EPA
United States
Environmental Protection
Agency

EPA/600/R-08/131 | January 2010 | www.epa.gov/ncea

Stressor Identification in an Agricultural Watershed: Little Floyd River, Iowa

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

EPA/600/R-08/131
January 2010

Stressor Identification in an Agricultural Watershed: Little Floyd River, Iowa

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

NOTICE

ABSTRACT

In Iowa, the use of the stressor identification (SI) protocol was prompted by stream impairments of the fish and benthic macroinvertebrate communities. The Little Floyd River was included on the 1998 303(d) list of impaired waterbodies based on a 1990 stream-use assessment. At that time, no fish were observed and only pollution-tolerant benthic macroinvertebrate species were present. Fish have since recolonized the stream.

The Little Floyd River is a third-order, warm-water stream located in the Northwest Iowa Loess Prairies Ecoregion. Land use in the watershed is dominated by row-crop agriculture and livestock production. Candidate causes for this biological impairment included flow alteration, substrate alteration, turbidity, altered basal food source, low dissolved oxygen concentrations, high temperature, and high ammonia concentrations.

The Iowa Department of Natural Resources (IDNR) characterized the impairment of the stream using chemical and biological samples, as well as observations of the physical habitat collected at four sites over the course of four years. The evidence was reanalyzed by comparing data among the four sites using a less impaired comparator site within the Little Floyd to determine the co-occurrence of stressors with benthic macroinvertebrates and fish. Other types of evidence used in the case were complete causal pathway, stressor response from other field studies, and stressor-response studies from laboratory data and other studies. IDNR identified the primary probable causes of biological impairment as deposited sediment and low dissolved oxygen. Based on the original SI completed by the IDNR, total maximum daily load (TMDL) for sediment and dissolved oxygen were submitted to and approved by the U.S. Environmental Protection Agency in 2005.

Preferred citation:
Haake, D.T. Wilton; K. Krier, T. Isenhart, J. Paul, A. Stewart, and S. M.Cormier. 2010. Stressor Identification in an Agricultural Watershed: Little Floyd River, Iowa. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Cincinnati, OH. EPA/600/R-08/131.

Cover photo:
Photo taken by IDNR in 2001 at Floyd River at station 3.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS cont.

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BMIBI	Benthic Macroinvertebrate Index of Biotic Integrity
CBOD	carbonaceous biochemical oxygen demand
CPUE	catch per unit effort
DELT	deformities, eroded fins, lesions, and tumors
DO	dissolved oxygen
EPT	phylogenetic Orders Ephemeroptera, Plecoptera and Trichoptera
FFG	functional feeding group
FIBI	Fish Index of Biotic Integrity
GPP	gross primary production
IAC	Iowa Administrative Code
IDNR	Iowa Department of Natural Resources
P:R	production-to-respiration ratio
REMAP	Regional Environmental Monitoring and Assessment Program
SI	stressor identification
TDS	total dissolved solids
TMDL	total maximum daily load
TSS	total suspended solids
UHL	University of Iowa Hygienic Laboratory
U.S. EPA	U.S. Environmental Protection Agency
WQC	water quality criteria
WWTP	wastewater treatment plant

PREFACE

This is a screening causal assessment of a biologically impaired stream in the state of Iowa. The case was investigated by the Iowa Department of Natural Resources (IDNR) after the Little Floyd River was listed as impaired on 303d lists and identified as requiring a determination of the total maximum daily load (TMDL) of the unknown pollutants causing the biological impairment. For this document, the causal analysis was restructured from the original TMDL (IDNR, 2005c) during a workshop at Canaan Valley, West Virginia in May of 2005 and in subsequent discussions. One difference from the original TMDL assessment is that comparisons are made within the Little Floyd River to a "less impaired" comparator site. The sampling, analysis, and conclusions are those of researchers who were employed by the IDNR. Comments appearing in text boxes were prepared by NCEA except where noted. NCEA provided editorial and formatting assistance to make the original IDNR report similar to four other case studies that were solicited as examples for practitioners of causal assessment. The reports and methods are posted on the Causal Analysis and Diagnosis Decision Information System EPA Website (www.epa.gov/caddis).

The Floyd River case study is one of five causal analyses 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 were not collected as a part of an investigation. Rather, most data were collected during routine monitoring done by the state or by other agencies for other purposes. It is common that these are the type of data upon which state agencies base their determinations. Resources for additional sampling are often unavailable. In fact, some reviewers commented that the data for the Little Floyd River case study was greater than what is typically available in many other situations. And yet, IDNR developed evidence to show that some causes co-occurred with the biological impairment, were a part of a larger causal chain of events, occurred at sufficient levels known to cause the observed effects, and were due to physical interactions that occurred after the introduction of stressors associated with land cover/land use changes following settlement. Although the amount and quality available evidence was not equivalent in for all candidate causes, it was enough to identify some probable causes and to suggest what additional, targeted data might greatly improve the confidence in the determination.

These cases, as all cases, could be improved 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. All of these case studies from the Canaan Valley Workshop defined the impairment based on a biological index rather than more specific impairments. This practice diminishes the ability to detect associations because summing the metrics dampens the overall signal from individual metrics and species that are responding differently to environmental conditions or stressors.

To address these and other issues, comment boxes have been inserted throughout the Little Floyd River case study to supply commentary or to suggest other approaches that could strengthen the case. The analyses in the cases cannot be modified as they are already a part of the Iowa's public record. It is our intention to link the case studies to relevant tools and guidance on the EPA website: www.epa.gov/caddis.

Overall, the case study of the Little Floyd presents a very realistic example of the difficulties of assigning specific cause to biological impairment. The Little Floyd Case Study is a good example of several strategic techniques to use when data are collected in different years, when the discrimination between acceptable streams and impaired streams is small, and when multiple stressors affect a stream's biological condition. Highlights include:

1. Defining the scope of the study based on different types of biological impairment (fish kill versus low biological index score).
2. Rationales for differentiating between deferred causes due to insufficient data or practical consideration and elimination of causes on the bases of logical implausibility.
3. Using limited data and data collected in different years.
4. Using encountered data that were developed for purposes other than causal analysis.
5. Using intermediate stressors to evaluate the causal pathways leading to the proximate causes.
6. Differential comparisons of sections of river using an internal comparator site and to regional reference sites. However, proper classification to account for natural variation needs to be an integral part of this process.
7. Assessment of a highly modified river in the agricultural Midwest.

Editor: Susan M. Cormier January 2010

AUTHORS, CONTRIBUTORS, AND REVIEWERS

AUTHORS

Danelle Haake
Iowa Department of Natural Resources
Des Moines, IA 50319

Current Affiliation:
Litzsinger Road Ecology Center;
9711 Litzsinger Road Ladue, MO 63I24, USA.
314-961-4410 (phone); 314-961-6825 (fax)
danelle.haalie@mobot.org

Tom Wilton
Water Monitoring and Assessment Section; Iowa Department of Natural Resources
502 East 9th Street; Des Moines, IA 50319-0034
515-281-8867(phone); 515-281-8895 (fax)
tom.wilton@dnr.iowa.gov;

Ken Krier
Water Monitoring and Assessment Section; Iowa Department of Natural Resources
502 East 9th Street; Des Moines, IA 50319-0034
[3]515-242-5184(phone); 515-281-8895 (fax)
ken.krier@dnr.state.ia.us

U.S. EPA EDITOR

Susan M. Cormier, Ph.D.
U.S. EPA, Office of Research and Development
Cincinnati, OH 45268

CONTRIBUTORS

Tom Isenhart
Iowa State University
Ames, IA 50011

John Paul
U.S. EPA, Office of Research and Development
Research Triangle Park, NC 27709

Athur J. Stewart
Oak Ridge Associated Universities
Oak Ridge, TN 37830
(865) 576-2312 (phone); (865) 574-4528 (fax)
arthur.stewart@orau.org

AUTHORS, CONTRIBUTORS AND REVIEWERS cont.

REVIEWERS

Don A. Essig, M.S.
Idaho Department of Environmental Quality
Boise, ID 83706

Marian Maas, Ph.D.
Private Consultant
Bellevue, NE 68123

Patrick A. O'Brien
Nebraska Department of Environmental Quality
Lincoln, NE 60509

EPA Reviewers

Evan Hornig
U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Washington, DC 20460

Robert Spehar
U.S. Environmental Protection Agency
Office of Research and Development
National Health and Environmental Effects Research Laboratory
Mid-Continent effects Research Laboratory
6201 Congdon Blvd.
Duluth, MN 55804

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

ACKNOWLEDGMENTS

We wish to thank Marian Maas for her support of the use of the stressor identification (SI) protocol in Iowa; Jerry Neppel, Jennifer Ender, and Jamie Mootz for their contributions in the collection and processing of data for IDNR's original SI; and the crew from the University of Iowa Hygienic Laboratory who continue to spend countless hours collecting data, analyzing samples, and identifying aquatic organisms for IDNR.

1. DEFINITION OF THE CASE

1.1. REGULATORY CONTEXT FOR THE CASE

In Iowa, the 1998 303(d) list identified impaired water bodies that were required to have a total maximum daily load (TMDL) report completed by a specified time, as stipulated in a U.S. Environmental Protection Agency (U.S. EPA) consent decree. Several of the waterbodies on the 1998 list were included based on biological impairments due to “unknown causes” or “unknown toxicity.” The Little Floyd River was among those.

The impairment of the Little Floyd River was identified during a 1990 stream-use assessment conducted by the Iowa Department of Natural Resources (IDNR). No fish were observed during that assessment and only pollution-tolerant benthic macroinvertebrate species were found. At that time, the cause of the biological impairment was reported as unknown. Follow-up monitoring was conducted in 1999, 2001, and 2002 to quantify the impairment; both fish and benthic macroinvertebrates were observed in these studies. (See Appendix A for methodology.)

In 2002 and 2004, the IDNR classified the designated use (Class B aquatic life) for the stream as “partially supporting.” These 305(b) water quality assessments were based on low scores on the Fish Index of Biotic Integrity (FIBI) and Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) (Wilton, 2004) from biological monitoring at multiple sites in the watershed over the course of several years. Methods for 305(b) assessments for the State of Iowa may be found on-line (IDNR, 2005a).

Comment 1. What are These Boxes For?

At various points in this document, comments have been inserted by the U.S. EPA editor and the authors. These comments are not meant to indicate that the IDNR causal analysis is in error. The Stressor Identification (SI) process does not address every possible option, nor does it provide details on implementation, so there are many opportunities for interpretation (U.S. EPA, 2000). The U.S. EPA encourages states and tribes to improve and interpret the methodology in ways that are appropriate to their circumstances. Hence, the comments are meant to assist other SI users by suggesting alternative approaches that may be applied to their cases.

By using the SI process, the IDNR sought to determine the cause of the biological impairment, which they did without involvement from the U.S. EPA, and U.S. EPA editor is grateful the IDNR is willing to share its experience from this case.

Some of the analyses and terminology have been reformatted to be consistent with the revised U.S. EPA guidance (2006). The final determination of the probable cause was unchanged from those found using the original process as applied by IDNR.

In 2004, the IDNR completed a stressor identification (SI) for biological impairments of the Little Floyd River to identify the causal agents that would require a TMDL. The IDNR completed the TMDL report in 2005. One of the IDNR’s major goals was to determine whether the biological impairment was caused by a pollutant requiring a TMDL or due to causes that do not require a TMDL, such as physical habitat alteration. Whether or not pollutant load reductions could correct impairment, IDNR expected that a complete SI would identify the key causal agents and pathways that would need to be addressed in order to allow the aquatic community to recover to a condition that supports the designated aquatic life use.

1.2. DESCRIPTION OF THE RIVER AND WATERSHED

The Little Floyd River watershed is located in the Northwest Iowa Loess Prairies (Ecoregion 47a), a gently undulating plain with a moderate to thick layer of fine loess soil (Griffith et al., 1994). The Loess Prairie is the highest, driest region of the Western Corn Belt Plains as it rises to meet the Northern Glaciated Plains of the Dakotas. Although loess covers most of the broad upland flats, ridges, and slopes, minor glacial till outcrops occur near the base of some of the side slopes. Silty clay loam soils have developed on the loess under native tall-grass prairie vegetation.

The Little Floyd River is a warm-water stream with little groundwater contribution. The water chemistry is typical of other streams within the Western Corn Belt Region: the water contains relatively high concentrations of dissolved solids, has a slightly alkaline pH, and has high concentrations of nitrate (see Appendix B, Tables B-1 and B-2).

Located in northwest O'Brien County, the Little Floyd River is a wadeable stream with a watershed area of 15,780 hectares (39,700 acres) (see Figure 1). The river flows southwest before joining the Floyd River 3 kilometers (km) southwest of the city of Sheldon. Annual row-crop agriculture, in a corn-soybean rotation, dominates current land use in the watershed (see Figure 2). Pastures are located predominantly in streamside areas that allow livestock direct access to water. In 2004, livestock in the watershed included approximately 7100 hogs, 2900 cattle, 630 turkeys, and 250 chickens.

Streamflow in the Little Floyd River is very responsive to rainfall and snowmelt; discharge can fluctuate by one order of magnitude within a few days of storms or snowmelt (see Figure 3). Annual stream low flows are less than 0.05 m^3/s. Extensive subsurface drainage tile within the watershed exacerbates flow variability. Portions of the river and tributary streams have been straightened, and almost all of the ephemeral watercourses have been converted into grass waterways or straightened into open drainage ditches to facilitate agricultural production.

The only permitted point source in the watershed is the city of Sanborn's wastewater treatment plant (WWTP). In addition to serving a population of 1350, the WWTP treats wastewater from a local dairy. The WWTP uses a conventional activated-sludge treatment process. Following sludge treatment, the effluent moves through several storage and treatment lagoon cells before discharge. Due to the storage capacity of these cells, discharge occurs only two or three times per year. The amount of wastewater was judged to be small compared to hog farm waste applied to fields adjacent to the river.

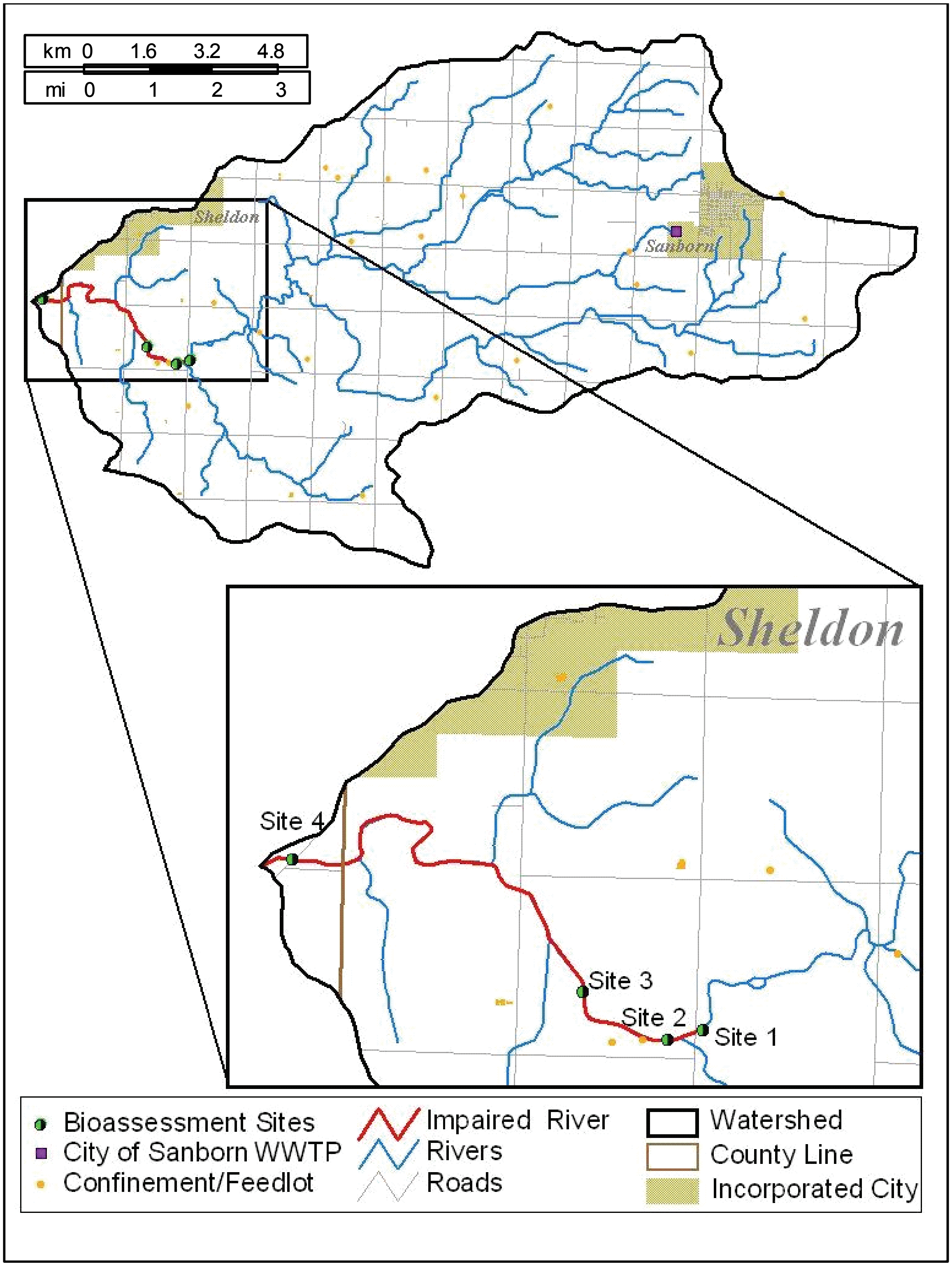


FIGURE 1

The Watershed of the Little Floyd River, Showing the Locations of the Impaired Segment, Bioassessment Sites, Livestock Operations, and Urban Areas

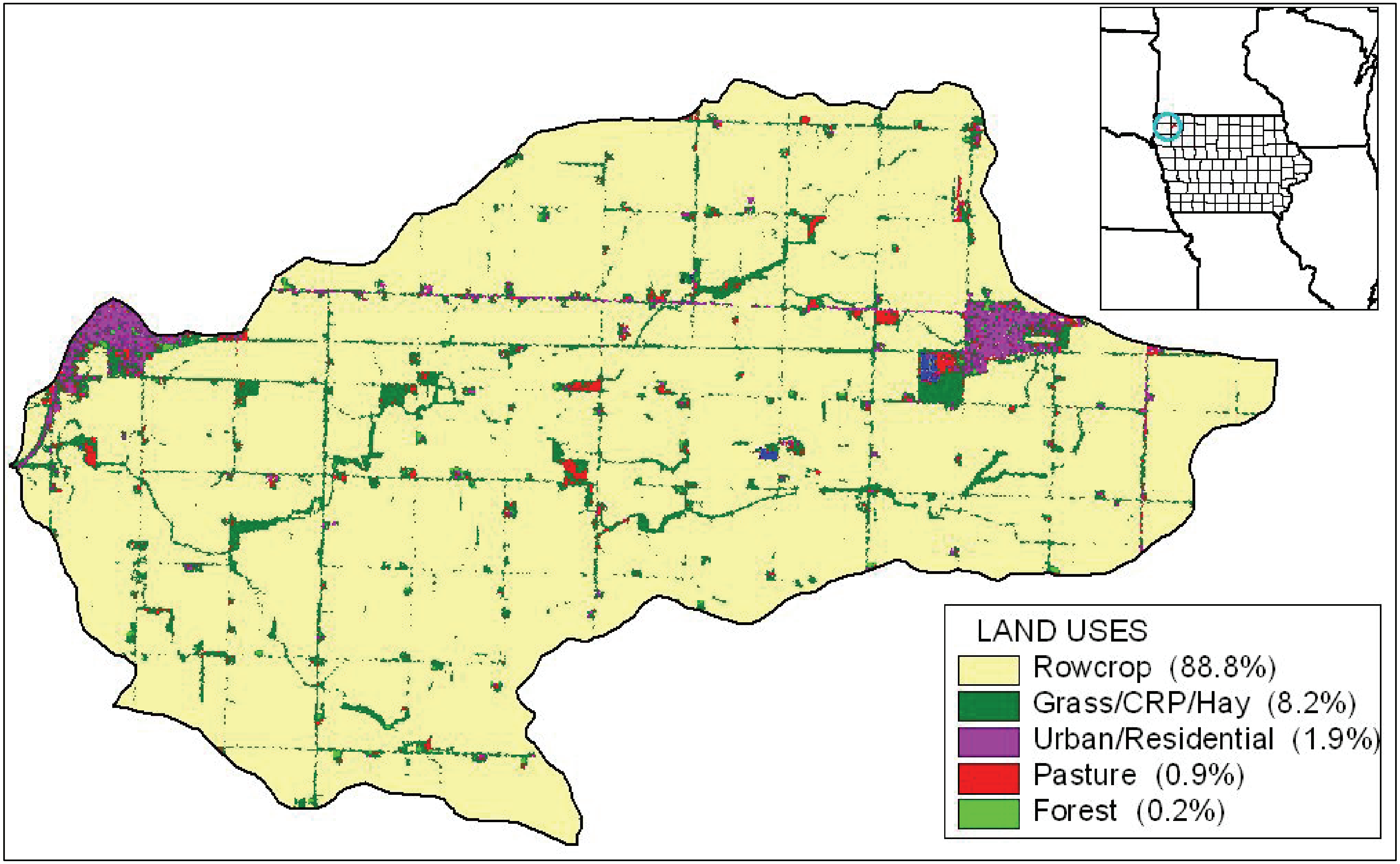


FIGURE 2

Land Uses in the Little Floyd River Watershed Based on 2002 Satellite Imagery

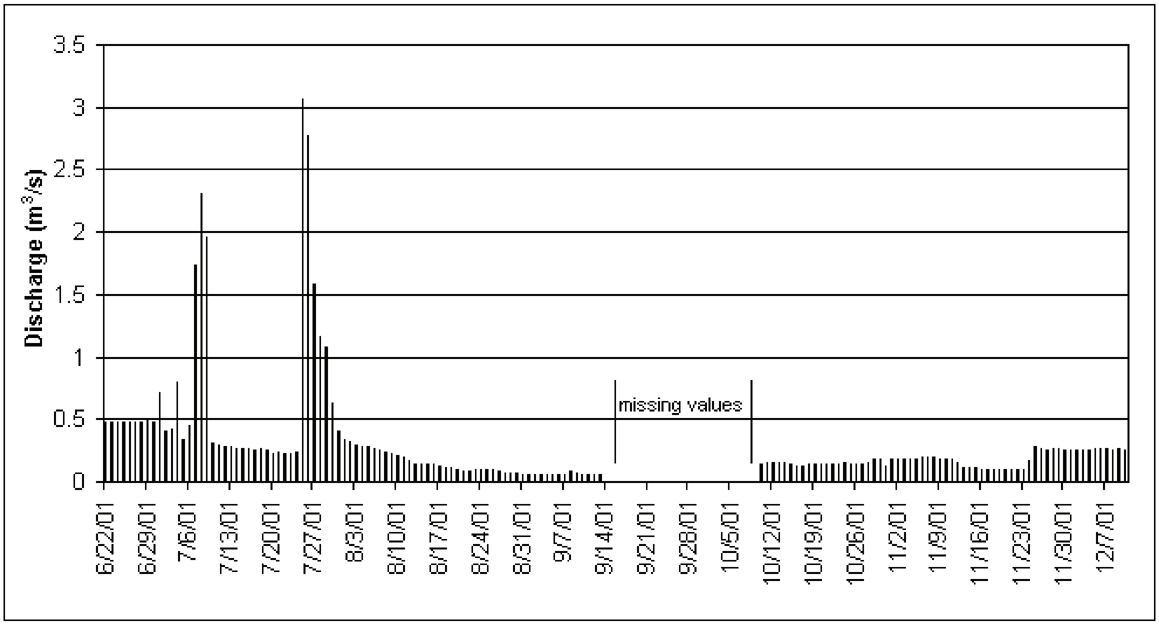


FIGURE 3

Discharge Measured in the Little Floyd River at Site 4 in 2001. Rainfall measurements within 29 km (18 miles) of the site ranged from 0.1 to 0.3 inches on July 7/8 and 1.4 to 3.1 inches on July 24/25.

There are several potential nonpoint sources of stressors in the Little Floyd River watershed. In particular, water quality may be impacted by the mishandling or over-application of animal wastes and fertilizers, even though agricultural nonpoint sources are not allowed to discharge waste into the Little Floyd River (see Comment 2).

> **Comment 2. Other Estimates of Exposure.** Although not developed in this case, information about the volume, extent, location, and management practices can be used to develop estimates of pollutant loadings and the likelihood of exposures.

1.3. SPECIFIC BIOLOGICAL IMPAIRMENT

The biological impairment of the Little Floyd River occurs in the lower 5.5 km of river. This is the only reach of the Little Floyd that is designated for Class B limited-resource (LR) aquatic life use (Wilton, 2004). Upstream areas are designated as general use and are protected only from acutely toxic conditions.

The IDNR has developed composite indices for fish (FIBI) and for benthic macroinvertebrates (BMIBI) (Wilton, 2004), with 12 metrics each (see Table 1). Index scores range from 0 to 100, with 100 representing the best conditions for fish and benthic invertebrates. Thresholds for biological impairment for fish and benthic

TABLE 1

Metrics Used for Iowa's Fish and Benthic Macroinvertebrate Indices. Macroinvertebrate metrics are based on either multihabitat samples (MH) or standard-habitat samples (SH).

Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI)	Fish Index of Biotic Integrity (FIBI)
1. Taxa Richness (MH)	1. # Native Fish Species
2. Taxa Richness (SH)	2. # Sucker Species
3. EPT Richness (MH)	3. # Sensitive Species
4. EPT Richness (SH)	4. # Benthic Invertivore Species
5. Sensitive Taxa (MH)	5. % 3 Dominant Fish Species
6. % 3 Dominant Taxa (SH)	6. % Benthic Invertivores
7. Biotic Index (SH)	7. % Omnivores
8. % EPT[a] (SH)	8. % Top Carnivores
9. % Chironomidae (SH)	9. % Simple Lithophil Spawners
10. % Ephemeroptera (SH)	10. Fish Assemblage Tolerance Index
11. % Scrapers (SH)	11. Adjusted Catch Per Unit Effort
12. % Dominant Functional Feeding Group (SH)	12. % Fish with DELT[b]

[a]EPT = Ephemeroptera, Plecoptera, Trichoptera.
[b]DELT = deformities, eroded fins, lesions, tumors.

macroinvertebrates in Iowa have been determined for each level IV ecoregion. For the Northwest Iowa Loess Prairies Ecoregion (47a), a FIBI score of less than 40 or a BMIBI score of less than 53 indicates impairment of the aquatic ecosystem. A site is considered impaired if the criteria are not met for either the FIBI or BMIBI. Further details may be found in Appendix A and in Biological Assessment of Iowa's Wadeable Streams (Wilton, 2004) (see Comment 3).

Comment 3. New Standards.
In 2006, the IDNR updated Iowa's water quality standards regarding warm-water stream aquatic life uses. When/if the U.S. EPA approves these changes, aquatic life use designations and water quality criteria protection will expand to a substantially greater number of stream miles within the Little Floyd River watershed.

In the impaired segment of the Little Floyd River, the IDNR conducted biological assessments at four sites over a 4-year period. Each site was sampled only once. Table 2 summarizes the resulting FIBI and BMIBI scores. Figure 1 shows the locations of the assessment sites.

TABLE 2

Index of Biotic Integrity Scores for Fish (FIBI) and Benthic Macroinvertebrates (BMIBI) in the Little Floyd River. Highlighted cells meet state biological criteria, others do not.

Site	Year	BMIBI	FIBI
Reference Criteria[a]		53	40
Site 1 (Upstream of Bridge)	1999	41	28
Site 2 (Downstream of Bridge)[b]	2001	65	39
Site 3 (REMAP Site)	2002	52	33
Site 4 (Downstream Site)	2001	34	41

[a]Criteria for the Northwest Iowa Loess Prairies ecoregion from Table 6-1 of Biological Assessment of Iowa's Wadeable Streams (Wilton, 2004).
[b]Based on the relatively high FIBI and BMIBI scores, Site 2 was selected as representative of less impaired conditions in the Little Floyd River for fish and macroinvertebrates.
REMAP = Regional Environmental Monitoring and Assessment Program.

1.4. SELECTION OF COMPARATOR SITE

The development of evidence depends on comparisons of the conditions at impaired locations with conditions at unimpaired or impaired laboratory, field, or modeled data. Comparisons within the same stream or watershed, from the case, provide a different and sometimes strong form of evidence. In this assessment, an internal comparator site was selected based on the year of collection, IBI scores, and the relative location along the stream reach. Site 2 (a "less-impaired" site) was selected as a comparator to assess causes of impairments of the benthic macroinvertebrate and fish assemblages. Site 2 was sampled in the same year as Site 4, reducing some year-to-year variation, and is upstream of Sites 3 and 4 (see Comment 4).

Comment 4. The Use of Metrics.
In the initial case, the IDNR selected the metrics believed to be most representative of the impairment based on which metrics scored the lowest at all sites. In future cases, the IDNR may compare individual metrics at sites in the impaired segment to the metrics at reference sites or compare metrics among sites in the watershed as described here.

The BMIBI score at Site 2 met Iowa's state biocriterion and was the greatest score among the four sites sampled in the Little Floyd River. Several invertebrate metrics within the BMIBI scored higher at Site 2 than at the other three sites (see Appendix B; Table B-3). These metrics provide insight into the aspects of the invertebrate community that need improvement. Site 2 has greater total taxa and Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa in both the multihabitat and standard-habitat samples than any of the other sample sites (see Figure 4). Additionally, Site 2 has the greatest metric scores for percent chironomids and percent scrapers, and the greatest metrics for the percent of individuals from both the top three dominant taxa and the dominant functional feeding group (FFG). Therefore, Site 2 was selected to compare to the other three sites on the Little Floyd River that were in poorer condition based on the BMIBI scores.

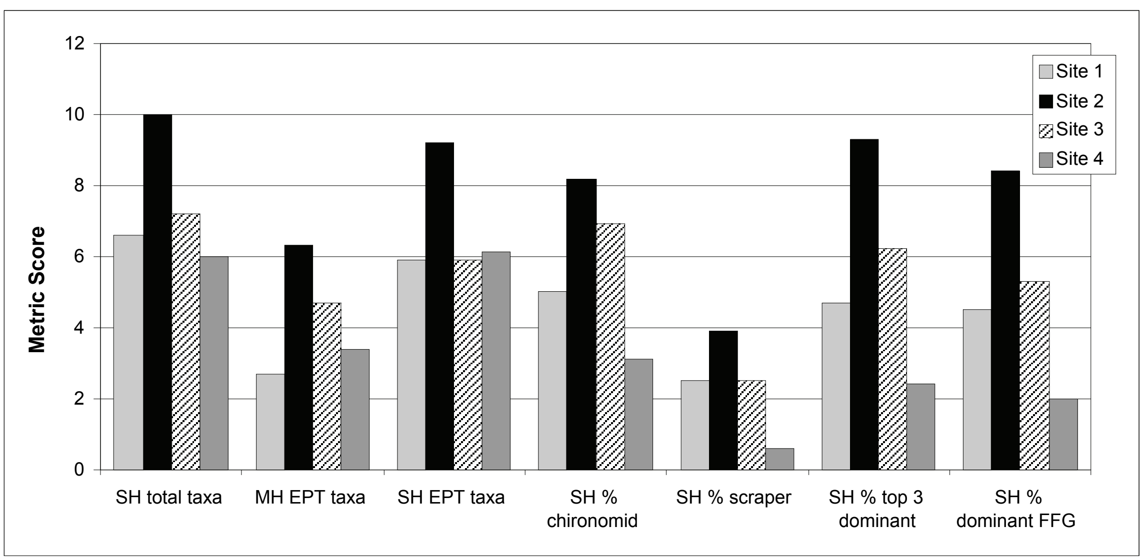


FIGURE 4

Invertebrate metrics at four sites along the Little Floyd River. (SH—standard habitat; MH —multihabitat; EPT—ephemeroptera, plecoptera, trichoptera; FFG—functional feeding group.)

Site selection for a fish assemblage comparator was less clear. While Site 4 had the only FIBI score that met the state biocriterion, Site 2 was selected as a better internal comparator for two reasons. First, the number of native species and sensitive species of fish, benthic invertivores, the percent of omnivores and tolerance values all had the highest scores at Site 2. Second, the remaining fish metrics for Site 2 were similar to or slightly higher than those for Site 4 with one exception: the adjusted catch per unit effort (CPUE) at Site 4 was greater than at Site 2 (see Figure 5). This difference in CPUE is attributed to the proximity of Site 4 to the confluence with the larger Floyd River and therefore not characteristic of the Little Floyd fish populations (see Comment 5).

The causal assessment, therefore, is limited to differential levels of stressors at Site 2 compared to the other three sites. Since fish metrics were qualitatively different among all four sites, a second assessment using metrics to describe the impairment would be needed to refine the assessment. Also, although a cause of stress was attributed to both the fish and benthic macroinvertebrate communities, the mechanism of action may be different.

Comment 5. Comparator Site. Clearly identifiable internal comparator sites and references sites are not always available. Some options are to use sites in nearby tributaries (see Willimantic Case) or regional sites (see Touchet and Bogue Homo Cases). Another novel approach is to use a site that is worse, a type of positive reference, sometime referred to as a “dirty reference” (see Clear Fork Case).

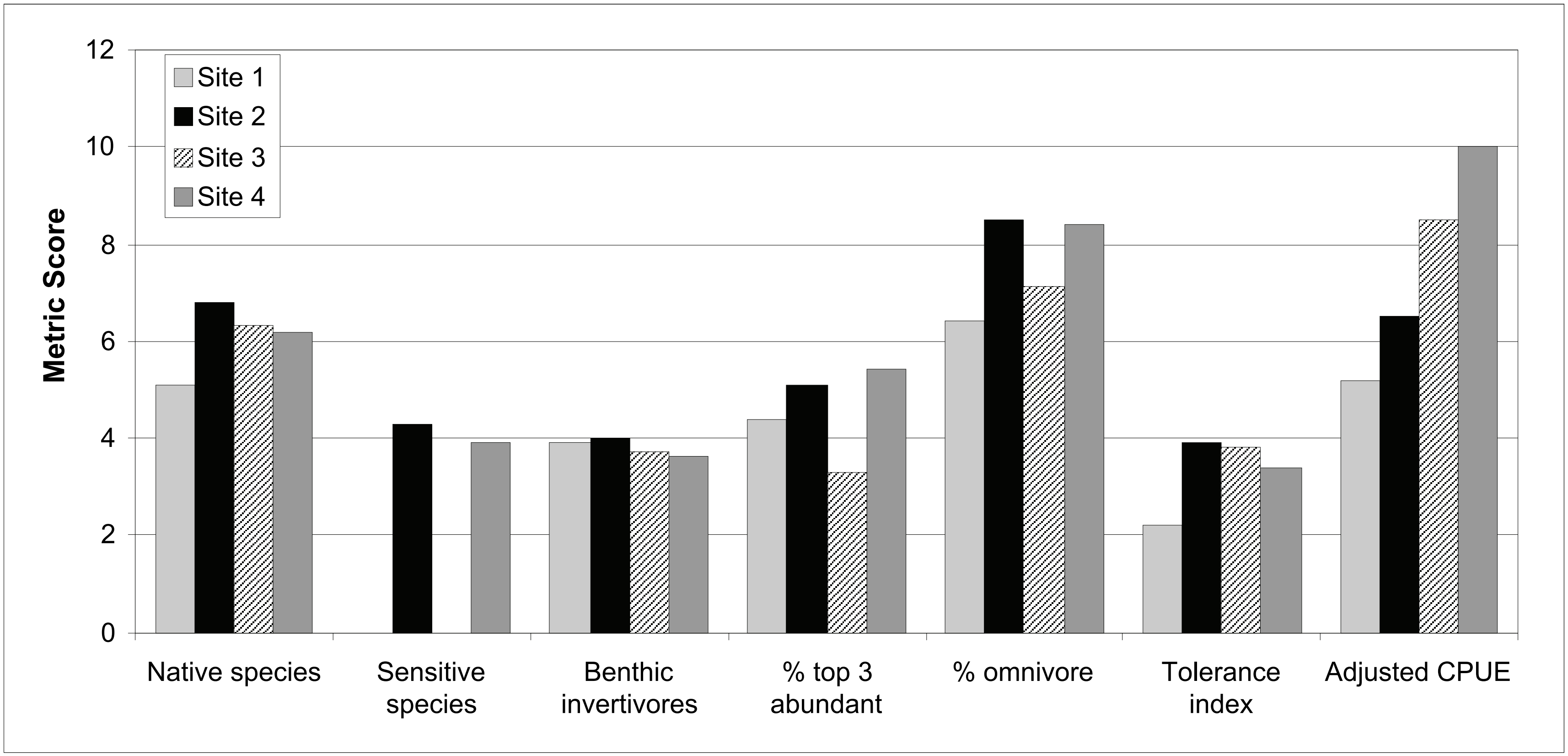


FIGURE 5

Fish Metrics at Four Sites Along the Little Floyd River (CPUE—Catch per Unit Effort). Note, there were no sensitive species at Site 1 or Site 3 (see Table B-3).

2. LIST THE CANDIDATE CAUSES

2.1. IOWA STANDARDIZED LIST OF CANDIDATE CAUSES

The candidate causes analyzed for this SI case were selected from a standard list of causes developed by the IDNR (2004) (see Comment 6). Because the SI process in Iowa is triggered by listings for biological impairments with unknown causes in the 303(d) list of impaired waters, the causes for the SI are linked to candidate cause possibilities for the 303(d) list as described in ADB+, Iowa's 305(b) assessment database (IDNR, 2005a).

> **Comment 6. Nutrients as Intermediate Stressors.**
> Nutrients are a contributing cause of algal growth leading to low dissolved oxygen. Phosphorous and nitrogen rarely act directly as toxicants. So, although nutrients are not identified as a proximate cause, they are part of the causal pathways for low dissolved oxygen, ammonia toxicity, and altered basal food source.

The IDNR standard list is generalized, but it does identify subcauses for most of the following parameters (e.g., arsenic [As] is included under metals):

- Metals
- Pesticide
- Ammonia
- Salinity
- Other nonmetal toxicant or biological toxin
- Basal food source alteration
- Exotic/introduced species
- Flow alteration
- Temperature
- Dissolved oxygen (DO)
- pH
- Conductivity/total dissolved solids (TDS)
- Turbidity/total suspended solids (TSS)
- Substrate alteration/increased sediment
- Habitat alteration

2.2. PRELIMINARY EVALUATION PROCESS

Using IDNR's standardized list, each candidate cause was either deferred or included for in-depth causal analysis (see Comment 7). Some candidate causes were deferred because preliminary analysis suggested that the exposures were within acceptable or expected ranges; others were deferred because there was not enough data to permit a more thorough analysis. By deferring, the IDNR ensured that if the SI did not determine the stressor or stressors with some certainty, the deferred candidate causes could be analyzed in greater detail at a later time. Causes were broken down into subcauses as needed.

2.1.1. Candidate Causes Deferred

Based on a preliminary analysis of Regional Environmental Monitoring and Assessment Program (REMAP) data from Site 3 (see Appendix B, Table B-4), the IDNR deferred metals as a candidate cause. Of the 10 metals sampled, all were below detection limits in the water samples and were well below levels of concern in the sediment. For example, concentrations of arsenic (As) and zinc (Zn) in the sediment were 2.2 and 38 mg/kg dry weight, respectively. Concentrations in sediment that may cause concern are 17 mg/kg dry weight for As and 270 mg/kg dry weight for Zn (Ingersoll et al., 2000).

The IDNR deferred pH as a candidate cause because the measured values were consistently between 7.5 and 8.7 (see Appendix B, Table B-1) similar to pH measured at ecoregion reference sites. Similarly, conductivity and TDS were deferred at a screening level because values for these parameters in the Little Floyd River were similar to or lower than those in the ecoregion reference dataset.

Comment 7. Elimination Versus Deferment. Many states have found it useful to use an iterative approach in which the most suspicious and very uncertain candidate causes are evaluated first. Other candidate causes are deferred until such time that the case is unresolved or that influential causes remain unidentified.

In the original study of the Little Floyd River, IDNR "eliminated" rather than "deferred" candidate causes as described in the U.S. EPA Stressor Identification Guidance of 2000. However, the U.S. EPA intended elimination to be used for only those candidate causes with irrefutable evidence of impossible exposure, for example, the effect was present before the candidate cause. In reality, such strong evidence is rarely available. Recognizing the benefits of an iterative analysis and retaining the distinction of very strong evidence, the U.S. EPA has clarified its guidance so that elimination of causes can be used as well as a "softer" exclusion in which the assessment of the candidate cause is "deferred." See Finalize List on the bottom Step 2.2 on the CADDIS website. The U.S. EPA editor has substituted the term *deferred* for the original 2005 IDNR's *elimination* when the intention was a postponement of analysis until new evidence indicates that additional investigation is warranted.

The IDNR deferred exotic/introduced species as a candidate cause because limited numbers of common carp (*Cyprinus carpio*) were the only nonindigenous aquatic species collected at any of the sites, and there was no known mechanism for carp to cause changes in the aquatic community observed in the Little Floyd River.

The IDNR deferred pesticides as a candidate cause because data for the Little Floyd River were limited to a single sample collected in August 2002. The concentrations of pesticides in this sample were below the detection limit for all parameters measured (see Appendix B, Table B-5). However, insecticides were not among the measured pesticides.

The IDNR deferred the other nonmetal toxics and salinity because there was not enough data to consider. At the completion of the SI, if probable causes of the biological impairment were not identified, additional data would need to be gathered to resolve the case.

2.1.2. Candidate Causes Analyzed

Candidate causes for the case are those that remain after the screening assessment. From the list of candidate causes, flow alteration, substrate alteration/increased sediment (including turbidity/TSS), altered basal food source, DO, temperature, and ammonia were evaluated by a formal weight-of-evidence analysis.

2.3. CONCEPTUAL MODELS

The IDNR developed conceptual models for each candidate cause to reflect known current and historical land uses, expected pathways of causation, and observed impacts to the ecological community. Figures 6–11 depict the models for this case (see Comment 8).

> **Comment 8. Generic Conceptual Models.**
> Generic conceptual models and narrative text describing them can be easily downloaded from the conceptual model library or select databases from the left navigation bar anywhere in CADDIS then choose conceptual models.

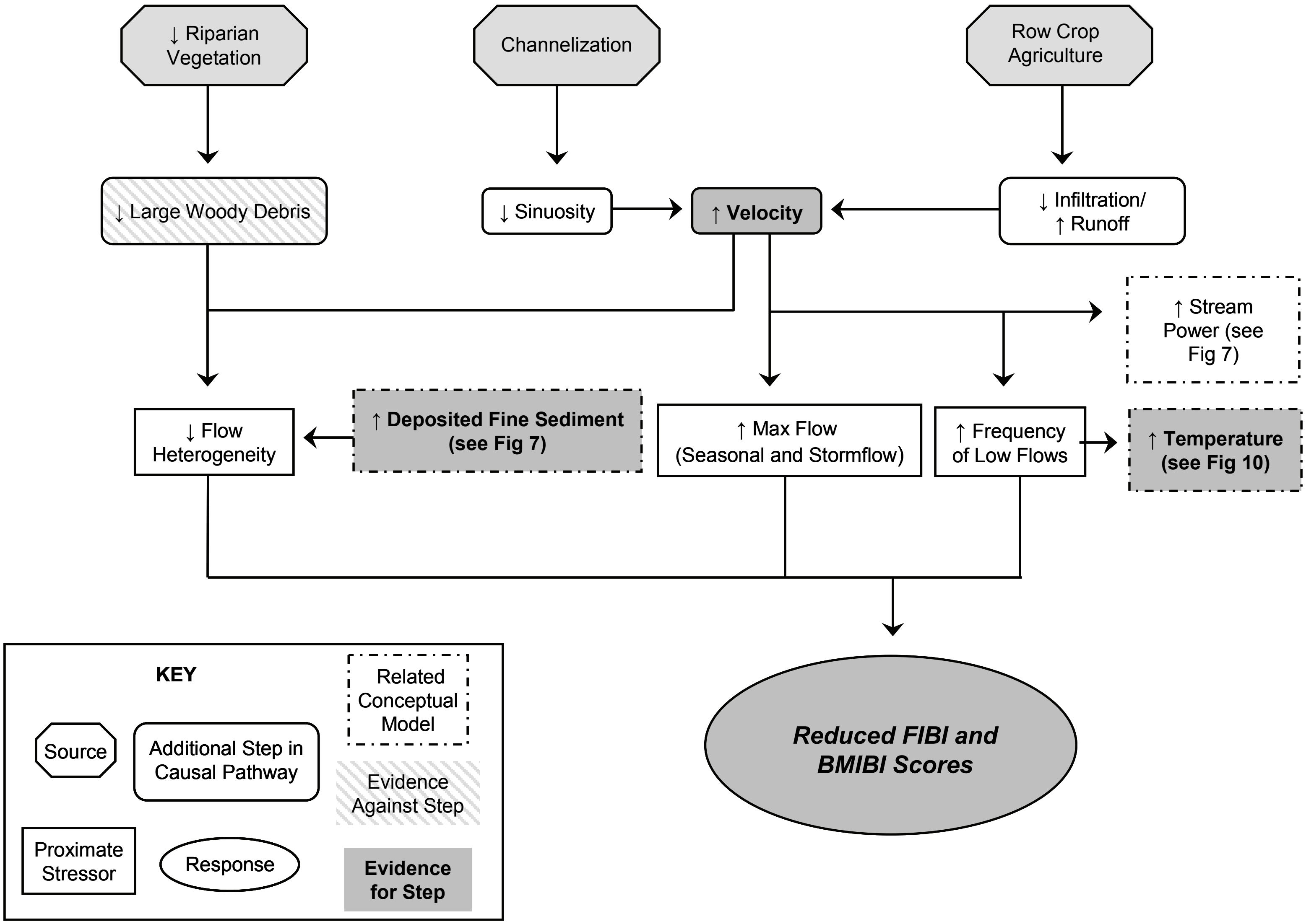


FIGURE 6

Conceptual Model for Altered Flow Regime Showing Evidence for and Against the Stressors and Steps in the Pathway

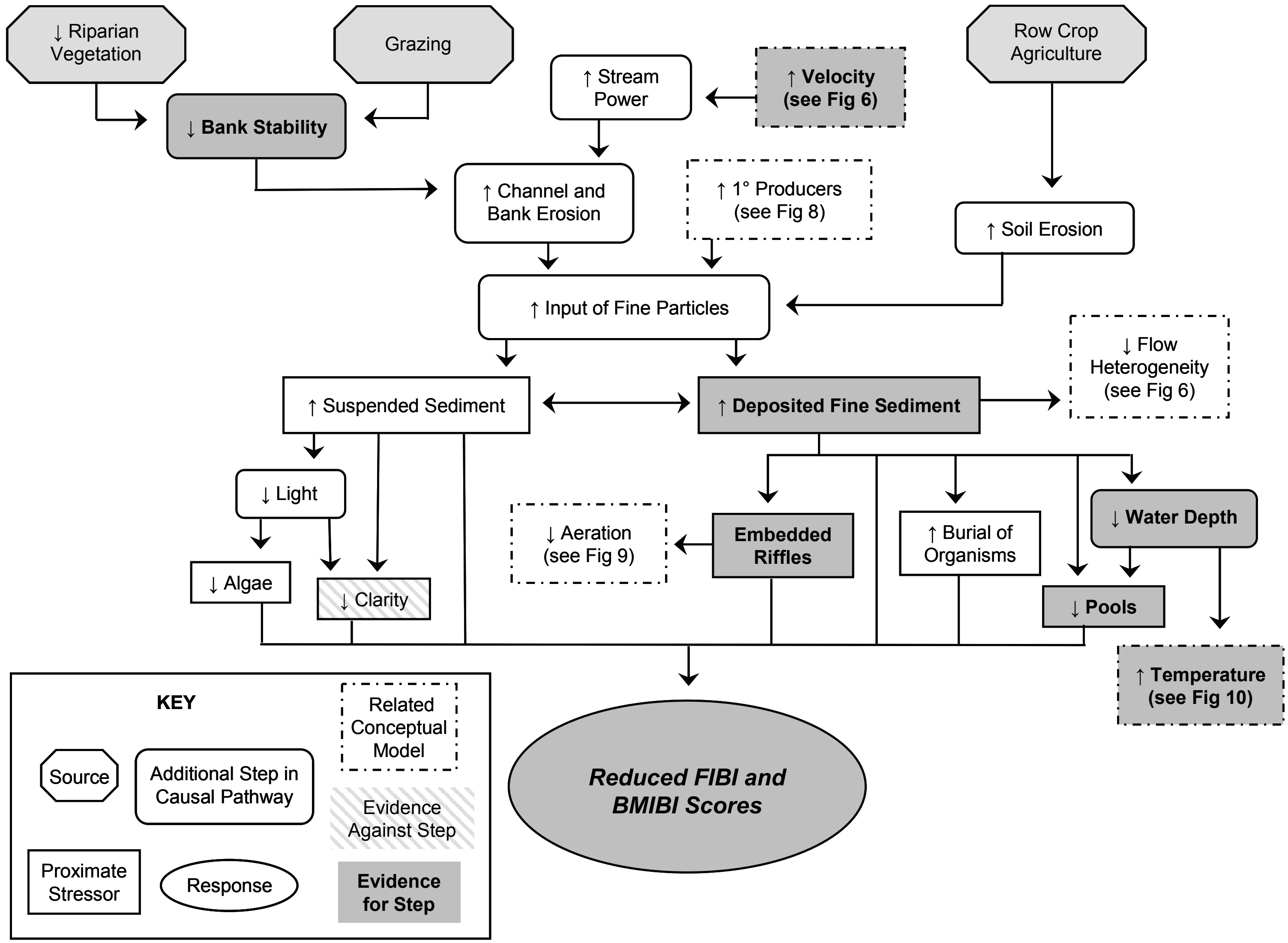


FIGURE 7

Conceptual Model for Increased Sediment Showing Evidence for and Against the Stressors and Steps in the Pathway

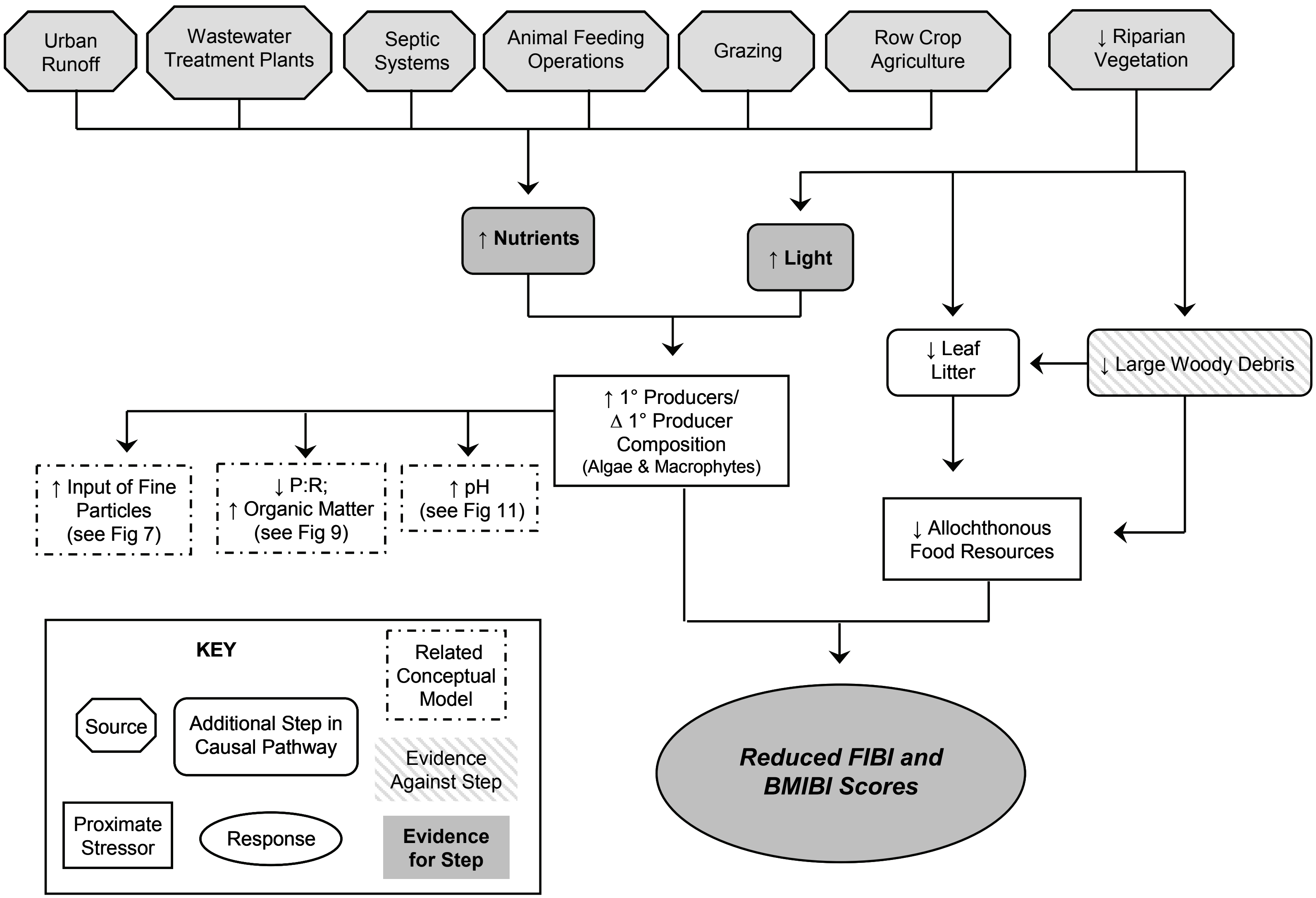


FIGURE 8

Conceptual Model for Altered Basal Food Source Showing Evidence for and Against the Stressors and Steps in the Pathway

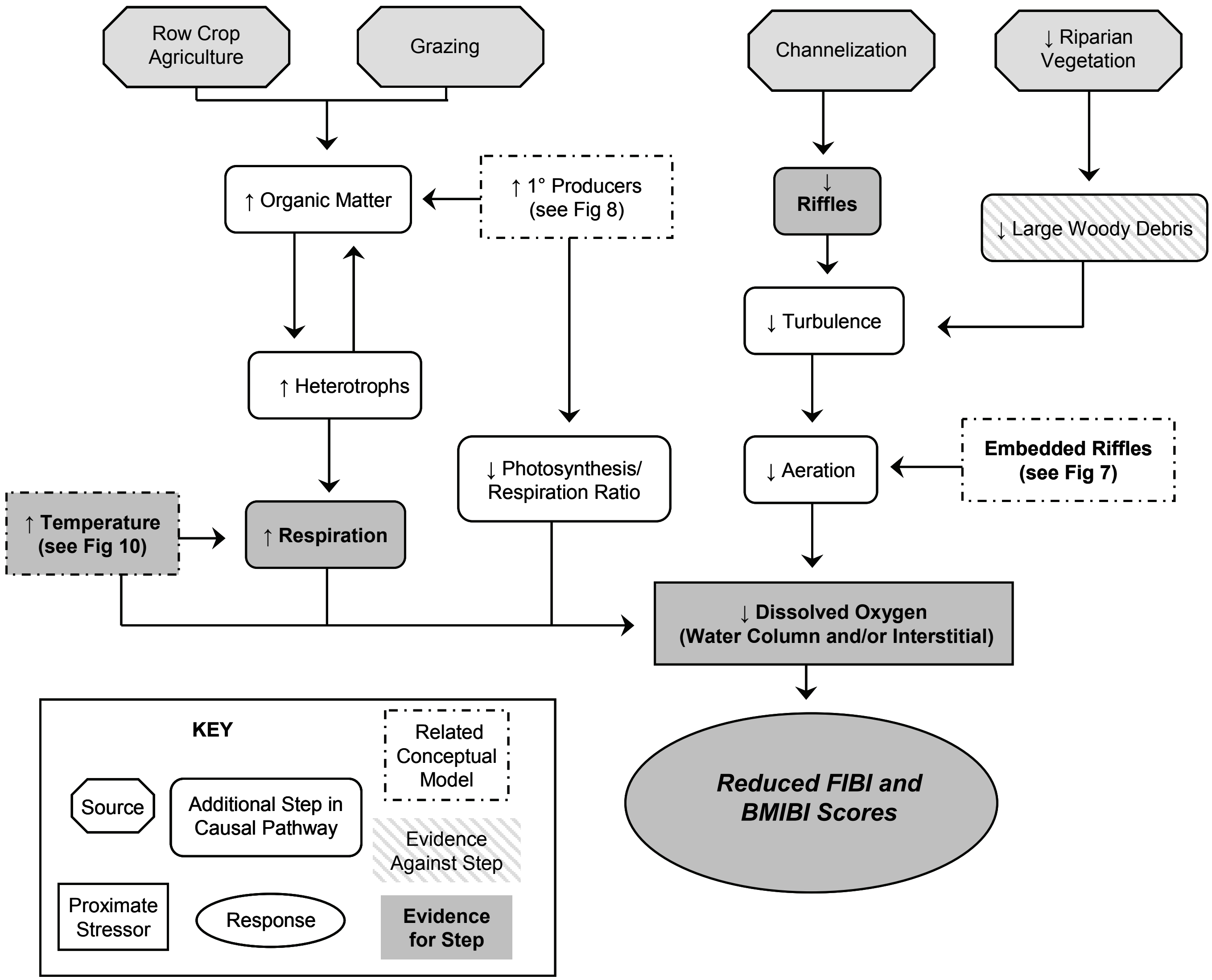


FIGURE 9

Conceptual Model for Decreased Dissolved Oxygen Showing Evidence for and Against the Stressors and Steps in the Pathway

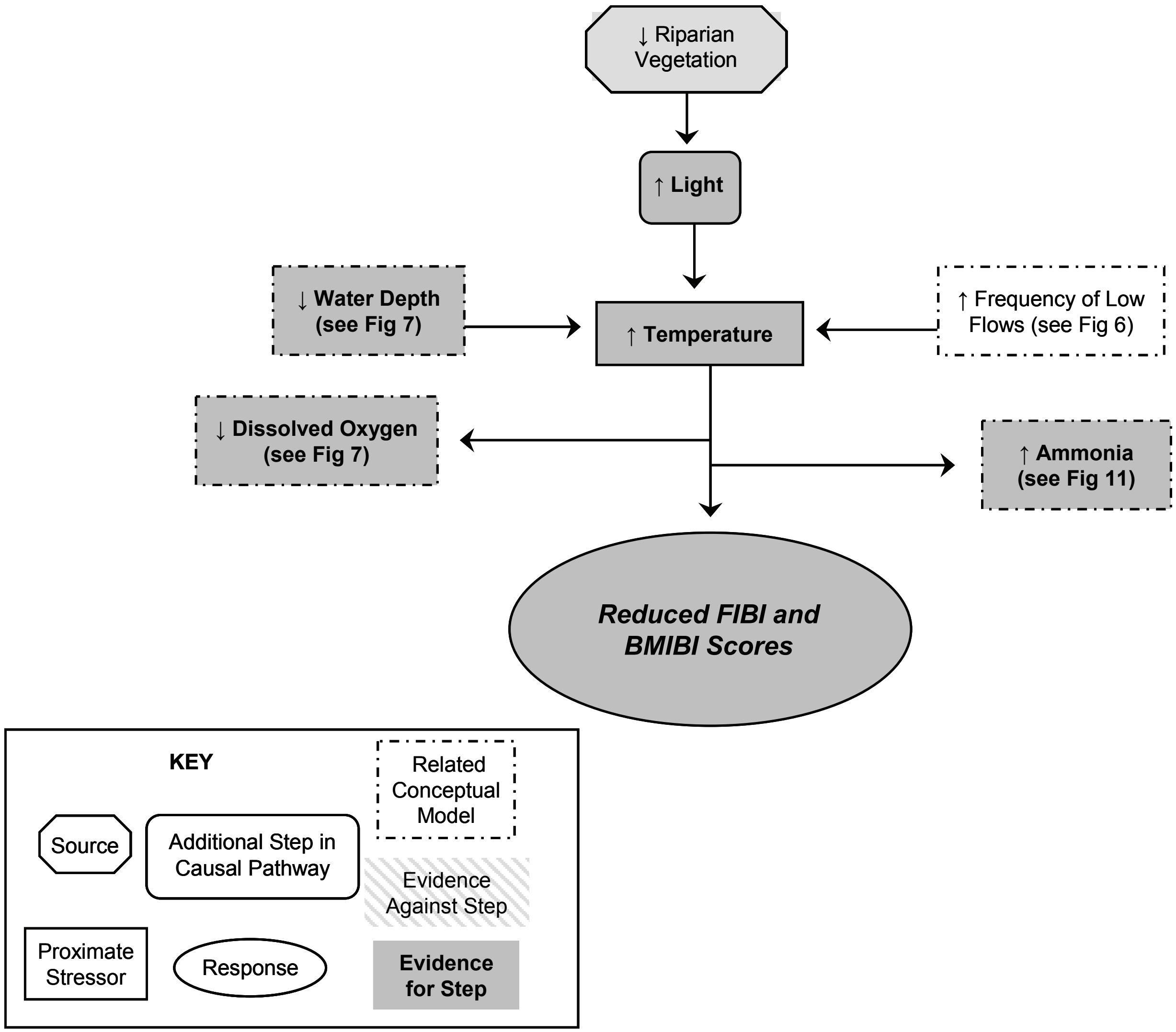


FIGURE 10

Conceptual Model for Increased Temperature Showing Evidence for and Against the Stressors and Steps in the Pathway

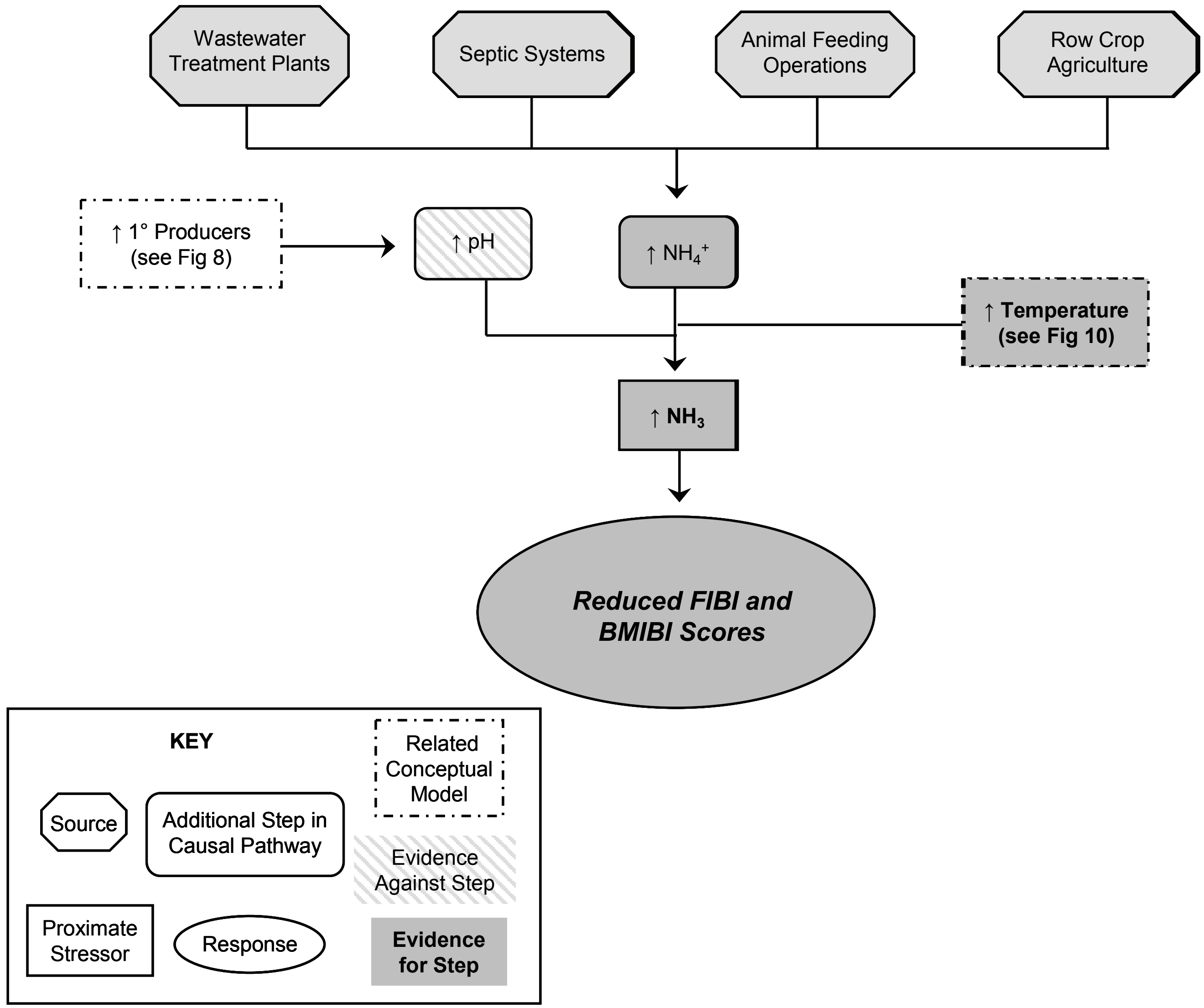


FIGURE 11

Conceptual Model for Increased Ammonia Showing Evidence for and Against the Stressors and Steps in the Pathway

3. EVALUATE DATA FROM THE CASE

3.1. SOURCES OF DATA FROM THE CASE

In the impaired segment of the Little Floyd River, the IDNR contracted the University of Iowa Hygienic Laboratory (UHL) to conduct biological assessments at four sites over a 4-year period. Each site was sampled only once. Despite the spatial and temporal variation in the samples, each assessment showed similar weaknesses in the biological assemblage as noted previously and in Table B-3.

Water chemistry data were collected at three of the biological assessment sites over the course of three years (see Appendix B, Tables B-1 and B-2). Because ecoregion reference data were collected only between midJuly and midOctober, data from the Little Floyd River outside this time-frame were excluded when comparing conditions in the Little Floyd to conditions at ecoregion reference sites. Mean values for chemical parameters for each site are used in the causal analysis (see Appendix C).

Diurnal variations of temperature and DO were measured at two of the sites (see Appendix B; Figure B-1). These data were used to determine if violations of the DO standard had occurred and to evaluate diurnal range in concentration. They were also used to evaluate temperature as a candidate cause in the Little Floyd River.

For the majority of the parameters described above, sites within the Little Floyd River were compared using the internal comparator (Site 2) as described in Section 1.3. For others, such as the analysis for co-occurrence, values for the four Little Floyd River sites were compared to the interquartile range (25^{th} to 75^{th} percentile) of values for reference sites within the Northwest Iowa Loess Prairies ecoregion.

It was possible that multiple stressors were acting independently upon the fish and invertebrates. However, although the calculated metrics listed in Table B-3 were separately maintained and analyzed, there were no pieces of evidence that were applied differently to fish and invertebrates. Thus, the same causes were likely to be identified for fish and invertebrates at the level of the index. The results may have been different if the analysis used individual fish or invertebrate metrics; however, this was not done during IDNR's study submitted for their TMDL report or in this edited version.

3.2. EVALUATION OF DATA FROM THE CASE

Two types of evidence were evaluated using data from the case: (1) spatial co-occurrence and (2) complete causal pathway.

3.2.1. Spatial/Temporal Co-occurrence

The analysis of associations began with the determination of the presence or absence of the proximate stressor. For both the fish and benthic macroinvertebrate communities, the levels of the stressors at Site 2 (internal comparator) were compared to levels at the other three sites in the watershed.

- If the stressor levels were greater at the comparator (Site 2) than at the other three, more-impaired sites (Sites 1, 3, and 4), this is evidence of co-occurrence. The strength is low because the association could be coincidental and was scored with a plus sign (+).
- If the relationship was reversed, then the evidence against the cause was strong because causes must co-occur with their effects and was therefore scored with three minus signs (- - -).
- If the relationship was supported at some sites but not at other sites, the evidence was uncertain and was scored zero (0).
- When data were not available for a measure at the less-impaired comparator site, there was no evidence (NE).

Table 3 provides a summary of the measures for which data were available at the comparator site. Table C-1 of Appendix C contains the complete analysis of co-occurrence.

3.2.2. Causal Pathway

The second type of evidence, complete causal pathway, used observations from the case to link potential source(s) to the proximate candidate cause(s). The first step in this process was to analyze evidence for each step in each of the causal pathways, similar to the examination of co-occurrence in Section 3.2.1.

For the Little Floyd River, to determine if each linkage in the causal pathway was present, the values at the impaired sites (Sites 1, 3, and 4) were compared to the levels of the intermediate stressors at the comparator site (Site 2) and the interquartile range (25^{th} to 75^{th} percentile) of values for reference sites within the Northwest Iowa Loess Prairies ecoregion (see Comment 9). For algal parameters, the mean from random statewide sites was used to represent the unimpaired condition. Table C-2 of Appendix C includes this analysis; Figures 6–11 graphically represent the causal pathways using conceptual models. When evidence weakens a pathway, that measure is depicted with hatch marks. When evidence strengthens a pathway, the measure is shaded a solid grey.

The second step was to determine the completeness of the pathways from watershed sources to each proximate stressor. All of the sources shown in the conceptual models were known to occur within the watershed (see Figures 1 and 2). Contributions of both point and nonpoint sources from the watershed (as described in Section 1.2) are roughly equivalent at all sites. However, data to evaluate all pathways was not available. Only a very few steps in the causal pathways presented in the conceptual models could be eliminated, such as a decrease in the amount of large

TABLE 3

Evidence of Spatial/Temporal Co-occurrence in the Little Floyd River, Iowa

Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
Increased Sediment (see Figure 7) Summary Score: Suspended: 0 Deposited: +					
Increased Suspended Sediment	TSS (mg/L)	38.8	M; 45.0; 33.3	NE; yes; no	0
Increased Deposited Fine Sediment	% total fines	75	95; 80; 85	yes; yes; yes	+
	% silt	32	58; 29; 42	yes; no; yes	0
	% sand	40	31; 47; 41	no; yes; yes	0
	% total coarse	25	5; 19; 15	yes; yes; yes	+
	% total gravel	19	3; 19; 11	yes; no; yes	0
Loss of Pool Depth	% reach as pool habitat	54	48; 50; 23	yes; yes; yes	+
	maximum depth (cm)	91	55; 73; 37	yes; yes; yes	+
Embedded Riffle	% riffles	11	0; 0; 5	yes; yes; yes	+

TABLE 3. cont.					
Decreased DO (see Figure 9)				Summary Score: +	
Decreased DO	lowest observed summer DO (mg/L) (daytime grab samples)	6.2	15.3; 6.2; 4.6	no; no; yes	0
	average summer DO (mg/L) before 10 am (daytime grab samples)	7.8	M; M; 8.1	NE; NE; no	0
	minimum DO (mg/L) (daytime grab samples)	6.2	15.3; 6.2; 3.3	no; no; yes	0
	ratio of highest to lowest summer DO	1.6	M; 1.8; 2.9	NE; yes; yes	+
Increased Temperature (see Figure 10)				Summary Score: +	
Increased Temperature	mean °C (summer grab samples)	17.5	18; 24; 21	yes; yes; yes	+
	maximum °C (summer grab samples)	25.7	18; 25.7; 27.6	no; no; yes	0

TABLE 3. cont.					
Increased Ammonia (see Figure 11)				Summary Score: +	
Increased Ammonia	mean ammonia (mg/L) (grab samples)	0.07	M; 0.19; 0.08	NE; yes; yes	+
	maximum ammonia (mg/L) (grab samples)	0.12	M; 0.32; 0.18	NE; yes; yes	+

M = missing.
NE = no evidence.

Comment 9. Quartiles Used for Stressor-Response.

The use of quartiles to evaluate exposure-response relationships from field data and relate them to site data is similar to the use of box plots. Both methods presume a monotonically increasing or decreasing relationship. If conditions are optimal at an intermediate level of the causal agent and suboptimal at both extremes, the technique is not appropriate. Further, comparison of site data to regional quartile ranges can support a candidate cause if the following are true:

1. Quartile ranges do not overlap between reference sites and a known population of impaired sites. If they do overlap, the reference sites may be affected by the stressor. Alternately, overlap may indicate that the stressor is not a significant cause in the region because the levels of response do not greatly differ between extremely high and low levels of the candidate cause.
2. Quartile ranges are small. If the cause is important, there should be a relatively consistent response. This criterion is weaker than Criterion 1, above.
3. The response at the impaired site falls within the quartile for the extreme level predicted by the causal hypothesis. This criterion was not evaluated in the Floyd River case except that the values for the index scores were below the reference criteria at some sites (see Table 2).
4. The level of the candidate cause at the impaired site falls within the appropriate regional extreme range. That is, if the biological response at the impaired site corresponds to the levels seen at an extreme level of the candidate cause (i.e., Criterion 3 is met), then the level of the candidate cause should be extreme at the impaired site. For example, the mean percent silt was 40, well outside the interquartile range for regional reference sites (6–20% silt).
5. Criteria 1–4 are true for most, if not all, of the response metrics that define the impairment. That is, if the candidate cause is responsible for the impairment, it should be associated with most or all of its component biological effects. The Floyd case did not examine metrics or other individual assessment endpoints.

woody debris. Conversely, few of the proximate stressors could be linked to sources by an entirely complete pathway.

Candidate causes were scored for complete causal pathway as follows:

- Evidence linking sources by at least one complete pathway (from source to proximate stressor) was scored with two plus signs (++).
- Evidence of at least some supported steps in the pathway was scored with a single plus sign (+).
- No evidence of an intermediate stressor for or against any part of the pathway was scored with a zero (0).
- Evidence that at least one step was missing in every causal pathways was scored with a minus sign (-).
- When data were not available to evaluate a candidate cause, no evidence (NE) was indicated and no evaluation was made.

Section 5 provides the scores for completeness of the causal pathway for the proximate stressors in this case.

4. EVALUATE DATA FROM ELSEWHERE

4.1. SOURCES OF DATA FROM OUTSIDE THE CASE

This case study included information from statewide water quality monitoring programs and from the scientific literature; that is, data from outside the Little Floyd River. The statewide monitoring data used in this case were from the Biological Assessment of Iowa's Wadeable Streams project, which monitored ecoregion reference sites (Wilton, 2004), and the REMAP (IDNR 2001c, 2002) data collected from random sites throughout the state.

It is important to note that most Iowa streams, both impaired and unimpaired, have been affected to some degree by anthropogenic changes in drainage patterns and/or channel morphology. Conditions in the Little Floyd River were compared to sites in the ecoregion and across the state that had similar land-use patterns.

4.2. EVALUATION OF DATA FROM OUTSIDE THE CASE

Two types of evidence were used to evaluate data from outside the case: stressor-response relationships from other field studies and stressor-response relationships from laboratory studies (referred to as stressor-response [other]). These types of evidence were used to establish whether the stressors are present in the Little Floyd River at levels that may be expected to elicit a biological response.

4.2.1. Stressor-Response Relationships from Other Field Studies

Data from field studies were used to develop stressor-response associations and then the measured levels of exposure in the Little Floyd River were evaluated to determine if they were sufficient to cause the observed biological effect. The interquartile range of values for the various stressors from ecoregion reference sites were compared to the values observed for the Little Floyd River. The mean value at statewide random sites was compared to the values in the Little Floyd River except where noted. Table B-6 of Appendix B contains data from ecoregion reference sites.

Values for the Little Floyd River were compared to the interquartile range of values for reference sites within the Northwest Iowa Loess Prairies ecoregion. In a few cases, such as measures of extreme temperature or DO, the maximum or minimum ecoregion reference value was used as a comparative value. For measures of algae and diurnal variations, the mean from random statewide sites were considered representative of the unimpaired condition because these parameters were not measured at the ecoregion reference sites.

Scoring for stressor-response from other field studies was based on a comparison between the mean or range of statewide or regional reference sites to the within-site means in the Little Floyd River for chemical parameters, or to the mean for all four Little Floyd River sites for habitat parameters (exceptions are noted in Table C-3):

- If exposure to the candidate cause was greater in the Little Floyd River than at the reference sites, the evidence for a plausible stressor-response was considered strengthened and scored with a plus sign (+).
- If the exposure levels in the Little Floyd River were less than or equal to the exposure at the reference sites, the evidence weakened that candidate cause and was scored with a minus sign (-).
- If the relationship was ambiguous, it received a zero (0).
- If data were not available to evaluate a candidate cause, then there was no evidence (NE) and no impact on the case.

Table 4 provides a summary of the measures for which data were available. The detailed analysis is available in Appendix C (see Table C-3). Section 5 gives the final score for the stressor-response for each proximate stressor.

4.2.2. Stressor-Response from Laboratory and Other Studies

Data from laboratory studies are the basis for stressor-response relationships that are used to derive most ambient water quality criteria (WQC) for chemicals (see Comment 10). Ambient WQC were used to judge if the DO levels or ammonia levels were sufficiently altered to cause the types of impairments observed in the Little Floyd. The Little Floyd River SI, as developed for the TMDL, relied in part on best professional judgment and Iowa water quality standards. The evidence from criteria documents for DO levels and ammonia levels strengthened these candidate causes because values at the impaired sites exceeded the criteria. For other stressors, such as pH, values at the site did not exceed the criteria, the evidence weakened that candidate cause and it was deferred (see Section 2.2.1).

Comment 10. Cautious Use of Water Quality Criteria.
In some cases, the IDNR uses water quality criteria (WQC) in lieu of a stressor-response curve to evaluate if exposures to a candidate cause were sufficient to cause the effects observed in the Little Floyd River. However, the SI process recommends great caution with this approach because the WQC *were <u>not</u> <u>developed to estimate likely effects for different intensities</u>* of stressors. The WQCs provide a single-point comparison rather than characterizing the stressor-response curve. Used individually, the WQC do not account for the combined exposures from multiple chemicals or factors in the field that increase or decrease their effects and they are not protective of all species in all situations. See Ambient Water Quality Criteria Documents as Literature Reviews and various sections on interpreting stressor-response information in the Analytical Tools section of the CADDIS Web site.)

4.2.2.1. *Dissolved Oxygen (+)*

Although DO concentrations measured in daytime sampling from the Little Floyd River are similar to those measured at the ecoregion reference sites, nighttime DO concentrations fall below the Iowa WQC for DO, which requires:

TABLE 4

Stressor-Response from Other Field Studies for Candidate Causes in the Little Floyd River, Iowa

Stressor Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Increased Sediment (see Figure 7)			Summary Score: Deposited: + Suspended: 0		
Increased Suspended Sediment	TSS (mg/L)	10–37 interquartile range (*n* = 8)	36 at Site 2 (mean; *n* = 7); 45 at Site 3 (mean; *n* = 3); 29 at Site 4 (mean baseflow and storm event; *n* = 10)	no; yes; no	0
Decreased Water Clarity	turbidity (NTU)	8–24 interquartile range (*n* = 8)	22 at Site 3 (mean; *n* = 3); 14 at Site 4 (mean; *n* = 5)	no; no	-
Decrease in Algal Growth	seston chlorophyll *a* (µg/L)	32 mean (*n* = 72)	20 at Site 3 (mean; *n* = 3); 9.7 at Site 4 (mean; *n* = 5)	yes; yes	+
	periphyton chlorophyll *a* (µg/cm^2)	32 mean (*n* = 72)	38–45 (range; *n* = 2)	no	-

TABLE 4. cont.

Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (n = 8) or Statewide Random Sites (n = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Stressor Response Relationship from Other Field Studies					
Decrease in Algal Growth cont.	sediment chlorophyll *a* ($\mu g/cm^2$)	27 mean (n = 72)	21–22 (range; n = 2)	yes	+
	gross primary production (GPP) and production-to-respiration ratio (P:R)	4.8, 0.68 (GPP, P:R), mean (n = 72)	10.9, 0.82 (GPP, P:R) at Site 3, (mean; n = 12 days); 4.4, 0.37 (GPP, P:R) at Site 4 (mean; n = 19 days)	no; yes	0
Increased Deposited Fine Sediment	% total fines	46–86 interquartile range (n = 8)	84 mean of all 4 sites (n = 4)	no	0
	% silt	6–20 interquartile range (n = 8)	40 mean of all 4 sites (n = 4)	yes	+
Loss of Pool Depth	% reach area as pool habitat	645 interquartile range (n = 8)	44 mean of all 4 sites (n = 4)	no	-
	maximum depth (cm)	76–88 interquartile range (n = 8)	64 mean of all 4 sites (n = 4)	yes	+

TABLE 4. cont.

Stressor Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (n = 8) or Statewide Random Sites (n = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Altered Basal Food Source (see Figure 8)					Summary Score: 0
Increased/ Altered Primary Producers	seston chlorophyll *a* (µg/L)	32 mean (n = 72)	20 at Site 3 (mean; n = 3); 9.7 at Site 4 (mean; n = 5)	no; no	-
	periphyton chlorophyll *a* (µg/cm^2)	32 mean (n = 72)	38–45 (range; n = 2)	yes	+
	sediment chlorophyll *a* (µg/cm^2)	27 mean (n = 72)	21–22 (range; n = 2)	no	-
	(GPP; g O_2/m^2/d)	4.8 mean (n = 72)	10.9 at Site 3, (mean; n = 12 days); 4.4 at Site 4 (mean; n = 19 days)	yes; no	0
Decreased DO (see Figure 9)					Summary Score: 0
Decreased DO	mean DO (mg/L) (daytime grab samples)	6.9–9.4 interquartile range (n = 8)	8.3 at Site 2 (mean; n = 7); 8.5 at Site 3 (mean; n = 3); 8.8 at Site 4 (mean baseflow and storm event; n = 10)	no; no; no	-

TABLE 4. cont.

Stressor Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Decreased DO cont.	minimum DO (mg/L) (daytime grab samples)	5.4 minimum (*n* = 8)	6.2 at Site 2 (*n* = 7); 6.2 at Site 3 (*n* = 3); 4.6 at Site 4 (*n* = 10)	no; no; yes	0
Increased Temperature (see Figure 10)					Summary Score: +
Increased temperature	mean temperature (°C) (grab samples)	15.1–19.8 interquartile range *n* = 8)	17.5 at Site 2 (mean; *n* = 7); 24 at Site 3 (mean; *n* = 3); 21 at Site 4 (mean baseflow and storm event; *n* = 10)	no; yes; yes	0
	maximum temperature (°C) (grab samples)	24.9 maximum (*n* = 8)	25.7 at Site 2 (*n* = 7); 25.7 at Site 3 (*n* = 3); 27.6 at Site 4 (*n* = 10)	yes; yes; yes	+
	mean temperature (°C)	19.8 mean (*n* = 72)	26.2 at Site 3; 23.2 at Site 4	yes; yes	+
	diurnal maximum temperature (°C)	26.2 mean (*n* = 72)	33.4 at Site 3; 30.4 at Site 4	yes; yes	+

TABLE 4. cont.

Stressor Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Increased Ammonia (see Figure 11)					Summary Score: +
Increased ammonia	ammonia nitrogen as N (mg/L)	0.085–0.10 interquartile range (*n* = 3)	0.06 at Site 2 (*n* = 7); 0.19 at Site 3 (*n* = 3); 0.08 at Site 4 (*n* = 10)	no; yes; no	0
	Maximum ammonia nitrogen as N (mg/L) (grab samples)	0.10 maximum sites (*n* = 3)	0.87 at Site 2 (*n* = 7); 0.32 at Site 3 (*n* = 3); 0.18 at Site 4 (*n* = 10)	yes; yes; yes	+

- A minimum DO of 5.0 mg/L for at least 16 hours of every 24-hour period; and
- A minimum DO of 4.0 mg/L at any time during every 24-hour period (IAC, 2004).

The U.S. EPA designed this standard as a protective measure for aquatic life and it reflects the levels of protection suggested in U.S. EPA's *Ambient Water Quality Criteria for Dissolved Oxygen* (1986). Table 5 summarizes the violations of these limits.

TABLE 5

Summary of Violations of Iowa's Water Quality Standard for DO in the Little Floyd River

Date(s)	Description of Violation
3/11/03	DO measurement of 3.3 mg/L at Site 4 (grab sample)
7/27/02, 7/28/02, 7/29/02, 7/30/02, 7/31/02, 8/1/02, 8/4/02, 8/5/02, 8/6/02	DO measurements below 5 mg/L for more than 8 hours at Site 3 (diurnal measurements 7/24/02 to 8/6/02; 13 d)
7/31/02, 8/1/02, 8/4/02	DO measurements below 4 mg/L at Site 3 (diurnal measurements 7/24/02 to 8/6/02; 13 d)
8/16/03, 8/17/03, 8/18/03, 8/19/03, 8/20/03, 8/21/03, 8/22/03, 8/24/03, 8/25/03, 8/26/03	DO measurements below 5 mg/L for more than 8 hours at Site 4 (diurnal measurements 6/24/03 to 7/1/03 and 8/12/03 to 8/26/03; 21 d)
8/16/03, 8/17/03, 8/18/03, 8/19/03, 8/20/03, 8/21/03, 8/25/03, 8/26/03	DO measurements below 4 mg/L at Site 4 (diurnal measurements 6/24/03 to 7/1/03 and 8/12/03 to 8/26/03; 21 d)

4.2.2.2. *Temperature (+)*

In the Little Floyd River, water temperatures changed more rapidly than 1°C per hour for some portions of all three diurnal sampling events in 2002 and 2003 (see Figure B 1). At Site 3, for example, the rate of temperature increase exceeded 8°C over a 4 hour period on both August 2 and 3, 2002. The IDNR judged thermal changes of 1°C per hour to be excessive. These rapid thermal fluctuations create highly stressful conditions for fish and macroinvertebrates and could be expected to negatively affect aquatic communities. In addition the Little Floyd River also had daily high temperatures that exceeded 32°C during one of the three diurnal sampling events; the maximum water temperature during this time was 33.4°C (see Comment 11).

Unfortunately, much of the literature dealing with fish thermal limitations focuses on cold-water species. This is especially true of the literature dealing with temperature fluctuations. However, in a study of the potential effects of climate change, Eaton and Scheller (1996) provide estimates of the maximum weekly average temperature tolerance of several fish species that inhabit the Little Floyd River. The maximum weekly average temperature measured in the Little Floyd River during diurnal sampling was 27.4°C. This is greater than or equal to the published tolerance levels for 4 of 11 fish species on their list found in the Little Floyd River.

Comment 11. Changes to State Limits.
When the original SI was completed, the Iowa Administrative Code (IAC) did not include specific limits on maximum temperature or the rate of temperature change. This standard was under development at that time. Recent revisions to the IAC, which apply to the Little Floyd River, include the following:
No heat shall be added to interior streams or the Big Sioux River that would cause an increase of more than 3°C. The rate of temperature change shall not exceed 1°C per hour. In no case shall heat be added in excess of that amount that would raise the stream temperature above 32°C (IAC, 2006).
Effect levels that were the basis for this decision were not used to evaluate this case.

4.2.2.3. *Ammonia (+)*

Iowa water quality standards include a specific numeric limit on ammonia in classified rivers and streams. Concentrations of ammonia in stream samples must be interpreted with respect to pH and temperature in order to determine if the concentrations violate Iowa water quality standards for aquatic life use (IAC, 2004). Iowa's water quality standards are based on the criteria developed by the U.S. EPA (1999).

During a rainstorm in November of 2003, ammonia concentrations exceeded the acute toxicity standard for ammonia. Concentrations of ammonia were measured at 4.9 mg/l and 6.5 mg/L under prepeak and postpeak flow conditions, respectively, with a temperature of 4.1°C and a pH of 8.2. These concentrations were the result of the over application of hog manure on a soybean field the day before the storm and caused a fish kill over a 6.6 km reach of the Little Floyd River (IDNR, 2005b) (see Comment 12).

Comment 12. Assessing Different Impairments.
Biological impairments can be due to different mechanisms and causes. IDNR strategically chose to investigate the cause of massive kills to wildlife separately from pervasive, chronic causes. The cause of the fish kill was identified as ammonia toxicity that resulted from a spill from a hog farm. This impairment was not the focus of this study.
Ammonia was also a candidate cause for the chronic impairment and implicated in the Little Floyd River. In addition to toxicity due to water column levels of ammonia, other causes that might be considered are: ammonia toxicity associated with deposited sediment, episodic formation of ammonia due to high levels of nitrate in reducing environments at high pH, and unreported, repeated episodic spills of waste.

5. IDENTIFY THE PROBABLE CAUSES

Four types of evidence are included in the SI for the Little Floyd River: (1) spatial co-occurrence, (2) complete causal pathway, (3) stressor-response relationships from other field studies, and (4) stressor-response from laboratory studies. Once a score was assigned for each candidate cause, an overall score for each type of evidence was determined for each candidate cause. Section 5 contains these final scores. See the CADDIS website (http://epa.gov/caddis/) for scoring protocols.

Since some types of evidence were composed of more than one piece of evidence, these were synthesized into a single score before comparing among different candidate causes. For example, co-occurrence for increased temperature includes four pieces of evidence that scored +, 0, and NE (see Table C-1). These four pieces of evidence were synthesized into a score of "+". Then the four types of evidence for each candidate cause were scored for consistency. Rules for scoring consistency among the different types of evidence for a candidate cause were as follows (U.S. EPA, 2007):

- A positive response in at least three types with no negative responses received two pluses (+ +).
- A positive response in two types with no negative responses scored one plus (+).
- The presence of both positive and negative responses received a minus (-).
- Two or more negative responses with no positive responses received two minuses (- -).
- Any other combination scored zero (0).

Table 6 shows the strength of evidence for each of these considerations across the proximate stressors defined in the conceptual models.

The IDNR examined evidence for each candidate cause in order to determine which of the causes were likely to have the greatest affect on the aquatic community. As shown in Table 6, of the seven candidate causes, the four most likely candidate causes are deposited sediment, decreased DO, increased temperature, and increased ammonia (shaded solid grey). These four candidate causes were then divided into primary causes for which IDNR developed TMDLs and secondary causes that were due to pollution, which would not require TMDLs. IDNR included both in their remediation plans.

TABLE 6

Strength of Evidence Tables for Little Floyd River

	Altered Flow Regime			Suspended Sediment			Deposited Sediment				Basal Food Source				
	Decreased Flow Heterogeneity	Increased Maximum Flow	Increased Frequency of Low Flows	Increased Suspended Sediment	Decreased Clarity	Decreased Algal Growth	Increased Deposited Fine Sediment	Loss of Pool Depth	Embedded Riffles	Burial of Organisms	Decreased Allochthonous Food Resources	Change in Primary Production	Decreased DO	Increased Temperature	Increased Ammonia
Co-occurrence	NE	NE	NE	0	NE	NE	+	+	+	NE	NE	NE	+	+	+
Complete Pathway	0	-	0	+	+	+	+	+	+	+	0	++	+	++	+
Stressor-Response (Field)	NE	NE	NE	0	-	0	+	0	NE	NE	NE	0	0	+	+
Stressor-Response (Other)	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	+	+	+
Consistency	0	0	0	0	-	0	++	+	+	0	0	0	++	++	++

NE = no evidence.

5.1. PROBABLE PRIMARY CAUSES

5.1.1. Deposited Sediment

IDNR identified excessive silt and sediment deposition in the Little Floyd River as a cause of the reduction in the FIBI and BMIBI scores. The levels of deposited sediment that were observed could limit the assemblages of benthic macroinvertebrates that require gravel substrates found in riffles for refugia and reproduction. Sediments could decrease habitat available to pool-dwelling fish and organisms that prefer coarse substrates.

IDNR determined that siltation and sedimentation was a problem based on habitat data collected by the IDNR/UHL biological assessment team (see Table 7). Total fine sediment was at the extreme end of the range for the regional reference sites at all four sites in the Little Floyd River. The percent silt is far greater than expected based on reference sites. The percent sand was within the expected range based on ecoregion reference data, but this is partially explained by the dominance of silt in the Little Floyd River. While the percentage of the Little Floyd River with pool habitats was greater than expected, the maximum depth in these pools was generally less than those at the ecoregion reference sites. Although embedded riffles were observed at seven of the ecoregion reference sites, riffles were generally lacking in the Floyd River and may be indicative of sedimentation problems beyond mere embeddedness. The lack of riffles limits the diversity of habitats available to aquatic organisms and thereby limits the diversity of the aquatic community.

TABLE 7

Details of Several Habitat Metrics for Deposited Fine Sediment

Parameter	9/14/99 Site 1	9/12/01 Site 2	8/22/02 Site 3	9/11/01 Site 4	Ecoregion 47a Reference
% total fines	95	75	80	85	46–86, 62, 59
% silt	58	32	29	42	6–20, 13, 13
% sand	31	40	47	41	29–59, 47, 44
% riffle	0	11	0	5	5–18, 13, 15
% pool	48	54	50	23	6–45, 24, 21.5
maximum depth (m)	1.8	3	2.4	1.2	2.5–2.9, 2.7, 2.7

Table 6 contains three measures under the umbrella of increased deposited sediment. This table documents the variety of evidence that pointed to the impacts of sediment in the Little Floyd River. The most convincing evidence for sediment as a proximate stressor was from the measurements of increased deposited fine sediment (see Table 6). Co-occurrence was supported, largely based on the lesser percent total fines and greater percent riffle and maximum depth at the internal comparator, Site 2, relative to the other three sites. The verified steps in causal pathway and stressor-response from the field further strengthened increased deposited fine sediment as a primary cause of biological impairment in the Floyd River. These findings resulted in a high level of consistency among both types and pieces of evidence.

Reduced pool depth and increased riffle embeddedness also lend support to deposited sediment as a probable cause of biological impairment. Co-occurrence was demonstrated and there was supporting evidence for steps in the causal pathway.

5.1.2. Low DO

Low levels of and fluctuations in concentrations of DO likely contribute to low FIBI and BMIBI scores in Iowa. DO measurements taken in the Little Floyd River over two separate 2-week periods in August of 2002 and 2003 using continuous dataloggers show that levels fluctuate by 7 mg/L or more over 24-hour periods (see Appendix B, Figure B-1). These extreme rates of change suggest that DO production during the day causes supersaturation while algal and bacterial respiration depletes DO when photosynthesis ceases at night.

In addition to diel fluctuations, the August data showed that DO levels fell below 4 mg/L for several hours each night for several nights each summer. Monthly grab samples collected by UHL showed that DO concentrations were within acceptable limits except for a measurement of 3.3 mg/L at Site 4 on March 11, 2003. On this day, DO at the Site 3 upstream site was 6.7 mg/L and the temperature was 0°C at both sites. These DO concentrations are equivalent to less than 50% saturation at Site 3 and less than 25% saturation at Site 4.

Concentrations of DO in August 2002 and 2003, particularly during the night and in the early morning, were at levels known to stress the species in the Little Floyd River. While the diel cycling of DO concentrations demonstrate that algae contribute to the DO stress, the evidence does not suggest that the Little Floyd River has higher than normal algal productivity for this ecoregion. For this reason, high summer temperatures may cause an increase in respiration rates or there may be additional sources of oxygen demand. Also, DO measurements in the water column may not adequately characterize DO concentrations on and near the Little Floyd River’s stream bed. The amount of deposited sediment could restrict aeration of substrates.

Three types of evidence strengthen DO as a probable cause of biological impairment in the Floyd River: (1) co-occurrence was demonstrated using the internal comparator; (2) steps in the causal pathway were supported; and (3) DO levels were likely to cause detrimental effects based on a plausible relationship between the stressor (DO) and the response (impaired biological assemblages) based on state standards. This resulted in a high degree of consistency for DO as a stressor in the Little Floyd River.

Iowa water quality standards (IAC, 2004) define the minimum level of DO allowed in Class B (LR) streams as 4.0 mg/L and that DO levels must be at least 5.0 mg/L for 16 hours of every 24-hour period. Independent of biological impairments and their causes, the documented violations of the DO standard are sufficient to include the Little Floyd River on Iowa's 303(d) list of impaired waterbodies. The Little Floyd River is in violation of these standards thus SI was not needed for regulatory action. However, the causal assessment confirmed that low DO was one among several probable causes. Therefore, remediation of low DO alone may not achieve the desired aquatic life uses.

5.2. PROBABLE SECONDARY CAUSES

5.2.1. High Temperature/Temperature Flux

The thermal conditions of the Little Floyd River include high temperatures and rapid, daily temperature fluctuations. These conditions contribute to the low FIBI and BMIBI scores for the River. Both monthly sampling and the diurnal samples from dataloggers deployed in the summers of 2002 and 2003 showed that daytime water temperatures exceeded 30°C. The summer measurements in both years also revealed temperature fluctuations that exceed 2°C per hour. Both of these conditions can be stressful to aquatic organisms.

There is evidence of increased heat in the Little Floyd River. The mean temperature of available measurements is higher at Sites 3 and 4 than Site 2 and the interquartile range for ecoregion reference sites. In addition, the maximum temperatures were greater at all three measured Little Floyd River sites compared to the maximum for ecoregion reference sites. Only one reference site had a water temperature greater than 20°C (observed in early August). At sites in the Little Floyd, temperatures above 20°C were recorded into September.

Co-occurrence was demonstrated with the benthic macroinvertebrate and fish community and a complete causal pathway was documented. A field stressor-response was verified using ecoregion data and statewide data. This allows a high level of consistency for the identification of water temperature as a stressor in the Little Floyd River.

Since there are no industrial thermal discharges in the watershed, causal pathways to increased temperature in the Little Floyd River result from poor riparian conditions and possibly reduced ground water base flow.

5.2.2. Ammonia

The Little Floyd River watershed supports a large number of livestock. This livestock produces a large quantity of manure that is applied to fields each year. Periodic leaks, spills, over-application, and lagoon failures throughout the watershed will have adverse effects on the biological community in the river.

High concentrations of ammonia have been detected infrequently in the Little Floyd River. Over-application of hog manure on October 31, 2003, caused a fish kill over a 6.6-km reach of the river. Following the application of manure, a storm and subsequent increased flow triggered the UHL event sampler on November 1. The pre- and postpeak samples registered ammonia concentrations of 4.9 and 6.5 mg/L. These concentrations, respectively, violate the chronic and acute limits in Iowa water quality standards and are associated with a known fish kill that included the three upstream sampling sites.

Minor manure releases and small fish kills may occur without notification being sent to IDNR. Without this notification, it is impossible to quantify the effects of ammonia and other manure-related parameters on the fish and benthic macroinvertebrate communities in the Little Floyd River. Smaller repeated exposures remain a potential cause.

Four types of evidence strengthen ammonia as a candidate cause: (1) co-occurrence, (2) steps in the causal pathway, (3) field stressor response, and (4) stressor-response based on Iowa's state standards. The evidence is consistent for ammonia in the water column as a probable chronic cause. Ammonia is sediment was not measured.

However, while the evidence supported ammonia as a candidate cause, the evidence was relatively weak and somewhat circumstantial. The concentrations of ammonia in the Little Floyd River were below the detection limit on most occasions, (see Appendix B; Table B-1). In fact, other than the high concentrations measured in association with the known fish kill, the highest concentration of ammonia measured in the Little Floyd was 0.32 mg/L, well below concentrations expected to cause impairments of the aquatic community. The evidence for increased sediment deposition and low DO were more compelling than the evidence for ammonia as a probable cause of biological impairment as measured by fish and benthic macro-invertebrate indices. Whereas, episodic fish kills were attributed to ammonia toxicity in a separate assessment.

5.3. UNSUPPORTED CAUSES

5.3.1. Altered Flow Regime

Data for the analysis of alteration of the flow regime in the Little Floyd River are limited (see Comment 13). A lack of historical flow data for both the impaired stream segment and the ecoregion reference sites prevented an analysis of co-occurrence and stressor response. There was evidence for several steps in the causal pathways, but without evidence for the proximate stressors, it is impossible to draw any conclusions about the relationship of the existing flow conditions to the impairment.

Despite this lack of data, altered flow regime is categorized as unsupported. This decision is based in part on the characteristics of the watershed. The land-use patterns and tile drainage systems in the Little Floyd River watershed and the channel alteration in the river itself are characteristic of the conditions of streams throughout the region which are not biologically impaired based on IDNR biological criteria. Furthermore, there are no impoundments or water withdrawals and flow is expected to be similar to other locations on the Little Floyd River. However, flow may affect other candidate causes.

Comment 13. Data Limitations.
Several of the potential stressors in the Little Floyd River case are reported as unsupported, that is, lacking evidence as the cause for the more severe impairment at other sites compared to Site 2. This is not to say that these causes are not affecting the stream community in a detrimental way. These stressors were not eliminated (i.e., refuted). Although the evidence suggests that they are not probable causes for the differences observed between Site 2 and the other three sites in the Little Floyd River, these causes may be responsible for other characteristics of the fish and invertebrate communities that were not evaluated in this assessment.
Also, had there been a weaker case for deposited sediment, DO, temperature, and ammonia as proximate stressors, the IDNR would have needed to collect additional data to allow a more thorough examination of co-occurrence and stressor response for altered flow regime and altered basal food source and suspended sediment in the Little Floyd River.

5.3.2. Suspended Sediment

Suspended sediment is considered an improbable cause for the biological impairment of the Little Floyd River. While the evidence within the causal pathway strengthened suspended sediment as a possible stressor, the data and evidence for co-occurrence and stressor response were unavailable, inconclusive, or contradicted suspended sediment as a stressor.

TSS and turbidity were evaluated to determine co-occurrence and stressor response for increased suspended sediment. TSS as measured at the less-impaired comparator site, (38.8 mg/L) was between the values for the more impaired sites (33.3 and 45.0 mg/L), therefore TSS evidence was scored as inconsistent. When comparing the Little Floyd River sites to reference sites within the ecoregion, the mean TSS concentration was outside the interquartile range at only one of the Little Floyd River sites. This evidence also is considered inconsistent. The measurements of turbidity from the Little Floyd River were all within the interquartile range of the ecoregion reference sites, contradicting the mechanism involving a decrease of water clarity.

Although the evidence from the Little Floyd River do not specifically point to problems of suspended sediment, the biological sampling period occurred during a limited time frame. Ecoregion reference values were collected only from July 15 to October 15 in order to have a consistent monitoring period during relatively low-flow periods. However, the higher concentrations of TSS that may be caused by spring storm events are not taken into account within the existing sampling regime (see Comment 14). Therefore, despite the classification of this stressor as unsupported, the available evidence was inadequate to fully eliminate increased suspended sediment as a potential seasonal stressor.

Comment 14. From a Reviewer.
Effects of TSS are concentration and duration dependent (see Newcombe and Jensen, 1996). Persistent levels of low TSS may be more important to aquatic life than the spikes in TSS due to spring freshets.
Nevertheless, in the Little Floyd River, TSS levels were similar to regional reference sites where no impairments were observed (U.S. EPA editor.)

5.3.3. Altered Basal Food Source

Data limitations for the altered basal food source allowed for the examination of only two types of evidence: causal pathway and stressor-response. While the relatively high phosphorus concentrations and decreased stream shading (see Table C-2) allowed a strong causal pathway for increased primary production, the inconsistent evidence of a plausible stressor-response relationship from the field from ecoregion data weakens altered basal food source as a likely cause of the described impairments (see Comment 9.

Seston and sediment chlorophyll measurements in the Little Floyd River were less than the mean values reported for statewide sites. However, the periphyton measurements were greater than the statewide mean. Changes in the basal food source seem to be due to the presence of increased periphytic algae. This change could influence the composition of the benthic macroinvertebrate and fish communities in the Little Floyd River. However, due to the small differences between the Little Floyd River sites and the statewide averages, an altered food resource is a weaker probable cause than low DO or increased temperature.

6. DISCUSSION AND HIGHLIGHTS

6.1. FROM SI TO TMDL

IDNR used the SI process to identify the probable causes for impairments in the Little Floyd River in order to fulfill their 303(d) reporting requirements. The probable causes determined in the SI were increased deposited sediment (particularly increased fine sediment, increased embeddedness of riffles, and reduced pool depth) and decreased concentrations of DO. Elevated temperature was designated as a secondary cause because it was not due to regulated pollutant sources in Iowa. Ammonia was not considered as the cause in this particular case, but was a pollutant of concern in the river and was treated separately as described below. For TMDL purposes, the terminology for these causes is siltation, organic enrichment/low DO, thermal modification, and un-ionized ammonia. However, the 303(d) listing and the TMDL for the Little Floyd River did not include all of these causes.

Based on the recommendations of the original SI, IDNR submitted a TMDL for Sediment and DO on April 25, 2005; it was approved by the U.S. EPA on June 6, 2005. The IDNR also listed un-ionized ammonia as a stressor in the SI primarily due to high concentrations associated with a known fish kill that occurred in the Little Floyd River in October 2003. Based on 305(b) assessment and 303(d) listing methodology (found in IDNR, 2005a), ammonia would not be included as a cause for an impaired waters listing but would be added to the 305(b) assessment. IDNR identified the source of the ammonia leading to this fish kill and took appropriate action independently from the TMDL process.

The thermal modification of the stream is strongly related to habitat alterations that have widened the channel and increased exposure of the water and stream sediments to sunlight. Although not included in the TMDL as a cause, the TMDL's implementation plan includes remediation components to address thermal stress, such as increasing riparian vegetation, that IDNR expects will improve the thermal regime of the Little Floyd River (see Comment 15).

Comment 15. Some Regulatory Approaches for Addressing Causes of Biological Impairments.

Some states do not have biological criteria. They rely on water quality criteria to protect aquatic resources through TMDL implementation. Some states do not have criteria for thermal inputs. They may rely on biological criteria to detect problems and then use a TMDL to address thermal stress along with other parameters. For example, High temperatures in the Little Floyd River were caused by nonpoint source changes in land uses due to removal and alteration of vegetation. At the time of the study, IDNR did not have state temperature criteria, but has since implemented them (see Comment 11). Therefore, the state chose to recommend actions in the TMDL that would lower temperature and thereby also increase DO and reduce sediment loading.

6.2. UNCERTAINTIES

The IDNR identified three uncertainties in the Little Floyd River case, predominantly related to the timing of data collection and the overall quantity of data:

(1) ecoregion reference data were collected only during summer periods, when low-flow conditions prevailed, (2) few data were available for ecoregion reference sites and for several parameters within the Little Floyd River and its watershed, (3) historical data on stream conditions is lacking both at ecoregion reference sites and in the Little Floyd River. At each ecoregion reference site, the IDNR measures biological, chemical, physical, and habitat parameters during each visit. The operating procedure dictates that these samples be collected between July 15 and October 15 each year. However, limiting sampling to this time period for developing stressor response relationships has the disadvantage of not capturing the full range of physical and chemical parameters that the resident community experiences over the course of a year. For example, high-flow conditions associated with snowmelt and spring rains may cause pulses of sediment, fertilizers, and pesticides—stressors that may have significant episodic effects on the biological assemblages.

An added degree of uncertainty in the SI for the Little Floyd River lies in the limited amount of ecoregion reference data. For the larger ecoregions across the state, the IDNR collected 20 to 40 reference samples. In the Northwest Iowa Loess Prairies ecoregion, the IDNR has collected only eight reference samples from six different sites. This limitation in reference data further increases the uncertainty of the values used for comparisons.

Within the dataset for the Little Floyd River itself, four very short reaches of stream were sampled and these were not necessarily sampled in the same year. The total evaluated length for habitat considerations was approximately 760 m. As shown in Figure 1, the impaired segment is nearly 5.5 km long and the waterways above this reach are extensive. This situation makes it difficult to assess the true condition of the stream and watershed as a whole. For example, data from the impaired segment indicate that stream bank erosion is minimal. However, general knowledge of the soils and topography of this region suggests the possibility that bank erosion may be occurring in the upper reaches of the watershed.

The limited historical dataset available for Iowa streams in general is problematic. Changes in stream channel sinuosity, stream gradient, and channel morphology are largely undocumented. The U.S. Geological Survey surface water gauges provide an extensive flow record for many larger streams and rivers. However, the lack of records for flow and other parameters in smaller streams like the Little Floyd River hinders causal analysis.

6.3. FUTURE PROJECTS

The IDNR has continued to use the SI approach to determine the causes of biological impairments in streams across the state. The results of this case are being used to better design future sampling in order to acquire sufficient data to assess the causal pathways in other impaired Iowa streams. Synoptic assessments are being used to supplement the data collected in the typical sampling regime. These assessments include full bioassessments used to determine FIBI and BMIBI scores, supplemented by several rapid bioassessments on both the mainstem and tributaries of the streams. For example, more recent biological sampling on a biologically impaired stream in eastern Iowa included three full bioassessments and 15 rapid bioassessments. IDNR expects that synoptic sampling will provide paired data and thus stronger evidence for co-occurrence in future SI efforts.

To improve the usability of the data from the rapid bioassessments, the IDNR has developed a supplemental datasheet for the rapid bioassessment sites. The new datasheet should provide valuable information about the condition of the stream as a whole. Some of the additional information collected for future SIs will fill gaps in the characterization of the causal pathways that increased uncertainty in the Little Floyd River case (for example, qualitative determinations of floodplain connectivity and leaf litter abundance). A portion of the new datasheet will provide supplemental measurements of parameters that are measured at full bioassessment sites but which are not normally captured in a rapid bioassessment (for example, stream shading, embeddedness, and abundance of woody debris).

In future SI cases, the IDNR also hopes to make more extensive use of the data available using Geographic Information Systems. Estimates of watershed soil loss (via the revised universal soil loss equation), land cover, livestock abundance, and other basin and subbasin statistics would compliment the SI process. Further, the comparison of geo-referenced aerial photographs and topographic maps generated over the years may provide insights into recent physical changes in the watershed.

6.4. CONCLUSION

The SI process as used by the IDNR has been a successful endeavor. This process made it possible for the IDNR to complete several TMDLs. These TMDLs include a rational and scientifically sound basis for improving the biological condition of impaired waterways. The SI and TMDL are the initial steps toward improving the conditions in the Little Floyd River and other streams. Implementation and continued monitoring are vital to the restoration of a sustainable community to biologically impaired waters such as the Little Floyd River.

7. REFERENCES

Eaton, J.G., Scheller, R.M. 1996. Effects of climate warming on fish thermal habitat in streams of the United States. Am. Soc. Limnol. Oceanogr. 41(5):1109–1115.

Griffith, G.E., Omernik, J.M., Wilton, T.F., et al. 1994. Ecoregions and subecoregions of Iowa: a framework for water quality assessment and management. J. Iowa Acad. Sci. 10(1):5–13.

IAC (Iowa Administrative Code). 2004. Chapter 567–61: water quality standards. [effective date 6/16/04].

IAC (Iowa Administrative Code). 2006. Chapter 567–61: water quality standards. [effective date 2/15/06].

IDNR (Iowa Department of Natural Resources). 2004. Draft protocol for stressor identification. Environmental Protection Division, TMDL and Water Quality Assessment Section; Des Moines, Iowa.

IDNR (Iowa Department of Natural Resources). 2005a. Methodology for Iowa's 2004 water quality assessment, listing, and reporting pursuant to Sections 305(b) and 303(d) of the federal Clean Water Act. Environmental Protection Division; Des Moines, Iowa. Available online at: http://wqm.igsb.uiowa.edu/WQA/303d/2004/2004FinalMethodology.pdf.

IDNR (Iowa Department of Natural Resources). 2005b. Fish kill data available online. Environmental Protection Division; Des Moines, Iowa. Available online at: http://wqm.igsb.uiowa.edu/wqa/fishkill.html.

IDNR (Iowa Department of Natural Resources). 2005c. Iowa Department of Natural Resources TMDL & Water Quality Assessment Section. Total Maximum Daily Load For Sediment and Dissolved Oxygen, Little Floyd River, Sioux and O'Brien Counties, Iowa. Available at: http://www.epa.gov/Region7/water/pdf/littlefloyd_riv_sediment_do_final060605.pdf.

Ingersoll, C.G., MacDonald, D.D., Wang, N., et al. 2000. Prediction of sediment toxicity using consensus-based freshwater sediment quality guidelines. U.S. Geological Survey final report for the U.S. Environmental Protection Agency. EPA 905/R-00/007. Available online at: http://www.cerc.usgs.gov/pubs/center/pdfdocs/91126.pdf.

Newcombe, C.P., and J.O.T. Jensen. 1996. Channel suspended sediment and fisheries: a synthesis for quantitative assessment of risk and impact. N. Am. J. Fish. Man. 16:693–727.

U.S. EPA (Environmental Protection Agency). 1986. Ambient water quality criteria for dissolved oxygen. Office of Water Regulations and Standards; EPA/440/5-86/003. Available online at http://www.sjrdotmdl.org/concept_model/bio-effects_model/documents/Chapman1986.pdf.

U.S. EPA (Environmental Protection Agency). 1999. 1999 Update of ambient water quality criteria for ammonia. Office of Water; EPA/822/R-99/014. Available online at http://www.fs.fed.us/fire/retardant/references/US_EPA_1999.pdf.

U.S. EPA (Environmental Protection Agency). 2000. Stressor identification guidance document. Office of Water; EPA/822/B-00/025. Available online at http://www.epa.gov/waterscience/biocriteria/stressors/stressorid.pdf.

U.S. EPA (Environmental Protection Agency). 2006. Causal Analysis/Diagnosis Decision Information System. www.epa.gov/caddis.

Wilton, T.F. 2004. Biological assessment of Iowa's wadeable streams. Project Report. Iowa Department of Natural Resources, Environmental Protection Division, TMDL and Water Quality Assessment Section. Des Moines, Iowa. Available at: http://wqm.igsb.uiowa.edu/wqa/streambio/index.html.

APPENDIX A

STATE OF IOWA METHODOLOGY

A.1. ECOREGION REFERENCE SITES

In Iowa, ecoregion reference sites represent contemporary stream conditions that are minimally disturbed by human activities. As they are used in bioassessment, reference sites define biological conditions against which other streams are compared. Therefore, they should not represent stream conditions that are anomalous or unattainable within the ecoregion.

Reference sites represent desirable, natural qualities that are attainable by other streams within the same ecoregion. IDNR evaluated a number of important watershed, riparian, and in-stream characteristics as part of the reference site selection process (Griffith et al., 1994; Wilton, 2004). Currently, there are 96 ecoregion reference sites used by IDNR for stream biological assessment purposes (see Figure A-1). Reference condition is the subject of a significant amount of research and development throughout the United States. The IDNR will continue to refine Iowa's reference condition framework as new methods and technologies become available.

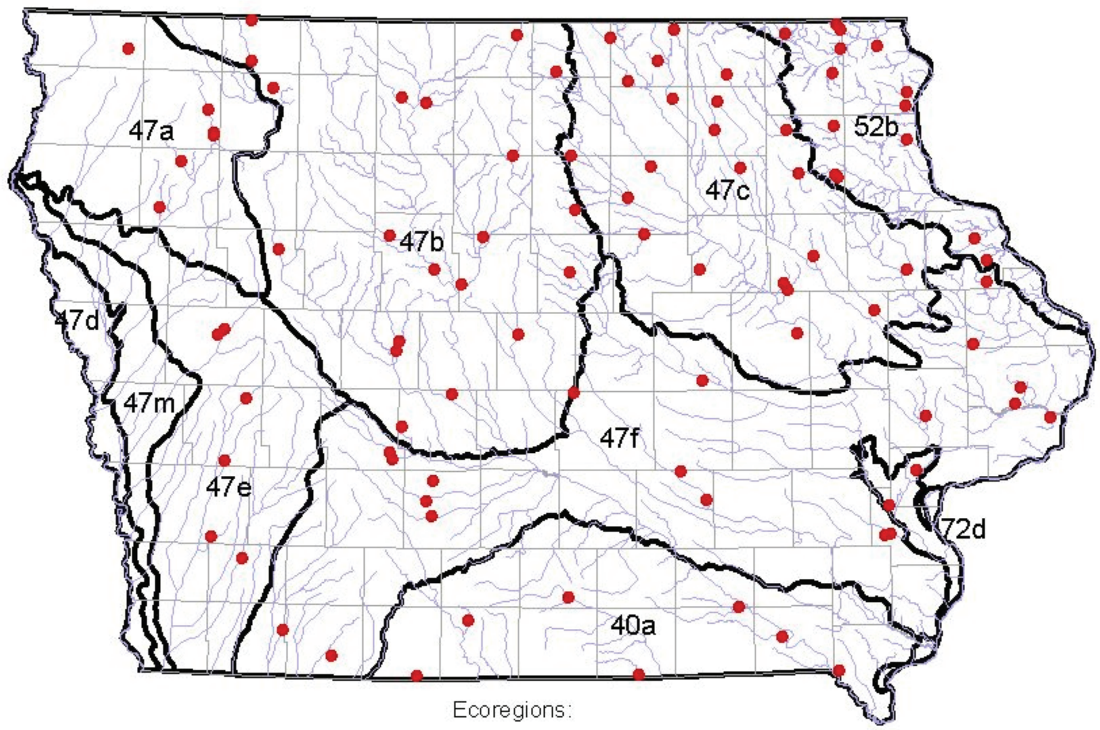
A.2. SAMPLING PROCEDURES

A.2.1. Biological and Habitat Parameters

The IDNR uses standard procedures for sampling stream benthic macroinvertebrates and fish assemblages to ensure data consistency between sampling sites and sampling years (IDNR, 2001a,b). Routinely, sampling is conducted during a 3-month index period (July 15–October 15) in which stream conditions and aquatic communities are relatively stable. A representative reach of stream ranging from 150–350 m is defined as the sampling area.

Two types of benthic macroinvertebrate samples are collected at each site: (1) Standard-Habitat samples are collected from rock or wood substrates in flowing water and (2) Multihabitat samples are collected by handpicking organisms from all identifiable and accessible types of benthic habitat in the sampling area. The multihabitat sample data improve the estimate of taxa richness for the entire sample reach. Benthic macroinvertebrates are identified in the laboratory to the lowest practical taxonomic endpoint.

IDNR samples fish using direct current (DC) electrofishing gear. In shallow streams, one or more battery-powered backpack shockers are used. A tote barge, generator-powered shocker is used in deeper, wadeable streams. Fish are collected in one pass through the sampling reach proceeding downstream to upstream. The number of individuals of each species is recorded, and individual fish are examined for



Ecoregions:

40(a) - Central Irregular Plains (Loess Flats and Till Plains)

47(a) - Western Corn Belt Plains (Northwest Iowa Loess Prairies) 47(b) - WCBP (Des Moines Lobe)

47(c) - WCBP (Iowan Surface) 47(d) - WCBP (Missouri Alluvial Plain) 47(e) - WCBP (Steeply Rolling Loess Prairies)

47(f) - WCBP (Rolling Loess Prairies) 47(m) - WCBP (Western Loess Hills)

52(b) - Driftless Area (Paleozoic Plateau) 72(d) - Central Interior Lowland (Upper Mississippi Alluvial Plain)

FIGURE A-1

Iowa Ecoregions and Wadeable Stream Reference Sites: 1994–2000. Little Floyd River is in Ecoregion 47a.

external abnormalities, such as deformities, eroded fins, lesions, and tumors (DELT). Most fish are identified to species in the field; however, small or difficult fish to identify are examined under a dissecting microscope in the laboratory.

IDNR evaluates physical habitat systematically at each stream sampling site. Different in-stream and riparian habitat variables are estimated or measured at 10 stream channel transects that are evenly spaced throughout the sampling reach. Summary statistics are calculated for a variety of physical habitat characteristics, and these data are used to describe the stream environment and provide a context for the interpretation of biological sampling results.

A.2.2. Physical and Chemical Parameters

Grab samples are collected and analyzed for a number of chemical parameters, including concentrations of ammonia nitrogen (as N), nitrate + nitrite nitrogen (as N), total Kjeldahl nitrogen (as N), 5-day carbonaceous biochemical oxygen demand (CBOD), total phosphate (as P), and TSS. Standard U.S. EPA-approved procedures are used in the analysis of all chemical constituents. Field measurements are recorded for flow, pH, DO, and water temperature.

Event monitoring involves the use of ISCO samplers equipped to determine stream stage. Composite samples are tested in the laboratory in the same way as grab samples. Field parameters are recorded when the samples are retrieved from the ISCO sampler.

Samples tested for pesticides and metals are collected and analyzed following REMAP protocols (IDNR, 2001c; IDNR 2002).

A.2.3. Biological Indices

Biological sampling data from ecoregion reference sites were used to develop a Fish Index of Biotic Integrity (FIBI) and a Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) (Wilton, 2004). The FIBI and BMIBI are described as multimetric or composite indices because they combine several individual measures or metrics. A metric is an ecologically relevant and quantifiable attribute of the aquatic biological community. A useful metric can be measured cost-effectively and reliably and responds predictably to specific environmental disturbances.

The FIBI and BMIBI indices each contain 12 metrics that reflect a broad range of aquatic community attributes (see Table A-1). Metric scoring criteria are used to convert raw metric data to normalized scores ranging from 0 (poor) to 10 (optimum). The normalized metric scores, which are weighted equally, are then combined to obtain the FIBI and BMIBI scores, which both have a possible scoring range from 0 (worst) to 100 (best). Table A-2 lists qualitative categories for FIBI and BMIBI scores. A detailed description of the FIBI and BMIBI development and calibration process is available on the IDNR Web page (http://wqm.igsb.uiowa.edu/wqa/streambio/index.html) (Wilton, 2004).

TABLE A-1

Data Metrics of the Benthic Macroinvertebrate Index of Biotic Integrity (BMIBI) and the Fish Index of Biotic Integrity (FIBI)

(BMIBI)	(FIBI)
1. MH-Taxa Richness	1. # Native Fish Species
2. SH-Taxa Richness	2. # Sucker Species
3. MH-EPT Richness	3. # Sensitive Species
4. SH-EPT Richness	4. # Benthic Invertivore Species
5. MH-Sensitive Taxa	5. % 3-Dominant Fish Species
6. % 3-Dominant Taxa (SH)	6. % Benthic Invertivores
7. Biotic Index (SH)	7. % Omnivores
8. % EPT (SH)	8. % Top Carnivores
9. % Chironomidae (SH)	9. % Simple Lithophil Spawners
10. % Ephemeroptera (SH)	10. Fish Assemblage Tolerance Index
11. % Scrapers (SH)	11. Adjusted Catch Per Unit Effort
12. % Dominant Functional Feeding Group (SH)	12. % Fish with DELTs

MH = Multihabitat sample.
SH = Standard-habitat sample.

TABLE A-2 Qualitative Scoring Guidelines for the BMIBI and FIBI		
Biological Condition Rating	BMIBI	FIBI
Poor	0–30	0–25
Fair	31–55	26–50
Good	56–75	51–70

BMIBI = Benthic Macroinvertebrate Index of Biotic Integrity.
FIBI = Fish Index of Biotic Integrity.

A.3. REFERENCES

Griffith, G.E., Omernik, J.M., Wilton, T.F., et al. 1994. Ecoregions and subecoregions of Iowa: a framework for water quality assessment and management. J. Iowa Acad. Sci. 10(1):5–13.

IDNR (Iowa Department of Natural Resources). 2001a. Biological sampling procedures for wadeable streams and rivers in Iowa. June 30, 1994 revised May 3, 2001. Environmental Protection Division, Water Resources Section; Des Moines, Iowa; 15 p. + appendices.

IDNR (Iowa Department of Natural Resources). 2001b. Habitat evaluation procedures for wadeable streams and rivers in Iowa. June 30, 1994 revised May 3, 2001. Environmental Protection Division, Water Resources Section; Des Moines, Iowa: 17 p. + appendices.

IDNR (Iowa Department of Natural Resources). 2001c. A probabilistic survey of Iowa's stream resources: R-EMAP project proposal. July 9, 2001. Environmental Protection Division, Water Resources Section; Des Moines, Iowa: 13 p. + appendices.

IDNR (Iowa Department of Natural Resources). 2002. Quality assurance project plan for a Region 7 REMAP project: a probabilistic survey of Iowa's wadeable streams. October 28, 2002. Environmental Protection Division, Water Resources Section; Des Moines, Iowa: 17 p. and appendices.

Wilton, T.F. 2004. Biological assessment of Iowa's wadeable streams. Project Report. Iowa Department of Natural Resources, Environmental Protection Division, TMDL and Water Quality Assessment Section; Des Moines, Iowa. Available at: http://wqm.igsb.uiowa.edu/wqa/streambio/index.html.

APPENDIX B

DATA SUMMARY

TABLE B-1

Monthly Monitoring Results for the Little Floyd River at Sites 2, 3, and 4

Collection Date	Flow Rate (cfs)	DO (mg/L)	Temp (°C)	pH	Ammonia Nitrogen as N (mg/L)	TKN as N (mg/L)	NO_3 + NO_2 as N (mg/L)	CBOD (20-d) (mg/L)	CBOD (5-d) (mg/L)
Site 2									
4/10/2001	34.5	10.2	6.8	8	<0.1	0.6	13	--	<2
5/8/2001	100	10.7	9.6	8	<0.1	<0.1	18	--	<2
6/12/2001	30	9.5	15.9	8	<0.1	<0.1	17	--	<2
7/17/2001	7	10	25.7	8.2	<0.1	0.5	10	--	<2
8/14/2001	3.7	7.5	17.9	8.1	<0.1	0.9	9.1	--	<2
9/12/2001	1.6	6.2	16	8.3	0.03	0.56	5	--	<2
10/9/2001	3.3	8.6	11.9	8.3	<0.05	1.1	4.7	--	2
11/13/2001	2.2	9.3	10.2	8.4	<0.05	0.67	4.4	--	<2
3/11/2003	0.6	6.7	0	7.8	0.12	0.44	5	3	--
4/8/2003	1.7	15.3	2.1	8.4	<0.05	0.68	4.5	8	--
5/13/2003	32.6	9.9	10.6	8.2	<0.05	0.62	13	6	--
6/10/2003	24	12.3	16.3	8.2	<0.05	0.7	13	35	--
7/8/2003	108.8	6.9	16.8	7.6	<0.05	1.7	7.6	20	--
8/12/2003	2.5	7	22.6	8.1	<0.05	0.74	4.8	5	--
9/9/2003	1.2	10	19.4	8.2	0.05	1.1	3	9	--
10/14/2003	1.9	9	8.8	8.1	<0.05	0.65	3.1	4	--

TABLE B-1. cont.									
Site 3									
7/24/2002	2	10.9	25	8.1	<0.05	1	3	--	--
8/6/2002	2	8.5	25.7	8.3	0.32	1.6	1.6	--	--
8/22/2002	5.6	6.2	21.3	8.1	0.21	11	1.6	--	--
Site 4									
3/13/2001	1.2	9.9	0	7.8	<0.1	0.2	6.5	--	3
4/10/2001	33.5	10.6	8.1	8.1	<0.1	0.7	13	--	<2
5/8/2001	107	10.5	10.9	8	<0.1	0.1	18	--	<2
6/12/2001	35	9.5	16.6	7.9	<0.1	<0.1	17	--	<2
7/17/2001	9	13.4	27.6	8.5	<0.1	<0.1	10	--	<2
8/14/2001	4.9	8.6	18.3	8.2	<0.1	0.7	9.2	--	<2
9/11/2001	1.9	12.2	22.1	8.5	0.04	0.87	3.8	--	3
10/9/2001	3.5	8.6	12.9	8.4	<0.05	0.9	4.9	--	2
11/13/2001	9	10.3	10.1	8.7	<0.05	0.6	3.9	--	<2
3/11/2003	0.6	3.3	0	7.7	0.18	1.2	5.9	4	--
4/8/2003	2	16	0.9	8.3	<0.05	0.4	4.6	7	--
5/13/2003	31.6	9.1	11.1	8.2	<0.05	0.63	13	5	--
6/10/2003	18	10.1	17.4	7.9	<0.05	0.83	13	30	--
7/8/2003	118.5	7	17.2	7.8	0.15	3	8.8	24	--
8/12/2003	7.3	9.9	25.8	8.2	0.06	0.9	4.3	4	--
9/9/2003	0.9	7.3	20.2	8.2	<0.05	0.69	2	7	--
10/14/2003	1.7	10.5	9.6	8.2	0.06	0.6	2.2	6	--

TABLE B-1. cont.						
Collection Date	Total Phosphorus as P (mg/L)	Orthophosphate as P (mg/L)	TSS (mg/L)	TVSS (mg/L)	Silica as SiO_2 (mg/L)	Specific Conduct-ance (µS/cm)
Site 2						
4/10/2001	0.1	<0.1	37	--	--	740
5/8/2001	0.1	0.14	18	--	--	780
6/12/2001	<0.1	0.03	6	--	--	760
7/17/2001	<0.1	0.07	6	--	--	730
8/14/2001	0.15	0.07	29	--	--	790
9/12/2001	0.16	0.05	15	--	--	700
10/9/2001	0.61	0.49	70	--	--	1300
11/13/2001	0.47	<0.05	28	--	--	1200
3/11/2003	0.06	0.06	2	<1	19	750
4/8/2003	0.12	<0.05	24	5	--	720
5/13/2003	0.09	0.06	13	3	--	820
6/10/2003	0.07	0.04	22	4	--	860
7/8/2003	0.87	0.54	220	37	--	440
8/12/2003	0.23	0.21	10	1	--	750
9/9/2003	0.28	0.12	97	13	--	700
10/14/2003	0.11	0.08	24	4	--	750
Site 3						
7/24/2002	0.23	0.14	16	3	22	--
8/6/2002	0.31	0.26	57	6	25	--
8/22/2002	0.28	0.12	62	8	21	--

TABLE B-1. cont.						
Site 4						
3/13/2001	0.3	0.2	6	--	--	760
4/10/2001	0.2	0.1	24	--	--	740
5/8/2001	0.1	0.12	21	--	--	770
6/12/2001	1.2	0.03	9	--	--	750
7/17/2001	<0.1	0.05	5	--	--	700
8/14/2001	0.22	0.03	14	--	--	820
9/11/2001	0.16	0.02	4	--	--	620
10/9/2001	0.41	0.37	28	--	--	1300
11/13/2001	0.49	0.53	8	--	--	1300
3/11/2003	0.12	0.06	37	5	18	810
4/8/2003	0.07	<0.05	11	2	--	700
5/13/2003	0.08	<0.05	25	4	--	820
6/10/2003	0.1	0.05	33	5	--	840
7/8/2003	0.84	0.44	260	46	--	540
8/12/2003	0.22	0.17	16	2	--	740
9/9/2003	0.15	0.08	41	7	--	690
10/14/2003	0.1	0.05	34	4	--	710

TKN = total Kjeldahl nitrogen.
TVSS = total volatile suspended solids.

TABLE B-2

Event Sampling Results at Site 4 in the Little Floyd River

Collection Date	Flow Rate (cfs)	DO (mg/L)	Temp (°C)	pH	Ammonia Nitrogen as N (mg/L)	TKN as N (mg/L)	NO_3 + NO_2 as N (mg/L)	CBOD (20-d) (mg/L)	CBOD (5-d) (mg/L)
3/28/2001	116.4	11.7	0.4	7.5	0.3	1.5	6.9	--	<2
7/25/2001	113	7.8	21.8	7.9	<0.1	1.6	13	--	2
4/9/2003	5.3	16.9	4.4	8.4	<0.05	0.48	3.9	2	--
11/1/2003 (grab)	1.6	10	4.1	8.2	--	--	--	--	--
11/1/2003 (prepeak)	--	--	--	--	4.9	5.6	4.7	26	--
11/1/2003 (postpeak)	--	--	--	--	6.5	9	4.7	40	--

Collection Date	Total Phosphorus as P (mg/L)	Orthophosphate as P (mg/L)	TSS (mg/L)	TVSS (mg/L)	Specific Conductance (µS/cm)
3/28/2001	0.6	0.5	38	--	430
7/25/2001	0.6	0.5	81	--	490
4/9/2003	0.06	<0.05	18	3	640
11/1/2003 (grab)	--	--	--	--	--
11/1/2003 (prepeak)	0.34	0.1	38	10	760
11/1/2003 (postpeak)	0.61	0.2	66	18	810

TABLE B-3

Metrics Calculated from the Biological Samples Collected from the Little Floyd River, 1999–2002

Metrics	9/14/1999 Site 1	9/12/2001 Site 2	8/22/2002 Site 3	9/11/2001 Site 4
Drainage Area (km^2)	109	119	142	154
Reach Length (m)	174	202	183	191
FIBI				
Total Fish (#)	1063	875	1068	2025
Total Species (#)	10	14	14	14
Native Species	5.1	6.8	6.3	6.2
Sucker Species	3.0	3.1	2.9	2.8
Sensitive Species	0	4.3	0	3.9
Benthic Invertivores	3.9	4.0	3.7	3.6
% Top 3 Abundant	4.4	5.1	3.3	5.4
% Benthic Invertivores	0.7	0.9	0.7	0.9
% Omnivore	6.4	8.5	7.1	8.4
% Top Carnivore	0	0	0	0
% Lithophilic Spawners	0	0	0	0
Tolerance Index	2.2	3.9	3.8	3.4
Adjusted CPUE	5.2	6.5	8.5	10
% DELT	0	0	0	0
BMIBI				
MH Total Taxa	4.0	6.1	4.8	5.6

TABLE B-3 cont.				
Metrics	9/14/1999 Site 1	9/12/2001 Site 2	8/22/2002 Site 3	9/11/2001 Site 4
SH Total Taxa	6.6	10	7.2	6.0
MH EPT Taxa	2.7	6.3	4.7	3.4
SH EPT Taxa	5.9	9.2	5.9	6.1
Metrics	9/14/1999 Site 1	9/12/2001 Site 2	8/22/2002 Site 3	9/11/2001 Site 4
MH Sensitive Taxa	0	1	2.9	1.9
SH Ephemeroptera %	3.6	5.0	5.6	2.7
SH EPT %	4.3	6.3	5.6	2.6
SH Chironomid %	5.0	8.2	6.9	3.1
SH Scraper %	2.5	3.9	2.5	0.6
SH Top 3 Dominant %	4.7	9.3	6.2	2.4
SH Dominant FFG %	4.5	8.4	5.3	2.0
Mod. Hilsenhoff Index	5	4.6	4.7	4.4

CPUE = catch per unit effort.
DELT = deformities, eroded fins, lesions, tumors.
EPT = Ephemeroptera, Plecoptera, and Trichoptera.
FFG = Functional feeding group.
MH = Multihabitat.
SH = Standard habitat.

TABLE B-4

Concentrations of Metals Found in Water and Sediment in the Little Floyd River at Site 3 on 8/22/2002. Probable effect concentrations for these substances are also given for comparison.

Parameter	Concentration in Water (mg/L)	Concentration in Sediment (mg/kg dry wt)	Probable Effect Concentration (mg/kg dry wt)*
Total Arsenic	<0.01	2.2	33
Total Cadmium	<0.001	<2	4.98
Total Chromium	<0.02	12	111
Total Copper	<0.01	8.8	149
Total Lead	<0.01	10	128
Total Mercury	<0.00005	<1	1.06
Total Nickel	<0.05	17	48.6
Total Selenium	<0.01	1.1	--
Total Silver	<0.01	<1	--
Total Zinc	<0.02	38	459

*Source: Ingersoll, C.G., MacDonald, D.D., Wang, N., et al. 2000. Prediction of sediment toxicity using consensus-based freshwater sediment quality guidelines. U.S. Geological Survey final report for the U.S. Environmental Protection Agency. EPA 905/R-00/007. Available online at: http://www.cerc.usgs.gov/pubs/center/pdfdocs/91126.pdf.

TABLE B-5

Concentrations of Common Pesticides and Pesticide Residue as Measured on 8/22/2002 at Site 3 in Sediments of the Little Floyd River.

Parameter	Concentration in Sediment (mg/kg)	Parameter	Concentration in Sediment (mg/kg)
Aldrin	<0.01	Endosulfan I	<0.01
alpha-BHC	<0.01	Endosulfan II	<0.01
alpha-Chlordane	<0.01	Endosulfan sulfate	<0.01
Aroclor 1016	<0.05	Endrin	<0.01
Aroclor 1221	<0.05	Endrin aldehyde	<0.01
Aroclor 1232	<0.05	Endrin ketone	<0.01
Aroclor 1242	<0.05	gamma-Chlordane	<0.01
Aroclor 1248	<0.05	Heptachlor	<0.01
Aroclor 1254	<0.05	Heptachlor epoxide	<0.01
Aroclor 1260	<0.05	Hexachlorobenzene	<0.01
beta-BHC	<0.01	Lindane (gamma-BHC)	<0.01
cis-Nonachlor	<0.01	Methoxychlor	<0.01
DDD	<0.01	Mirex	<0.01
DDE	<0.01	Pentachloroanisole	<0.01
DDT	<0.01	Propachlor	<0.01
delta-BHC	<0.01	Toxaphene	<0.1
Dieldrin	<0.01	trans-Nonachlor	<0.01

TABLE B-6

Habitat and Water Quality Measurements in the Little Floyd River and in the Ecoregion 47a (Northwest Iowa Loess Prairies) Reference Sites (Reference Samples 73 and 78 Represent a Single Site Sampled Twice [8/27/2002 and 7/9/1996] and 75 and 79 Represent a Single Site Sampled Twice [8/14/2001 and 8/3/1995])

Site #	Date	Stream Width (m)	Width: Depth	Std Dev Depth (cm)	Avg Thalweg Depth (cm)	Avg Depth (cm)	Max Depth (cm)	Riffle %	Pool %	Run %	Avg Embedd-edness	% Embedd-edness
LF1	9/14/1999	4.8	25	7	19	12.	55	0	48	52	NA	NA
LF2	9/12/2001	3.6	7.96	21	45	24	91	11	54	36	0	NA
LF3	8/22/2002	5.9	13.15	14	44	28	73	0	50	50	2	21–40
LF4	9/11/2001	5.2	22.99	7	23	12	37	5	23	71	0	NA
73	8/27/2002	2.7	8.33	11	33	23	82	9	62	29	2.5	41–60
74	9/14/2001	7.6	18.15	16	42	23	113	16	52	32	2	21–40
75	8/14/2001	5.2	13.98	11	37	21	82	14	23	62	2.5	41–60
76	10/7/1998	21.5	49.72	12	43	20	76	4	5	91	0	41–60
77	9/5/1996	9.4	41.62	6	23	11	46	4	0	96	0	NA
78	7/9/1996	4.2	11.60	13	36	24	79	18	23	59	0	41–60
79	8/3/1995	6.9	14.01	13	49.	26	107	28	20	52	0	41–60
80	8/2/1995	11.4	30.99	12	37	19	76	18	7	75	0	41–60

TABLE B-6. cont.

Site #	% Horizontal + %Vertical	% Moderate + % Undercut	Left Horiz. %	Left Moderate %	Left Vert.%	Left Undercut %	Right Horiz.%	Right Moderate %	Right Vert.%	Right Under-cut%
LF1	40	50	20	20	30	10	20	70	10	0
LF2	35	50	30	30	10	0	30	70	0	0
LF3	15	35	0	0	0	0	0	70	30	0
LF4	25	50	20	20	10	0	20	80	0	0
73	10	40	10	70	20	0	10	90	0	0
74	35	15	60	40	0	0	70	20	10	0
75	12.5	37.5	10	90	0	0	40	60	0	0
76	22.5	27.5	30	60	10	0	30	40	20	10
77	0	0	0	0	0	0	0	0	0	0
78	0	0	0	0	0	0	0	0	0	0
79	0	0	0	0	0	0	0	0	0	0
80	0	0	0	0	0	0	0	0	0	0

TABLE B-6. cont.														
Site #	Total Coarse	Total Fines	Clay %	Silt %	Sand %	Soil %	Gravel %	Cobble %	Boulder %	Rip Rap %	Detritus/ Muck %	Wood %	Bed-rock %	Other Sub-strate %
LF1	5	95	6	58	31	0	3	2	0	0	0	0	0	0
LF2	25	75	3	32	40	0	19	0	0	6	0	0	0	0
LF3	19	80	4	29	47	0	19	0	0	0	0	0	0	1
LF4	15	85	2	42	41	0	11	0	0	4	0	0	0	0
73	56	43	0	26	17	0	31	17	8	0	0	0	0	1
74	38	62	0	13	43	6	32	4	2	0	0	0	0	0
75	43	55	2	22	31	0	20	21	2	0	1	1	0	0
76	7	93	0	13	80	0	5	2	0	0	0	0	0	0
77	4	94	0	4	90	0	4	0	0	0	0	0	0	2
78	66	28	0	6	22	0	52	10	4	0	0	0	0	6
79	36	56	6	6	44	0	8	28	0	0	2	2	0	4
80	32	66	0	14	52	0	26	6	0	0	2	0	0	0

TABLE B-6. cont.

Site #	Lt Bare Bank (%)	Rt Bare Bank (%)	Lt Buffer Width	Lt Buffer Avg	Rt Buffer Width	Rt Buffer Avg	Instream Cover (%)	Woody Debris (%)	Avg Canopy Shade	Canopy Std Dev	Canopy Max Shade	Canopy Min Shade
LF1	48	36	--	100	--	52	1	0	5	12	60	0
LF2	49	64	--	8	--	8	12	0	15	22	41	5
LF3	11	22	--	42	--	22	8	0	12	21	32	2
LF4	41	42	--	33	--	33	2	4	6	12	15	0
73	7	5	--	100	--	100	28	0	5	8	18	0
74	53	90	--	93	--	100	2	29	27	23	53	0
75	17	24	--	100	--	100	2	0	13	24	32	0
76	60	63	40-100+	0	50-100+	0	2	18	29	34	52	6
77	73	88	100+	0	100+	0	0	18	6	11	16	0
78	24	36	100+	0	100+	0	6	0	24	35	48	0
79	29	31	80-100	0	50–100	0	7	7	17	20	23	1
80	53	42	100+	0	<10	0	1	4	6	17	26	0

TABLE B-6. cont.

Site #	Ammonia Nitrogen as N (mg/L)	Atrazine Screen (μg/L)	DO (mg/L)	Field pH	Field Temp (°C)	Flow Rate (cfs)	NO_2 + NO_3 as N (mg/L)	Specific Conductance (μS/cm)	TKN (mg/L)	TDS (mg/L)	TSS (mg/L)	Total Hardness (mg/L as $CaCO_3$)	Total Phosphorus as P (mg/L)	Turbidity (NTU)
LF1	--	--	15.3	8.1	18	0.7	--	--	--	--		--	--	--
LF2	0.03	--	6.2	8.3	16	1.6	5	700	0.56	--	15	--	0.16	--
LF3	0.21	--	6.2	8.1	21.3	5.6	1.6	--	11	350	62	--	0.28	33
LF4	0.04	--	12.2	8.5	22.1	1.9	3.8	620	0.87		4	--	0.16	--
73	M	0.14	9.50	7.3	18.5	27.0	9.40	770	0.40	410	11	375	0.10	10
74	M	0.12	8.90	7.1	17.5	19.0	12.00	880	0.60	480	56	430	0.10	31
75	M	0.28	8.70	7.9	14.3	5.0	9.90	730	0.40	410	38	400	0.10	26
76	M	0.14	7.30	7.4	20.0	8.0	0.50	870	0.90	490	33	410	0.10	18
77	M	0.10	10.20	8.3	12.7	38.5	7.40	720	0.80	490	14	370	0.10	8
78	0.10	0.14	9.20	8.3	19.0	5.5	9.00	850	0.65	520	7	420	0.02	4
79	0.10	0.11	6.80	8.1	18.3	11.8	5.10	680	1.20	420	23	330	0.02	18
80	0.07	0.09	5.40	7.9	24.9	1.6	5.90	760	0.90	450	9	400	0.05	9

NTU = Nephlometric Turbidity Units.

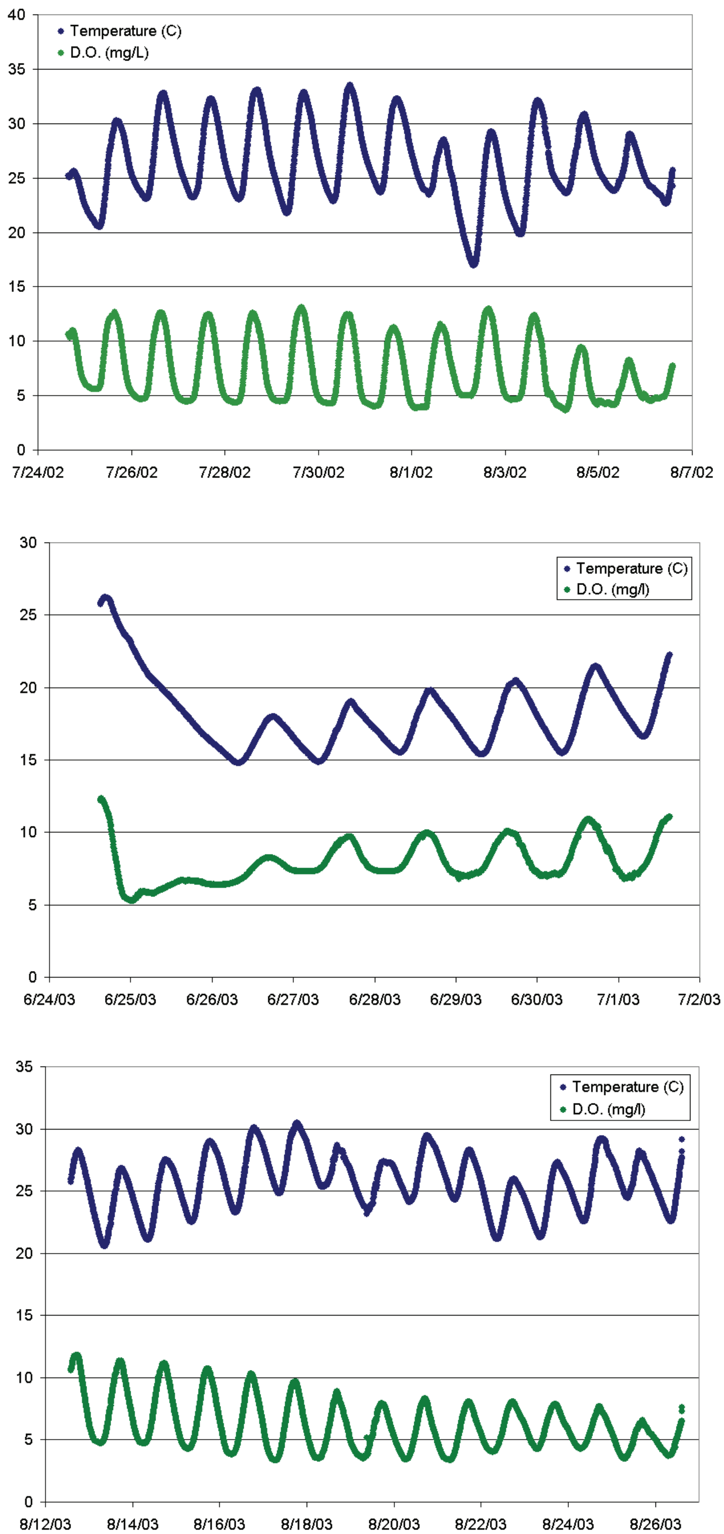


FIGURE B-1

Diurnal Temperature and Dissolved Oxygen Measurements in the Little Floyd River (a) at Site 3 from July 24 to August 6, 2002; (b) at Site 4 from June 24 to July 2, 2003; and (c) at Site 4 from August 12 to August 27, 2003

APPENDIX C

ANALYSIS OF EVIDENCE TABLES

TABLE C-1

Evidence of Spatial/temporal Co-occurrence in the Little Floyd River, Iowa

Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
Altered Flow Regime (see Figure 6)				NE	
Decreased Flow Heterogeneity				NE	NE
Increased Maximum Flow				NE	NE
Increased Frequency of Low Flows				NE	NE
Increased Sediment (see Figure 7)				Suspended: 0	Deposited: +
Increased Suspended Sediment	TSS (mg/L)	38.8	Missing (M); 45.0; 33.3	NE; yes; no	0
Decreased Clarity	turbidity (NTU)	M	M; 21.6; 12.7	NE	NE
Decreased Algal Growth	seston chlorophyll *a* (µg/L)	M	M; 19.7; 7.4	NE	NE
	periphyton chlorophyll *a* (µg/cm^2)	M	M; 42; M	NE	NE

TABLE C-1. cont.					
Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
	sediment chlorophyll *a* ($\mu g/cm^2$)	M	M; 22; M	NE	NE
	gross primary production (GPP)	M	M; 11; 4.4	NE	NE
	production to respiration ratio (P:R)	M	M; 0.82; 0.37	NE	NE
Increased Deposited Fine Sediment	% total fines	75	95; 80; 85	yes; yes; yes	+
	% silt	32	58; 29; 42	yes; no; yes	0
	% sand	40	31; 47; 41	no; yes; no	0
	% total coarse	25	5; 19; 15	yes; yes; yes	+
	% total gravel	19	3; 19; 11	yes; no; yes	0
Loss of Pool Depth	% reach as pool habitat	54	48; 50; 23	yes; yes; yes	+
	maximum depth (cm)	91	55; 73; 37	yes; yes; yes	+
Embedded Riffle	% riffles	11	0; 0; 5	yes; yes; yes	+
	embeddedness rating	M	M; 21–40%; M	NE	NE
Burial of Organisms				NE	NE

TABLE C-1. cont.					
Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
Altered Basal Food Source (see Figure 8)					NE
Increased/ Altered Primary Producers	seston chlorophyll *a* (µg/L)	M	M; 19.7; 7.4	NE	NE
	periphyton chlorophyll *a* (µg/cm^2)	M	M; 42; M	NE	NE
	sediment chlorophyll *a* (µg/cm^2)	M	M; 22; M	NE	NE
	GPP	M	M; 11; 4.4	NE	NE
	P:R	M	M; 0.82; 0.37	NE	NE
Decreased Allochthon-ous Resources				NE	NE
Decreased DO (see Figure 9)					+
Decreased DO	lowest observed summer DO (mg/L) — daytime grab samples	6.2	15.3; 6.2; 4.6	no; no; yes	0

TABLE C-1. cont.

Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
	average summer DO (mg/L) before 10 am — daytime grab samples	7.8	M; M; 8.1	NE; NE; no	0
	lowest observed summer DO (mg/L) — continuous monitoring	M	M; 4.5; 3.5	NE	NE
	minimum DO (mg/L) daytime grab samples	6.2	15.3; 6.2; 3.3	no; no; yes	0
	ratio, highest to lowest summer DO	1.6	M; 1.8; 2.9	NE; yes; yes	+
Increased Temperature (see Figure 10)				+	
Increased Temperature	mean ^{o}C from summer grab samples	17.5	18; 24; 21	yes; yes; yes	+
	maximum ^{o}C from summer grab samples	25.7	18; 25.7; 27.6	no; no; yes	0
	diurnal mean (^{o}C)	M	M; 26.2, 25.7	NE	NE
	diurnal maximum (^{o}C)	M	M; 33.4; 30.4	NE	NE

TABLE C-1. cont.					
Spatial/Temporal Co-occurrence					
Proximate Stressor	Measure of Exposure	Measurement at Internal Comparator (Site 2)	Measurements at Sites 1, 3, and 4, Respectively	Evidence of Co-occurrence	Score
Increased Ammonia (see Figure 11)				+	
Increased Ammonia	mean ammonia (mg/L) from grab samples	0.07	M; 0.19; 0.08	NE; yes; yes	+
	maximum ammonia (mg/L) from grab samples	0.12	M; 0.32; 0.18	NE; yes; yes	+

NE = indicates no evidence.

<table>
<tr><td colspan="5">TABLE C-2

Evidence Used to Assess Complete Causal Pathway for Candidate Causes in the Little Floyd River, Iowa</td></tr>
<tr><td colspan="5">Complete Causal Pathway</td></tr>
<tr><td>Step in Pathway</td><td>Measure of Exposure</td><td>Measurement at Comparator and Ecoregion Reference Sites</td><td>Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River</td><td>Score for Pathway/ Step</td></tr>
<tr><td colspan="4">Altered Flow Regime (see Figure 6)</td><td>0</td></tr>
<tr><td rowspan="4">Decrease in Large Woody Debris</td><td rowspan="2">% woody debris</td><td>S2: 0</td><td>S1,3,4: 0; 0; 4</td><td>-</td></tr>
<tr><td>IR: 0–18 (n = 8)</td><td>1.0 mean of all 4 sites</td><td>0</td></tr>
<tr><td rowspan="2">% wood substrate</td><td>S2: 0</td><td>S1,3,4: 0; 0; 0</td><td>0</td></tr>
<tr><td>IR: 0–0.25 (n = 8)</td><td>0 at all 4 sites</td><td>0</td></tr>
<tr><td>Increased Deposited Fine Sediment</td><td colspan="3">See spatial/temporal co-occurrence for increased sediment.</td><td>+</td></tr>
<tr><td rowspan="3">Decreased Infiltration, Increased Runoff</td><td>% annual row crop</td><td rowspan="3"></td><td rowspan="3"></td><td rowspan="3">NE</td></tr>
<tr><td>% perennial vegetation</td></tr>
<tr><td>% urban</td></tr>
</table>

TABLE C-2. cont.				
Complete Causal Pathway				
Step in Pathway	Measure of Exposure	Measurement at Comparator and Ecoregion Reference Sites	Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River	Score for Pathway/ Step
Decreased Sinuosity	sinuosity (stream length/straight line)	IR: 1.3–2.4 (*n* = 5)	Avg. approx. 1.5 (3 of 4 sites channelized); 49% (Site 3) and 9% (Sites 1&2) decrease in sinuosity ca. 1970s; no change from 1992 to 2002.	0
Increased Velocity	stream gradient (ft/mi)	IR: 5–20 (*n* = 5)	Sample site avg. approx. 5; However, 17% overall main channel slope increase since 1964.	-
Increased Sediment (see Figure 7) Suspended: + Deposited: +				
Decreased Bank Stability	% vertical bank	S2: 5	S1,3,4: 20; 15; 5	+
		IR: 0–6.3 (*n* = 8)	11.3 mean of all 4 sites	+
Increased Velocity	See causal pathway for altered flow regime.			+
Increased Stream Power				NE
Increased Channel and Bank Erosion	% horizontal + % vertical bank	S2: 35	S1,3,4: 40; 15; 25	0
		IR: 0–20 (*n* = 8)	14.4 mean of all 4 sites	-
	% bare bank	S2: 56	S1,3,4: 42; 16; 41	-
		IR: 28–64 (*n* = 8)	40 mean of all 4 sites	-
Increased Primary Producers	See spatial/temporal co-occurrence for altered basal food source.			NE
Increased Soil Erosion	revised universal soil loss equation measurements		16,300 tons/year (2 tons/acre/year)	NE

TABLE C-2. cont.				
Complete Causal Pathway				
Step in Pathway	Measure of Exposure	Measurement at Comparator and Ecoregion Reference Sites	Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River	Score for Pathway/ Step
Increased Input of Fine Particles				NE
Decreased Light				NE
Decreased Water Depth	average depth (cm)	S2: 24	S1,3,4: 12; 28; 12	0
		IR: 20–23 (*n* = 8)	19 mean of all 4 sites	+
	average thalweg depth (cm)	S2: 45	S1,3,4: 19; 44; 23	+
		IR: 35–42 (*n* = 8)	33 mean of all 4 sites	+
	standard deviation of depth (cm)	S2: 21	S1,3,4: 7; 14; 7	+
		IR: 11–13 (*n* = 8)	12 mean of all 4 sites	-
Altered Basal Food Source (see Figure 8)				++
Increased Nutrients	nitrate + nitrite (mg/L)	IR: 5.3–9.8 (*n* = 8)	5.7 at Site 2 (mean; *n* = 7); 2.1 at Site 3 (mean; *n* = 3); 5.5 at Site 4 (mean baseflow and storm event; n = 10)	-
	total phosphorus (mg/L)	IR: 0.03–0.10 (*n* = 8)	0.23 at Site 2 (mean; *n* = 7); 0.27 at Site 3 (mean; *n* = 3); 0.26 at Site 4 (mean baseflow and storm event; *n* = 10)	+
Increased Light	% shade	S2: 15	S1,3,4: 5; 12; 6	+
		IR: 6–25 (*n* = 8)	9.5 mean all 4 sites	0
	standard deviation of % shade	S2: 22	S1,3,4: 12; 21; 12	+
		IR: 15.5–26.3 (*n* = 8)	16.8 mean of all 4 sites	0

TABLE C-2. cont.				
Complete Causal Pathway				
Step in Pathway	Measure of Exposure	Measurement at Comparator and Ecoregion Reference Sites	Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River	Score for Pathway/ Step
Decrease in Large Woody Debris	See causal pathway for altered flow regime.			-
				0
				0
				0
Decreased Leaf Litter				NE
Decreased DO (see Figure 9)				+
Increased Primary Producers	See spatial/temporal co-occurrence for altered basal food source.			NE
Increased Organic Matter				NE
Increased Heterotrophs				NE
Increased Temperature	See spatial/temporal co-occurrence for increased temperature.			+
Increased Respiration	community respiration	7.8 mean from statewide random sites (n = 72)	13.3 at Site 3, (mean; n = 12 d); 11.1 at Site 4 (mean; n = 19 d)	+
Decreased Production: Respiration Ratio	production-to-respiration ratio (P:R)	0.68 mean from statewide random sites (n = 72)	0.82 at Site 3, (mean; n = 12 days); 0.37 at Site 4 (mean; n = 19 d)	0

TABLE C-2. cont.				
Complete Causal Pathway				
Step in Pathway	Measure of Exposure	Measurement at Comparator and Ecoregion Reference Sites	Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River	Score for Pathway/ Step
Decrease in Large Woody Debris	See causal pathway for altered flow regime.			-
				0
				0
				0
Decreased Riffles	% riffle	S2: 11	S1,3,4: 0; 0; 5	+
		IR: 5–18 (n = 8)	4.0 mean of all 4 sites	+
Decreased Turbulence				NE
Embedded Riffles	See spatial/temporal co-occurrence for increased sediment.			+
Decreased Aeration	reaeration coefficient			NE
Increased Temperature (see Figure 10)				++
Increased Light	See causal pathway for altered basal food source.			+
				0
				+
				0
Decreased Water Depth	See causal pathway for increased sediment.			0
				+
				+
				+
				+

TABLE C-2. cont.				
Complete Causal Pathway				
Step in Pathway	Measure of Exposure	Measurement at Comparator and Ecoregion Reference Sites	Measurements at the More-impaired Sites (1, 3, and 4) or Overall in the Little Floyd River	Score for Pathway/ Step
				-
Increased Frequency of Low Flows	See spatial/temporal co-occurrence for altered flow regime.			NE
Increased Ammonia (see Figure 11)				+
Increased Primary Producers	See spatial/temporal co-occurrence for altered basal food source.			0
Increased pH	mean pH from grab samples	IR: 7.3–8.3 (n = 8)	8.2 at Site 2 (mean; n = 7); 8.2 at Site 3 (mean; n = 3); 8.2 at Site 4 (mean baseflow and storm event; n = 10)	-
	pH range from grab samples	IR: 7.1–8.3 (n = 8)	8.1–8.3 at Site 2 (n = 7); 8.1–8.3 at Site 3 (n = 3); 7.8–8.5 at Site 4 (n = 10)	-
Increased NH_4^+	NH_4^+ (mg/L)			NE
Increased Temperature	See spatial/temporal co-occurrence for increased temperature.			+

S2 = Site 2 Less-Impaired Comparator.
S1, 3, 4 = Sites 1, 3, and 4.
IR = Interquartile Range for Regional Reference Sites.
NE = No evidence.

TABLE C-3 Stressor-Response from Other Field Studies for Candidate Causes in the Little Floyd River, Iowa					
Stressor-Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Altered Flow Regime (see Figure 6)					NE
Decreased Flow Hetero-geneity				NE	NE
Increased Maximum Flow				NE	NE
Increased Frequency of Low Flows				NE	NE
Increased Sediment (see Figure 7) Suspended: 0 Deposited: +					
Increased Suspended Sediment	TSS (mg/L)	10–37 interquartile range (*n* = 8)	36 at Site 2 (mean; *n* = 7); 45 at Site 3 (mean; *n* = 3); 29 at Site 4 (mean baseflow and storm event; *n* = 10)	no; yes; no	0
Decreased Clarity	turbidity (NTU)	8–24 interquartile range (*n* = 8)	22 at Site 3 (mean; *n* = 3); 14 at Site 4 (mean; *n* = 5)	no; no	-

TABLE C-3. cont.					
Stressor-Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Decrease in Algal Growth	seston chlorophyll *a* (μg/L)	32 mean (*n* = 72)	20 at Site 3 (mean; *n* = 3); 9.7 at Site 4 (mean; *n* = 5)	yes; yes	+
	periphyton chlorophyll *a* (μg/cm^2)	32 mean (*n* = 72)	38–45 (range; *n* = 2)	no	-
	sediment chlorophyll *a* (μg/cm^2)	27 mean (*n* = 72)	21–22 (range; *n* = 2)	yes	+
	gross primary production (GPP) and production to respiration ratio (P:R)	4.8, 0.68 (GPP, P:R), mean (*n* = 72)	10.9, 0.82 (GPP, P:R) at Site 3, (mean; *n* = 12 days); 4.4, 0.37 (GPP, P:R) at Site 4 (mean; *n* = 19 days)	no; yes	0
Increased Deposited Fine Sediment	% total fines	46–86 interquartile range (*n* = 8)	84 mean of all 4 sites (*n* = 4)	no	0
	% silt	6–20 interquartile range (*n* = 8)	40 mean of all 4 sites (*n* = 4)	yes	+
Loss of Pool Depth	% reach area as pool habitat	6–45 interquartile range (*n* = 8)	44 mean of all 4 sites (*n* = 4)	no	-

TABLE C-3. cont.

Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (n = 8) or Statewide Random Sites (n = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Stressor-Response Relationship from Other Field Studies					
	maximum depth (cm)	76–88 interquartile range (n = 8)	64 mean of all 4 sites (n = 4)	yes	+
Embedded Riffles	embedded-ness rating	41–60% at 6 of the 8 sites	not available (possibly could not be determined due to lack of gravel)	NE	NE
Burial of Organisms				NE	NE
Altered Basal Food Source (see Figure 8)					0
Increased/ Altered Primary Producers	seston chloro-phyll *a* (µg/L)	32 mean (n = 72)	20 at Site 3 (mean; n = 3); 9.7 at Site 4 (mean; n = 5)	no; no	-
	periphyton chloro-phyll *a* (µg/cm^2)	32 mean (n = 72)	38–45 (range; n = 2)	yes	+
	sediment chloro-phyll *a* (µg/cm^2)	27 mean (n = 72)	21–22 (range; n = 2)	no	-
	(GPP) (g O_2/m^2/d)	4.8 mean (n = 72)	10.9 at Site 3, (mean; n = 12 days); 4.4 at Site 4 (mean; n = 19 days)	yes; no	0

TABLE C-3. cont.					
Stressor-Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Decreased Allochthonous Food Resources				NE	NE
Decreased DO (see Figure 9)					0
Decreased DO	mean DO (mg/L) from daytime grab samples	6.9–9.4 interquartile range (*n* = 8)	8.3 at Site 2 (mean; *n* = 7); 8.5 at Site 3 (mean; *n* = 3); 8.8 at Site 4 (mean baseflow and storm event; *n* = 10)	no; no; no	-
	minimum DO (mg/L) from daytime grab samples	5.4 minimum from ecoregion reference sites (*n* = 8)	6.2 at Site 2 (*n* = 7); 6.2 at Site 3 (*n* = 3); 4.6 at Site 4 (*n* = 10)	no; no; yes	0
	diurnal mean DO (mg/l)		7.2 at Site 3; 7.9, 6.2 at Site 4	NE	NE
	diurnal minimum DO (mg/l)		4.5 at Site 3; 5.3, 3.5 at Site 4	NE	NE

TABLE C-3. cont.					
Stressor-Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
Increased Temperature (see Figure 10)					+
Increased Temperature	mean temp. (°C) from grab samples	15.1–19.8 interquartile range (*n* = 8)	17.5 at Site 2 (mean; *n* = 7); 24 at Site 3 (mean; *n* = 3); 21 at Site 4 (mean baseflow and storm event; *n* = 10)	no; yes; yes	0
	maximum temp. (°C) from grab samples	24.9 maximum (*n* = 8)	25.7 at Site 2 (*n* = 7); 25.7 at Site 3 (*n* = 3); 27.6 at Site 4 (*n* = 10)	yes; yes; yes	+
	diurnal mean temp. (°C)	19.8 mean (*n* = 72)	26.2 at Site 3; 23.2 at Site 4	yes; yes	+
	diurnal maximum temp. (°C)	26.2 mean (*n* = 72)	33.4 at Site 3; 30.4 at Site 4	yes; yes	+
Increased Ammonia (see Figure 11)					+
Increased Ammonia	ammonia nitrogen as N (mg/L)	0.085–0.10 interquartile range (*n* = 3)	0.06 at Site 2 (*n* = 7); 0.19 at Site 3 (*n* = 3); 0.08 at Site 4 (*n* = 10)	no; yes; no	0

TABLE C-3. cont.					
Stressor-Response Relationship from Other Field Studies					
Proximate Stressor	Measure of Exposure	Measurement at Regional Reference Sites (*n* = 8) or Statewide Random Sites (*n* = 72)	Measurements in the Little Floyd River	Evidence of Stressor Response	Score
	max ammonia nitrogen as N (mg/L) from grab samples	0.10 maximum for regional reference sites (*n* = 3)	0.12 at Site 2 (*n* = 7); 0.32 at Site 3 (*n* = 3); 0.18 at Site 4 (*n* = 10)	yes; yes; yes	+

NE = No evidence.