \ = 1987-1991, ( ) = 1992, [ ] = 1998. Zero = below detection.

Criteria are also available for ammonia (USEPA 1998b) (Table 7-8). The toxicity of total ammonia (which includes NH_3 and NH_4^+) varies with pH. Dehydration of ammonium ion (NH_4^+) to un-ionized ammonia is controlled by ambient pH, such that excess hydroxide ions (high pH) increase the concentration of the more toxic, un-ionized form. Hard water streams of the Eastern Corn Belt Plains (ECBP) typically have pH from 7.5-8; in the summer, during maximum photosynthesis in nutrient enriched waters, pH may rise above 9.0. In 1998, pH values ranged between 7.4 and 8.4, and appeared to be independent of location. Total ammonia concentrations at RM 5.8 through 2.7 would have exceeded the ammonia criterion for water having a pH 8.0 to 8.5 in 1992 (Table 7-8). In 1998, the criterion would have been exceeded at pH 8.5.

Ohio's criteria for dissolved oxygen (causal candidate #5) are 4.0 mg/l for warm water, and 3.0 mg/l for modified warm water. In 1992, no locations had dissolved oxygen below the modified warm water criterion, and only RM 2.7 had dissolved oxygen concentrations below the warm water criterion, based on a single measurement. However, in 1987, continuous data were collected by Datasonde (in-stream Hydrolab) and violations were detected at Impairments A and B (Table 7-8).

Ohio's proposed state-wide criterion for modified warm-water habitat for nitrate and nitrite is 1.6 mg/L for wadeable streams in the ECBP having a drainage greater than 20 mi^2 and less than 200 mi^2. For total phosphorus, the proposed state-wide criterion for modified warm-water habitat is 0.28 mg/L (Rankin et al. 1999). These are exceeded at RM 5.8 (Table 7-8).

A state-wide study by Yoder and Rankin (1995b) indirectly examined the plausibility of specific community changes associated with nine types of sources, including waste water treatment plants, industrial point sources, conventional municipal sources, combined sewer overflows, channelization, and agricultural non-point sources. They found that deformities, erosions, lesions, tumors and anomalies (DELTA) in fish were associated with industrial discharges (Yoder and Rankin 1995b) and nutrient enrichment (Rankin et al. 1999). In the Little Scioto, the greatest % DELTA values are associated with the greatest nutrient concentrations. Among macroinvertebrates, the loss of Tanytarsini midges and the increase of *Cricotopus sp.* are both associated with industrial discharges (Yoder and Rankin 1995b). In the Little Scioto, the disappearance of Tanytarsini midges and an increase in *Cricotopus* are associated with Impairment C.

Table 7-8. Comparison of the reported concentration of water quality parameters (mg/L) with exceedances.

Sediment Parameter Criteria mg/L	RM 7.9 [RM 7.1]	RM 6.5	RM 5.8 [RM 6.2]
Ammonia[a] 0.57 mg/L at pH 8.5 1.27 mg/L at pH 8.0	(<0.05) [0.11, <0.05]	(0.1)	(1.2) [0.35, 0.69]
Dissolved Oxygen[b] 3.0 mg/L for MWH	{4.6-2.8}* (7.9, 5.7)	{7.2-1.9}* (NA)	{8.3, 4.2} (8.23, 4.21)
Nitrate-nitrite[c] 1.6 mg/L	(1.4) [0.73, <0.1]	(0.8)	(8.1)* [0.33, 2.37]*
Total phosphorus[d] 0.28 mg/L	(0.07) [0.36*, 0.13]	(0.09)	{1.65}* (2.17)* [1.9, 1.21]*

No Entry = No data for that year. { } = 1987, ( ) = 1992, [ ] = 1998.
[a] USEPA (1998b) recommended ammonia criterion
[b] OEPA (1994) dissolved oxygen criterion
[c] Rankin et al. (1999) proposed nitrate-nitrite criterion
[d] Rankin et al. (1999) proposed total phosphorus criterion
* Exceedance of criterion
Dissolved oxygen values are maximum and minimum. Ammonia, nitrate-nitrite, total phosphorus measured in August and October, 1998.

7.9   Characterize Causes: Strength of Evidence

Strength of evidence analysis uses all of the evidence generated in the analysis phase to examine the credibility of each remaining candidate cause. The causal considerations for the strength of evidence analyses used three types of evidence: case-specific evidence, evidence from other situations or biological knowledge, and evidence based on multiple lines of evidence (Section 4.3.3). All the evidence was evaluated for consistency or coherence with the hypothesized causes.

CHARACTERIZE CAUSES

Eliminate   Diagnose   Strength of Evidence

Identify Probable Cause
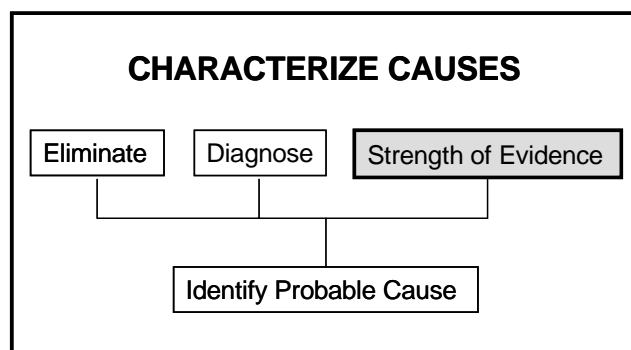
The results of the strength of evidence analysis are presented in Tables 7-9 to 7-11. Following the strength of evidence analysis, the candidate causes are characterized (Table 7-12). This involves describing the causal evidence and identifying the probable cause.

Table 7-9. Strength of evidence analysis for the three candidate causes of Impairment A, RM 7.9.

Causal Considera-tion	Evidence	Score	Evidence	Score	Evidence	Score
Case-Specific Considerations						
	Habitat Alteration		Metals Contamination		Nutrient Enrichment	
Co-occurrence	Compatible: At and below RM 7.9, the habitat of the Little Scioto is altered as a result of channelization. The degree of habitat alteration remains about the same to the mouth of the river. The upstream reference is not channelized and habitat is good.	+	Compatible: All sediment metal concentrations were slightly higher at RM 7.9 compared to upstream.	+	Compatible: N was elevated by 0.2mg/L in 1992 compared to upstream. P is the same or decreases compared to upstream.	+
Temporality	No evidence	NE	No evidence	NE	No evidence	NE
Consistency of Association	No evidence	NE	No evidence	NE	No evidence	NE
Biological Gradient	Not applicable: Other downstream candidate causes interfere with this consideration.	NA	Not applicable: Other downstream candidate causes interfere with this consideration.	NA	Not applicable: Other downstream candidate causes interfere with this consideration.	NA
Complete Exposure Pathway	Evidence for all steps: The fish and invertebrates inhabit the channelized reach where the habitat is altered. Channel was deepened. DO was depressed. Substrate was embedded.	++	Incomplete evidence: No internal concentrations of metals were measured. Metals were present in sediment and exposure could occur from ingestion or by respiration of epibenthic water or sediment particles or through the food chain.	+	Incomplete evidence: Fish and invertebrates inhabit stream where nutrients are elevated. Concentrations of algae or chlorophyll *a* were not measured.	+
Experiment	No evidence.	NE	No evidence.	NE	No evidence.	NE

NE = no evidence; NA = not applicable/not available

Table 7-9 (continued). Strength of evidence analysis for the three candidate causes of Impairment A, RM 7.9.

Causal Considera-tion	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge						
	Habitat Alteration		Metals Contamination		Nutrient Enrichment	
Plausibility: Mechanism	Increased Relative Weight: Plausible: Artificially deepened channel allows larger sized fish to survive.	+	Increased Relative Weight: Implausible: No known mechanism for metals. Metals usually cause a decrease in the relative weight of fish (Eisler 2000b).	-	Increased Relative Weight: Implausible: N is a nutrient for algal growth. Greater production of algae could provide additional food, increasing fish growth. However, the mechanism is implausible because N is generally not limiting (Allan 1995).	-
	Increased DELTA: Not known: No obvious mechanism other than stress.	0	Increased DELTA: Implausible: Metals do not cause fin erosion and lesions (Eisler 2000b).	-	Increased DELTA: Plausible: Nutrients are believed to create conditions that favor opportunistic pathogens and fungi that cause lesions, fin erosion and interfere with wound healing.	+
	Loss of species: Plausible: Embedded sediments remove forage, reproductive, and cover habitats for benthic fish including darters and benthic invertebrates including mayflies. Low DO is not tolerated by many species (Karr and Schlosser 1977, Yount and Niemi 1990, Rankin 1995).	+	Loss of species: Plausible: Metals are known to cause lethal and sub-lethal effects to invertebrates and fish that can extirpate species from a site (Eisler 2000b). Metals usually cause a decrease in the relative weight of fish (Eisler 2000b).	+	Loss of species: Plausible: Switching to an autochthonous energy source could alter species survival and community composition of fish and invertebrates.	+
Plausibility: Stressor-Response	Increased Relative Weight: No evidence.	NE	Increased Relative Weight: Not applicable: Implausible mechanism.	NA	Increased Relative Weight: Not applicable: Implausible mechanism.	NA
	Increased DELTA: No evidence.	NE	Increased DELTA: Not applicable: implausible mechanism.	NA	Increased DELTA: Inconcordant: magnitude of nutrient change too small to cause effect.	-
	Loss of species: No evidence. No quantitative evidence. Habitat alteration associated with channelization is generally believed to be an all or none situation affected by it's spatial extent and severity.	NE	Loss of species: Inconcordant. No metals exceeded *Hyalella azteca* PEL values in 1987, 1992 or 1998. The TEL value for arsenic was exceeded only in 1992. Metals cumulative toxic units exceeded PEL in 1992, but only by 0.1 units (USEPA 1996b).	-	Loss of species: Inconcordant. The magnitude of nutrient change was too small to account for the dramatic shifts in invertebrate and fish metrics. Proposed nitrogen criterion for Ohio was not exceeded (Rankin et al. 1999).	-

NE = no evidence; NA = not applicable/not available

Table 7-9 (continued). Strength of evidence analysis for the three candidate causes of Impairment A, RM 7.9.

Causal Considera-tion	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge (cont'd)						
	Habitat Alteration		Metals Contamination		Nutrient Enrichment	
Consistency of Association	Increased Relative Weight: In most places.	++	Increased Relative Weight: Many exceptions.	-	Increased Relative Weight: No evidence.	NE
	Increased DELTA: In most places.	++	Increased DELTA: Many exceptions.	-	Increased DELTA: Many exceptions. At many sites in Ohio, DELTA was not increased by these levels of N (Rankin et al. 1999).	-
	Loss of species: In most places. Moderate increase in DELTA and loss of species are commonly associated with habitat alteration associated with channelization (Yoder and Rankin 1995b). Increased Relative Weight is also commonly increased with deepened channels (Personal Observation). Agricultural areas with channelization having similar stressors showed decreases in IBI and ICI component metrics (Edwards et al. 1984, Sheilds et al. 1998).	++	Loss of species: Many exceptions. At other sites in Ohio with similar metals concentrations, Relative Weight and DELTA were not increased and species were abundant. Personal observation of Ohio database.	-	Loss of species: Many exceptions. At many sites in Ohio, IBI and ICI scores were high at these levels of N (Rankin et al. 1999). High IBI and ICI cannot be achieved when many species are lost.	-

NE = no evidence; NA = not applicable/not available

Table 7-9 (continued). Strength of evidence analysis for the three candidate causes of Impairment A, RM 7.9.

Causal Considera-tion	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge (cont'd)						
	Habitat Alteration		Metals Contamination		Nutrient Enrichment	
Specificity of Cause	Increased Relative Weight: One of a few: Deep channels or pools required for larger fish. Relative weight of fish is significantly correlated with drainage area, a surrogate for channel depth (Norton 1999).	++	Increased Relative Weight: Not applicable: Implausible mechanism.	NA	Increased Relative Weight: Not applicable: Implausible mechanism.	NA
	Increased DELTA: One of many.	0	Increased DELTA: Not applicable: Implausible mechanism.	NA	Increased DELTA: One of many.	0
	Loss of species: One of many.	0	Loss of species: One of many.	0	Loss of species: One of many.	0
Analogy	Not applicable	NA	Not applicable	NA	Not applicable	NA
Experiment	Increased Relative Weight: No evidence	NE	Increased Relative Weight: No evidence	NE	Increased Relative Weight: No evidence	NE
	Increased DELTA: No evidence	NE	Increased DELTA: No evidence	NE	Increased DELTA: No evidence	NE
	Loss of species: Concordant: Artificial riffle and pools improved invertebrate assemblage in the channelized Olentangy River (Edwards et al. 1984), and fish in Mississippi River (Sheilds et al. 1998).	+++				
Predictive Performance	No evidence	NE	No evidence	NE	No evidence	NE
Considerations from Multiple Lines of Evidence						
	Habitat Alteration		Metals Contamination		Nutrient Enrichment	
Consistency of Evidence	Increased Relative Weight: All consistent.	+++	Increased Relative Weight: Inconsistent: Implausible mechanism.	---	Increased Relative Weight: Inconsistent: Magnitude of change inconsistent with magnitude of effect.	---
	Increased DELTA: All consistent.	+++	Increased DELTA: Inconsistent: Implausible mechanism.	---	Increased DELTA: Inconsistent: Magnitude of change inconsistent with magnitude of effect.	----
	Loss of species: All consistent.	+++	Loss of species: Inconsistent - Although metals are present, the concentrations are unlikely to cause species extirpation.	---	Loss of species: Inconsistent: Magnitude of change inconsistent with magnitude of effect.	---
Coherence of Evidence	Increased Relative Weight, Increased DELTA, Loss of species: None.	0	Increased Relative Weight, Increased DELTA, Loss of species: None.	0	Increased Relative Weight, Increased DELTA, Loss of species: None.	0

NE = no evidence; NA = not applicable/not available

Table 7-10. Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score
Case-Specific Considerations				
	PAH contamination		Metals Contamination	
Co-occurrence	Compatible: Sediment PAH concentrations were several orders of magnitude greater at RM 6.5 than upstream (Table 13).	+	Compatible: Lead, chromium, copper and mercury concentrations in sediment were two to ten times greater at RM 6.5 than upstream. Cadmium and zinc were also greater, but to a lesser degree.	+
Temporality	No evidence	NE	No evidence	NE
Consistency of Association	No evidence: only one location.	NE	No evidence: only one location.	NE
Biological Gradient	Not Applicable: Other candidate causes downstream interfere with this consideration.	NA	Not Applicable: Other candidate causes downstream interfere with this consideration.	NA
Complete Exposure Pathway	Actual evidence for all steps: PAHs were present in the sediment, and bottom-feeding fish and benthic invertebrates are typically exposed to sediment contaminants. Both BAP and NAPH metabolites were found in fish. EROD, a detoxifying enzyme known to be induced by PAH, was elevated.	+ +	Actual evidence for all steps: Metals were present in sediment and exposure could occur from ingestion or by respiration of epibenthic water of sediment particles or through the food chain. Zinc and lead were detected in fish tissues.	+ +
Experiment	No evidence	NE	No evidence	NE

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge				
	PAH contamination		Metals Contamination	
Plausibility: Mechanism	Decreased relative weight: Plausible: PAHs are known to reduce growth. Toxic compounds can shorten life span resulting in smaller fish (Eisler 2000a).	+	Decreased relative weight: Plausible: Metals are known to reduce growth. Toxic compounds can shorten life span resulting in smaller fish (Eisler 2000b).	+
	Increased DELTA: Plausible: PAHs are known to cause eroded barbels, fin erosion, lesions and internal and external tumors (Eisler 2000a).	+	Increased DELTA: Implausible: Metals do not cause fin erosion and lesions	-
	Decreased species: Plausible: PAHs are known to be toxic and cause reproductive impairments which could extirpate species (Eisler 2000a).	+	Decreased species: Plausible: Metals are known to cause lethal and sub-lethal effects to invertebrates and fish that can extirpate species from a site (Eisler 2000b).	+
Plausibility: Stressor-Response	Decreased relative weight: Concordant: Toxic levels are consistent with decreased fish growth.	+	Decreased relative weight: Ambiguous. Toxic levels are consistent with decreased fish growth (Eisler 2000b).	0
	Increased DELTA: Quantitatively consistent: PAHs are at levels that cause tumors and other DELTA.	+++	Increased DELTA: Not applicable: mechanism is implausible.	NA
	Decreased species: Quantitatively consistent: The *Hyalella azteca* PEL's were exceeded for all PAHs. The cumulative PAH toxic units ranged between 339 to 18,820 times the PEL value (USEPA 1996b).	+++	Decreased species: Quantitatively consistent. Lead exceeded *Hyalella azteca* PEL values in 1988-1991 and 1992 and chromium in 1992. The cumulative toxic units values for all metals range from 1.6 to 5.1 (USEPA 1996b).	+++
Consistency of Association	Decreased relative weight: In most places: Decreased relative weight is associated with complex toxic exposures (Yoder and Rankin 1995b).	++	Decreased relative weight: In most places: Decreased relative weight is associated with complex toxic exposures (Yoder and Rankin 1995).	++
	Increased DELTA: Invariant: Tumors and other DELTA are associated with fish exposed to high concentrations of PAH in fresh and marine waters (Albers 1995).	+++	Increased DELTA: Not applicable.	NA
	Decreased species: Invariant: At more than 25 locations associated with PAH contamination that exceeded exposure criteria in Ohio, IBI and ICI scores were below 30 (Cormier et al. 2000a). IBI and ICI are known to be depressed even when habitat quality is high (Cormier et al. 2000b, OEPA 1992a). IBI and ICI scores of less than 30 only occur when some species are extirpated.	+++	Decreased species: In most places: Hickey and Clements (1998) reviewed changes in invertebrate community associated with metals in water column.	++
Specificity of Cause	Decreased relative weight: One of many.	0	Decreased relative weight: One of many.	0
	Increased DELTA: One of many. PAHs are known to cause external lesions seen at Impairment B.	0	Increased DELTA: Not applicable.	NA
	Decreased species: One of many.	0	Decreased species: One of many.	0

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge (cont'd)				
	PAH contamination		Metals Contamination	
Analogy	Not applicable	NA	Not applicable	NA
Experiment	Decreased relative weight: Concordant: Following dredging in the Black River, Ohio, the age structure of the brown bullheads increased (Baumann and Harshbarger 1995).	+++	No evidence: No references sought.	NE
	Increased DELTA: Concordant: In the Black River Ohio, removal of PAHs by dredging resulted in lower levels of DELTA (Baumann and Harshbarger 1995) and PAH bile metabolites (Lin et al. submitted).	+++	No evidence: No references sought.	
	Decreased species: Concordant: Following dredging the composition of species at this site also changed (Baumann, pers. comm.).	+++	No evidence: No references sought.	
Predictive Performance	No evidence	NE	No evidence	NE
Considerations from Multiple Lines of Evidence				
	PAH contamination		Metals Contamination	
Consistency of Evidence	Decreased relative weight: All consistent.	+++	Decreased relative weight: All consistent.	+++
	Increased DELTA: All consistent.	+++	Increased DELTA: Multiple inconsistencies.	---
	Decreased species: All consistent.	+++	Decreased species: All consistent.	+++
Coherence of Evidence			Increased DELTA: No known explanation.	0

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Case-Specific Considerations						
	Ammonia Toxicity		Low Dissolved oxygen/High BOD		Nutrient Enrichment	
Co-occurrence	Compatible: Ammonia concentration was doubled relative to Impairment A.	+	Compatible: In 1992, BOD was double the upstream value and the lowest DO levels measured were 0.9 mg/L less than upstream.	+	Compatible: Compared to RM 7.9, P was elevated by 0.02 mg/L. N was less.	+
Temporality	No evidence	NE	No evidence	NE	No evidence	NE
Consistency of Association	No evidence: Only one location.	NE	No evidence: Only one location.	NE	No evidence: Only one location.	NE
Biological Gradient	Not applicable: Other downstream candidate causes interfere with this consideration.	NA	Not applicable: Other downstream candidate causes interfere with this consideration.	NA	Not applicable: Other downstream candidate causes interfere with this consideration.	NA
Complete Exposure Pathway	Evidence for all steps: Fish and invertebrates inhabited stream where ammonia was present.	+ +	Evidence for all steps: Fish and invertebrates inhabited stream where conditions of low DO and high BOD occurred.	+ +	Evidence for all steps: Fish and invertebrates inhabit stream where P was elevated.	+ +
Experiment	No evidence	NE	No evidence	NE	No evidence	NE

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge						
	Ammonia Toxicity		Low Dissolved oxygen/High BOD		Nutrient Enrichment	
Plausibility: Mechanism	Decreased relative weight: Plausible: Ammonia toxicity could reduce growth and survival. Low survival could alter the age structure resulting in smaller, younger fish.	+	Decreased relative weight: Plausible: Stress could reduce growth and survival. Low survival could alter the age structure resulting in more smaller, younger fish.	+	Decreased relative weight: Implausible: Increased nutrients are usually associated with increased algal growth that augment the energy available for growth.	_
	Increased DELTA: Plausible: Ammonia has been associated with anomalies (Dyer, pers. comm.).	+	Increased DELTA: Not known: No known mechanism.	0	Increased DELTA: Plausible: Nutrients are believed to create conditions that favor opportunistic pathogens and fungi that cause lesions, fin erosion, and interfere with wound healing (Rankin et al. 1999).	+
	Decreased species: Plausible: Ammonia is known to be toxic to fish and invertebrates (USEPA 1998b).	+	Decreased species: Plausible: Low DO can kill fish and invertebrates (Allan 1995).	+	Loss of species: Plausible: Switching to an autochthonous energy source could alter species survival and community composition for fish and invertebrates (Allan 1995).	+
Plausibility: Stressor-Response	Decreased relative weight: No evidence.	NE	Decreased relative weight: No evidence.	NE	Decreased relative weight: Inconcordant.	-
	Increased DELTA: No evidence.	NE	Increased DELTA: Not applicable.	NA	Increased DELTA: Inconcordant.	-
	Decreased species: Inconcordant: The ammonia concentrations were not great enough to cause the dramatic effects seen at Impairment B. Ammonia criteria were not exceeded. (USEPA 1998b).	-	Decreased species: DO levels are below Ohio criteria for MWH (OEPA 1992b).	+	Decreased species: Inconcordant: The magnitude of P change was not great enough to cause dramatic effects seen at Impairment B. Proposed P criterion was not exceeded (Rankin et al. 1999).	-

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge (cont'd)						
	Ammonia Toxicity		Low Dissolved oxygen/High BOD		Nutrient Enrichment	
Consistency of Association	No evidence	NE	No evidence.	NE	Decreased relative weight: Many exceptions.	-
					Increased DELTA: Many exceptions: DELTA are associated with increased P at many sites in Ohio, but at a higher concentration of P (Rankin et al. 1999).	-
					Decreased species: Many exceptions: Reduced species are associated with many sites in Ohio increased P, but at a higher concentration (Rankin et al. 1999).	-
Specificity of Cause	Decreased relative weight: One of many.	0	Decreased relative weight: One of many	0	Decreased relative weight: Not applicable	NA
	Increased DELTA: One of many.	0	Increased DELTA: Not applicable.	NA	Increased DELTA: One of many.	0
	Decreased species: One of many.	0	Decreased species: One of many.	0	Decreased species: One of many.	0
Analogy	Not applicable	NA	Not applicable	NA	Not applicable	NA
Experiment	No evidence: No reference sought.	NE	No evidence: No reference sought.	NE	No evidence: No references sought.	NE
Predictive Performance	No evidence	NE	No evidence	NE	No evidence	NE

NE = no evidence; NA = not applicable/not available

Table 7-10 (continued). Strength of evidence analysis for the five candidate causes of Impairment B, RM 6.5.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Considerations from Multiple Lines of Evidence						
	Ammonia Toxicity		Low Dissolved oxygen/High BOD		Nutrient Enrichment	
Consistency of Evidence	Decreased relative weight: All consistent.	+++	Decreased relative weight: Most consistent.	+	Decreased relative weight: Many inconsistencies.	_ _ _
	Increased DELTA: All consistent.	+++	Increased DELTA: Many inconsistencies: No known mechanism.	_ _ _	Increased DELTA: Many inconsistencies: Magnitude of change inconsistent with magnitude of effect.	_ _ _
	Decreased species: Inconsistent: Magnitude of change inconsistent with magnitude of effect.	_ _ _	Decreased species: Most consistent.	+	Decreased species: Many inconsistencies: Magnitude of change inconsistent with magnitude of effect.	_ _ _
Coherence of Evidence	Decreased species: No known explanation.	0	Increased DELTA: No known explanation.	0	Decreased relative weight, Increased DELTA, Decreased species: No known explanation.	0

NE = no evidence; NA = not applicable/not available

Table 7-11. Strength of evidence analysis for the three candidate causes of Impairment C, RM 5.7.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Case-Specific Considerations						
	Metals Contamination		Ammonia Toxicity		Nutrient Enrichment	
Co-occurrence	Uncertain: There were only slight changes in metal concentrations in sediment at RM 5.7 compared to RM 6.5. Only copper and zinc increased slightly and possibly cadmium. All others declined.	0	Compatible: Ammonia concentrations were 10X or greater than at RM 6.5. from RM 5.7 to RM 2.7	+	Compatible: Total phosphorus and nitrogen concentrations are elevated at RM 5.7 through 2.7. P values are more than 24X greater than at RM 6.5 and more than 10X greater for nitrogen than upstream.	+
Temporality	No evidence	NE	No evidence	NE	No evidence	NE
Consistency of Association	Similar patterns of fish and invertebrate communities are seen at RM 5.7, 4.4 and 2.7	+	Similar patterns of fish and invertebrate communities are seen at RM 5.7, 4.4 and 2.7.	+	Similar patterns of fish communities are seen at RM 5.7, 4.4 and 2.7.	+
Biological Gradient	Increased DELTA: Strong and monotonic: From RM 5.7 to RM 0.4, copper and mercury are strongly correlated with % DELTA.	++	Increased DELTA: None: No correlation of ammonia with % DELTA.	-	Increased DELTA: Strong and monotonic: % DELTA was moderately correlated with BOD, N and P.	++
	Decreased Tanytarsini: Strong and monotonic: The decline in % tanytarsini was also strongly correlated with copper and mercury.	++	Decreased Tanytarsini: None: No correlation of ammonia with the decline in % Tanytarsini.	-	Decreased Tanytarsini: Strong and monotonic: BOD, nitrate-nitrite and phosphorus were all strongly correlated with decline in % Tanytarsini midges and the ICI.	++

NE = no evidence; NA = not applicable/not available

**Table 7-11 (continued).** Strength of evidence analysis for the three candidate causes of Impairment C, RM 5.8

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Case-Specific Considerations (cont'd)						
	Metals Contamination		Ammonia Toxicity		Nutrient Enrichment	
Complete Exposure Pathway	Incomplete evidence: Lead and zinc were detected in water samples (OEPA 1992a). In sediment, many metals were detected. No internal concentrations of metals were measured. Water hardness may have reduced metal availability.	+	Evidence for all steps: Ammonia levels measured in water column, so exposure possible for fish and invertebrates. Ammonia is directly discharged into streams by point sources. Temperature and pH conditions are favorable for forming unionized ammonia, the toxic form of ammonia. Conditions are favorable for conversion of nitrites to ammonia (low DO).	+ +	Incomplete evidence: Nutrient and phosphorus concentrations were measured in water column, and would be available for algal, fungal and bacterial growth. Neither algal nor chlorophyll *a* concentrations, the direct effect of nutrient enrichment, nor bacterial concentrations were not measured.	+
Experiment	No evidence.	NE	No evidence.	NE	No evidence.	NE
Considerations Based on Other Situations or Biological Knowledge						
Plausibility: Mechanism	Increased DELTA: Implausible: Metals do not cause fin erosion and lesions (Eisler 2000b).	-	Increased DELTA: Plausible: Ammonia has been associated with DELTA (Dyer, pers. comm.).	+	Increased DELTA: Plausible: Nutrients are believed to create conditions that favor opportunistic pathogens and fungi that cause lesions, fin erosion and interfere with wound healing (Rankin et al. 1999).	+
	Decreased Tanytarsini: Plausible: Metals are known to cause lethal and sub-lethal effects to invertebrates that can extirpate species from a site. In a literature review, lead and copper were associated with mortality and other metals with mortality, reproduction, growth and behavior changes (Eisler 2000b).	+	Decreased Tanytarsini: Plausible: Ammonia is toxic to benthic macroinvertebrates (USEPA 1998b).	+	Decreased Tanytarsini: Increased nutrients are known to change community structure primarily by changing the food source (Allan 1995).	+

NE = no evidence; NA = not applicable/not available

Table 7-11 (continued). Strength of evidence analysis for the three candidate causes of Impairment C, RM 5.8.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Considerations Based on Other Situations or Biological Knowledge (cont'd)						
	Metals Contamination		Ammonia Toxicity		Nutrient Enrichment	
Plausibility: Stressor-Response	Increased DELTA: Not applicable. Mechanism not plausible.	NA	Increased DELTA: Concordant	+	Increased DELTA: Quantitatively consistent: %DELTA consistent with associations of P concentrations found in streams throughout Ohio (Rankin et al, 1999)	+++
	Decreased Tanytarsini: Ambiguous: The cumulative toxic units exceed PEL by 1.5 to 2.8 times in 1988/91 and 1992, respectively. The cumulative toxic units for PEL decreased compared to upstream in 1988/91 and 1992. In 1998, cumulative PEL was 3.5 times greater than at Impairment B, but this occurred after the impairment had already occurred (USEPA 1996b).	0	Decreased Tanytarsini: Quantitatively consistent: Ammonia concentrations are in a plausible range to cause toxic effects especially on warm, sunny days. Conservatively, ammonia was two times the USEPA chronic criteria (USEPA 1996b).	+++	Decreased Tanytarsini: Concordant. Nutrient criteria are proposed for Ohio and were exceeded at RM 5.7 through RM 0.4 for both nitrate-nitrite and phosphorus. At RM 5.7, nitrogen concentration was five times the proposed criterion value. P concentration was more than seven times the proposed phosphorus criterion (Rankin et al. 1999).	+
Consistency of Association	Increased DELTA: Many exceptions. Ohio EPA database.	-	Increased DELTA: In most places (Rankin et al. 1999).	++	Increased DELTA: In most places (Rankin et al. 1999).	+ +
	Decreased Tanytarsini: No evidence.	NE	Decreased Tanytarsini: No evidence.	NE	Decreased Tanytarsini: No evidence.	NE
Specificity of Cause and Effect	Increased DELTA: Not applicable.	NA	Increased DELTA: One of a few.	++	Increased DELTA: One of a few.	++
	Decreased Tanytarsini: One of many.	0	Decreased Tanytarsini: One of many.	++	Decreased Tanytarsini: One of many.	++
Analogy	Not applicable	NA	Not applicable	NA	Not applicable	NA
Experiment	No evidence	NE	No evidence	NE	No evidence	NE
Predictive Performance	No evidence	NE	No evidence	NE	No evidence	NE

NE = no evidence; NA = not applicable/not available

Table 7-11 (continued). Strength of evidence analysis for the three candidate causes of Impairment C, RM 5.8.

Causal Consideration	Evidence	Score	Evidence	Score	Evidence	Score
Considerations from Multiple Lines of Evidence						
	Metals Contamination		Ammonia Toxicity		Nutrient Enrichment	
Consistency of Evidence	Increased DELTA: Multiple inconsistencies.	---	Increased DELTA: Most consistent.	+	Increased DELTA: All consistent.	+++
	Decreased Tanytarsini: Most consistent. Although metals are toxic the magnitude and type of effect do not seem to indicate that metals caused either the increase % DELTA or shifts in invertebrate metrics. However, mercury and copper are both significantly correlated with % DELTA and % tanytarsini.	0	Decreased Tanytarsini: Most consistent. Ammonia may have toxic effects, but % DELTA not likely to be caused by ammonia. No biological correlation.	+	Decreased Tanytarsini: All consistent. Reasonable evidence to suspect that nitrogen and phosphorus are creating conditions that favor opportunistic pathogens. Proposed criteria values are exceeded and high % DELTA consistent with effects seen even in the absence of toxics. Shifts in invertebrate metrics more uncertain.	++
Coherence of Evidence	Increased DELTA: No known explanation.	0	Increased DELTA: Biological gradient based on few observations and may be confounded by other stressors downstream.	0		
		0	Decreased Tanytarsini: Biological gradient based on few observations and may be confounded by other stressors downstream.	0		

NE = no evidence; NA = not applicable/not available

7.10 Characterize Causes: Identify Probable Causes

Impairment A (RM 7.9). At RM 7.9, there is a decline in IBI and ICI that is characterized by an increase in the relative weight of fish and percent DELTA, a decreased number of fish and species of fish, and a decreased percentage of mayflies. Candidate Causes #2, PAH, #4, ammonia, and #5, low DO/BOD were eliminated (Tables 7-5 and 7-6). Candidate causes #1, habitat alteration, #3, metal

CHARACTERIZE CAUSES

| Eliminate | Diagnose | Strength of Evidence |

Identify Probable Cause

contamination, and #6, nutrient enrichment, were evaluated in a strength of evidence analysis (Tables 7-9, 7-10 and 7-11). An artificially deepened channel was identified as the probable cause for an increase in the relative weight of fish. An embedded stream bed was identified as the probable cause for decreased numbers and species of fish and decreased percentage of mayflies. The stream bed may have been susceptible to becoming embedded due to a lower gradient than upstream. The probable cause for the low but measurable increase in percent DELTA remained uncertain. The strength of evidence analysis strongly supports this causal relationship. The quality of the data is high, and the consistency of the evidence is good.
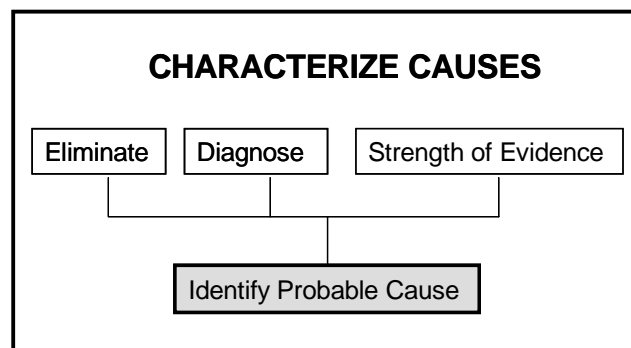
Impairment B (RM 6.5). At RM 6.5, there is a further decline in the IBI and ICI. Specific impairments include an increase in % DELTA, a decrease in the relative weight and numbers of species of fish, and an additional decrease in percent mayflies. Habitat alteration was eliminated as a candidate cause (Tables 7-5 and 7-6). In the strength of evidence analysis a single probable cause, PAHs, was found to be sufficient to cause all of the specific impairments (Tables 7-10 and 7-12). Habitat alteration continued to impair the site but was not the cause of the increased DELTA, decreased relative weight, or the additional decline in the number of species. The strength of evidence analysis strongly supports this causal relationship. The quality of the data is high, and the consistency of the evidence is very good.

Impairment C (RM 5.7). At RM 5.7, there is a notable further increase in % DELTA and a decrease in % Tanytarsini. Altered habitat and PAH still cause impairments, but since the level of alteration remains about the same or decreases, these candidate causes were eliminated (Tables 7-5 and 7-6). In the strength of evidence analysis, nutrient enrichment, candidate cause #6, was identified as the probable cause for both impairments. Nevertheless, ammonia toxicity may still be important. We have moderate confidence in this characterization.

The causal characterization of the Little Scioto River could be strengthened by evidence from published literature that reports associations applying to plausible mechanism and stressor-response, consistency of association, specificity, and others. It was not the intent of this document to prepare an exhaustive list of appropriate evidence, but such a resource is certainly needed to make these types of evidence accessible for future characterizations. This case study does demonstrate the stressor identification process and the importance of clearly presenting the reasoning and evidence.

Table 7-12. Causal characterization.

Impairment A - RM 7.9	Impairment B - RM 6.5	Impairment C - RM 5.7
Probable Cause: Habitat Alteration	Probable Cause: PAH Contamination	Probable Cause: Nutrient Enrichment
Increased Relative Weight: Is probably caused by the artificial deepening of the channel that allows larger fish to live there. Increased DELTA: The percentage of DELTA is commonly associated with channelized streams, but the specific aspect of the channelization that increased DELTA is unknown. Loss of species: Many factors could contribute to the loss of fish and benthic invertebrate species; however, embedded substrates seem to be the most likely stressor since upstream locations had even lower DO levels and yet had a greater variety of fish and invertebrate species. Although metals are present, the likelihood of response at the these concentrations are low. Furthermore, the types of changes in the community, especially an increase in the relative weight of fish, is very unlikely with the candidate cause of metals. Although P levels are slightly higher, effects are not associated with these phosphorous concentration elsewhere and they do not exceed Ohio's proposed criteria values for effects. Candidate Causes #2, PAH, and #4, Ammonia, were eliminated because levels were the same or lower than upstream. Candidate Cause #5, Low DO /BOD , was also eliminated as an overall pathway; however, low DO associated with channelization may still play a roll especially in DELTA. Siltation and deepened channel are consistent with Impairment A. The magnitude of the alteration and clear difference from upstream location strongly support this cause.	A single cause is likely for the three manifestations of Impairment B: decreased relative weight, increased DELTA, and decreased species: The probable cause of Impairment B is toxic levels of PAH-contaminated sediments. All of the evidence support PAH contamination as the cause. There is a complete exposure pathway at the location and clear mechanism of action for each of the effects. The single most convincing piece of evidence is that the cumulative toxic units of PAH were more than 300 times the probable effects level. Metals are at sufficient concentrations to cause effects; however, they were sometimes at levels close to upstream levels and were less than 2% as toxic as the lowest cumulative toxic units of PAH. Metal concentrations are high enough that they should be considered a potentially masked cause. Candidate cause #5 is unlikely because even greater levels of BOD did not cause reduction of dissolved oxygen downstream. Candidate Causes #4, Ammonia, and #6, Nutrient Enrichment, are unlikely given that state criteria levels were met and the much stronger evidence for PAH. Habitat alteration continues to impair the site, but it is not the cause of the increased DELTA, decreased relative weight, or the additional decline in the number of species.	At Impairment C increased % DELTA and % Tanytarsini may have different causes. Increased DELTA in fish is probably caused by increased P and NO$_x$. Nutrients, especially P, have been associated with increased fin erosion and lesions but some uncertainty exists since P acts indirectly. Ammonia is slightly higher than at Impairment B and exceeded ammonia criteria values. Biological gradients were absent; however, this may have been a statistical artifact given the number of sites available to perform the analysis and potential interference from other stressors downstream. Metals are considered unlikely because surface lesions are only occasionally noted as effects from long term exposure and only some metal concentrations were slightly greater than at Impairment B. Metal concentrations are high enough that they should be considered a potentially masked cause. The probable cause of extirpation of Tanytarsini at Impairment C is more uncertain because less is known about the natural history and stressor response relationships of these benthic invertebrates. Candidate cause #6, nutrient enrichment, still seems to be the most likely cause since all of the strength of evidence considerations were consistent. PAH contamination and habitat alteration continue to impair the site, but they are not the cause of the increased % DELTA or extirpation of Tanytarsini. The causal characterization at Impairment C is less certain, but the strength of evidence favors cause #6, increased nutrients.

7.11   Discussion

An important, practical aspect of this study is that even though the primary cause was identified in each case, it is obvious that other causes are also present that would constrain the biological community if the dominant cause was removed. For instance, if PAHs could be independently removed from the river, metals might be high enough to

impair the biological assemblage. Likewise, if metals were removed, habitat alteration would still affect the biological community and would lower IBI and ICI scores at Impairments B and C.

Another issue is the impact of habitat alteration and its influence on modifying the assimilative capacity of the river. In other words, if the physical habitat were improved, would the impacts of PAH contamination be lessened? At Impairment B, this is unlikely based on evidence from at least one river elsewhere that has very good physical habitat qualities, yet has an impoverished biological community replete with high levels of % DELTA due to high PAH concentrations (OEPA 1992b, Cormier et al. 2000b). The strength of evidence analysis can provide these insights for the next step in managing ecosystems, which is to find ways to identify and apportion the sources for the identified causes and then take action to restore and protect the resource.

At Impairment C, a physical habitat that included wetlands, riparian wetlands, and riparian cover might improve the assimilative capacity of the river by providing sinks for the nutrient and ammonia loadings. However, since PAH and metals contamination are still high at Impairment C, removal of nutrient loading alone would result in only a very small improvement in biological condition.

At Impairment B, nutrient enrichment was retained as a candidate cause, even though the increase in phosphorous was minute. Nutrient enrichment was an unlikely cause, but the reasons for it being improbable come from ecological knowledge from examples in other watersheds, not from evidence that permits elimination. The reason nutrient enrichment was retained was because it failed to meet the criteria for elimination. The strength of evidence is the proper way to show this evidence.

There are other uncertainties. Wet weather flow data was not available for review. Events, especially near the combined sewer overflow at RM 6.0, could be undetected sources of candidate causes. Downstream from Impairment C, persistent impairments may have other causes. For instance, BOD is elevated at RM 5.8; however, its effects are usually associated with a certain lag time that results in low DO.

The results from this particular causal analysis could have several practical applications. If it is determined that the river conditions must be improved due to state regulations, federal TMDL (total maximum daily load) rules, citizen action, or other reasons, one option is to remove or decrease all potential stressors identified in the causal analysis; that is, remove both channel modification as well as water and sediment contamination. However, there may be intermediate pathways that may be more cost effective. Factors that should be considered in choosing an option include the desired or expected level of improvement in river condition, and the usefulness of the river's resources versus the cost to restore the river. Another factor to consider is the mode of restoration. For instance, both PAH and metal remediation may require dredging of the contaminated sediments. Knowing which agents (PAH, metals, or a combination of the two) may satisfy our curiosity, but it may not change the management action or ecological outcome. However, it might be determined that knowing the cause is important for assigning the financial responsibility for clean-up. In the latter case, additional information may be needed, especially if restoration costs are high.

7.12   References

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Table 7-13. Fish metrics for the Little Scioto River 1987 and 1992.*

Response	River Mile							
	{9.2} (9.2)	(7.9)	{6.5} (6.5)	{6.0} (5.7)	(4.4)	(3.1)	{2.7} (2.7)	{0.1} (0.3)
Total No. of Species	{19.3} (22)	(13)	{13} (8.3)	{10} (10.7)	(7.7)	{3.3}	{6} (7.7)	{8.7} (9.7)
No. of Darter Species	{5 } (5.5)	(0)	{0} (0)	{0} (0)	(0)	{0}	{0} (0)	{0.3}
No. of Sunfish species	{3} (4)	(5)	{3} (2.3)	{1.3} (3.3)	(3)	{0.7}	{0.3} (2.7)	{1} (2.7)
No. of Sucker Species	{1.3} (2.5)	(3)	{1} (1.3)	{1.7} (1.7)	(1)	{0.7}	{1} (1.3)	{2} (2.7)
No. of Intolerant Species	{1} (1)	0	{0} (0)	{0} (0)	(0)	{0}	{0} (0)	{0}
Percent Tolerant Species	{35.45} (60.69)	(69.12)	{82.43} (85.12)	{94.28} (68.14)	(82.75)	{98.2}	{94.95} (70.85)	{63.41} (38.64)
Percent Omnivores	{33.28} (56.21)	(44.95)	{57.2} (56.72)	{72.47} (46.84)	(71.37)	{94.15}	{85.4} (51.77)	{62.72} (31.92)
Percent Insectivores	{53.41} (35.96)	(53.07)	{40.99} (39.77)	{16.73} (47.8)	(21.91)	{3.95}	{10.18} (42.51)	{32.74} (55.36)
Percent Pioneering Species	{35.49} (69.85)	(28.41)	{22.52} (26.39)	{21.94} (21.76)	(17.81)	{4.05}	{7.58} (23.31)	{5.33} (22.1)
No. of Individuals	{808.5} (1104.6)	(206)	{416.3} (335)	{237.33} (174)	(137)	{84.7}	{237.33} (94)	{78} (75)
Percent Simple Lithophilic Species	{21.24} (12.8)	(18.19)	{2.7} (42.69)	{24.01} (26.2)	(31.45)	{5.88}	{9.14} (24.91)	{19.01} (28.08)
Percent DELTA	{0.13} (0.14)	(1.64)	{0.0} (9.98)	{16.46} (14.51)	(22.37)	{32.8}	{14.22} (10.99)	{16.19} (10.04)
Relative Weight	{4.021} (8.6)	(74.9)	{34.2} (38.7)	{29.773} (17.031)	(7.2)	{10.7}	{24.482} (6.3)	{46.079} (21.1)
IBI	{332 (33)	(23)	{24} (19)	{14} (19)	(18)	{12}	{13} (19)	{14} (25)

Table 7-14. Macroinvertebrate metrics for the Little Scioto River 1987 and 1992.*

Response	River Mile							
	{9.2} (9.2)	(7.9)	{6.5} (6.5)	{5.8} (5.7)	(4.4)	{3.2}	{2.7} (2.1)	{0.4} (0.4)
Total Number of Macroinvertebrates	{773} (1464)	(1952)	{1116} (2815)	{207} (1600)	(1899)	{763}	{1779} (5242)	{645} (1151)
Total No. of Taxa collected at a Site, both Qualitative and Quantitative	{51} (47)	(38)	{38} (29)	{26} (32)	(27)	{28}	{28} (41)	{24} (37)
Total No. of Quantitative Taxa	{34} (36)	(30)	{25} (18)	{13} (18)	(20)	{14}	{13} (23)	{16} (26)
No. of Mayfly Taxa	{6} (7)	(2)	{3} (2)	{2} (2)	(1)	{1}	{0} (2)	{2} (3)
No. of Caddisfly Taxa	{2} (3)	(0)	{2} (0)	{0} (0)	(1)	{0}	{0} (3)	{0} (1)
No. of Dipteran Taxa	{19} (20)	(18)	{15} (12)	{7} (13)	(13)	{10}	{11} (14)	{12} (16)
No. of Qualitative EPT Taxa	{10} (8)	(1)	{2} (0)	{1} (1)	(0)	{4}	{2} (6)	{1} (2)
Percent Mayfly Taxa	{56.016} (58.811)	(16.393)	{20.251} (5.009)	{3.382} (2)	(0.263)	{1.573}	{0} (0.114)	{1.24} (3.215)
Percent Caddisfly Taxa	{4.657} (6.557)	(0)	{0.179} (0)	{0} (0)	(0.053)	{0}	{0} (0.267)	{0} (0.087)
Percent Tanytarsini Midges	{1.552} (3.347)	(3.381)	{4.48} (2.345)	{0.966} (0)	(0)	{3.67}	{0} (0.343)	{0} (2.085)
Percent Dipterans	{26.132} (32.445)	(74.795)	{55.018} (57.336)	{37.198} (91)	(74.829)	{95.937}	{23.834} (97.138)	{61.085} (91.659)
Percent Non-insects	{7.762} (1.639)	(5.43)	{20.43} (37.549)	{56.039} (7)	(21.959)	{0.524}	{75.998} (2.461)	{37.674} (4.344)
Percent Tolerant Organisms	{4.916} (8.607)	(15.061)	{37.993} (77.371)	{61.353} (67.75)	(54.766)	{20.315}	{89.545} (29.569)	{52.713} (59.34)
Percent *Cricotopus*	{0.388} (0.48)	(0)	{6.631} (0)	{0} (8)	(2.53)	{0}	{4.947} (0.301)	{4.961} (2.172)
ICI	{40} (38)	(16)	{22} (8)	{8} (6)	(10)	{8}	{4} (18)	{6} (18)

* { } = 1987; ( ) = 1992

Table 7-15. QHEI metrics for the Little Scioto River 1987 and 1992.*

Metric	River Mile								
	{9.2} (9.2)	(7.9)	{6.5} (6.5)	{6.0}	(5.7)	(4.4)	{3.1}	{2.7} (2.7)	{0.1} (0.3)
Substrate	{18} (16)	(5)	{1} (1)	{1}	(1)	(5)	{1}	{1} (5)	{1} (5)
Cover	{10} (14)	(10)	{9} (11)	{13}	(10)	(10)	{11}	{13} (11)	{12} (9)
Cover Types	{3} (6)	(4)	{2} (6)	{6}	(4)	(4)	{4}	{6} (6)	{5} (6)
Channel	{18} (17)	(10)	{6} (10)	{10}	(10)	(10)	{11}	{10.5} (10)	{10} (7)
Riparian	{9} (6)	(5.5)	{4} (4)	{4}	(6)	(6)	{5}	{6} (8)	{8} (5.5)
Pool	{8} (11)	(8)	{6} (8)	{8}	(9)	(6)	{6}	{8} (8)	{8} (8)
Riffle	{5} (6)	(0)	{0} (0)	{0}	(0)	(0)	{0}	{0} (0)	{0} (0)
Gradient	{6} (6)	(4)	{4} (4)	{4}	(4)	(2)	{2}	{2} (2)	{4} (4)
QHEI	{74} (76)	(42.5)	{30} (38.5)	{40}	(40)	(39)	{36}	{40.5} (42)	{43} (38.5)

* { } = 1987; ( ) = 1992

Table 7-16. Average concentrations of selected sediment organic compounds (mg/kg) in the Little Scioto River, Ohio, by river mile in 1987, 1991, 1992 and 1998.*

Compound	River Mile								
	[11.1]	/9.42\ (9.5) [9.21]	(7.9)	/7.15 \ [7.09]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.7) [2.65]	(0.4)
Acenaphthene	[0.59]ND	(ND) [0.7]ND	(ND)	[0.047]J	{14.8} (ND) [760]J	/150\ (5) [5]	(4.3)	{1.3} (ND) [0.930]J	(ND)
Anthracene	[0.59]ND	(ND) [0.70]ND	(ND)	[0.037]J	{66.8} (ND) [100]J	/360\ (27.1) [41]	(7.9)	{2.3} (ND) [3.7]	(3.3)
Benzo(a)anthracene	[0.072]J	(ND) [0.043]J	(ND)	/15\J [0.059]J	{44.7} /15\J (8.2)J [310]J	/185\ (16.5) [42]J	(6.9)	{4.3} (2)J [8.2]	(15.8)
Benzo(b)fluoranthene	[0.068]J	(ND) [0.052]J	(ND)	/25\ [0.051]J	{23.6} /20\J (18.1) [200]J	/215\ (16.8) [95]	(6.9)	{2.0} (1.6)J [12]	(13.8)
Benzo(k)fluoranthene	[0.058]J	(ND) [0.052]J	(ND)	[0.046]J	{213.2} (9.9)J [160]J	(12.87) [80]	(4.6)	{21.3} (ND) [10]	(10.5)

Table 7-16 (continued). Average concentrations of selected sediment organic compounds (mg/kg) in the Little Scioto River, Ohio, by river mile in 1987, 1991, 1992 and 1998.*

Compound	River Mile								
	[11.1]	/9.42\ (9.5) [9.21]	(7.9)	/7.15 \ [7.09]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.7) [2.65]	(0.4)
Benzo(ghi)perylene	[0.052]J	(ND) [0.044]J	(ND)	/10\J [0.030]J	{144.1} (49.5) [150]ND	/65\ (11.2) [19]	(4.9)	{16.5} (ND) [13]	(6.9)
Benzo(a)pyrene	[0.067]J	(ND) [0.053]J	(ND)	/10\J [0.043]J	{141.1} (14.8)J [210]J	/125\ (15.8) [14]	(7.2)	{11.4} (ND) [12]	(11.5)
Chrysene	[0.087]J	(ND) [0.065]J	(ND)	/15\J [0.081]J	{119.5} /15\J (16.5) [390]J	/305\ (20.8) [13]	(9.9)	{9.7} (1.6)J [13]	(ND)
Dibenzo(a,h)anthracene	[0.59]ND	(ND) [0.7]ND	(ND)	[0.56]ND	{33.3} (ND) [150]ND	(4.6) [16]ND	(ND)	{2.1} (ND) [3.7]	(3.3)
Fluoranthene	[0.19]J	(ND) [0.097]J	(ND)	/20\J [0.20]J	{78.4} /50\ (8.2)J [100]J	/550\ (37.6) [44]J	(13.5)	{6.3} (ND) [14]	(22.4)

Table 7-16 (continued). Average concentrations of selected sediment organic compounds (mg/kg) in the Little Scioto River, Ohio, by river mile in 1987, 1991, 1992 and 1998.*

Compound	River Mile								
	[11.1]	/9.42\ (9.5) [9.21]	(7.9)	/7.15 \ [7.09]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.7) [2.65]	(0.4)
Fluorene	[0.590]ND	(ND) [0.70]ND	(ND)	[0.059]J	{18.3} (ND) [830]J	/200\ (7.0) [20]	(4.0)	{1.2} (ND) [0.98]J	(ND)
Indeno(1,2,3-cd)pyrene	[0.045]J	(ND) [0.037]J	(ND)	/5\J [0.56]ND	{156.0} (13.2)J [150]ND	/60\ (14.5) [16]	(6.6)	{18.6} (ND) [10]	(10.5)
Naphthalene	[0.59]ND	(ND) [0.70]ND	(ND)	[0.56]ND	{22.9} (ND) [260]	/70\ (4.6) [18]J	(ND)	{1.6} (ND) [0.28]J	(ND)
Phenanthrene	[0.14]J	(ND) [0.053]J	(ND)	[0.11]J	{88.3} /40\J (ND) [230]	/470\ (24.1) [38]J	(12.9)	{2.0} (ND) [2.8]J	(2.6)J

* { } = 1987; / \ = 1991; ( ) = 1992; [ ] = 1998

Table 7-16 (Continued). Average concentrations of selected sediment organic compounds (mg/kg) in the Little Scioto River, Ohio, by river mile in 1987, 1991, 1992 and 1998.*

Compound	River Mile								
	[11.1]	/9.42\ (9.5) [9.21]	(7.9)	/7.15 \ [7.09]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.7) [2.65]	(0.4)
Pyrene	[0.2]J	(ND) [0.1]J	(ND)	/15\J [0.2]J	{67.5} /30\J (ND) [810]J	/405\ (23.8) [32]J	(10.2)	{5.2} (ND) [10]	(17.5)

{ } = 1987 data from OEPA 1988, sample depth unknown
/ \ = 1991 data from OEPA 1992a, sample depth unknown
( ) = 1992-93 data from OEPA 1994, sample from 1-6" except RM 7.9 sample from 8-12"
[ ] = 1998 data from OEPA unpublished, sample depth unknown
J is an estimated value that is above zero but below the practical quantitation limit.

Table 7-17. Average concentrations (mg/kg) of selected metals in sediment from the Little Scioto River, Ohio, by river mile in 1987, 1991,1992 and 1998.*

Metal	River Mile								
Arsenic	[11.1]	/9.4\ (9.5) [9.2]	(7.9)	/7.2\ [7.1]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.67) [2.65]	(0.36)
	[3.6]J	/<10\ (<10) [8.3]J	(12.4)	/<10\ [6.0]J	{11.2} /<10\ (<10) [10.8]J	/<10\ (13.8) [9.8]J	(11.3)	{9.49} (<10) [9.0]	(<10)
Cadmium	[0.1]ND	/<1.0\ (<1.0) [0.1]ND	(<1.0)	/<1.0\ [0.2]	{1.8} /3.4\ (<1.0) [0.1]ND	/1.0\ (<1.0) [2.0]	(10.5)	{4.39} (1.0) [1.4]	(1.6)
Chromium	[8.1]J	/5.8\ (7.3) [14.3]J	(13.6)	/13.2\ [8.9]	{47.6} /415\ (208) [32.3]J	/39.2\ (60.9) [50.4]	(302)	{134} (71.2) [77.1]	(48.6)
Copper	[15.7]	(7.4) [24.1]	(17.2)	[22.9]	{68} (79) [39.2]	(56.0) [133]	(76.8)	{83} (42.4) [79.3]	(24.5)
Lead	[23.8]	/<10\ (12.1) [20.4]	(19.1)	/25.5\ [24]J	{170} /175.5\ (172) [46.4]	/59.5\ (84.6) [220]J	(93.4)	{160} (108) [180]J	(38)
Mercury	[0.1]ND	/<0.1\ (<0.1) [0.2]J	(<0.1)	/<0.1\ [0.1]J	/0.3\ (0.33) [0.3]J	/0.2\ (0.2) [0.6]J	(0.8)	(0.12) [0.4]J	(<0.1)

Table 7-17 (continued). Average concentrations (mg/kg) of selected metals in sediment from the Little Scioto River, Ohio, by river mile in 1987, 1991,1992 and 1998.*

Metal	River Mile								
	[11.1]	/9.4\ (9.5) [9.2]	(7.9)	/7.2\ [7.1]	{6.5} /6.6\ (6.5) [6.6]	/5.8\ (5.8) [6.2]	(4.4)	{2.7} /2.7\ (2.67) [2.65]	(0.36)
Zinc					{187}			{760}	
	[48.2]	(30.6) [81.4]	(79.0)	[66.6]	(173) [89.2]	(141) [280]J	(226)	(408) [316]J	(96.8)

{ } = 1987 data from OEPA 1988, sample depth unknown
/ \ = 1991 data from OEPA 1992, sample depth unknown
( ) = 1992-93 data from OEPA 1994, sample from 1-6" except RM 7.9 sample from 8-12"
[ ] = 1998 data from OEPA unpublished, sample depth unknown
J is an estimated value that is above zero but below the practical quantitation limit.

Table 7-18. Average concentrations of selected water chemistry parameters (mg/L) in the Little Scioto River, Ohio, by river mile in 1987, 1992 and 1998.*

Compound	River Mile								
	[11.1]	(9.2) [9.2]	{7.9} (7.9)	[7.1]	{6.5} (6.5) [6.2]	{5.8} (5.8)	{4.4} (4.4)	{2.7} (2.7) [2.7]	{0.4} (0.4)
Ammonia	[0.1,0.3]	(<0.05) [<0.05,<0.05]	(<0.05)	[0.11, <0.05]	(0.12)	(1.16) [0.35, 0.69]	(1.44)	(2.10) [0.67, 1.1]	(0.58)
Dissolved oxygen**		(12.2, 8.8)	{4.6, 2.8} (7.9, 5.7)		{7.27, 1.9}	{8.3, 4.2} (8.23, 4.21)	{8.8, 3.2} (5.2, 4.3)	{6.67, 2.0} (4.1, 3.0)	{6.74, 2.5} (5.6, 4.4)
BOD	[<2.0, 6.6]	(1.0) [<2.0, <2.0]	(1.0)	[<2.0, 2.1]	(2.3)	(4.7) [4.6,13]	(4.2)	(3.5) [3.3, 4.1]	(2.2)
Nitrate-nitrite, NO$_x$	[0.7,3.3]	(1.2) [0.4, 0.2]	(1.4)	[0.73, <0.1]	(0.8)	(8.1) [0.33, 2.37]	(6.6)	(4.5) [3.5, 0.9]	(4.47)
Phosphorus, total P	[0.5,0.6]	(0.06) [1.8, 0.1]	(0.07)	[0.36, 0.13]	(0.09)	{1.65} (2.17) [1.9, 1.21]	(1.96)	{2.71} (1.80) [1.18, 1.31]	(1.34)
Hardness, CaCO$_3$	[222,250]	(329) [275, 269]	(327)	[281, 407]	(389)	(278) [224, 261]	(280)	(306) [228, 210]	(320)

*   { } = 1987 (OEPA 1988b; ( ) = 1992-1993 (OEPA 1994) [ ] = 1998 (OEPA August and October, unpublished data).
** Dissolved Oxygen   {maximum, minimum}, data from 1987 (OEPA, 1988b).
    (maximum, minimum from box plots), data from 1992 (OEPA, 1994.

Table 7-19. PAH concentrations at nearest upstream location and locations of impairments (mg/kg). (*Hyalella azteca* sediment effects concentrations, PEL and TEL, normalized to sediment WET weight.)

Chemical		PAH sediment concentration			
PEL	TEL	Nearest Upstream Location	Impairment A	Impairment B	Impairment C
Benzo(a)pyrene (BAP) 0.32	0.03	(0) [0.053] #	(0) [0.043] #	/141.1\ * (14.8) * [210] *	/125\ * (15.8) * [14] *
Naphthalene (NAPH) 0.14	0.02	(0) [0]	(0) [0]	/22.9* (0) [260]*	/70\ * (4.6) * [18] *
Fluorene 0.15	0.01	(0) [0]	(0) [0.059] #	(0) [830] *	/200\ * (7) * [20] *
Phenanthrene 0.41	0.02	(0) [0.053] #	(0) [0.11] #	(0) [230] *	/470\ * (24.1) * [38] *
Anthracene 0.17	0.03	(0) [0]	(0) [0.037] #	(0) [100] *	/360\ * (27.1) * [41] *
Fluoranthene 0.32	0.04	(0) [0.097] #	(0) [0.2] #	(8.2) * [100] *	/550\ * (37.6) * [44] *
Pyrene 0.49	0.02	(0) [0.076] #	(0) [0.16] #	(0) [810] *	/405\ * (23.8) * [32] *
Benzo[a]anthracene 0.28	0.03	(0) [0.043] #	(0) [0.059] #	(8.2) * [310] *	/185\ * (16.5) * [42] *
Chrysene 0.41	0.02	(0) [0.065] #	(0) [0.081] #	(16.5) * [390] *	/305\ * (20.8) * [13] *
Benzo(g,h,i)perylene 0.25	0.01	(0) [0.044] #	(0) [0.03] #	(49.5) * [150] *	/65\ * (11.2) * [19] *

(*) exceeds PEL and TEL; (#) exceeds TEL. / \ = 1987-1991, ( ) = 1992, [ ] = 1998. Zero = below detection; No Entry = No data for that year

Table 7-20. Metals concentrations at nearest upstream location and locations of impairments (mg/kg). (*Hyalella azteca* sediment effects concentrations, PEL and TEL, normalized to sediment wet weight.)

Chemical		Nearest Upstream Location	Impairment A	Impairment B	Impairment C
PEL	**TEL**				
As		/5\	/8\	/11.2\ #	/8\
		(5)	(12.4) #	(0)	(13.8) #
48.4	10.8	[8.3]	[6]	[10.8] #	[9.8]
Cd		/0.5\	/0.5\	/1.8\ #	/1\
		(0.5)	(0.5)	(0.5)	(0.5)
3.2	0.58	[0]	[0.2]	[0.1]	[2] #
Cr		/5.8\	/13.2\	/47.6\ #	/39.2\ #
		(7.3)	(13.6)	(208) *	(60.9) #
119.4	32.3	[14.3]	[8.9]	[32.3] #	[50.4] #
Cu				/68\ #	
		(7.4)	(17.2)	(79) #	(56) #
101.2	28	[24.1]	[22.9]	[39.2] #	[133] *
Pb				/170\ *	/59.5\ #
		(12.1)	(19.1)	(172) *	(84.6) *
81.7	37.2	[20.4]	[24]	[46.4] #	[220] *
Zn				/187\ #	
		(30.6)	(79)	(173) #	(141) #
544	98.1	[81.4]	[66.6]	[89.2] #	[280] #

(*) exceeds PEL and TEL; (#) exceeds TEL. *ND= not detected, NA = not available, / \ = 1987-1991, ( ) = 1992, [ ] = 1998. Zero = below detection; No Entry = No data for that year

APPENDIX A
OVERVIEW OF WATER MANAGEMENT PROGRAMS SUPPORTED BY THE SI

Appendix A

Overview of Water Management Programs Supported by the SI

The following sections describe several major water management programs and how the SI process can support them.

A.1 Water Quality Assessment Reports Under CWA Section 305(b)

In 1987, EPA's Office of Water recommended that regulatory authorities increase the use of biological monitoring to better characterize aquatic systems. State and Tribal agencies were directed to protect the fishable and swimmable goals of the Clean Water Act. Under Section 305(b), States, Territories, the District of Columbia, interstate water commissions, and participating American Indian Tribes are required to assess and report on the quality of their waters (USEPA 1997). The results of 305(b) assessments are not raw data, but rather are statements about the degree to which each waterbody supports the uses designated in state or tribal water quality standards. Each State and Tribe aggregates these assessments and extensive programmatic information in a 305(b) report, which is a detailed document usually including information from multiple agencies. EPA then uses individual 305(b) reports to prepare a biennial National Water Quality Inventory Report to Congress. This report is the primary vehicle for informing Congress and the public about water quality conditions in the United States.

Most of the information contained in 305(b) assessments is based on data collected and evaluated by states, tribes, and other jurisdictions over the two-year period immediately preceding issuance of the report. The Report to Congress contains national summary information about water quality conditions in rivers, lakes, estuaries, wetlands, coastal waters, the Great Lakes, and groundwater. The report also contains information about public health and aquatic ecosystem concerns, water quality monitoring, and state and federal water pollution management programs.

States and Tribes base their 305(b) water quality determinations on whether waterbodies are clean enough to support basic uses, such as aquatic life, swimming, fishing, and drinking supply. These uses, along with appropriate national criteria and anti-degradation statements, are part of the water quality standards set by each state or tribe to protect its waters. These standards must be approved by EPA.

Water quality for each individual use is rated as either:

- ► Good/Fully Supporting
- ► Good/Threatened
- ► Fair/Partially Supporting
- ► Poor/Not Supporting
- ► Poor/Not Attainable

For waterbodies with more than one use, information is consolidated into a summary use support designation of general water quality conditions. These uses are characterized as either:

- ▸ Good/Fully Supporting All Uses
- ▸ Good/Threatened for One or More Uses
- ▸ Impaired for One or More Uses

Once a state or tribe has determined, under section 305(b), that a waterbody is impaired for one or more uses, the state or tribe is required to identify the source and cause of impairment. Some causes are much easier to identify than others. For example, a case where impairment is caused by a specific chemical from a point source discharge might be straightforward and easily analyzed. Monitoring programs, however, must deal with impacts caused not only by chemical toxicity, but also conventional pollutants (e.g., temperature, pH and dissolved oxygen) and anthropogenic pollutants from non-point sources. Monitoring agencies need the ability to evaluate the relative impact that a particular pollutant or other stressor has on the biological integrity of a receiving water.

A.2   303(d) Lists and TMDLs

Section 303 of the 1972 Clean Water Act requires States, Territories and authorized Tribes to establish water quality standards and Total Maximum Daily Loads TMDLs) for EPA review and approval. Water quality standards identify the uses for each waterbody (e.g., drinking water supply, contact recreation, aquatic life support) and the water quality criteria to support that use. Water quality criteria can be either numeric (e.g., no more than 10 μg/L of copper) or narrative (e.g., nutrients are not to exceed levels which cause an imbalance of aquatic flora and fauna). Water quality standards also include antidegradation policies to prevent deterioration of existing high quality waters.

Under Section 303(d), States, Territories and authorized Tribes must identify impaired waters and establish TMDLs for these waters. Impaired waters are those that do not meet applicable water quality standards, even after point sources of pollution have installed the minimum required levels of pollution control technology. States, Territories and authorized Tribes are required to submit their list of impaired every two years.

States, Territories and authorized Tribes are required to establish priority rankings for impaired waters on the 303(d) lists and develop TMDLs for these waters. A TMDL specifies the maximum amount of a pollutant that a waterbody can receive and still meet water quality standards, and allocates pollutant loadings among point and nonpoint pollutant sources. EPA must approve or disapprove lists and TMDLs established by States, Territories and authorized Tribes. If a State, Territory or authorized Tribe submission is inadequate, EPA must identify the impaired waters and establish the TMDL.

TMDLs are a critical component of the water quality program. They provide the analytic underpinning for watershed decisions and promote integrated program planning, implementation, and funding. For example, controlling sediment and/or nutrient loadings can protect aquatic habitat, wetlands, endangered species, and drinking water sources. As requirements are strengthened and public communication emphasized, sound procedures for identifying stressors and management solutions will become more important.

Development of a TMDL varies based on numerous factors including environmental setting, waterbody type, source type/behavior, and pollutant type/behavior. However, TMDL development generally includes the following activities:

1. Problem Identification: characterization of the impairment and identification of the pollutant causing the impairment;

2. Identification of Water Quality Targets: establishment of the TMDL endpoint or target value, which is typically the applicable numeric water quality criterion or a numeric interpretation of the narrative water quality standard;

3. Source Assessment: estimation of the point, nonpoint and background sources of pollutants of concern, including magnitude and location of sources;

4. Allocations: identification of appropriate wasteload allocations for point sources and load allocations for nonpoint sources;

5. Link Between Numeric Target(s) and Pollutant(s) of Concern: Analysis of the relationship between numeric target(s) and identified pollutant sources. For each pollutant, describes the analytical basis for conclusion that sum of wasteload allocations, load allocations, and margin of safety does not exceed the loading capacity of the receiving water(s).

6. Calculation of the explicit or implicit margin of safety for each pollutant and description of accounting for seasonal variations and critical conditions in the TMDL.

A.2.1 Causes for Impairment: Pollutants and Pollution

Waterbodies are impaired by a variety of stressors. Recent data indicate that the top causes for impairment include sedimentation/siltation/turbidity and suspended solids (16%), nutrients (13%), pathogens (13%), and dissolved oxygen (10%). These stressors are often associated with sources or activities that fall under the Clean Water Act definition of *pollutant*, or *pollution*. Pollution is defined in Section 502(19) as the "man-made or man-induced alteration of the chemical, physical, biological, and radiological integrity of water."

Section 303(d) requires the identification and listing of all impaired waterbodies regardless of the origin or source of the pollution or pollutant. Current regulations require that TMDLs be calculated only for *pollutants*. Pollutants are defined in Section 502(6) as "dredged spoil, solid waste, incinerator residue, sewage, garbage, heat, and industrial, municipal, and agricultural waste discharged into water."

Both *pollution* and *pollutants* are "stressors" that can be identified and evaluated using the SI process. Under current regulations, those calculating TMDLs will benefit directly from guidance on identifying stressors considered *pollutants* under the Clean Water Act. The SI guidance can also assist in establishing the causal linkage between a pollutant and the biological impairment, and thus provide a basis for the development of a TMDL. For example, if a pollutant causes ecosystem changes that alter the fish community, the

altered biological community is an impairment that can be traced to a pollutant for which a TMDL can be calculated.

A.2.2 EPA Actions to Implement the TMDL Program

In an effort to speed the Nation's progress toward achieving water quality standards and improving the TMDL program, EPA began, in 1996, a comprehensive evaluation of EPA's and the states' implementation of their Clean Water Act section 303(d) responsibilities. EPA convened a committee under the Federal Advisory Committee Act, composed of 20 individuals with diverse backgrounds, including agriculture, forestry, environmental advocacy, industry, and state, local, and tribal governments. The committee issued its recommendations in 1998. These recommendations were used to guide the development of proposed changes to the TMDL regulations, which EPA issued in draft in August, 1999. After a long comment period, hundreds of meetings and conference calls, much debate, and the Agency's review and serious consideration of over 34,000 comments, the final rule was published on July 13, 2000. However, Congress added a "rider" to one of their appropriations bills that prohibits EPA from spending FY2000 and FY2001 money to implement this new rule. The current rule remains in effect until 30 days after Congress permits EPA to implement the new rule. TMDLs continue to be developed and completed under the current rule, as required by the 1972 law and many court orders. The regulations that currently apply are those that were issued in 1985 and amended in 1992 (40 CFR Part 130, section 130.7). These regulations mandate that states, territories, and authorized tribes list impaired and threatened waters and develop TMDLs.

A.2.3 Stressor Identification and the TMDL Program

EPA developed the SI process to assist water resource managers in identifying and delineating stressors causing biological impairments to waterbodies. While not all water quality impairments listed under 303(d) are linked directly to biological components of waterbodies, a sample of submittals from 19 states indicate that approximately one-half of waterbodies listed as impaired under 303(d) are not meeting biological designated uses (e.g., aquatic life, cold water fishery). The SI process will have direct utility to States, Tribes, and EPA by providing sound approaches to evaluating the causes of biological impairments under the TMDL Program.

As used in the SI process, the term *stressor* is synonymous with the terms *pollutant* and *pollution* which, under Section 303(d), are considered causes of impairment. The identification of *pollutant* stressors resulting in biological impairment to waterbodies, and the diagnostic evaluation of the sources of these stressors, is an essential first step in calculating Total Maximum Daily Loads under Section 303(d) of the Clean Water Act. For *pollution* stressors (e.g., habitat degradation, water control structures), for which TMDLs are not calculated, SI results can be used to identify the sources of the pollution for use in alternative watershed management activities.

A.3   State/Local Watershed Management

Since 1991, EPA has promoted a watershed protection approach to help address the nation's remaining water resource challenges (USEPA 1991a). The watershed approach is an integrated, holistic strategy for protecting and managing surface water and groundwater resources by watershed, a naturally defined hydrologic unit. For any given watershed, the approach considers not only the water resource; such as a stream, river,

lake, estuary, or aquifer; but all of the land from which water drains into that resource. The watershed approach uses all aspects of water resource quality—physical (e.g., temperature, flow, mixing, habitat); chemical (e.g., conventional and toxic pollutants, such as nutrients and pesticides); and biological (e.g., health and integrity of biotic communities, biodiversity).   EPA's Office of Water has worked to orient and coordinate point source, non-point source, surface water, wetlands, coastal, groundwater, and drinking water programs within a watershed context.

The watershed approach is not a program but a way to organize programs, so that the use of SI will vary with the program conducting the investigation. The watershed approach, however, can facilitate an SI investigation since information is already integrated from various sources, such as point source discharges and non-point source runoff. This integrated information can help investigators make sense of disturbances through knowledge of potential sources of stressors that might feed into that location or might affect the food source or some other essential ecosystem component by affecting the natural continuum (Vannote et al. 1980).

The challenge for identifying stressors for watershed-based programs is proper scaling. Even though the SI may be initiated by a program using the watershed approach, the impairment may not be watershed wide. Impairment to the biological system may be difficult to determine on a watershed scale. Similarities among biota tend to follow ecoregions, rather than watersheds. Several ecoregions may exist within a watershed, especially where elevation differences are great. The biota within any given ecoregion may respond differently to a given stressor than the biota within a neighboring ecoregion. Accurate scaling of the problem is important any time a biological impairment is found, but especially with the watershed approach, to ensure that the information is used to full advantage in identifying and characterizing stressors.

A.4   Non-point Source 319 Management

The 1987 Water Quality Act Amendments to the Clean Water Act added section 319, which established a national program to assess and control non-point source (NPS) pollution. Under this program, states and tribes are asked to assess their NPS pollution problems and submit their assessments to EPA. The assessments included a list of navigable waters within the State or Tribal Territories, which without additional action to control NPS pollution, cannot reasonably be expected to attain or maintain applicable water quality standards or the goals and requirements of the Clean Water Act. Section 319 also requires identification of categories and subcategories of NPS pollution that contribute to impairment of waters, descriptions of procedures for identifying and implementing best management practices, control measures for reducing NPS pollution, and descriptions of State, Tribal, and local programs used to abate NPS pollution.

NPS programs need to identify and control NPS pollutants. Since NPS pollutants can be difficult to trace, identifying the source of these pollutants is probably the greatest challenge for NPS programs. The SI process can help investigators obtain greater confidence that stressors have been accurately identified. Attributing responsibility to a particular source can be very straightforward and obvious or very difficult. Mechanisms used to attribute responsibility need to be assessed for each situation, and common sense should be used. For example, runoff may be obviously coming from one farm. In another situation, runoff may encounter multiple potential sources of pollution, including a poultry farm, a cattle feedlot, and an abandoned mine. In the latter situation, if nutrient

loading is the identified stressor, attributing responsibility between the poultry farm and cattle feedlot may be difficult, but ruling out the abandoned mine would be simple.

A.5   Permitting Programs

A.5.1 NPDES Permits

All discrete sources of wastewater are required to obtain a National Pollutant Discharge Elimination System (NPDES) permit (or State equivalent) that regulates the facility's discharge of pollutants. This approach to controlling and eliminating water pollution is focused on pollutants determined to be harmful to receiving waters and sources of such pollutants. Authority for issuing NPDES permits is established under Section 402 of the CWA. A summary of the Water Quality-based "Standards to Permits" Process for Toxics Control (adapted from the Technical Support Document for WQ-based Toxics Control, TSD, USEPA 1991a) lists nine steps:

1.   Define water quality objectives, criteria, and standards;

2.   Establish priority waterbodies;

3.   Characterize effluent - chemical-specific or Whole Effluent Toxicity (WET);
       a) evaluate for excursions above standards,
       b) determine reasonable potential, and
       c) generate effluent data;

4.   Evaluate exposure (critical flow, fate modeling, and mixing) and calculate wasteload allocation;

5.   Define required discharge characteristics by the waste load allocation;

6.   Derive permit requirements;

7.   Evaluate toxicity reduction and/or investigate indicator parameters (as needed, for permits containing WET monitoring or limits);

8.   Issue final permit with monitoring requirements – average monthly and maximum daily average weekly for publicly operated treatment works) limits; and

9.   Track compliance.

Sometimes the monitoring requirements include biological assessment of the receiving water. The permit can contain a reopener clause to allow the limits and monitoring requirements to be adjusted if biological impairment is found in the receiving water.

The SI guidance is somewhat analogous in function to the Toxicity Reduction Evaluation (TRE) and Toxicity Identification Evaluation (TIE) guidance used in Step 7 above (USEPA 1988a,b,c, 1991b, 1993a,b). In the permitting process, toxicity is controlled through limits for specific chemicals and limits for whole effluent toxicity. When permit monitoring shows that an effluent has toxicity above the amount allowed by the permit, the discharger is often required to conduct a TRE to determine if a simple solution exists

for reducing the toxicity, e.g., housekeeping procedures for cleaning fluids, or pH buffering of the effluent. If the solution is not apparent from the TRE, additional TIE procedures may be required. TIE procedures guide investigators through additional data collection to determine the toxic component(s) of the waste stream. These procedures include both aquatic toxicity methods and chemistry methods.

When WET or chemical testing show that the effluent is toxic, this does not mean that an impairment will necessarily be found in the aquatic biota within the zone of influence of the discharge. Effluent limits include safety factors in their calculations. The waste load allocation (Step 4, above) is calculated based on worst-case estimations. For example, effluent limits for toxicity or for a toxic chemical are based on low-flow conditions in streams and rivers (often the lowest seven-day flow in a ten-year period). Effluent limits may be exceeded, a TRE/TIE conducted, and the problem solved without incurring measurable impairment in the receiving water biota. The current trend is to lessen this safety buffer by customizing water quality-based permit limits to local conditions through such mechanisms as dynamic modeling of waste load allocation (USEPA 1991a) and recalculation of water quality standards or use of the water-effects ratio (USEPA 1994).

Conversely, ambient biological assessments may show impairment in the aquatic biota below a permitted discharge without a measured permit limit exceedence. The role of the effluent in causing the impairment is not readily apparent in this case. The effluent stream could have been toxic during periods when toxic parameters were not being measured; effluent toxicity tests could have been insufficiently sensitive through inappropriate selection of test organisms or operator error; or impairment could have been caused by stressors other than effluent discharge. Accurate attribution of responsibility can be very critical in NPDES permitting cases, both for fairness and success in stressor control. A SI should be conducted to distinguish effects caused by the effluent discharge and effects from other stressors.

A.5.2 Cooling Tower Intake 316(b) Permitting

Under section 316(b) of the CWA, any NPDES permitted discharger which intakes cooling water must not cause an adverse environmental impact to the waterbody. To determine if a cooling water intake structure is causing adverse environmental impacts to the waterbody, the overall health of the waterbody should be known. Where biological impairments are found, stressor identification procedures should help investigators identify the different stressors causing the waterbody to be impaired, including the intake structure. A high degree of certainty is needed.

A.5.3 Dredge and Fill Permitting

Under Section 401 of the CWA, different types of federal permitting activities (such as wetlands dredge and fill permitting) require a certification that there will be no adverse impact on water quality as a result of the activity. This certification process is the 401 Water Quality Certification. Under Section 404 of the CWA, the discharge of dredge and fill materials into a wetland is illegal unless authorized by a 404 Permit. The 404 Permit must receive a 401 Water Quality Certification.

Stressor identification procedures will help investigators identify the different types of stress an activity may place on water quality that can then be addressed through conditions in the 401 Certification. Stressor identification procedures may help to

identify unanticipated stress from a dredge and fill activity on water quality or the biological community after the activity is underway. Stressor identification procedures may also help in pre-permitting evaluations of the potential impacts of 404 permitting by assessing different potential stressors on the wetland in advance.

A.6   Compliance and Enforcement

Since 1972, Section 309 of the Clean Water Act has provided statutory authority for a range of enforcement responses for entities or individuals who fail to comply with the Act. At the extreme end of this range, actions can result in criminal penalties. EPA has national and regional programs in place to investigate and prosecute cases. States and Tribes may have their own compliance and enforcement investigation programs.

A.6.1 Investigations

When a violation occurs, an investigator must first ascertain what must be done to achieve compliance with the Clean Water Act. Under a Section 309 order, the violator must come in full compliance with the Clean Water Act; which, under Article 101, directs the restoration and maintenance of the biological integrity of the nation's waters. When non-compliance is due to biological impairment or non-attainment of biological integrity, the investigator must determine the cause of the impairment before implementing a program to restore biological integrity and achieve compliance. This is a direct use of the SI process.

The degree of environmental harm is a very important factor that investigators and judges evaluate when assessing criminal penalties. The SI process should be helpful in determining whether the causes of impairment are consistent with the causes that would likely have resulted from the source under investigation. The SI process can also help to determine the likelihood that one stressor versus another caused the impairment. In cases where separation of stressor mechanisms is fairly clear cut, the SI process can help investigators determine the significance of the available evidence in determining whether the alleged stressor caused the noted environmental harm. However, the SI process is limited to evaluating causes. If more than one stressor or source are involved, allocating the relative contribution of each stressor or source to the environmental harm may require additional tools, such as allocation methodologies, that are beyond the scope of this document.

A.6.2 Enforcement Proceedings

In an enforcement action, the enforcement official seeks for a court to order the defendant to cease the harmful action, or give injunctive relief. Identifying the causes of impairment is a crucial step in identifying the actions that would constitute injunctive relief. The SI process should benefit enforcement officials and expert witnesses by helping them identify responsible stressors and organize cogent evidence supporting the identified causal scenario. The SI process adds uniformity to the organization and analysis of data.

A special program that is often used to grant injunctive relief is the Supplemental Environmental Project (SEP). Under this program, a judge may allow a defendant to improve the environment in lieu of paying a portion of a federal fine to the National Treasury. The environmental benefit gained through an SEP may not directly alter the harm that the defendant caused originally, but is seen as alternate compensation. For

example, rather than paying a fine of $1 million, a defendant might pay a $600,000 fine and build a bike path with a 30-foot riparian buffer zone (for runoff reduction) along the impacted creek, or even a neighboring stream.

When the SI process identifies multiple stressors as the cause of impairment, the information can still be valuable to the SEP program because the alternate stressors may help direct compensatory action. If, for example, the SI process identifies a stressor scenario with two stressors working in conjunction and the defendant is responsible for only one of the two stressors, a judge might approve a plan for the defendant to use resources to conduct an SEP project that reduces the second stressor, in lieu of a portion of the fine.

Targeting resources is very important to investigation and enforcement efforts. EPA often uses 303d lists of impaired waterbodies to target these efforts. The SI process can supplement the information in the 303d lists so that stressors may be targeted within targeted waterbodies. Targeting may also be important in assessing future legislative needs when mechanisms for stressor control are inadequate in national rules and policies, and in current state and tribal statutes. Targeting stressors for increased control may identify changes to instigate.

A.7   Risk Assessment

Risk assessment is a scientific process that includes stressor identification, receptor characterization and endpoint selection, exposure assessment, stress-response assessment, and risk characterization (USEPA 1998a, Suter 1993). Risk management is a decision-making process that combines human-health and ecological assessment results with political, legal, economic, and ethical values to develop and enforce environmental standards, criteria, and regulations. Risk assessment can be performed on a site-specific basis, or can be geographically-based (e.g., watershed scale). It can be used to assess human health or ecological risks.

Results of bioassessment studies can be used in watershed ecological risk assessments to develop broad-scale empirical models of biological responses to stressors. Such models can be combined with exposure information to predict risk from specific stressors and anticipate the success of management actions. Accurate stressor identification is an integral part of this process and can help ensure that management actions are properly targeted and efficient in producing the desired results.

A.8   Wetlands Assessments

Although few states have fully incorporated wetlands into water quality standards or biological assessment programs, a growing number have started to develop biological assessment methods for wetlands. During the past five years, several state and federal agencies have independently started to develop bioassessment methods for wetlands. Minnesota, Montana, North Dakota, and Ohio have been pioneers among the states. The Biological Resource Division of the U.S. Geological Service, Wetlands Science Institute of the Natural Resources Conservation Service, and EPA have been the leading federal agencies.

The SI process and tools specific to wetlands investigations are very much needed by wetlands managers. In recent 305(b) Reports, states identified sedimentation, nutrient enrichment, fill and drainage, pesticides, and flow alterations as the major causes of

wetlands degradation. Biological assessment methods will allow resource managers to evaluate the condition of wetlands and may provide some indication of the types of stressors involved. Once bioassessment methods are completed and incorporated into monitoring programs, wetlands may be listed as impaired due to biological impairment. SI methods will be needed to identify stressors causing biological impairment so that resource managers can better remedy the problems. More information about wetland bioassessments is available at the EPA Wetlands Division web page (www.epa.gov/owow/wetlands).

A.9   Preservation and Restoration Programs

Preservation and restoration programs like the National Estuary Program and the Superfund Program can also benefit from the SI process.

A.9.1 National Estuary Program

The National Estuary Program (NEP) was established in 1987 by amendments to the Clean Water Act to identify, restore, and protect nationally significant estuaries of the United States. Unlike traditional regulatory approaches to environmental protection, the NEP targets a broad range of issues and engages local communities in the process. The program focuses not only on improving water quality in an estuary, but also on maintaining the integrity of the whole system, its chemical, physical, and biological properties, and its economic, recreational, and aesthetic values.

The NEP is designed to encourage local communities to take responsibility for managing their own estuaries. Each NEP is made up of representatives from federal, state and local government agencies responsible for managing the estuary's resources, as well as members of the community -- citizens, business leaders, educators, and researchers. These stakeholders work together to identify problems in the estuary, develop specific actions to address those problems, and create and implement a formal management plan to restore and protect the estuary. Twenty-eight estuary programs are currently working to safeguard the health of some of our nation's most important coastal waters.

The SI process should be useful to the NEP, and other preservation programs, by helping stakeholders identify sources and causes of impairments. This information would feed into the development of a management plan.

A.9.2 Superfund

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), commonly known as Superfund, was enacted in 1980 (and amended in 1986) for hazardous waste cleanup.   This law created a tax on the chemical and petroleum industries and provided federal authority to respond to releases or threatened releases of hazardous substances that may endanger public health or the environment. The money collected from the taxation went to a trust fund for cleaning up abandoned or uncontrolled hazardous waste sites. CERCLA also established prohibitions and requirements for closed and abandoned hazardous waste sites; defined liability of persons responsible for releases of hazardous waste at these sites; and established funding for cleanup when no responsible party could be identified.

Since the basis for actions is whether the hazardous substance may endanger public health or the environment, identifying the stressor(s) causing environmental harm is

important. For cleanup sites where other stressors (e.g., habitat alteration) are also likely causes of impairment, any cleanup and ecosystem recovery plans would need to take into account the effects of these stressors. Allocating the amount of responsibility that may be attributed to each stressor is beyond the scope of the SI process, but knowledge of any additional stressors that may be causing effects can be valuable in determining expected outcomes of recovery activities.

APPENDIX B
WORKSHEET MODEL

Appendix B

Worksheet Model

The following pages contain a worksheet model that may be used with the SI process. This is only an example and may not fit every case without alterations.

B.1 Instructions for Using the Worksheet Model

This worksheet follows the SI process outlined in this document. The worksheet was designed to be flexible. At certain points, the user will be asked to stop (STOP) and consider the evidence gathered thus far, in order to determine whether the process is complete or requires further analysis. For detailed guidance, the user will need to refer to the sections of the document that are cited at each step.

1. To begin, write the name of the investigator and date for reference.

2. Fill in the appropriate information in *Unit I: List Candidate Causes*. To determine the types of information to include throughout the worksheet, please refer to the cited sections of the document.

3. Summarize and document the data and analyses in *Unit II, Part A*. Then, you may use either of the following options:

    ▸ Option 1: Analyze the strongest evidence. If you feel that you have enough **case specific** data to eliminate some causes, analyze this data using *Unit II, Part B* and proceed to *Unit III, Step 1: Eliminate Alternatives*. Note: You may also look at other types of evidence that can be used for elimination in *Unit II, Parts C and D*. To do this, fill in only the blanks in Parts C and D that are designated by the letter **E** (for elimination) under the heading *Associated Causal Characterization Method in Unit III*. Review this additional evidence to see if it allows you to eliminate any alternatives.

    ▸ If you still have more than one likely causal scenario that could not be readily eliminated, or if you want to thoroughly review all evidence, proceed to *Unit II, Parts C and D*. Complete relevant sections of *Parts C and D* for each candidate cause that you listed in *Unit I*. Then proceed to *Unit III* and characterize the cause using diagnosis or strength of evidence, as appropriate (described under #4 below).

    ▸ Option 2: List all available evidence in *Unit II* before going on to *Unit III: Characterize Causes*. Using either option, you may still choose to do additional iterations if the available evidence is insufficient.

    ▸ Go to *Unit III, Characterize Causes*. For those candidate causes listed in *Unit I* that were not eliminated while analyzing the evidence listed in *Unit II* (i.e., those causes not designated as *E* in *Parts C and D* under the heading *Associated Causal Characterization Method in Unit III*), complete *Step 1: Eliminate Alternatives* and try to further eliminate

causes. **Analyze this evidence carefully**; if the evidence is not strong enough to eliminate a candidate, it still may be useful for the strength of evidence analysis. Using the worksheet in *Unit III, Step 1*, determine:

▸ If the primary cause is so dominant that it masks the effects of others, then re-evaluate whether the other stressors should be retained. A cause should not be eliminated if it is potentially masked. Instead, strength of analysis should be used.

▸ If only one candidate cause remains, go to *Unit IV: Sufficiency of Evidence*. Note: You still may want to look at the diagnostic and strength of evidence information to strengthen your case. If so, go to *Unit III*, *Step 2*.

▸ If more than one candidate cause remains, go to *Unit III*, *Step 2* to look for diagnostic evidence.

▸ If no candidate causes remain, go to *Unit V*. You will need to do another iteration with more information.

▸ Next, try diagnosis. Look for evidence designated as *D* under the column labeled *Associated Causal Characterization Method in Unit III* in *Unit II*, *Part C* tables. Using the worksheet in *Unit III, Step 2*, determine:

▸ If only one candidate cause remains, go to *Unit IV: Sufficiency of Evidence*. Note: You may still want to do a strength of evidence analysis to strengthen your case. If so, go to *Unit III*, *Step 3*.

▸ If more than one candidate cause remains, go to *Unit III*, *Step 3* (*Strength of Evidence Analysis*).

▸ If no candidate causes remain go to *Unit V* and do another iteration with more information.

▸ Many investigators will want to complete the strength of evidence analysis even if elimination or diagnosis have identified the stressor. **This part of the SI process helps determine how strong a case an investigator can make for a particular stressor.** Look for evidence designated as *S* under the column labeled *Associated Causal Characterization Method in Unit III* in *Unit II Part C,* and also consider the evidence gathered in *Part D*. Analyze this evidence carefully using the worksheet in *Unit III, Steps 3, 4, and 5*.

▸ *Unit III Steps 3, 4*, and *5* allow the investigator to compare evidence, side-by-side, for candidate causes. The step used depends on the type of evidence. Scores are assigned to each candidate cause to reflect that cause's relevance to each causal consideration. (For more detailed information on comparing stressors, refer to the sections cited in the worksheets). Compare scores among the candidate causes, and then go to *Unit IV*, *Sufficiency of Evidence*.

▸ List the most likely cause in *Unit IV*, and determine if the evidence is sufficient for the intended use.

▸ If yes, your SI is complete, report results.

▸ If no, go to *Unit V, Reconsider Impairment*.

▸ Reconsider whether the impairment was real and describe the results.

▸ If no, your SI is complete, report results.

▸ If yes, go to *Unit VI, Collect More Data*.

▸ Determine whether all reasonable causes were analyzed.

▸ If no, complete *Unit VI, Follow-on 1* to determine whether additional scenarios should be analyzed (back to *Unit I*), or whether the process should be ended and the results reported as inconclusive.

▸ If yes, go to *Unit VI, Follow-on 2* to determine whether additional data should be collected and another iteration begun (back to *Unit I*), or whether the process should be ended and the results reported as inconclusive.

Stressor Identification Worksheet

Investigator _____   Date Completed_____

UNIT I. LIST CANDIDATE CAUSES	
	Results / Notes
Describe the impairment. *(see Chapter 2.2)*	
Make a map. (Unit I part A) *(see Chapter 2.2)*	
Define the Scope of the Investigation. *(see Chapter 2.3)*	
List the candidate causes *(see Chapter 2.4)*	
Develop a conceptual model for the case. (Unit I, part B) *(see Chapter 2.5)*	
Candidate Causes	
# 1.	
# 2.	
# 3.	

Go to Unit II, Analyze Evidence.

UNIT I. LIST CANDIDATE CAUSES

Part A. Make a map to document geographic features relevant to the analysis.

- Draw a map or insert map of study area.
- Include natural and man-made features such as dams, sources, tributaries, landfills, dredge areas, jetties, sand bars, waterfalls, wetlands, salt water intrusion, etc. See Chapter 2.2.
- Show location of impairment.

UNIT I. LIST CANDIDATE CAUSES

Part B. Make a conceptual model of the case.

- Draw a conceptual model of the case. See Chapter 2.5.
- Include hypothesized sources, stressors and important environmental processes that lead to the impairment.
- Label candidate causes.

UNIT II

Part A. Summarize and document associations between the candidate cause and the effect from the case.
   • Insert tables, graphs and/or figures of relevant data. See Chapter 3.1.
   • Insert statistical analyses including correlations, geographic associations, etc. See Chapter 3, textbox 3-2.
   • You may want to look at other types of evidence that can be used for elimination in Unit II, Part B and C.

If you feel that you have enough case specific data to eliminate some causes, proceed to Unit III Step 1 (Eliminate Alternatives). If not, proceed to Unit II Part B.

UNIT II			
Part B. Measurements associated with the causal mechanism (Chapter 3.3).			

- Evidence can be used for Elimination (**E**) Diagnosis (**D**) or Strength of Evidence (**S**), as noted below.
- Prepare a separate table for each candidate cause.
- Use this as a reminder of types of data that could be used in the analysis. Not all questions may be appropriate.

Candidate Cause: _____

Example Questions:	Yes/No/ Question Not Relevant	Associated Causal Characterization Method in Unit III*	Supporting Analysis
Are symptoms or other responses specific to or characteristic of a type of stressor found in organisms from the impaired community?		D, S	
Are there internal measures of exposure (e.g., body burdens, biomarkers) found in organisms from the impaired community?		E, D, S	
Is an intermediate product of an ecological process present?		E, S	
Do distributions of stressors and receptors coincide?		E, S	

Example Questions:	Yes/No/ Question Not Relevant	Associated Causal Characterization Method in Unit III*	Supporting Analysis
Have there been expected changes in the abundance of predators, prey, or competitors?		S	
Are there expected effects on other receptors?		S	
Other			

*E = Elimination; D = Diagnosis; S = Strength of Evidence

If you feel that your evidence can be used to identify the cause through diagnosis, go to Unit III, Step 2. If not, continue with the analysis of evidence in Unit II Parts C and D.

UNIT II

Part C. Associations of effects mitigation with manipulation of causes (Chapter 3.4).

- Evidence can be used for elimination ONLY if it is from the site.
- Prepare a separate table for each candidate cause.
- Use this as a reminder of the types of data that could be used in the analysis. Not all questions may be appropriate.

Candidate Cause: _____

Questions:	Yes/No/ Information not available/ Question not Applicable	Asso-ciated Causal Charac-teriza-tion Method in Unit III*	Supporting Analysis
Does elimination of the source reduce or eliminate the effect?		S, E	
Does the introduction of previously unexposed organisms result in an effect?		S	
Does the isolation of organisms from one cause reveal the effects of others?		S	

Questions:	Yes/No/ Information not available/ Question not Applicable	Asso-ciated Causal Charac-teriza-tion Method in Unit III*	Supporting Analysis
Does the testing of chemical fractions of site media result in toxicity being associated with a particular fraction (i.e., TIE)?		S	
Other			

*E = Elimination; D = Diagnosis; S = Strength of Evidence

If you have enough data to determine the cause, proceed to Unit III Step 1 (Elimination) or Step 2 (Diagnosis) or Step 3 (Strength of Evidence), as appropriate. If not or uncertain, proceed to Unit II Part D.

UNIT II

Part D. Using effects data from elsewhere (Chapter 3.2).

- Use this table to incorporate data from other situations that support the analysis. Not all questions may be appropriate for a given candidate cause.
- This evidence is applicable to Strength of Evidence **(S)** characterization method.
- Prepare a separate table for each candidate cause.

Candidate Cause: _____

Type of Candidate Cause	Characterization of Exposure (Intensity, Time, and Space)	Data Available? Yes (note location of data)/No	Exposure-Response (E-R) Relationship	E-R Available? Yes (note location of data) /No/Not Relevant	Would effects be expected at the environmental conditions seen in the case? (Yes/No)	Location of supporting analysis
Chemical	What is the concentration in the medium at the site?		What is the concentration-response relationship (seen in the lab or the field)?			
	What is the internal concentration in organisms at the site?		What is the internal external concentration-response relationship (seen in the lab or the field)?			
	What is the concentration in the biomarker at the site?		What is the biomarker-response relationship?			

Type of Candidate Cause	Characterization of Exposure (Intensity, Time, and Space)	Data Available? Yes (note location of data)/No	Exposure-Response (E-R) Relationship	E-R Available? Yes (note location of data) /No/Not Relevant	Would effects be expected at the environmental conditions seen in the case? (Yes/No)	Location of supporting analysis
Effluent	What is the dilution of the effluent at the location of the impairment?		What are the laboratory test (i.e., WET) results from 100% effluent or diluted effluent?			
Contaminated ambient media	What were the location and time of collection and the results of analyses?		What are the results of laboratory tests of ambient media?			
Habitat	What are the structural attributes of the habitat?		Are empirical models available that relate habitat characteristics to biological responses ?			
Water Withdrawal or Drought	Are hydrograph readings and summary statistics (e.g., 7Q10) available?		What are the results of instream flow models (e.g., IFIM)?			
Thermal Energy	Are temperature records available?		What are the thermal tolerances of the impacted organisms?			

Type of Candidate Cause	Characterization of Exposure (Intensity, Time, and Space)	Data Available? Yes (note location of data)/No	Exposure-Response (E-R) Relationship	E-R Available? Yes (note location of data) /No/Not Relevant	Would effects be expected at the environmental conditions seen in the case? (Yes/No)	Location of supporting analysis
Siltation (Suspended)	What is the total suspended solids (TSS) concentration?		What is the concentration-response relationship (seen in the lab or field)?			
Siltation (Bed-load)	What is the degree of embeddedness and texture of the silt?		Are empirical models available to characterize the effects?			
Dissolved Oxygen and Oxygen-Demanding Contaminants (e.g., BOD, COD)	Review the dissolved oxygen data (esp. predawn).		What is the concentration-response relationship (from lab or other field studies)?			
	Review the BOD, COD data from the source.		Are there oxygen demand models that can be used to predict effects?			
Excess Mineral Nutrients	What were the dissolved mineral nutrient concentrations?		What is the concentration-response relationship (from lab or other field studies)?			
			Are there nutrient/eutrophication models that can be used to predict effects?			

Type of Candidate Cause	Characterization of Exposure (Intensity, Time, and Space)	Data Available? Yes (note location of data)/No	Exposure-Response (E-R) Relationship	E-R Available? Yes (note location of data) /No/Not Relevant	Would effects be expected at the environmental conditions seen in the case? (Yes/No)	Location of supporting analysis
Nonindigenous Species	Is a nonindigenous species present or abundant?		Are ecological models available to characterize the effects?			
Pathogen	Is a pathogen present? If so, is it abundant?		Are any symptoms or diseases observed?			
Other						

Go to Unit III, Characterize Causes.

Unit III. Characterize Causes

Step 1. Eliminate Alternatives (Section 4.1.1) *and compare supporting evidence where causes were eliminated.*

- For each candidate cause indicate Yes, No, No Evidence (NE), or Not Applicable (NA).
- If more than one stressor is necessary for a cause to be sufficient (i.e., temperature and dissolved oxygen), indicate response for each stressor.
- Use extra pages for more than 3 candidate causes.
- Provide comments as necessary.

Case-Specific Consideration	Candidate Cause # 1 (Yes / No / NE / NA)	Candidate Cause # 2 (Yes / No / NE / NA)	Candidate Cause # 3 (Yes / No / NE / NA)
Temporal Co-occurrence Did the effect precede the stressor in time? *(If the effects preceded a proposed cause and effects are not obscured by another sufficient cause, then it cannot be the primary cause.)*			
Temporal Gradient Did the effect increase or decrease over time in association with an increase or decrease in the stressor? *(If the effect increases or decreases over time without a corresponding increase or decrease in the stressor, then the stressor cannot be the primary cause.)*			

Case-Specific Consideration	Candidate Cause # 1 (Yes / No / NE / NA)	Candidate Cause # 2 (Yes / No / NE / NA)	Candidate Cause # 3 (Yes / No / NE / NA)
Spatial Co-occurrence Is there an upstream/downstream conjunction of candidate cause and effect? *(If the effect occurs upstream of the source or does not occur regularly downstream, e.g., is distributed spatially independently of a plume, sediment deposition areas, etc., and effects are not obscured by another sufficient cause, then the candidate cannot be the primary cause).*			
Co-occurrence with Reference Site(s) Is there a reference site/impaired site conjunction of candidate cause and effect? *(If the cause occurs at reference sites as well as the impaired sit, it can be eliminated.)*			
Spatial Gradient Does the effect increase or decrease across a given region in association with an increase or decrease in the stressor? *(If the effect increases or decreases over a given region without a corresponding increase or decrease in the stressor, then the stressor cannot be the primary cause.)*			

Case-Specific Consideration	Candidate Cause # 1 (Yes / No / NE / NA)	Candidate Cause # 2 (Yes / No / NE / NA)	Candidate Cause # 3 (Yes / No / NE / NA)
Biological Gradient Is a decrease in the magnitude or proportion of an effect seen along a decreasing gradient of the stressor? *(A constant or increasing level of effect with decreasing exposure would eliminate a cause.)*			
Complete Exposure Pathway, Question 1: Is there evidence that the stressor did not co-occur with, contact, or enter the receptor(s) showing the effect? *(If there is no route of exposure, or, for appropriate stressors, if tissue burdens or other measures of exposure were not found to occur in affected organisms, the cause may be eliminated.)*			
Complete Exposure Pathway, Question 2: Is there evidence that a necessary intermediate step in the causal chain of events did not occur? *(If a link in a known chain of events can be shown to be missing, the cause may be eliminated.)*			

Case-Specific Consideration	Candidate Cause # 1 (Yes / No / NE / NA)	Candidate Cause # 2 (Yes / No / NE / NA)	Candidate Cause # 3 (Yes / No / NE / NA)
Experiment, Temporality Did the effects continue when the candidate cause was removed (allowing for rates of recovery)? *(If effects continue despite elimination of the candidate cause, that cause can be eliminated.)*			
Other			

After completing Step 1 (above) for each candidate cause listed in Unit I:

- If only **one** candidate cause remains, elimination is definitive. Go to Unit IV.
- If **more than one** candidate cause remains, go back to Unit II, Part B. If Unit II Part B is complete, go to Unit III Step 2.
- If **no** candidate causes remains, go to Unit V.

UNIT III

Step 2. Characterize cause using diagnostic evidence (Section 4.1.2).

- If diagnostic evidence was found in Unit II Part D, determine if the evidence is sufficient to define the cause using this table.
- If evidence is not sufficient to diagnose the cause, it may still be used in the strength of evidence in Unit III Step 3.
- Use extra pages for more than 3 candidate causes.

Candidate Cause	Type of Diagnostic Evidence	Description of Evidence
# 1		
# 2		
# 3		

After completing Step 2 for all causes remaining after the elimination step (Step 1):

- If diagnosis is definitive. Go to Unit IV.
- If **diagnosis is uncertain**, go back to Unit II Parts B, C and D. **If Unit II Parts B, C, and D are complete, proceed to Unit III Step 3.**

UNIT III						
Step 3. Analyze strength of evidence (Section 4.1.3) *for Case-Specific Considerations.* • Use extra pages for more than 3 candidate causes.						
Causal Considerations and possible scores	**Candidate Cause # 1**		**Candidate Cause # 2**		**Candidate Cause # 3**	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Co-occurrence Compatible (+), Uncertain (0), Incompatible (- - -), No evidence (NE) *(The stressor has either contacted the affected organisms, their food source, or some parameter that can affect the organisms.)*						

Causal Considerations and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Temporality Compatible (+), Uncertain (0), Incompatible (- - -), No evidence (NE) *(A cause must always precede its effects.)*						
Consistency of Association Invariant (++), In many places and times (+), At background frequencies (-), No Evidence (NE) *(The repeated observation of a similar relationship of the effect and candidate cause in different places and times.)*						

Causal Considerations and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Biological Gradient Strong and monotonic (+++), Weak or other than monotonic (+), None (-), Clear association but wrong sign (- - -), Not applicable (NA) *(The effect increases in a regular manner with increasing exposure.)*						

Causal Considerations and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Complete Exposure Pathway Evidence for all steps (++), Incomplete evidence (+), Ambiguous (0), Some steps missing or implausible (-), No evidence (NE) *(The stressor co-occurs with or contacts the receptor(s).)*						

Causal Considerations and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Experiment Experimental studies Concordant (+++), Ambiguous (0), Inconcordant (- - -) No evidence (NE) *(Toxicity tests or other controlled experimental studies demonstrated that the candidate cause can induce the observed effect.)*						

UNIT III						
Step 4. Analyze strength of evidence *(Section 4.1.3)* ***using Evidence from Other Situations or from Biological Knowledge.*** • Use extra pages for more than 3 candidate causes.						

Causal Consideration and possible scores	**Candidate Cause # 1**		**Candidate Cause # 2**		**Candidate Cause # 3**	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Plausibility: Mechanism Evidence of Mechanism (++), Plausible (+), Not Known (0), Implausible (-) *(It is plausible that the effect resulted from the cause given what is known about the biology, physics, and chemistry of the candidate cause, the receiving environment, and the affected organisms.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Plausibility: Stressor-Response Quantitatively consistent (+++), Concordant (+), Ambiguous (0), Inconcordant (-), No evidence (NE) *(Given a known relationship between the candidate cause and the effect, effects would be expected at the level of stressor seen in the environment.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Consistency of Association Invariant (+++), In most places (++), In some places (+), At background frequency (-), Not applicable (NA) *(The repeated observation of the effect and candidate cause is similar in different places and times.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Analogy: Positive Analogous cases: Many or few but clear (++), Few or unclear (+), None (0) *(The hypothesized relationship between cause and effect similar to other well-established cases.)*						
Analogy: Negative Analogous cases: Many or few but clear (- -), Few or unclear (-), None (0) *(The hypothesized relationship between cause and effect is dissimilar to other well-established cases.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Specificity of Cause* *Note: only applicable if the cause is plausible or is consistently associated with the effect.* Only possible cause (+++), One of a few (+), One of many (0), Not applicable (NA) *(The effect observed at the site is known to have only one or a few known causes.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Experiment Experimental studies: Concordant (+++), Ambiguous (0), Inconcordant (- - -), No evidence (NE) *(Toxicity tests or other controlled experimental studies demonstrated that the candidate cause can induce the observed effect.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Predictive Performance Prediction: Confirmed specific or multiple (+++), Confirmed general (++), Ambiguous (0), Failed (- - -), No evidence (NE) *(The candidate cause has any initially unobserved properties that were predicted to occur and the prediction was subsequently confirmed at the site.)*						

	UNIT III					
Step 5. Analyze strength of evidence *(Section 4.1.3)* ***based on multiple lines of evidence.*** • Use extra pages for more than 3 candidate causes.						
Causal Consideration and possible scores	**Candidate Cause # 1**		**Candidate Cause # 2**		**Candidate Cause # 3**	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Consistency of Evidence All consistent (+++), Most consistent (+), Multiple inconsistencies (- - -) *(The hypothesized relationship between the cause and effect is consistent across all available evidence.)*						

Causal Consideration and possible scores	Candidate Cause # 1		Candidate Cause # 2		Candidate Cause # 3	
	Evidence and Literature Citation	**Score**	**Evidence and Literature Citation**	**Score**	**Evidence and Literature Citation**	**Score**
Coherence of Evidence Evidence: Inconsistency explained by a credible mechanism (+), No known explanation (0) **No entry if all consistent** *(A mechanistic conceptual model explains any apparent inconsistencies among the lines of evidence.)*						

Compare evidence among the candidate causes, then go to Unit IV to summarize your findings.

IV. SUFFICIENCY OF EVIDENCE (Chapter 4.2)

Most Likely Candidate Cause: _____

Is Evidence Sufficient for the Management Purpose?

☐ **YES   SI COMPLETE, REPORT RESULTS**   ☐ **NO   GO TO UNIT V, RECONSIDER IMPAIRMENT**

Summary of Characterization		
Candidate Cause	**Cause**	**Reasoning & Confidence**
# 1.		
# 2.		
# 3.		

V. RECONSIDER IMPAIRMENT
Does Biological Impairment Really Exist?
(Section 5.1)

Reconsider the impairment by auditing the quality of the methods used to generate and manage the data, by using better analysis tools, and by eliminating any suspicious data or analyses.
Describe Reconsideration:

_____

_____

_____

_____

Were effects real?

☐ **NO** **SI COMPLETE, REPORT RESULTS.**

☐ **YES** **GO TO UNIT VI, COLLECT MORE INFORMATION.**

VI. COLLECT MORE INFORMATION (Section 5.2)

Were all reasonable causes analyzed?

      □ **NO**   **Go to Follow-on 1.**

      □ **YES**   **Go to Follow-on 2.**

Follow-on 1:   **Make sure that all reasonable causes were analyzed.**

- **If additional scenarios are indicated, repeat process, beginning at Unit 1.**

- **If a good faith effort was implemented with reasonable time and resource expenditures, consult management goals and determine if the process should be ended with inconclusive results.**

    SI COMPLETED, REPORT RESULTS AS INCONCLUSIVE.

Follow-on 2:   **Look at the supporting evidence in Unit II, Analyze Evidence.**

- **Prioritize information needs for likely candidate causes, collect new information and repeat the process, beginning at Unit 1.**

- **If a good faith effort was implemented with reasonable time and resource expenditures, consult management goals and determine if the process should be ended with inconclusive results.**

    SI COMPLETED, REPORT RESULTS AS INCONCLUSIVE.

APPENDIX C
GLOSSARY OF TERMS

Appendix C

Glossary of Terms

Ambient monitoring: All forms of monitoring conducted beyond the immediate influence of a discharge pipe or injection well and may include sampling of sediments and living resources.

Ambient waters: water bodies that are in the environment.

Analogy: a comparison of two things, based on their similarity in one or more respects. In SI, the criterion of an analogy refers specifically to similar causes.

Bioassessment (biological assessment): evaluation of the condition of an ecosystem that uses biological surveys and other direct measurements of the resident biota.

Biocriteria (biological criteria): numerical values or narrative expressions that describe the reference biological condition of aquatic communities inhabiting waters of a given designated aquatic life use. Biocriteria are benchmarks for evaluation and management of water resources

Biogenic: produced by biological processes. For example, organic acids produced by decomposition of plant litter are biogenic acids.

Biological gradient: a regular increase or decrease in a measured biological attribute with respect to space (e.g., below an outfall), time (e.g., since a flood), or an environmental property (e.g., temperature).

Biomarker: contaminant-induced physiological, biochemical, or histological response of an organism.

Body burden: the concentration of a contaminant in a whole organism or a specified organ or tissue.

Candidate cause: a hypothesized cause of an environmental impairment which is sufficiently credible to be analyzed.

Categorical regression: regression analysis in which the dependent variable is defined by a categorical scale rather than as a count or continuous variable.

Causal analysis: a process in which data and other information are organized and evaluated using quantitative and logical techniques to determine the likely cause of an observed condition.

Causal mechanism: the process by which a cause induces an effect.

Causal relationship: the relationship between a cause and its effect.

Causal association: a correlation or other association between measures or observations of two entities or processes which occurs because of an underlying causal relationship.

Causal evidence: the results of an analysis of data to reveal an association between the environmental condition and a candidate cause.

Causal inference: the component of a causal analysis that is specifically concerned with the interpretation of the evidence to determine the most likely cause.

Causal characterization: a step in the stressor identification process in which the proposed cause is described, the evidence for its causal relationship to the impairment is summarized, and uncertainties are presented.

Causal considerations: logical categories of evidence that are consistently applied to support or refute a hypothesized cause. A causal consideration (e.g., biological gradient) is evaluated using causal evidence (e.g., a regression of benthic invertebrate diversity against sediment PCB concentration).

Cause: 1. that which produces an effect (a general definition). 2. a stressor or set of stressors that occur at an intensity, duration and frequency of exposure that results in a change in the ecological condition (a SI-specific definition).

Co-occurrence: the spatial co-location of the candidate cause and effect.

Coherency of evidence: the final consideration in a strength of evidence analysis. If the results of all of the causal considerations in a strength of evidence analysis are not consistent, they may still be coherent, if a mechanistic conceptual or mathematical model explains the apparent inconsistencies.

Complete exposure pathway: the physical course a stressor takes from the source to the receptors (e.g., organisms or community) of interest. (Evidence for a complete exposure pathway is case-specific and may include measurements such as body burdens of chemicals, presence of parasites or pathogens, or biomarkers of exposure.)

Concentration-response model: a quantitative (usually statistical) model of the relationship between the concentration of a chemical to which a population or community of organisms is exposed and the frequency or magnitude of a biological response.

Consideration: see Causal consideration.

Consistency of association: the degree to which an effect and candidate cause have been determined to co-occur in different places or times.

Consistency of evidence: the degree to which the causal considerations in a strength of evidence analysis are in agreement concerning a candidate cause.

Diagnostic analysis: a type of causal analysis in which effects that are characteristic of a particular cause are used to determine whether that candidate cause may be responsible for an impairment.

Diagnostic protocol: a standard procedure for performing a diagnostic analysis.

Dilution ratio: the ratio of the stream flow to the wastewater flow

Ecoepidemiology: the study of the nature and causes of effects on ecological systems.

Endpoint species: a species that is the object of an assessment or test.

Eutrophication: enrichment of a water body with nutrients, resulting in high levels of primary production, often leading to depletion of dissolved oxygen.

Experiment: the manipulation of a candidate cause by eliminating a source or altering exposure so as to evaluate its relationship to an effect.

Expert judgement: a method of causal inference based on the knowledge and skill of the assessors rather than a formal method.

Exposure: the co-occurrence or contact of a stressor and the resource that becomes impaired.

Exposure-response relationships: a qualitative or quantitative (usually statistical) model of the relationship between an exposure metric (e.g., the concentration of a chemical or the abundance or an exotic species) to which a population or community of organisms is exposed and the frequency or magnitude of a biological response.

Impairment: a detrimental effect on the biological integrity of a water body that prevents attainment of the designated use.

Indirect causation: the induction of effects through a series of cause-effect relationships, so that the impaired resource may not even be exposed to the initial cause.

Indirect effects: changes in a resource that are due to a series of cause-effect relationships rather than to direct exposure to a contaminant or other stressor.

Inferential logic: a process for reasoning from the evidence to a necessary and specific conclusion.

Initial response: the response of an organism, population or community to direct exposure to a stressor.

Intermediate processes: processes that occur between the occurrence of a stressor in an ecosystem and the induction of the effect of concern. For example, the reduction in algal abundance is an intermediate process between the introduction of a non-native filter feeder and the reduction in abundance of native planktivorous species.

Internal exposure: exposure of an organism to bioaccumulated contaminants.

Logic of abduction: inference from data to the hypothesis that best accounts for the data.

Mechanism: the process by which a system is changed.

Necropsy: a post-mortem examination or inspection intended to determine the cause of death or the nature of pathological changes.

Negative evidence: evidence that tends to refute a candidate cause.

Opportunistic: having the ability to exploit newly available habitats or resources.

Pathogens: organisms that are capable of inducing a disease in a susceptible host.

Plausibility:	the degree to which a cause and effect relationship would be expected, given known facts.
Positive evidence:	evidence that tends to support a candidate cause.
Predictive performance:	the degree to which a candidate cause has led to predictions concerning conditions in the receiving system which have been subsequently confirmed by observation or measurement.
Principal cause:	the cause that makes the largest contribution to the effect.
Pseudoreplication:	the treatment of multiple samples that are subject to the same treatment as replicates for statistical purposes. For example, multiple samples of benthic invertebrates taken in a channelized stream are pseudo- replicates because they are not independent. True replicates would be taken from different channelized streams.
Publicly Owned Treatment Works (POTW):	a water treatment facility, as defined by Section 212 of the Clean Water Act, that is used in the storage, treatment, recycling, and reclamation of municipal sewage or industrial wastes of a liquid nature and is owned by a municipality or other governmental entity. It usually refers to sewage treatment plants.
Receptors:	organisms, populations, or ecosystems that are exposed to a contaminant or other stressor.
Replicate:	(a) one of a set of independent systems which have been randomly assigned a treatment; or (b) to generate a set of such systems.
Source:	an origination point, area, or entity that releases or emits a stressor. A source can alter the normal intensity, frequency, or duration of a natural attribute, whereby the attribute then becomes a stressor.
Spatial gradient:	a graded change in the magnitude of some quantity or dimension measured on a transect
Specificity:	the quality of being specific rather than general.
Specificity of cause:	only one candidate cause or a few similar causes can induce the observed effect.
Specificity of effect:	one type of effect is characteristically induced by a candidate cause. The absence of that effect is evidence for eliminating the candidate cause.

*Strength-of-evidence
analysis:* an inferential process that uses all relevant evidence in a systematic process to determine which candidate cause is most likely to have induced the effect of concern.

Strength of association: the size of the effect produced by an increment in the candidate cause. A candidate cause that is associated with a large change in the level of effect is more likely to be the true cause than one that is weakly associated.

Stressor: any physical, chemical, or biological entity that can induce an adverse response.

*Supplemental
Environmental Project
 (SEP):* a special program that is often used to grant injunctive relief.

Symptomatology: a set of signs of the action of a causal agent on organisms. A set of symptoms with a common cause constitutes a symptomatology.

Temporal relationship: the relationship between the time of occurrence of a candidate cause and of the effect of concern.

Temporal gradient: a graded change in the magnitude of some quantity or dimension measured over time.

*Total Maximum Daily
Load (TMDL):* the total allowable pollutant load to a receiving water such that any additional loading will produce a violation of water-quality standards.

*Toxicity Reduction
Evaluation (TRE):* a site-specific study conducted in a stepwise process designed to identify the causative agent(s) of effluent toxicity, isolate the sources of toxicity, evaluate the effectiveness of toxicity control options, and then confirm the reduction in effluent toxicity.

*Toxicity Identification
and Evaluation (TIE):* a process that identifies the toxic components of an effluent or ambient medium by a process of chemically manipulating the effluent or medium and testing the resulting material.

APPENDIX D
LITERATURE CITED

Appendix D

Literature Cited

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Index