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An Iterative Approach for Identifying the Causes of Reduced Benthic Macroinvertebrate Diversity in the Willimantic River, Connecticut

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

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

NOTICE

ABSTRACT

Calculations using data from the toxicity monitoring reports from a permitted publically owned treatment works (POTW) indicated a high probability of exceeding Connecticut's water quality criteria for copper, lead, and zinc in the Willimantic River downstream in Stafford Springs, Connecticut. In 1998, a 2.4-km segment of the Willimantic River in northeastern Connecticut was listed as impaired by the Connecticut Department of Environmental Protection (CT DEP) based on the review of Aquatic Toxicity Monitoring Reports from the POTW. Subsequent monitoring by the CT DEP in the autumn of 1999 confirmed the biological impairment. However, biological impairment was also found upstream from the discharge. This case study outlines the logical arguments used to determine the cause of the biological impairments upstream of the Stafford POTW discharge and is an application of the U.S. Environmental Protection Agency's Stressor Identification (SI) Guidance.

The specific biological impairment was defined as low numbers of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa at a site on the Middle River, and low numbers of EPT and non-EPT taxa downstream on the Willimantic River. The magnitude of the measured Candidate Causes were judged to be insufficient to cause the severe impairment observed at the site and therefore an episodic release was hypothesized. Additional biological sampling revealed the origin of the impairment, a raceway that co-occurred at the most upstream area of the biological impairment, and a gray discharge was discovered. The episodic toxic discharge was confirmed as the probable cause after rerouting the illicit discharge and observing an increase in number of EPT and non-EPT taxa at two impaired locations. Three years after rerouting the illicit discharge, the impaired sites reached acceptable biological conditions as defined by the State's Department of Environmental Protection. Episodic releases of toxic effluent released during batch processing in the textile mill were identified as the probable cause of reduced numbers of taxa at both sites. However, sediment embeddedness could have been a contributing factor at one of the locations, and heat stress, and fine organic matter may have impeded the rates of recovery at both sites and may continue to cause lesser impacts to the aquatic life in the stream.

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Cover photograph:
Susan Cormier, U.S. EPA, Cincinnati OH. Middle River near its confluence with Furnace Brook in Stafford Springs, Connecticut. Photo taken October 2000.

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BOD	5-day biological oxygen demand
CT DEP	Connecticut Department of Environmental Protection
DO	dissolved oxygen
EC_{20}	the highest tested concentration causing less than 20% reduction in growth, fecundity or survivorship in a chronic test with a daphnid species
EPT	phylogenetic Orders Ephemeroptera, Plecoptera and Trichoptera
HR	Hop River
LCV	lowest chronic values
MATC	Maximum Acceptable Total Concentration
NPDES	National Pollution Discharge Elimination System
POTW	Publicly Owned Treatment Works
RBP	Rapid Bioassessment Protocols
SI	Stressor Identification
SOE	strength-of-evidence
SR	Skungamaug River
TKN	total Kehldahl nitrogen
TMDL	total maximum daily load
TN	total nitrogen
TR	Tenmile River
TT	total taxa richness
U.S. EPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
WQC	water quality criteria

PREFACE

This is a causal assessment of a biologically impaired river in the state of Connecticut. The assessment was done by the CT DEP after they listed the Middle River and Willimantic River on the State's 303(d) list of impaired waters. A determination of the total maximum daily load to meet water quality standards was then required. The sampling, analysis, and conclusions are those of researchers who were employed by the CT DEP at the time of the assessment. The text was reorganized and formatted for the U.S. Environmental Protection Agency (U.S. EPA) publication during a workshop at Canaan Valley, West Virginia in May of 2005. Only comments indicating alternative approaches and suggestions were prepared by the National Center for Environmental Assessment (NCEA). NCEA provided editorial and formatting assistance to make the original CT DEP report similar to four other case studies that were solicited as examples for other practitioners of causal assessment. The analyses in the case cannot be modified because they are already a part of the State of Connecticut's public record.

The Willimantic River case study is several causal assessments that were completed prior to 2005 by states. These cases were used to support state programs that required that the probable cause of a biological impairment be determined. Data for these cases are generally part of a monitoring program not necessarily designed for causal assessment, limited by resources, and often dependent on encountered data. And yet, some causes can be identified as co-occurring with the biological impairment, part of a larger causal chain of events, occurring at sufficient levels known to cause the observed effects, and coherent with general ecological and scientific theory related to physical interactions that have occurred post European settlement. In some cases, manipulation of the cause altered the biological effect. Although none of the cases has evidence of similar quality for all Candidate Causes, evidence for some Candidate Causes is enough to identify probable causes or to suggest what additional, targeted data might greatly improve the confidence in the determination.

These cases could be improved with more resources, but represent the state of the capability and analysis that was available in 2005. Since then, additional analytical tools and databases have become more readily available and states, tribes, and territories continue to reduce the uncertainty of the analysis. However, in many cases, the information was adequate for basing a decision.

To demonstrate causal relationships, most of the case studies, including the Willimantic River case study, used biological metrics. This practice can diminish the ability to detect associations because summing dampens the overall signal from individual taxa and species that are responding differently to environmental conditions or stressors. However, CT DEP did use changes in the presence and abundance of individual species after the assessment to confirm possible mechanisms and to monitor recovery after removal of an elicit discharge. This case study is linked to relevant tools and guidance on the U.S. EPA Web site: www.epa.gov/caddis.

The Willimantic case is relatively simple and did not need the level of detail presented here for decision making. The detail is present to illustrate the various types of evidence that can be developed and used in causal assessments. Different ways of presenting the evidence are also shown including detailed inferential text explanations, diagrams with annotated text boxes, detailed strength-of-evidence tables, and comparative tables. Rarely are all these formats necessary, rather they are examples of what could be done. In addition, text boxes have been inserted throughout the Willimantic River case study to supply commentary, useful links, or to suggest other approaches that could strengthen other similar cases.

The Willimantic Case Study is a good example of several strategic techniques:

1. preliminary analysis leading to the collection of additional information (Iteration);
2. biological screening methods to bracket an impairment and its sources;
3. comparing and evaluating multiple potential causes;
4. confirmation of the probable cause by monitoring after manipulating exposure;
5. displaying the evidence by annotating evidence onto diagrams of causal pathways;
6. recognition of different styles for providing information, which are presented for comparison;
7. illustration of the advantages of using genus-level data in the analysis; and
8. the case is relevant to: metals toxicity, impoundments (low-level dams), hydrologic alteration, urban point sources versus nonpoint sources of pollution, habitat alteration, and aquatic multimedia evaluations, applications of 303(d) listings and categories 2 and 4c, adaptive management, and using monitoring information to make informed regulatory decisions.

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The participants of the 2002 U.S. EPA Biological Advisory Board are recognized for their contribution to the first iteration of the case study in which it was judged that the effect was due to an unknown episodic source below MR1 and above MR3. We also thank the CT DEP for their efforts to restore the stream and their continued research into causal relationships that affect stream condition.

1. INTRODUCTION

This case study used an iterative approach to identify stressors in the Willimantic River. Impairments were characterized by reduced diversity of benthic invertebrates based on the Connecticut Department of Environmental Protection's (CT DEP) evaluation system. The case study was initiated as part of total maximum daily load (TMDL) development for a segment of the river below a permitted point source discharge. The Stressor Identification (SI) process was applied as part of that effort (U.S. EPA, 2000b; Suter et al., 2002). The first iteration of the SI process was conducted in early 2000, using data collected in 1999. We found that there was no strong evidence for any of the Candidate Causes. However, the nature and distribution of effects pointed toward an unknown local cause in one section of a tributary of the river. In preparation for a second iteration of causal analysis (2001), samples were collected in the reach that was believed to contain a source. A previously unknown intermittent effluent was identified and corrected. After the presumed source of impairment had been corrected, the diversity of benthic macroinvertebrates increased over a 2-year period. A second iteration of causal analysis in the spring of 2003 confirmed that the illicit effluent had been the cause of impairment. Consequently, this case study shows how SI can be used within a TMDL program to identify localized causes of impairment.

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

This case study provides an opportunity to illustrate several effective strategies:

1. Preliminary analysis leading to the collection of additional information (Iteration).
2. Biological screening methods to bracket an impairment and its sources.
3. Comparing and evaluating multiple potential causes.
4. Confirmation of the probable cause by monitoring after manipulating exposure.
5. Displaying the evidence by annotating causal pathways on conceptual models.
6. Recognition of different styles for providing information, which are presented for comparison.
7. Recognition of different styles for providing information.
8. Recognition of rates of change following a manipulation or management action.

2. DEFINE THE CASE

2.1. REGULATORY CONTEXT FOR CASE STUDY

The CT DEP used the SI process to help address requirements of Section 303(d) of the Clean Water Act (see Comment 2). Specifically, they found it useful for the identification of the causes of impairment enabling appropriate management actions to be taken so that the body of water met its designated uses.

Comment 2. Regulatory Authority. Relevance of Causal Assessment to the Clean Water Act is available on the CADDIS Website.

A 2.4-km section of the Willimantic River, from 0.8 km downstream from Route 190 (approximate location of the Stafford Publicly Owned Treatment Works [POTW]) to the confluence of Bonemill Brook, was listed on the 1998 303(d) list of Connecticut water bodies not meeting water quality standards (CT DEP, 2004b) (see Comment 2) (see Figure 1). This determination was based on chemistry data for the Stafford POTW that had been submitted to the CT DEP as part of Aquatic Toxicity Monitoring Reports. Calculations made by the CT DEP using the chemistry data revealed a high probability of exceeding Connecticut's water quality criteria (WQC) for copper(Cu), lead (Pb), and zinc (Zn) downstream of the POTW outfall. On the basis of these calculations, the CT DEP initiated a biological assessment in the autumn of 1999. This monitoring confirmed biological impairment downstream of the Stafford POTW. However, biological impairment was also found upstream from the POTW outfall. This observation led to a need to identify a cause that could account for the unexpected, up stream impairment. This case study examines only the moderate impairments at MR3 and WL1. Therefore, to develop different types of evidence, these sites are compared to sites immediately upstream that are less impaired and to unimpaired sites. For example, the site on Roaring Brook (RB1) is the best quality site in the watershed. The Middle River site (MR1) is the nearest upstream site in 1999 from the moderately impaired site MR3. Furnace Brook site FB2 and MR3 are the nearest upstream sites in 1999 from the most upstream Willimantic site WL1. As new sites are sampled the nearest available comparison site may be closer to the impaired site (see Comment 3).

Comment 3. Orienting the Assessment. Maps do not need to be fancy. Hand-drawn sketches can often be the fastest most efficient means to organize what is known and unknown. Recent advances in geographic information systems, however, have become readily accessible even since the beginning of this investigation. Some landscape attributes to consider are roads, dams, discharges, water withdrawals and returns. In this case, unmapped underground channels became very important and were only revealed by reconnaissance.

2.2. DESCRIPTION OF THE WATERSHED

The Willimantic River Watershed encompasses an area of approximately 582.7 km^2 in southern Massachusetts and northeastern Connecticut (see Figure 1). The Watershed's headwaters are located in Massachusetts; streams within the

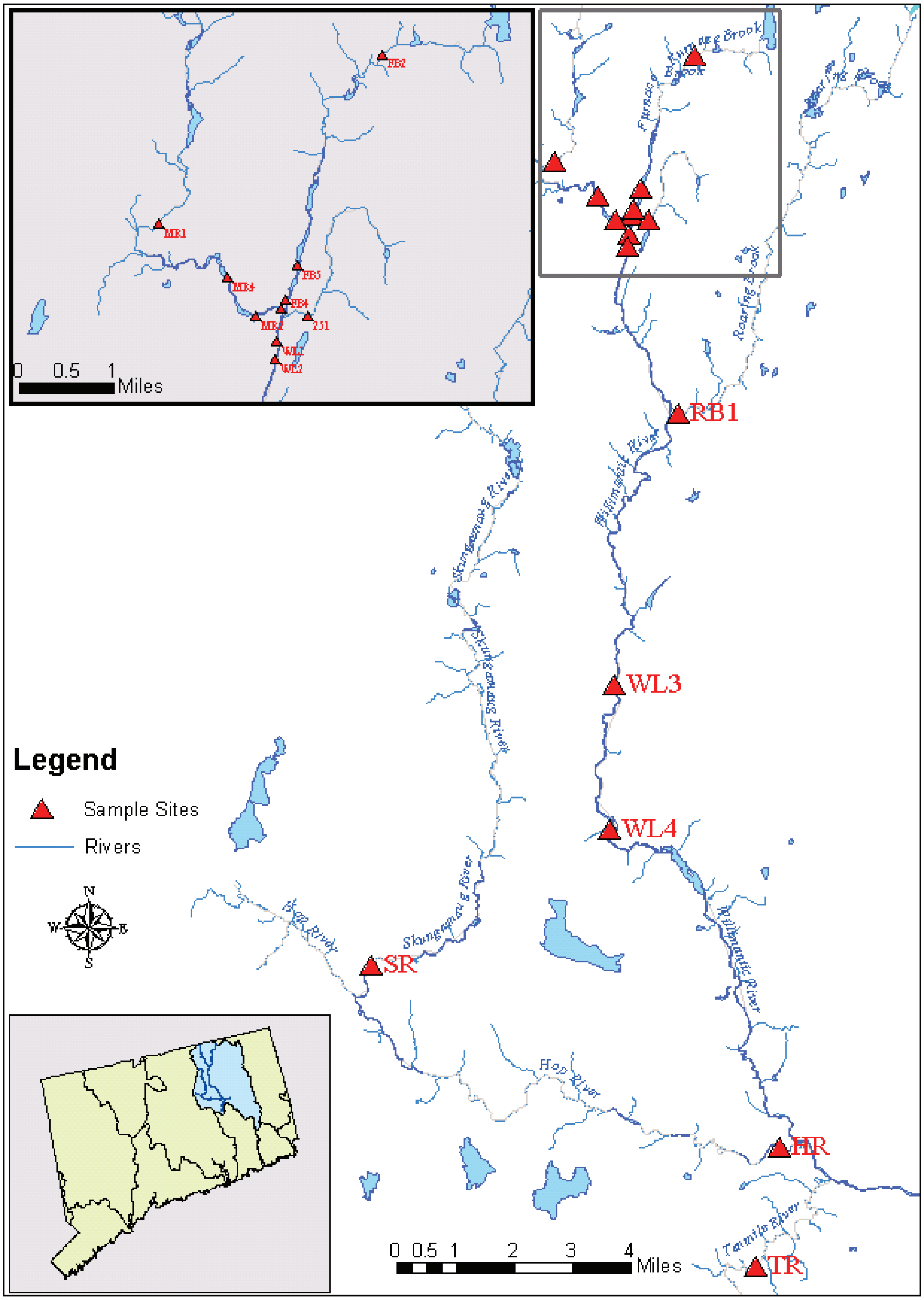


FIGURE 1

Willimantic Basin Depicted in Lower Left Insert of Connecticut. Sampling sites on the Willimantic River are depicted on the larger map. An enlarged insert of sampling sites near Stafford Springs appears in upper left insert.

watershed flow southward to Connecticut. At Stafford Springs, Connecticut, the Middle River and Furnace Brook join to form the Willimantic River. The Willimantic River then flows approximately 40 km before joining the Natchaug River; this confluence creates the Shetucket River. The Willimantic River and its tributaries have been used for power generation and waste disposal since the 1700s. Impoundments were built along the river and its tributaries to generate power for mills that became established in the towns of Willimantic, Stafford, and Stafford Springs, Connecticut. Many of these mills operated along the river for centuries; some are still active today. As a result, natural stream flow in the Willimantic River is regulated by the Staffordville Reservoir, located in the upper Furnace Brook Basin. The Staffordville Dam was built in the late 1800s to store and supply water for power generation and industrial uses. Today, power and industrial needs have declined in importance, and water recreation has become the primary use of the Staffordville Reservoir.

Figure 2 provides a schematic of the river, showing the locations of dams, major dischargers to the POTW, and sampling locations. The causal analysis focused on the lower sections of Middle River and Furnace Brook and the first few kilometers below their confluence that forms the Willimantic River. These sections of the rivers contained benthic invertebrate assemblages that did not meet Connecticut's minimum water quality standards for supporting aquatic life (see Section 2.4).

Throughout this impaired segment, the river has a moderately steep gradient. Average stream widths are approximately 6- to 9-m depths are generally less than 0.3 m. The dominant substrate in the segment is a mix of boulders, cobble, gravel, and sand (see Table A-7). Physical features of the Middle River, Furnace Brook, and the Willimantic River sampling locations near Stafford Springs are shown in photographs (see Figures 3-5)**.**

2.3. POTENTIAL SOURCES OF STRESSORS IN THE WILLIMANTIC RIVER

Potential sources of stressors that could affect aquatic life in the River include known point sources such as National Pollution Discharge Elimination System (NPDES)-permitted outfalls, inferred but ill-defined sources such as agricultural runoff, and unknown sources, such as unreported accidents or illicit outfalls. Unknown sources were hypothesized based on land uses and on the occurrence of stressors or their effects.

The watershed of the river segment being investigated is predominantly forested. However, the area includes a lightly developed residential area and a business center to the north, in Stafford Springs. This residential section of the Willimantic River is bordered by Nye-Holman State Forest to the west and portions of Nipmuck State Forest to the east. Small farms with cattle and orchards are scattered throughout the watershed; they are more prevalent in the Furnace Brook Watershed. The study area is located within the business center of Stafford Springs, in the town of Stafford (see Figures 2-5); it covers approximately 155 km^2, making it the third largest municipality

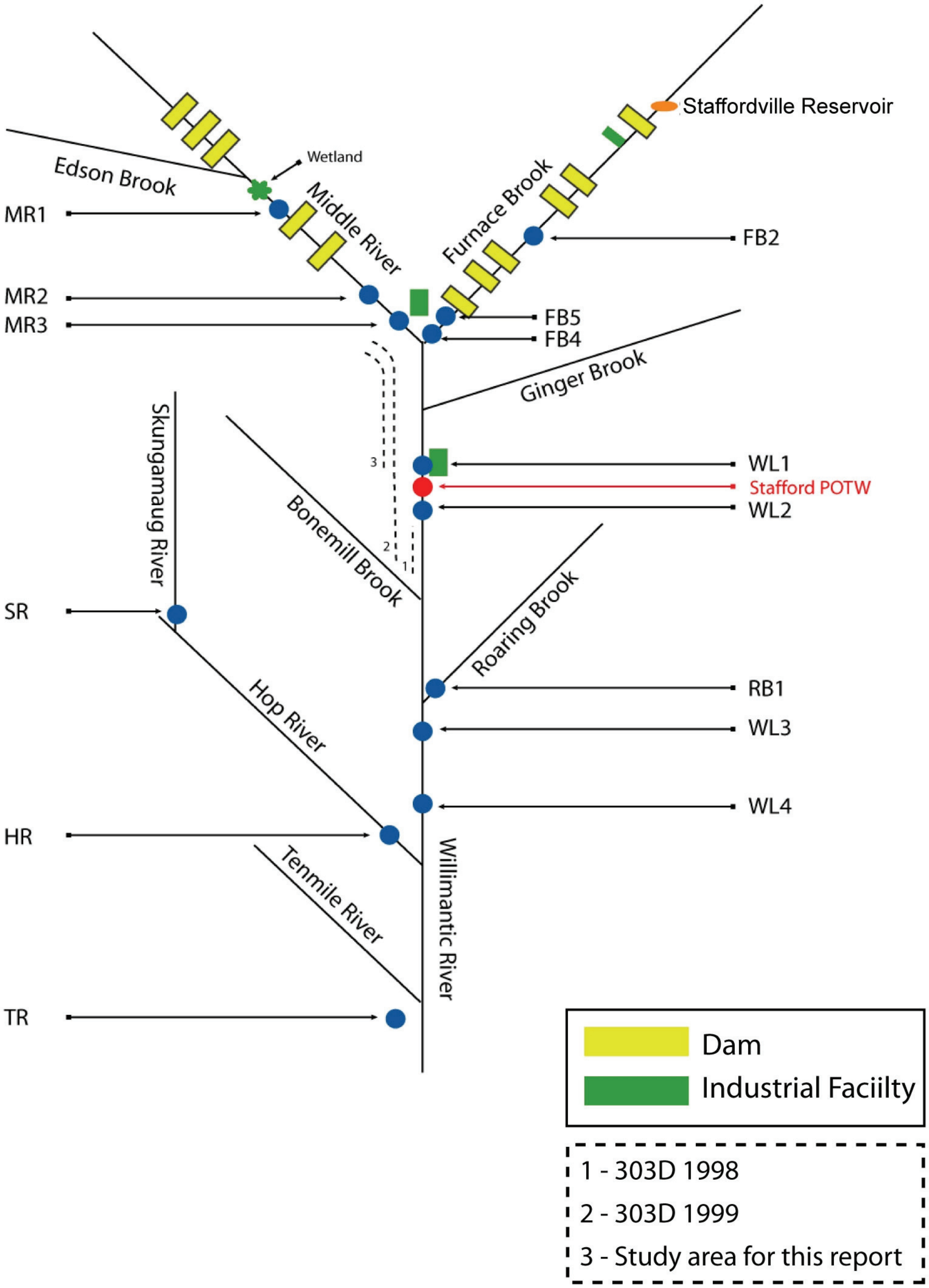


FIGURE 2

Schematic of Study Area in the Willimantic Watershed. Blue dots are sampling locations. Broken lines below MR3 indicate sections of river listed as impaired in 1996 (1), 1999 (2), and the study area (3). MR3, WL1, and WL2 are impaired.

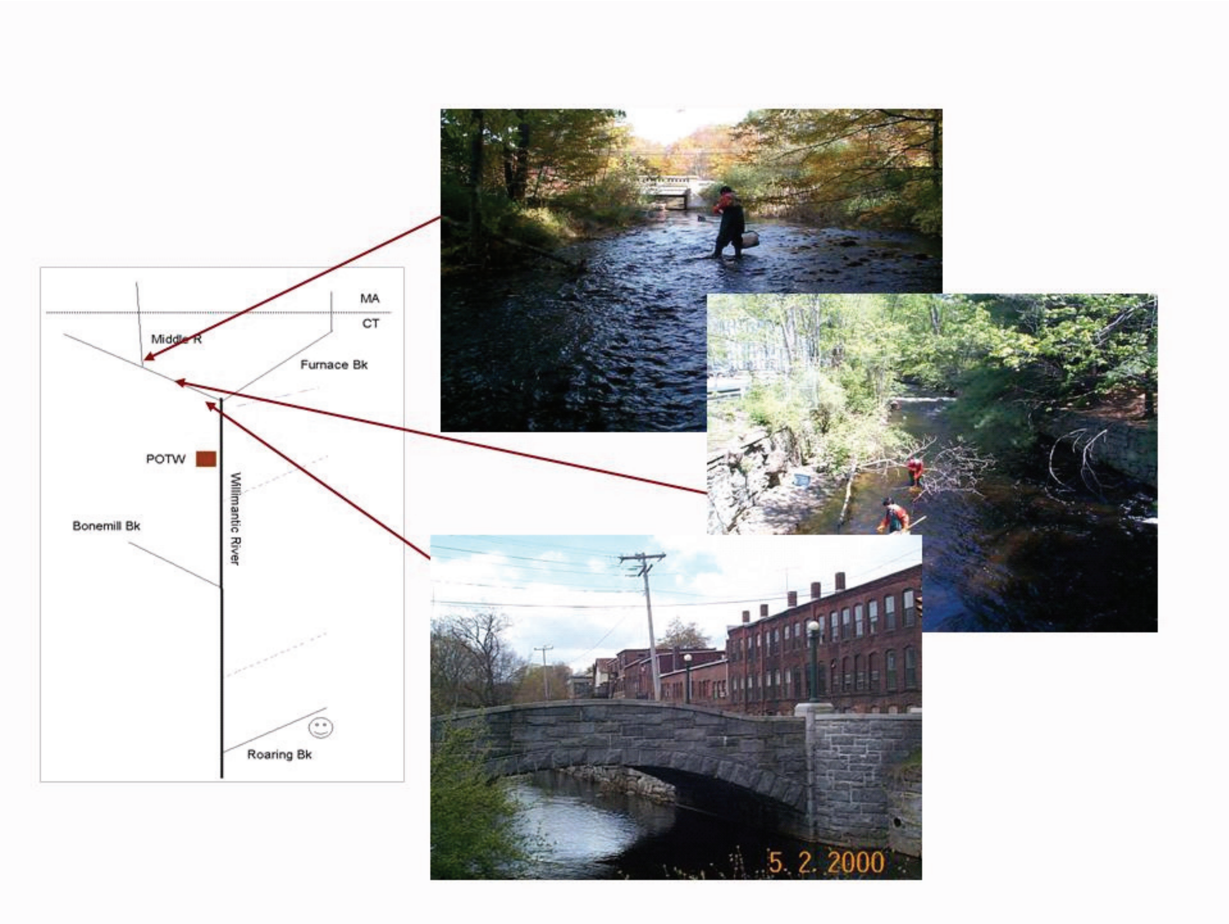


FIGURE 3

Features of Sampling Locations in the Middle River near Stafford Springs

FIGURE 4

Features of Sampling Locations in Furnace Brook near Stafford Springs

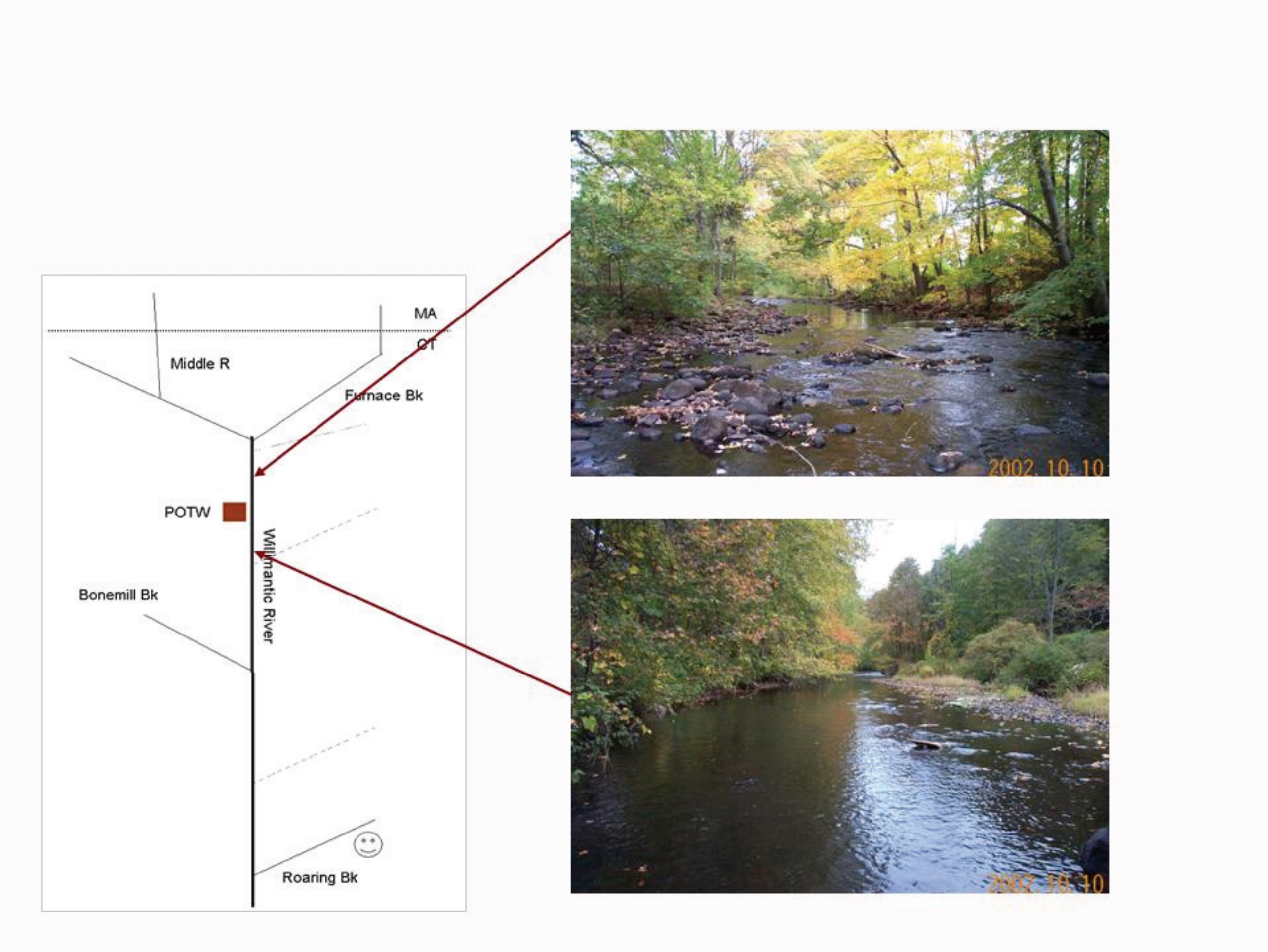


FIGURE 5

Features of Sampling Locations in Willimantic River near Stafford Springs by area in Connecticut. However, the population density of Stafford is low (~75 people per km^2; CT DEP, 2001), with most of these people living in Stafford Springs. Stormwater runoff and the effects of urbanization in the study area are suspected to contribute to the degradation of water quality in the 2.4-km 303(d)-listed segment.

One known point source and several nonpoint sources of pollution are associated with the Willimantic River (see Figure 2). The Stafford POTW is the only permitted point source within the study area. Five impoundments are located on the Middle River tributary to the Willimantic River, at km 9.2, 6.4, 4.8, 1.6, and 0.6 (see Figure 2). A ball-bearing production factory is located at km 0.6 and a woolen mill at 0.8 km. Although the manufacturing facilities may contribute to nonpoint source pollution of the rivers, they currently discharge wastes to the Stafford POTW. Six impoundments are located on the Furnace Brook tributary to the Willimantic River at km 7.2, 5.8, 4.2, 1.9, 1.0, and 0.5. There are also two circuit board facilities (located at km 6.4 and 4.0) and the same woolen mill facility associated with the Middle River (located at km 0.8) also were noted (see Figure 2). During the mid-1960s, Furnace Brook was channelized and lined from its mouth to just below the first impoundment (approximately 0.2 km). In Middle River and Furnace Brook, the impoundments retain a substantial amount of silt. Farm animals and geese have access to some of the impoundments. The sources associated with the main stem of the Willimantic River (downstream of the Furnace Brook and Middle River confluence) are (1) a filter manufacturer (at km 0.6) and (2) the Stafford POTW (located at km 0.8). Several additional nonpoint sources to the Willimantic River are associated with the municipality of Stafford Springs. These nonpoint sources include atmospheric deposition of pollutants and stormwater runoff from the city's impervious surfaces and lawns that can carry pollutants such as oil, deicing salts, pesticides, garbage, and lawn and garden chemicals (CT DEP, 2001).

2.4. SPECIFIC BIOLOGICAL IMPAIRMENT

Biological impairments of Connecticut's wadeable streams are assessed using primarily benthic macroinvertebrate data (CT DEP, 2004a). In 1999, macroinvertebrate data were collected following protocols in U.S. Environmental Protection Agency's (U.S. EPA) Rapid Biological Protocol 3 methodology (Plafkin et al., 1989). The state used a reference data set for determining impairment. In the case of the Willimantic River, the reference data set was from Roaring Brook, a tributary of the Willimantic River (see Figure 6).

The CT DEP invertebrate index, which was used to determine impairment, is calculated as a percentage of the score derived from seven metric values (see Table 1). A range of 0–6 points are assigned to each metric at each sampling location, and the points are summed to provide the total scores for the locations. The score for the Willimantic River was expressed as a percentage compared to the score at the reference area (Roaring Brook in this case). The CT DEP uses these percentages to evaluate the degree of impairment; the lower the percentage, the greater the impairment. In 1999, the lowest scores in the rivers were at and immediately

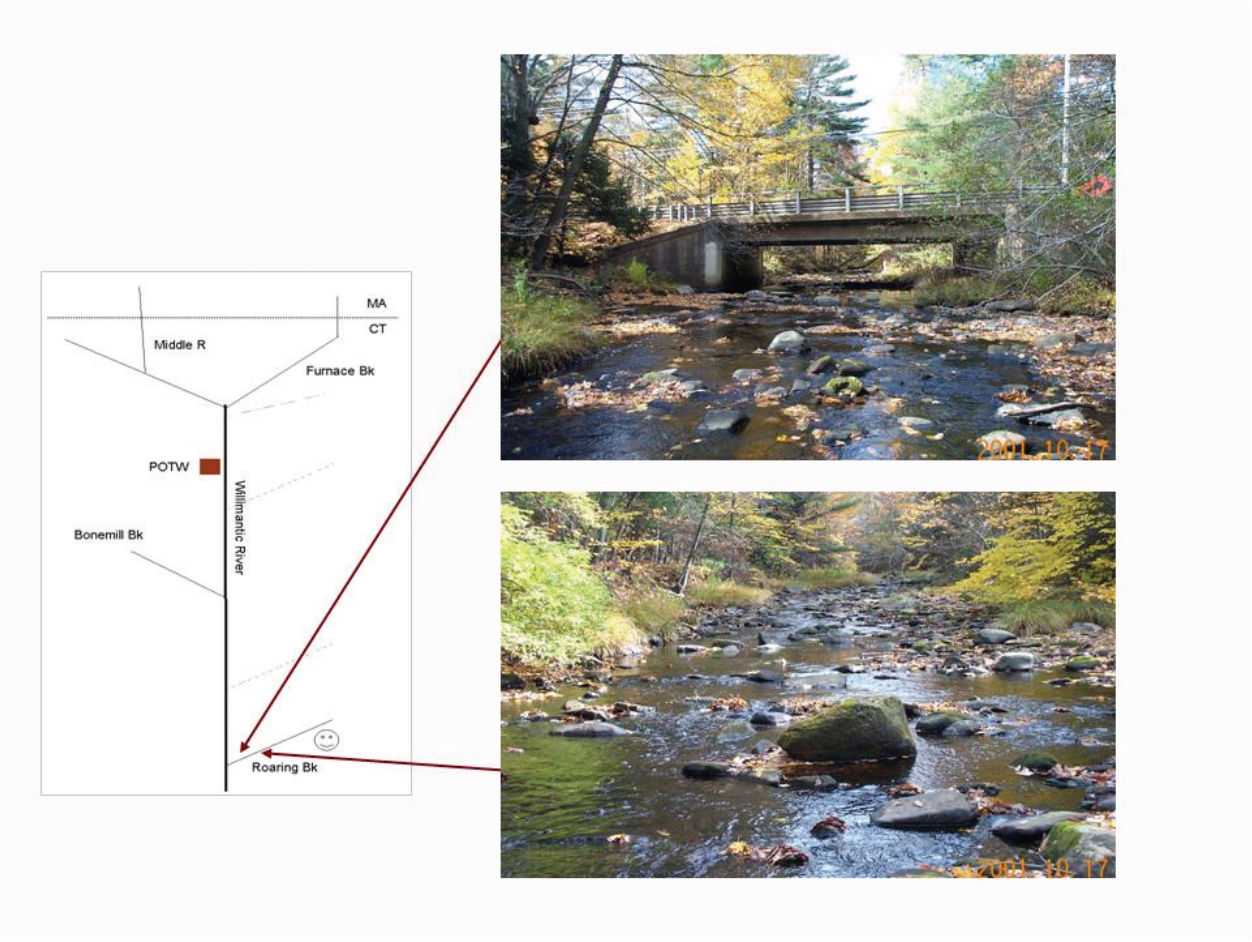


FIGURE 6

Features of Sampling Locations in Roaring Brook, Used as a Reference Area for the Willimantic Stressor Identification Case Study

TABLE 1

Benthic Macroinvertebrate Metrics Used to Calculate the Connecticut Invertebrate Index

Metric	Definition	The Invertebrate Index Increases When the Metric
Total Taxa	The total number of taxa found in the sample(s)	Increases
HBI (Modified)	Summarizes the overall pollution tolerance of the benthic macroinvertebrate community	Decreases
Scraper/Filterer	The number of scraper organisms divided by the number of filterer organisms	Increases
EPT/Chironomidae	The ratio of EPT to Chironomidae individuals	Increases
% Dominant Taxa	Measures the dominance of the single most abundant taxon	Decreases
EPT Index	Number of EPT taxa	Increases
Community Loss	Measures the loss of benthic species between a reference station and the station of comparison	Decreases

downstream of Stafford Springs. These locations included MR3 on the Middle River tributary to the Willimantic, WL1 in the Willimantic above the Stafford POTW and WL2 in the Willimantic below the POTW (see Figure 2). The CT DEP classified these three sites as Moderately Impaired. The other eight sites that were sampled in 1999 were classified as Nonimpaired, Slightly/Moderately Impaired, or Slightly Impaired (see Figure 2, Table 2).

TABLE 2

Individual Metric Values and Metric Scores Available at Beginning of Assessment

Metric/Stations	RB1	FB2	MR1	MR3	WL1	WL2	WL3	WL4	SR	HR	TR
Data Values											
Taxa Richness	33	24	32	23	10	12	30	23	29	35	37
HBI (Modified)	2.37	4.42	3.63	5.24	4.95	4.41	3.13	3.68	3.14	2.97	2.79
FFG-Scraper/Filterer	1.79	0.07	5.56	0.06	0.01	0.01	2.33	2.40	0.84	0.78	5.06
EPT/Chironomidae	26.5	111	9.09	14.7	93.0	19.2	10.8	14.8	46.0	18.7	31.5
% Dominant Taxa	0.31	0.30	0.13	0.46	0.77	0.48	0.22	0.43	0.23	0.11	0.27
EPT Index	25	13	17	9	5	5	17	16	16	21	18
Community Loss	NA	0.79	0.66	1.00	2.80	2.33	0.53	0.83	0.55	0.40	0.46
Metric Scores											
Taxa Richness	6	4	6	4	0	0	6	4	6	6	6
HBI (Modified)	6	2	2	0	0	2	4	2	4	4	4
FFG-Scraper/Filterer	6	0	6	0	0	0	6	6	4	4	6
EPT/Chironomidae	6	6	2	4	6	4	2	4	6	4	6
% Dominant Taxa	2	4	6	0	0	0	4	0	4	6	4
EPT Index	6	0	0	0	0	0	0	0	0	4	2
Community Loss	6	4	4	4	2	2	4	4	4	6	6
Index Score	38	20	26	12	8	8	26	20	28	34	34
% of Reference	100	53	68	32	21	21	68	53	74	89	89
Impairment Category	Ref	SL/ Mod	SL	Mod	Mod	Mod	SL	SL/ Mod	SL	N	N

HBI = Modified Hilsenhoff Biotic Index.
FFG = functional feeding group.
Ref = Watershed Reference.
SL = Slightly Impaired.
Mod = Moderately Impaired.
N = Not Impaired.

Causes of impairments can be discerned, in part, by their biological effects, as reflected in the metrics used to calculate the index score (see Table 1). For this case study, metrics were selected based on (1) the independence of the metric, and (2) the unconfounded nature of the measurement (see Table 3).

TABLE 3

Choosing Metrics

Metric Selection Considerations	Rationale
1. The magnitude of the change, from the nearest upstream site or reference site	Large effects are more important and more likely to be nonrandom
2. The independence of the metric	Some indices and other metrics are not independent of each other, because they include the same taxa abundances or other attributes
3. The different patterns of change from site to site	Metrics that vary in different patterns may be changing due to different causes, therefore, they should be retained so that the causes can be distinguished
4. The unconfounded nature of the measurement	Percentages and proportional abundances should be avoided because changes may be due to changes in the denominator or referent rather than the numerator, so it is not clear what biological change requires a causal explanation
5. The relevance of the measurement to ecological processes or environmental values	Metrics that reflect designated uses, important processes, or stakeholder interest should be retained
6. The highest practical level of specificity	Narrowly defined effects increase the likelihood of identifying the cause

Five of the seven metrics used in the index were not used in this causal analysis. The Community Loss metric was excluded because it closely reflects the inverse of the Taxa Richness Metric (Consideration 2, above). Four proportional metrics (the modified Hilsenhoff Biotic Index, the ratio of Scrapers to Filterers, the ratio of Ephemeroptera, Plecoptera, and Trichoptera [EPT] individuals to individuals in the Chironomidae, and Percent of Dominant Taxa) were not used because of confounding (Consideration 4). Original unprocessed data could have been used to develop measurements of the impairment using specific taxonomic groups (Consideration 6), but these were not available at the beginning of the study. However, species-level data for presence and abundance were examined at the end of the case study, when they became available to the U.S. EPA. We found these data to be useful for evaluating the confidence in the causal analysis (see Comment 4).

The two metrics retained for use in characterizing biological impairment were total taxa richness (TT) and the EPT index. The TT score includes EPT, so the number of EPT taxa was subtracted from TT to give the number of non-EPT taxa. Actual numbers of taxa, rather than the relative percentage of taxa, were used in the evaluation to retain as much information as possible.

2.4.1. Number of EPT Taxa

The impairment at MR3 was defined as a reduced number of EPT taxa, compared to the number of EPT taxa found at MR1. One component of the impairment at WL1 was an incremental decline in EPT taxa, compared to the number of EPT taxa found at MR3 (see Table 4). The number of EPT taxa was originally developed as an indicator of organic loading and associated declines in concentrations of dissolved oxygen (DO) (cf. Lenat, 1987). However, EPT taxa can be sensitive to other stressors (Wallace et al., 1996).

Comment 4. Retrospectives on Defining Impairment. Although not available at the time, macroinvertebrate data for the Willimantic sites indicate that impairment also could have been defined based on either the presence of individual species or the number of individual organisms at the reference and impaired sites. For example, most EPT taxa at the impaired sites were filter-feeding caddisflies (hydropsychids; up to 75% of EPT taxa at WL1) some of which are known to be tolerant of metals, and which are commonly found below impoundments (Cain and Luoma, 1998; Kiffney and Clements, 2002). Additionally, the relative abundance of taxa changed at MR3 and WL1 after the illicit discharge was rerouted. This change provided evidence for improvement at the impaired sites, above and beyond changes that occurred in the numbers of taxa at the sites. For example, during the second year after rerouting, the abundance of the hydorpsychid caddisfly *Cheumatopsyche* at MR3 decreased, relative to the reference site. Furthermore, we found no stoneflies initially at the impaired sites, but one stonefly species was found at MR3 after the discharge had been rerouted. This important detail—the addition of a new order, Plecoptera, rather than just another filter-feeding hydropsychid caddisfly—would be unremarkable if reported only as an increase of one EPT species, and the reduced abundance of *Cheumatopsyche* would not be noticed at all.

TABLE 4

Biological Impairment Based on Autumn 1999 Sampling (see Table A-8)

	MR1	MR3	Difference MR1 to MR3	WL1	Difference MR3 to WL1
Number of EPT Taxa	17	9	Decrease	5	Decrease
Number of Non-EPT Taxa	15	14	Similar	5	Decrease

2.4.2. Number of Non-EPT Taxa

The number of non-EPT taxa were similar at the upstream reference MR1 (15) and at MR3 (14). Furthermore, there were more non-EPT taxa at MR3 than at the reference site, RB1 (8) (see Table A-8). For this reason, we considered the number of non-EPT at MR3 to be nonimpacted.

About 30% fewer non-EPT taxa were found at WL1, compared to MR1 or MR3 (see Table 4). Although many non-EPT taxa can tolerate low or even moderate levels of common pollutants, severe reductions in the numbers of taxa can indicate biological impairment due to toxic and nontoxic causes. DeShon (1995) and Ohio EPA (1987) concluded that reduced number of taxa may reflect more monotonous habitat structure. This generalization is relevant where stressors, such as sedimentation, are natural habitat features. Whatever the mechanism, the reduced number of non-EPT taxa at WL1 suggests that conditions at this location may differ from those at MR1 or MR3.

3. LIST THE CANDIDATE CAUSES

3.1. SIMILARITY OF SITES

The occurrence of impairment upstream of the POTW prompted an evaluation of the causes of impairment at MR3 and WL1, sites above the POTW. Because the index score at WL1 was less than that of the more upstream but impaired MR3, the causes of impairment at MR3 and WL1 were analyzed separately (see Table 4). WL1 is upstream of the POTW outfall and downstream of the confluence of Middle River and Furnace Brook and it was possible that MR3 (upstream of the confluence) and WL1 (downstream of both Middle River and Furnace Brook) were being influenced by different stressors. The following scenarios were considered for explaining the apparent greater impairment at WL1 relative to MR3:

1. The magnitude of the same stressor increases from MR3 to WL1;
2. an additional stressor occurs at WL1 but not at MR3;
3. measurement error, rather than a change in stressor type or stressor level, accounted for the difference; or
4. completely different causes for impairments occur at MR3 and WL1.

Likewise, the biological impairment at WL2, downstream from WL1 and the POTW, could be due to the same stressor, a related stressor, or a completely different stressor. However, in this case study, we focused on identifying the cause(s) of the unexpected impairments upstream from the POTW at sites MR3 and WL1. The CT DEP continued to sample below the Stafford POTW and separately developed a TMDL for the Willimantic River from the Stafford POTW to Bonemill Brook (see Comment 5).

Comment 5. A TMDL was Developed while the Causal Analysis was Performed.

In 2001, the TMDL was adopted for copper, lead, and zinc. NPDES permit limits for the Stafford POTW were revised based on this TMDL. CT DEP personnel worked with the Stafford POTW personnel to gain a better understanding of influent metals loading to the POTW. Administrative orders were issued to industrial users of the POTW to study ways to reduce loadings. A privately owned water company voluntarily agreed to remove zinc from the water distribution system. In 2002, aquatic life standards were met at WL2, a year before improvements were noted in upstream locations.

3.2. SELECTION OF CANDIDATE CAUSES

Six Candidate Causes of the biological impairments at sites MR3 and WL1 were hypothesized based on the available data described above, information on land use in the Willimantic River Watershed, and common causes of impairments in streams. Habitat loss associated with undercut banks, woody debris, stream geomorphology, and geological substrates also were proposed as Candidate Causes. Although biological sampling was restricted to riffles, stream geomorphology, and geological substrates were considered in conjunction with analysis of embedded substrates. The six Candidate Causes that were considered are listed below.

1. toxicity from metals, ammonia (NH_3), or an undefined mixture of substances;
2. removal of organisms by high-flow events;
3. loss of interstitial habitat due to settled particles;
4. asphyxiation due to low levels of DO;
5. heat stress; and
6. taxa loss due to altered feeding resources from impoundment retention of leaf litter and exportation of fine particles.

The rationale for including each of these Candidate Causes is given in Table 5. Conceptual models were developed for each of the Candidate Causes (see Figures 9-14). Each of the conceptual models was annotated with information derived from the causal analysis; the annotated conceptual models presented with the final evidence in Section 6, Identify the Probable Cause.

When multiple sources were hypothesized, pathways were identified and numbered for analysis of evidence for causal pathways (see the strength-of-evidence [SOE] tables in Appendix B). In the course of performing the causal analyses, Candidate Cause 1 was divided into more specific Candidate Causes (sustained and episodic) as well as pathways based on the type of source. Those specific Candidate Causes are

1.1a—Sustained exposure to metals or ammonia from nonpoint sources;

1.1b—Sustained exposure to metals or ammonia from a point source; and

1.2—Episodic exposure to undefined toxicants.

TABLE 5

Rationale for Including Each Candidate Cause of Impairment

Rationale for Inclusion	Elaboration of the Rationale
Candidate Cause 1: Toxicity from Metals, Ammonia, or a Complex Mixture	
Metals and NH_3 were detected in the stream and there were potential sources of toxic compounds: the impoundments, nonpoint sources, and unknown point sources. Toxic chemicals can lower diversity of benthic invertebrates through long-term exposures as well as through periodic or episodic exposures. Periodic or episodic events could easily be missed if sampling is infrequent and, therefore, monitoring data might not reveal these sources. The pathways of periodic/episodic and long-term exposures are the same (see Section 6, Figure 9).	There are no permitted discharges directly into the Willimantic River above the Stafford POTW. Therefore, Sustained Exposures of invertebrates at MR3 and WL1 (upstream of the POTW) could occur only as a result of (1) “inplace” contaminated sediment or continually resuspended or leached contaminants from sediments and/or (2) undocumented and nonpermitted direct discharges. Since the sources are not characterized, the pathways in Figure 9 are not evaluated separately. Rather, NH_3 and the eight measured metals are evaluated independently of source. Organics and other unmeasured chemicals are not evaluated, because of the lack of source or concentration information. Periodic or Episodic Exposures could occur if (1) upstream contaminated sediment behind impoundments is mobilized during major storms; and/or (2) surface runoff from roads, lawns, and farms carries road salt, oil, metals, pesticides, and other chemicals into the stream during storms that occur after an extended dry period; and/or (3) accidental releases and other episodic releases from unknown point sources, which have not been diverted to the POTW. NH_3 can occur in streams by direct discharge or through the nitrogen cycle. NH_3 can be formed by decomposition of organic amines or by conversion of nitrate to nitrite and nitrite to ammonium (NH_4^+) in the presence of low DO. NH_4^+ is converted to the more toxic form of NH_3 at high pH, usually above 7.5. In the Willimantic Watershed, potential sources of organic amines, NH_3, NH_4^+, or NO_2 include aerial deposition, animal wastes, fertilizer, failed septic tanks, and inadequate waste treatment.

Comment 6. How Others Have Demonstrated Metal Toxicity.

Although not necessary for the Willimantic case, linkages between metals (or toxics) aggregations and effects to benthic macroinvertebrates are often difficult to establish. Transfer of contaminants can be mediated by presence of biofilm that entrains high concentrations of toxics and is consumed by BMIs. The difficulty in linking presence of toxics concentrations with biological response can result in weak correlations or conclusions. One consideration for determining toxics transfer is to suggest/perform *in situ* measurements of overlooked media (e.g., biofilm) that clarifies the relationship between toxics presence and bioavailability. The following literature citation provides an example for how much detail about the food chain is necessary in order to substantiate cause-and-effect linkages for toxics transfer.

Farag, A.M., D.F. Woodward, J.N. Goldstein, W. Brumbaugh and J.S. Meyer. 1998. Concentrations of metals associated with mining waste in sediments, biofilm, benthic macroinvertebrates, and fish from the Coeur d’Alene River Basin, Idaho. Archives of Environmental Contamination and Toxicology 34:119−127.

TABLE 5 cont.

Rationale for Inclusion	Elaboration of the Rationale
Candidate Cause 2: Removal of Organisms During High Flows	
High-flow events can occur in this part of the system because Furnace Brook and Middle River are channelized and this shortens the flow path, reduces roughness, and increases gradient, resulting in increased velocity. Also, impervious surfaces are greater in the town's center and can increase the variance in flow at MR3 and WL1. Two causal pathways were considered: increased hydrological flow due to increased impervious surfaces during spring high flows or during storms and a more local removal of organisms at stormwater outfalls (see Section 6, Figure 10).	Benthic macroinvertebrates are morphologically and behaviorally adapted to a range of flow conditions. However, stream flow can become so powerful that it flushes macroinvertebrates downstream (Holomuzki and Biggs, 2003). Scouring of geologic substrates can also dislodge macroinvertebrates making them more vulnerable to removal (Kilbane and Holomuzki, 2003). In the Willimantic River, the hydrology is modified by impoundments, which have continuous flow over their dams and channelization, especially in and around Stafford Springs where Furnace Brook is modified with concrete armament of the streambed and banks for the last 0.2 km. Middle River is armored with stone walls or granite rip rap and channelized in the lower 0.6 km, but it maintains a natural cobble and gravel streambed. The surrounding area is characterized by steep hillsides and a moderate amount of impervious surfaces in the town of Stafford Springs. While flooding has occurred in Stafford Springs, it is now controlled by a reservoir at km 7.2 and a deepened and controlled channel of the river through the town.

TABLE 5 cont.

Rationale for Inclusion	Elaboration of the Rationale
Candidate Cause 3: Loss of Interstitial Habitat Due to Settled Particles	
Sedimentation is a common cause of impairment in streams, and there was evidence of increased embedded substrates at MR3 and WL1. Causal pathways involving several sources were considered: impoundments, unknown sources, winter road treatments with sand, bank failure, and streambed scour (see Section 6, Figure 11).	Benthic macroinvertebrates occupy a variety of habitat and feeding niches, including spaces between rocks and gravel of all sizes. Highly to moderately graded streams, like the Willimantic River, can be expected to have well sorted substrates that are free from mud, silt, and excess algal growth, thereby permitting well aerated water to flow through the spaces between the substrate particles. When flows are unnaturally low and there are sources of material that becomes embedded in the substrates, the interstitial spaces, and habitats under rocks can be eliminated and sensitive organisms can be smothered or excluded, and intergravel oxygen can be depressed asphyxiating sensitive species. Potential sources of embedding materials in the Willimantic River include clays, silts, and sands from failed or scoured stream banks, release of water containing resuspended sediments (Whiles and Dodds, 2002;) and organic matter from impoundments (Wisconsin Department of Natural Resources, 2001), agricultural and silvicultural erosion, construction erosion, sand from road treatment, animal waste, industrial waste, and excess algal growth in the stream or impounded areas. Delivery of material can increase during high-flow events and deposition increased in some areas or during periods of slower flow. Conversely, scouring due to increased stream power combined with insufficient sources of new sediment can result in insufficient sediment in older, urban systems where impervious surfaces increase stream power during periods of high rainfall and where impoundments and armored streambed and banks restrict addition of sediment. This pathway was not proposed as a Candidate Cause because substrates were known to be moderately embedded at MR3 and WL1.

TABLE 5 cont.

Rationale for Inclusion	Elaboration of the Rationale
Candidate Cause 4: Asphyxiation Due to Low Dissolved Oxygen	
There were potential sources that could lower DO in the Willimantic and low DO is a common cause of biological impairment. Four causal pathways were considered: organic enrichment, nutrient enrichment, deoxygenated water from the impoundments and channel modification (see Section 6, Figure 12).	Benthic macroinvertebrates vary in their requirements for DO for survival (Nebeker, 1972). Ephemeroptera and Plecoptera are some of the more sensitive taxonomic orders. DO concentrations can be reduced by bacterial respiration when the water column or the stream's substrates are enriched with allochthonous organic matter or decaying algae, which result from elevated nutrient levels. Potential sources of nutrients or organic matter include aerial deposition, fecal waste from water fowl, farm animals, fertilizers, and failed septic systems. DO is less soluble in warmer water, therefore, inadequate shading can increase heating and result in lower DO concentrations. DO can become depleted in low-flow conditions where a lack of turbulence prevents the water column from reoxygenating. Impoundments can reduce DO concentrations by reducing stream velocity and aeration, and although not observed could interrupt flow during droughts. Impounded water can become stratified with the colder hypolimnion becoming deoxygenated due to bacterial respiration while warmer temperatures reduce oxygen solubility in the epilimnion. Either way, impoundments can reduce DO in water released to the stream.
Candidate Cause 5: Temperature Stress	
Impoundments and channel modifications potentially raise the temperature of the water. Temperature could also become elevated in the river and impoundments from lack of canopy cover and increased surface area available to absorb solar radiation, or from transferred heat from impervious surfaces to stormwater runoff (11) (Wisconsin Department of Natural Resources, 2001) (see Section 6, Figure 13).	Benthic macroinvertebrates in New England streams are adapted to cool temperatures in the summers and below-freezing temperatures in the winters. While warmer water temperatures can be tolerated for short periods of time, long-term elevation in stream temperature or abrupt temperature increases from stormwater can kill sensitive species or cause them to be replaced by less sensitive species. Also, elevated temperatures can indirectly contribute to other causes of impairment (Candidate Causes 3, 4, 6). Warmer temperatures can increase algal production causing a shift in the food resource or contributing to embedded substrates (Candidate Causes 3 and 6), reduce the solubility of DO (Candidate Cause 4) and generally increase stress and food requirements.

TABLE 5 cont.

Rationale for Inclusion	Elaboration of the Rationale
Candidate 6: Taxa Loss Due to Altered Food Resources	
Impairments occurred in an urban area where conditions might favor algal growth (Whiles and Dodds, 2002) and decrease the supply of decaying leaves. Four causal pathways were considered: (1) the reduction of leafy and woody debris and addition of algae by reservoirs and impoundments, (2) nutrient enrichment from multiple sources leading to algal growth, (3) organic matter release from farms, and (4) the reduction of leafy and woody debris from deforested stream banks (see Section 6, Figure 14).	The assemblages of macroinvertebrates can change when an allochthonous resource (e.g., woodland debris) is replaced by an autochthonous food resource (algae or bacteria) or by addition of allochthonous fine particulate or dissolved organic matter. Algal and bacterial growth is promoted by nutrient enrichment, especially phosphorous, warmer temperatures and adequate light. In extreme cases, nutrient enrichment can lead to toxicity due to NH_3 (Candidate Cause 1), algal growth filling interstitial spaces (Candidate Cause 3), and bacterial respiration causing low DO (Candidate Cause 4). Runoff carrying aerial deposition, animal waste, fertilizer, and failed septic tanks is a potential source of nutrient and organic matter enrichment in the Willimantic Watershed.

4. EVALUATE DATA FROM THE CASE

4.1. SOURCES OF DATA FROM THE CASE

All data used in the causal analysis reported here were provided by the CT DEP and were collected during either routine monitoring or special sampling events. Tables A-1 through A-2 contain sampling times, locations, and measurements. Fish, benthic macroinvertebrates, habitat data, and water samples were collected throughout the Willimantic River Basin. Data from 15 of these locations (see Figure 1) were used for this study. The watershed reference site, RB1, is located on Roaring Brook, a tributary of the Willimantic River, 5.6 km downstream from Stafford Springs. The Roaring Brook Watershed has a drainage area that is roughly the same size as the Middle River and similar geology. At RB1, the stream is well aerated by riffles and the substrates are dominated by cobbles; the substrates are slightly embedded, and many of the cobbles support periphyton. Stream banks at RB1 are not particularly high, so when the stream floods, it connects with a forested flood plain on both sides of the stream (see Comment 7).

Comment 7. Sites Used for Comparisons.
This case study examines the greatest impairment (MR3, WL1, WL2) based on # of EPT taxa and invertebrate index score. Therefore, when developing different types of evidence, moderately impaired sites (MR3 or WL1) are compared to sites immediately upstream that are less impaired and with other unimpaired sites. For example, the site on Roaring Brook (RB1) is the best quality site in the watershed. The Middle River site (MR1) is the nearest upstream site in 1999 from the moderately impaired site MR3. In 2002, MR2 is the nearest upstream site to MR3. Furnace Brook site FB2 and MR3 are the nearest upstream sites in 1999 from the most upstream Willimantic site (WL1). As new locations are sampled the analysis shifts to using MR2 and FB5 when appropriate.
A more inclusive study that also examined the slight impairments throughout the watershed can be found in U.S. EPA (2003b) and implicates the presence of dams in the watershed.

Four sampling sites were located on the Willimantic River: WL1 (0.64 km), WL2 (0.97 km), WL3 (12 km), and WL4 (20 km). Three sites were located on the Middle River: MR1 (3.2 km), MR2 (0.6 km), and MR3 (0.16 km). We also used three sites on Furnace Brook: FB2 (3.9 km), FB4 (0.16 km), and FB5 (0.32 km) (see Figure 2). River kilometers are based on the distance from the confluence of Furnace Brook and Middle River. We also used data from three other tributaries: the Skungamaug River (SR), Hop River (HR), and Tenmile River (TR). These three tributaries flow into the Willimantic downstream from the study area (see Figure 2). In 2000, after this case study had started, sampling was performed at two additional sites—one on Middle River (MR2; 0.6 km) and one on Furnace Brook (FB5; 0.16 km).

On the Middle River, benthic invertebrates were sampled at MR2, a riffle located downstream from the last impoundment on the Middle River. MR2 is below a small impoundment and adjacent to a ball-bearing factory and upstream from the impaired site, MR3. On Furnace Brook, additional sampling occurred at FB5 downstream from the last impoundment on Furnace Brook, adjacent to the woolen mill, and upstream from a concrete flood abatement channel and WL1.

Sampling occurred more frequently at RB1, MR3, WL1, and WL2 than at the other sites. The additional data from these four sites permitted some temporal analyses using data from the autumn of 1999, 2000, 2001, and 2002, and spring of 2000 and 2001. The types of samples collected, and the sampling dates and locations, are provided in Tables A-1 through A-2. All sample data collected by the CT DEP are given in Appendix A; a hydrograph for the Willimantic located several kilometers downstream from the study locations is shown in Figure A-1.

Macroinvertebrates were sampled using modified versions of the U.S. EPA's level III Rapid Bioassessment Protocols (RBP) (CT DEP, 1999; Plafkin et al., 1989; Barbour et al., 1999). The primary macroinvertebrate assessment period in Connecticut is the autumn, although spring samples sometimes are collected to supplement autumn data for special studies. Each sampling event involved sampling 2 m^2 of stream riffle habitat with a kick net (12 kicks); all organisms within a 200-organism subsample were identified to the lowest practicable taxonomic level for calculating macroinvertebrate metrics. Only the macroinvertebrate data were used as indicators of biological impairment because fish were not collected from all 14 sites.

The CT DEP collected data on water chemistry and habitat quality at most sites in 1999 and in some succeeding years (see Appendix A) (see Comment 8). No chemical analysis of sediment was available due to cost. Data used in the analyses included measures of substrate composition (percentage of boulders, cobble, gravel, and sand) and three metrics from the CT DEP habitat index: embeddedness, water velocity, and bank stability. Ambient water chemistry measurements were from grab samples collected between 0800 and 1600 hours. Water column chemical and physicochemical parameters used in the analysis included temperature, DO, total solids, turbidity, organic nitrogen, NH_3, total Kjeldahl nitrogen (TKN), nitrate (NO_3), nitrite (NO_2), total phosphorous (P), 5-day biological oxygen demand (BOD) and the concentrations of total and dissolved aluminum (Al), cadmium (Cd), chromium (Cr), Cu, iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn). No data on organic chemicals or pesticides were available. Total nitrogen (TN) was calculated by summing TKN, NO_3, and NO_2 (the levels of NH_3 were too low to contribute much). Discharge data for the

Comment 8. Analyzing Sparse Causal Data.

Abundant data is usually preferred, but in most cases costs restrict monitoring to grab samples on a few occasions. Sparse data contains information and should be analyzed conservatively. There is a difference between using sparse data to determine next steps and the data needs for testing scientific hypotheses.

Traditional statistical approaches to data interpretation are often not appropriate for causal analysis. Field data rarely meet the assumptions and requirements of statistical tests, which were designed for the analysis of experimental results, and causal analysis requires multiple lines of evidence indicating stressor influence on biological responses. Traditional hypothesis testing does not indicate whether or not the stressor influenced the response, it only indicates whether variability in the response is greater than one would expect from random variation.

See CADDIS for tips on analyzing data, especially paired data.

study period was obtained from a gauging station on the Willimantic River at Coventry, CT (see Figure A-1).

4.2. EVALUATION OF DATA FROM THE CASE

A causal analysis using data from 1999, the first iteration, indicated that none of the Candidate Causes were of sufficient magnitude to account for the observed effects. The analysis led to a specific strategic sampling plan that was implemented between 2000 and 2002 (second iteration and confirmation). Data evaluations for both the first iteration performed in early 2000 and the second iteration in 2003 are presented in this section. Tables A-1 through A-2 show which data were available for each iteration. The conceptual models (see Figures 9-14) indicate the year the data were collected. In some cases, new evidence was found that changed the causal analysis. The final form of evidence (second iteration) is depicted in the conceptual models.

4.2.1. Spatial/Temporal Co-occurrence

Under most circumstances, the biological effect was expected to be observed where and when the cause was observed, but not where and when the cause was absent (see Comment 9). Because data were not available for all years at every site, only the autumn 1999 data were used except where noted for temperature and DO. For both iterations, associations were determined for co-occurrence by comparing the concentration or level of a proximal cause at sites MR3 and WL1 (where the impairments occurred) to the nearest upstream location or to the watershed reference site (see Tables 6 and 7). That is, if the levels of the stressors were greater (i.e., concentrations of toxicants or temperature were greater or the concentrations of DO were less) at an impaired segment than at the comparator segments, the Candidate Cause and the impairment were said to co-occur. We compared conditions at MR3 to conditions at MR1 and RB1; for WL1, comparisons were made to MR3 and FB2. Co-occurrence could be used to eliminate Candidate Causes and to evaluate the SOE. A choice had to be made whether to use total or dissolved, and mean, median, or maximum levels, for the water chemistry parameters. We used a conservative approach: we selected maximum total-metal concentrations to evaluate potential exposure because extreme events could cause the effects and there were few samples (see Comment 10).

Comment 9. Types of Evidence.
The terminology used here is adapted from human epidemiology as described in the CADDIS. Definitions of the terms are in italics when a new type of evidence is introduced throughout this case.

Comment 10. Cautions for Co-occurrence.
Evidence of spatial/temporal co-occurrence should be evaluated with caution when multiple sufficient causes may be present, and when the objective of the analysis is to identify all contributing causes. For example, candidate causes occurring upstream may mask the effects of candidate causes occurring farther downstream, even though those candidates may be contributing to the observed effects. Also, exposures can occur from sediment or ingested food, which are often not analyzed during monitoring programs to save costs. See CADDIS for more on Co-occurrence.

Some benthic macroinvertebrate data were available for the spring of 1999 and 2000; we compared these data to data from the autumn collections in 1999,

TABLE 6

Causal Analysis I and II: Spatial Co-occurrence for MR3 (Autumn 1999 except where noted)

Spatial Co-occurrence

Candidate Cause		MR1 Reference	RB1 Reference	MR3 Impaired Site	Adverse Change for MR3 Compared to References	
1: Toxics	Total Metals and Ammonia (mg/L)					
		MR1	RB1	MR3	MR1	RB1
	Al	0.080	0.037	0.101	Yes	Yes
	Cd	0	0	0	No	No
	Cr	0	0	0.005	Yes	Yes
	Cu	0.004	0.004	0.005	Yes	Yes
	Fe	0.395	0.208	0.695	Yes	Yes
	Ni	0	0.001	0	No	No
	Pb	0.001	0	0.001	No	Yes
	Zn	0.006	0.004	0.011	Yes	Yes
	NH_3	0.100	0.100	0.100	No	No
2: High Flow	No Evidence					
		MR1	RB1	MR3	MR1	RB1
3: Embeddedness	% Silt Covered Substrate[a]	0–25%	0–25%	50–75%	Yes	Yes
4: Low Dissolved Oxygen	Minimum Dissolved Oxygen (mg/L)	7.32[c]	10.17[c]	8.91[c]	No	Yes
5: Temperature Stress	Maximum Temperature	22.56°C[c]	17.28°C[b]	23.41°C[c]	Yes	Yes
6: Altered Food Resource	No Measurements					

[a] Metrics from Table A-7 converted to percentages according to Plafkin et al. (1989).
[b] 8/28/00.
[c] 7/23/01.

TABLE 7

Causal Analysis I and II: Spatial Co-occurrence for WL1 (Autumn 1999 except where noted)

Spatial Co-occurrence						
Candidate Cause		MR3 reference	FB2 reference	WL1	Adverse Change Compared to Each Reference	
1: Toxics	Total Metals and Ammonia (mg/L)					
		MR3	FB2	WL1	MR3	FB2
	Al	0.158	0.058	0.098	No	**Yes**
	Cd	0	0	0	No	No
	Cr	0.005	0	0.003	No	**Yes**
	Cu	0.005	0.005	0.005	No	No
	Fe	0.695	0.495	0.608	No	**Yes**
	Ni	0	0.001	0	No	No
	Pb	0.001	0.001	0.001	No	No
	Zn	0.011	0.005	0.01	No	**Yes**
	NH_3	0.1	0.0001	0.1	No	**Yes**
2: High Flow	No Measurements					
		MR3	FB2	WL1	MR3	FB2
3: Embeddedness	% Silt Covered Substrate[a]	50–75%	0–25%	50–75%	No	**Yes**
4: Low Dissolved Oxygen	Minimum Dissolved Oxygen (mg/L)	8.91[c]	8.29[c]	8.78[c]	**Yes**	**No**
5: Temperature Stress	Maximum Temperature	23.41°C [c]	23.13°C [c]	22.53°C [c]	No	No
6: Altered Food Resource	No Measurements					

[a]Metrics from Table A-7 converted to percentages according to Plafkin et al. (1989).
[b]8/28/00.
[c]7/23/01.

2000, 2001, and 2002 (see Figures 7 and 8). We assumed that rainfall and concomitant flows were greater in the springtime than in the summer or autumn; this assumption seemed reasonable, given the river's hydrograph (see Figure A-1). Water temperature, dilution of toxic substances, and especially phenology of stream communities may confound this association.

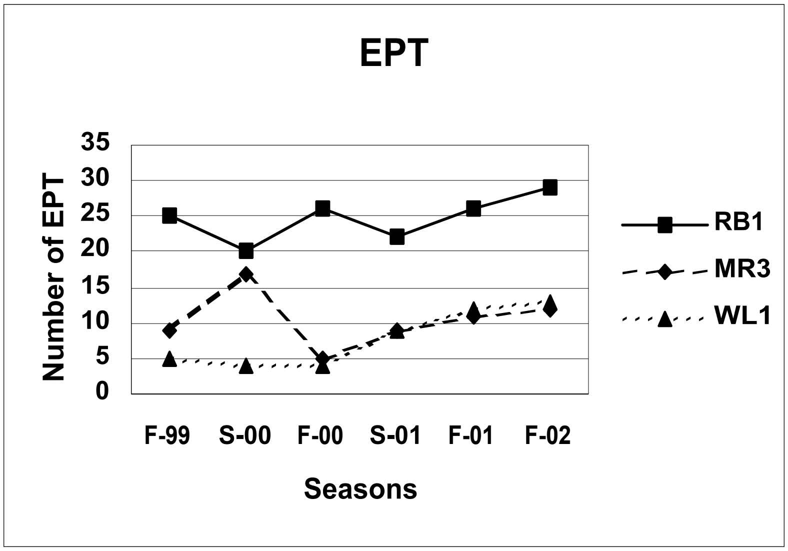


FIGURE 7

Number of EPT from Autumn and Spring Sampling (1999–2002). The watershed reference (RB1) consistently had higher scores for number of EPT taxa than MR3 or WL1.

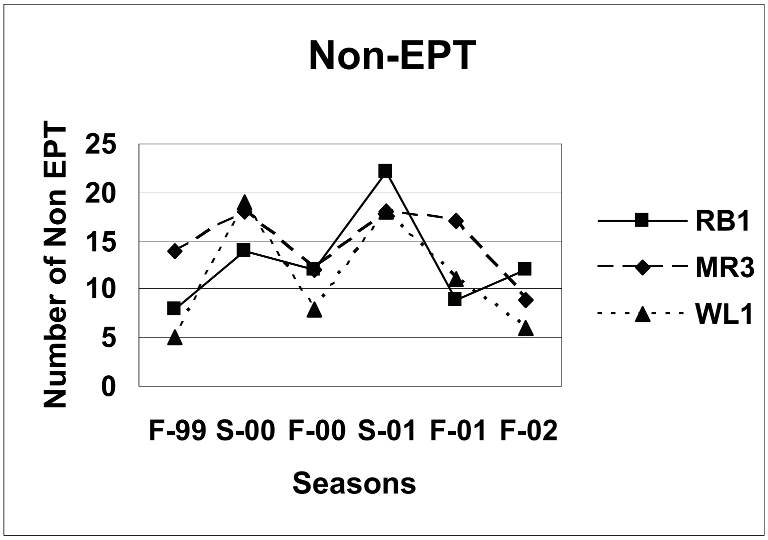


FIGURE 8

Number of Non-EPT Taxa for Autumn and Spring Sampling (1999–2002). Numbers of non-EPT taxa at RB1, MR3, and WL1 increase during spring sampling.

4.2.2. Stressor-Response Relationship from the Field

It was expected that as exposure to a cause increased, the intensity or frequency of the biological effect would have increased; and vice versa. Stressor-response relationships could strongly support or weaken a Candidate Cause, if data were available that were continuous in time or space and that were not confounded by other variables or new stressors. There were no data sets of this type. However, stressor-response relationships for some stressors were cautiously evaluated using data from sites throughout the upper portion of the watershed that had similar habitats to the impaired locations.

The stressor-response relationship from the field was used with some skepticism, because (1) many variables could confound the analysis and (2) only 12 of the sites had both biological and physical-chemical data suitable for comparison. We evaluated associations for non-EPT taxa and EPT taxa, with Candidate Causes or surrogates, using data from the following sites: MR1, MR2, MR3, WL1, WL2, WL3, WL4, FB2, RB1, SR, HR, and TR. Data were checked for normalcy with a Kolmogorov-Smirnov test. Pearson's correlations were used for parametric data and Spearman's for nonparametric data. Results of these univariate linear correlations are presented numerically in Table 8.

Variables correlating strongly with the number of EPT taxa (Pearson's; $p < 0.1$) were further analyzed by multiple stepwise regressions (SigmaStat, 1997). The variables included in these analyses were DO, NH_3, BOD, Fe, Zn, Cr, Cu, temperature at the time of biological sampling (October 1999), and maximum summer temperature (recorded either in August 2000 or July 2001). The only variable entered into the EPT regression model using forward selection was Zn. None of the other variables significantly improved the model.

Variables with strong correlations to the number of non-EPT taxa (Pearson or Spearman coefficients) were analyzed by multiple stepwise regressions (SigmaStat, 1997). Variables included in this analysis were turbidity, Zn, and temperature at the time of biological sampling (October 1999). The only variable entered into the model using forward selection was temperature. None of the other variables significantly improved the model (see Comment 11).

Comment 11. Cautions for Stressor-Response from the Field.
Statistical tests of these relationships should be interpreted cautiously: these tests are very sensitive to sample size, the impaired ecosystem and treatment are not replicated, and stressor levels are not randomly assigned. Multiple stressors frequently co-occur and can result in confounding. For more stressor response from the filed see CADDIS.

4.2.3. Causal Pathway

This refers to the sequence of events, from release of a stressor at its source to the effect of interest. Steps in the pathways linking sources to the cause can serve as

TABLE 8

Correlations of Numbers of EPT and Non-EPT Taxa with Physical and Chemical Stream Variables (Autumn 1999 data except where noted)

Physical and Chemical Variables	Correlation Coefficient	R^2	Normal Distribution[a]	Stressor-Response from the Field for the Case Supports a Candidate?
Correlations with EPT				
Dissolved Oxygen	0.517	0.267	Yes	Yes
Dissolved Cr	–0.647	0.419	No	Yes
Dissolved Cu	–0.481	0.231	No	Yes
Dissolved Fe	–0.597	0.356	Yes	Yes
Dissolved Zn	–0.769	0.591	Yes	Yes
BOD	–0.510	0.260	No	Yes
Ammonia	0.550	0.303	No	No for toxicity, yes for nutrients
Autumn Temperature (1999)	–0.521	0.271	Yes	Yes
Summer Temperature (2001–2002)	–0.613	0.376	No	Yes
Correlations with Non-EPT				
Autumn Temperature (1999)	–0.581	0.3380	Yes	Yes
Turbidity	–0.487 (–0.560)[b]	0.237 (–0.314)[b]	No	Ambiguous
Dissolved Zn	–0.469	0.2200[c]	Yes	Yes

[a] Normal distributions were determined using Kolmogorov-Smirnov test. Pearson Correlation was used for normally distributed variables. Spearman Correlation was used for non-normal variables.

[b] Correlation without the outlying value of 22 NTU.

[c] Zn was included because the *p*-value for Spearman Correlation was less than 0.1.

supplementary or surrogate indications that the cause and the biological effect were likely to have co-occurred. Many individual pieces of evidence were used to weaken or support pathways depicted in the conceptual models (see Figures 9−14). Data were compared using measurements taken during the autumn of 1999, when biological, physical, and chemical data were simultaneously obtained from many sites. A pathway was strengthened if the evidence was compatible after comparing with both references; it was weakened if any comparison indicated incompatible evidence for the causal pathway.

Comment 12. Caution with Causal Pathway. Save data directly related to the proximate stressor for analysis under spatial/temporal co-occurrence or evidence of exposure or biological mechanism, as these types of evidence carry more weight. Keep in mind that causal pathway evidence cannot refute the case for a candidate cause, because although critical steps in some pathways may appear to be absent, there may be unknown pathways or unknown system dynamics operating. More on Causal Pathway.

Brief descriptions of data used to assess the exposure pathway are shown (along with other evidence) on separate models for individual causal pathways (see Comment 13). This approach is illustrated for MR3 in Figures 9−14. A weakened connection in the pathway is illustrated by an *arrow bisected by a "~" where the pathway is weakened* and an associated text explanation is presented in a box beside it. *A refuted connection in the pathway is illustrated with an "X" superimposed over the connection* along with a text box describing the evidence. Evidence supporting a causal pathway simply appears in a box to the side of the model. Dates indicate the year in which the data for the evidence were collected. Although some exposure pathways could be eliminated based on refutation, no Candidate Causes could be eliminated because at least one causal pathway remained possible for each of the six Candidate Causes. Therefore, evidence for all causal pathways is presented in the SOE analysis (see SOE Appendix B) and the separate conceptual models (see Figures 9−14).

Comment 13. Communication Tip. The CT DEP scientists and managers found the annotated conceptual models particularly helpful for discussing this case. The visual display enabled the group to "see" the presence of relationships and the evidence corroborating or discounting possible causes. Based on that experience, they suggest structuring the analyses around the conceptual models that serve as both an analytical and a communication tool.

4.2.4. Manipulation of Exposure

Management actions that increased or decreased exposure to a cause at the impaired locations are expected to increase or decrease the biological effect. No manipulations were performed before the first iteration (2000 sample). However, new data were collected in 2001 after a point source had been discovered and eliminated. This was an experiment in that a causal relationship between the effluent and the biological response was hypothesized and removal of the effluent constituted, in effect, an unreplicated, experimental treatment. The numbers of EPT and non-EPT taxa at each site, before and after the manipulation, are depicted in Figures 7 and 8. The associations were relevant to several Candidate Causes. After eliminating the illicit discharge, mean concentrations of total Cr, Fe, and Ni decreased at MR3 and WL1 compared to levels observed in 1999 (see Table 9). The concentrations of dissolved Al, Cr, and Pb also decreased at these sites, compared to 1999 levels, both in 2001 and 2002 (see Table 10). These data are relevant to the Candidate Cause 1.2, a toxic mixture and were evaluated when the management action was being evaluated in the second iteration (see Comment 14).

Comment 14. Caution with Unreplicated Manipulation.

Uncertainty in the data can be introduced when other events, natural factors, or other causes co-occur with the variable being manipulated; sampling designs such as before-after-control-impact (BACI) can help control for some of these factors. Recovery rates and treatment effectiveness also are sources of uncertainty and should be taken into account when analyzing results. There was concern that the initial recovery in this case was due to a "wet" year, but the recovery has persisted through 2007. More on manipulation of exposure see CADDIS.

4.2.5. Verified Prediction

Knowledge of a cause's mode of action permits prediction and subsequent confirmation of previously unobserved effects, or as in this case, knowledge about the effects predicted an episodic exposure of a severe nature. It was the opinion of the scientists attending a workshop in the spring of 2000 that the paucity of taxa observed at the site occurred only in situations of severe stress. None of the measured Candidate Causes were at levels expected to cause the severe effects observed at the site. The workshop participants predicted that an episodic exposure was occurring and its location could be determined by sampling downstream from the first impoundment on the Middle River. In 2000, the CT DEP returned to the Middle River and sampled at MR3, and at a new site, MR2. During the sampling, they observed a gray discharge from 19th century raceway that was hidden by vegetation at the location where the impairment was first evident, that is, at MR3.

TABLE 9

Causal Analysis II: Changes in Annual Mean Total Metal Concentrations Before and After a Management Experiment, Removal of an Illicit Discharge in Autumn of 2001

Metal in µg/L	Site	1999–2000	2001	2002	Decrease Both Years?
Al	MR3	107	76	107	
	WL1	116	62	94	Yes
	WL2	110	109	94	Yes
Cd	MR3	1	1	0	
	WL1	1	1	0	
	WL2	1	1	0	
Cr	MR3	3	0	0	Yes
	WL1	2	0	0	Yes
	WL2	1	0	0	Yes
Cu	MR3	3	9.5	4	
	WL1	4	5.2	6	
	WL2	8	15	7	
Fe	MR3	645	572	636	Yes
	WL1	592	497	583	Yes
	WL2	560	472	534	Yes
Pb	MR3	2	1.7	2	
	WL1	5	1.7	3	Yes
	WL2	3	2.7	3	
Ni	MR3	1	0.5	0	Yes
	WL1	1	0.2	0	Yes
	WL2	1	1.5	0	
Zn	MR3	10	8	10	
	WL1	11	7	8	Yes
	WL2	13	13	14	

Sample dates: 10/13/99, 11/29/99, 2/9/00, 5/16/00, 8/2/00, 10/3/00, 3/19/01, 5/1/01, 7/23/01, 10/17/01, 6/13/02, 10/10/02, and 10/23/02.

TABLE 10

Manipulation of Exposure: Changes in Mean Dissolved Metal Concentrations Before and After Removal of an Illicit Discharge in Autumn of 2001

Metal in µg/L	Site	1999–2000	2001	2002	Decrease Both Years?
Al	MR3	107	76	90	Yes
	WL1	116	62	78	Yes
	WL2	110	89	89	Yes
Cd	MR3	1	1	0	
	WL1	1	1	0	
	WL2	1	1	0	
Cr	MR3	2	0	0	Yes
	WL1	2	0	0	Yes
	WL2	1	0	0	Yes
Cu	MR3	3	2.5	3	
	WL1	3	3	3	
	WL2	6	4	4	Yes
Fe	MR3	532	439	538	
	WL1	478	412	483	
	WL2	444	371	442	Yes
Pb	MR3	1	0.7	0	Yes
	WL1	2	0.7	1	Yes
	WL2	1	0.7	1	
Ni	MR3	0	0.5	0	
	WL1	0	0.25	0	
	WL2	1	1	0	
Zn	MR3	9	6	8	Yes
	WL1	7	6.5	7	
	WL2	12	10	10	Yes

Sample dates: 10/13/99, 11/29/99, 2/9/00, 5/16/00, 8/2/00, 10/3/00, 3/19/01, 5/1/01, 7/23/01, 10/17/01, 6/13/02, 10/10/02, and10/23/02.

5. EVALUATE DATA FROM ELSEWHERE

5.1. SOURCES OF DATA FROM ELSEWHERE

Measurements from the MR3 and WL1 were compared with relationships from published laboratory studies of DO, nutrients, temperature, sediment, NH_3, and selected metals (see Tables 11-13).

5.2. EVALUATION OF DATA FROM ELSEWHERE

5.2.1. Stressor-Response Relationships from Laboratory Studies or Other Field Studies

The plausibility that exposure at the site was sufficient to cause the observed effects was evaluated by comparing paired exposure and response measurements at the site with exposure-response relationships from controlled experiments and from field observations.

5.2.1.1. Toxicity of Metals and Ammonia

The stressor-response association was evaluated for sustained exposures first by screening against chronic benchmark concentrations and then by evaluating the chemicals retained by the screens relative to the conditions in the Willimantic. The screening was accomplished by calculating hazard quotients, for the mean concentrations of chemicals of interest divided by a set of benchmark values. The aquatic life criteria developed by the CT DEP, the lowest chronic values (LCV) for daphnids (Suter, 1996), and the test EC_{20} for daphnids (Suter, 1996) were selected for this purpose. The test EC_{20} is defined as the highest tested concentration causing less than 20% reduction in growth, fecundity or survivorship in a chronic test with a daphnid species" (Suter, 1996). The EC_{20} for Fe was not used because it was based on a test assuming "acidic iron-containing waste water" (Dave, 1984). Hence, the LCV for Fe, taken from a test conducted at neutral pH by Biesinger and Christensen (1972), was used to calculate total toxicities (see Table 11). Since these benchmarks are designed to be protective, quotients less than 1.0 indicate that the chemical is unlikely to cause effects on invertebrates at the observed mean concentration. Metals also may have combined toxic effects, and a mixture may be toxic even if none of its constituents are toxic. To account for this possibility, we assumed concentration additivity, and the hazard quotients for the metals were summed for each benchmark at each location to obtain a hazard index. A hazard index less than 1.0 indicates that the mixture of measured metals is unlikely to cause effects, even with sustained exposure. Table 12 presents the screening results obtained using this approach and Table 11 presents the individual hazard indices and the sums of the partial toxicities. For the two iterations, the mean dissolved metals concentrations for sites from 1999–2000 were used in the analyses (see Tables A-4 through A-5).

TABLE 11

Plausible Stressor Response Evaluated by Sum of Partial Toxicity Based on Benchmarks for Test EC_{20} Values and Daphnids Lowest Chronic Values and Mean Ambient Concentrations from 1999–2000

	Test EC_{20} (µg/L)	MR1 Reference	MR3 Impaired	WL1 Iimpaired	Lowest Chronic Value (µg/L)	MR1 Reference	MR3 Impaired	WL1 Impaired
Al	540	0.043	0.198	0.215	1900	0.043	0.056	0.061
Cd	0.75	0.533	0.667	0.667	0.15	2.667	3.333	3.333
Cr	0.5	0.400	4.000	3.400	<44	0.005	0.045	0.039
Cu	0.205	10.732	12.195	13.171	0.23	9.565	10.870	11.739
Fe	-	***0.119***	***0.121***	***0.109***	4380	0.119	0.121	0.109
Ni	45	0.004	0.007	0.007	<5	0.040	0.060	0.060
Pb	-	***0.000***	***0.065***	***0.146***	12.3	0.000	0.065	0.146
Zn	-	***0.139***	***0.184***	***0.154***	46.73	0.139	0.184	0.154
Total		***11.971***	***17.437***	***17.868***		12.578	14.735	15.642

Bolded italics are based on lowest chronic value for Fe, Pb, and Zn because no Test EC_{20} was available.

TABLE 12

Plausible Stressor Response Evaluated by Comparison of Mean Water Concentrations for 1999–2000 to Water Quality Benchmarks for Dissolved Metals and Ammonia for Daphnids, and Connecticut's Chronic Criteria Values

	CT Values (µg/L)	Daphnids[a] (µg/L)	Test EC_{20}[b] (µg/L)	MR1 Reference	Exceeded at MR1	MR3 Impaired	Exceeded at MR3	WL1 Impaired	Exceeded at WL1
Al	None[c]	1900	540	82.2	No	107	No	116	No
Cd	0.62	0.15	0.75	0.4	Yes	0.5	Yes	0.5	Yes
Cr	100	<44	0.5	0.2	No	2	Yes	1.7	Yes
Cu	4.8[d]	0.23	0.205	2.2	Yes	2.5	Yes	2.7	Yes
Fe	None[c]	4380	-	522.8	No	532	No	478	No
Ni	88	<5	45	0.2	No	0.3	No	0.3	No
Pb	1.2	12.3	-	0	No	0.8	No	1.8	Yes
Zn	58.2	46.73	-	6.5	No	8.6	No	7.2	No
NH_3	1430–2470[e]	630	-	120	No	100	No	100	No

[a] Benchmarks for lowest chronic value for daphnids (Suter, 1996).
[b] Benchmarks for the lowest daphnid test EC_{20} value (Suter, 1996).
[c] Connecticut has not adopted WQC for Al or Fe.
[d] CT water quality criteria value based on field stressor-response associations.
[e] CT chronic Criteria Value for ammonia at 0–25°C.
Mean value calculations Tables A-4 through A-5.

5.2.1.1.1. ***Select metals.*** Copper concentrations were well above the screening benchmarks. The actual toxicity of Cu varies with hardness, dissolved organic matter, and the species exposed. The Willimantic waters are soft (10–27 mg/L as calcium carbonate [$CaCO_3$]), suggesting high Cu toxicity. However, the only study of the effects of variable hardness on chronic toxicity of Cu to invertebrates shows no consistent relationship (U.S. EPA, 1985a), so correction is inappropriate. While no measurements of organic acids or dissolved organic matter for the river are available, the light yellow color throughout the watershed suggests that organic acids (e.g., humic, tannic) are likely present (authors' observations). The presence of elevated organic acids could bind Cu. If binding of Cu to organic acids was occurring, the bioavailability and toxicity of Cu would be reduced (Winner, 1984). The possibility that Cu is binding to dissolved organic matter is consistent with the fact that the dissolved and total concentrations do not significantly differ (see Tables A-3 through A-5). Organic acid associated metals are soluble and are not available to bind to suspended particles. Hence, the bioavailable Cu concentration may be considerably lower than the dissolved concentration in the Willimantic.

Because impairment was characterized as a reduced number of EPT taxa, Cu toxicity data for aquatic insects are particularly relevant to the causal analysis. The only such chronic toxicity data are from a life cycle test of the caddisfly *Clistoronia magnifica* in neutral water at a hardness of 26 mg/L as $CaCO_3$ (Nebeker et al., 1984). That test yielded a Maximum Acceptable Total Concentration (MATC) of 10.4 µg Cu/L; at this concentration, adult emergence was reduced by ~50%. The value of 10.4 µg/L may not be conservative, as suggested by the greater sensitivity of *Daphnia* (see the screening values) and the relatively large effect. However, these considerations are judged to be more than balanced by the fact that the taxon and water hardness of this test are appropriate to the site. Hence, the 10.4 µg/L value is judged to be an adequate estimate of the threshold for Cu toxicity. This value also is higher than the range of concentrations of dissolved Cu reported at the impaired sites (1–5 µg/L; Table 12, Tables A-4 through A-5). Furthermore, the CT DEP water quality standard for Cu of 4.8 µg/L is based on the relationship between Cu concentration and community impairment (CT DEP, 1990). None of the sites in the Willimantic had mean concentrations that exceeded this standard.

Cr occurs in the environment in trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) forms. The low screening value (daphnid EC_{20} of 0.5 µg/L) is based on a test of the hexavalent form, which is far more toxic than the trivalent form (Suter, 1996). Cr^{6+} is reduced to Cr^{3+} in the presence of organic matter, which is apparently abundant in the Willimantic based on observed water color (above). Further, Cr^{3+} forms strong bonds with negative ions (Eisler, 2000), so the bioavailability of Cr in the Willimantic was expected to be low. The only chronic toxicity data for Cr and invertebrates are from tests of *Daphnia magna*. The most relevant result is a MATC of 66 µg/L for Cr^{3+} at 52 mg/kg hardness (U.S. EPA, 1985b). This value is much greater than the range of dissolved Cr concentrations reported from the impaired locations in the Willimantic (<1–4 µg/L; Tables A-4 through A-5). There was no adjustment for hardness, because there was no clear trend in chronic toxicity to *D. magna* as a function of hardness (U.S. EPA, 1985b). Hence, based on the expectation that Cr in the Willimantic is predominantly trivalent, the observed concentrations were judged insufficient to cause toxicity.

Cd was not detected in dissolved form at a detection limit of 1 µg/L. However, concentrations of total Cd were 1 µg/L at two of the sites. The detection-limit concentration is greater than the LCV and EC_{20} for *Daphnia* spp. (see Table 12). The influence of dissolved organic matter on the bioavailability of Cd is unclear. Some authors report that the toxicity of Cd is reduced by the presence of dissolved organic matter (Eisler, 2000); Winner (1984), though, found no effect of humic acid on Cd toxicity to *Daphnia pulex*. However, that test was conducted in moderately hard water, in which Ca could displace Cd (Winner, 1984), whereas Willimantic water is quite soft. Hence, depending on the actual concentration of dissolved Cd, the actual effect of Willimantic water chemistry on the bioavailability of Cd and the sensitivity of the EPT species relative to *Daphnia*, Cd may or may not have elicited toxic effects (see Comment 15).

Comment 15. Comment on Toxicity Values.

The stressor-response relationships used for metals are those that were readily available in 2000, when this causal analysis was conducted. Since then, more test data have appeared in the literature and have been used to derive chronic concentration-response relationships and acute species sensitivity distributions for specific water chemistries (Shaw-Allen and Suter, 2005). In addition, draft Cu criteria based on the biotic ligand model have been proposed (U.S. EPA, 2003a). However, this type of evidence was not updated because the causal analysis did not depend on it.

5.2.1.1.2. *Ammonia.*

Connecticut's freshwater chronic criteria for NH_3 vary with temperature (2.47–1.01 mg/L at 0–25^{o}C). Chronic toxic values for exposure of invertebrates to NH_3 range from 1.23–44.9 mg/L (U.S. EPA, 1999) (see Table 12). NH_3 toxicity is a function of pH and temperature, but corrections are not necessary in this case, because measured levels of NH_3 were well below even the lowest chronic value for any pH or temperature that was likely to occur in the watershed.

5.2.1.2. Low Dissolved Oxygen

Minimum observed oxygen concentrations at all locations were well above the Connecticut chronic aquatic life criterion of 5.0 mg/L (see Table 13). The minimum measured values also were higher than or slightly less than the high oxygen requirements of some Ephemeroptera from cold water streams (7.6 mg/L) (Nebeker, 1972; U.S. EPA, 1986).

5.2.1.3. Nutrients and Altered Food Resources

Nutrient levels were compared to U.S. EPA default criteria (U.S. EPA, 2000a) and U.S. Geological Survey (USGS) nutrient background values (Smith et al., 2003) for the Eastern Coastal Plains. The default criteria and background values for nutrients are based on the frequency distribution of water concentrations; they were not developed by association with biological endpoints. Hence, these are not stressor-response relationships. However, if concentrations of total P and TN exceeded either

TABLE 13

Causal Analysis I and II: Plausible Stressor Response Evaluated by Comparison of U.S. EPA Default Criteria for Total Phosphorous, Total Nitrogen, and Biological Oxygen Demand and CT Dissolved Oxygen Criteria Values to Mean Ambient Concentrations for 1999–2000

Measure	Criteria Values	MR1 Reference	Benchmark Exceeded?	MR3 Impaired	Benchmark Exceeded?	WL1 Impaired	Benchmark Exceeded?	FB2 Reference	Benchmark Exceeded?
Mean Total Phosphorus (µg/L)	23.75[a]	13	No	20	No	30	Yes	17	No
Mean Total Nitrogen[b] (µg/L)	610[a]	883	Yes	667	Yes	720	Yes	1158	Yes
Mean BOD (mg/L)	>7.0	1.1	No	1.1	No	1.2	No	0.67	No
Lowest Dissolved Oxygen (mg/L)	<5.0[c]	7.32	No	8.91	No	8.78	No	8.29	No

[a] Nutrient ecoregion XIV, U.S. EPA (2000a).
[b] Total nitrogen calculated as TKN+nitrate+nitrite.
[c] CT Chronic Criteria Value for DO.
Calculations Tables A-4 through A-5, Total Phosphorus, Total Nitrogen, and BOD.

background levels or U.S. EPA default criteria, then the stream reach would be among those streams having the greatest nutrient levels and, therefore, the greatest chance of nutrient-induced effects. These values and the values measured at the two impaired locations are given in Table 13.

5.2.1.4. Elevated Temperature

Species-specific tolerance values for heat stress were not found. However, temperatures above 20ºC have been reported to have lethal effects on some species. The LT50 (temperature causing 50% mortality) for Ephemeroptera, *Deleatidium autumnale* (New Zealand) was 21.9ºC (Cox and Rutherford, 2000). Water temperatures above 20ºC also reduce the growth efficiency of *Hyalella azteca* (Panov and McQueen, 1998). Temperature increases of 5ºC caused by impoundments also were associated with reduced numbers of invertebrate taxa in Wisconsin streams (Lessard et al., 2000).

6. IDENTIFY THE PROBABLE CAUSE

Each Candidate Cause and each the type of evidence presented in the previous two sections was evaluated and scored to identify the probable cause. The consistency and credibility of the case was evaluated based on the scores and the most compelling lines of evidence were identified and displayed on the conceptual model diagrams. Finally, the Candidate Causes were compared to determine which were more probable and which were unlikely based on the evidence.

To conduct the analysis, we organized evidence for each of the six Candidate Causes, in three ways: this text, a summary scoring table, SOE tables, and corresponding conceptual models for impairment at MR3. Although SOE analysis was carried out for both MR3 and WL1 (U.S. EPA 2003b), we restrict the discussion here to MR3 because the analysis for WL1 indicated that these were a similar contiguous impairment. However, the analysis of WL1 was useful for documenting that similarity and excluding the effect from the confluence with Furnace Brook. The SOE tables for MR3 in Appendix B (see Tables B-1 through B-6) are summarized in Table 14. This section presents the types of evidence that were important to the identification of the Candidate Cause. All types of evidence are evaluated in the SOE tables (see Appendix B).

6.1. MR3 CANDIDATE CAUSE 1, TOXICITY FROM METALS, AMMONIA, OR COMPLEX MIXTURES

Candidate Cause 1 was divided into two types: sustained exposures (1.1) and episodic exposures (1.2). Sustained exposures were further divided into types of toxicants, NH_3, and metals. Evidence that weakened or strengthened each causal pathway is described in SOE Table B-1 and depicted in Figure 9. Measurements of metal and NH_3 concentrations were too infrequent to characterize episodic exposures, but concentrations from grab samples from the water column were used to assess the toxicity of sustained exposures.

6.1.1. First Iteration

The first iteration evaluated sustained exposures because data were unavailable to assess episodic exposures. Evidence for a toxic pathway was weak, and evidence supporting other Candidate Causes was even weaker. Neither of these alternatives seemed likely to have caused such a low overall invertebrate index score. This result suggested the need for additional data to complete the analysis. Because grab samples did not indicate a likely cause, the workshop participants predicted that an episodic exposure was occurring and its location could be determined by sampling at MR2 downstream from the first impoundment on the Middle River and upstream from MR3. The evidence used to make this determination is summarized in Box 1.

TABLE 14

Scoring of Evidence for Site MR3 from the Second Iteration

	Metals	NH_3	Flow	Silt	Low DO	Heat	Food	Episodic Mix
Types of Evidence that Use Data from the Case								
Spatial/Temporal Co-occurrence	+	– – –	– – –	+	– – –	– – –		
Causal Pathway	– – –	– – –	+	–	0	0	+	0
Stressor-Response from the Field	+	– – –		–	+	+	+*	
Manipulation of Exposure								+++
Verified Predictions								+++
Types of Evidence that Use Data from Elsewhere								
Stressor-Response from Other Field Studies	–			+		+	–	
Stressor-Response from Laboratory	0	– – –			0	++		
Evaluating Multiple Types of Evidence								
Consistency of Evidence	–	– – –	–	–	–	–	+*	+++
Reasonable Explanation of the Evidence	0	0	0	0	0	0		

* Positive for the forested stream bank pathway only.

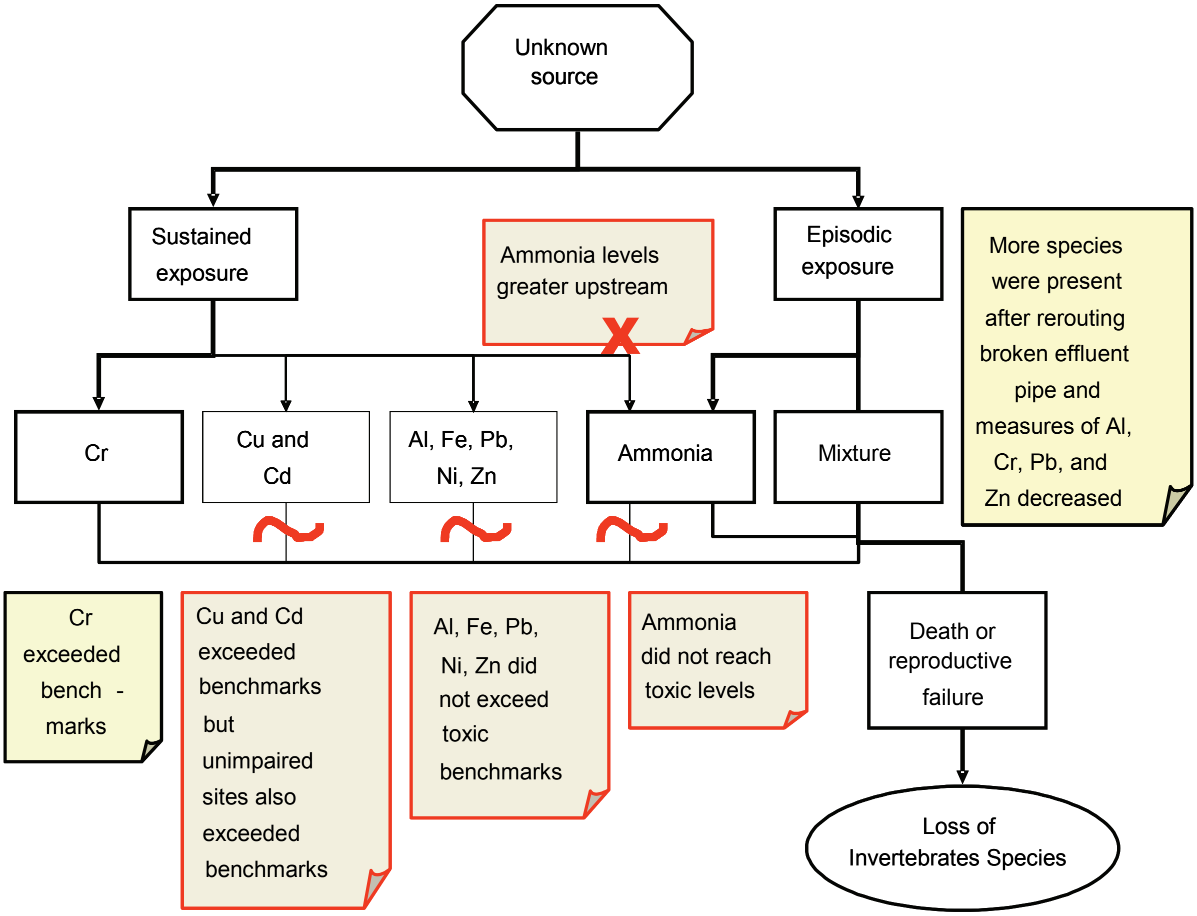


FIGURE 9

Annotated Conceptual Model of Impairment Caused by Toxic Contamination

Box 1. Evidence from First Iteration.

Co-occurrence of Stressor and Impairment—Maximum concentrations of total Al, Cr, Cu, Fe, and Zn increased from MR1 to MR3. The remaining metals (Cd, Ni, and Pb) were either undetected, or were found at concentrations equal to those found at MR1 (see Table 6, Table B-4). Mean concentrations of dissolved of Al, Cd, Cr, Cu, Fe, Ni, and Zn were greater at MR3 than at MR1 (see Table 12, Table B-4). Measured concentrations of NH_3 were similar at MR1, RB1 and MR3—0.1 mg/L (see Table 6). Hence, metals, but not NH_3, co-occurred with the impairment.

Stressor-Response from the Field—Concentrations of Cr, Zn, and Fe correlated negatively with EPT taxa ($r = -0.77$, -0.65, and -0.60, respectively; $n = 12$ in each case). The correlation for Cu and EPT taxa was weaker ($r = -0.48$). Variables that correlated with EPT ($p < 0.1$) were then analyzed in a multiple stepwise regression (SigmaStat, 1997). Variables used in this analysis included DO, NH_3, BOD, Fe, Zn, Cr, Cu, water temperature at the time of biological sampling (October 1999), and maximum summer water temperature recorded (either in August 2000 or July 2001). The only variable entered into the model using forward selection was Zn. None of the other variables significantly improved the model. Hence, a stressor-response relationship was found for metals at the site, but the evidence is weak because it is based on correlations and includes data from tributaries.

Stressor-Response Relationships from Laboratory Studies or Other Field Studies—This type of evidence was ambiguous, but was judged to weakly support the candidate cause. Concentrations of metals did not exceed aquatic life criteria for Connecticut (see Table 12). However, screening benchmarks were exceeded for Cu, Cr, and Cd. The hazard index (sum of toxic units) for metals at MR3 was 15, using LCVs, and 17 using test EC_{20} values (see Table 11). Several inconsistencies and sources of uncertainty were noted. For example, although Cu concentrations exceeded LCV and test EC_{20} values, so did Cu levels in all other sites in the watershed (see Tables B-3 through B-5). Total Cd was detected on four of six sampling dates at MR3, and dissolved Cd was detected on three of six, sampling dates. Thus, sustained exposures to these metals may not have occurred (see Table A-4). All but two sites (FB2 and FB4) exceeded the screening benchmarks for Cd. The hazard index at MR1 was 13 using LCV and 12 using test EC_{20} values (see Table 11); this difference did not seem large enough to have caused the impairment. These discrepancies might be explained by the visible dissolved organics in the stream, which would decrease bioavailability, and in the case of Cr, reduce the metal to a relatively non-toxic state. There were important data gaps:

Many chemicals were not measured;
Sediment analyses were not available to assess toxicity;
Information was not available to assess episodic mixtures as a cause.

6.1.2. Second Iteration

Biological sampling at MR2 was not impaired and indicated that the impairment was not due to the upstream impoundment. New sampling at MR3 revealed the origination of the biological impairment and a 19th century mill raceway was discovered behind vegetation. On the day of sampling, a grey discharge was observed from the raceway, which was traced to a broken sewer line from a nearby mill that held an NPDES permit for release to the POTW of organic waste, metals, and NH_3. The mill operated by batch productions and releases were not continuous. The previously unknown and apparently toxic episodic effluent was identified as the primary cause of the impairment at MR3. The evidence used to make this determination is summarized in Box 2.

Box 2. Evidence from Second Iteration.

Co-occurrence of Stressor and Impairment—The measurements of somewhat elevated metal concentrations at the impaired sites may be residual material from episodic releases. In particular, they may be released from contaminated sediments. However, this is speculation.

Causal Pathway—For the second iteration, additional sampling resulted in the identification of an episodic source. This evidence is considered to be an instance of verified prediction (below) and is not scored as part of the causal pathway to avoid double counting.

Manipulation of Exposure—After the illicit discharge had been rerouted, additional samples were collected in 2001 and 2002. The concentrations of total Cr, Fe, and Zn decreased at MR3, compared to levels measured in 1999 (see Table 9). Dissolved concentrations of Al, Cr, Pb, and Zn also declined further in 2001 and 2002 (see Table 10). This evidence is not strong, because the sampling events apparently did not correspond temporally to the discharge episodes. The relatively small declines in aqueous metal concentrations are likely due instead to declines in metal releases from sediment and other contaminant sinks. The mean postrestoration number of EPT increased by 4.5 taxa at MR3; it increased downstream, as well (see Table 10). The mean number of non-EPT taxa in 2001and 2002 declined, but the lower value was still similar to the mean value for the watershed reference site (RB1) measured in 1999−2002 (see Table 10). The unauthorized discharge was identified as the probable cause, based largely on the results of the restoration experiment. However, the CT DEP continued to monitor the sites.

Verified Prediction—During the first iteration, the authors predicted that an unknown source, probably episodic, occurred upstream of MR3. In the autumn of 2000, biological samples were collected from several sites above MR3 to try to identify the upstream extent of the impairment, using the method of Plafkin et al. (1989). Sampling effort was directed particularly to MR2, downstream from the nearest impoundment and upstream from MR3. The benthic invertebrate communities at MR2 scored higher than those at MR3. This result effectively eliminated non-point sources as a possible factor: the effects were induced within a very short distance (Candidate Cause 1.1a). During the autumn of 2000 biological sampling, an unauthorized gray discharge was discovered upstream from MR3. This discharge was traced from a raceway to a broken effluent pipe from the woolen mill that irregularly discharged material during batch processes at the mill. The pipe was repaired, and the effluent was rerouted to the POTW. Although the discharge was not chemically analyzed, the effluent was associated with an NPDES permit that allowed discharge of metals, organic matter, acid, and ammonia to the POTW (Candidate Cause 1.2).

6.2. MR3 CANDIDATE CAUSE 2, REMOVAL OF ORGANISMS DURING HIGH FLOWS

The SOE for this Candidate Cause is described and scored in Table B-2 and pertinent evidence is presented with the conceptual model (see Figure 10).

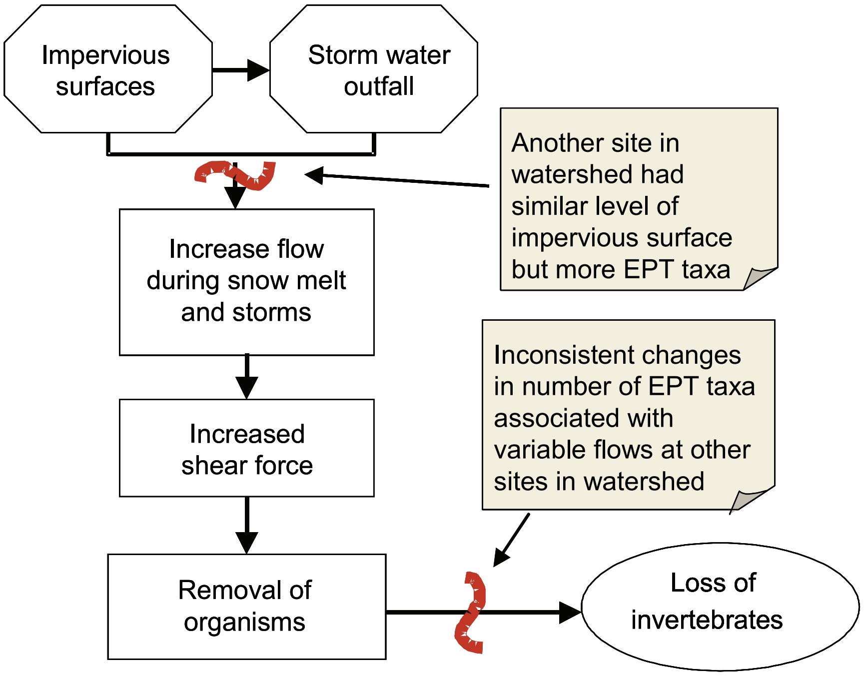


FIGURE 10

A Conceptual Model of Impairment Caused by the Removal of Organisms by Storm Flow, with Annotations Concerning Relevant Evidence

6.2.1. First Iteration

No storm-flow hydrologic data were available at MR3 and nonstorm flows (see Table 15) were not considered relevant, so the assessment was limited to surrogate measures and examination of aspects of the causal pathway. The pattern of EPT taxa (fewer in autumn, more in the following spring) was incompatible with high flows, which generally are greatest and most common in the spring, as a cause of impairment. The pattern was coherent with flow as a cause of toxicant dilution.

6.2.2. Second Iteration

Analysis of this Candidate Cause was not repeated in 2003, because the removal of the illicit discharge was believed to have changed the invertebrate assemblage at MR3 and no new hydrologic evidence became available. The proximity of MR3 with the unimpaired MR2 also suggested that permanent removal by strong hydrologic forces were unlikely. The evidence used to make these determinations is summarized in Box 3.

Box 3. Removal by Strong Flow.

Co-occurrence of Stressor and Impairment—In Connecticut, rainfall and hydrological flow typically are greatest in the spring (see Figure A-1). Neither of the seasonal patterns of rainfall and biological response observed with EPT (see Figure 7) or non-EPT (see Figure 8) taxa supported the possibility that high-flow conditions removed organisms. More non-EPT taxa were found in the spring than later, and this finding is incompatible with removal of organisms by high-flow events. The seasonal pattern of non-EPT taxa at the watershed reference site also was similar to that at MR3, further weakening the idea that hydrological modifications at MR3 caused the impairment.

The temporal patterns in EPT (see Figure 7) were more difficult to interpret because they varied with flow in an inconsistent manner. We expected to find a lower number of EPT taxa at MR3 in the spring, if high flows were removing organisms. However, cooler temperatures and dilution of toxics also coincide with high flows, potentially confounding the association. The phenology of insect emergence is yet another confounding factor. Larval stages of different benthic macroinvertebrates are present in the stream at different times of the year, making it difficult to compare the communities between seasons.

Causal Pathway—Evidence was available for three points in the causal pathway.

Impervious surface source—The proportion of impervious surface is greater at MR3 than at MR1.

Stormwater outfall source—The raceway that conveyed the illicit discharge may also serve as a stormwater discharge.

Both sources—Large substrate particle sizes are consistent with high flow velocities. The proportion of boulders and cobble was greater at MR3 than at MR1 but not greater than at RB1 (see Table 15).

6.3. MR3 CANDIDATE CAUSE 3, LOSS OF INTERSTITIAL HABITAT DUE TO SETTLED PARTICLES

The SOE for this Candidate Cause is described and scored in Table B-3 and pertinent evidence is presented with the conceptual model (see Figure 11).

TABLE 15

Measurement Relevant to Causal Pathway for MR3 (Autumn 1999)

Candidate Causes	Data	Upstream Reference MR1	Watershed Reference RB1	Impaired Site MR3	Pathway Supported Compared To Both References
1, 3, 4, 5, 6	Habitat Index % of Reference	68	100	32	**Yes**
4, 6	BOD (mg/L)	1.1	1.1	1.6	**Yes**
5, 6	Tree Canopy	Moderate	High	Low	**Yes**
2, 3	Bank Stability	10	8	10	No
2, 3	Velocity	15	16	11	No
2	Stormwater Outfalls	No	No	Yes	**Yes**
2, 5	% Impervious Surface	Low	Low	Moderate	**Yes**
1, 4	Illicit Discharge	Absent	Absent	Present	**Yes**
2, 3	Substrate Composition				
	% Boulder	0	33	25	No
	% Cobble	25	33	50	No
	% Gravel	50	33	13	No
	% Sand	25	0	12	No
4, 6, 3	Nutrients: Concentration (mg/L)				
	Organic Nitrogen	0.4	0.4	0.4	No
	NH_3	0.1	0.1	0.1	No
	TKN	0.4	0.4	0.4	No
	Nitrate	0.1	0.1	0.1	No
	Nitrite	0.05	0.05	0.05	No
	Total Phosphorus	0.01	0.02	0.01	No
	Total Solids	58	77	74	No
	Total Suspended Solids	5	4	0	No

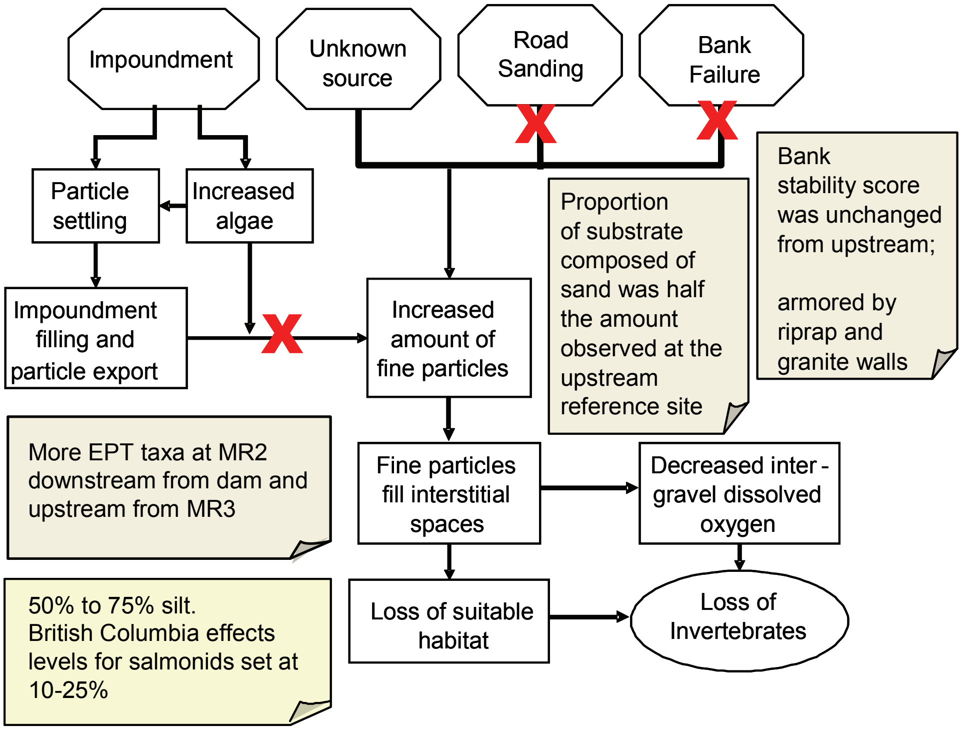


FIGURE 11

Annotated Conceptual Model Used to Examine Stressors Associated with Embedded Substrates from Sedimentation

6.3.1. First Iteration

Embeddedness probably is not the major cause of the impairment at MR3. However, the degree of embeddedness at MR3 was greater than at MR1, and, indeed, greater at MR3 than at most sites sampled in the watershed. Of the ten other sites sampled in the watershed, 70% had both less embeddedness and greater numbers of EPT taxa than at MR3.

6.3.2. Second Iteration

No new data concerning silt deposition or embeddedness were obtained for additional analysis of this Candidate Cause in 2000–2002. For this reason, this Candidate Cause was not explored in more detail in the second iteration. There is no data available to determine if the level of embeddedness decreased when the numbers of taxa increased in 2001–2003.

Box 4. Embededness.

Co-occurrence of Stressor and Impairment—The embeddedness score from the CT DEP habitat index indicated an increase in embeddedness compared to the upstream site (MR1) (see Table 6). However, another site, SR, had the same embeddedness score (11) but supported 16 EPT taxa. Also, the proportion of sand at MR3 was less than at MR1, where the diversity of EPT was greater (see SOE Table B-3). However, between 50% and 75% of the boulders, cobble, and gravels were embedded at MR3 versus 0–25% at MR1 and RB1 (see Table 6). Hence, co-occurrence is weakly compatible.

Causal Pathway—No substantial sources of fine silty material (e.g., from bank failure or road sand application) were observed. The banks of the stream, from the impoundment to MR3, were stabilized with granite boulders and constructed walls.

Stressor-Response from the Field—Embeddedness was not correlated with EPT taxa.

Stressor-Response from Other Field Studies—The reported 50–75% embedded substrate at MR3 is considered “fair,” and could be expected to have some impact on the composition of the benthic macroinvertebrate communities (Plafkin et al., 1989).

6.4. MR3 CANDIDATE CAUSE 4, ASPHYXIATION DUE TO LOW LEVELS OF DISSOLVED OXYGEN

The SOE for this Candidate Cause is described and scored in Table B-4 and pertinent evidence is presented with the conceptual model (see Figure 12).

6.4.1. First Iteration

Only fall samples were available. Additional sampling during hot summer days or predawn was recommended to determine concentrations of DO when conditions were most likely to create low-oxygen problems.

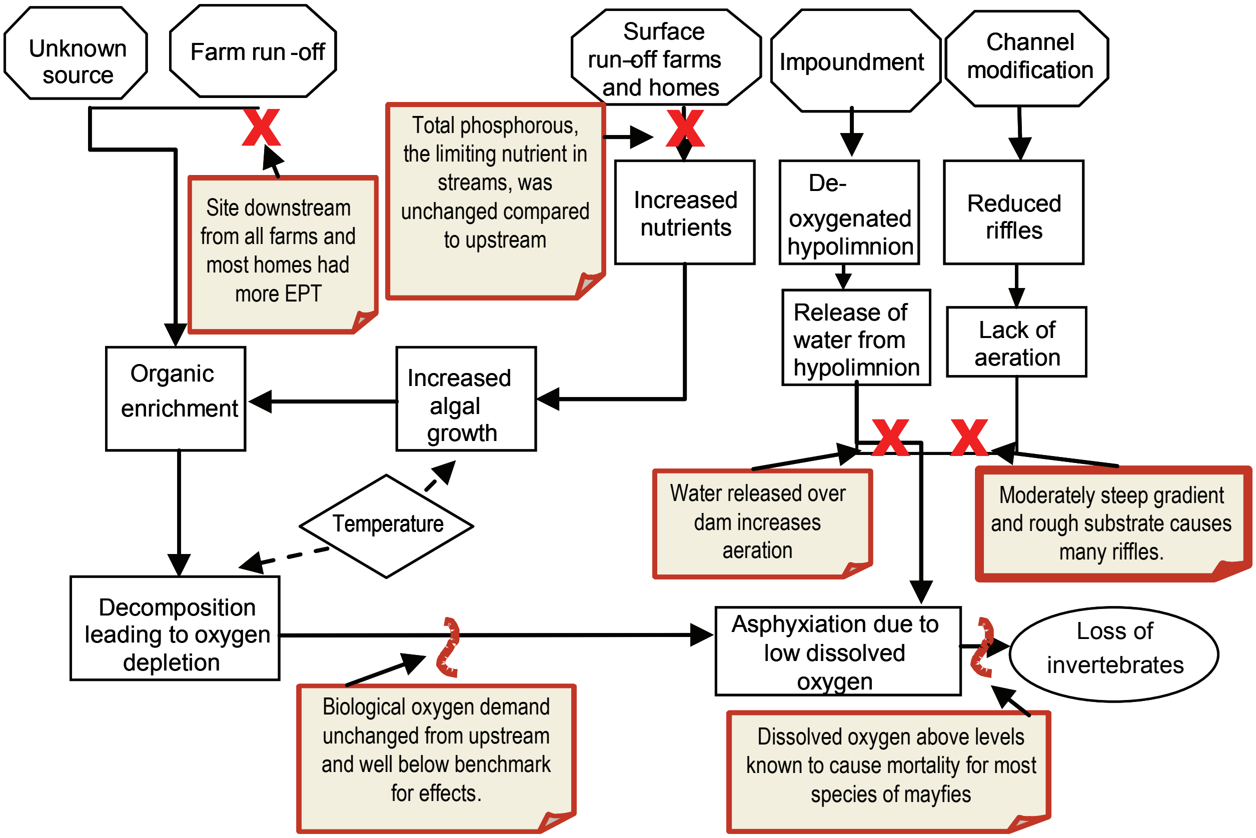


FIGURE 12

Annotated Conceptual Model Used to Examine Stressors Associated with Low Dissolved Oxygen

6.4.2. Second Iteration

Asphyxiation due to low levels of DO probably did not account for the lower numbers of benthic macroinvertebrate taxa because other sites had lower concentrations of DO—but reasonably large numbers of taxa.

Box 5. Low Dissolved Oxygen.

Co-occurrence of Stressor and Impairment—Summertime levels of DO were not measured in 1999.

Causal Pathway—All pathways except for an unknown source of organic matter are blocked by contrary evidence (see Table B-4).

Second Iteration

Co-occurrence of Stressor and Impairment—In 2000 and 2001, the lowest measured concentration of DO at MR3 was 8.9 mg/L (see Table 6). This concentration was greater than those at the upstream site MR1, where the lowest concentration was 7.3 mg/L. Hence, the candidate cause and effect did not co-occur.

Stressor-Response Relationships from the Field—DO levels correlated weakly with EPT taxonomic richness (see Table 8).

Stressor-Response from the Laboratory—All measurements were well above the Connecticut standard of >5.0 mg/L. The measured concentrations also were above or just below the EC_{30} levels for reduced emergence of mayflies (7.0 and 9.0 mg/L, respectively; Nebeker, 1972). However, DO concentrations were not measured continuously or during periods when it would be expected to be low.

6.5. MR3 CANDIDATE CAUSE 5, MORTALITY DUE TO HEAT STRESS

This SOE for this Candidate Cause is described and scored in Table B-5 and pertinent evidence is presented with the conceptual model (see Figure 13).

6.5.1. First Iteration

No temperature measurements were available during the summer. Additional sampling concomitant with hot summer days were recommended to determine water temperature when the warmest temperatures were most likely to occur (see Comment 16).

Comment 16. Locating Stressor-Response Data. Finding relevant stressor-response literature and data is often time consuming. Causal Analysis/Diagnosis Decision Information System has attempted to make this easier by providing lists of useful reviews and demonstrated methods for deriving stressor-response relationships from data collected by state and federal monitoring programs.

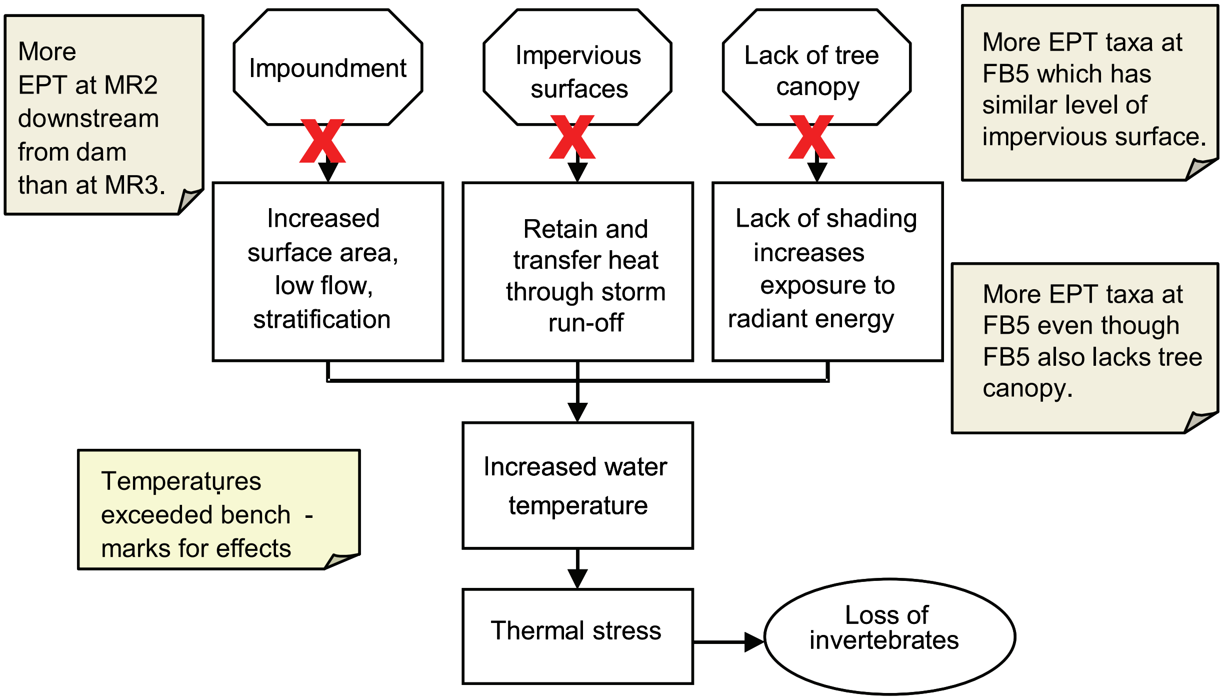


FIGURE 13

Annotated Conceptual Model Used to Examine Stressors Associated with Elevated Temperature

6.5.2. Second Iteration

Stream temperatures were measured in 2003. Based on many reported effects on stream invertebrates at temperatures exceeding 20°C, heat stress could limit the number of EPT taxa at MR3. However, temperatures on the same summer days were consistently warmer at MR2 (an upstream location which was more shaded but nearer to the dam) than at MR3. Therefore, heat stress was not likely to be the predominant cause of the impairment. It is possible that temperature could interact with other stressors at MR3 to heighten the impact of those stressors.

Box 6. Heat Stress.

First Iteration

Co-occurrence of Stressor and Impairment—Temperature was not measured during the summer of 1999, so no assessment of summertime temperatures could be made in the first iteration, except to note that temperatures at MR3, even in October, were slightly warmer than at RB1and MR1 (see Table A-6).

Second Iteration

Co-occurrence of Stressor and Impairment—The warmest temperature measured at MR3 was 23.4°C in July 2001 (see Table 6). The temperatures at two upstream sites on the same date (MR1 upstream, and MR2 downstream from the impoundment), were 22.6°C and 24.5°C, respectively (see Table A-6). Since the number of EPT taxa was greater at MR2 than at MR3, co-occurrence was not observed, so temperature probably cannot account for the impairment. Measurements were not necessarily made on the hottest day or extended series of hot days. The duration, maximum temperatures, and the rapidity with which temperature changes occurred in stormwater runoff was not known.

Stressor-Response from the Field—Correlations were relatively strong between temperature and the number of EPT taxa, both in the autumn (1999; $r = -0.52$) and summer ($r = -0.61$) (see Table 8). Numbers of non-EPT taxa (again, 1999 data), also correlated strongly with autumn temperatures ($r = -0.58$), and temperature was the only variable that entered in a step-wise multiple regression model.

Stressor-Response from Other Field Studies—Impoundment-induced temperature increases of 5°C were associated with decreased *Plecoptera* (Lessard, 2000). However, the temperature differences at MR3 were not that great.

Stressor-Response from the Laboratory—Temperatures at and above 20°C can adversely affect many species of aquatic invertebrates. For example, a temperature of 21.9°C caused 50% mortality for the mayfly *Deleatidium autumnale* (New Zealand) (Cox and Rutherford, 2000), and the growth rate of the amphipod *Hyalella azteca* declines substantially above 20°C (Panov and McQueen, 1998). Numerous other examples can be presented.

6.6. MR3 CANDIDATE CAUSE 6, TAXA LOSS DUE TO ALTERED FOOD RESOURCES

The SOE for this Candidate Cause is described and scored in Table B-6 and pertinent evidence is presented with the conceptual model (see Figure 14).

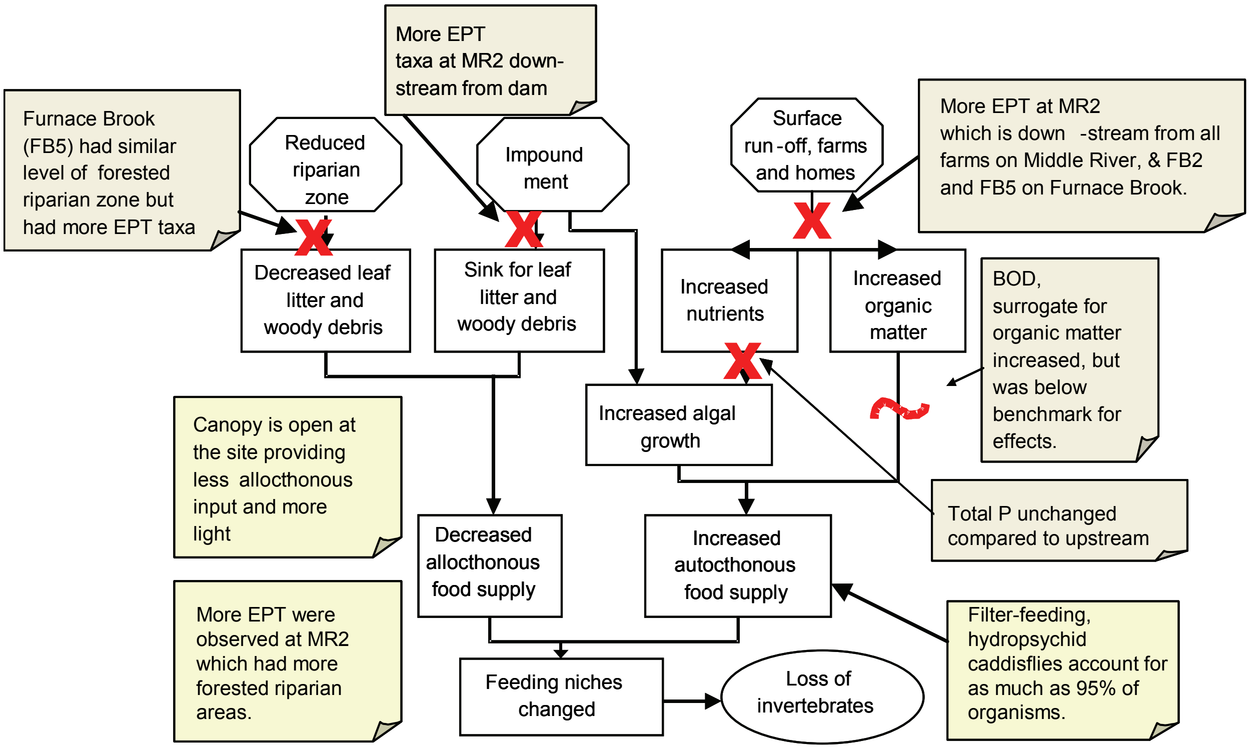


FIGURE 14

Annotated Conceptual Model Used to Examine Stressors Associated with Altered Food Resources

6.6.1. First and Second Iterations

Low inputs of allocthonous organic matter could contribute to a smaller number of EPT taxa at MR3, and the impoundments could be a source of dissolved and suspended organic matter. Sites in the watershed below impoundments had greater proportions of hydropsychid caddisflies than sites distant from impoundments. Also, WL3, located below the wastewater outfall, continues to have a very high proportion of hydropsychid caddisflies (see Table A-8).

Nevertheless, EPT taxa increased in numbers to levels observed at MR2 after the illicit discharge was eliminated, and the two sites were thought to be similar with respect to allocthonous inputs, even though some trees are present on both banks at

MR2. Hydropsychid filter-feeding caddisflies still dominated the assemblage but were more diverse and proportionately similar at MR2, MR3, and WL1, suggesting that the continued higher proportion of hydropsychids is shared among the sites and does not account for the differences observed in 1999–2000.

Box 7. Altered Food Resources.

Co-occurrence of Stressor and Impairment—No measurements were made of possible food resources, such as algal concentrations or allocthonous organic matter.

Causal Pathway—Measures were available to evaluate the causal pathways (see Tables 6 and 16, SOE Table B-6). The second iteration used the same 1999 data, but included information from MR2 collected in 2000 and background values published in U.S. EPA (2000a) and by Smith et al. (2003).

With respect to algal growth, phosphorous rather than nitrogen is most commonly the limiting nutrient in high gradient streams. Phosphorous was not elevated compared to the upstream sites (MR1 and MR2) or the tributary reference site (RB1). However, measurements were not taken continuously, and episodic events may occur. Additionally, total P concentrations were at natural background concentrations for streams in the Eastern Coastal Plains, including the Willimantic basin (Smith et al., 2003). Further, U.S. EPA default criteria for P and N (U.S. EPA, 2000a) were not exceeded. TN concentrations were near the upper limit for natural background concentrations for streams in the Eastern Coastal Plain (Smith et al., 2003), and the default U.S. EPA criteria for TN was exceeded (see Table 13). However, TN concentration was 175 µg greater at the upstream location (MR1) than at MR3 and a larger number of taxa was found at the site where TN levels were higher.

Input of allocthonous organic matter, such as leaves, was expected to be low near MR3 because (a) few trees occur within the riparian zone in the town of Stafford Springs and (b) impoundments upstream of MR3 would reduce downstream transport of leaves. However, trees line both banks at MR1 and MR2.

We found almost no evidence for organic enrichment at MR3. A very small increase in the mean total solids (+0.16 mg/L) and organic nitrogen (+0.04 mg/L) was noted, compared to the nearest upstream site (MR1). These values were thought to be too low to account for the impairment.

TABLE 16

Measurements Relevant to Causal Pathway for WL1 (Autumn 1999)

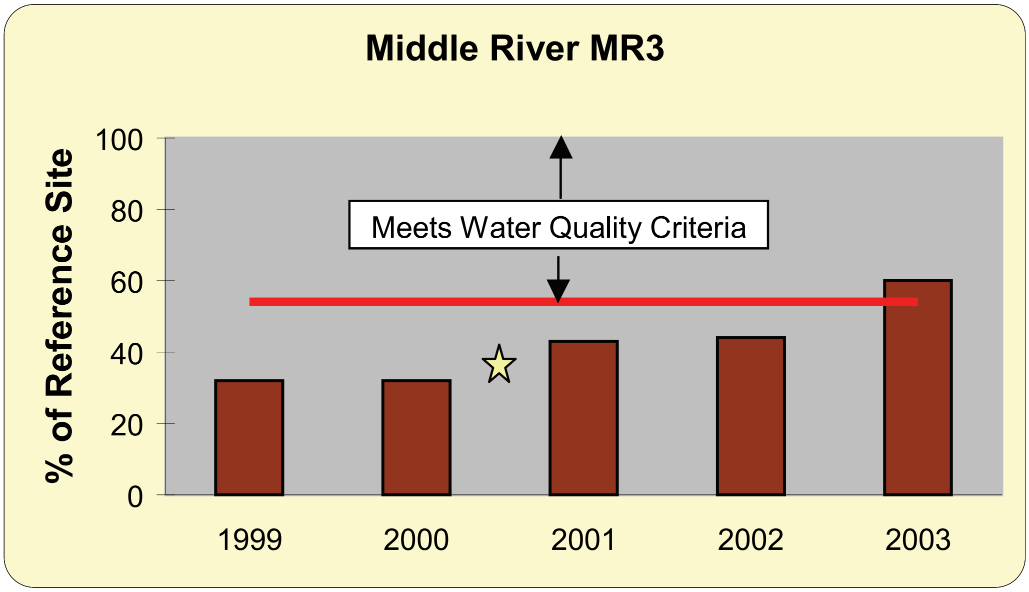
Candidate Causes	Data	MR3	FB2	WL1	Pathway Supported Compared to Both References?
		Upstream Reference Sites		Impaired Site	
1, 3, 4, 5, 6	Habitat Index % of Reference	0.32	0.53	0.26	**Yes**
4, 6	BOD	1.6	1.0	1.7	**Yes**
5, 6	Tree Canopy	Low	Moderate	High	No
2, 3	Bank Stability	10	9	6	**Yes**
2	Stormwater Out Falls	Yes	No	No	No
2, 5	% Impervious Surface	Moderate	Low	High	**Yes**
1, 4	Illicit Discharge	Present	Absent	Absent	No
2, 3	Substrate Composition				
	% Boulder	25	60	60	No
	% Cobble	50	20	30	No
	% Gravel	13	10	10	No
	% Sand	12	0	0	No
4, 6, 3	Nutrient Concentration (mg/L)				
	Organic Nitrogen	0.4	0.8	0.4	No
	NH_3	0.1	0	0.1	No
	TKN	0.4	0.8	0.4	No
	Nitrate	0.1	0.6	0.1	No
	Nitrite	0.05	0	0.05	No
	Total Phosphorus	0.01	0.02	0.08	**Yes**
	Total Solids	74	110	74	No
	Total Suspended Solids	0	5	0	No

7. COMPARISON OF CANDIDATE CAUSES

After both iterations had been completed, we concluded that episodic toxicity was the probable dominant cause for the reduced number of EPT and non-EPT taxa at MR3 and WL1 (see Table 4). The strongest piece of evidence supporting this Candidate Cause was a manipulation of exposure: after an illicit discharge was removed, the benthic macroinvertebrate communities downstream recovered, at least partially, to a condition that was judged to be acceptable by the State of Connecticut. Our confidence in this probable cause was further enhanced by 2003 monitoring data obtained after the second iteration was completed (see Figure 15) showing further recovery of the benthic invertebrate community (see Comment 17). However, sediment alterations associated with the discharge and heat stress could have been contributing factors and may be responsible for the stream not reaching the same level as the watershed reference site. The continued dominance of hydropsychid caddisflies also suggests an abundance of very fine particulates as a food source, which are commonly associated with areas downstream from impoundments (see Table A-8).

Comment 17. Additional Confirmation.
Targeted monitoring in 2006–2007 reported in the 305(b) report for 2008 indicated that all stream segments were fully supporting for aquatic life except the concrete channel of Furnace Brook through the center of Stafford Springs (CT DEP, 2008).

By 2003, the difference between MR3 and WL1 was negligible suggesting that the slightly greater impairment at WL1 may have been due to sampling variation or greater vulnerability of the site to the toxic mixture rather than an additional cause.



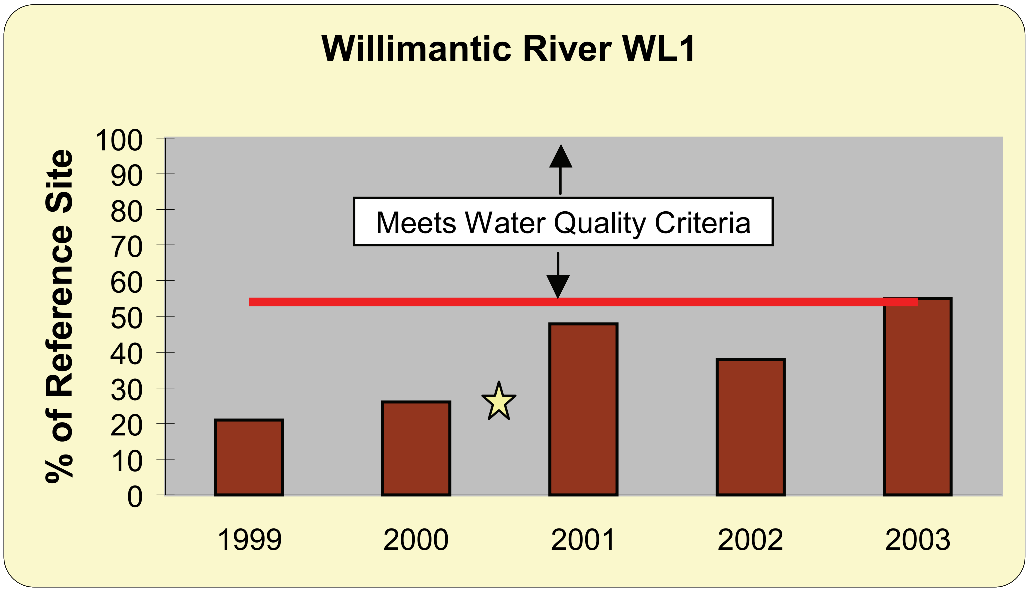


FIGURE 15

Macroinvertebrate Scores for MR3 and WL1 Relative to the Reference Area RB1. As the figure indicates, biological water quality criteria were met at these locations in 2003, approximately 2.5 years after the illicit discharge was identified and corrected. Removal of illicit discharge occurred in the autumn of 2000.

8. HIGHLIGHTS

The following highlights and lessons learned emerged from this case study.

1. Preliminary analysis leading to the collection of additional information (Iteration).
2. Biological screening methods to locate the origination of an impairment and its sources.
3. Comparing and evaluating multiple potential causes.
4. Confirmation of the probable cause by monitoring after manipulating exposure.
5. Displaying the evidence by annotating causal pathways on conceptual models.
6. Recognition of different styles for providing information.
7. Recognition of rates of change following a manipulation.

The stressor identification process can proceed in an iterative way linked to adaptive management strategies for watersheds.

This case study is an example of iterative assessment, monitoring, and management as a type of adaptive management. Adaptive management is the practice of using models or logic to suggest an appropriate management action, carrying out that action experimentally, monitoring the results of the action, and using the results of monitoring to modify the model or logic and recommend modified or additional actions. In this case, the logical process was SI and the preliminary inference was that some relatively severe source of impairment occurred on Middle River below MR1. The process and inference led to additional focused biological monitoring, discovery of the intermittent source, and the management action of removing the source. Subsequent monitoring of the results of that action showed recovery of downstream invertebrate communities.

Biological screening methods to bracket an impairment and its sources.

After the initial screening of available data, the first iteration led to investigative bioassessment to locate and demonstrate impairment caused by a previously unidentified source. The case study demonstrated the importance of a sufficient number of strategically selected monitoring sites. Sometimes the “obvious” answer, (e.g., that the Stafford Springs POTW was causing biological impairment at WL2) is incorrect. If CT DEP had not collected data from a number of locations above the POTW, and logically analyzed the evidence, the biological impairment might have been attributed solely to the POTW and, thus, thought to have been solved by the TMDL-driven changes to the permit limits.

Comparing and evaluating multiple potential causes.

U.S. EPA's SI Process is a formal process to evaluate data and assist investigators with determining the most probable cause of a measured biological impairment. One objective of a formal process is that it forces the investigators to look at the data objectively and avoid bias to the decisions made about the data to support the most probable cause of impairment. For example, biological metrics were adequate for discovering the cause of a severe, unknown impairment. However, it also suggests that genus-level data, rather than the composited metrics used in this study, may be necessary for deciphering impairments from more difficult cases.

One value of a process such as SI is that many people from different areas of expertise can have input to listing Candidate Causes and offer suggestions about how the causal mechanism may be working in the environment to cause the biological impairment observed in systems such as the Willimantic River. The annotated conceptual models were useful for achieving a shared understanding of the conditions in the systems and the causes contributing to impairment.

Displaying the evidence by annotating causal pathways on conceptual models.

CT DEP has found that annotating conceptual models with text boxes that provide data to support or weaken causal pathways is a valuable tool that they continue to find useful in preparing additional SI investigations. Conceptual model diagrams have been useful in explaining the logic used to determine the most probable cause of biological impairments to the public, resource managers, and other scientists. CT DEP has approximately 90 waterbodies listed on the 2004 Connecticut List of Waterbodies Not Meeting Water Quality Standards for not meeting aquatic life use goals with unknown causes. CT DEP believes that by giving conceptual models a central role, they can implement the SI methodology more efficiently.

Therefore, conceptual diagrams, annotated with case information, were especially useful in two ways: (1) analyzing possible stressor-response relationships and (2) communicating results of the investigation to stakeholders, managers, the public, and other investigators.

Recognition of different styles for providing information, which are presented for comparison.

Although the CT DEP found the annotated conceptual models to be very effective, there is no optimal way for communicating all the data or even the most important evidence used to decide the case. A combination of some diagrams and tables with text probably reaches most people. Analysts seemed to prefer tables of data and table of inferences. Decision makers preferred synthesis provided by conceptual models. However, some reviewers wanted each inferential step be described in text. No one wanted all of these formats. Serving so many different communication styles suggests that more than one report is needed: (1) An executive summary with diagrams or brief tables for decision making. (2) Another longer report with detailed analytical and inferential methods. Appendices for data were welcomed by all groups as an assurance of transparency and quality, although many would likely not examine them closely.

Recognition of rates of change following a manipulation.

Recovery rates of benthic invertebrate communities vary and such variation should be factored in to expectations following the implementation of management actions. In this study, the CT DEP expected a more rapid recovery after the illicit discharge was removed. It took several years for recovery possibly due to residual metals in the sediments. Recovery rates vary depending on a number of factors. Expectations on the part of managers need to account for such variations. Based on reviews of the literature and discussions with CT DEP personnel, the following factors will be particularly important:

- The type of material creating the impairment (e.g., oil, metals, sediment, organic enrichment)—it is likely that materials vary in persistence and in the number of stresses they cause; for example, discharges of oil can result in both toxicity and physical effects (e.g., smothering or coating);
- The type of environment—high-energy environments are more likely to disperse introduced materials, reducing exposures than are low-energy environments; in some cases, high energy can lead to mixing pollutants deeper into sediments and can actually increase the residence time of the material;
- The availability of colonizing organisms—a source of organisms either through transport, swimming, or egg-laying is necessary for recolonization; the rate of recolonization may be related to the location and extent of source areas for organisms relative to the extent and magnitude of the impairment;
- The types of organisms—some species may be more sensitive to the stressors than others and their ability to recolonize may reflect changing conditions;
- The presence of other stressors—in aquatic systems there may be multiple stressors and interactions among them; therefore, the rate of recovery following control of one stressor might appear slow because other stressors are present.

Comment 18. Strong Causes and Effects.
This SI worked well because the stress was sufficiently severe and well defined to trip the sensitivity of the RBP methods (for two metrics). Confident diagnosis of more complex and less clear-cut stressor scenarios would likely require more rigorous and detailed biological data as was needed to evaluate the residual effects not related to the episodic toxic releases.

The case investigated the cause of less EPT diversity at MR1 and WL1. However, a watershed study also assessed the cause of less diversity throughout the watershed compared to the site with the greatest diversity, at Roaring

Brook, RB1. This study determined that additional causes were associated with the numerous old-mill impoundments that altered flow, altered the food base, and increased stream temperatures (U.S. EPA 2003b).

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APPENDIX A
DATA TABLES

TABLE A-1

List of Biological and Habitat Measurements Used in Analyses

	RB1	FB2	FB4	FB5	MR1	MR2	SB	MR3	WL1	WL2	WL3	WL4	SR	HR	TR
Invertebrate Data	A 99 S 00 A 00 S 01 A 01 A 02	A 99	n	S 00	A 99	S 00	S 00	A 99 S 00 A 00 S 01 A 01 A 02	A 99 S 00 A 00 S 01 A 01 A 02	A 99 S 00 A 00 S 01 A 01 A 02	A 99	A 99	A 99	A 99	A 99
Habitat Parameters Embeddedness, Velocity, Bank Stability, Scour/Depth, Total Habitat Score **Substrate Composition** Bedrock, Boulder, Cobble, Gravel, Sand etc.	A 99	A 99	A 99	n	A 99	n	n	A 99	A 99	A 99	A 99	A 99	A 99	A 99	A 99

A = Autumn; S = Spring followed by sampling years 1999−2002; n = no sampled.

TABLE A-2

Water Quality Parameters Used in Analyses

	RB1	FB2	FB4	MR1	MR2	MR3	WL1	WL2	WL3	WL4	SR	HR	TR
Dissolved Oxygen and Temperature	99 00 01 02	99 00 01		99 00 01	00 01	99 00 01 02	99 00 01 02	99 00 01 02	99 00 01 02	99 00 01	99 00	99 00	99 00
Metals: Al, Cd, Cr, Cu, Fe, Ni, Pb, Zn, Organic Nitrogen, TKN, Nitrate, Nitrite, Total Phosphorus, BOD	99 00 01 02	99	99	99	00	99 00 01 02	99 00 01 02	99 00 01 02	99	99	99	99	99

TABLE A-3

Water Chemistry for Red Brook, Skungamaug River, Hop River, and Ten Mile River

Parameter	Units	RB1				SR	HR	TR
		10/14/99	10/03/00	10/17/01	10/23/02	11/01/99	10/26/99	10/26/99
Aluminum, Total	ppm	0.037	0.058	0	0.035	0.065	0.072	0.1
Cadmium, Total	ppm	0	<0.001	<0.001	0	0	0	0
Chromium, Total	ppm	0	0	0	0	0	0.001	0
Copper, Total	ppm	0.004	0.008	0.005	0.007	0.006	0.004	0.006
Iron, Total	ppm	0.208	0.34	0.113	0.17	0.383	0.21	0.393
Lead, Total	ppm	0	0.002	0.002	0.003	0.001	0	0
Nickel, Total	ppm	0.001	0.001	0	0	0	0.001	0
Zinc, Total	ppm	0.004	0.005	0.004	0.003	0.003	0.003	0.003
Aluminum, Dissolved	ppm	0.037	0.058	0	0.035	0.065	0.062	0.09
Cadmium, Dissolved	ppm	0	<0.001	<0.001	0	0	0	0
Chromium, Dissolved	ppm	0	0	0	0	0	0	0
Copper, Dissolved	ppm	0.002	0.003	0.005	0.004	0.003	0.003	0.003
Iron, Dissolved	ppm	0.145	0.283	0.099	0.162	0.215	0.178	0.35
Lead, Dissolved	ppm	0	0.002	0	0.001	0	0	0

TABLE A-3 cont.

Parameter	Units	RB1				SR	HR	TR
		10/14/99	10/03/00	10/17/01	10/23/02	11/01/99	10/26/99	10/26/99
Nickel, Dissolved	ppm	0.001	0.001	0	0	0	0	0
Zinc, Dissolved	ppm	0.004	0.003	0.003	0.003	0.003	0.003	0.003
Ammonia Nitrogen	ppm	<0.1	<0.1	<0.1	0.1	<0.1	<0.1	<0.1
Nitrate as Nitrogen	ppm	0.1	0.8	1	13	0.3	0.2	0.1
Nitrite as Nitrogen	ppm	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Organic Nitrogen	ppm	0.4	0.1	0.5	0	0.5	0.5	0.6
Phosphate as P, Total	ppm	0.02	0.03	0.08	0.04	0.04	0.01	0.1
Solids, Total	ppm	77	82	130	120	84	77	88
Solids, Total Suspended	ppm	4	2	7	<1	3	3	3
TKN	ppm	0.4	0.1	0.5	0.1	0.5	0.5	0.6
Turbidity	NTU	1.1	1.2	0.5	0.5	0.8	0.9	1.2
BOD	ppm	1.1	<1	<1	<1	1.2	<1	<1

TABLE A-4

Water Chemistry Data for Furnace Brook and Middle River

Parameter	Units	FB2	FB4	MR1	MR2	MR3			
		10/13/99	10/13/03	10/13/99	10/03/00	10/13/99	10/03/00	10/17/01	10/23/02
Aluminum, Total	ppm	0.058	0.052	0.08	0.109	0.101	0.115	0.019	0.078
Cadmium, Total	ppm	0	0	0	<0.001	0	<0.001	<0.001	0
Chromium, Total	ppm	0	0	0	0	0.005	0.002	0	0
Copper, Total	ppm	0.005	0.006	0.004	0.003	0.005	0.004	0.005	0.005
Iron, Total	ppm	0.495	0.46	0.395	0.948	0.695	0.928	0.663	0.5
Lead, Total	ppm	0.001	0.001	0.001	0.002	0.001	0.002	0.001	0.002
Nickel, Total	ppm	0.001	0	0	0.006	0	0.001	0	0
Zinc, Total	ppm	0.005	0.004	0.006	0.007	0.011	0.01	0.004	0.007
Aluminum, Dissolved	ppm	0.058	0.052	0.08	0.109	0.101	0.115	0.019	0.078
Cadmium, Dissolved	ppm	0	0	0	0.001	0	0.001	0.001	0
Chromium, Dissolved	ppm	0	0	0	0	0.004	0.002	0	0
Copper, Dissolved	ppm	0.005	0.004	0.003	0.003	0.003	0.004	0.005	0.003
Iron, Dissolved	ppm	0.375	0.315	0.365	0.805	0.528	0.805	0.493	0.409
Lead, Dissolved	ppm	0	0	0	0.002	0	0.002	0	0

TABLE A-4 cont.

Parameter	Units	FB2	FB4	MR1	MR2	MR3			
		10/13/99	10/13/03	10/13/99	10/03/00	10/13/99	10/03/00	10/17/01	10/23/02
Nickel, Dissolved	ppm	0	0	0	0	0	0	0	0
Zinc, Dissolved	ppm	0.005	0.003	0.005	0.006	0.011	0.008	0.004	0.006
Ammonia Nitrogen	ppm	0	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	<0.1
Nitrate as Nitrogen	ppm	0.6	0.2	<0.1	<0.1	<0.1	0.1	0.1	0.1
Nitrite as Nitrogen	ppm	0	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Organic Nitrogen	ppm	0.8	0.6	0.4	0.1	0.4	0.2	0.3	0.2
Phosphate as P, Total	ppm	0.02	0.01	0.01	0.03	0.01	0.05	0.21	0.05
Solids, Total	ppm	110	46	58	50	74	48	72	94
Solids, Total Suspended	ppm	5	0	5	2	0	3	7	<1
TKN	ppm	0.8	0.6	0.4	0.1	0.4	0.2	0.4	0.2
Turbidity	NTU	1.5	1.7	1.1	3	1.9	3	1.5	1.3
BOD	ppm	1	<1	1.1	<1	1.6	<1	<1	<1

TABLE A-5

Water Chemistry Data for Willimantic River

Parameter	WL1				WL2				WL3	WL4
	10/13/99	10/03/00	10/17/01	10/23/02	10/13/99	10/03/00	10/17/01	10/23/02	10/14/99	10/14/99
Aluminum, Total	0.098	0.107	0.007	0.059	0.111	0.109	0.01	0.077	0.809	0.096
Cadmium, Total	0	<0.001	<0.001	0	0	<0.001	<0.001	0	0	0
Chromium, Total	0.003	0.001	0	0	0.002	0	0	0	0.003	0
Copper, Total	0.005	0.004	0.005	0.007	0.012	0.008	0.006	0.012	0.007	0.005
Iron, Total	0.608	0.868	0.537	0.488	0.573	0.798	0.481	0.467	0.97	0.383
Lead, Total	0.001	0.002	0.001	0.004	0.001	0.004	0.001	0.005	0.001	0.002
Nickel, Total	0	0.001	0	0	0.001	0.001	0	0	0.003	0.001
Zinc, Total	0.01	0.007	0.004	0.006	0.019	0.008	0.011	0.011	0.011	0.008
Aluminum, Dissolved	0.098	0.107	0.007	0.051	0.111	0.109	0.01	0.071	0.283	0.076
Cadmium, Dissolved	0	<0.001	< 0.001	0	0	<0.001	<0.001	0	0	0
Chromium, Dissolved	0.003	0.001	0	0	0.002	0	0	0	0.001	0
Copper, Dissolved	0.003	0.004	0.005	0.003	0.007	0.008	0.006	0.004	0.005	0.003
Iron, Dissolved	0.468	0.733	0.449	0.433	0.428	0.668	0.41	0.452	0.353	0.193

TABLE A-5 cont.

Chemical Parameter	Units	WL1				WL2				WL3	WL4
		10/13/99	10/03/00	10/17/01	10/23/02	10/13/99	10/03/00	10/17/01	10/23/02	10/14/99	10/14/99
Lead, Dissolved	ppm	0	0.002	0	0.003	0	0.004	0	0.002	0	0
Nickel, Dissolved	ppm	0	0	0	0	0.001	0.001	0	0	0.002	0.001
Zinc, Dissolved	ppm	0.009	0.006	0.004	0.006	0.018	0.008	0.011	0.011	0.008	0.007
Ammonia Nitrogen	ppm	<0.1	<0.1	0.1	0.2	<0.1	0.1	<0.1	<0.1	<0.1	<0.1
Nitrate as Nitrogen	ppm	0.1	0.1	0.7	1.3	0.7	1	0.7	1.4	0.5	0.3
Nitrite as Nitrogen	ppm	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Organic Nitrogen	ppm	0.4	0.1	0.4	0.4	0.6	0.6	0.8	0.7	0.4	0.4
Phosphate as P, Total	ppm	0.08	0.04	0.03	0.01	0.07	0.06	0.19	0.02	0.06	0.09
Solids, Total	ppm	74	44	82	82	85	76	100	140	100	97
Solids, Total Suspended	ppm	0	0	6	<1	1	12	4	4	17	5
TKN	ppm	0.4	0.2	0.5	0.6	0.6	0.7	0.8	0.8	0.4	0.4
Turbidity	NTU	2	2.7	1.2	1.6	2	3	1.3	1.8	22	3
BOD	ppm	1.7	<1	<1	<1	1.5	<1	<1	<1	1.3	1

TABLE A-6

Dissolved Oxygen (mg/L), Total Dissolved Solids (mg/L), and Temperature (°C) for All Sites

Physical Parameters		10/23/02	10/10/02	7/23/01	10/3/00	8/28/00	8/22/00	11/1/99	10/26/99	10/14/99	10/13/99
RB1	Dissolved oxygen	12.72		10.17	12.03	10.25					11.1
	Total dissolved solids	0.113		0.099	0.087	0.083					
	Water temperature	5.63		17.19	11.09	17.28					12.09
MR1	Dissolved oxygen			7.32		7.44					9.26
	Total dissolved solids			0.05		0.041					
	Water temperature			22.56		21.09					12.11
MR2	Dissolved oxygen			8.19	10.47						
	Total dissolved solids			0.06	0.051						
	Water temperature			24.51	14.62						
MR3	Dissolved oxygen	11.1	11.26	8.91	10.71	9.17					9.85
	Total dissolved solids	0.074	0.067	0.025	0.055	0.064					
	Water temperature	8.95	13.8	23.41	14.06	21.59					12.48

TABLE A-6 cont.

Physical Parameters		10/23/02	10/10/02	7/23/01	10/3/00	8/28/00	8/22/00	11/1/99	10/26/99	10/14/99	10/13/99
FB2	Dissolved oxygen			8.29		8.7					10.05
	Total dissolved solids			0.066		0.055					
	Water temperature			23.13		21.31					11.7
WL1	Dissolved oxygen	11.49	11.67	8.78	10.85	9.24					9.32
	Total dissolved solids	0.075	0.07	0.059	0.055	0.054					
	Water temperature	8.62	14.37	22.53	13.85	21.15					13.4
WL2	Dissolved oxygen	11.31	11.89	9.06	11.62	9.4					10.73
	Total dissolved solids	0.16	0.14	0.104	0.093	0.107					
	Water temperature	9.84	15.31	22.02	14.05	20.98					14.22
WL3	Dissolved oxygen		12.52	9.15		9.71				10.21	
	Total dissolved solids		0.146	0.094		0.08					
	Water temperature		13.02	20.76		19.76				13.55	

TABLE A-6 cont.

Physical Parameters		10/23/02	10/10/02	7/23/01	10/3/00	8/28/00	8/22/00	11/1/99	10/26/99	10/14/99	10/13/99
WL4	Dissolved oxygen			8.3		8.6				10.09	
	Total dissolved solids			0.101		0.088					
	Water temperature			21.87		21.38				13.72	
SR	Dissolved oxygen						10.45	11.35			
	Total dissolved solids						0.081				
	Water temperature						14.56	11.67			
HR	Dissolved oxygen						9.77		11.81		
	Total dissolved solids						0.081				
	Water temperature						17.02		9.06		
TR	Dissolved oxygen						9.01		11.22		
	Total dissolved solids						0.068				
	Water temperature						16.28		9.08		

TABLE A-7

Connecticut Habitat Index and Metric Scores for All Sites from Autumn 1999

Site	RB1	MR1	FB2	MR3	WL1	WL2	WL3	WL4	SR	HR	TR
Substrate	16	20	16	15	15	20	16	20	11	20	16
Embedded	16	20	16	11	11	16	16	15	11	16	16
Velocity	16	15	10	11	16	11	10	11	16	15	16
Alteration	15	15	12	12	11	11	11	8	12	12	12
Scour/Dep	12	12	15	7	8	12	7	15	7	15	12
Pool/Riffle	15	11	15	11	12	11	7	11	11	12	12
Bank Stab	8	10	9	10	6	9	6	9	10	9	9
Bank Veg	8	10	9	9	6	9	9	10	9	10	10
Cover	6	9	8	8	6	8	8	9	8	9	8
Total Score	112	122	110	94	91	107	90	108	95	118	111
Substrate Composition											
% Bedrock	0	0	10	0	0	0	0	0	0	0	0
% Boulder	33	0	60	25	60	0	10	0	60	5	20
% Cobble	33	25	20	50	30	75	70	70	15	60	60
% Gravel	33	50	10	13	10	20	10	15	10	30	20
% Sand	0	25	0	12	0	5	10	15	15	5	0
Silt	0	0	0	0	0	0	0	0	0	0	0
Clay	0	0	0	0	0	0	0	0	0	0	0
Detritus	0	0	0	0	0	0	0	0	0	0	0
Muck	0	0	0	0	0	0	0	0	0	0	0
Marl	0	0	0	0	0	0	0	0	0	0	0

Note: Silt, Clay, Detritus, Muck, Marl not estimated.

TABLE A-8

Invertebrate Data (Autumn only)

	RB1				SR	HR	TR
	1999	2000	2001	2002	1999	1999	1999
EPT taxa	25	26	26	27	16	20	21
Non-EPT taxa	8	12	9	14	13	15	19
% Hydropsychid	8	11	5	13	8	18	2
*C-F taxa	7	8	5	6	3	9	8
% C-F	16	15	10	16	24	32	6
Score	38	38	42	38	28	34	36
% of RB1	1	1	1	1	74	89	89

	FB2	MR1	MR2	MR3			
	1999	1999	2000	1999	2000	2001	2002
EPT taxa	13	17	13	9	5	11	12
Non-EPT taxa	10	15	10	14	12	17	3
% Hydropsychid	55	4	27	80	72	49	43
C-F taxa	6	4	10	9	7	8	7
% C-F	67	8	68	84	83	52	68
Score	20	26	20	12	12	18	14
% of RB1	53	68	53	32	32	43	44

	WL1				WL2				WL3	WL4
	1999	2000	2001	2002	1999	2000	2001	2002	1999	1999
EPT taxa	5	4	12	13	5	5	11	9	19	16
Non-EPT taxa	5	9	11	6	7	7	15	15	13	7
% Hydropsychid	96	85	42	43	92	94	71	85	6	24

TABLE A-8 cont.										
	WL1				WL2				WL3	WL4
	1999	2000	2001	2002	1999	2000	2001	2002	1999	1999
*C-F taxa	5	3	8	7	4	4	10	8	5	6
% C-F	97	85	62	66	92	94	76	63	15	24
Score	8	10	20	12	8	10	18	22	30	20
% of RB1	21	26	48	38	21	26	43	58	79	53

*C-F: number of collector and filterer taxa.

TABLE A-9

Invertebrate Data (Spring only)

	RB1		MR3		FB5	WL1		WL2		SB
	2000	2001	2000	2001	2000	2000	2001	2000	2001	2000
EPT taxa	20	21	15	9	12	3	9	5	5	11
Non-EPT taxa	14	23	20	18	13	20	18	21	21	12
% Hydropsychid	1	5	18	14	18	31	15	9	10	5
*C-F taxa	10	10	-	10	8	-	10	-	59	3
% C-F	13	13	-	74	44	-	57	-	59	21
Score	42	42	22	18	20	12	18	10	20	-
% of RB1	1	1	52	43	48	29	43	24	48	-

*C-F: number of collector and filterer taxa.

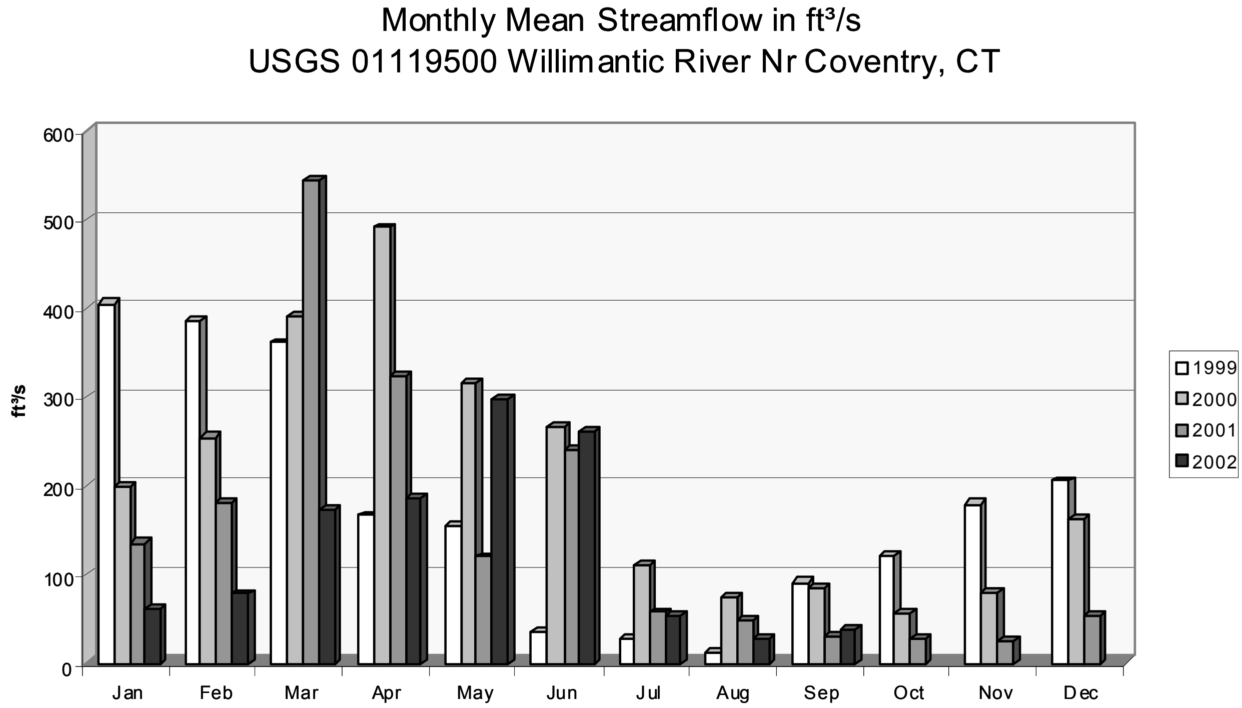

FIGURE A-1

Discharge Data for the Willimantic River at Coventry, CT: Monthly Mean Stream Flow (ft^3/s) from a USGS Gauging Station Near Coventry, CT. Discharge is greatest in the spring and lowest in the late summer and early autumn.

APPENDIX B
STRENGTH-OF-EVIDENCE TABLES FOR ALL CANDIDATE CAUSES AT MR3 AND WL1

Evidence for the first iteration of causal analysis is labeled 2000.

New evidence that was added for the second iteration is labeled 2003.

In addition to the standard types of evidence, these strength-of-evidence tables contain comparisons of each Candidate Cause to the others and summary characterizations of the level of confidence in the strength-of-evidence analysis.

Each distinct line of evidence is assigned a score based on the following system.

+++	Convincingly supports
++	Strongly supports
+	Somewhat supports
0	Neither supports nor weakens
−	Somewhat weakens
− −	Strongly weakens
− − −	Convincingly weakens
NE	No evidence
NA	Not applicable

TABLE B-1

Summary of the Strength-of-Evidence Analysis for Candidate Cause 1 at MR3. Specific Candidate Causes are 1.1a Sustained exposure to metals or ammonia from nonpoint sources, 1.1b Sustained exposure to metals or ammonia from a point source, and 1.2 Episodic exposure to undefined toxicants.

Candidate Cause 1	Toxicity from Metals or Ammonia	
	Evidence	Interpretation
Case-Specific Considerations		
Co-occurrence	2000: 1.1 Highest values for total Al, Cr, Cu, Fe, and Zn increased from site MR1 to MR3. The remaining metals, Cd, Ni, and Pb, were undetected or remained at the same concentration relative to MR1 (see Table 6).	+ Compatible
	2000: 1.1 Ammonia did not increase compared to MR1 (see Table 6).	− − − Incompatible
Stressor-Response in the Field	2000: Zn, Fe, and Cr were negatively correlated with EPT taxa (see Table 8), but data from tributaries were used and the relationship was not modeled.	+ Strong but not spatially contiguous
	2000: Ammonia was correlated with EPT but with the wrong sign (see Table 8).	− − − Clear association but wrong sign
Causal Pathway	2000: 1.1a Nonpoint source of sustained metal or ammonia toxicity: None identified.	NE No Evidence
	2003: 1.1a Nonpoint sources of sustained metal or ammonia toxicity: EPT taxa at MR2 were more numerous than at MR3, thereby demarcating location of impairment and independence from most nonpoint sources. Nonpoint source therefore unlikely.	− − − Source absent
	2000: 1.1b Unknown point sources of sustained metal or ammonia toxicity: None characterized.	NE No Evidence
	2000: 1.2 Source of episodic toxicity from complex mixture. None known.	NE No Evidence
	2003: 1.2 Source of episodic toxicity from complex mixture: Identification of the illicit source would count as partial evidence of the causal pathway, but is used instead as a verified prediction. To avoid double counting of evidence it is given a zero (0) score here.	0 Partial Evidence

TABLE B-1 cont.

Candidate Cause 1	Toxicity from Metals or Ammonia	
	Evidence	Interpretation
Manipulation of Exposure	2003: 1.2 Unknown episodic source of toxicity from a mixture: Ruptured waste line repaired in 2000. EPT diversity increased in 2001 (see Figure 7). In 2001 and 2002, mean concentrations of total Cr, Fe, and Ni (see Table 9) and of dissolved Al, Cr, Pb, and Zn (see Table 10) decreased at MR3 compared to concentrations in 1999.	+++ Compatible
Verified Prediction	2003: 1.2 Unknown source of episodic toxicity from complex mixture: During the first iteration, the assessors predicted that a toxic source would be found above MR3. In 2000, an illicit discharge from a mill permitted to discharge metals and organic matter to POTW was found in the predicted reach. Chemical characteristics of grey discharge are unknown.	+++ Specific predictions are confirmed
Considerations Based on Other Situations or Biological Knowledge		
Mechanistically Plausible Cause	Metals, organic compounds, and ammonia may be toxic to invertebrates and cause loss of species.	+ Plausible
Stressor-Response from Laboratory Studies	2000: 1.1 Unknown source of sustained metal and ammonia toxicity: Screening benchmarks were exceeded by observed concentrations of Cd, Cu, and Cr (see Table 12). However, the apparently high humic acid levels (based on water color) would reduce the bioavailability of Cu, Cr, and possibly Cd, and reduce Cr to the trivalent form. Ammonia was well below toxic levels (see Table 12).	0 Ambiguous
	2000: 1.2 Source of episodic toxicity from complex mixture: None known.	NE No Evidence
Stressor-Response from Other Field Studies	2000: Measured copper (Cu) concentrations did not exceed Connecticut's numerical water quality criterion (see Table 12), which is based on the association of Cu concentrations with biological criteria. Hence, the observed Cu concentrations are not consistently associated with biological impairments. Equivalent information is unavailable for other chemicals.	− Inconsistent

TABLE B-1 cont.

Candidate Cause 1	Toxicity from Metals or Ammonia	
	Evidence	Interpretation
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	2000: 1.1a: Nonpoint sources of sustained metals or ammonia toxicity.	− Inconsistent
	2000: 1.1b Point source of sustained metals or ammonia toxicity.	− Inconsistent
	2000: 1.2 Source of episodic toxicity from complex mixture.	NE No Evidence
	2003: 1.2 Source of episodic toxicity from complex mixture.	+++ All Consistent
Reasonable Explanation of the Evidence	2000: 1.1a Nonpoint sources of sustained metals or ammonia toxicity.	0 No known explanation
	2000: 1.1b Unknown point source of sustained metal or ammonia toxicity. The observed slightly elevated concentrations may have been residues of episodic releases from an unknown point source above MR3 or an unmeasured chemical.	+ Inconsistency explained by a credible mechanism
	2000: 1.2 Source of episodic toxicity from complex mixture: It is possible that there is an unknown source or unmeasured chemical.	+ Inconsistency explained by a credible mechanism
	2003: 1.2 Source of episodic toxicity from complex mixture: all consistent and consideration is unnecessary.	NA Not applicable
Comparison Among Candidate Causes	2000: Before remediation 1.2 Unknown source of episodic toxicity from complex mixture: The loss of species was so great, and the identified causes were so weak, that a highly toxic unknown source was hypothesized and lead to further studies in lower MR. 2003: After Remediation: 1.2: After removal of a toxic point source, EPT taxa increased clearly demonstrating the role of the discharge in causing the impairment.	+++ Strongest sufficient cause

TABLE B-1 cont.		
Candidate Cause 1	Toxicity from Metals or Ammonia	
	Evidence	Interpretation
Characterization and Level of Confidence		
Cause of Impairment	2003 1.2 Acute episodic toxicity from complex mixture is the probable cause, based on severity of effects and presence of potential industrial sources.	Probable
Uncertainty	Unknown chemicals may be responsible for residual effects. The remediated source was not characterized. Effects of water chemistry on toxicity are uncertain. Sensitivities of benthic invertebrates to contaminants are poorly known.	Uncertainty very low
Potential Additional Causes and Actions	Continue biological monitoring, examine details of species requirements from those present and those absent. Measure other chemicals. Monitor remaining Candidate Causes.	

TABLE B-2

Summary of the Strength-of-Evidence Analysis for Candidate Cause 2 at MR3

Candidate Cause 2	Removal of Organisms During High Flow		
	Evidence	Interpretation	
Case-Specific Considerations			
Spatial Co-occurrence	NE: Hydrologic flow regime at MR3 not available.	NE	No Evidence
Temporal Co-occurrence	2000: There were more EPT and non-EPT taxa at MR3 in the spring compared to the previous autumn (see Figures 7 and 8). Flows are higher in the spring than autumn. Temperature and life cycle may confound this association.	− − −	Incompatible
Causal Pathway	2000: 2.1 Impervious surface pathway: Proportion of impervious surface is greater at MR3 than at MR1. The proportion of boulders and cobble is greater at MR3 than at MR1 but not compared to RB1. High proportions of boulder (25%) and cobble (50%) indicate erosional area. However, embeddedness increased compared to upstream suggesting high flow velocity is episodic as occurs with large storm events (see Table 15).	+	Partial Evidence
	2003: 2.2 Stormwater outfall pathway: A raceway above MR3 may be connected to a source of stormwater.	0	Ambiguous
Considerations Based on Other Situations or Biological Knowledge			
Mechanistically Plausible Cause	High flow velocity has been shown to selectively remove invertebrate taxa depending on substrate morphology (Kilbane and Holomuzki, 2003).	+	Plausible
Considerations Based on Multiple Lines of Evidence			
Consistency of Evidence	2000: 2.1 Impervious surface and 2.2 Stormwater outfall: There are greater numbers of non-EPT and EPT taxa in the spring when rainfall and stream discharge is greatest.	−	Inconsistent
Reasonable Explanation of the Evidence	2000: 2.1 Impervious surface and 2.2 Stormwater outfall	0	No known explanation
Comparison Among Candidate Causes	2003: High flows are coherent with dilution of toxics and absence of impairment at MR3 during higher spring flows. Acute episodic toxicity from complex mixture is the more probable cause.	− − −	Impairment attributed to another cause

TABLE B-2 cont.		
Candidate Cause 2	Removal of Organisms During High Flow	
	Evidence	Interpretation
Characterization and Level of Confidence		
Cause of Impairment	2003: High flow is an improbable cause because biological scores were highest during spring when flow is highest. In fact, the opposite situation, low flow, is coherent with less dilution and increased toxicity of a toxic discharge.	Improbable
Uncertainty	None.	Uncertainty very low
Potential Additional Causes and Actions	Consider if Low flow—rather than high flow—is contributing to incomplete recovery. Continue monitoring benthic assemblage for improvement.	

TABLE B-3

Summary of the Strength-of-Evidence Analysis for Candidate Cause 3 at MR3

Candidate Cause 3	Loss of Interstitial Habitat Due to Settled Particles	
	Evidence	Interpretation
Case-Specific Considerations		
Co-occurrence	2000: Embeddedness increased compared to the nearest upstream site (MR1) (score declined from 20 to 11, corresponding to an increase from 0−25% to 50−75% silt covered substrate) (see Table 6). Based on the habitat RBP (Plafkin et al., 1989), MR3 is among the three sites in the watershed with the most embedded substrates: that is, 50 to 75% of gravels, cobbles, and boulders were surrounded by fine sediment. In contrast, at MR1 and RB1, no more than 25% of substrate is surrounded by fine sediment.	+ Compatible
Stressor-Response in the Field	2000: Increased embeddedness was not correlated with the number of EPT taxa (not shown).	- Inconsistent
Causal Pathway	2000: 3.1 Impoundment pathway: Several impoundments are located on the Middle River. Although impoundments trap silt and larger particles, they may be sources of fine organic particles (algae and waterfowl manure) or, if nearly filled, may export silt during high flows.	+ Partial evidence
	2003: 3.1 Impoundment pathway: Impairment did not occur at MR2 which is upstream from MR1 and nearer to the impoundment.	− − − Source absent
	2000: 3.2 Unknown sources pathway: Total suspended solids (0 mg/L) decreased compared to the upstream site at MR1 (5 mg/L) and less than at RB1 (see Table 15).	− Some steps missing
	2000: 3.3 Road sanding pathway: Percentage of sand as substrate decreased compared to MR1 (see Table 15).	− Some steps missing
	2000: 3.4 Bank failure pathway: Bank failures were nearly impossible due to armoring of the channel and none were observed (see Table 15). No upstream bank failures were observed. Material from further upstream is likely to be trapped by impoundments.	− − − Source absent

TABLE B-3 cont.

Candidate Cause 3	Loss of Interstitial Habitat Due to Settled Particles	
	Evidence	Interpretation
Considerations Based on Other Situations or Biological Knowledge		
Mechanistically Plausible Cause	Studies have linked declines in macroinvertebrate numbers to increases in sediment load and decreases in substrate particle size (Swank and Crossely, 1988; Richards and Bacon, 1994; Waters, 1995; Grubaugh et al., 1996; Angradi, 1999; Wisconsin Department of Natural Resources, 2001; Whiles and Dodds, 2002). However, some fine sediment is important for some organisms (Pennak, 1989; Murphy and Meehan, 1991; Thorp and Covich, 1991).	+ Plausible
Stressor-Response from Laboratory Studies	No appropriate laboratory studies were readily available for EPT.	NE No Evidence
Stressor-Response from Other Field Studies	Studies have linked declines in macroinvertebrate numbers to increases in sediment load and decreases in substrate particle size (Swank and Crossely, 1988; Richards and Bacon, 1994; Waters, 1995; Grubaugh et al., 1996; Angradi, 1999; Wisconsin Department of Natural Resources, 2001; Whiles and Dodds, 2002).	+ Qualitatively consistent
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	2000: 3.1 Impoundment: Incomplete exposure pathway: Site located between the impoundment and MR3 had greater number of EPT taxa.	− Inconsistent
	2000: 3.2 Unknown sources: Lack of quantitative evidence hinders assessment, but the number of EPT is greater at most sites with less embeddedness.	+ Consistent
	2000: 3.3 Road sanding and 3.4 Bank failure. Incomplete exposure pathways.	− Inconsistent
Reasonable Explanation of the Evidence	3.1 Inconsistencies concerning impoundment, 3.3 Road sanding and 3.4 Bank failure: unexplained.	0 No known explanation

TABLE B-3 cont.		
Candidate Cause 3	Loss of Interstitial Habitat Due to Settled Particles	
	Evidence	Interpretation
Comparison Among Candidate Causes	Although the evidence for embedded substrates from an unknown source was fairly consistent, there was no gradient of EPT taxa richness associated with levels of embeddedness. The increase in EPT taxa following rerouting of the illicit discharge clearly shows that embedded substrate was not the major cause the impairment. However, the grey water discharge may have contained suspended solids that contributed to embeddedness at the site. At SR, a site with 50−75% of substrata surrounded by fine silt, there were more EPT taxa than at MR3 indicating that EPT can occur at the levels of embeddedness observed at MR3.	− Other causes stronger
Characterization and Level of Confidence		
Cause of Impairment	Loss of interstitial habitat due to settled particles.	Improbable
Uncertainty	None.	Very Low
Potential Additional Causes or Actions	None.	

TABLE B-4

Summary of the Strength-of-Evidence Analysis for Candidate Cause 4 at MR3

Candidate Cause 4	Asphyxiation Due to Low Dissolved Oxygen	
	Evidence	Interpretation
Case-Specific Considerations		
Co-occurrence	2000: No summertime measurements of dissolved oxygen were available at MR3. In October and November, readings were 9.85 and 13.25 mg/L, respectively. The RB1 reading for October was 11.1 mg/L. No measurements were available for MR1 (see Table A-6).	0 Ambiguous
	2003: In 2000, a measurement in August was 9.17 mg/L, and, in July of 2001, 8.91 mg/L; the lowest value for RB1 was 10.17 mg/L in July 2001. However, on both dates dissolved oxygen was less at MR1 (7.32 on July 2001 and 7.44 mg/L on August 2000) (see Table A-6). No measurements of intragravel dissolved oxygen were taken.	− − − Incompatible
Stressor-Response in the Field	2000: Concentrations of dissolved oxygen in the autumn were weakly positively correlated with the number of EPT taxa (see Table 8).	+ Weak
	2000: BOD was weakly negatively correlated with the number of EPT taxa (see Table 8).	+ Weak
Causal Pathway	2000: 4.1 Organic enrichment pathway: Mean BOD was higher than MR1 (MR3, 1.6; MR1, 1.1 mg/L) (see Table 15).	+ Partial Evidence
	2000: 4.2 Nutrient enrichment pathway: Mean total phosphorous and mean BOD were unchanged or less at MR3 than at MR1. Algal growth was not measured.	− Source absent
	2000: 4.3 Deoxygenated hypolimnetic pathway: Water released over impoundments with aeration.	− Source absent
	2000: 4.4 Channel modification pathway: Moderate gradient with continuous riffles and aeration makes low dissolved oxygen unlikely.	− Source absent
Manipulation of Exposure	2003: 4.1 Organic enrichment and 4.2 nutrient Enrichment pathway: Mean BOD decreased slightly (1.1 to 1.02 mg/L) following removal of illicit discharge (see Table A-4).	0 Ambiguous

TABLE B-4 cont.

Candidate Cause 4	Asphyxiation Due to Low Dissolved Oxygen	
	Evidence	Interpretation
Considerations Based on Other Situations or Biological Knowledge		
Mechanistically Plausible Cause	Many aquatic insects require high levels of dissolved oxygen to survive (Eriksen, 1991).	+ Plausible
Stressor-Response from Laboratory Studies	2003: The two lowest concentrations of dissolved oxygen were recorded in the summer but were well above the CT DEP Water Quality Standard of >5.0 mg/L of dissolved oxygen (MR3: 9.17, 8.91 mg/L). The Test EC_{30} based on a 30% reduced emergence for three mayflies, *Leptophlebia nebulosa*, *Baetisca lauentian*, and *Ephemera simulans*, ranged between 7.0 mg/L and 9.0 mg/L (Nebeker, 1972). The observed concentrations were near the upper end of this range.	0 Ambiguous
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	All of the causal pathways were interrupted in some fashion.	− A few inconsistencies
Reasonable Explanation of the Evidence	No known explanation.	0 No known explanation
Comparison Among Candidate Causes	Even if the discharge was a contributing cause to low dissolved oxygen, it is now removed. High levels of embeddedness and weak correlation with dissolved oxygen and BOD may reflect another related causal mechanism: low intergravel gas exchange.	− Impairment attributed to another cause
Characterization and Level of Confidence		
Cause of Impairment	Low dissolved oxygen in the water column is unlikely.	Very Improbable
Uncertainty	May have been an intermittent event but not very likely.	
Potential Additional Causes and Actions	Measure intragravel dissolved oxygen.	

TABLE B-5

Summary of the Strength-of-Evidence Analysis for Candidate Cause 5 at MR3

Candidate Cause 5	Mortality Due to Elevated Temperature		
	Evidence	Interpretation	
Case-Specific Considerations			
Co-occurrence	2000: Water temperature had not been measured in the summer at site MR3 or MR1.	NE	No Evidence
	2003: Of the few measurements taken at MR3, the greatest (23.41°C) was recorded July 2001. At MR2, on the same date where the number of EPT and Non-EPT were greater the temperature was even warmer, 24.51°C (see Table A-6).	− − −	Incompatible
Stressor-Response in the Field	2003: The number of EPT taxa was negatively correlated with summer temperature ($r = -0.61$) and with autumn temperatures ($r = -0.52$) (see Table 8).	+	Strong
Causal Pathway	2000: 5.1 Heat stratified within impoundment pathway: Middle River has several impoundments, one near the impairment at MR3. Water temperature could become elevated in the impoundments due to increased surface area exposed to solar radiation.	+	Partial evidence
	2003: 5.1 Heat stratified within Impoundment pathway*:* MR2 is closer to the impoundment and yet number of EPT at MR2 was greater than at MR3.	−	Some Steps Missing
	2000: 5.2 Heated stormwater pathway: Amount of impervious surfaces is greater at MR3 than at MR1. Temperatures not measured during storms	+	Partial evidence
	2003: 5.2 Heated stormwater pathway: A raceway at MR3 may carry stormwater.	0	Ambiguous
	2000: 5.3 Lack of canopy pathway: Shading is continuous upstream from the site and below the dam but becomes more open at the site.	+	Partial evidence
	2003: 5.3 Lack of canopy pathway: MR2 was shaded and MR3 was not, yet temperatures were greater at MR2 in July 2001 than at MR3.	−	Some Steps Missing

TABLE B-5 cont.

Candidate Cause 5	Mortality Due to Elevated Temperature	
	Evidence	Interpretation
Considerations Based on Other Situations or Biological Knowledge		
Mechanistically Plausible Cause	Invertebrates are known to have differing temperature tolerances and excessive temperatures can reduce survival and fecundity (Cox and Rutherford, 2000; Panov and McQueen 1998; Oberlin and Blinn, 1997).	+ Plausible
Stressor-Response from Laboratory Studies	2003: The maximum temperature at MR3 was 23.41°C. Temperatures this high may be lethal to some aquatic insects. For example, the LT50 for *Deleatidium autumnale* (New Zealand) was 21.9°C (Cox and Rutherford, 2000).	++ Quantitatively Consistent
Stressor-Response from Other Field Studies	Impoundment-induced temperature increases of 5°C were associated with decreased *Plecoptera* (Lessard, 2000).	+ Qualitatively Consistent
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	2000: 5.1 Heat stratified within impoundment pathway: Pathway improbable because impairment did not occur at MR2 (2000) downstream from impoundment. In July 2001, temperature at MR2 was greater than MR3.	− Inconsistent
	2000: 5.2 Heated stormwater pathway: More EPT taxa at FB5 which has similar level of impervious surface.	− Inconsistent
	2000: 5.3 Lack of canopy: Number of EPT at MR2 (2000) and MR3 (2001−2002) were the similar even though MR2 was shaded and MR3 was not.	− Inconsistent
	2003: Temperatures were higher at MR2, which had more EPT taxa.	− Inconsistent
Reasonable Explanation of the Evidence	5.1 Heat stratified within impoundment pathway.	0 No known explanation
	5.2 Heated stormwater pathway: The raceway at MR3 may carry stormwater and impervious surfaces are greater at MR3 than at MR1, and slightly greater than at MR2. Hence, temperature effects may be intermittent.	0 Credible explanation for new pathway
	5.3 Lack of canopy pathway.	0 No known explanation

TABLE B-5 cont.

Candidate Cause 5	Mortality Due to Elevated Temperature	
	Evidence	Interpretation
Comparison Among Candidate Causes	Although elevated water temperature could cause the impairment, the increase in EPT taxa following rerouting of the illicit discharge clearly shows that the grey water discharge was the probable cause of reduced numbers of EPT taxa compared to MR2.	– – – Impairment attributed to another cause
Characterization and Level of Confidence		
Cause of Impairment	Impairment attributed to another cause.	Improbable
Uncertainty	Episodically high temperatures possible.	Uncertainty low
Potential Additional Causes and Actions	None.	

TABLE B-6

Summary of the Strength-of-Evidence Analysis for Candidate Cause 6 at MR3

Candidate Cause 6	Taxa Loss Due to Altered Food Resources	
	Evidence	Interpretation
Case-Specific Considerations		
Co-occurrence	2000−2003: No estimates of woody debris or leaf packs.	NE No Evidence
	2000−2003: Neither algae nor chlorophyll a measured.	NE No Evidence
Stressor-Response in the Field	2000: Mean TKN, nitrate, nitrite, total phosphorous, organic nitrogen were not correlated with EPT.	− Inconsistent
	2000: Concentrations of ammonia were positively correlated with EPT (see Table 8).	++ Strong
	2000: BOD was weakly negatively correlated with EPT. Algae, which increase BOD, are not a favored food of EPT (see Table 8). Therefore, the negative correlation may be due to a common cause, increased algal levels.	+ Weak
Causal Pathway	2000: 6.1 Impoundment pathway: Middle River has several impoundments; one is near the impairment at MR3.	+ One step present
	2003: 6.1 Impoundment pathway: Number of EPT taxa was greater at MR2, which is nearer the impoundment.	− A missing step
	2000: 6.2 Nutrient pathway: PO_4 is limiting nutrient in streams. Mean total phosphorus, ammonia, nitrate, TKN, and nitrite were unchanged compared to MR1.	− A missing step
	2000: 6.3 Organic matter pathway: TSS was less, but BOD increased and total solids was intermediate compared to watershed reference sites MR1 and RB1 (see Table 15).	− A missing step
	2000: 6.4 Forested stream bank pathway: Deciduous trees occur on both stream banks at MR1 and MR2 where the number of EPT taxa is greater than at MR3.	+ Partial evidence

TABLE B-6 cont.

Candidate Cause 6	Taxa Loss Due to Altered Food Resources	
	Evidence	Interpretation
Considerations Based on Other Situations or Biological Knowledge		
Mechanistically Plausible Cause	Organic enrichment and altered food source are recognized as important parameters that alter invertebrate assemblages (Hilsenhoff, 1987; Shieh et al., 2002). Invertebrates have different feeding strategies and preferences that affect competition and survival.	+ Compatible
Stressor-Response from Other Field Studies	2000: 6.2 Nutrient pathway: Concentrations of total phosphorous at MR3 did not exceed U.S. EPA default criteria (see Table 13) (U.S. EPA, 2000a). U.S. EPA recommended concentrations for total nitrogen were exceeded at MR3 but, also, at MR1 (see Table 13). Total phosphorous and nitrogen were within background levels for the Eastern Coastal Plains including the Willimantic drainage and therefore would not be among the streams with the greatest nutrient concentrations and the greatest chance of nutrient induced effects (Smith et al., 2003). These data sets were not available to determine if nutrient levels were associated with few EPT or non-EPT taxa.	− Incompatible
	2000: 6.3 Organic matter pathway: BOD did not exceed U.S. EPA criteria (see Table 13).	− Incompatible
	2000: 6.4 Forested stream bank pathway.	NE No Evidence
	2000: 6.5. Reservoir pathway.	NE No Evidence
Considerations Based on Multiple Lines of Evidence		
Consistency of Evidence	6.1 Impoundment, 6.2 Nutrient, and 6.3 Organic matter pathways: Causal pathways were interrupted in some fashion or increase in concentrations of stressor was insufficient to cause the effect.	− Inconsistencies
	6.4 Forested stream bank pathway: The lack of woody debris and leaf packs compared to the watershed reference site and the fact that the impairment did not occur at MR2 which had forested stream banks, supports this causal pathway.	+ Consistent

TABLE B-6 cont.		
Candidate Cause 6	Taxa Loss Due to Altered Food Resources	
	Evidence	Interpretation
Reasonable Explanation of the Evidence	6.1 Impoundment.	0 No Known Explanation
	6.2 Nutrient, and 5.3 Organic matter pathways: there could be an intermittent point source that was undetected and nutrients unmeasured.	0 Inconsistency explained by a new pathway
	6.4 Forested stream bank pathway.	NA Not Applicable
Comparison Among Candidate Causes	The increase in EPT taxa following rerouting of the illicit discharge clearly shows that an altered food resource did not cause the decline in EPT compared to MR2.	– – – Impairment attributed to another cause
Characterization and Level of Confidence		
Cause of Impairment	Taxa Replacement or Loss due to Altered Food Resources.	Improbable
Uncertainty	None.	Uncertainty very low
Potential Additional Causes and Actions	None.	