# DETERMINING PROBABLE CAUSES OF ECOLOGICAL IMPAIRMENT IN THE LITTLE SCIOTO RIVER, OHIO, USA: PART 1. LISTING CANDIDATE CAUSES AND ANALYZING EVIDENCE

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Abstract—The Little Scioto River in north-central Ohio, USA, is considered to be biologically impaired based on the results of fish and invertebrate surveys. The causes for these impairments were evaluated by means of a formal method. Two of the impairments identified on the stream reach were characterized in detail to support the causal assessment. A list of six candidate causes was developed that included habitat alteration, polycyclic aromatic hydrocarbon contamination, metals contamination, low dissolved oxygen, ammonia toxicity, and nutrient enrichment. Evidence for the causal evaluation was developed with data from the site that associated each candidate cause with the biological responses. Evidence was also developed that drew on data from other locations and laboratory studies, including comparisons of site exposures with screening values and criteria. The formal method increased the transparency of the assessment; candidate causes were clearly listed and the pathways by which they may have produced effects were shown. Analysis of the evidence maximized the utility of available data, which were collected as part of monitoring and research programs rather than to specifically support a causal assessment. This case study illustrates how the stressor identification method can be used to draw conclusions from available data about the most likely causes of impairment and to show what additional studies would be useful.

Keywords—Biological assessment Ecoepidemiology Ohio, USA Macroinvertebrates Fish

## INTRODUCTION

As the use of biological surveys and monitoring increases, interest has increased in improving methods for determining the causes of observed biological impairments. Although causes of impairments are often obvious to trained investigators, a formal method can improve the ability to communicate the evidence and logic that formed the basis of conclusions about cause. A formal method provides additional benefits, including minimizing opportunities for lapses in logic, improving the likelihood that obscure causes are identified, strengthening the defense of the results, and increasing confidence when undertaking costly remediation.

The case study described in this paper and its companion [1] followed a process described in the U.S. Environmental Protection Agency's stressor identification guidance [2] and Suter et al. [3]. This paper describes the beginning phases of a causal evaluation prompted by a biological impairment detected through monitoring of fish and macroinvertebrate assemblages. It presents the background of the investigation, the development of the list of candidate causes, and the organization and analysis of available evidence. The companion paper then uses the evidence to characterize causes by elimi-

nating impossible causes, and comparing the strength of evidence supporting the remaining causes [1].

This case study is not meant to illustrate an ideal causal assessment. The data used were those that were available from various studies, none of which were designed to support the stressor identification process and none of which were as extensive or intensive as we would have liked. However, the data are typical of the early phases of many investigations. This case study illustrates how the stressor identification method can be used to draw conclusions from available data about the most likely causes of biological impairment and to show what additional studies would be useful.

#### MATERIALS AND METHODS

#### Study area

The Little Scioto case study involves a 15-km reach of a river in north-central Ohio, USA, near Marion. The Little Scioto River drains primarily farmland in the northeastern quadrant of the Eastern Corn Belt Plains ecoregion (Fig. 1) [4]. The soils in this area are glacial till overlying limestone, dolomite, and shale bedrock. The water table has been lowered in much of the watershed by extensive use of tile drainage in crop fields.

Many point and nonpoint sources of pollutants are associated with the Little Scioto River (Fig. 1). Point sources include a wastewater treatment plant and combined sewer overflows that enter between 9.5 km and 10.5 km, respectively, upstream of the confluence with the Scioto River (Rkm 9.5

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Fig. 1. Map of the Little Scioto River, Ohio, USA, showing sites where fish were sampled. Approximate locations of significant physical features, tributaries, and point-source inputs are noted. The locations where impairments A and B were observed are also shown. The small inset shows the location of the study area in the state of Ohio. CSO = combined sewer overflow.

and 10.5). Nonpoint sources include runoff from agricultural land uses and from the city of Marion. Releases also may originate from several industrial areas, including an abandoned wood treatment plant, a landfill, an appliance plant, and a rail facility [5]. Finally, the stream was channelized in the early 1900s starting from Rkm 15 and continuing downstream to the confluence with the Scioto River.

# Data from the case

Information useful for causal evaluations can come from the case at hand, from other similar cases, and from biological knowledge [3]. Data from the Little Scioto included chemical analyses (sediment, water, and fish tissue), qualitative rankings of physical habitat quality, and biological surveys. The data were collected as part of Ohio Environmental Protection Agency (Ohio EPA) monitoring programs, and U.S. Environmental Protection Agency methods development research (Table 1). Data from 1992 provided the principal basis of the evaluation because they were collected during the same year as the biological data that were used to establish the impairment. In addition, the chemistry and habitat data were collected at locations that were considered spatially equivalent to the locations where the biological data were collected. Results from all 1992 sampling efforts are shown in Ohio EPA [5]. Relevant data also were available from sampling conducted during 1987, 1991, and 1998 [6-8]. These additional years of data were used to verify that the values observed in 1992 were not atypical, to evaluate the extent of year-to-year variability that might be expected, and to provide insights into uncertainties and gaps in the 1992 data. All years of data are shown in the U.S. Environmental Protection Agency stressor identification guidance document [2].

*Biological surveys.* The fish and macroinvertebrate assemblages both were surveyed at seven locations, by means of methods described by the Ohio EPA [9–11]. Macroinvertebrate communities were sampled with Hester–Dendy artificial sub-

Table 1. Summary of sampling locations for 1992 data collected in the Little Scioto River, Ohio, USA [5]

		Site designation and sample location (km upstream from confluence with Scioto River)							
Sample medium	Parameters <sup>a</sup>	Upstream site Site A Site B		Site B	Downstream sites			Number of samples per location	
Fish assemblage Macroinvertebrate assemblage	IBI and MIWB metrics ICI metrics	14.9 14.9	12.7 12.7	10.5 10.5	9.2 9.2	7.1 7.1	4.4 3.4	0.48 0.65	2–3 1
Sediment	Volatile chemicals semivol- atile chemicals, metals and cyanide, PCBs and pesticides	15.3	12.7	10.5	9.4	7.1	4.4	0.65	1–3
Water	Metals, phenolic com- pounds, ammonia, ni- trates and nitrites, total phosphorus, hardness, and biochemical oxygen demand	14.9	12.7	10.5	9.4	7.1	4.4	0.65	1
Water	Dissolved oxygen	14.8	12.7		9.4	7.1	4.4	0.58	Measured continu- ously over 3 d
Physical habitat Fish tissue	QHEI metrics Pesticides, PCBs, metals, semivolatile compounds, and percent lipid	14.9 14.9	12.7	10.5 10.5	9.2	7.1	4.4 4.4	0.48	2–5 composite sam- ples
White sucker bile	PAH metabolites		12.7	10.5	9.2	7.1	4.4	0.48	5–7

<sup>a</sup> IBI = index of biotic integrity; MIWB = modified index of well being; ICI = invertebrate community index; PCB = polychlorinated biphenyl; QHEI = qualitative habitat evaluation index; PAH = polycyclic aromatic hydrocarbon.



Fig. 2. Values of the fish index of biotic integrity (IBI) (**A**) and benthic macroinvertebrate invertebrate community index (ICI) (**B**) in the Little Scioto River, OHIO, USA, in 1992 [5]. WWH = warm-water habitat; MWH = modified warm-water habitat.

strate samplers, deployed for a six-week period. Fish were sampled by electrofishing. The upstream site at Rkm 2 was sampled in two passes over 250 m by a wading method. All other sites were sampled by electrofishing from a boat in three passes over 500 m of stream [11].

The Ohio EPA used the biological survey data to calculate the index of biotic integrity (IBI) and invertebrate community index (ICI) [9–11]. Average IBI and ICI values are shown in Figures 2A and B, respectively. A subset of metrics, described below, was used to characterize the impairments.

Sediment chemistry. Methods for the collection and analysis of sediment samples are described in Ohio EPA [5,12]. Finegrained sediment samples were collected in the upper 15 cm of bottom material at each location with decontaminated stainless steel scoops. Samples from 45 to 60 cm were taken with Teflon<sup>®</sup> core samplers. Sediment samples were analyzed for metals and cyanide, volatile organic chemicals, semivolatile organic chemicals (including polycyclic aromatic hydrocarbons [PAHs]), pesticides, and polychlorinated biphenyls. The full list of analytes is provided in Ohio EPA [5]. Volatile organic chemicals, pesticides, and polychlorinated biphenyls were generally not detected in the upper 15 cm. The exceptions were at Rkm 9.4 where 4,4'-dichlorodiphenyldichloroethane and 4,4'-dichlorodiphenyldichloroethylene were detected at concentrations considered to be elevated over background concentrations. Results for selected PAHs and metals are shown in Table 2. In addition, field investigators noted that a black material with a creosote odor occurred just below the surface layer of sediments from site B to Rkm 7.1 [5].

*Water chemistry.* Methods for the collection and analysis of surface water samples are described in Ohio EPA [5,12]. One water sample was taken at each location. Water samples were analyzed for metals, phenolic compounds, ammonia, nitrates and nitrites, total phosphorus, hardness, and biochemical oxygen demand (BOD). The full list of analytes is provided in Ohio EPA [5]. Dissolved oxygen measurements were taken at six locations over a 3-d period with Datasonde continuous monitors. Water hardness ranged from 278 to 329 mg/L CaCO<sub>3</sub>. Metals were detected in the water column at only a few locations. Copper was detected at site B at a concentration of 15  $\mu$ g/L. Lead was detected at 3  $\mu$ g/L at site B and one of the downstream sites. Zinc was detected only at sites downstream of site B at concentrations between 12 and 18  $\mu$ g/L. Concentrations of other selected parameters are shown in Table 3.

*Physical habitat quality.* The quality of the habitat was characterized with the qualitative habitat evaluation index [13]. The qualitative habitat evaluation index incorporates ordinal scores of habitat attributes including substrate type and quality; in-stream cover type and amount; channel morphology; riparian width and quality and bank erosion; pool and riffle characteristics including depth, current, pool morphology, substrate stability, and riffle embeddedness; and stream gradient. The scores for channel morphology, degree of siltation, and degree of substrate embeddedness are particularly relevant for this case and are shown in Table 4. Higher scores indicate higher quality sites.

Table 2. Average sediment concentrations (mg/kg dry wt) of selected polycyclic aromatic hydrocarbons and metals [5]. Samples were taken from the upper 15 cm<sup>a</sup>

	TT .			Downstream site (river km)				
Chemical	site	Site A	Site B	9.4	7.1	4.4	0.65	
Anthracene	ND <sup>b</sup>	ND	ND	27.1	7.9	ND	3.3	
Benzo[a]anthracene	ND	ND	8.2°	16.5	6.9	$2^{\circ}$	15.8	
Benzo[ghi]perylene	ND	ND	49.5	11.2	4.9	ND	6.9	
Benzo[ <i>a</i> ]pyrene	ND	ND	14.8°	15.8	7.2	ND	11.5	
Chrysene	ND	ND	16.5	20.8	9.9	1.6 <sup>c</sup>	ND	
Fluoranthene	ND	ND	8.2°	37.6	13.5	ND	22.4	
Fluorene	ND	ND	ND	7.0	4.0	ND	ND	
Cr	7.34	13.6	208	60.9	302	71.2	48.6	
Cu	7.44	17.2	79	56	76.8	42.4	24.5	
Pb	12.1	19.1	172	84.6	93.4	108	38	
Zn	3.06	79	173	141	226	408	96.8	
Hg	ND	ND	0.33	0.24	0.79	0.12	ND	

<sup>a</sup> Polycyclic aromatic hydrocarbons shown were either significantly correlated with biological responses, or exceeded sediment quality guidelines. <sup>b</sup> ND = Not detected.

<sup>c</sup> Estimated values (below quantitation limit).

Table 3. Concent	rations (mg/L) of	chemical in water	[5]. All data are	from 1992 except as	noted
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	¥¥.			Downstream sites (river km)			
Chemical	Upstream site	Site A	Site B	9.4	7.1	4.4	0.65
BOD <sup>a</sup>	1	1	2.3	4.7	4.2	3.5	2.2
Nitrate and nitrite	1.22	1.44	0.81	8.1	6.6	4.5	4.47
Ammonia	$ND^{b}$	ND	0.12	1.16	1.44	2.1	0.58
total phosphorus	0.06	0.07	0.09	2.17	1.96	1.8	1.34
Dissolved oxygen (minimum)	8.8	5.7	NAc	4.2	4.3	3.0	4.4
•• • • •	NA <sup>d</sup>	2.8 <sup>d</sup>	1.9 <sup>d</sup>	4.2 <sup>d</sup>	3.2 <sup>d</sup>	$2.0^{d}$	2.5 <sup>d</sup>

<sup>a</sup> BOD = biochemical oxygen demands.

 $^{b}$  ND = not detected.

 $^{\circ}$  NA = no data were collected.

<sup>d</sup> Data collected in 1987 by using continuous monitors over 3 d [6].

*Fish tissue chemistry*. Fish were collected at three locations for whole-body analyses of pesticides, polychlorinated biphenyls, metals, semivolatile compounds, and percent lipid (Table 1) [5,14,15]. Zinc was detected in common carp (*Cyprinus carpio*) from the upstream site (79.6 mg/kg), site B (68.3 mg/kg), and at Rkm 4.4 (15.8 mg/kg). Lead was detected in white suckers (*Catostomus commersoni*) at site B (81.4 mg/kg) and Rkm 4.4 (0.34 mg/kg). Bile samples from white suckers were analyzed for benzo[*a*]pyrene and naphthalene-type metabolites; results are shown in Figure 3.

*Experiments*. Ireland et al. [16] performed studies in which *Ceriodaphnia dubia* was experimentally exposed in situ chambers to epibenthic water in the Little Scioto at the upstream site and at Rkm 7.1.

#### Data from other similar cases and biological knowledge

Inferences concerning causes may be supported by data from laboratory studies or studies at other sites of the effects of the agents that comprise the candidate causes. For the chemical contaminants, we relied on numeric water quality criteria, benchmark values, summaries of aquatic toxicity data, and studies of similarly polluted rivers in Ohio. For the habitat factors, we relied on published reviews. These sources constitute evidence if they provide exposure–response relationships that can be related to exposures at the site or to the responses that constitute the distinct impairments.

#### Data analyses

The data from the Little Scioto and other cases were analyzed to define the impairment for the causal evaluation, to develop a list of candidate causes, and to examine associations between candidate causes and biological responses. This body of evidence was then used to characterize causes, as described in the companion paper [1].

## Defining the impairment

The term impairment is used in two senses in this paper. First is the official definition used by the state of Ohio to determine that a stream is not achieving its designated use. Impairment under this official definition provides the impetus for performing a causal analysis; it means that a community is in an unacceptable condition. Second, within officially impaired waters, it is necessary to identify impairments in terms of distinct and reasonably consistent sets of biological responses that are likely to have an identifiable cause. The causal analysis is facilitated by characterizing these distinct impairments in detail.

In Ohio, biological impairment is officially defined by standard multimetric indices including the IBI, the ICI, and the modified index of well being [11]. These indices were used to identify the reaches of the Little Scioto River that were considered to have impaired aquatic assemblages in comparison to the criteria for warm-water habitat (WWH) or modified warm-water habitat (MWH) designated uses.

The Little Scioto River is considered WWH above site A (Rkm 12.7), a designation shared by the majority of Ohio's rivers and streams [17]. This designation is narratively defined as supporting a balanced, reproducing aquatic community. Quantitatively, the minimum criteria required to be in attainment of WWH standards are defined as the 25th percentile values of reference condition scores for a given index, site type, and ecoregion.

The Little Scioto River is classified as MWH at and below site A (see Fig. 1). The MWH criteria are based on comparisons to a different reference condition than are used for the WWH criteria [17]. The MWH designation is a nonfishable aquatic life use, and is designed to protect streams that have been too physically impacted, or modified, to meet WWH standards. Modified warm-water habitat streams are unlikely to recover

Table 4.	Selected	physical	habitat	quality	scores	[5]

	T				Downstream s	sites (river km)	
Parameter	site	Site A	Site B	9.4	7.1	4.4	0.65
Channel	17	10	10	10	10	10	7
Substrate embeddedness <sup>a</sup>	2	1	1	1	1	1	1
Silt <sup>b</sup>	2	1	1	1	1	1	1

<sup>a</sup> Levels of embeddedness were scored from most to least: 1 = extreme; 2 = moderate; 3 = low; 4 = none.

<sup>b</sup> Levels of silt were scored from most to least: 1 = heavy; 2 = moderate; 3 = normal; 4 = free.



Fig. 3. Bile metabolites ( $\mu$ g/mg protein) measured in white suckers from the Little Scioto River, Ohio, USA, in 1992. Median levels of polycyclic aromatic hydrocarbon (PAH) metabolites below river kilometer 10.5 were as much as four times greater than the exposure criteria (dashed horizontal line), which are upper limits of concentrations considered background for the state of Ohio. The number of fish sampled is shown above each bar. Vertical lines are standard errors. A = site A; B = site B; BaP = benzo[a]pyrene; NAPH = naphthalene.

sufficiently to meet WWH designation. Consequently, MWH criteria are typically lower than WWH criteria. Modified warm-water habitat streams are able to support permanent assemblages of species tolerant of low DO, elevated ammonia and nutrient levels, increased siltation, and poor quality habitat.

To characterize distinct impairments, the responses of the biological assemblages were analyzed further by evaluating the changes in individual metrics at different locations on the Little Scioto. If the responses of an assemblage at different locations are distinct, then different causes are likely to be operating. Metrics selected for this analysis exhibited sensitivity to changing conditions along the stream. Because a shorter length of stream was sampled at the upstream location, we selected fish metrics that were expressed as a proportion of the fish sampled, or could be normalized to distance sampled. For fish (Fig. 4), we used the weight of fish normalized to a 1 km distance (relative fish weight) and the percent of fish having deformities, eroded fins, lesions, or tumors (DELT anomalies). For invertebrates, we used the percentage of individuals that were mayflies (percent mayflies), and the percentage of individuals that were from taxa considered to be tolerant of stress (percent tolerant invertebrates). Metrics not included were based on only a few organism counts (e.g., number of sunfish species), were unaltered over the stream reach (e.g., percentage of intolerant fish species), or were known to be correlated with confounding variables (e.g., the percentage of pioneering fish species is highly correlated with stream size). To simplify the analyses, we did not include metrics that were highly correlated with those already selected.

Distinct impairments were identified by comparing the value of individual metrics at adjacent locations, and by evaluating the general trend of metrics over the stream reach. Impairments were considered to be different if the metric values changed substantially, or if an overall trend changed direction. The determination was based on inspection of plots and professional judgment.



Fig. 4. Changes in fish and macroinvertebrate metrics in the lower 15 km of the Little Scioto River, Ohio, USA, 1992. Normalized values were calculated by dividing the value at the each site by the highest value for all sites. IBI = index of biotic integrity; ICI = invertebrate community index; DELT = deformities, eroded fins, lesions, or tumors; U = upstream site; A = site a; B = site B.

#### Development of the list of candidate causes

The list of candidate causes and associated conceptual models were developed by examining the available data on stressors and sources, by discussing the case with Ohio EPA biologists and other experts, and by applying our professional experience of stressors and their effects.

# Analyzing evidence

Suter et al. [3] discussed four types of associations that provide evidence useful for causal evaluations: associations between measurements of causes and effects, combining site exposure data with effects data from elsewhere, associations of measurements with mechanisms, and associations of the mitigation of effects with mitigation of exposure. The following paragraphs discuss these types of evidence.

Spatial associations between measurement of the candidate causes and effects were analyzed with data from the 15-km stream reach. Although we sought to use biological and chemical data from the same locations, in some cases, chemical measurements were recorded at a location that did not exactly coincide with the location of biological assessment (e.g., Rkm 15.3 and Rkm 14.9, for sediment chemistry and fish assemblage, respectively). Table 1 shows sampling locations that were considered equivalent by Ohio EPA biologists. Although sediment samples were taken at depth, we used only sediment chemistry values collected from the top 15 cm.

We used data from the site to analyze the relationship between candidate causes and response. The first analysis was limited to the measurements taken at the upstream site, site A, and site B. We evaluated whether any measurement associated with a candidate cause was detected at sites A or B and whether measurements indicated lower environmental quality as compared with the closest upstream location. Because each comparison used only two values, we used simple differences to conclude whether environmental quality improved or declined.

Data from all seven sites were used to correlate measurements associated with candidate causes and the biological responses. Spearman's rank correlations were used because many variables were not normally distributed. Chemistry concentrations that were below detection limits were given the lowest rank. Because our objective was to conservatively include causes, we used a p value of <0.10 to conclude that a correlation was significantly different from that expected from random processes.

We compared site exposure data to available criteria and screening values. Sediment screening values were available for PAHs and all of the metals except for mercury, although not for the community parameters or species of greatest interest for this study. Sediment effect concentrations are expressed as threshold effect level (TEL) and probable effect level (PEL) [18]. The interpretation is that toxicity to test organisms (Hyalella azteca and Chironomus riparius) would rarely occur at sediment concentrations below the TEL but would frequently occur above the PEL. Both TELs and PELs were developed from a large database consisting of chemistry data from fieldcollected sediments and associated toxicity test results. Because most of the sediments in the database contained multiple chemicals, these values should be interpreted with caution. Sediment effect concentrations developed for H. azteca and C. riparius were considered, but only the H. azteca values were used because C. riparius was always less sensitive. We used a modified toxic unit approach to account for exposure to multiple chemicals; the ratios of sediment concentration to PELs were added for the PAHs and for the metals.

We compared aqueous ammonia and metal concentrations with U.S. Environmental Protection Agency ambient water quality criteria, which are based on laboratory tests measuring effects on survival, growth, and reproduction [19,20]. The total ammonia criterion (which includes  $NH_3$  and  $NH_4^+$ ) varies with pH. High concentrations of hydroxide ions (i.e., high pH) increase the dehydration of the ammonium ion ( $NH_4^+$ ) to the more toxic unionized form ( $NH_3$ ). In 1998, pH values in the Little Scioto ranged between 7.4 and 8.4 and seemed to be independent of location [8].

We compared DO concentrations with Ohio EPA criteria. Ohio's criteria for minimum DO concentrations are 4.0 mg/L and 3.0 mg/L for WWH and MWH streams, respectively [21]. Nutrient concentrations were compared with Ohio's proposed statewide criterion [22]. The criterion for nitrate and nitrite is 1.6 mg/L for wadeable, WWH streams in the Eastern Corn Belt Plains having a drainage greater than 50 km<sup>2</sup> and less than 500 km<sup>2</sup>. For total phosphorus, the proposed state-wide criterion for MWH is 0.28 mg/L.

The third type of evidence is based on analyses of causal mechanisms and pathways. In the best case, available data would document a stressor's course from its sources and precursors, to the environmental medium, to contact or co-occurrence with organisms, into the organisms (when appropriate), to distinct physiological responses observed in exposed organisms. If a necessary step in the pathway can be shown to be missing, the candidate cause can be eliminated from consideration. In the Little Scioto, exposure pathways were evaluated primarily by examining the colocation of the available data representing different steps of the pathways. If no data were available for an intermediate step, the step may have occurred.

For the toxic chemical stressors, sediment concentrations were available. The exposure pathway was assumed to be complete for benthic invertebrates, which are in direct contact with the contaminated sediments, but may also be exposed via water or food to chemicals that have partitioned from the sediment into these other media. For fish, the pathway could be shown to be complete if water or food items were shown to be con-

 Table 5. Summary of the distinct impairments considered in the Little

 Scioto River, Ohio, USA

Response	Impairment A: Change in assemblage at site B relative to upstream site	Impairment B: Additional changes in assemblage at site B relative to site A
Fish Relative fish weight DELT <sup>a</sup> anomalies	Increased Increased	Decreased Increased
% Mayflies % Tolerant taxa	Decreased Increased	Decreased Increased

<sup>a</sup> DELT = deformities, eroded fins, lesions, or tumors.

taminated or if the fish themselves contained the chemicals or metabolites. As discussed above, data were available on fish tissue concentrations of chemicals and metabolites. For PAHs, we concluded that the pathway was complete if the PAH metabolite data indicated that exposure had occurred. For metals, fish tissue concentrations were available only at site B. Because data were not available for food items, we conservatively concluded that the pathway was complete if metals were found in the sediments.

The analysis of the exposure pathway for nutrients was straightforward; we assumed exposure occurred where nutrients were detected in the water column. The DO pathway was evaluated by examining colocation with precursors, such as BOD.

Symptoms that occur in response to exposure to a particular candidate cause can provide strong evidence of the mechanism. Deformities, fin erosion, tumors, and lesions on fish are potentially useful for this purpose. For example, some pathologies have been linked with toxic substances (e.g., liver tumors with PAHs) and others (e.g., skin lesions) with certain bacteria [23,24]. Unfortunately, insufficient detail was available during the case study to use anomalies to distinguish among these stressors.

#### RESULTS

# Defining the impairments

Of the seven sites sampled in 1992, the highest IBI score was 33 (out of a possible score of 60); it occurred at the upstream site (Fig. 2A). This score translates to a fair ranking according to WWH standards [17]. The remaining sites were described as severely impaired, with IBI scores between 25 and 12 (the lowest possible IBI score) [5,17]. Similarly, the ICI met WWH aquatic life use standards at the upstream site with a score of 38 (Fig. 2B). The ICI score from site A downstream was below MWH aquatic life use standards.

Examination of the spatial distribution of the IBI, ICI, and component metrics indicates that at least two distinct impairments occurred (Table 5). Impairment A was measured at site A (Rkm 12.7) where a marked drop in both the IBI and ICI occurred relative to the upstream site. Relative fish weight and DELT anomalies increased. For the invertebrates, the percentage of mayflies decreased, and the percentage of tolerant invertebrates increased. Impairment B was observed at site B (Rkm 10.5) and corresponded with an additional decrease in both the IBI and the ICI. Compared with the upstream site, the relative weight of fish and DELT anomalies increased. For invertebrates, the percentage of mayflies decreased, and the



Fig. 5. A conceptual model of the six candidate causes for the Little Scioto River, Ohio, USA. Potential sources are shown in the topmost rectangles. Potential stressors and interactions are located in ovals. Candidate causes are numbered 1 through 6. Note that some causes are associated with more than one stressor or more than one step. The impairments are shown in the lowest rectangle. DO = dissolved oxygen; BOD = biochemical oxygen demand; PAH = polycyclic aromatic hydrocarbon; UV = ultraviolet; DELT = deformities, eroded fins, lesions, or tumors.

percentage of tolerant invertebrates increased. Compared with the changes in metric values between the upstream site and site A, the changes measured between sites A and B are distinctive enough to suggest that either additional or completely different causes may be influencing the assemblage at site B. In particular, fish weight increased between the upstream site and site A, but then decreased again at site B. The other metrics indicated that environmental quality declined further. Relative to site A, at site B the DELT anomalies increased, and percentage of tolerant invertebrates increased; the percentage of mayflies decreased. To analyze the different causes influencing the assemblage at site B, we defined impairment B in terms of the additional changes relative to site A.

The biological assessment data for the remaining locations indicate that distinct effects also occur at the downstream locations. As discussed in the stressor identification guidance document [2], midges in the tribe Tanytarsini disappear completely at Rkm 9.2, with a concurrent increase in the percentage of *Cricotopus* spp. Further downstream, the pattern of impairment remained generally similar, with the possibility of intensification at Rkm 7.1 and some improvement in metric scores occurring at Rkm 4.4 and Rkm 0.65. The causal analysis of the impairment observed at Rkm 9.2 is discussed in the stressor identification guidance document [2], but is not included here for the sake of brevity.

## List of candidate causes

As discussed in Suter et al. [3], the causal evaluation focuses on the stressors that may actually contact organisms living in the stream, rather than the sources of those stressors. These stressors include toxic chemicals and excessive nutrients in the water column and sediment as well as physical stressors such as smothering of sediments. To simplify the analysis, we grouped stressors that emanate from the same source and would be expected to occur together. We separated toxic substances from nutrients or physical stressors, and further separated the toxic substances into chemical classes. Based on the knowledge of the sources and effects, six candidate causes were identified. A conceptual model of these candidates is provided in Figure 5.

*Habitat alteration.* Habitat alteration, resulting from channelization, includes several interacting stressors. Channelization can alter biological communities by changing the physical structure of the stream and the flow characteristics of the water, ultimately lowering DO, increasing siltation, and reducing substrate complexity. This complex suite of stressors also includes decreased woody debris, which reduces available substrate and changes the energy source; altered flow characteristics; increased erosion of banks; embedded substrates; increased channel depth; loss of pools; and loss of riffles that oxygenate water and transport sediment [25–28].

*PAHs and Metals.* Biological impairment also could have been caused by toxic stress. Historically, the river has provided a means of waste disposal for various industries, whose effluents have contained metals, PAHs, and creosote. Waste materials also may have been buried in the landfill downstream of site B [5]. All are potentially toxic to aquatic life, and some have the ability to bioaccumulate through the food web [29,30]. Thus, two candidate causes emerge: PAH exposure and metal exposure. Exposure of fish and macroinvetebrates to these contaminants could occur through food, water, and direct contact with sediment. Exposure to ultraviolet light enhances the toxicity of PAHs in sediments and water to both fish and invertebrates [16,31–33].

Ammonia toxicity. Ammonia is directly discharged into streams by point sources [34,35]. Ammonia also can be formed as the result of nutrient enrichment. When DO levels are low, nitrates are reduced to ammonium ion. Some of the ammonium ion is converted to unionized ammonia, which is toxic to aquatic organisms [34]. The proportion of total ammonia present as unionized ammonia is a function of pH, and the proportion increases as pH increases. Moreover, pH may rise during periods of high photosynthetic rates from bicarbonate depletion. High concentrations of nutrients often lead to increased algal growth rates, and the conversion of ammonium to unionized ammonia is expedited [36].

High BOD and low DO. Depletion of dissolved oxgyen commonly occurs from organic enrichment [37]. Organic enrichment is the most common cause of increased BOD [28]. Potential sources of excess organic matter within the study area include a wastewater treatment plant and several combined sewer outfalls, as well as upstream, nonpoint sources. Organic matter also is produced by excess algal growth from nutrient enrichment [36], and nocturnal respiration of algae contributes further to DO depletion. Because no chlorophyll a or algal biomass data were collected in this study, the pathway of DO depletion can be traced back only to BOD.

Nutrient enrichment. The sixth and final candidate cause is a less extreme form of nutrient enrichment. Primary production and organic matter loading to the sediments are increased, but not enough to reduce DO. This can cause changes in fish and benthic macroinvertebrate assemblages, including changes in dominant species, and greatly increased abundance and biomass [22,36-38]. This form of nutrient enrichment is also associated with DELT anomalies [22].

## Evidence for causal characterization

Although high-quality data are essential, they are not, in themselves, causal evidence. Causal evidence consists of relationships between the observed effects (impairments) and the candidate causes. This section shows how data are associated with the candidate causes and then with data concerning effects.

# Associating candidate causes with effects by using data from the site

The relationships between candidate causes and effects were analyzed by using data from the site. Two approaches were used. First, we evaluated whether the measurements relevant to each candidate cause were spatially colocated with biological responses. Second, we examined whether biological responses were correlated with an increase or decrease in the candidate cause. All of these analyses evaluated the candidate causes by using the associated measurements shown in Table 6.

Five of the candidate causes, habitat alteration, metals, low DO, and nutrient enrichment were detected at site A and so were colocated with impairment A (Table 7). However, only four had decreased quality as compared with the upstream site: habitat alteration, metals, high BOD and low DO, and nutrient enrichment. All candidate causes were observed at site B. All of the candidate causes except for habitat alteration exhibited decreased quality at site B as compared with site A; habitat alteration was unchanged.

The second analysis evaluated whether changes in candidate causes were correlated with the biological responses along the entire study reach of the Little Scioto (Table 8). Relative fish weight decreased with increased concentrations of ammonia, nutrients, and BOD. Relative fish weight increased with increasing DO concentrations. The percentage of fish having DELT anomalies increased and the percentage of mayflies decreased with increasing concentrations of two of the metals,

Table 6. Available measurements relevant to each candidate cause

Candidate cause	Relevant measurements
Habitat alteration	Channel score; embeddedness subscore; siltation subscore; dissolved oxygen
PAH <sup>a</sup> toxicity	PAH concentrations in sedi- ments; bile metabolites in fish
Metal toxicity	Metal concentrations in sedi- ments, water, and fish tissue
Ammonia toxicity	Ammonia concentrations in wa- ter
Low dissolved oxygen and high biological oxygen demand	Dissolved oxygen, biological oxygen demand of water
Nutrient enrichment	Nitrate and nitrite concentra- tions in water; total phospho- rus concentrations in water

<sup>a</sup> PAH = polycyclic aromatic hydrocarbon.

ammonia, DO, BOD, and nutrients. Finally, the percentage of tolerant invertebrates increased with increasing concentrations of PAHs and metals.

# Combining exposure data from the site with effects data from elsewhere

The analyses presented in this section combined exposure data from the Little Scioto River with effects data from other studies. The objective was to evaluate whether stressors in the Little Scioto River were present in sufficient amounts that effects would be expected.

Habitat alteration. Although some studies have been performed on the depth of channel required for the survival of larger species, and the amount of siltation that reduces survival of invertebrates, none of the actual response relationships were readily available for direct comparison with existing conditions. Furthermore, data from the Little Scioto were not available for the actual depth of the stream or quantitative measure of stream embeddedness with which to make comparisons. However, reviews indicate that many community metrics are drastically altered by channelization but anomalies are not [39].

Table 7. Spatial colocation of candidate causes with impairments A and B

Candidate cause	Detected at site A?	Decreased quality at site A compared with upstream site?	Detected at site B?	Decreased quality at site B compared with site A?
Habitat alteration	Yes	Yes	Yes	No <sup>a</sup>
PAHS <sup>6</sup> Matala	No	NO Vac	Yes	Yes
Ammonia	Yes	No	Yes	Yes
High BOD and low dissolved oxygen	Yes	Yes	Yes <sup>d</sup>	Yes <sup>d</sup>
Nutrient enrichment	Yes	Yes	Yes	Yes

<sup>a</sup> Although dissolved oxygen concentrations declined between sites A and B in 1987, no change occurred in the habitat alteration metrics. <sup>b</sup> PAHs = polycyclic aromatic hydrocarbons.

<sup>c</sup> BOD = biochemical oxygen demand.

<sup>d</sup> Dissolved oxygen concentrations declined between site A and B (based on 1987 data), and BOD increased (based on 1992 data).

Table 8. Correlation coefficients and associated p values (in italics) between selected measurements and biological responses. Correlations (underlined) are significant at p = 0.10. Stressor measurements with no significant correlations (e.g., channel, silt, and embeddedness scores, fluoranthene, and mercury) are not shown. n = 7 except where noted<sup>a</sup>

	Relative fish weight	DELT anomalies	Percent mayflies	Percent tolerant invertebrates
Anthracene <sup>b</sup>	-0.45	$\frac{0.77}{0.066}$	-0.45	0.45
Benzo[a]anthracene <sup>b</sup>	-0.29 0.45	0.59 0.16	-0.41 0.29	$\frac{0.88}{0.034}$
Benzo[ghi]peryleneb	0.074 <i>0.89</i>	0.33 <i>0.44</i>	$-0.11 \\ 0.75$	$\frac{0.96}{0.021}$
Benzo[a]pyrene <sup>b</sup>	-0.037 0.89	0.44 0.30	-0.19 0.62	$\frac{0.93}{0.026}$
Chrysene <sup>b</sup>	-0.33 0.39	0.63 <i>0.13</i>	$-0.48 \\ 0.22$	$\frac{0.74}{0.076}$
Fluoranthene <sup>b</sup>	-0.26 0.50	0.63 <i>0.13</i>	-0.33 0.39	$\frac{0.74}{0.076}$
Fluorene <sup>b</sup>	-0.45 0.26	$\frac{0.76}{0.070}$	$-0.45 \\ 0.26$	$\frac{0.81}{0.053}$
Cr <sup>b</sup>	$-0.5 \\ 0.20$	$\frac{0.75}{0.073}$	$-\frac{0.71}{0.073}$	0.61 <i>0.15</i>
Cu <sup>b</sup>	-0.29 0.46	0.64 <i>0.13</i>	-0.54 0.18	$\frac{0.82}{0.049}$
Pb <sup>b</sup>	-0.39 0.31	0.54 0.20	-0.64 0.11	0.68 <i>0.11</i>
Zn <sup>b</sup>	$-\frac{0.71}{0.073}$	$\frac{0.75}{0.073}$	$-\frac{0.89}{0.026}$	0.43 <i>0.31</i>
BOD <sup>c</sup>	$-\frac{0.72}{0.070}$	$\frac{0.92}{0.027}$	$-\frac{0.81}{0.042}$	0.59 <i>0.16</i>
Nitrate and nitrite <sup>c</sup>	$-\frac{0.75}{0.06}$	$\frac{0.86}{0.040}$	$-\frac{0.75}{0.060}$	0.14 <i>0.76</i>
Total ammonia <sup>c</sup>	$-\frac{0.72}{0.019}$	$\frac{0.88}{0.034}$	$-\frac{0.99}{0.014}$	0.31 <i>0.48</i>
Total phosphorus <sup>c</sup>	$-\frac{0.75}{0.06}$	$\frac{0.96}{0.020}$	$-\frac{0.86}{0.032}$	0.54 <i>0.20</i>
Minimum DO <sup>c, d</sup>	$\frac{0.89}{0.055}$	$-\frac{0.77}{0.074}$	$\frac{0.94}{0.041}$	$-0.6 \\ 0.16$

<sup>a</sup> DELT = deformaties, eroded fins, lesions, or tumors; BOD = biochemical oxygen demand; DO = dissolved oxygen.

<sup>b</sup> Concentrations measured in sediment.

<sup>c</sup> Concentrations measured in water.

 $^{d}n = 6.$ 

*PAHs and metals.* Tables 9 and 10 compare the TEL and PEL values with the sediment concentrations of PAHs and metals measured at the upstream site and sites A and B. No sediment quality values were exceeded at the upstream site or site A. At site B, five PAHs exceeded the PEL values and the metals exceeded either the TEL or the PEL. The cumulative toxic units for PAHs and metals both exceeded 1.0 at site B,

although the PAH toxic units were substantially higher. Table 11 compares aqueous concentrations of copper, lead, and zinc with the ambient water quality criteria for these chemicals. No criteria were exceeded.

In other studies, PAHs and metals have been associated with anomalies and tumors in fish. Polycyclic aromatic hydrocarbons characteristically cause liver tumors in fish at relatively low concentrations, but that effect is unlikely to be detected by examinations for DELT anomalies. However, PAHs also have been associated with more obvious anomalies at high concentrations in sediment (21,200 mg total PAH/kg) [40]. Lead causes spinal deformities [29], which would be included in the DELT index if sufficiently severe. Chromium, copper, and zinc are not reported to cause DELT anomalies [29]. Fin erosion and deformities were found in fish collected from the Ottawa River in Lima, Ohio, USA, in the presence of elevated levels of a mixture of metals (primarily chromium, lead, and zinc) and PAHs [41]. Subsequent studies confirmed that the fish had taken up the PAHs [15,42]. These studies did not exclude other pollutants, such as nitrogen compounds, as the cause of anomalies. However, they did indicate that these anomalies occur in white suckers from a metal- and PAH-contaminated river in the absence of physical habitat modification. Hence, in the absence of information concerning the actual anomalies at site B, the elevated DELT anomalies provide weak evidence for PAHs or lead as causes of impairment B.

Studies in the western United States and New Zealand indicate that species richness and abundance of mayflies are sensitive to metal contamination, although some of the streams are also impacted by low pH [43–45]. Hence, the decline in mayflies is consistent with effects of mixed metals.

*Ammonia, DO, and nutrient enrichment.* Table 11 compares measurements of ammonia, DO, nitrogen, and phosphorus at sites A and B with available criteria. No criteria were violated at any location in 1992. However, because DO was not measured at site B in 1992, we evaluated data collected in 1987 with the same methods. In 1987, DO levels were lower than criteria at both sites A and B. In Ohio, nutrient enrichment has been associated with DELT anomalies, particularly at sites influenced by point sources [46].

# Measurements associated with causal mechanisms and pathways

For several candidate causes, evidence was available for intermediate steps in causal pathways. The pathways for habitat alteration and nutrient enrichment did not include any intermediate steps, and so these candidate causes are not discussed in this section.

PAH. For fish, the exposure pathway for PAHs was con-

 Table 9. Polycyclic aromatic hydrocarbon (PAH) concentrations (mg/kg) at the upstream site, site A, and site B (Hyalella azteca sediment effects concentration, probable effect level [PEL], and threshold effect level [TEL], normalized to sediment dry wt)

			РАН	PAH sediment concentration			
Chemical	TEL	PEL	Upstream site	Site A	Site B		
Benzo[ <i>a</i> ]pyrene	0.03	0.32	$ND^{a}$	ND	14.8 <sup>b</sup>		
Fluoranthene	0.04	0.32	ND	ND	8.2 <sup>b</sup>		
Benzo[b]anthracene	0.03	0.28	ND	ND	8.2 <sup>b</sup>		
Chrysene	0.02	0.41	ND	ND	16.5 <sup>b</sup>		
Benzo[ghi] perylene	0.01	0.25	ND	ND	49.5 <sup>b</sup>		
Cumulative toxic units based on PEL			ND	ND	339.4		

<sup>a</sup> ND = below detection.

<sup>b</sup> Exceeds PEL and TEL.

Table 10. Metals concentrations (mg/kg) at the upstream site, site A, and site B (threshold effect level [TEL] and probable effect level [PEL] values are for *Hyalella azteca* and are normalized to sediment dry wt)

Chemical	TEL	PEL	Upstream site	Site A	Site B
Cr	32.3	119.4	7.3	13.6	208ª
Cu	28	101.2	7.4	17.2	79 <sup>b</sup>
Pb	37.2	81.7	12.1	19.1	172ª
Zn	98.1	544	30.6	79	173 <sup>b</sup>
Cumulative toxic units based on PEL			0.28	0.66	4.94

<sup>a</sup> Exceeds PEL and TEL.

<sup>b</sup> Exceeds TEL.

cluded to be incomplete at site A, and complete at site B. Biomarkers of naphthalene and benzo[*a*]pyrene are elevated from site B to the mouth of the river, providing evidence that the exposure pathway is complete at these locations. Concentrations of metabolites were above background for the state of Ohio from site B downstream to the confluence with the Scioto River [15].

*Metals.* Data gaps for site A made the evaluation of exposure pathways more difficult for the metals. Data from fish tissue confirm the uptake of lead and zinc at site B.

*Ammonia.* As shown in Figure 5, several interweaving pathways exist by which unionized ammonia can be produced in the river and cause effects. Because unionized ammonia is formed at high pH, and the pH of samples collected from the Little Scioto in 1998 ranged from 7.5 to 8, the presence of total ammonia also provides evidence of the toxic form. Total ammonia was detected at site B, but not at site A.

*High BOD and low DO.* Dissolved oxygen can be depleted by high BOD because of the bacterial respiration associated with allochthonous organic matter or decaying algal mats. Nocturnal algal respiration can further decrease DO concentrations. We have measurements of several relevant parameters: nitrates and nitrites, total phosphorus, BOD, and DO concentrations. Algal concentration was not measured. This exposure pathway was considered complete under two scenarios: BOD is elevated and DO is reduced compared with the upstream site; or, if BOD data are unavailable, nitrates and nitrites and phosphorus are elevated and assumed to cause algal growth, and DO is reduced as compared with the upstream site. At site A, DO is reduced, but BOD is unchanged, so that the exposure pathway is considered incomplete. Site B is more difficult to evaluate because data are scanty and are used from different years. In 1992, BOD was slightly elevated. Although DO was not measured in 1992, in 1987, DO was lower at site B than at the upstream site. Thus, the pathway was considered to be complete.

# Associations of effects with mitigation or manipulation of causes

The experimental field exposures showed that epibenthic water downstream of site B reduced survival of *C. dubia* when exposed to ultraviolet radiation but survival was not reduced when the experimental chambers were shaded [16]. Because PAH toxicity is increased by ultraviolet radiation, this study established that PAHs cause toxicity in the Little Scioto, albeit at a site with higher concentrations than measured at site B.

If the DELT anomalies are liver tumors, then the reduction in such tumors in fish from the Black River after reductions in PAH levels provides supporting evidence that PAHs cause these anomalies in Ohio rivers [47].

#### DISCUSSION

The application of the first steps of the causal inference methodology method to the Little Scioto case study illustrates how information can be organized to support causal evaluations in complex cases. This paper illustrates the complexities of distinguishing distinct impairments, identifying candidate causes, hypothesizing the relationships between candidate causes and effects, obtaining and summarizing data, and converting the data into evidence concerning causation.

Table 11. Comparison of the reported concentration of water quality parameters with available criteria. All data are from 1992 unless noted otherwise

Criterion (units)	Upstream site	Site A	Site B
Ammonia (mg/L) <sup>a</sup> ; 0.57 at pH 8.5, 1.27 at pH 8.0	$ND^{b}$	ND	0.12
Dissolved oxygen (mg/L) <sup>c</sup> : 3.0 for MWH <sup>d</sup>	8.8	5.7	NA
	NA <sup>e</sup>	$2.8^{\mathrm{f},\mathrm{g}}$	1.9 <sup>f,g</sup>
Nitrate–nitrite (mg/L) <sup>h</sup> : 1.6	1.2	1.4	0.8
Total phosphorus (mg/L) <sup>i</sup> : 0.28	0.06	0.07	0.09
Copper $(\mu g/L)^{j}$ : 21	ND	ND	15
Lead $(\mu g/L)^{f}$ : 7.7	ND	ND	3
Zinc $(\mu g/L)^{j}$ : 190	ND	ND	ND

<sup>a</sup> U.S. Environmental Protection Agency (U.S. EPA) ammonia criterion [19].

<sup>b</sup> ND = not detected.

<sup>c</sup> Ohio EPA dissolved oxygen criterion [21].

<sup>d</sup> MWH = modified warm-water habitat.

- <sup>e</sup> NA = no data.
- <sup>f</sup> U.S. EPA ambient water quality criteria; 4-d averages at hardness of 200 mg/L [20].

<sup>g</sup> Violates criteria.

<sup>h</sup> Proposed nitrate-nitrite criterion [22].

<sup>i</sup> Proposed total phosphorus criterion [22].

<sup>j</sup> Minimum dissolved oxygen concentrations from continuous monitoring over 3 d in 1987 [6].

Where multiple sources and stressors are present, different stressors may dominate at different locations and result in multiple distinct impairments. In the Little Scioto, the patterns of the biological community at sites A and B were different enough to define two distinct impairments. However, the stressors overlapped in space and time so that the impairments were not independent. Our solution to the complexity was to define impairment B in terms of the incremental responses between sites A and B. In this way, the candidate causes that were responsible for the distinct responses could be determined. However, at the conclusion of the investigation, all cumulative stressors responsible for the impairment noted at site B would need to be considered.

Disaggregation of the multimetric indices aided the ability to distinguish different impairments. Specific definition of the impairments increases the likelihood that stream reaches with potentially different causes can be selected for analysis. In this case study, if IBI and ICI were used solely rather than the component metrics, the differences between impairments A and B would have been ambiguous or even unnoticed.

We hypothesized seven candidate causes for this section of the Little Scioto River. The development of the list is clearly a critical step in causal assessments. If the true cause is not included, then the impairment may be attributed to the wrong candidate. In the worst case, expensive remedial actions might target the wrong stressor, and result in no improvement. On the other hand, if the list of candidates is too large, then a large amount of effort may be spent on very unlikely causes. In the worst case, the assessment may become so complicated that the true cause is missed in the confusion. In the Little Scioto, the list of candidate causes may have been expanded to include overfishing, habitat alteration from boating, increased hydrologic variance from impervious surfaces in the watershed, and toxic chemicals from spills. Overfishing was not included because fishermen avoid this river because the fishing is poor, a fish consumption advisory is in effect, and fish are tainted with an oily odor. For similar reasons, boating recreation also was considered to be unlikely. Chemical exposure from spills could have been considered, but the biological impairments had been observed in both 1987 and 1992, and so were considered to be long-term, rather than acute, changes. Spills of materials into the Little Scioto and its tributaries have included red food dye, oil, paint waste, and sewage sludge, but none were reported between July 1990 and the 1992 sampling season [5].

Limitations of water sampling and analysis also raise concerns that some candidate causes may have been missed. Although sediments were analyzed for persistent pesticides, other pesticides frequently detected in agricultural streams by the U.S. Geological Survey (e.g., atrazine and chlorpyrifos) were not included [48]. In this study, water quality data for wet weather flows were not available. Storm events could contribute toxic chemicals, nutrients, and flow extremes, especially through the combined sewer overflow at Rkm 9.7. Some pathways may have been incompletely specified. For example, organic chemical and ammonia concentrations may have been sufficiently high that the chemical oxygen demand may have contributed to low DO, but no data were available to provide evidence for this pathway. Finally, some potential causes may be missed because they are obscure or not influential relative to the identified candidate causes. Remediation of one cause may reveal others that were masked.

The development of the conceptual model was a useful step

in communicating among the work group members. As noted in other papers, conceptual models can serve to illuminate causal relationships [49,50]. Although the information in a conceptual model may seem self-evident after the fact, the authors have found that scientific specialists are likely to miss causal pathways unless they participate in the development of a conceptual model by a multidisciplinary team.

Data quality issues included the potential for sampling, analytical, and transcriptional errors. Detection limits, particularly in media that are difficult to analyze such as sediment and fish, may be above levels of concern. In this study, the quantification limits of most of the PAHs were above the sediment quality concentrations. For this reason, the toxic potential of PAHs was likely underestimated.

Only data from 1992 were presented in this paper to simplify the presentation. In reality, all available years of data should be used. Data were available for the Little Scioto for 1987, 1991, and 1998. In most cases, these data supported the conclusions reached with 1992 data, increasing our confidence in our analyses. The exception was DO data, which violated criteria in 1987, but not in 1992. In addition, PAH concentrations tended to increase with depth at site B and the downstream locations, indicating that their toxic potential would be greater if these sediments became accessible.

The analysis was limited by the fact that data were not collected for the purpose of the investigation. Establishing causation is more demanding than determining that a water body is chemically or biologically impaired. For causal analysis, sampling for characterization of habitat, stressors, and responses must be effectively collocated in space and time. An additional issue in this investigation is that fish were sampled at the upstream site by a wading method, whereas all other sites were sampled by the boating method. Because the boating method uses more electrical power, it may sample more larger fish in deeper pools, although, in shallow areas such as the upstream site, more power would make a minimal difference in the size or number of fish sampled. Still, the difference in relative fish weight between the upstream site and site A may be partially due to the difference in sampling method. Finally, sampling with sufficient intensity to characterize variance also is important. In this study, we had low confidence in the aqueous chemical concentrations, which likely are more variable than sediment concentrations. In addition, although we believe that the patterns of biological responses in impairments A and B are real, the fact that each impairment is characterized at only one point is a significant weakness.

Although more samples at more times and locations would be highly useful, designing studies to support causal analysis is not simply a matter of increasing expense and effort. Often, if impairments and candidate causes are well defined, it will be possible to focus studies on a few critical mechanistic studies that clearly eliminate or implicate a cause. Toxicity identification evaluation studies are one example [51]. Liver pathology studies of the fish are another.

This analysis also illustrates the importance of pausing between the collection of data and the determination of the most likely cause to organize the data into causal evidence. Particularly when existing data are used, important relationships among types of data are likely to be overlooked if this step is skipped. Further, organizing the data as causal evidence simplifies the determination of the causes. Finally, proceeding through the assessment in a stepwise and deliberate manner can help communicate the reasoning and evidence that underlie the intuitive leaps that often characterize causal assessments [1].

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