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USING HISTORICAL BIOLOGICAL DATA TO EVALUATE STATUS AND TRENDS IN THE BIG DARBY CREEK WATERSHED (OHIO, USA)

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Abstract—Assessment of watershed ecological status and trends is challenging for managers who lack randomly or consistently sampled data, or monitoring programs developed from a watershed perspective. This study investigated analytical approaches for assessment of status and trends using data collected by the Ohio Environmental Protection Agency as part of state requirements for reporting stream quality and managing discharge permits. Fish and benthic macroinvertebrate metrics collected during three time periods (1979–1981, 1986–1989, 1990–1993) were analyzed for the mainstem of Big Darby Creek, a high-quality warm-water stream in central Ohio, USA. Analysis of variance of transformed metrics showed significant differences among time periods for six fish metrics. In addition, significant positive linear trends were observed for four metrics plus the index of biotic integrity score, and negative linear trends for two fish metrics. An analysis of a subset of sites paired by location and sampled over the three periods reflected findings using all available data for the mainstem. In particular, mean estimates were very similar between the reduced and full data sets, whereas standard error estimates were much greater in the reduced subset. Analysis of serial autocorrelation patterns among the fish metrics over the three time periods suggests changes in the nature of stressors over time. A comparison within the most recent time period showed significantly better condition for Big Darby Creek mainstem than for Hellbranch Run (the easternmost subwatershed), after adjusting for watershed size. The consistency of paired and nonrandomized results suggested that either type of data might be judiciously used for this watershed assessment. Results indicated that overall biological condition of the mainstem of the Big Darby Creek watershed has significantly improved since the early 1980s.

Keywords—Bioindicators Biomonitoring Spatial autocorrelation Watershed management

INTRODUCTION

The Big Darby watershed, a high-quality warm-water stream system in central Ohio, USA, is one of five sites nationwide selected by the U.S. Environmental Protection Agency to demonstrate approaches to geographically based risk assessment [1]. The watershed is a national and state scenic river and was one of the original 12 locations designated by The Nature Conservancy as a Last Great Place in the Western Hemisphere [2]. The primary goal of the stakeholders is to protect and maintain native stream communities of the Big Darby Creek ecosystem [3]. To measure attainment of this goal, stakeholders selected the diversity and abundance of fish, benthic macroinvertebrates, and molluscs as indicators of stream community condition. The rationale for the selection of these indicators is described in detail in the accompanying problem formulation document [1].

Two papers in this issue of *Environmental Toxicology and Chemistry* present ecoregion-level analytical studies of the biological data: Gordon and Majumder [4] develop empirical models that forecast ecological quality under different land use scenarios, whereas Norton et al. [5] evaluate important factors that discriminate among sites, using the biological data. This study, in contrast, evaluates historic fish and benthic macroinvertebrate data to determine if these indicators have changed over time or differ geographically within the watershed.

Abundant assessment data exist for the Darby Creek watershed: fish assemblage data exist from the 1950s, and benthic macroinvertebrate data exist from the 1960s [6,7]. The Ohio Department of Natural Resources [8] has recorded declines in the total number of fish species over the past 40 years; however, longitudinal profiles of the stream suggest that, more recently, improvement has occurred in fish assemblage measures at specific locations in the watershed (Ohio Environmental Protection Agency [OEPA] draft report). This study attempts to quantify the status of native fish and benthic macroinvertebrate assemblages among subwatersheds of the Big Darby Creek ecosystem, and to determine whether significant changes have occurred over time at the watershed level. The statistical treatment of nonrandom and spatially autocorrelated sampling data is explored and provides a template for examining status and change in other watersheds.

METHODS

Biological community assessment endpoints

The OEPA implemented a biomonitoring program in the 1970s [9–11], establishing fish and benthic indices of community status. The index of biotic integrity (IBI) and the invertebrate community index (ICI) [9,10,12] were developed from methods established by Karr et al. [13]. The IBI consists of 12 metrics that evaluate different aspects of the fish assemblage's taxa richness, diversity, habitat niche, and pollution

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Table 1. Description of component metrics of the fish index of biotic integrity (IBI)

IBI metric name	Abbreviation	Metric description	Expected response to increased disturbance
Relative abundance	MODNUMS	Number of fish in a sample, adjusted for sampled area	Decrease
Total species richness	NUMSPEC	Number of native species in sample (excluding hybrids)	Decrease
Darter richness	DARTERS	Number of darter species	Decrease
Sunfish richness	SUNFISH	Number of sunfish species	Decrease
Sucker richness	SUCKERS	Number of sucker species	Increase
Intolerant abundance	INTOLS	Number of stress-intolerant species	Decrease
Percent tolerant	TOLPERC	Percent of fish that are stress-tolerant	Increase
Percent top carnivores	TOPCARN	Percent of fish considered top carnivores	Decrease
Percent insectivores	INSECT	Percent of fish that are insectivorous	Decrease
Percent omnivores	OMNIVOR	Percent of fish considered omnivorous	Increase
Percent simple lithophils	SMPL_LP	Percent clean-gravel spawners	Decrease
Percent DELT anomalies	DELTA	Percent of fish with deformities, eroded fins, lesions, or tumors	Increase
Percent round suckers	RDSUCKPC	Percent round-bodied suckers	Decrease

tolerance, whereas the ICI's 10 metrics evaluate benthic macroinvertebrate assemblage structure. The scales of the IBI and ICI are both from 0 to 60, with higher scores indicating better condition. Several of the fish taxa-richness metrics have been scaled to the maximum expected species richness for a given drainage area [14]. The details of the construction, scaling, and validation of the metrics and indexes for benthic macroinvertebrates and fish are given in the above references; however, descriptions of the metrics are provided in Tables 1 and 2.

Historical biological surveys

The biological data comprising the IBI and ICI metrics have been collected using consistent methodology since the late 1970s [10]. Sites within the watershed were selected based on the expectation that they were representative of stream condition at impaired and less-impacted stream segments. Sampling locations were selected on the basis of best professional judgment by experts in fish and benthic macroinvertebrate community evaluation in the OEPA, and sample collection and processing are described elsewhere [15]. Many sampling areas were revisited during the period of interest; however, only a few sampled locations were established as permanent monitoring stations by the state agency.

Data assessment

All data for this assessment were obtained from databases maintained by the OEPA. At the outset of the risk assessment, biologists, statisticians, and those involved in designing the study and collecting the data collaborated to identify data to be used for the temporal and subwatershed comparisons. Fish and benthic macroinvertebrate assemblage data were used for this study. Mollusc data were not used because data sets were restricted to only a few locations in the watershed. For the subwatershed assessment, only data from 1990 to 1993 were sufficiently abundant to permit statistical comparisons. For the temporal comparisons, three periods were identified with enough data for evaluation: 1979 to 1981, 1986 to 1989, and 1990 to 1993; however, only the mainstem of Big Darby Creek had sufficient data for the temporal analysis. Important covariates were also ascertained, including the stream mile location and watershed drainage area at the site.

Because stream sampling locations were not randomly selected during the three evaluation time periods, a separate analysis was conducted using only data collected from sites within 0.2 stream miles of each other. Comparing the conclusions from both sets of analyses permitted an evaluation of bias resulting from different sampling regimes during different time periods.

Statistical analysis

All statistical calculations were performed using SAS/STAT® software (SAS Institute, Cary, NC, USA). Data were transformed to enhance homogeneity of variance and normality of error residuals. Differences among subwatersheds in

Table 2. Component metrics of the benthic invertebrate community index (ICI)

ICI metric name	Abbreviation	Metric description	Expected response to increased disturbance
Total taxa richness	NUMTAXA	Total number of taxa in sample	Decrease
Mayfly richness	NUMMAY	Total number of mayfly taxa in sample	Decrease
Caddisfly richness	NUMCAD	Total number of caddisfly taxa in sample	Decrease
Dipteran richness	NUMDIPT	Total number of dipteran taxa in sample	Increase (variable)
Percent mayfly	PERMAY	Percent mayfly individuals in sample	Decrease
Percent caddisfly	PERCAD	Percent caddisfly individuals in sample	Decrease
Percent Tanytarsini	PERTANY	Percent Tanytarsini individuals in sample	Decrease
Percent other dipterans and noninsects	PEROTHDI	Percent non-Tanytarsini dipteran and noninsect individuals in sample	Increase (variable)
Percent tolerant	PERTOLN	Percent tolerant individuals in sample	Increase
Qualitative EPT[a] taxa	QUALEPT	Number of EPT taxa in sample	Decrease

[a] EPT = Ephemeroptera, Plecoptera, and Trichoptera.

the component metrics of the IBI and ICI were evaluated using analysis of variance of the transformed data. Levene's test [16] was used to evaluate the homogeneity of variances in a simple analysis of variance model of mean differences across time periods and subwatersheds. Analysis of covariance was used to test for differences among time periods, after adjusting the model for the covariate watershed drainage area. The Tukey–Kramer multiple comparison procedure [17] with an experimentwise alpha of 0.05 was used to evaluate the significance of differences in least squares means of the biological metrics across time periods or subwatersheds. Means and confidence intervals were back-transformed for reporting in original units. For the time period trend evaluation, a linear trend was tested using orthogonal polynomials (−1, 0, 1) for the earliest through latest time periods, respectively [18]. For the comparison among time periods, the residuals from each successive addition to the linear model were tested for first-order serial autocorrelation. A Durbin–Watson statistic was calculated to test for serial autocorrelation among adjacent points along the stream [17], using an alpha of either 0.05 or 0.01.

RESULTS

Site selection

A map of the Big Darby watershed is included in Cormier et al. [1]. In the Big Darby watershed during the historical period of interest (late 1970s to 1990s), more than 157 sites were evaluated for fish assemblage status, whereas benthic macroinvertebrates were assessed at approximately 60 sites. A preponderance of the fish assemblage data were obtained from the Big Darby mainstem (n = 121). Most data in the Little Darby and Hellbranch Run subwatersheds were obtained during the early 1990s (n = 25 and 11, respectively); therefore, the subwatershed comparison was restricted to data collected from 1990 to 1993. For both the Big Darby and Little Darby subwatersheds, sampling locations were dispersed along the entire stream length; however, sampling was focused along the lower half of the Hellbranch Run subwatershed.

In the Big Darby Creek subwatershed, the majority of biological assessment data were collected during one of three time periods: 1979 to 1981, 1986 to 1989, and 1990 to 1993 (n = 46, 37, 38, respectively, for fish, and n = 14, 8, 18, respectively for benthic macroinvertebrates). Therefore, data were grouped into one of these three time periods for the evaluation of trends. No seasonal differences occurred in sample collection during the three time periods (data not shown). Sites were located consistently either upstream or downstream of known point sources; however, the OEPA's site selection tended to be slightly closer to the point source in the latest time period.

A companion analysis among time periods was conducted using only sites located within 0.2 river miles of a site in a previous time period, in order to evaluate bias in sampling across time periods. Fourteen such sites were identified in time periods 1979 to 1981 and 1990 to 1993, and 12 sites in time period 1986 to 1989. The locations of all sample points used in the paired and unpaired data analyses are shown in Figure 1. Neither the locations of the sites along the stream length nor the watershed drainage area at a site differed between paired and complete data sets for any time period ([T] always less than 1.6, p always greater than 0.12, data not shown).

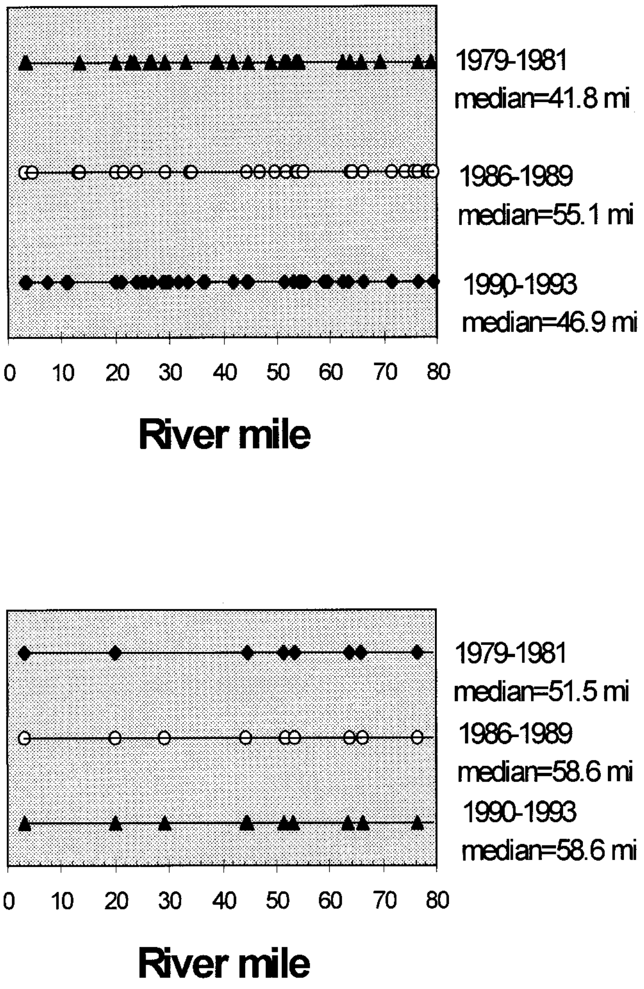

Fig. 1. River mile sampling locations for time period comparisons, Big Darby Creek mainstem. Locations for complete data set are in top panel, and for paired locations in the bottom panel.

Comparison of subwatersheds

To improve homogeneity of variance and normality of the data, the metrics for number of sucker species, and percentage top carnivores, round-bodied suckers, tolerant individuals, and fish with deformities, fin erosion, lesions, and tumors (DELT anomalies) were adjusted using a square root transformation. All other data were normal with homoscedastic residuals when expressed in raw form across subwatersheds or time periods.

The fish assemblages of the Big Darby ecosystem showed numerous significant differences across subwatersheds (Fig. 2 and Table 3), when no adjustment was made for drainage area differences among the three subwatersheds. Compared to the Little Darby or Hellbranch Run subwatersheds, the Big Darby mainstem had significantly greater mean numbers of darters, sunfish, intolerant species, total numbers of species, and individuals, as well as significantly greater mean percentages of round-bodied suckers, top carnivores, insectivores, and simple lithophils (Table 3). A significantly lower percentages of tolerant fish and fish DELT anomalies were found in the Big Darby compared to the Little Darby and Hellbranch subwatersheds. The mean IBI score was significantly greatest in the Big Darby mainstem (51.9), and was progressively lower in the Little Darby and Hellbranch watersheds (48.7 and 41, respectively).

The watershed drainage area at a given site in the stream

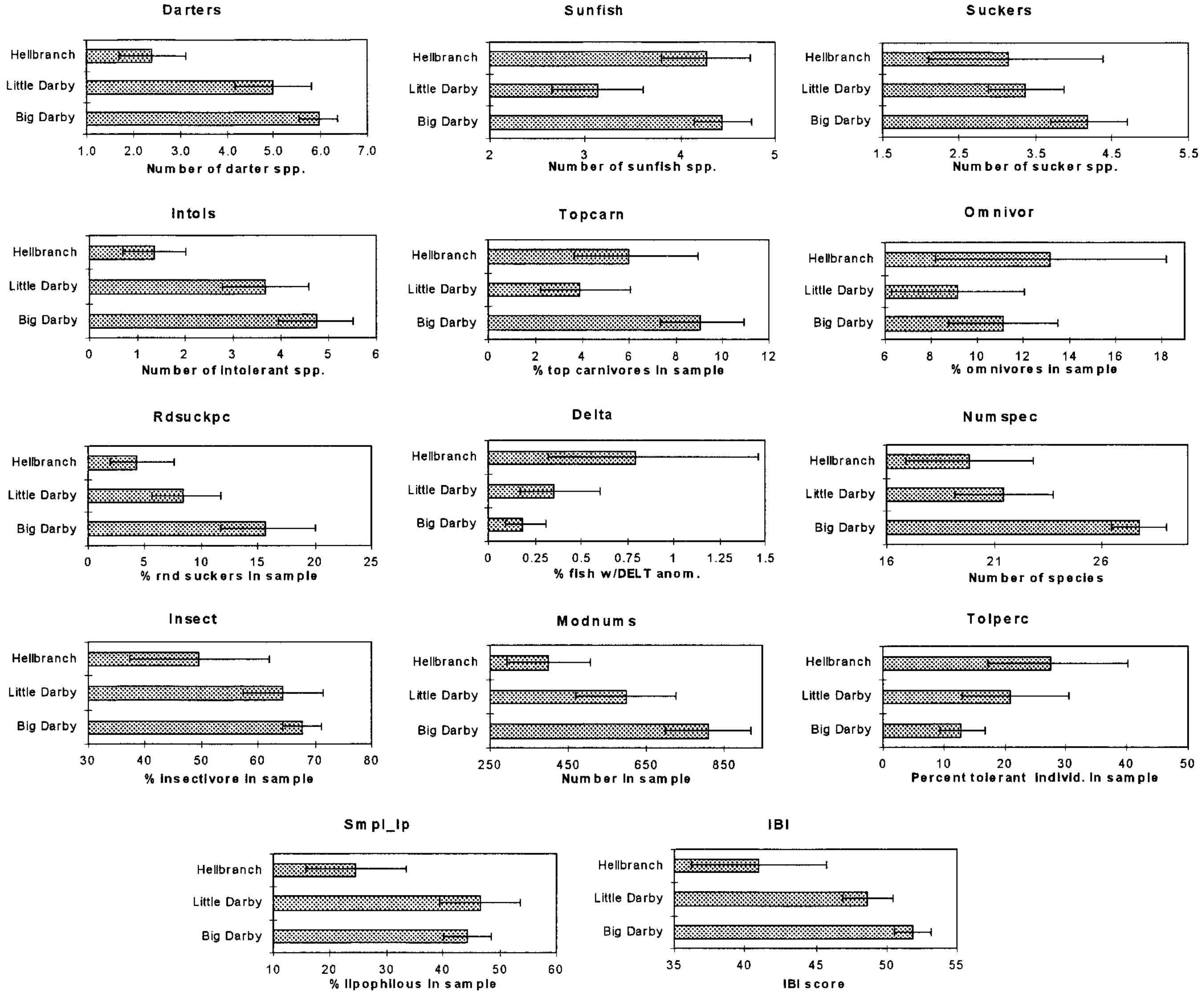

Fig. 2. Results of subwatershed analyses of variance for the 13 fish metrics and index of biotic integrity scores (1993 data). Mean value (±2 SE) shown in graph.

Table 3. Results of *F* tests and Tukey–Kramer multiple comparison procedures for differences in the index of biotic integrity metrics among subwatersheds, before and after controlling for the effect of watershed drainage area. All comparisons are made at a two-sided familywise α of 0.05. For metrics in bold type, watershed drainage area was a highly significant ($p < 0.001$) covariate[a]

	F (*p* value) for subwatershed and multiple comparison tests			
Metric	Subwatershed only		Subwatershed + drainage area	
DARTERS	23.22 (<0.0001)	BD > LD > HB	18.14 (<0.0001)	(BD = LD) > HB
SUNFISH	13.02 (<0.0001)	(BD = HB) > LD	10.63 (<0.0001)	(BD = HB) > LD
SUCKERS	3.36 (0.0405)	BD = LD = HB	**0.32 (0.7259)**	**BD = LD = HB**
INTOLS	10.24 (<0.0001)	(BD = LD) > HB	**5.04 (0.0090)**	**BD = (LD > HB)**
TOPCARN	7.63 (0.0010)	(BD > LD) = HB	**2.66 (0.0768)**	**BD = LD = HB**
OMNIVOR	1.23 (0.2977)	BD = LD = HB	**4.52 (0.0143)**	**(BD > LD) = HB**
RDSUCKPC	8.61 (0.0004)	BD > (LD = HB)	**0.76 (0.4695)**	**BD = LD = HB**
DELTA	4.94 (0.0098)	(BD < HB) = LD	4.79 (0.0112)	(BD < HB) = LD
NUMSPEC	19.19 (<0.0001)	BD > (LD = HB)	**5.87 (0.0044)**	**BD > (LD = HB)**
INSECT	6.53 (0.0025)	(BD = LD) > HB	3.31 (0.0425)	BD = (LD > HB)
MODNUMS	8.31 (0.0006)	BD > (LD = HB)	9.07 (0.0003)	BD > (LD = HB)
TOLPERC	3.96 (0.0234)	(BD < HB) = LD	**0.71 (0.4944)**	**BD = LD = HB**
SMPL_LP	9.30 (0.0003)	(BD = LD) > HB	**8.43 (0.0005)**	**BD = (LD > HB)**
IBI	21.46 (0.0001)	BD > LD > HB	**10.73 (0.0001)**	**(BD = LD) > HB**

[a] Metric names are defined in Table 1. BD = Big Darby subwatershed ($n = 38$); LD = Little Darby subwatershed ($n = 25$); HB = Hellbranch subwatershed ($n = 11$).

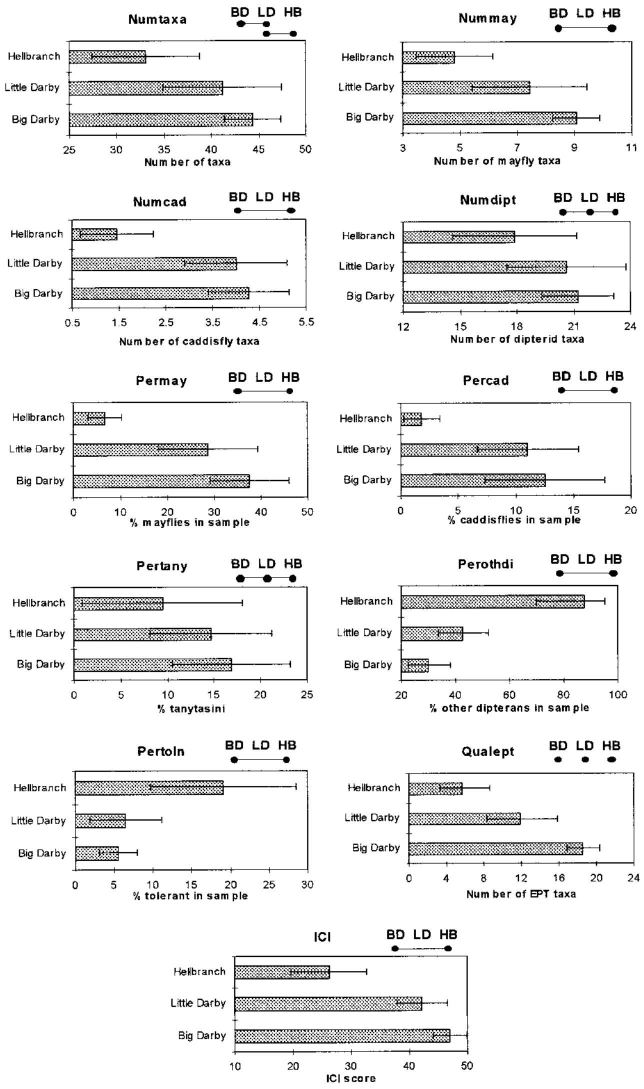

Fig. 3. Results of subwatershed analyses of variance for the 10 benthic macroinvertebrate metrics and invertebrate community index scores (1993 data). Mean value (±2 SE) shown in graph. Result of Tukey–Kramer multiple comparisons procedure is in top right corner of each graph: lines connecting points indicate no significant difference between pair of group means.

reach was a highly significant covariate for the IBI and for several of the metrics, including sucker, intolerant species, and total native species richness, and the percentages of top carnivores, omnivores, round-bodied suckers, and tolerant individuals. Moreover, the median drainage areas at sampling locations were also significantly different among subwatersheds at 202.5, 83, and 30 km^2 in the Big Darby, Little Darby, and Hellbranch runs, respectively ($p < 0.0001$). Inclusion of drainage area in the analysis of covariance model changed the significance, and in some cases, the conclusions regarding differences among subwatersheds (Table 3). For example, differences for the round-bodied sucker metric were eliminated after the inclusion of drainage area. The Big Darby subwatershed was not significantly different from Hellbranch Run after including drainage area with respect to the intolerant species richness, sucker richness, round-bodied sucker percent individuals, percent insectivorous individuals, percent tolerant individuals, and the percent simple lithophils.

The benthic macroinvertebrate metrics, like the fish metrics, showed significant differences among subwatersheds (Fig. 3). The total taxa, mayfly, and caddisfly richness, and the percent abundances of mayfly and caddisfly were significantly greater in the Big Darby mainstem than in Hellbranch subwatershed. The Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) taxa richness metric was successively larger in Little Darby and Big Darby than in Hellbranch ($p < 0.05$). Overall ICI scores were significantly higher in Little Darby and Big Darby than in Hellbranch. Metrics indicating impairment (percent other dipterans and percent tolerant organisms) were significantly higher in Hellbranch than in the Little Darby or Big Darby subwatersheds. These results indicate consistently higher-quality conditions in Big Darby mainstem than in the Hellbranch Run, with the Little Darby subwatershed tending to be more similar to the Big Darby mainstem. Because of the small sample size, drainage area was not incorporated into the linear model for the benthic macroinvertebrate metrics. However, scores for 8 of 10 metrics are calibrated by drainage area in OEPA calculations of ICI scores to account for differences in occurrence and distribution of taxa affected by stream size or order [10,12].

Comparison of time periods and test of linear trend

Data were grouped into three time periods (1979–1981, 1986–1989, 1990–1996) for the analysis of change over time within the watershed, using analysis of variance and a test of linear trend. Sufficient data for the analysis of trends over time were available only for the fish metrics in the Big Darby mainstem.

Many of the measured aspects of the fish communities showed significant and consistent improvement in the most recent time period (Fig. 4 and Table 4). The mean IBI score was significantly greater in 1990 to 1993 than in the late 1970s, at 52 and 47.5, respectively. Darter and total species richness, number of intolerant species, and the percentage of round-bodied suckers all were significantly greater in 1990 to 1993 than in 1979 to 1981 ($p < 0.05$), whereas the percentage of omnivores was significantly lower in 1990 to 1993. The percentage of insectivorous fish significantly decreased from the late 1970s to the early 1990s, and the percentage of fish with DELT anomalies increased significantly.

The test for linear trend in fish metrics showed a significant increase in darter, intolerant, and total species richness metrics, an increase in the total number of individuals and percentage of DELT anomalies, and a decrease in the percentage of omnivores over the observed time period (Table 4). The analysis of variance results in Table 4 also indicate generally improved condition in the latest time period. The darter, sunfish, sucker, intolerant and total species richness, the total number of individuals, IBI score, and the percentages of top carnivores and round-bodied suckers each show significant increases in the latest time periods over at least one of the earlier time periods. However, the proportion of DELT anomalies also significantly increased since the late 1970s.

The mean drainage areas at the sampled locations in the Big Darby mainstem were not significantly different among the three time periods ($F = 2.14$, $p = 0.1223$). However, as found in the subwatershed analysis, drainage area was a highly significant covariate for the IBI and some of its metrics (sucker, intolerant and total species richness, and percentages of omnivore, round-bodied sucker, insectivore, and tolerant individuals). Incorporation of drainage area into the model did not greatly alter the significance of differences among time periods

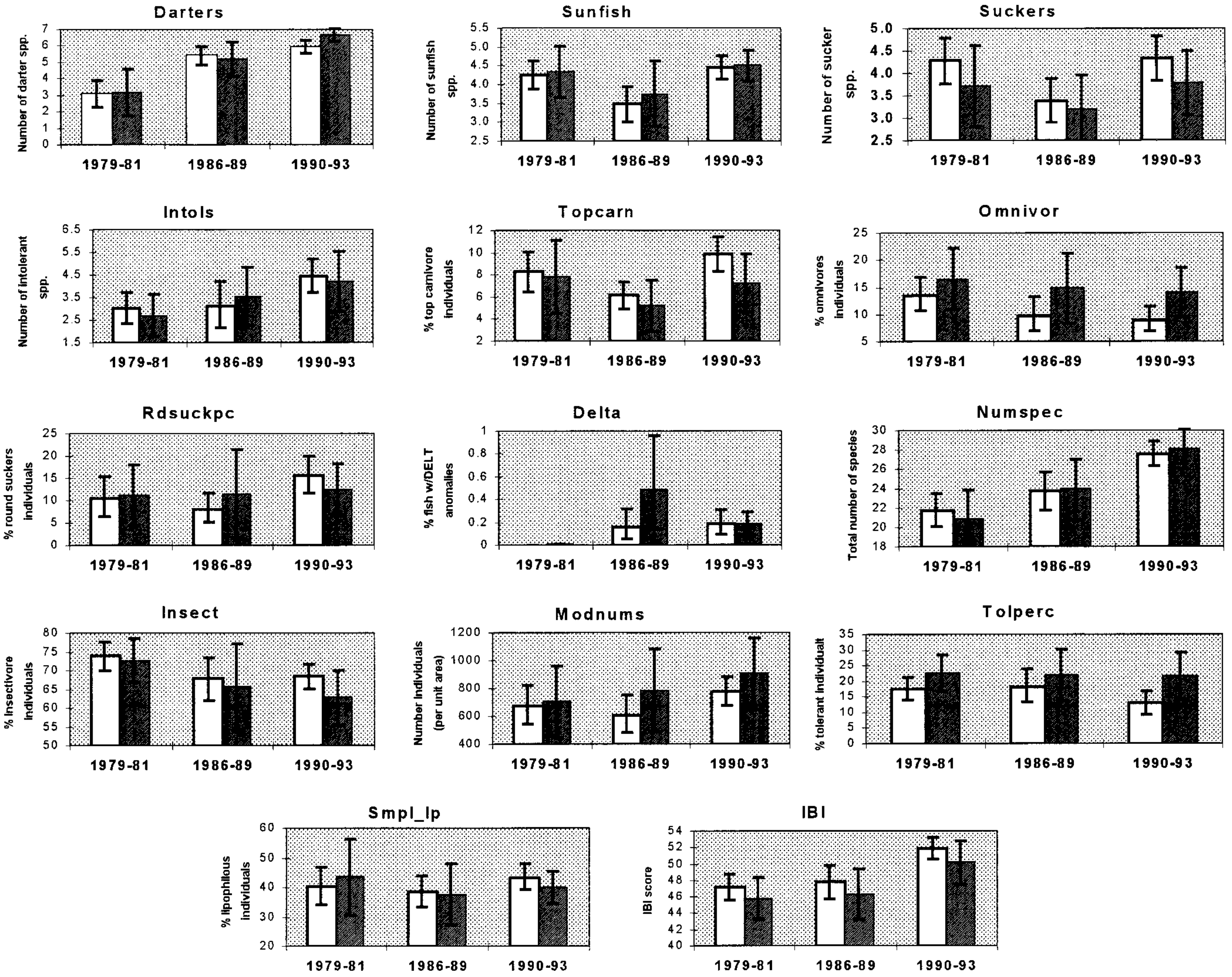

Fig. 4. Comparisons among time periods for the index of biotic integrity and its component metrics in the mainstem of Big Darby watershed, 1979 to 1993. White bars show mean values (±2 SE) for complete data set, and hashed bars indicate means (±2 SE) for data from sites paired throughout the time periods.

Table 4. Results of linear trend test, *F* tests, and Tukey–Kramer multiple comparison procedures for differences in the index of biotic integrity metrics along time periods within the Big Darby mainstem, before and after controlling for the effect of watershed drainage area. Comparisons in boldface indicate that watershed drainage area is a highly significant covariate ($p < 0.002$). All comparisons are made at a two-sided familywise α of 0.05[a]

		Test for time period & multiple comparison results			
	Test for linear trend (df = 1,119)	Time period only (df = 2,118)		Time period + drainage area (df = 2,117)	
Metric	F ($p > F$)	F ($p > F$)	Result	F ($p > F$)	Result
DARTERS	42.18 (<0.0001)	24.3 (<0.0001)	79 < (86 = 90)	23.3 (<0.0001)	79 < (86 = 90)
SUNFISH	0.25 (0.6178)	6.61 (0.0019)	86 < (79 = 90)	6.83 (0.0016)	86 < (79 = 90)
SUCKERS	0.00 (0.9989)	5.28 (0.0064)	86 < (79 = 90)	**2.62 (0.073)**	**79 = 86 = 90**
INTOLS	5.60 (0.0196)	5.05 (0.0079)	(79 = 86) < 90	**6.26 (0.0026)**	**(79 = 86) < 90**
TOPCARN	1.50 (0.225)	5.26 (0.0065)	79 = (86 < 90)	4.50 (0.0131)	79 = (86 < 90)
OMNIVOR	5.24 (0.0239)	3.27 (0.0416)	79 = 86 = 90	**4.77 (0.0102)**	**79 > (86 = 90)**
RDSUCKPC	2.44 (0.1213)	4.15 (0.0181)	79 = (86 < 90)	**3.53 (0.0325)**	**(79 < 86) = 90**
DELTA	29.12 (<0.0001)	18.4 (<0.0001)	79 < (86 = 90)	18.2 (<0.0001)	79 < (86 = 90)
NUMSPEC	23.56 (<0.0001)	13.6 (<0.0001)	(79 = 86) < 90	**14.9 (<0.0001)**	**79 < 86 < 90**
INSECT	3.68 (0.0576)	2.76 (0.0677)	79 = 86 = 90	**1.66 (0.1941)**	**79 = 86 = 90**
MODNUMS	1.06 (0.3061)	1.54 (0.2188)	79 = 86 = 90	1.45 (0.2389)	79 = 86 = 90
TOLPERC	2.53 (0.1153)	3.49 (0.0337)	79 = 86 = 90	**4.34 (0.0152)**	**86 = (79 < 90)**
SMPL_LP	0.50 (0.4824)	0.72 (0.4905)	79 = 86 = 90	0.72 (0.4887)	79 = 86 = 90
IBI	15.44 (<0.0001)	10.6 (<0.0001)	(79 = 86) < 90	11.3 (<0.0001)	(79 = 86) < 90

[a] Metric names are defined in Table 1. 79 = 1979–1981 (n = 38); 86 = 1986–1989 (n = 46); 90 = 1990–1993 (n = 38).

Table 5. Spatial autocorrelation in index of biotic integrity metric data for the Big Darby mainstem. First-order autocorrelation (ρ) and significance level (*p*) are tabulated before and after controlling for drainage area. All comparisons are made at a two-sided α of 0.05, indicated in boldface[a]

	Durbin–Watson residual test for first-order serial autocorrelation					
	Time period only			Time period + drainage area		
Metric	79–81 (ρ, *p*)	86–89 (ρ, *p*)	90–93 (ρ, *p*)	79–81 (ρ, *p*)	86–89 (ρ, *p*)	90–93 (ρ, *p*)
DARTERS	0.009, >0.05	0.094, >0.05	0.002, >0.05	0.004, >0.05	0.057, >0.05	0.065, >0.05
SUNFISH	0.208, >0.05	0.158, >0.05	**0.649, <0.01**	0.110, >0.05	−0.022, >0.05	**0.330, <0.05**
SUCKERS	0.078, >0.05	0.231, >0.05	**0.303, <0.01**	0.070, >0.05	0.240, ind[b]	**0.205, <0.05**
INTOLS	−0.047, >0.05	**0.538, <0.01**	**0.660, <0.01**	−0.051, >0.05	**0.275, <0.05**	**0.325, <0.05**
TOPCARN	−0.070, >0.05	**0.266, <0.05**	**0.457, <0.01**	−0.066, >0.05	0.180, >0.05	**0.306, <0.05**
OMNIVOR	0.239, >0.05	0.137, >0.05	**0.245, <0.05**	**0.300, <0.05**	−0.086, >0.05	−0.001, >0.05
RDSUCKPC	0.138, >0.05	**0.489, <0.01**	**0.572, <0.01**	0.047, >0.05	**0.288, <0.05**	−0.101, >0.05
DELTA	−0.023, >0.05	0.139, >0.05	−**0.463, <0.01**	0.141, >0.05	0.139, >0.05	−**0.477, <0.01**
NUMSPEC	−0.005, >0.05	**0.304, <0.05**	**0.448, <0.01**	−0.068, >0.05	0.222, >0.05	**0.366, <0.01**
INSECT	0.115, >0.05	0.197, >0.05	**0.367, <0.01**	0.157, >0.05	0.036, >0.05	0.228, >0.05
MODNUMS	−0.151, >0.05	**0.419, <0.01**	**0.028, >0.05**	−0.048, >0.05	**0.397, <0.01**	0.082, >0.05
TOLPERC	0.227, >0.05	**0.580, <0.01**	**0.628, <0.01**	0.043, >0.05	**0.214, <0.05**	0.180, >0.05
SMPL_LP	−0.013, >0.05	**0.325, <0.01**	0.107, >0.05	−0.016, >0.05	**0.326, <0.01**	0.114, >0.05
IBI	−0.060, >0.05	**0.409, <0.01**	**0.247, <0.05**	−0.062, >0.05	**0.281, <0.05**	−0.157, >0.05

[a] Metric names are defined in Table 1. 79 = 1979–1981 (*n* = 38); 86 = 1986–1989 (*n* = 46); 90 = 1990–1993 (*n* = 38).

[b] Indeterminate.

(Table 4). The specific comparisons among the time periods were similar to the model unadjusted for drainage area: darter, sunfish, intolerant, and total species richness, as well as percentages of top carnivores and DELT anomalies, and the overall IBI scores were all significantly greater in the 1990 to 1993 time period than in at least one of the earlier periods. The percentage omnivores and tolerant individuals was significantly lower in the latest time period. Percent insectivores and simple lithophils, sucker richness, and modified total numbers of individuals did not differ significantly among the time periods after controlling for drainage area. Drainage area was very highly correlated with river mile (Pearson's $\rho = -0.998$) for the entire combined data set in the Big Darby subwatershed.

Spatial analysis

A spatial analysis between adjacent points in the three time periods was possible because the distribution of distances between adjacent points was not significantly different for the three time periods (Fig. 1). The median adjacent point distances were 0.7, 1.1, and 0.8 miles, respectively, for the first, middle, and latest time periods.

The degree and significance of serial autocorrelation between adjacent sites differed among the three time periods (Table 5). In the linear model adjusted for time period only, neither the IBI nor any of the metrics were serially autocorrelated for the first time period ($p > 0.05$). In the second time period, the IBI and 7 of the 13 component metrics showed significant positive autocorrelation, and in the most recent time period, 11 of the 13 metrics and the IBI scores were significantly autocorrelated. The autocorrelations also tended to become increasingly significant over the three time periods (Table 5).

Incorporating drainage area into the regression model reduced the degree and significance of the Durbin–Watson autocorrelation measure (Table 5) for most metrics. Just five of the component IBI metrics in the second time period showed significant autocorrelation, and all but two metrics had reduced degree and significance in the autocorrelation. In the latest time period, 6 of the 13 metrics were significantly autocorrelated, with reduced significance in most cases (Table 5).

DISCUSSION

Benthic and fish indices of integrity, and their component metrics, demonstrate superior biotic condition in the Big Darby compared to the Little Darby and Hellbranch subwatersheds. Explanations for the increased biotic integrity are likely to be quite complex. For example, controlling an important covariate, drainage area at the sampled location in the watershed, somewhat mitigated these subwatershed differences. Most richness and proportional fish metrics have been shown to be influenced by the size of the watershed. The OEPA, when constructing fish IBIs, adjusts some metrics for differences in drainage area [10]. However, in the current study, darter, sunfish, and top carnivore richness, as well as the total number of individuals and percentages of simple lithophil individuals and individuals with DELT anomalies were unaffected by drainage area. Several of these metrics are likely to be influenced by other major factors not considered in this study, such as proximity to point source discharges (in the case of DELT anomalies) [19] and substrate type or quality (for percent simple lithophils).

Hellbranch Run is a much smaller subwatershed than Little Darby Creek, which is smaller than Big Darby Creek. Therefore, some degree of the differences among the fish metrics and IBI is likely to be due to these drainage area differences. Thus, when considering the hypothesis of subwatershed differences in the Big Darby risk assessment, expectations for the fish metrics should take into account differences due to watershed size [20]. Although the Ohio IBI is adjusted for watershed size, a residual effect of drainage area seems to remain in the fish biotic index for the Big Darby watershed, perhaps because of correlations between drainage area and other important variables, such as habitat or location of point sources.

The sensitivity of benthic metrics to drainage area differences could not be considered in this study because of a small sample size; analysis of the unadjusted data suggests that conditions in the Big Darby mainstem are superior to those in Hellbranch Run. After controlling for subwatershed differences in drainage area, it is evident that most fish metrics were not significantly better in the Big Darby mainstem compared to Hellbranch Run. The total number of species, the total number of nontolerant individuals (modified for sampling area), and darter richness were significantly greater in the Big Darby. The percent of fish with DELT anomalies was significantly lower in the Big Darby mainstem than in Hellbranch Run, perhaps due to a heavier concentration of point and nonpoint source toxic contaminants in the latter system.

The second hypothesis of interest for the Big Darby watershed risk assessment was a test of changes in the watershed over time [1]. Because insufficient data were available to consider the entire watershed, evaluation focused on the mainstem of Big Darby Creek. The results of the present study suggest that conditions in the Big Darby watershed mainstem have generally improved since the late 1970s. These changes were not likely due to site selection biases during the three different time periods, because the mean values and especially the trends observed were very similar between the complete data set and the data set limited to sites that were paired across time periods (Fig. 4). Differences in the IBI and component metrics over time suggest that much of the improvement has occurred since the mid-1980s. Although the mean watershed drainage areas did not differ significantly among the three time periods, incorporation of the drainage area into the overall model generally increased the significance of the time period effect, as is often observed in analysis of covariance [17]. Drainage area was an especially important covariate for reducing the degree of serial autocorrelation among the sites.

Serial autocorrelation, in which adjacent points are more or less likely to be similar in value as points far apart, is generally measured for data collected along a time series. However, the analogy of streams to time is quite good: both are unidimensional, especially in the case of smaller streams, and may be assumed to be predominantly unidirectional in influence. A large body of statistical analysis has developed to measure and incorporate serial autocorrelation in time series data [17,21], including the use of the Durbin–Watson test. The strengths of evaluating and incorporating serial autocorrelation into linear models are well known: data that are serially autocorrelated artificially deflate the model variance. Autocorrelation in data sets frequently suggests the influence of unincorporated important covariates [17]. In this model, serial autocorrelation was greatly reduced for most metrics by the inclusion of the watershed area.

The differential patterns in spatial autocorrelation for the three time periods are not attributable to different distances between adjacent points (data not shown), suggesting that a change has occurred in the underlying influences on stream biota. The lack of significant spatial autocorrelation in the first time period may suggest that isolated influences (such as localized pollutant inputs, or the presence of barriers in the stream) were highly influential during the late 1970s. More recently, as low-head dams have been removed in the streams and point source discharges have been mitigated, more diffuse influences on the watershed may be contributing to the higher correlations among adjacent sites. The percentage of individuals with DELT anomalies was the sole measure that showed significant negative autocorrelation, indicating that adjacent sites were less likely to be similar than sites far apart. This result was probably due to extremely localized point sources that were spatially separated, because this metric responds primarily to inputs of toxic contaminants [19].

An important result of this study is the finding that river mile location may effectively substitute for drainage area at a site. Linear models employed in this study for the time period comparisons could have used either river mile location or drainage area with equivalent results. Similar equivalent measures could include cross-sectional volume at a site, or stream order. These substitutions may be important when watershed drainage area information is unavailable.

In this study we have provided an example of a retrospective evaluation of the status and trends of biological community integrity, which accounts for some of the potential biases that may exist in nonrandomly sampled data. Although the modeling approach is powerful, careful consideration of the approach in sample design, collection, and data analysis is required to provide meaningful and unbiased tests of hypotheses of interest such as those considered in the Big Darby Creek watershed risk assessment.

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