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DETERMINING THE CAUSES OF IMPAIRMENTS IN THE LITTLE SCIOTO RIVER, OHIO, USA: PART 2. CHARACTERIZATION OF CAUSES

SUSAN M. CORMIER,*† SUSAN B. NORTON,‡ GLENN W. SUTER II,§ DAVID ALTFATER,∥ and BERNIE COUNTS∥
†U.S. Environmental Protection Agency, National Exposure Research Laboratory, 26 Martin Luther King Drive, MS 642, Cincinnati, Ohio 45268
‡U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC 20460
§U.S. Environmental Protection Agency, National Center for Environmental Assessment, Cincinnati, Ohio 45268
∥Ohio Environmental Protection Agency, Ecological Assessment Section, Groveport, Ohio 43125, USA

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Abstract—Two stream reaches in the Little Scioto River (OH, USA) were characterized for the causes of impairments measured at two locations. By inductive inference, six candidate causes were winnowed down to three and five candidate causes for each of the two stream reaches. Using a formal strength-of-evidence process, a single cause was determined. At the most upstream location, habitat alterations, including fine-textured substrates and low DO, were characterized as the probable causes for an increased percentage of anomalies of fish, a decreased percentage of mayflies, and an increased percentage of tolerant macroinvertebrates. An increase in the relative weight of fish was attributed to an artificially narrow, deepened channel. Approximately 2 km downstream, polycyclic aromatic hydrocarbon (PAH)-contaminated sediments were identified as the cause for both fish and macroinvertebrate impairments. Causal characterization using first elimination and then a strength-of-evidence approach narrowed and defined the causes of ecological impairment even in this situation, where many complex and interacting candidate causes existed. Applying a formal method highlighted types of data and associations that can strengthen and present a more convincing determination of the causes of impairment.

Keywords—Causation   Stressor   Fish   Invertebrate

INTRODUCTION

A process for characterizing the causes of biological impairments to ecosystems and presenting the evidence is described in the U.S. Environmental Protection Agency's (U.S. EPA) Stressor Identification Guidance Document [1] and by Suter et al. [2]. In this process, hypothesized candidate causes are refuted in the elimination step. Remaining causes are then characterized by diagnosis or strength of evidence. The strength-of-evidence step sorts evidence into three categories: data from the site, associations between data at the site and data from elsewhere, and consistency across all lines of evidence. It evaluates each candidate cause with respect to each type of evidence and then determines the most likely cause.

In this case study, we present a causal characterization for two sites that fail to meet the state of Ohio's biological criteria that are based on multimetric indices for fish and macroinvertebrates. To simplify discussion, we refer to two distinct biological impairments as impairments A and B as described in Norton et al. [3]. These impairments are defined relative to the upstream site (site U) at river kilometer (Rkm) 14.9. Impairment A occurs near Rkm 12.7 (site A) and includes an increase in the relative weight of fish; an increase in the percentage of deformities, erosion, lesions, and tumors (DELT) of fish; a decrease in the percentage of mayflies; and an increase in the percentage of tolerant macroinvertebrates. Impairment B occurs near Rkm 10.5 (site B) and includes an increase in the relative weight of fish compared to the upstream site and a decrease compared with impairment A, an increase in the percentage of DELT, a decrease in the percentage of mayflies, and an increase in the percentage of tolerant macroinvertebrates. See part I of the case study in Norton et al. [3] for a detailed discussion of the impairments.

We hypothesized six candidate causes for these impairments: habitat alteration, polycyclic aromatic hydrocarbon (PAH) contamination, metal contamination, organic enrichment resulting in low dissolved oxygen, ammonia toxicity, and nutrient enrichment. We analyzed evidence for each of these candidate causes using four broad categories: associations between measurements of candidate causes and effects from the site, associations using effects data from elsewhere with exposure data from the site, measurements associated with a causal mechanism, and associations of effects with mitigation or manipulation of causes. These associations are presented in detail in Norton et al. [3].

In this sequel, we used the four types of associations as evidence to eliminate candidate causes where possible and then applied a strength-of-evidence approach to identify the most likely cause from those remaining after elimination. Because impairment B occurs downstream from an existing impairment, we evaluated candidate causes for the incremental impairment at site B from three perspectives: as an incremental increase of the same cause identified for impairment A, as a different and sufficient cause independent of impairment A, and, if the different cause was not sufficient, then as a combination of candidate causes.

METHODS AND DATA SOURCES

The methods for elimination, diagnosis, and strength-of-evidence characterization for the causes of biological impair-

* To whom correspondence may be addressed (cormier.susan@epa.gov).
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ment are described in the U.S. EPA's Stressor Identification Guidance Document [1], and Suter et al. [2]. Candidate causes were eliminated if the evidence indicated that they did not co-occur with the biological impairment, if the biological impairment decreased with increasing influence of the candidate cause, or if the exposure pathway was incomplete. In the Little Scioto River, gradients of biological responses are not sufficiently well defined to allow for elimination; however, we cautiously used the presence and absence of biological gradients in the strength-of-evidence analysis. Different causes may be responsible for impairments at sited A and B and at other sites downstream to the mouth of the river. In fact, no gradient of recovery was observed; both the degree of biological impairments and the concentrations of environmental contaminants increased or remained relatively constant.

Diagnosis is the identification of causes based on characteristic signs or symptoms [2]. No evidence strong enough to support diagnosis was available for any of the candidate causes.

Strength-of-evidence analysis uses all the evidence generated in the analysis phase to examine the credibility of each remaining candidate cause [3]. The causal considerations for the strength-of-evidence analyses were organized into three types of considerations: case-specific evidence, evidence from other situations or biological knowledge, and evidence based on multiple lines of evidence. This last consideration evaluated the consistency of the evidence and, when inconsistent, the coherence of the evidence in support of the hypothesized causes. We conducted the strength-of-evidence analysis by using the causal considerations discussed in U.S. EPA [1] and Suter et al. [2] to array the information relevant to each cause and impairment. We used table 6 in Suter et al. [2] to score each piece of evidence by assigning it pluses, minuses, zeros, no evidence, or not applicable. For evidence of biological gradient, we used a p value of < 0.05 for a strong association and for a weak association $0.05 > p < 0.10$. Scores were assigned as follows: strong (+++), $r \geq 0.90$, weak (+), $r \geq 0.70$ and < 0.90; ambiguous (0), $r < 0.70$; and weak correlations with the wrong sign (−). No score of triple minus was assigned since no correlations showed strong associations with a counterintuitive sign. Finally, we evaluated the scores together with our confidence in the underlying data to reach a conclusion. The scores were not summed; they formed the basis of comparison and were used to identify the most compelling pieces of evidence.

Data pertinent to the study were obtained from the Ohio EPA and U.S. EPA as described by Norton et al. [3]. The associations derived from these data were used in this sequel to eliminate candidate causes or were considered in a strength-of-evidence process to determine the most likely causes of the impairments. It is important to recognize that the evidence was derived from data that were not collected for the purpose of this causal analysis.

RESULTS

Characterize cause: Elimination

The two impairments (A and B) are discussed here in relation to the elimination of specific causes. Conclusions about which candidate causes remained for each impairment are also listed.

Impairment A. The PAHs and ammonia were not elevated at site A relative to the upstream site; thus, we eliminated these candidate causes. Dissolved oxygen (DO) concentrations were about 30% lower than upstream, but biochemical oxygen demand (BOD) concentrations were not different from the upstream site, and thus we eliminated high BOD/low DO as a candidate cause.

Table 1. Candidate causes remaining after elimination

	Impairment A	Impairment B
Habitat alteration	X	
PAH contamination[a]		X
Metal contamination	X	X
Ammonia		X
Low BOD/low DO[b]		X
Nutrient enrichment	X	X

[a] PAH = polycyclic aromatic hydrocarbon.
[b] DO = dissolved oxygen; BOD = biochemical oxygen demand.

We retained habitat alteration, metal contamination, and nutrient enrichment as candidate causes because they co-occurred with impairment A and increased compared to upstream locations. Habitat alteration was evident by lower values for channel and substrate scores and lower DO concentrations compared to the upstream site. All metals measured in sediments were slightly greater at site A compared to the upstream site; however, aqueous concentrations of metals were below detection limits at site A and the upstream site. Nitrates and Nitrites (NO_x) increased from 1.2 to 1.4 mg/L, and total phosphorus (P) increased from 0.06 to 0.07 mg/L. Although these nutrient shifts were small, they precluded logical elimination of nutrient enrichment as a candidate cause.

Impairment B. At this site, we eliminated only one candidate cause, habitat alteration. At site B, the fish and invertebrate metrics changed even though the channel, substrate embeddedness, and silt scores remained the same. Because channel modification was unchanged, it is unlikely that habitat alteration is the cause of the decline in the concentration of DO. Furthermore, DO levels increase downstream of site B even though the stream is still channelized.

We were unable to eliminate the remaining candidate causes. The PAH contaminants in sediments were present and were elevated above concentrations at site A. Exposure to these organic chemicals was demonstrated by internal concentrations of PAH metabolites in fish and induction of ethoxyresorufin-*O*-deethylase activity. The metals chromium, copper, lead, and mercury were elevated in sediment compared to upstream concentrations at site A. Lead and copper were detected in water samples. Fish tissues had detectable levels of lead and zinc. The DO levels were lower at site B than at site A in 1987, and BOD levels were greater than upstream locations in 1992. Data within the same years were not available. Ammonia concentrations were also greater, and total P concentrations were 0.02 mg/L greater. Therefore, five candidate causes remained after elimination: PAH contamination, metal contamination, ammonia toxicity, high BOD/low DO, and nutrient enrichment.

Table 1 summarizes the candidate causes that remained after the elimination process. Only those causes remaining needed to be evaluated by diagnostic or strength-of-evidence characterization.

Characterize cause: Diagnosis

Although no evidence was strong enough to support diagnosis, the pattern of community change was considered to be suggestive and was used later in the strength-of-evidence characterization.

The deformities, fin erosion, physical lesions, and tumors (DELT) on fish are pathologies that are also potentially subject to diagnosis. Some DELT are strongly associated with known toxic substances and others with bacterial infections [4,5]. However, DELT cannot be used to distinguish among toxic substances unless specific anomalies are identified, and even these may be too nonspecific to diagnose without additional information (e.g., histopathology).

Characterize causes: Strength of evidence

The results of the strength-of-evidence analysis are presented in Tables 2 and 3 to permit comparisons of the quality of the evidence across candidate causes. The scores are combined in two shorter tables (4 and 5) in order to more easily see the scoring patterns. Following the strength-of-evidence analysis, we characterized the candidates by describing the most compelling pieces of evidence and identifying the probable cause(s).

Characterize causes: Identify probable causes

Impairment A. We identified habitat alterations as the probable causes of impairment A. Here we describe the evidence for each of the four distinct impairments: an increase in the relative weight of fish, an increase in the percentage of DELT, a decrease in the percentage of mayflies, and an increase in the percentage of tolerant macroinvertebrates. We eliminated the candidate causes PAH, ammonia, and high BOD/low DO for all four distinct impairments because they were not elevated compared to the nearest upstream location. Diagnosis was not applied because of a lack of specific symptomology. In a strength-of-evidence characterization (Table 2), we evaluated the candidate causes: habitat alteration, metal contamination, and nutrient enrichment.

The increase in the relative weight of fish at the site is probably caused by an artificially deepened channel that allows larger fish to survive at site A. Unaltered streams are broad, shallow, and meandering. At site A, the stream channel is cut in a straight, deep, narrow watercourse in the relatively flat landscape in order to lower the water table to drain the soils for farming. Quantitative evidence was not found to support this cause, but it still seemed more likely when compared to the two other candidate causes, which had inconcordant evidence. Metal contamination is an improbable cause because controlled laboratory studies reported decreases in growth rather than increases [6]. Nutrient enrichment is unlikely because the increase in nutrient concentrations was minute and not likely to increase the plant biomass that could be converted to fish biomass.

The cause of the increase in DELT remains uncertain but seems to be related to general stress associated with altered habitat, including low DO [5]. The minimum DO concentration decreased by 3.1 mg/L from the upstream site to site A. Nutrient enrichment is an unlikely cause because only minute increases in the concentrations of nutrients were observed compared with the upstream site. Rankin et al. [7] did report an association for DELT and total P in Ohio streams but at higher levels of DELT than the 1.5% detected at site A. The significant biological gradients between the percentage of DELT and total P appeared to have a greater bearing on impairments downstream, where concentrations of P and the percentage of DELT are much greater. Metal contamination is an unlikely cause because the concentrations of metals in sediment at site A were quite low and may not be readily bioavailable because of the low solubility of metals in hard water (327 mg/L at site A). Aqueous concentrations of metals in water were below ambient water quality criteria.

Many habitat alterations could contribute to the decrease in the percentage of mayflies; however, low substrate texture and low DO seemed to be the most likely causes for this distinct impairment. The biological gradient between low DO and the percentage of mayflies was significant. The greatest change in the percentage of mayflies and concentration of DO occurred from the upstream site to site A. Although metal concentrations were elevated, they were so low that even cumulatively they did not reach a toxic level. Nutrient enrichment is unlikely because the increase in the concentration of nutrients is not likely to increase the plant biomass and change the type of food source and assemblages of macroinvertebrates.

The shift in the percentage of tolerant macroinvertebrates was small and less representative of impairment A than the other three distinct impairments but was consistent with the evidence presented for mayflies except that no significant correlations occurred between the percentage of tolerant macroinvertebrates and any water quality parameter. This may suggest that substrate quality more than water quality influenced the proportion of tolerant macroinvertebrates. We concluded that the small increase in the percentage of tolerant macroinvertebrates is due to habitat alterations, but a more specific cause could not be determined.

Fine substrate texture, low DO, and a deepened channel associated with channel modification are consistent with impairment A. The magnitude of the alteration and clear difference from the unimpaired upstream location strongly support this cause, and inconcordant stressor–response evidence weakens the other candidate causes.

Impairment B. We found PAH contamination to be sufficient to cause all of the specific biological impairments at site B. Here we describe the evidence for each of the four distinct impairments: a decrease in the relative weight of fish, an additional increase in the percentage of DELT, an additional decrease in the percentage of mayflies, and an additional increase in the percentage of tolerant macroinvertebrates. Habitat alteration was eliminated as a candidate cause for the incremental impairment and changes in fish and macroinvertebrates at site B because the impairments increased and changed while scores for substrate and channel remained unchanged relative to site A. Dissolved oxygen decreased, but this could not be attributed to habitat alteration because channel, embeddedness, and silt scores were unchanged. The decrease in DO was evaluated as a candidate cause associated with increased organic matter. Diagnosis was not applied because of a lack of specific symptomology. In a strength-of-evidence characterization (Table 3), we evaluated the candidate causes: PAH contamination, metal contamination, ammonia toxicity, low DO resulting from organic enrichment (high BOD/low DO), and nutrient enrichment.

Several lines of evidence support PAHs as the cause of the decline in relative weight and increase in DELT anomalies associated with impairment B. The measurement of PAH metabolites in fish tissue provides evidence of a complete exposure pathway. The concentrations of the contaminants in sediments were at levels known to cause DELT anomalies [8,9]. Reduced growth and declines in weight are sublethal effects that occur at concentrations lower than those associated with anomalies. The percentage of DELT weakly correlated with PAH. Correlations with the other biological metrics may

Table 2. Strength-of-evidence analysis for the three candidate causes of impairment A[a]

Casual consideration	Habitat alteration: Evidence	Habitat alteration: Score	Metals contamination: Evidence	Metals contamination: Score	Nutrient enrichment: Evidence	Nutrient enrichment: Score
Case-specific considerations						
Co-occurrence	Compatible: at and below site A the habitat of the Little Scioto is altered as a result of channelization. The degree of habitat alteration remains about the same to the mouth of the river. Substrate scores and DO are lower than upstream. The upstream site is not channelized, and habitat is good [3].	+	Compatible: all sediment metal concentrations were slightly higher at site A compared to the upstream site [3].	+	NO_x was elevated by 0.2mg/L compared to the upstream site. Total *P* was elevated compared to the upstream site by 0.01 mg/L [3].	+
Temporality	No evidence	NE	No evidence	NE	No evidence	NE
Consistency of association	No evidence	NE	No evidence	NE	No evidence	NE
Biological gradient	Increased relative weight: none for channel or sediment, but low DO was weakly correlated.	+	Increased relative weight: weak correlation with zinc	+	Increased relative weight: clear association but wrong sign: weak correlations with NO_x and total P, but relative weight decreases with increasing nutrient concentrations.	−
	Increased % DELT: none for channel or sediment, but low DO was weakly correlated.	+	Increased % DELT: weak correlations with chromium and zinc.	+	Increased % DELT: weak correlation with NO_x, strong with total P.	+++
	Decreased % mayflies: none for channel or sediment, but low DO was strongly correlated.	+++	Decreased % mayflies: weak correlations with chromium and zinc.	+	Decreased % mayflies: weak correlations with both NO_x and total P.	+
	Increased % tolerant organisms: ambiguous [3]	0	Increased % tolerant organisms: weak correlation with copper [3].	+	Increased % tolerant organisms: ambiguous [3]	0
Complete exposure pathway	Not applicable: no known intermediate steps.	NA	No evidence: internal concentrations of metals were not measured.	NE	No evidence: concentrations of algae or chlorophyll *a* were not measured.	NE
Experiment	No evidence	NE	No evidence	NE	No evidence	NE
Considerations based on other situations or biological knowledge						
Plausibility: mechanism						
	Increased relative weight: plausible: artificially deepened channel allows larger sized fish to survive.	+	Increased relative weight: implausible: no known mechanism for metals. Metals usually cause a decrease in the relative weight of fish [6,13–15].	−	Increased relative weight: plausible: NO_x and total P are nutrients that can increase algal growth. Greater production of algae could provide additional food, increasing fish growth.	+
	Increased DELT: plausible: no obvious mechanism other than stress due to low DO. DO decreased by 3.1 mg/L [5].	+	Increased DELT: plausible: metals cause fin erosion and lesions [4]. Pb, Cu, Zn cause deformities [6,13–15].	+	Increased DELT: plausible: nutrients may create conditions that favor opportunistic pathogens and fungi that cause lesions and fin erosion and that interfere with wound healing.	+
	Decreased % mayflies and increased % tolerant organisms: plausible: fine sediments provide poor forage, reproductive, and cover habitats for benthic invertebrates, including many mayflies. Low DO is not tolerated by many species [16–18].	+	Decreased % mayflies and increased % tolerant organisms: plausible: metals are known to cause lethal and sublethal effects to invertebrates [6,13–15].	+	Decreased % mayflies and increased % tolerant organisms: plausible: switching to an autochthonous energy source could alter species survival and community composition of invertebrates.	+

Table 2. Continued

Casual consideration	Habitat alteration: Evidence	Score	Metals contamination: Evidence	Score	Nutrient enrichment: Evidence	Score
Plausibility: stressor–response	Increased relative weight: no evidence	NE	Increased relative weight: not applicable: implausible mechanism	NA	Increased relative weight: inconcordant: NO_x does not limit algal growth in most Ohio streams [7,19]. Magnitude of nutrient change too small to cause effect.	−
	Increased DELT: no evidence	NE	Increased DELT: inconsistent: deformities, including lordoscoliosis, are reported at water hardness greater than 200 mg/L for 850 μg/L lead and 160 μg/L for zinc [15]. No metals were detected in water at site A.	−	Increased DELT: inconcordant: Ohio's proposed nitrogen and phosphorus criteria were not exceeded. Criteria are protective of fish [7].	−
	Decreased % mayflies and increased % tolerant organisms: no evidence	NE	Decreased % mayflies and increased % tolerant organisms: inconcordant: no metals exceeded *Hyalella azteca* PEL values. Cumulative toxic index was not exceeded [20]. Hickey and Clements [21] reviewed invertebrate species richness, particularly of mayflies, which declined in association with metals in water column; however, concentrations at site A were probably less than concentrations reported by Hickey and Clements.	−	Decreased % mayflies and increased % tolerant organisms: inconcordant: magnitude of nutrient change too small to account for change of invertebrate metrics. NO_x does not limit algal growth in most streams [19]. Nutrients in Virginian streams are associated with algal growth that decrease numbers of EPT taxa, but at higher concentrations than at site A [22]. Ohio's proposed nitrogen and phosphorus criteria were not exceeded [7].	−
Consistency of association	No evidence	NE	No evidence	NE	No evidence	NE
Specificity of cause	Increased relative weight: one of many	0	Increased relative weight: not applicable: implausible mechanism	NA	Increased relative weight: one of many	0
	Increased DELT: one of many	0	Increased DELT: one of many	0	Increased DELT: one of many	0
	Decrease % mayflies and increased % tolerant organisms: one of many	0	Decrease % mayflies and increased % tolerant organisms: one of many	0	Decrease % mayflies and increased % tolerant organisms: one of many	0
Analogy	Not applicable	NA	Not applicable	NA	Not applicable	NA
Experiment	No evidence	NE	No evidence	NE	No evidence	NE
Predictive performance	No evidence	NE	No evidence	NE	No evidence	NE
Considerations from multiple lines of evidence						
Consistency of evidence	Increased relative weight: all consistent	+++	Increased relative weight: multiple inconsistencies: implausible mechanism	−−−	Increased relative weight: multiple inconsistencies: biological gradient is in the wrong direction; implausible stressor response.	−−−
	Increased DELT: all consistent	+++	Increased DELT: most consistent: stressor response unlikely	+	Increased DELT: most consistent: magnitude of changes inconsistent with magnitude of effect.	+
	Decreased % mayflies and increased % tolerant organisms: all consistent	+++	Decreased % mayflies and increased % tolerant organisms: most consistent: although metals are present, the concentrations are unlikely to be toxic.	+	Decreased % mayflies and increased % tolerant organisms: most consistent: magnitude of changes inconsistent with magnitude of effect.	+
Coherence of evidence	Not applicable	NA	Increased relative weight, increased DELT, decrease % mayflies and increased % tolerant organisms: no explanation.	0	Increased relative weight, increased DELT: decrease % mayflies and increased % tolerant organisms: no explanation.	0

[a] NE = no evidence; NA = not applicable; DO = dissolved oxygen; DELT = deformities, erosion, lesions, and tumors; NO_x nitrogen oxide; P = phosphorus; PEL = probable effects level; EPT = Ephemeroptera, Plecoptera, Trichoptera.

Table 3. Strength-of-evidence analysis for the five candidate causes of impairment B[a]

	PAH contamination		Metals contamination	
Causal consideration	Evidence	Score	Evidence	Score
Case-specific considerations				
Co-occurrence	Compatible: sediment PAH concentrations were several orders of magnitude greater at site B than site A [3].	+	Compatible: lead, chromium, copper, zinc, and mercury concentrations in sediment were 2 to 10 times greater at site B than at site A [3]. In surface water grab samples, copper and lead were greater at site B than at site A. Other metals were below detection limit.	+
Temporality	No evidence	NE	No evidence	NE
Consistency of association	No evidence: only one location	NE	No evidence: only one location	NE
Biological gradient	Decreased relative weight: ambiguous	0	Decreased relative weight: weak correlation with zinc	+
	Increased % DELT: weak correlation with anthracene and fluorene	+	Increased % DELT: weak correlations with chromium and zinc	+
	Decreased % mayflies: ambiguous	0	Decreased % mayflies: weak correlations with chromium and zinc	+
	Increased % tolerant organisms: correlations with all PAH except anthracene, strong correlations with benzo[*ghi*]perylene and benzo[*a*]pyrene [3].	+++	Increased % tolerant organisms: weak correlation with copper	+
Complete exposure pathway	Fish metrics: actual evidence for all steps: both BAP and NAPH metabolites were found in fish [3].	++	Fish metrics: Actual evidence for all steps: zinc and lead were detected in fish tissues [3].	++
	Invertebrate metrics: no evidence	NE	Invertebrate metrics: no evidence	NE
Experiment	Fish metrics: no evidence	NE	Fish metrics: No evidence	NE
	Invertebrate metrics: concordant: field exposures in the Little Scioto showed that epibenthic water downstream of site B reduced survival of *C. dubia* when exposed to UV radiation, but survival was not reduced when experimental chambers were shaded [23].	+++	Invertebrate metrics: no evidence	NE
Considerations based on other situations or biological knowledge				
Plausibility: mechanism	Decreased relative weight: plausible: PAHs are known to reduce growth and shorten life span, resulting in smaller fish [9].	+	Decreased relative weight: plausible: metals are known to reduce growth. Toxic compounds can shorten life span, resulting in smaller fish [6,13–15].	+
	Increased DELT: plausible: PAHs are known to cause eroded barbels, fin erosion, lesions, and internal and external tumors [9].	+	Increased DELT: plausible: metals cause fin erosion and lesions [4]. Pb, Zn, Cu cause deformities [6,13–15].	+
	Decreased % mayflies and increased % tolerant organisms: plausible: PAHs are known to cause reproductive impairments, which could decrease % mayflies and favor tolerant species [9].	+	Decreased % mayflies and increased % tolerant organisms: plausible: metals are known to cause lethal and sublethal effects to invertebrates that could decrease % mayflies and favor tolerant [6].	+
Plausibility: stressor–response	Decreased relative weight: no evidence	NE	Decreased relative weight: inconsistent: copper at site B was 15 mg/L, and lead was 3 μg/L. Ambient water quality criteria for lead, copper, and zinc (7.7 μg/L, 21 μg/L, 190 μg/L, water hardness 200 mg/L, respectively) are not exceeded.	−
	Increased DELT: quantitatively consistent: PAHs were at levels that cause tumors and other DELT [8].	+++	Increased DELT: inconsistent: lordoscoliosis is reported at 850 μg/L lead at a hardness of 353 mg/L. Hardness at site B was 389 mg/L. Lead at site B was 3 μg/L. Copper at site B was 15 mg/L. Also, ambient water quality criteria for lead and copper (7.7 μg/L and 21 μg/L, water hardness 200 mg/L, respectively) were not exceeded.	−

Table 3. Extended

Ammonia toxicity		High BOD/low DO		Nutrient enrichment	
Evidence	Score	Evidence	Score	Evidence	Score
Compatible: ammonia concentration was doubled relative to impairment A [3].	+	Compatible: in 1992, BOD was double the upstream value, and in 1987, the minimal DO levels measured were 0.9 mg/L less than upstream [3].	+	Compatible: compared to site A, total P was elevated by 0.02 mg/L. NO_x was less [3].	+
No evidence	NE	No evidence	NE	No evidence	NE
No evidence: only one location	NE	No evidence: only one location	NE	No evidence: only one location	NE
Decreased relative weight: weak correlation	+	Decreased relative weight: weak correlations with BOD and DO	+	Decreased relative weight: weak association with NO_x and total P with wrong sign.	−
Increased % DELT: weak correlation	+	Increased % DELT: strong correlation with BOD, weak with low DO	+++	Increased % DELT: weak correlation with NO_x, strong with total P	+++
Decreased % mayflies: strong correlation	+++	Decreased % mayflies: weak correlation with BOD, strong with low DO	+++	Decreased % mayflies: weak correlations with both NO_x and total P	+
Increased % tolerant organisms: ambiguous [3]	0	Increased % tolerant organisms: ambiguous [3]	0	Increased % tolerant organisms: ambiguous [3]	0
Not applicable: no known intermediate steps	NA	Not applicable: no known intermediate steps	NA	No evidence: concentrations of algae and chlorophyll *a* were not measured.	NE
No evidence	NE	No evidence	NE	No evidence	NE
Decreased relative weight: plausible: ammonia toxicity is known to reduce growth and survival. Low survival could alter the age structure, resulting in smaller fish and more juvenile fish [29,30].	+	Decreased relative weight: plausible: stress could reduce growth and survival [5]. Low survival could alter the age structure, resulting in smaller fish and more juvenile fish.	+	Decreased relative weight: implausible: increased nutrients are usually associated with increased algal growth that augment the energy available for growth.	−
Increased DELT: plausible: reports of histopathologies of gills and internal organs, but not of DELT. Stress could increase susceptibility to opportunistic pathogens [5].	+	Increased DELT: plausible: chronic low DO may cause stress, which then increases growth deformities or susceptibility to opportunistic pathogens and fungi [5,7].	+	Increased DELT: plausible: nutrients are believed to create conditions that favor opportunistic pathogens and fungi that cause lesions and fin erosion and that interfere with wound healing [5,7].	+
Decreased % mayflies and increased % tolerant organisms: plausible: ammonia is known to be toxic to invertebrates [29].	+	Decreased % mayflies and increased % tolerant organisms: plausible: low DO can kill fish and invertebrates [19].	+	Decreased % mayflies and increased % tolerant organisms: plausible: switching to an autochthonous energy source could alter species survival and community composition for fish and invertebrates [19].	+
Decreased relative weight: inconcordant: in several species, reduced growth was reported at greater than 0.5 mg/L [29]. At site B, ammonia levels were near 0.2 mg/L.	−	Decreased relative weight: concordant	+	Decreased relative weight: not applicable: implausible mechanism	NA
Increased DELT: inconcordant: most studies reported histopathology to gills or internal organs at these or higher concentrations [29]. Reports of gross anomalies similar to those found at site B were not found.	−	Increased DELT: concordant	+	Increased DELT: inconcordant: concentrations of NO_x and P are at or below background levels. Nitrogen does not limit algal growth in most streams [19]. Ohio's proposed criteria for nitrogen and phosphorus criteria were not exceeded [7].	−

Table 3. Continued

Causal consideration	PAH contamination: Evidence	Score	Metals contamination: Evidence	Score
	Decreased % mayflies and increased % tolerant organisms: quantitatively consistent: the *Hyalella azteca* PELs were exceeded for all PAHs. The cumulative PAH toxic units were 339 times the PEL value [20].	+++	Decreased % mayflies and increased % tolerant organisms: quantitatively consistent: Hickey and Clements [21] reviewed invertebrate species richness, particularly of mayflies, which declined in association with metals in water column; however, water quality concentrations at site A are uncertain, and they reported declines in taxa rather than % mayflies. Lead and chromium exceeded *H. azteca* PEL values. The cumulative toxic unit values for all metals was 4.9 times the PEL value [20].	+++
Consistency of association	Decreased relative weight: no evidence	NE	Decreased relative weight: no evidence	NE
	Increased DELT: invariant: tumors and other DELT are associated with fish exposed to high concentrations of PAH in fresh and marine waters [24]. Increased DELT was associated with complex toxic exposures [12].	+++	Increased DELT: no evidence	NE
	Decreased % mayflies and increased % tolerant organisms: invariant: at more than 25 locations associated with PAH contamination that exceeded exposure criteria in Ohio, ICI scores were below 30 [25]. ICI scores of less than 30 occur only when mayflies are decreased and tolerant species are relatively abundant. IBI and ICI are known to be depressed even when habitat quality was good [26,27].	+++	Decreased % mayflies and increased % tolerant organisms: no evidence	NE
Specificity of cause	One of many	0	One of many	0
Analogy	Not applicable	NA	Not applicable	NA
Experiment	Decreased relative weight: concordant: following dredging in the Black River, Ohio, the age and weight of brown bullheads increased. The Black River is larger and deeper than the Little Scioto [17].	+++	Decreased relative weight: no evidence	NE
	Increased DELT: concordant: in the Black River, Ohio, removal of PAHs by dredging resulted in lower levels of liver tumors [8] and PAH bile metabolites [28].	+++	Increased DELT: no evidence	NE
	Decreased % mayflies and increased % tolerant organisms: no evidence	NE	Decreased % mayflies and increased % tolerant organisms: no evidence	NE
Predictive performance	No evidence	NE	No evidence	NE
Considerations from multiple lines of evidence				
Consistency of evidence	Decreased relative weight: all consistent	+++	Decreased relative weight: most consistent	+
	Increased DELT: all consistent	+++	Increased DELT: most consistent	+
	Decreased % mayflies and increased % tolerant organisms: all consistent	+++	Decreased % mayflies and increased % tolerant organisms: all consistent	+++
Coherence of evidence	Not applicable	NA	Decreased relative weight, increased DELT: no known explanation	0

[a] NE = no evidence; NA = not applicable/not available; DO = dissolved oxygen; NO_x = total nitrate-nitrite; P = phosphorus; BOD = biochemical oxygen demand; DELT = deformities, erosion, lesions, and tumors; PAH = polycyclic aromatic hydrocarbons; BAP = benzo[*a*]pyrene; NAPH = naphthalene; PEL = probable effects level; ICI = invertebrate community index; IBI = index of biotic integrity; MWH = modified warmwater habitat.

Table 3. Extended Continued

Ammonia toxicity		High BOD/low DO		Nutrient enrichment	
Evidence	Score	Evidence	Score	Evidence	Score
Decreased % mayflies and increased % tolerant organisms: inconcordant: the ammonia concentrations were not great enough to cause the dramatic effects seen with impairment B. Ammonia criteria were not exceeded [29].	−	Decreased % mayflies and increased % tolerant organisms: concordant: DO levels are below Ohio criteria for MWH [31].	+	Decreased % mayflies and increased % tolerant organisms: inconcordant: the magnitude of change in P from site A was not great enough to cause dramatic effects seen with impairment B. Proposed P criterion was not exceeded [7].	−
No evidence	NE	No evidence	NE	No evidence	NE
Decreased relative weight: one of many	0	Decreased relative weight: one of many	0	Decreased relative weight: not applicable	NA
Increased DELT: one of many	0	Increased DELT: one of many	0	Increased DELT: one of many	0
Decreased % mayflies and increased % tolerant organisms: one of many	0	Decreased % mayflies and increased % tolerant organisms: one of many	0	Decreased % mayflies and increased % tolerant organisms: one of many	0
Not applicable	NA	Not applicable	NA	Not applicable	NA
No evidence	NE	No evidence	NE	No evidence	NE
No evidence	NE	No evidence	NE	No evidence	NE
Decreased relative weight: most consistent: ammonia concentrations were lower than those reported to cause effects.	+	Decreased relative weight: all consistent	+++	Decreased relative weight: multiple inconsistencies: implausible mechanism.	−−−
Increased DELT: most consistent: magnitude of change was inconsistent with magnitude of effect.	+	Increased DELT: all consistent	+++	Increased DELT: most consistent: magnitude of change was inconsistent with magnitude of effect.	+
Decreased % mayflies and increased % tolerant organisms: most consistent: magnitude of changes was inconsistent with magnitude of effect.	+	Decreased % mayflies and increased % tolerant organisms: all consistent	+++	Decreased % mayflies and increased % tolerant organisms: multiple inconsistencies: magnitude of change was inconsistent with magnitude of effect.	+
Decreased % mayflies and increased % tolerant organisms: ammonia levels were out measured at sediment/water interface and may be higher than in the water column.	+	Not applicable	NA	Decreased relative weight, increased DELT, decreased % mayflies and increased % tolerant organisms: no known explanation.	0

Table 4. Summary of strength of evidence analysis of impairment A

Causal consideration	Habitat	Metals	Nutrient
Case-specific considerations			
Co-occurrence	+	+	+
Temporality	NE[a]	NE	NE
Consistency of association	NE	NE	NE
Biological gradient			
Increased relative weight	+	+	−
Increased % DELT[b]	+	+	+++
Decreased % mayflies	+++	+	+
Increased % tolerant	0	+	0
Complete exposure pathway	NA[c]	NE	NE
Experiment	NE	NE	NE
Considerations based on other situations or biological knowledge			
Plausibility: mechanism			
Increased relative weight	+	−	+
Increased % DELT	+	+	+
Decreased % mayflies and increased % tolerant	+	+	+
Plausibility: stressor–response			
Increased relative weight	NE	NA	−
Increased % DELT	NE	−	−
Decreased % mayflies and increased % tolerant	NE	−	−
Consistency of association	NE	NE	NE
Specificity of cause			
Increased relative weight	0	NA	0
Increased % DELT	0	0	0
Decreased % mayflies and increased % tolerant	0	0	0
Analogy	NA	NA	NA
Experiment	NE	NE	NE
Predictive performance	NE	NE	NE
Considerations from multiple lines of evidence			
Consistency of evidence			
Increased relative weight	+++	−−−	−−−
Increased % DELT	+++	+	+
Decreased % mayflies and increased % tolerant	+++	+	+
Coherence of evidence	NA	0	0

[a] NE = no evidence.
[b] DELT = deformities, erosion, lesions, and tumors.
[c] NA = not applicable/not available.

be obscured by additional changes occurring further downstream.

Similarly, several lines of evidence support PAH contamination as the cause of decline in the percentage of mayflies and the increase in the percentage of tolerant macroinvertebrates. Although no direct measurement was available to demonstrate exposure of invertebrates, we assumed that if fish were exposed, benthic invertebrates were also exposed. The PAH levels were at concentrations known to be toxic to benthic macroinvertebrates; in fact, the cumulative toxic units of PAH were more than 300 times the probable effects level. The percentage of tolerant invertebrates increased sharply from site A to site B and was strongly correlated with all the PAHs except anthracene. Overall, the consistency of the evidence is very good.

The evidence for low DO as a cause was fairly consistent, but the precursors of the low DO and the magnitude of the effect were uncertain. Low DO may contribute to stress that results in lesions or reduced relative weight. Among the seven sites on the Little Scioto, both the fish metrics correlated weakly with DO. In 1987, the criteria for DO were exceeded at site B. However, the decrease in DO concentrations from site A to site B (0.9 mg/L) was moderate, whereas the changes in percentage of DELT anomalies, relative weight of fish, and decrease in the percentage of mayflies were large. In addition, further downstream, changes in the percentage of DELT and relative weight were equally great, but DO concentrations increased. Finally, the role of BOD as a precursor is questionable because higher concentrations of DO occur at greater BOD concentrations at downstream locations. It is possible that the incremental decrease in the percentage of mayflies is related to a further decline in the concentration of DO. However, the highly toxic, PAH-laden sediments seem to be a more probable cause.

Applying stressor–response information weakened the cases for the other three candidate causes. None of the available criteria for ammonia, metals, total P, or NO_x were exceeded. The cumulative toxic units calculated for metals in sediments exceeded 1 but were only 1.4% of those for PAHs. Only zinc and lead were detected in fish tissue analysis. Total P correlated strongly with the percentage of DELT anomalies; however, the incremental change in total P was small, and the correlation was probably reflective of much higher concentrations of total P and higher incidence of DELT anomalies at downstream locations. Similarly, the percentage of mayflies was very strongly correlated with ammonia concentrations for the entire stream, but ammonia concentrations were 10 times greater downstream, and the correlation appeared to be relevant to downstream impairments rather than impairments at site B. The percentage of tolerant macroinvertebrates showed no correlation with any water quality parameter while showing consistent correlations with PAH.

Table 5. Summary of strength-of-evidence analysis of impairment B

Causal consideration	PAH[a]	Metals	NH_3	BOD/DO[b]	Nutrient
Case-specific considerations					
Co-occurrence	+	+	+	+	+
Temporality	NE[c]	NE	NE	NE	NE
Consistency of association	NE	NE	NE	NE	NE
Biological gradient					
Decreased relative weight	0	+	+	+	−
Increased % DELT[d]	+	+	+	+++	+++
Decreased % mayflies	0	+	+++	+++	+
Increased % tolerant macroinvertebrates	+++	+	0	0	0
Complete exposure pathway	++	++	NA[e]	NA	NE
Experiment	+++	NE	NE	NE	NE
Considerations based on other situations or biological knowledge					
Plausibility: mechanism					
Decreased relative weight	+	+	+	+	−
Increased % DELT	+	+	+	+	+
Decreased % mayflies and increased % tolerant	+	+	+	+	+
Plausibility: stressor–response					
Decreased relative weight	NE	−	−	+	NA
Increased % DELT	+++	−	−	+	−
Decreased % mayflies and increased % tolerant	+++	+++	−	+	−
Consistency of association					
Decreased relative weight	NE	NE	NE	NE	NE
Increased % DELT	+++	NE	NE	NE	NE
Decreased % mayflies and increased % tolerant	+++	NE	NE	NE	NE
Specificity of cause					
Decreased relative weight	0	0	0	0	NA
Increased % DELT	0	0	0	0	0
Decreased % mayflies and increased % tolerant	0	0	0	0	0
Analogy	NA	NA	NA	NA	NA
Experiment					
Decreased relative weight	+++	NE	NE	NE	NE
Increased % DELT	+++	NE	NE	NE	NE
Decreased % mayflies and increased % tolerant	NE	NE	NE	NE	NE
Predictive performance	NE	NE	NE	NE	NE
Considerations from multiple lines of evidence					
Consistency of evidence					
Decreased relative weight	+++	+	+	+++	−−−
Increased % DELT	+++	+	+	+++	+
Decreased % mayflies and increased % tolerant	+++	+++	+	+++	+
Coherence of evidence	NA	0	+	NA	0

[a] PAH = polycyclic aromatic hydrocarbons.
[b] BOD/DO = biochemical oxygen demand/dissolved oxygen.
[c] NE = no evidence.
[d] DELT = deformities, erosion, lesions, and tumors.
[e] NA = not applicable/not available.

DISCUSSION

Use of the causal analysis approach described in the Stressor Identification Guidance Document [1] and in Suter et al. [2] permitted us to determine the causes of biological impairments in the Little Scioto River with reasonable confidence. Most important, it led to the identification of distinct impairments within an officially impaired river segment and a way to evaluate and discriminate among physical and chemical causes.

This causal characterization demonstrates that some basic applications of logic can help narrow down a rather complex list of candidate causes and might in other cases be sufficient to select restorative or protective actions. At minimum, it highlights those types of data that need to be collected and can point to types of associations that may be needed to strengthen and present a more convincing determination of the causes of impairment. For instance, the format helps organize complex ecological information into three main activities: describing the impairment and listing candidate causes, assembling the data and analyzing associations between candidate causes and impairments, and using logical arguments to identify causes. In presenting the arguments for identifying the causes, refutation is clearly separated from diagnosis and strength of evidence. These are complex activities, and using a formal process helps ensure more complete documentation of the integrated thought processes used by ecologists, toxicologists, and resource managers to determine causes of impairment.

It is worth noting that the more precise the identification of the impairment, the more likely that the list of candidate causes can be reduced in the elimination step and that a cause can be ultimately identified in later steps. Specific definition of the impairments increases the likelihood that stream reaches with potentially different causes can be recognized and evaluated. In this case study, if Index of Integrity and Invertebrate community index were used rather than the constituent or component metrics, differences between impairments A and B would have been ambiguous. Likely causes could not have been determined or narrowed down to only a few likely causes.

Also, different causes for different specific impairments at the same site could not be characterized.

Further definition of the component metrics could have been useful. Characterization of the specific DELT anomalies is an obvious example. It would have also been useful to know whether the increases in percentage tolerant macroinvertebrates were due to increases in specific tolerant taxa or declines in intolerant taxa.

Many readers probably realize that some of the causes remaining after the elimination process are very unlikely. However, to accurately reflect the logical process that we use and because only mean data or few data are available, these causes are retained. In so doing, the elimination step is stronger, and the entire process possesses greater integrity. For instance, nutrient enrichment is retained as a candidate cause for impairment A even though the increase in total P and NO_x is minute. The reason it is retained is because it fails to meet criteria for elimination, namely, a reduction or unchanging concentration of the candidate causes. Some may argue that the amount of change in the concentration of NO_x and total P would not be statistically different and therefore could be eliminated. However, unless a large sampling program is implemented, the power to detect real differences would remain small and unreliable. More important, the most compelling reason for nutrient enrichment being an improbable cause comes from ecological knowledge about the amounts of nutrients that would be needed for effects to occur. This knowledge comes from other watersheds and is not grounds for refutation at this site. The proper way to show this type of evidence is by the strength-of-evidence procedure.

In this particular case study, the diagnostic analysis is not effective for identifying any causes. However, in other studies, it has been a decisive tool for determining the causes of fish kills [10]. In fish kills, pathological evidence is usually particularly useful. For instance, surface lesions may be associated with bacterial infections, whereas liver tumors may be associated with PAH. For alterations to community structure, diagnostic evidence is less well documented and studied. This is being rectified by a number of researchers who are trying to improve the specificity and confidence in using patterns of biological metrics as a diagnostic tool [11,12].

The causal characterization of the Little Scioto River could be strengthened by additional evidence from the literature that could be used to evaluate the plausibility of mechanisms and stressor–response relationships, consistency of associations, specificity of causes, and results of experiments. The use of readily available literature in this case study makes it representative of the use of the method by scientists in regulatory agencies given current resources. However, it would be desirable to assemble the voluminous but diffuse results of field studies of impaired waters and combine them with information from the open literature to make these types of evidence accessible for future characterizations.

To rigorously apply the method described by U.S. EPA [1] and Suter et al. [2] requires discipline to guard against the intuitive leaps that often characterize conclusions about cause. Scientists with long experience in particular streams can quickly identify causes on the basis of their knowledge of the system and its history. However, a formal process can help document the critical pieces of the evidence used to draw conclusions, increase consistency among investigations, and increase confidence that the true cause has been identified.

This case study demonstrates the usefulness of combining multiple lines of evidence and different types of information using a flexible but formal process described by the Stressor Identification Guidance Document [1]. The process that is used here may be useful for determining the causes of impairments not only to streams but for other resource types. Furthermore, research designs may benefit from considering methods for eliminating alternative hypothesis that depend on inductive reasoning rather than experiments alone.

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