PREDICTIVE MODELS ATTRIBUTE EFFECTS ON FISH ASSEMBLAGES TO TOXICITY AND HABITAT ALTERATION

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Abstract. Biological assessments should both estimate the condition of a biological resource (magnitude of alteration) and provide environmental managers with a diagnosis of the potential causes of impairment. Although methods of quantifying condition are well developed, identifying and proportionately attributing impairment to probable causes remain problematic. Furthermore, analyses of both condition and cause have often been difficult to communicate. We developed an approach that (1) links fish, habitat, and chemistry data collected from hundreds of sites in Ohio (USA) streams, (2) assesses the biological condition at each site, (3) attributes impairment to multiple probable causes, and (4) provides the results of the analyses in simple-to-interpret pie charts. The data set was managed using a geographic information system. Biological condition was assessed using a RIVPACS (river invertebrate prediction and classification system)-like predictive model. The model provided probabilities of capture for 117 fish species based on the geographic location of sites and local habitat descriptors. Impaired biological condition was defined as the proportion of those native species predicted to occur at a site that were observed. The potential toxic effects of exposure to mixtures of contaminants were estimated using species sensitivity distributions and mixture toxicity principles. Generalized linear regression models described species abundance as a function of habitat characteristics. Statistically linking biological condition, habitat characteristics including mixture risks, and species abundance allowed us to evaluate the losses of species with environmental conditions. Results were mapped as simple effect and probable-cause pie charts (EPC pie diagrams), with pie sizes corresponding to magnitude of local impairment, and slice sizes to the relative probable contributions of different stressors. The types of models we used have been successfully applied in ecology and ecotoxicology, but they have not previously been used in concert to quantify impairment and its likely causes. Although data limitations constrained our ability to examine complex interactions between stressors and species, the direct relationships we detected likely represent conservative estimates of stressor contributions to local impairment. Future refinements of the general approach and specific methods described here should yield even more promising results.

Key words: bioassessment; biological-resource impairment; ecological, ecotoxicological, and exposure modeling; effect and probable-cause pie charts; environmental management; habitat degradation; integrated effects; Ohio (USA) streams; species composition; species sensitivity distribution.

INTRODUCTION

Prevention and minimization of adverse alterations to ecosystems are the principal goals of environmental management. Ecosystem degradation is caused by one or more physical and chemical stressors operating together (Baird and Burton 2001), which produce a typical sequence of biotic change with increasing stress (Davies and Jackson 2006). Some human-caused stressors have no natural counterparts, whereas other stresses represent alterations in naturally occurring factors.

Assessing the degree of alteration and assigning causality often requires a wide array of tools. Methods for measuring the magnitude of biotic degradation in aquatic communities are well developed (e.g., Karr 1981, Moss et al. 1987), but diagnoses of probable causes rely on various combinations of expert judgment, application of multivariate statistics, and weighting of evidence. Unfortunately, these methods often require a great deal of expertise to use and interpret, and their results are often difficult to communicate. Further, mixtures of potentially toxic compounds are often not a part of such assessments. Although identification of the causes of
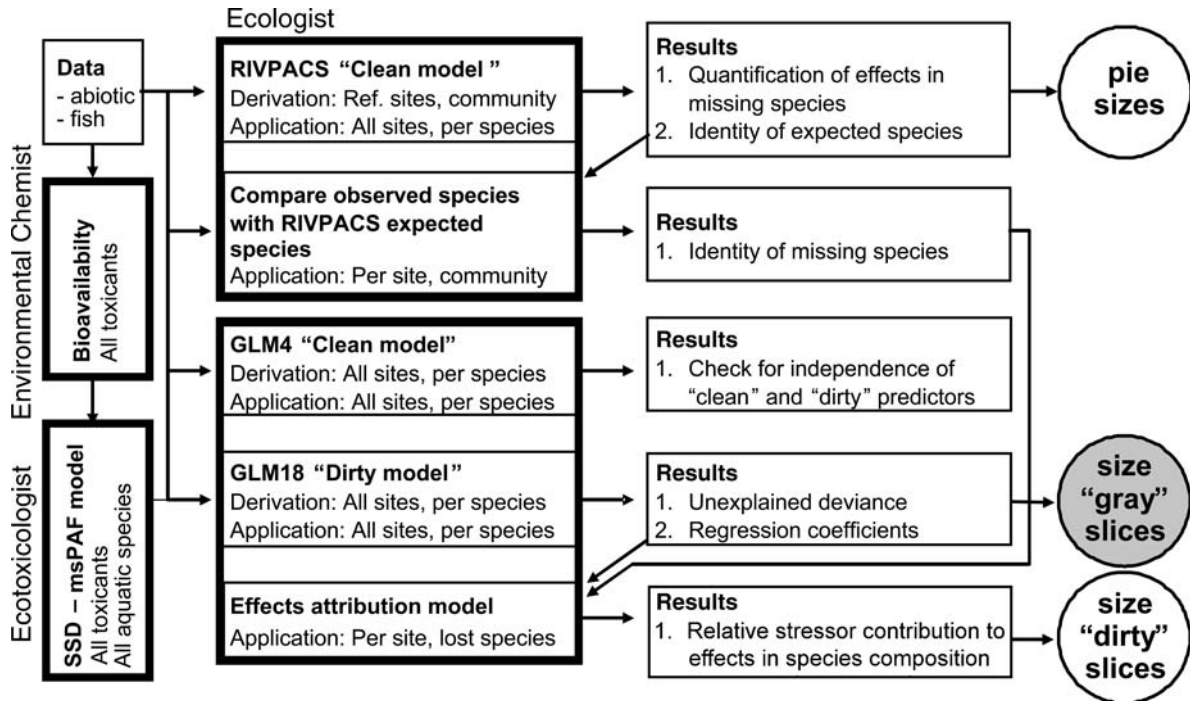
Fig. 1. Schematic outline of the steps in data analysis needed to derive the degree of impact per site (pie sizes), the unexplained deviance slice, and the statistical association of impact to the predictors (other slice sizes).

biological impairment may always require application of sophisticated tools, there may be more elegant and effective ways of presenting complicated information. A method is needed that communicates both the magnitude of impairment and the likely relative importance of different stressors in that impairment. In this paper, we describe such a method.

Our purpose is to describe a general eco-epidemiological diagnostic framework for linking measures of ecological impairment with likely causes (Bro-Rasmussen and Løkke 1984). The specific method combines ecological, ecotoxicological, and exposure modeling to provide statistical estimates of the probable effects of different natural and anthropogenic stressors on stream fish assemblages. We show the outcome of such analyses as easily understood pie diagrams. We initially conducted this analysis with anonymous sites and species as a way of ensuring a "double blind" procedure. Once we created maps showing species loss and likely stressors, we then examined the literature to determine if the observed patterns were consistent with previous observations.

Schematic outline

We used a large (1552 sites) data set consisting of measures of fish species composition and abundance, habitat descriptors, and water chemistry to assess how well we could link local impairment to site information. All data were placed into a geographic information system (GIS) to facilitate data management and linkage

with various statistical and graphic programs. In the following sections we describe the data set, analyses, and methods in which results were simplified to effect and probable-cause pie charts (hereafter, EPC). A schematic outline of the analytical steps is presented in Fig. 1.

Data Sources and Assumptions

River network data

We obtained baseline data for Ohio rivers (see Plate 1) from the U.S. Environmental Protection Agency's (U.S. EPA) reach file Version 1 (RF1) (USEPA 1992a). This file presents rivers as a series of connected line segments at a scale of 1:500,000. However, the data do not have network features, which are essential for establishing up-down stream relationships. We therefore used ARC/INFO version 7.0.4 (Environmental Systems Research Institute [ESRI], Redlands, California, USA) to create a river network from the RF1 line file within a GIS database (Dyer et al. 2000).

Fish data

We used survey data provided by the Ohio Environmental Protection Agency (Ohio EPA), Columbus, Ohio, USA, for 98 native and 19 introduced fish species (Trautman 1981, Barbour et al. 1999) for the years 1990–1996. Prior to analysis all sample counts were standardized to ≤300 individuals by randomly resampling any original field count >300 individuals to 300 individuals. Resampling was done without replacement to mimic how a large field sample of fish would be

PLATE 1. Fish reference site located on the East Fork of the Little Miami River, near Batavia, southwest Ohio (USA). The Little Miami River is a National and State Scenic River. Photo credit: Donna Morrall.

subsampled manually. If the original sample contained <300 individuals, we used the original count.

Physical-habitat data

Local, site-specific, fish habitat data were provided by the Ohio EPA. Habitat data included sampling location (latitude, longitude), drainage area above each sample site, and the individual metrics used to derive Ohio EPA's qualitative habitat evaluation index (QHEI; Rankin 1989). Briefly, the QHEI is derived from seven metrics scored by expert judgment: substrate, in-stream cover (cover), channel quality (channel), riparian/erosion condition (riparian), pool, riffle, and vertical gradient (slope). In addition to the QHEI metrics, the number of modified warm-water habitat attributes (WWATR, data set range 0–9) were included as an indicator of the degree to which sites conformed to reference conditions. Reference WWATR included: no channelization; silt-free substrates; boulders, cobbles. and gravel in substrate; moderate to high sinuosity; low overall riffle embeddedness; presence of fast current and eddies; presence of varied cover; and a maximum depth >40 cm. The number of modified WWATR provided an antithesis metric to the other habitat factors, indicative of altered sites.

We extracted locations of wastewater treatment plants (WWTP) from U.S. EPA's Needs Survey (USEPA 1989) and Permit Compliance System (PCS; USEPA 1992b)

databases, which included a total of 567 Ohio WWTP facilities discharging to RF1 river reaches.

Chemical-habitat data

Measured water chemistry.—We extracted ambient water-chemistry data for Ohio streams from U.S. EPA's STORET database (USEPA 1995). Parameters were: total metal concentrations (Cd, Cu, Pb, Ni, Zn), dissolved oxygen, hardness, total ammonia, pH, and total suspended solids. Too few data for organic contaminants, BOD, and inorganic nutrients (P and N) were available for use in this study. Water-chemistry data were retrieved for the years 1990–1996, the same time period over which data on fish assemblages were compiled. The median and 90th-percentile concentrations for each water-chemistry parameter were determined per site.

Calculation of cumulative effluent.—We obtained mean flow data for all receiving waters from U.S. EPA's RF1 river file (USEPA 1992a). We combined these values with flow data obtained from municipal WWTPs to estimate dilution factors and cumulative percentage WWTP effluent. We used cumulative percentage WWTP effluent as a surrogate measure of persistent wastewater constituents within stream reaches. Percentage cumulative effluent was calculated as the ratio of WWTP flow to receiving-stream flow for headwater segments. For all other segments, WWTP flow included not only contri-

TABLE 1. Annual U.S. consumption volumes for five chemicals used as ingredients in consumer products as well as wastewater treatment plant (WWTP) removals and first-order river loss rates, as used for GIS-ROUT model estimations for riverine concentrations in Ohio, USA.

	U.S. usage		WWTP removal (%)						
Chemical†	National average (metric tons)	Per capita per day (g)	Activated sludge	Oxidation ditch	Rotating biological contactor	Lagoon	Trickling filter	Primary	In-stream degradation (d^{-1})‡
Triclosan	600[1]	0.0062	95[1]	95[1]	95[1]	95[1]	80[1]	30[1]	0.264[2]
LAS (C12)	303 458[3]	3.137	99[4]	99[4]	98[4]	98[4]	80[4]	27[4]	0.7[2]
AE (C13-E3.1)	141 976[3]	1.467	99[4]	99[4]	99[4]	99[4]	96[4]	18.9[4]	31.2[2]
AES (C13.45-E1.5S)	268 077[3]	2.771	98[4]	98[4]	98[4]	98[4]	93[4]	0[4]	24[2]
Boron	4536[3]	0.0467	0[4]	0[4]	0[4]	0[4]	0[4]	0[4]	0[5]

† The five consumer-product ingredients are triclosan, linear alkylbenzene sulfonate (LAS), alcohol ethoxylates (AE), alcohol ethoxylate sulfates (AES), and boron. Items in parentheses below chemical names refer to the average alkyl and ethoxylate chain lengths, respectively. Data sources are denoted by numerical superscripts: 1, McAvoy et al. (2002); 2, T. Federle and E. Schwab (*unpublished manuscript*); 3, SRI (2002); 4, McAvoy et al. (1998); 5, Dyer and Caprara (1997).

‡ First-order river loss (degradation fraction per day). Data are average national rate constants.

butions from facilities on those river segments, but also contributions from facilities upstream (e.g., main stem, tributaries) of those segments.

Estimation of household product chemicals in rivers.—
We used the GIS-ROUT model (Dyer and Caprara 1997, Wang et al. 2000, 2005) to estimate riverine concentrations of chemicals derived from household products. GIS-ROUT is a national-scale model that assumes a per capita use per day of product ingredients to determine WWTP loads, derives effluent concentrations by estimating removal of these chemicals as a function of treatment type, and then predicts receiving water concentrations in all RF1 river reaches by accounting for dilution, first-order losses, and upstream contributions via the simultaneous routing of reaches (Wang et al. 2000). We estimated environmental concentrations of five consumer-product ingredients (triclosan, linear alkylbenzenesulfonate (LAS), alcohol ethoxylates (AE), alcohol ethoxylate sulfates (AES), and boron) at both mean and critical low flows (7Q10). Loadings and treatment-plant removal varied substantially for each compound as did in-stream losses (Table 1).

Exposure modeling of toxicants.—

1. *Heavy metals.*—The toxicity of heavy metals for fish is strongly associated with the dissolved fraction in ionized form (Sorensen 1991), which depends on water hardness. We estimated the bioavailable fractions of Cd, Cu, Ni, Pb, and Zn in each reach using Ohio EPA's hardness-based criteria (Ohio EPA 1996).

2. *Household product chemicals.*—We considered the modeled concentrations of the household-product chemicals boron, AE, AES, LAS, and triclosan to be entirely bioavailable.

3. *Total ammonia.*—Unionized ammonia (NH_3) is 100 times more toxic for fish than the ammonium ion (NH_4^+) (USEPA 1999). We expressed total ammonia as the 90th percentile value of total ammonia values measured at a site. We estimated NH_3 from total ammonia following methods given in USEPA (1999). Because ionization of ammonia is dependent on pH and

temperature, we used site-specific median pH and assumed a constant temperature of 12°C in the calculations.

METHODS

Risk estimation of chemicals and mixtures

Instead of using separate compound concentrations, we calculated two summary indicators of risk based on the bioavailable fractions of the studied chemicals to depict the potential influence of various contaminants on fish: (1) the multi-substance potentially affected fraction (msPAF) of species for chemicals of industrial and geochemical origin (NH_3, Cd, Cu, Ni, Pb, and Zn), and (2) the msPAF of species for household-product constituents (boron, AE [alcohol ethoxylates], AES [alcohol ethoxylate sulfates], LAS [linear alkylbenzene-sulfonates], and triclosan). This procedure provides indicators for potential toxic stress, while minimizing the number of parameters to the assessment (entering the concentrations of individual contaminants would imply added degrees of freedom and reduction of statistical power). Converting the exposure concentrations of each contaminant to a msPAF value required two steps.

We first used species sensitivity distributions (SSD) (Posthuma et al. 2002) to estimate toxic risk for each compound (Fig. 2). Toxic risk is expressed as the potentially affected fraction (PAF) of species at a given concentration. A SSD is defined by a log-logistic function in which alpha (α) specifies its median and beta (β) its slope. We used laboratory aquatic toxicity data from the U.S. Environmental Protection Agency ECOTOX database (USEPA 2002) to construct SSDs for ammonia and heavy metals. Our goal was to derive SSDs for chronic effects. Since chronic-toxicity data were scarce compared to the availability of acute-toxicity test data, even for common chemicals like heavy metals and ammonia, we estimated chronic SSDs by first calculating acute SSDs and then applied an assessment factor of 10 (i.e., left-shifted) following De Zwart (2002).
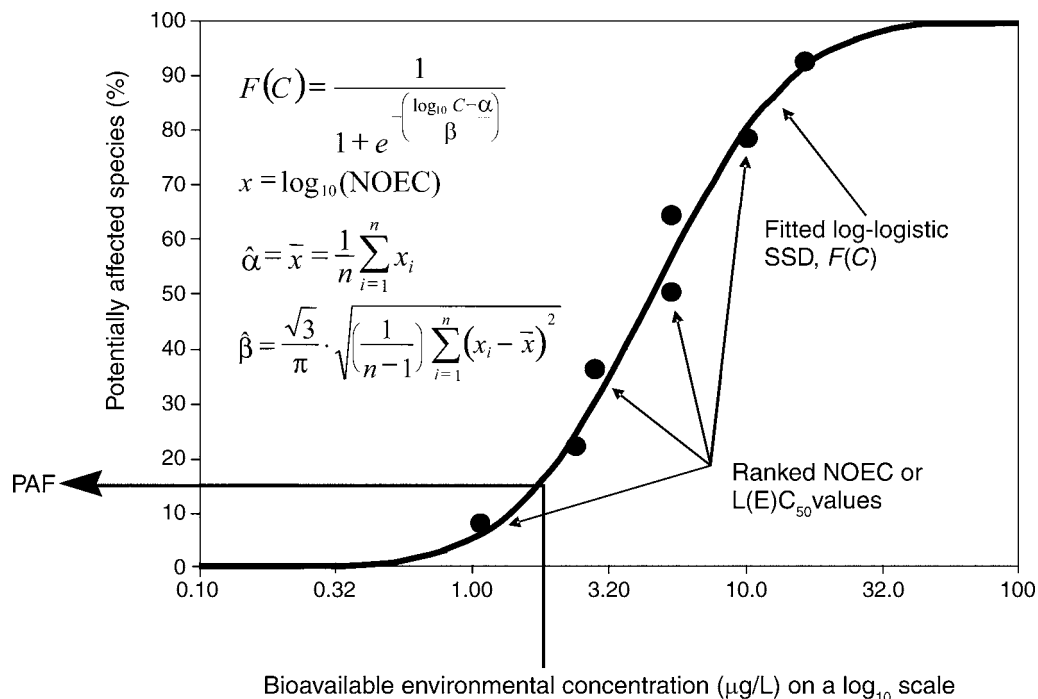
$$F(C) = \frac{1}{1 + e^{-\left(\frac{\log_{10} C - \alpha}{\beta}\right)}}$$

$$x = \log_{10}(\text{NOEC})$$

$$\hat{\alpha} = \bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i$$

$$\hat{\beta} = \frac{\sqrt{3}}{\pi} \cdot \sqrt{\left(\frac{1}{n-1}\right)\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

Fitted log-logistic SSD, $F(C)$

Ranked NOEC or L(E)C$_{50}$ values

PAF

Bioavailable environmental concentration (µg/L) on a $\log_{10}$ scale

FIG. 2. Derivation of a potentially affected fraction (PAF) of species from aquatic toxicity data used to build a species sensitivity distribution (SSD) per chemical. Each SSD consisted of a log-logistic model where α and β correspond to the median and slope, respectively. Key to abbreviations: n, number of species; NOEC, no-observed-effect concentration; L(E)C$_{50}$, median lethal or effective concentration; $F(C)$, fraction affected.

That is, we estimated the chronic SSD for metals and ammonia from acute toxicity data by applying $\alpha_{chr} = \alpha_{acu} - 1$ and $\beta_{chr} = \beta_{acu}$. The assessment factor we used is different from and far more robust than a standard acute-to-chronic ratio for individual species. The derivation of surfactant (LAS, AE, AES) SSDs required normalization to mean surfactant structures following a procedure based on quantitative structure–activity relationships (Van de Plassche et al. 1999). Hence, for

all compounds we derived chronic α and β values (Table 2) to estimate potential risk. To account for both direct and indirect effects, we included data on fish toxicity, as well as on toxicity for other species that may constitute the food supply of fish (algae and invertebrates).

We then combined the PAF values for individual compounds within both industrial chemicals and household-product categories to derive msPAF values. In doing so, we assumed that all compounds had different

TABLE 2. Chronic (chr) aquatic species sensitivity distributions (SSD) information needed to calculate the potentially affected fraction (PAF) of species for each toxicant, with Kolmogorov-Smirnov test for goodness-of-fit by the log-logistic model.

Toxicant†	α_{chr}‡	β_{chr}§	Number of species‖	D_{max}¶	P	Logistic model
Ammonia (µg/L)	2.42	0.39	14	0.17	0.78	accepted
Cadmium (µg/L)	1.98	0.72	134	0.08	0.41	accepted
Copper (µg/L)	1.10	0.60	33	0.07	0.99	accepted
Nickel (µg/L)	2.50	0.66	19	0.15	0.74	accepted
Lead (µg/L)	2.29	0.31	18	0.16	0.68	accepted
Zinc (µg/L)	2.26	0.70	58	0.08	0.82	accepted
Boron (mg/L)	1.15	0.38	20	0.18	0.50	accepted
AE (mg/L)	−0.18	0.33	22	0.08	1.00	accepted
AES (mg/L)	−0.34	0.34	10	0.25	0.50	accepted
C12LAS (mg/L)	0.16	0.27	19	0.14	0.78	accepted
Triclosan (µg/L)	1.18	0.45	11	0.23	0.52	accepted

† Key to abbreviations: AE, alcohol ethoxylates; AES, alcohol ethoxylate sulfates; C12LAS, linear alkylbenzenesulfonate with 12 C alkyl group.
‡ Average log toxicity (midpoint of SSD curve).
§ Slope of SSD curve.
‖ All species in the SSD: fish, algae, and invertebrate species.
¶ Kolmogorov-Smirnov maximum deviation statistic.

TABLE 3. Statistical attributes of measured and modeled abiotic predictors and their use in RIVPACS and general-linear models.

Variable	Abbreviation	Units	Percentile				
			5th	25th	50th	75th	95th
Habitat characteristics							
Latitude	LAT	degrees	39.23	39.73	40.14	41.1	41.46
Longitude	LONG	degrees	−84.47	−83.88	−83.03	−81.98	−80.97
Log(drainage area)	logDA	km^2	1.18	1.96	2.54	3.11	3.77
Log(gradient)	logGRAD	m drop/km	−0.80	−0.32	0.03	0.36	0.77
Alterations§							
Channel	CHANNEL	range 0–20	6.5	12.5	15.25	17	19
Cover	COVER	range 0–20	6	11	13	15	18
Pool	POOL	range 0–12	5	8	9.5	11	12
Riffle	RIFFLE	range 0–8	1.5	3	4.5	6	7
Riparian	RIPARIAN	range 0–10	3.5	4.75	6	7	9
Substrate	SUBSTR	range 0–20	6	12.5	15	17	19
No. modified warm-water habitat attributes	WWATR	range 0–9	1	2	4	5	7
Measured chemistry							
Dissolved oxygen (median)	DOMED	mg O$_2$/L	4.96	6.82	7.82	8.71	10.39
pH (median)	PHMED	std. units	7.5	7.81	8	8.17	8.4
Hardness (median)	HARDMED	mg CaCO$_3$/L	138.5	236	290.5	343	411.3
Total suspended solids (median)	TSSMED	mg/L	5	8	15	26	58
Total ammonia (90th percentile)	NH3	mg N/L	0	0	0.08	0.22	2.8
Total heavy metals	HM						
Total cadmium (90th percentile)		μg/L	0	0	0	0	0.8
Total copper (90th percentile)		μg/L	0	0	0	0	17
Total lead (90th percentile)		μg/L	0	0	0	0	50
Total nickel (90th percentile)		μg/L	0	0	3	5	15.3
Total zinc (90th percentile)		μg/L	0	17	32	60	170
Calculated effluent and modeled ecotoxicity							
Percentage cumulative effluent (median)		%	0	0.37	2.01	4.91	20.42
msPAF$_{NH3\text{-}Metals}$‖ (90th percentile)	PNH3HM90	%	0	0.07	0.11	0.21	0.43
msPAF$_{HH}$¶ (low flow)	PHHL	%	0.01	0.03	0.08	0.14	0.36

† Key to model abbreviations: RIVPACS, river invertebrate prediction and classification system predictive model; GLM4, generalized linear model with four predictors; GLM18, GLM with 18 predictors. In the data field, an "X" means used in the model specified in the column heading; a blank cell means not used in the model.

‡ See grouping in Figs. 7, 8, and 9.

§ High values indicate less alteration in the particular habitat aspect.

‖ Multi-substance potentially affected fraction (PAF) of species based on species sensitivity distributions for ammonia and heavy metals, including cadmium, copper, lead, nickel, and zinc.

¶ Multi-substance PAF of species based on species sensitivity distributions for the household (HH) product constituents, boron, AE (alcohol ethoxylates), AES (alcohol ethoxylate sulfates), LAS (linear alkylbenzene sulfonate), and triclosan.

toxic modes of action and calculated both msPAF values as though responses were additive and species were uncorrelated in their sensitivity for the different toxicants (de Zwart and Posthuma 2005).

Data integration

We used an imputation scheme (Dyer and Wang 2002) to associate biological samples with the nearest habitat and chemistry samples. Briefly, we created a point coverage of the locations of water chemistry, fish, and habitat samples in ARC/INFO, which was overlaid on the RF1 river network. We then divided the RF1 (U.S. EPA's reach file version 1) river network into segments whose boundaries were defined by significant changes in hydrologic features, such as the confluence of WWTP (wastewater treatment plant) discharges and tributaries. We combined segments <30 m long with the next downstream segment. Finally, we assigned each sample to a river segment, and associated biological

samples with the nearest habitat and chemistry samples within a segment. Applying this procedure resulted in a total of 1552 river sampling sites with both fish-survey data and geographical information (latitude, longitude, slope, and drainage area). About 45% (695) of these sites had complete biological, habitat, and chemical data representation (Table 3).

Site classification

Ohio EPA classified 114 of the 1552 sites as least-altered reference sites (Stoddard et al. 2006) based on best professional judgment (Rankin 1989), of which 60 sites had complete data as described above. For sites with complete data, abiotic variables were usually only weakly correlated with one another, if at all (see correlation structure in Appendix A). Most significant ($P < 0.001$) correlations were expected, e.g., slope was negatively correlated with drainage area ($r = -0.57$), the cumulative amount of effluent ($r = -0.21$), and

TABLE 3. Extended.

Model[†]			
RIVPACS	GLM4	GLM18	Group[‡]
X	X	X	habitat
X	X	X	habitat
X	X	X	habitat
X	X	X	habitat
		X	habitat
		X	habitat
		X	habitat
		X	habitat
		X	habitat
		X	habitat
		X	habitat
		X	chemistry
		X	chemistry
		X	chemistry
		X	chemistry
		X	effluent
		X	toxicity
		X	toxicity

suspended solids ($r = -0.17$) and positively correlated with dissolved oxygen ($r = 0.18$) and pH ($r = 0.13$). Most habitat characteristics were positively correlated with one another ($r = 0.34$–0.54), indicating that altered sites had been degraded in a variety of ways. The number of modified warm-water habitat attributes was negatively correlated with all other habitat factors ($r = -0.38$ to -0.67), because it represents the count of lower scores in the other habitat factors.

Ecological and statistical techniques

Quantifying the amount of biological alteration and identifying likely causes of impairment required two main series of analyses, each of which consisted of several steps (Fig. 1).

Quantifying biological condition and impairment: RIVPACS modeling.—Comparison of the observed fauna with that expected to occur in the absence of human-caused stress provides a basis for quantifying the biological condition of potentially stressed ecosystems. Given a standard sampling effort, RIVPACS-type models (Moss et al. 1987, Hawkins 2006) estimate the probabilities of capturing (PC) each species in the regional species pool at each local site assuming reference conditions. The PC values are used to estimate the number of species expected (E) at a site. The ratio of observed (O) to expected taxa (O/E) at a site provides an

indicator of condition that is easy to interpret (i.e., the proportion of expected species that were present). Only species native to the state of Ohio were used to build the model used here. Any exotic species that had been introduced to Ohio were tracked, but not used in assemblage-level assessments, i.e., calculations of O/E, which therefore provided a measure of the integrity of the native fish fauna. The mechanics of RIVPACS models have been thoroughly described elsewhere (e.g., Wright et al. 2000, Hawkins and Carlisle 2001) and we give only a brief description here.

We derived a RIVPACS model with only four predictors of assemblage composition (latitude, longitude, drainage area, and stream- channel slope). These predictors are considered to be surrogates for naturally occurring factors (e.g., temperature, substrate type), and are needed when predicting the biota that should have occurred at sites prior to modification of the actual causal factors by human activity. In this sense, RIVPACS is a strictly empirical modeling approach. For the model we developed, latitude and longitude were surrogates for historical biogeographic factors and broad-scale factors associated with ecoregion. Drainage area was a surrogate for the multiple environmental features that change with increasing stream size, and stream slope was likely a surrogate for both current speed and substrate character. We evaluated a model that used ecoregions as predictors, but it performed less well than the model based on latitude and longitude.

We built the RIVPACS-type model from data collected at the 114 reference sites and then applied the model to all 1552 sampling sites. We used estimates of PC for each species at each site to estimate the expected number of species at a site by summation of PC over all species with a PC ≥ 0.5. We then determined how many of the species predicted to occur at a site with PC ≥ 0.5 were actually observed and calculated the ratio O/E as a measure of departure from expectation in missing species. Models that use PC ≥ 0.5 are typically more precise than models based on PC > 0 (Hawkins 2006) and base assessments on the more common and more reliably sampled and modeled species.

From the output of each RIVPACS assessment, we could also identify the specific species that were expected but missing at each site. We used this information for the attribution of impact to probable causes (see *Identification of likely causes. . . ,* below).

When applied to sets of non-reference sites, RIVPACS output can also be summarized to identify those taxa that were either found at more sites than expected ("increasers") or fewer sites than expected ("decreasers"). The number of sites at which a species is expected is estimated as the sum of PC's for that species across all assessed sites. The ratio of observed/expected sites (S_O/S_E) describes a species overall type of response to stressors at these sites. We calculated these ratios for all species. To evaluate the RIVPACS assessment, we compared S_O/S_E values with species-specific tolerance

information extracted from the seven sources (Karr et al. 1986, Ohio EPA 1987, Plafkin et al. 1989, Simon 1991, Lyons 1992, Hall et al. 1996, and Halliwell et al. 1999) that were summarized in Barbour et al. (1999). We arbitrarily assigned all taxa with S_O/S_E values > 1.25 as tolerant, those with S_O/S_E values < 0.75 as intolerant, and those with values between 0.75 and 1.25 as neutral or intermediate, terms used in Barbour et al. (1999). We then compared these tolerant, intermediate, and intolerant assignments to the similar three-category assignments compiled in Barbour et al. (1999) and then noted if one or more of the seven sources they used differed with our assignments.

The O/E ratios per site were the basis for calculating the radius of the effect and probable-cause (EPC) pie charts in which a larger radius implies more missing species and a larger impairment. To calculate the radius, impairment was expressed on an absolute scale of 0 to 1. However, by applying the O/E method, O can theoretically exceed E because of sampling or prediction error. This could result in pie sizes $(1 - O/E)$ that are negative. Furthermore, this way of scaling may also result in a positive pie size that would imply impairment even when all species that are expected are actually observed. To address this issue we considered any species with PC $\geq$ 0.5 as expected to occur and counted these species as an alternative way of estimating E and calculating pie sizes. This alteration resolved both problems of negative pie size and the potential problem of implying impairment when no species were missing. This approach did not compromise the analyses because estimates of E based on counting species with PC $\geq$ 0.5 and summing all PC $\geq$ 0.5 were strongly correlated ($r^2 = 0.98$).

Construction of GLM models.—We used generalized linear models (GLM; McCullagh and Nelder 1989) to quantify the associations between each of the 117 fish species and the environmental variables. We initially planned to model the presence/absence of species using binomial logistic GLM regression, but this approach did not explain much of the variance. We therefore used Poisson GLM regression to quantify associations between species abundances and environmental variables, which yielded substantially better results.

We built two sets of models from data collected at the 695 sites with full data representation. We used one set of models (GLM4) to predict species abundances from the same four natural predictors used in the RIVPACS model. The form of those models was

$$\ln(A_i) = a_i + b_i(\text{LAT}) + c_i(\text{LONG}) + d_i(\log\text{DA})$$
$$+ e_i(\log\text{GRAD})$$

where A_i = the predicted abundance of species i, LAT = latitude, LONG = longitude, DA = drainage area, and GRAD = gradient. We then constructed another set of models (GLM18) to describe species responses to both natural gradients and the other variables (Table 3). These models took the following form:

$$\ln(A_i) = a_i + b_i(\text{LAT}) + c_i(\text{LONG}) + d_i(\log\text{DA})$$
$$+ e_i(\log\text{GRAD}) + f_{1,i}(\text{DOMED}) + f_{2,i}(\text{DOMED}^2)$$
$$+ g_{1,i}(\text{HARDMED}) + g_{2,i}(\text{HARDMED}^2) + \cdots$$
$$+ r_{1,i}(\text{PHHL}) + r_{2,i}(\text{PHHL}^2) + s_{1,i}(\text{PNH3HM90})$$
$$+ s_{2,i}(\text{PNH3HM90}^2)$$

where DOMED stands for median dissolved oxygen; HARDMED is median water hardness; PHHL is msPAF$_{\text{HH}}$(low flow), i.e., the multi-substance, potentially affected fraction (msPAF) of species for household-product chemical constituents; and PNH3HM90 is msPAF for NH_3 and heavy metals, 90th percentile.

We forced all GLM18 models to use the four natural variables that were used in the RIVPACS and GLM4 models. We then added both linear and quadratic forms of the seven habitat-related and seven contaminant-related variables to the models by a stepwise procedure. The stepwise procedure used the Bayesian information criterion (BIC; Schwarz 1978) to restrict the addition of terms to those that had a significant contribution to the overall model ($P < 0.05$), based on Type I evaluation of sums of squares. Calculations were conducted with S-Plus 2000, Professional Release 3 (MathSoft, Cambridge, Massachusetts, USA). Predictor variables that were not selected by this procedure received a regression coefficient of zero value.

We considered, but did not include interactions of predictor variables on the response of species into our analyses. Several types of interactions are likely to occur. Some assemblages may be more sensitive than others, leading to interactions among natural and stressor variables. Jointly acting stressors may be more damaging than the sum of individual effects (synergistic effects). There may be a limit to the extent of degradation, i.e., once a portion of the fauna is lost to one stressor, the assemblage may be insensitive to other stressors (antagonistic effects). We did not include interactions in our analyses at this time for three reasons. (1) With only 695 observations, the addition of interaction terms would reduce statistical power due to added degrees of freedom, and would violate "Simpson's rule of thumb". This rule is also known as the "curse of dimensionality" (Bellman 1961), which implies that to minimize error in regression analysis, an absolute minimum of 10 observations are needed for each predictor variable (Vaughan and Ormerod 2003). Safer interpretations of this rule of thumb mention a requirement of 20, 50 or even 100 observations per predictor. (2) There is little pre-existing knowledge available to guide us on what predictors are likely to interact in their effect. The restrictions implied by dimensionality would only allow us to make a haphazard selection of a subset of the possible interactions. (3) By not considering interactions, estimates of stressor contribution to the overall effect on species assemblages likely are conservative. Therefore, attributions of different stressors to biological impairments are likely underestimates of their actual contribution.

We created both full and null models for each species. Null models were of the form ln(abundance) = a, where a is the mean abundance of the species across all sites. GLM output consisted of regression coefficients, degrees of freedom, and deviance residuals for both the full (DEV_f) and null models (DEV_n). We used explained deviance (ED) as a measure of the explanatory capacity of each model, where $ED = (DEV_n - DEV_f)/DEV_n$.

The objective of the GLM modeling was to isolate the likely effects of different stressor variables on fish abundance. As in the RIVPACS models, we needed to distinguish between the effects of stressor variables on fish and effects associated with natural factors. However, direct regression of the differences between observed abundances and those expected from the GLM4 models, i.e., $[\ln(O_i) - \ln(E_i)]$, on the 14 stressor variables resulted in significant convergence problems. To avoid this problem, we fit GLM18 models directly to the $\ln(O_i)$ data but included the abundance predicted by the GLM4 model ($E_{i,GLM4}$) as a way of accounting for naturally occurring variation. For example, $\ln(O_{i,GLM18}) = \ln(E_{i,GLM4}) + GLM18$ modeled effects of the stressors that are potentially of anthropogenic origin. This approach is only valid when the natural and other stressor variables are not substantially correlated, otherwise the values of the regression coefficients would not be independent of one another.

Identification of likely causes of impairment.—We used statistically significant associations between species abundances and stressor variables to identify likely causes of biological impairment. While we recognize that such associations do not necessarily imply causation we use the term "cause" in this restricted sense in the remainder of the paper.

We linked the abundances of individual species and the stressors occurring at individual sites as follows:

1) *Predicted abundance.* We applied the calibrated GLM18 regression models to predict the abundance of species i at any site ($E_{i,GLM18}$) as a function of both the naturally occurring and stressor conditions occurring at a site.

2) *Unexplained variance (unknown causes).* We calculated the unexplained variance in species abundances at each site as the departure from a linear association between observed $[\ln(O_i)]$ and expected $[\ln(E_{i,GLM18})]$ abundances over all species. We expressed unexplained variation as $100 - (r^2 \times 100)$, and included this value as one of the slices in the EPC pie diagrams. We realize that the unexplained variance may contain model error, the influence of unknown environmental factors, and may also reflect the omission of factor interactions in our analyses.

3) *Identity of missing species.* The RIVPACS model output allowed us to identify those species that were expected at $PC \geq 0.5$ but not observed at the 695 Ohio river sites.

4) *Associations with different stressor variables.* If a species was missing at a site, as a possible consequence of unfavorable levels of some or all stressors, the contribution of those stressor variables in the GLM18 model prediction should be negative. For example, if species i is missing at site x because of a lack of dissolved oxygen, the value of $(f_{1,i} \cdot DOMED_x + f_{2,i} \cdot DOMED_x^2)$ should be negative. The relative potential influence of each stressor variable is simply that stressor's negative contribution divided by the sum of all negative stressor contributions for missing species. These proportions along with the unexplained variance were used to size the pie slices in the EPC graphs.

5) *Aggregation over sites.* We aggregated site-based estimates to derive insight regarding the overall regional importance of different stressors. We calculated regional values as simple averages of the percentages of variation in abundances associated with different measured factors observed at individual sites. These percentages were used along with percentage unexplained variation to construct a regional summary EPC pie graph.

We recognize that variables used in this study are in part composites, and need not be purely of natural or anthropogenic origin. In particular, we recognize that stressor attribution does not require only human-induced changes. For example, the local pH can be determined by both natural causes (e.g., humic acids) and human-related causes (e.g., acid mine drainage).

Note that the RIVPACS model also identifies species that are not expected at a site, but were nonetheless observed. The attribution model can be adjusted to also identify the likely causes for such additions by evaluating just the positive contributions of individual stressor variables in the GLM 18 models for unexpected species observed. However, because of length and complexity limitations, we do not present these complementary assessments in this paper.

RESULTS AND DISCUSSION

RIVPACS modeling

Latitude, longitude, drainage area, and stream-channel slope accounted for 51% of the variation in observed species richness at reference sites (Fig. 3). Although the RIVPACS model accounted for a considerable amount of natural variation in species composition and richness among reference sites, at least some of the unexplained variation was likely associated with natural factors that we did not or could not measure, factors that were only partly associated with the surrogate predictors, or incomplete representation in the reference data set of the fish fauna found in certain types of streams. For example, some species may have been introduced into water bodies at which they were historically absent and thus partly disassociated with factors that naturally controlled their distribution. In contrast, the natural distribution of some species may be largely the result of biogeographic accidents, and such species with small populations would be especially difficult to model as a function of environmental conditions. These errors in RIVPACS assessments must therefore be considered when inferences are made both on overall biological
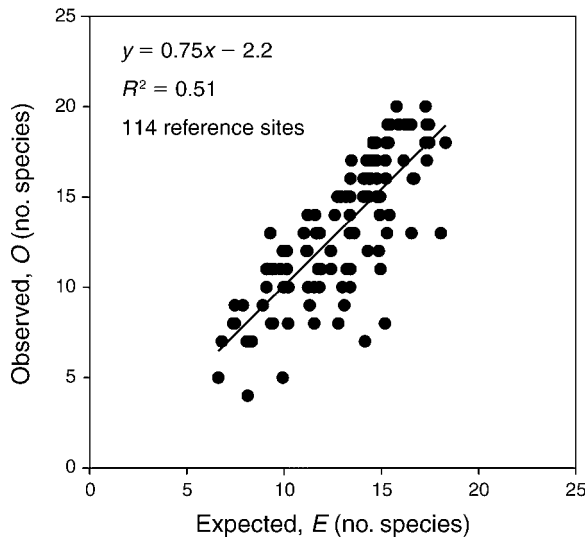
FIG. 3. The goodness-of-fit of the RIVPACS model, based on 114 reference sites.

condition and apparent abundance change of specific species.

O/E values (the ratio of number of species observed [O] at a site vs. the number expected [E]) of 0.8 and 1.2 approximately corresponded to the 10th and 90th percentiles of the reference-site values and were used as error thresholds for inferring if non-reference sites were biologically impaired (Fig. 4). Of the 1438 non-reference sites, ~50% were in non-reference condition ($O/E < 0.8$) and we considered 23% of sites as being severely impaired ($O/E < 0.5$).

Although little quantitative information was available from the literature on which to base comparisons, the RIVPACS assessments of individual species responses

(Appendix B) were more often than not consistent with qualitative assignments found in the literature (Trautman 1981, Barbour et al. 1999). However, for several species, our assignments of species sensitivities were somewhat different than that inferred from the literature. Of the 98 taxa native to Ohio and the 19 introduced species, our assignments agreed with the literature for 63 species and disagreed with 33. Disagreements never involved differences of more than one category (e.g., tolerant [T] in one and intolerant [I] in the other). The RIVPACS-based estimates showed that 17 species native to Ohio were found at more sites than expected. Our analyses imply that these species should be tolerant species, and the majority of them presumably expanded into streams that they did not historically occupy as habitat conditions were altered. This interpretation assumes that the reference-site data used to train the model were representative of all the assessed sites. Although we know this assumption to be imperfect, we attempted to minimize this type of problem by excluding any site from analysis whose predictor-variable values were outside the experience of the model. In other cases, human introductions helped spread species into streams they would have historically not inhabited. For example, the gizzard shad (*Dorsoma cepadianum*), whose habitat typically includes lakes, oxbows, sloughs, and large, slow rivers, was never predicted by the model to occur at any site with a probability of capture > 0.5, but it was found in 499 samples, a likely consequence of both escape from suitable human created habitats (e.g., reservoirs) and their generally high tolerance. The RIVPACS-based assessments also flagged as tolerant any nonnative species that was introduced into these streams and that was captured in a sample. For example, as an exotic
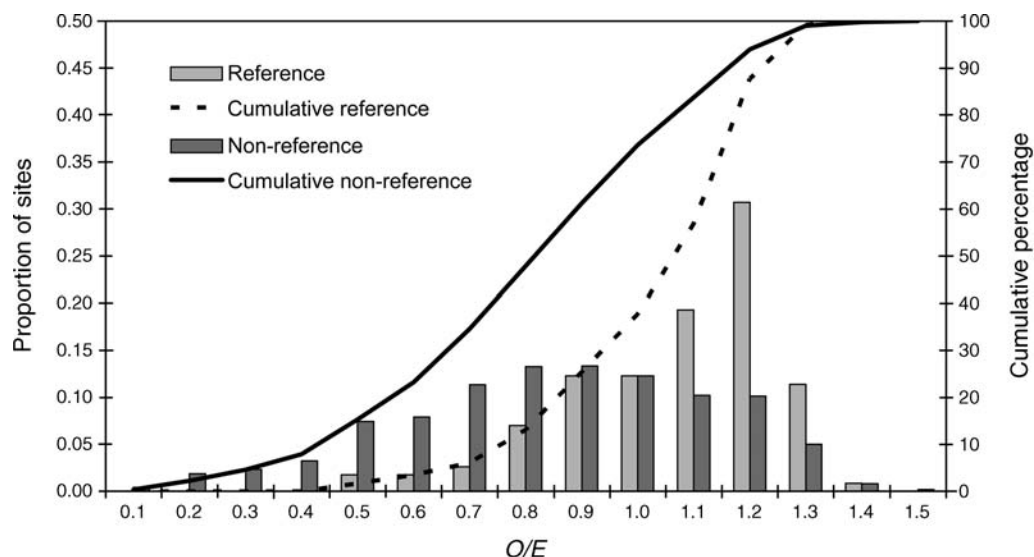

FIG. 4. The distribution of O/E values (the ratio of observed to expected abundances of species) generated by the RIVPACS model for both reference sites ($n = 114$) and potentially impacted sites ($n = 1438$).
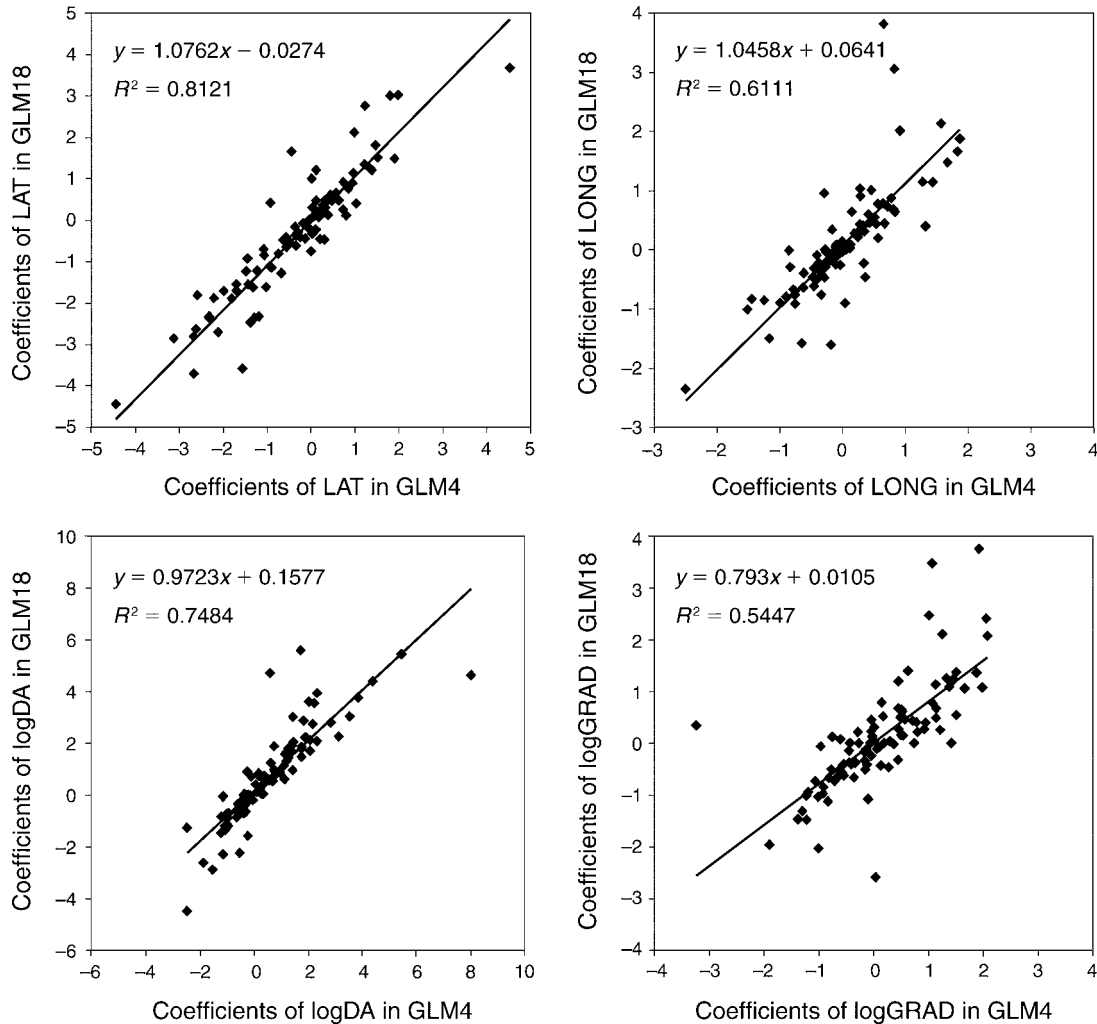
Fig. 5. Comparison of the regression coefficients for 96 different species for the four chosen natural descriptors: latitude (LAT), longitude (LONG), log(drainage area) (logDA), and log(gradient) (logGRAD) in the two sets of generalized linear models, GLM4 and GLM18.

species, the common carp (*Cyprinus carpio*) was predicted to occur at no sites, but it was found at 803 sites. In this case, the high S_O/S_E value (the ratio of observed/expected sites) was a consequence of both its introduction and subsequent spread and it being tolerant of the altered conditions in many Ohio streams.

Forty species were found at substantially fewer sites than predicted by the model and were considered to be likely sensitive species, i.e., decreasers. Some of these species were predicted to be widespread given their distribution among different types of reference sites.

GLM modeling and the derivation of slice sizes

Response of species abundances to natural and stressor variables.—We were able to produce two sets of generalized linear models, GLM4 and GLM18, for 96 of the 117 species assessed by the RIVPACS model. The species for which we could not construct models had low

numbers of occurrences in the 695-site data set. The regression coefficients for the natural predictors in both sets of models were generally similar (Fig. 5) implying that the values of the four natural and the 14 potential predictors of habitat deterioration and contamination were not seriously correlated, a necessary condition for assessing the effects of stressor variables with the approach we used. Only logGRAD (log(gradient)) had significantly different GLM4 and GLM18 regression coefficients (slope = 0.79), which is not surprising in view of its observed correlation with some of the other predictors. However, logGRAD had little effect on overall GLM model predictions (1%). Much more natural variation in species abundances was associated with variation in latitude (50%) and longitude (46%) than with drainage area (4%). The remaining correlations in the predictor data set were mainly associated with habitat factors. These correlations may have
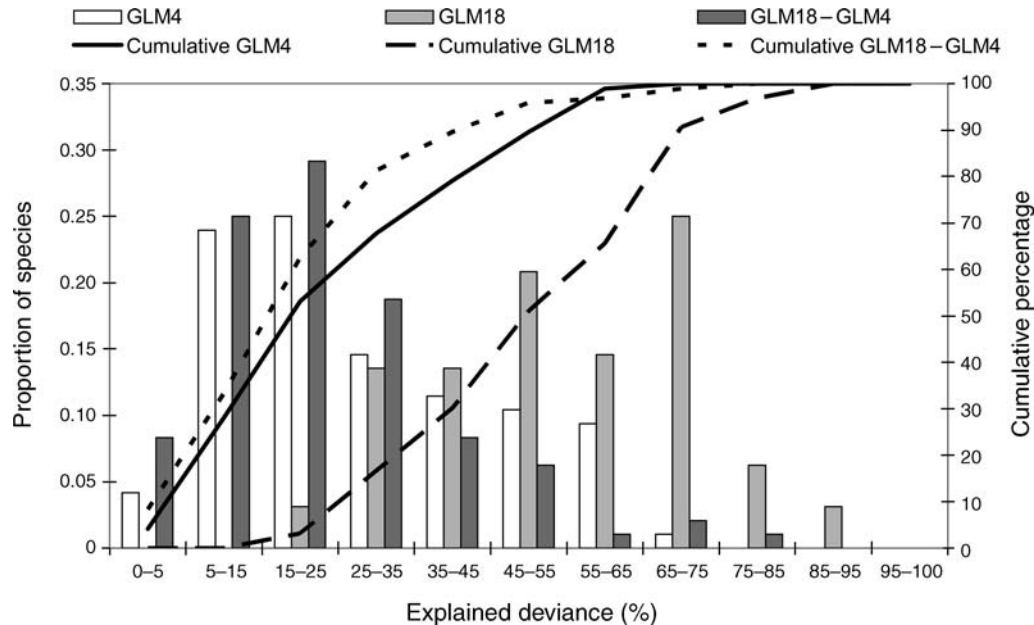
FIG. 6. Frequency distributions over 96 species of explained deviance in the GLM4 and GLM18 models as well as the difference in explained deviance between the two models.

introduced some bias in the attribution of effects to individual habitat factors. In the final presentation of the results, we therefore grouped the different habitat factors into a single pie slice. In view of these considerations, we are confident that our combined use of GLM4 and GLM18 models described realistic responses of species to the different stressors.

Explanatory capacity of the GLM models.—GLM4 models explained between 15% and 25% of the variation in species abundances, whereas the GLM18 models explained between 45% and 55% of abundances (Fig. 6). The difference in the amount of variation associated with GLM4 and GLM18 models indicated that, on average, stressor variables influenced species abundances by about 15–25% above that associated with the four natural variables.

Geographic patterns in likely causes of impairment.— Sites that were highly impaired by loss of species occurred throughout Ohio (Fig. 7) and in both urban and rural areas. Stressors associated with water chemistry were most often associated with species loss, followed by degraded habitat, percentage cumulative effluent, and ecotoxicity. Sites with deviating stressor attribution were first identified by double-blind analysis (anonymous sites and species). Mapping of sites with stress-associated loss of species uncovered that particular combinations of stressors were regionalized throughout Ohio. Our inferences regarding the types of stressors affecting species in these regions were generally consistent with previous analyses as illustrated in the following four examples (Fig. 7).

A) *Black River.* The Black River, located in northern Ohio, west of the city of Cleveland, consists of two branches, East and West. Impairment was greater in the West Branch than the East Branch, and water chemistry appeared to be the dominant stressor, followed by habitat alteration, ecotoxicity, and percentage municipal effluent. The smaller amount of impairment observed at the East Branch was mostly associated with water chemistry and modified habitats. These results agree with Ohio EPA's assessment of the biological impairment of the Black River and selected tributaries (Ohio EPA 1994a). More recently, Ohio EPA's section 305b report (2000) specifically indicated that the primary stressors for the West Branch were heavy silt loads and bank erosion as a result of row-crop agriculture. Failing septic systems were also identified as potential sources of impairment. In contrast, the East Branch was noted as having a high-quality fish assemblage, typically attaining the state's warm-water habitat criteria. Unknown factors dominated likely stressors in the city of Elyria and downstream to Lake Erie. At these sites, U.S. EPA judged nutrients, organic enrichment, and flow alteration from combined sewer overflows and storm sewers as the primary causes for impact (USEPA 1998), factors that we could not include in our analyses.

B) *Cuyahoga River.* Located in northeastern Ohio, the Cuyahoga River flows through the major metropolitan areas of Akron and Cleveland. Minimal impairment was evident in the headwaters of the Cuyahoga (upstream of City of Akron) with most measured degradation associated with water chemistry and unknown factors. However, from Akron downstream, impairment greatly increased, and our assessment identified water chemistry, habitat alteration, municipal effluent, and toxicity from metals and ammonia as likely causes. Ohio EPA
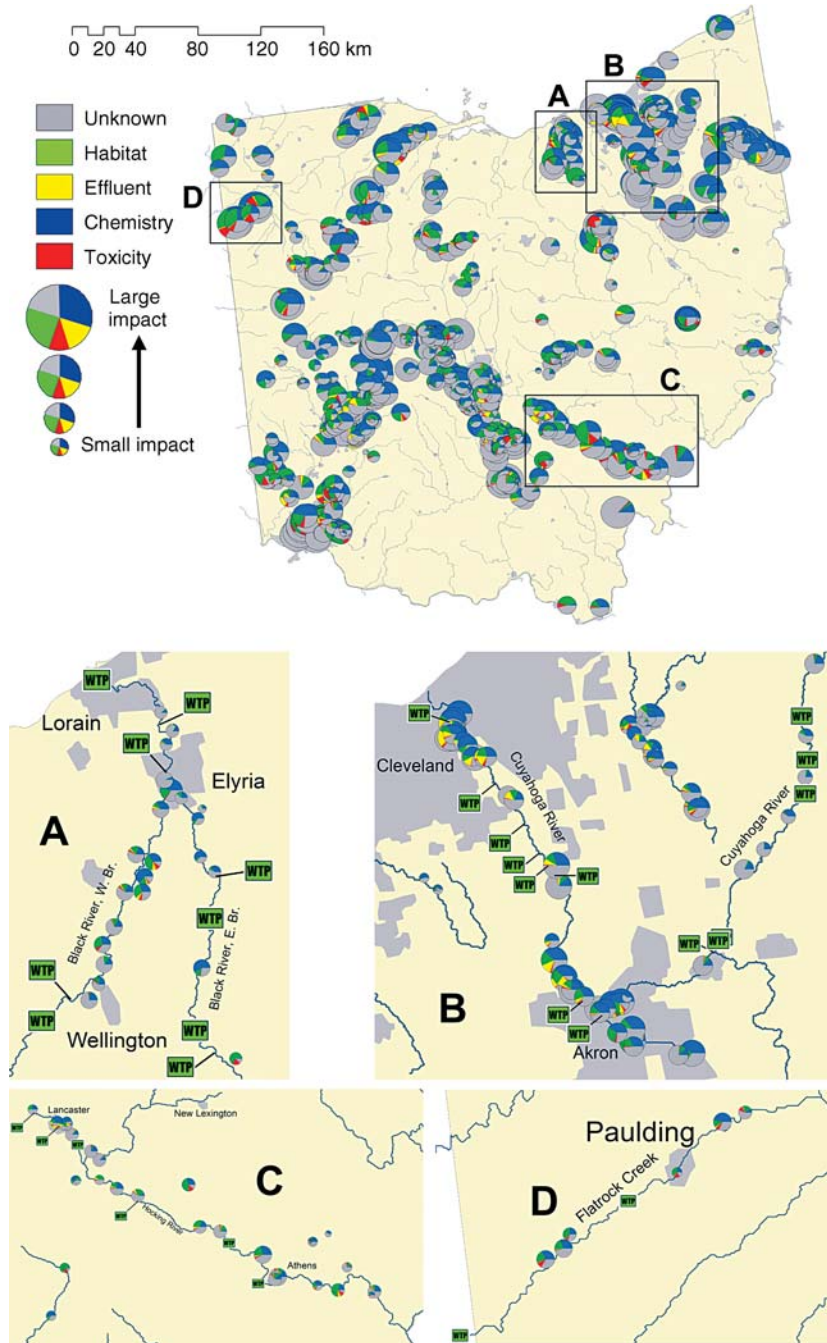
FIG. 7.   Effect and probable cause (EPC) pie charts for 695 sites in Ohio (USA). The size of each pie is proportional to impact (i.e., large pie = large impact). The size of the slice is relative to probable cause. Stressors are grouped in four main types (see color key) for ease of interpretation. See Table 3 for type and descriptions of stressors. (A)–(D) identify locations of four selected Ohio rivers for which more detailed pie charts are shown: (A) Black River, (B) Cuyahoga River, (C) Hocking River, and (D) Flatrock Creek. WTP indicates a waste water treatment plant.

(1994b) identified habitat degradation and exposure to pollutants (land use, number of spills, and number of combined sewer overflows and storm sewer overflows) as likely stressors.

C) *Hocking River*. Located in southeastern Ohio, the biological impairment in the upper Hocking River has

been primarily associated with the irregularities of wastewater treatment in the town of Lancaster (Ohio EPA 1997). Throughout the entire mainstem, siltation, habitat alterations, organic enrichment, nutrients, metals, and pH have been associated with the greatest number of impaired reaches (USEPA 1998). Down-
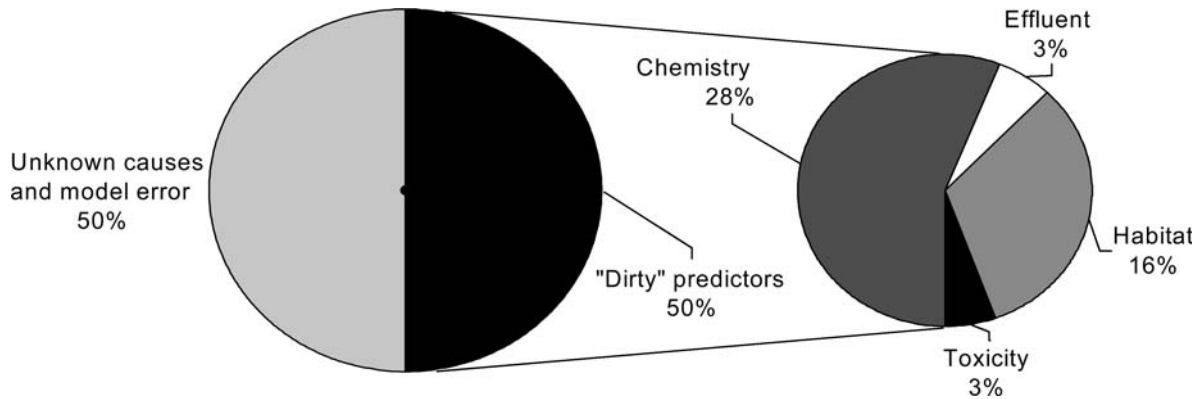
F‌IG. 8. Average loss of fish species in 695 river sites in Ohio (USA) is 40%. This figure shows the likely causes.

stream of Lancaster to the Ohio River, increased levels of metals and silt have been attributed to mining and streambank modifications. Although our analyses attributed ~50% of impairment to unknown factors, our results generally agreed with Ohio EPA reports and indicated that effluent was a key factor in the upper Hocking River and that the ecotoxicity of metals was more important downstream. Our results were also consistent with Ohio EPA's conclusion regarding the importance of streambank modifications in the lower Hocking.

D) *Flatrock Creek.* A small tributary to the Auglaize River, Flatrock Creek is located in northwestern Ohio. According to Ohio EPA, biological impairment in Flatrock Creek, upstream of Paulding, was attributed to organic enrichment and flow alterations (USEPA 1998). Our analyses also indicated that much of the impairment was associated with alterations in water chemistry and habitat modifications. Our methods also identified the toxicity of mixtures in these reaches, of which household-product ingredients were the primary mixture components.

Ohio-wide overview.—Aggregation of all results over all sampling sites showed that, for the state of Ohio as a whole, 40% of fish species were missing from standard samples relative to reference-site expectations (Fig. 8). A large proportion (50%) of biological effects was associated with unknown factors and model error. The remaining 50% of effects was associated with variation in stressors, most of which was related to alteration in water chemistry and habitat (28% and 16%, respectively). The combined toxicity of metals, ammonia, and household-product chemicals, as well as the cumulative input of treated sewage effluents, were associated with 3% of the biological degradation. The high proportion of unexplained effects is understandable because other stressors known to affect aquatic ecosystems, such as alteration in stream flow, pesticides, industrial discharges, input of cooling water, and fishing activities, were not included in our analyses.

Conclusions

We have shown how integration of different assessment tools can be applied to identify both the magnitude and likely causes of biological impairment, given the variability in species composition and species abundances that occurs naturally. Our proposed method combines ecological, ecotoxicological, and exposure modeling to provide both a measure of impact and statistical estimates of the probable effects of different potential stressors on stream fish assemblages. Although a set of statistical analyses was required, a fair proportion of variance was accounted for, and the end product could be presented as simple effect and probable-cause pie charts, facilitating both interpretation and communication of results and decision-making. Constraints imposed by statistical power limited our ability to address interactions among variables, implying that attribution of effects to likely causes is conservative.

The most innovative aspect of this study involved linking different types of models, all of which have been individually applied in the past for many purposes. Applying these models in concert yielded results that generally matched the interpretation of experts who assess and manage Ohio surface waters. Since our analyses were blind to both previous assessments of impairment and inferences regarding the probable causes of impairment at specific sites, the match of our results with other data demonstrates that this approach may provide a robust means of assessing the likely causes of biological impairment in freshwater ecosystems.

Further testing of the approach should include expanding the data set and methods to other stress factors (e.g., pesticides and biological stressors), further discrimination of human-induced and natural variation for composite parameters, addressing the issue of stressor interactions, and inclusion of other biological endpoints (e.g., alteration of invertebrate or algal assemblages). A primary focus of future work should be on reducing the unexplained variance and model error in the approach. Because of the many degrees of

freedom (many predictors) relative to the number of sites in this study, it was not possible to apply a scheme of external validation, something that must be conducted in future work. We also need to evaluate how well the approach can be applied to other data sets. Further testing should also determine if the predicted responses to particular types of perturbation are consistent with known autecological data for different species, such as the quantitative pollution-tolerance values that are being developed by various researchers (e.g., Yuan and Norton 2003). In summary, the results of this study were encouraging and provide a foundation on which future refinement of both the general approach as well as specific methods used can be built.

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APPENDIX A

Scatterplot and correlation matrix of the abiotic input data, comprising the 18 different predictors for the RIVPACS and GLM models (*Ecological Archives* A016-043-A1).

APPENDIX B

Table comparing the ecological sensitivities (tolerant, intolerant and neutral) of individual fish species estimated from the RIVPACS model assessments with literature sensitivity values summarized in Barbour et al. (1999) (*Ecological Archives* A016-043-A2).