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Biotic Index Tested for Ability to Assess Water Quality of Missouri Ozark Streams[1]

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Abstract

The Biotic Index (BI), a numerical index that combines the pollution tolerance of benthic insects with estimates of community structure, effectively discriminated among differences in the macroinvertebrate assemblages of eight Missouri Ozark streams of differing water quality. The BI at a given site is affected by spatial and temporal differences in the benthic community but not by species that occur in only small numbers. Six kick-net samples provided sufficient data for estimating an average BI value, and for detecting statistically significant differences in the values between two sites. Differences in the BI among sampling sites were supported by differences in the taxonomic composition of the benthic communities and were statistically related to stream water chemistry. The BI was more sensitive and less variable than diversity indices for discriminating differences in stream water quality.

Inventories of stream water quality usually include quantitative estimates of the benthic macroinvertebrates because their special characteristics make them valuable environmental indicators (Hynes 1970). They are especially useful under conditions of intermittent or mild organic enrichment when altered water quality is not readily detectable by conventional chemical surveys (Chutter 1972). Although there is general agreement that organic enrichment tends to restrict the number of species and simultaneously increase the density of tolerant species (Keup et al. 1967; Gaufin 1973), there is no single accepted method of data analysis or a generally agreed upon standard criterion by which this community can be used to interpret water quality (Bartsch and Ingram 1966; Howmiller and Scott 1977).

Two common approaches toward assessing stream environmental quality by means of benthic macroinvertebrates are the use of the animals as indicator organisms and the evaluation of community diversity. Assessments of the effects of pollution sometimes are based on the presence or absence of an indicator species having a known tolerance to organic enrichment (Sladecek 1973); however, such assessments frequently are considered insufficient because

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the information provided by an analysis of the community is considered necessary (Richardson 1929; Gaufin and Tarzwell 1952). Although community diversity, based on the structure and composition of taxa in the benthic community, frequently is used and lends itself to mathematical analyses (Wilhm and Dorris 1968; Pielou 1975; Kaesler et al. 1978), it disregards the ecological sensitivities of the organisms composing the community. Another disadvantage of this approach is that not all communities, even though undisturbed by human activities, have an inherently high diversity. Consequently, it is not always possible to equate specific ranges in diversity values with environmental conditions or degree of damage to the ecosystem (Wilhm 1970; Howmiller and Scott 1977; Kaesler 1978).

Several authors have attempted to combine the indicator-organism and community-diversity approaches to provide a method of data analysis and valid criteria for interpreting benthic communities as indicators of water quality (Beck 1955; Brinkhurst et al. 1968; Howmiller and Scott 1977). One means of analyzing the environmental quality of streams based on benthic macroinvertebrates is the Biotic Index (BI) as proposed by Chutter (1972) and modified by Hilsenhoff (1977). This index is a measure of the pollution of lotic environments by readily oxidizable allochthonous organic matter and its breakdown products. It summarizes the deviation of the observed community of animals from the community that would be expected if the water were unenriched (Chutter 1972). This summary is made by integrating the biology, natural history, and tolerance to organic pollution of the individual species collected. The BI combines the ecological affinity of the species with estimates of community structure to provide a numerical index.

To calculate the BI, one must assign to each species a pollution-tolerance value related to its occurrence in streams of known water quality (Chutter 1972; Hilsenhoff 1977). Assigned values, designated a_i, are whole numbers ranging from 0 for species characteristically found in clean streams to 5 for species inhabiting extremely polluted waters; values from 1 to 4 are assigned to species associated with intermediate degrees of pollution tolerance (Hilsenhoff 1977). The index is calculated by multiplying the number of individuals of each taxon by its pollution tolerance value. The sum of the products is divided by the total number of individuals collected in the sample.

Because the BI has had only limited application (Chutter 1972; Hilsenhoff 1977; Coetzer 1978), its potential for evaluating stream water quality requires further examination. We sought to determine if, by using the BI as described by Hilsenhoff (1977), we could discriminate effectively among differences in water quality in selected Missouri Ozark streams whose environmental condition had been estimated, a priori, from previously collected data. We established three objectives: (1) to determine the ability of the BI to discriminate among differences in water quality in selected Missouri streams, and to relate the BI to those differences; (2) to analyze the variation of the BI within and among sampling sites; and (3) to propose criteria by which water quality in Missouri Ozark streams can be evaluated, and to compare these with an index commonly used to evaluate benthic macroinvertebrate communities.

Methods

Fifteen samples of the aquatic insect community were collected between 1 June and 19 December 1978 at each of 11 study sites in eight streams in the White and Gasconade river basins in southwest and south-central Missouri (Table 1). These streams were selected, on the basis of available data on water chemistry and benthic macroinvertebrates, to represent the range of conditions found in Missouri Ozark streams (Dieffenbach and Ryck 1976; Duchrow 1976; Ryck 1976). Urban, pasture, and forest land made up 94 to 100% of the area of each watershed. On the basis of available data, we believed that these streams covered a wide range from nearly pristine, with no apparent human perturbations (Spring Creek, Bryant Creek, and North Fork of the White River), to slightly enriched (sites 1, 2, and 3 in Finley Creek, Potters Creek, and sites 1 and 2 in James River), to grossly enriched (Wilson Creek and Big Piney River—both of which receive treated municipal sewage). During this study, Smart (1980) concurrently collected water quality data at the same sites (Table 1).

We collected aquatic insects from the stones-in-current biotope (Chutter 1972) in an aquatic kick net having a D-frame (46 × 20 cm) and a

TABLE 1.—*Location and geometric means of water chemistry variables from sites on Missouri Ozark streams between June and December 1978.*

Preliminary assessment of condition and site[a]	Chlorophyll *a* plankton (mg/m^3)	NH_3-N (mg/m^3)	NO_2-N (mg/m^3)	NO_3-N (mg/m^3)	Total P (mg/m^3)	Total particulate P (mg/m^3)	Alkalinity (mequivalents/liter)	Cl^- (equivalents/liter)	K^+ (equivalents/liter)	Na^+ (equivalents/liter)	$SO_4^=$ (equivalents/liter)	JTU[b]
Nearly pristine												
Spring Creek	2.1	1.9	1.7	118	14.1	3.1	3.50	23	56	44	17	1.6
Bryant Creek	3.5	2.9	2.9	143	28.1	14.2	4.05	38	67	63	23	4.0
North Fork, White River	2.5	1.9	2.4	100	18.8	9.1	3.66	37	60	60	85	1.9
Slightly enriched												
Finley Creek, site 1	2.8	3.6	4.3	331	38.0	18.3	3.22	138	84	163	77	3.9
Finley Creek, site 2	5.2	2.8	5.1	448	87.8	18.4	3.03	138	99	191	65	4.0
Potters Creek	2.1	2.4	2.3	185	15.5	5.8	2.11	73	63	92	73	1.6
James River, site 1	4.9	5.1	5.2	149	47.8	22.3	2.85	151	120	175	130	5.4
James River, site 2	10.2	3.2	6.6	405	81.7	42.4	3.05	176	106	233	213	11.9
Grossly enriched												
Wilson Creek	2.6	26.7	32.9	2,406	5,531	1,029	2.90	2,140	680	1,768	903	4.2
Big Piney River	84.1	1,228	44.8	49	1,584	506	5.11	482	226	1,020	230	14.2

[a] No water chemistry data for Finley Creek, site 3.

[b] Jackson Turbidity Units.

net (46 × 20 × 25.4 cm) with 8 meshes/cm and 1-mm openings. At each site two sets of three kick samples were collected—one near shore, one near the middle of the stream, and one between the two. To collect each kick sample, we disturbed an area of about 0.40 m^2 to a depth of 10 cm.

From 50 to 75 organisms were sorted and picked in the field from each set of samples, yielding 100–150 specimens per site. Hilsenhoff (1977) reported that about 100 organisms constituted an adequate sample for estimating the BI at a site, and that the most abundant taxon(s) should not exceed 25 in the sample. About 30 minutes were spent collecting at each site. It was not always possible to collect 100 organisms from a site: treated sewage effluent severely reduced populations in Wilson Creek, and scouring occasionally decreased populations at several other sites. At the Big Piney River site, sewage effluent caused the benthic community to be heavily dominated by two taxa; consequently we usually collected only about 50 organisms there.

To determine the amount of variation in the aquatic insect community within a site on a given stream, we sampled four riffles and one chute within a 450-m reach of Spring Creek on 30 September 1978. Twenty samples (each the composite of three kick samples) were collected from five transects. The samples were returned to the laboratory for sorting and enumeration. Data collected by this procedure were compared with field-sorted samples to determine if about 100 organisms would indeed adequately estimate the BI.

We identified organisms to species when possible, using the most appropriate keys available, and had the identifications verified by specialists (Tracy 1979).

Two biological indices were calculated for each sample to evaluate water quality: an approximation of the Shannon-Weaver index of species diversity (Wilhm and Dorris 1968) and the Biotic Index (Chutter 1972; Hilsenhoff 1977). These indices were calculated as follows:

$$\text{Species diversity} = \sum_{i=1}^{S} (n_i/N)\log_2(n_i/N);$$

$$\text{Biotic Index} = \sum_{i=1}^{S} n_i \cdot a_i/N;$$

where N is the total number of individuals in the sample, n_i is the number of individuals in the ith species (taxon), a_i is the pollution tolerance value for the ith species, and S is the number of species.

For the BI, pollution tolerance values (a_i) were adopted from Hilsenhoff (1977) or were assigned according to the tolerance ranges of specific taxa for water quality variables (Table 2), including chlorides, nitrogen, phosphorus, and biochemical oxygen demand (Roback 1974; Harris and Lawrence 1978; Hubbard and Peters 1978; Surdick and Gaufin 1978). About 45% of the organisms could not be identified to species; about half of these were chironomids. Organisms not identified to species were assigned a_i values corresponding to the most pollution-tolerant species of that genus (Hilsenhoff 1977). The a_i values are, therefore, considered to be conservative estimates.

Taxonomic comparisons were made among collections of benthic invertebrates by the percent-similarity index of Pielou (1975):

$$\text{Percent similarity} = 200\sum_{i=1}^{S} \min P_{ix} \cdot P_{iy};$$

where P_{ix} and P_{iy} are the quantities of species (taxon) i at stations x and y, as proportions of the total quantity of all species at the two stations combined, and S is number of species.

Results and Discussion

Aquatic Insect Communities

We collected 119 taxa of aquatic insects during the study; 45 to 66 were collected from the pristine to moderately enriched sites and 22 were collected from each of the grossly enriched sites (Table 3). Within any single collection, the number of taxa varied from 2 to 32, depending on the site and date of collection.

Of the aquatic insects identified, the values of a_i were 0 for 18 taxa (clean-water association), 1 for 30 taxa, 2 for 37 taxa, 3 for 22 taxa, 4 for 10 taxa, and 5 for 2 taxa (pollution-tolerant organisms). Some 71% of the organisms collected had a_i values of 2 or less, indicative of overall good water quality in most of these streams.

Biotic Index

Within a site, the variance in the BI depends on the spatial distribution of benthic insects and the effects of temporal fluctuations on the composition of the benthic community. The major source of variance in the BI among sites, however, is attributed to differences in water quality; differences in BI among sites should reflect the degree of nutrient or organic enrichment of the waters. These sources of variance in the BI were examined.

Within-Site Variability

The mean BI at a single site on a given sampling date, estimated at five locations in Spring Creek, ranged from 1.34 to 1.90, and the standard error of the mean (reflecting within-site variation) was less than 0.12 at all locations (Table 4). Mean BI values at four locations did not differ significantly from one another (range 1.71–1.90) but were significantly greater ($P < 0.05$) than the mean BI value of 1.34 at location 3. This difference was caused by large numbers of *Helicopsyche borealis* and *Optioservus sandersoni* (both having a_i values of 1) relative to the total number of organisms collected. The proportions of these two species in the total collection accounted for 64% of the variability in the BI among locations in Spring Creek (Spearman rank correlation coefficient $r_s = 0.80$; $P < 0.01$).

Substratum differences among the locations probably accounted for the differences in species distribution (Hynes 1970; Rabeni and Minshall 1977). The substratum at location 3 was predominantly pebble, whereas those at the other locations were more varied, composed of cobble and gravel. Also, location 3 was completely shaded by a tree canopy, whereas the

TABLE 2.—*Pollution tolerance values* (a_i) *of aquatic insects inhabiting Missouri Ozark streams, assigned by B. H. Tracy. Values of* a_i *range from 0 (pollution-intolerant) to 5 (very pollution-tolerant).*

Taxon	a_i
Ephemeroptera	
Ephemerellidae	
Eurylophella aestiva	0
Serratella serratoides	1
Ephemeridae	
Ephemera varia	1
Hexagenia limbata	3
Heptageniidae	
Epeorus spp.	0
Heptagenia maculipennis	2
Stenacron gildersleevi	2
Stenonema femoratum	3
S. integrum	3
S. luteum	1
S. pulchellum	2
Leptophlebiidae	
Choroterpes spp.	2
Habrophlebiodes spp.	1
Leptophlebia spp.	2
Neoephemeridae	
Neoephemera spp.	1
Polymitarcyidae	
Ephoron album	1
Siphlonuridae	
Siphlonurus marshalli	2
Odonata	
Coenagrionidae	
Argia apicalis	2
A. plana	3
A. tibialis	3
Enallagma civile	4
Gomphidae	
Dromogomphus spp.	3
Erpetogomphus designatus	2
Gomphus vastus	1
Ophiogomphus rupinsulensis	1
Progomphus obscurus	1
Stylogomphus albistylus	1
Macromiidae	
Didymops transversa	2
Macromia spp.	2
Plecoptera	
Capniidae	
Allocapnia granulata	1
A. vivipara	2
Leuctridae	
Zealeuctra claasseni	0
Perlidae	
Neoperla spp.	2
Perlinella drymo	0
P. ephyre	0
Perlodidae	
Hydroperla crosbyi	2
Isogenoides varians	1
Pteronarcyidae	
Pteronarcys dorsata	1
P. pictetti	1
Taeniopterygidae	
Taeniopteryx burksi/maura	2
T. metequi	1
T. nivalis	1
Coleoptera	
Dryopidae	
Helichus lithophilus	3
Elmidae	
Optioservus sandersoni	1
Stenelmis beameri	2
S. convexula	2
S. exigua	2
S. lateralis	2
S. sexlineata	3
Limnichidae	
Lutrochus laticeps	1
Diptera	
Athericidae	
Atherix lantha	2
Ceratopogonidae	
Forcipomyia spp.	1
Chironomidae	
Ablabesmyia spp.	3
Procladius spp.	4
Stenochironomus spp.	1
Diptera, continued	
Thienemanniella spp.	1
Tribelos spp.	1
Empididae	
Hemerodromia spp.	4
Roederiodes spp.	4
Simuliidae	
Simulium spp.	4
Tanyderidae	
Protoplasa fitchii	1
Tipulidae	
Erioptera spp.	3
Trichoptera	
Brachycentridae	
Brachycentrus lateralis	1
Glossosomatidae	
Agapetus spp.	1
Hydropsychidae	
Hydropsyche arinale	1
H. frisoni	3
H. orris	3
H. sclaris	3
Macronema carolina	1
Symphitopsyche (*bifida* group)	3
S. piatrix	0
Leptoceridae	
Setodes spp.	1
Limnephilidae	
Pseudostenophylax spp.	2
Philopotamidae	
Wormaldia spp.	1
Polycentropidae	
Polycentropus centralis	0
Rhyacophilidae	
Rhyacophila fenestra	0
R. lobifera	2
Lepidoptera	
Pyralidae	
Petrophila spp.	2

canopy was more open at the other locations. Our data, however, suggested that the within-site variability of the BI caused by the spatial distribution of benthic insects was only a small source of error in sites with a uniform substrate.

At eight of the sampling sites (Table 3) the BI showed a significant linear decrease over time (regression analysis, $P < 0.01$). The BI's were highest in June and lowest in December (see range of values in Table 3). The BI at these sites decreased an average of 0.5 unit (range 0.3 to 0.9), which accounted for about 50% (r^2 range 0.41 to 0.87) of the variation in the BI within a site during the study.

This decrease in the BI over time seems re-

TABLE 3.—*Total taxa and Biotic Index (BI) values for Missouri Ozark streams, June–December 1978.*

Site	N	Total taxa		BI		Standard error	Coefficient of variation (SD/mean)· 100
		Number	Range per collection	Mean	Range		
Spring Creek	15	58	18–27	1.62	1.21–1.98[a]	0.058	14.20
Bryant Creek	15	57	13–25	2.05	1.72–2.35[a]	0.049	9.26
North Fork, White River	15	61	11–31	2.10	1.90–2.25	0.026	4.74
Finley Creek, site 1	15	63	19–29	2.22	2.01–2.63[a]	0.051	9.01
Finley Creek, site 2	15	57	20–29	2.03	1.73–2.31[a]	0.048	9.36
Finley Creek, site 3	15	66	18–32	2.19	1.78–2.53[a]	0.059	10.50
Potters Creek	15	58	19–24	2.24	1.65–2.69[a]	0.079	13.84
James River, site 1	15	65	18–32	2.34	2.06–2.51[a]	0.033	5.56
James River, site 2	11	45	18–25	2.28	2.11–2.43	0.035	5.26
Wilson Creek	11	22	6–15	2.98	2.40–3.61[a]	0.106	4.03
Big Piney River	15	22	2–12	4.77	3.99–5.00	0.083	6.71

[a] Biotic Index values decreased significantly with time ($P < 0.01$).

lated to the seasonal dynamics within the benthic community. The level of identification given to all taxa was consistent throughout the study and we found no difference in the number of taxa collected at these sites over time. However, between June and December the composition of the benthic fauna, as estimated by percent similarity comparisons, changed by 40 to 70% depending on the site. Organisms identifiable only to genus (such as *Cheumatopsyche* sp., *Baetis* sp., *Caenis* sp., *Heptagenia* sp., and others) made up over 50% of the June collections but only 30% of the December collections. Inasmuch as organisms identified to genus were assigned a_i values corresponding to the most pollution-tolerant species of that genus, the BI probably was overestimated at sites where a high proportion of the sample was composed of organisms identifiable only to genus—as was true in our early collections. When only the organisms identified to species were used to calculate the BI, the BI values were about 0.4 unit lower, and showed no significant change over time (regression analysis, $P > 0.05$).

TABLE 4.—*Variation in the Biotic Index (BI) at five locations, Spring Creek, Missouri, 30 September 1978 (based on four samples per location).*

Location	Mean BI	Range	SD	SE	Coefficient of variation[a]
1	1.71	1.52–1.85	0.17	0.08	9.94
2	1.88	1.80–1.97	0.08	0.04	4.26
3	1.34	1.20–1.61	0.19	0.09	14.18
4	1.81	1.58–1.92	0.16	0.08	8.84
5	1.90	1.67–2.19	0.25	0.12	13.16

[a] (Standard deviation ÷ mean) × 100.

The BI was stable over time at three sites—James River site 2, North Fork of the White River, and Big Piney River—and none of the variance in the index could be related to time. The coefficient of variation at these sites was less than 7%.

Among-Site Comparisons

Differences in BI's among the 11 sites were significant (analysis of variance, $P < 0.01$). In orthogonal comparisons ($\alpha = 0.05$) of mean BI values made on the basis of prior water chemistry and macroinvertebrate data collected from these streams (Dieffenbach and Ryck 1976; Duchrow 1976; Ryck 1976), the values for Wilson Creek (2.98) and Big Piney River (4.77) were significantly different ($P < 0.05$) from one another and from the other sites. Spring Creek, representing nearly pristine conditions, had a mean BI (1.62) significantly lower than the values at all the other sites. Mean BI's for the North Fork of the White River (2.11) and Bryant Creek (2.05) did not differ. Mean BI's for the Finley Creek sites (site 1, 2.22; site 2, 2.03; site 3, 2.29) did not differ significantly ($P > 0.05$), but collectively they were significantly lower than mean BI's for Potters Creek (2.24) and the two James River sites (site 1, 2.34; site 2, 2.28). There were no statistical differences between mean BI's for Potters Creek and James River sites.

Water Quality Categories and the Biotic Index

Based on statistical differences among our sites and the criteria of Chutter (1972) and Hilsenhoff (1977), we present preliminary criteria for evaluating water quality in streams of the Missouri Ozarks on the basis of BI values. These criteria classify Ozark streams as ranging from clean, unpolluted waters ($BI < 1.75$) to polluted waters ($BI \geqslant 3.25$); two intermediate categories—slightly enriched ($1.75 < BI < 2.50$) and enriched ($2.50 < BI < 3.25$)—represent conditions of transition between these extremes. Using these criteria, one can evaluate the water quality at the sites we studied as follows:

Clean, unpolluted—Spring Creek;
Slightly enriched—North Fork of the White River; Bryant, Finley, and Potters creeks; James River;
Enriched—Wilson Creek;
Polluted—Big Piney River.

The delineations provided by these criteria seem reasonable, as judged by earlier water quality data from these streams and the water chemistry data collected during the study (Table 1). Differences in BI's among streams were significantly correlated with an increase in the concentration of certain stream chemicals associated with nutrient enrichment (Table 5). The BI's were highest in Wilson Creek and the Big Piney River, which are enriched by point-source additions of municipal sewage, as indicated by the water chemistry data (Table 1). There were no known point-source inputs of sewage to the other streams; there, water chemistry was influenced by land-use practices on the watersheds. The concentrations of the chemicals correlated with the BI (Table 5) increased with increases in the proportional areas of urban and pasture land on a watershed and decreased as the area of forest land increased (Smart 1980). Therefore, human disturbances on these watersheds influenced the chemical composition of the stream water, and the BI values reflected these disturbances.

TABLE 5.—*Spearman's rank correlation coefficients between the geometric mean of water quality variables and the mean Biotic Index (BI) from sites on Missouri Ozark streams (N = 10), June–December 1978. Asterisks denote * P < 0.05 or ** P < 0.01.*

Water quality variable	Correlation with BI
Ammonia N	0.84**
Nitrite N	0.78**
Total N	0.70**
Total P	0.64**
Total particulate P	0.77**
Chloride	0.83**
Potassium	0.81**
Sodium	0.76**
Sulfate	0.91**
Turbidity	0.67*

Differences in BI values among streams generally supported our a priori opinions of stream water quality. Exceptions were the North Fork of the White River and Bryant Creek, which were previously considered pristine (like Spring Creek), but, as judged by water chemistry data collected during the study and the BI values (Table 3), probably represented an intermediate condition between Spring Creek and enriched waters. During the study the concentrations of most water chemistry variables were generally lower in Spring Creek than at the other sites (Table 1), and concentrations of Cl, Na, turbidity, and planktonic chlorophyll *a* were significantly lower in Spring Creek than in the North Fork of the White River, and Bryant Creek (analysis of variance, $P < 0.01$). These differences probably were related to land-use practices on the watersheds. Forest land covered about 86% of the Spring Creek watershed but only about 60% of the other two watersheds (Smart 1980).

The proposed water quality categories also are substantiated by differences in the taxonomic composition of the benthic communities. Using the percent-similarity index to compare benthic communities among sites, we found that only 2% of the organisms collected from Big Piney River (classified as polluted) and 10% of those collected from Wilson Creek (enriched) were present at the other sites (Table 6). Of the organisms collected at these sites during the study, 97% in the Big Piney River and 76% in Wilson Creek had pollution tolerance values (a_i) of 3 or more, indicating poor water quality conditions in these streams. An average of 39% of the organisms collected in Spring Creek (classified as clean) were found in the slightly enriched streams. Within the streams classified as slightly enriched, 44% of the organisms were common to all the communities.

Despite the general agreement between the

TABLE 6.—*Mean percent-similarity values for faunal compositions in Missouri Ozark streams.*

Stream	Spring Creek	Bryant Creek	North Fork, White River	Finley Creek			Potters Creek	James River		Wilson Creek
				Site 1	Site 2	Site 3		Site 1	Site 2	
Bryant Creek	53									
North Fork, White River	50	61								
Finley Creek, site 1	38	47	48							
Finley Creek, site 2	25	37	32	46						
Finley Creek, site 3	37	47	47	60	60					
Potters Creek	43	37	37	46	30	44				
James River, site 1	38	41	45	56	44	56	43			
James River, site 2	27	30	34	41	35	46	36	51		
Wilson Creek	7	6	8	10	16	16	11	14	12	
Big Piney River	2	2	2	2	2	3	2	2	1	5

BI and our prior opinion of stream conditions, the proposed criteria describe a continuum of water quality that is difficult to subdivide. There were no clear discontinuities upon which to base separations in water quality in the streams because changes did not occur at sharply defined places. Many intermediate conditions existed within each of the nomenclatural categories tentatively proposed. This intermediacy was indicated by statistically significant differences among BI values even within the proposed categories. Therefore, waters that have a mean BI within the midrange of the respective categories can be classified with greater confidence than waters with BI's near the extremities of the range.

Sampling Considerations

To estimate a BI value, we field-sorted about 100 organisms as recommended by Hilsenhoff (1977), except that during the within-site variability study at Spring Creek, we collected complete samples. The Spring Creek samples resulted in mean BI values (1.34 to 1.90, Table 4) similar to those for the entire period of study (1.21 to 1.98, Table 3), even though the number of specimens in a collection was 2 to 20 times greater. Of the 62 taxa of insects collected in the larger samples, about 84% were represented in the field-sorted samples. The taxa not normally collected were rare forms, not present at all locations in Spring Creek, and composing less than 1% of the organisms in the samples. These data suggest that thorough samples of faunal composition are not necessary to determine a BI value for a site because the BI is not greatly influenced by species that do not occur in the community in significant numbers (Hilsenhoff 1977; Kawasaki and Pollack 1980). Therefore, for purposes of biological monitoring, estimates of faunal composition taken by a standardized collection technique should be adequate for the estimation of the BI of a stream and for comparison with estimates made from similar collections.

Detecting statistically significant differences in the BI values between two sites and estimating width of the confidence intervals about a BI value from a given site depend on the number of samples collected. To determine the number of samples per site required to detect differences and estimate confidence intervals, we used an estimate of within-site variability over time from a pooled estimate (variance $[s^2] = 0.05$; df = 146).

The criterion used for considering the desired precision at a specific site was that a sample of values for a site (at the 95% confidence interval) must yield an estimated average BI value within 0.25 of the actual BI value. The 0.25 value was selected because it is 10% of the middle BI value of 2.5. For example, when $N = 6$, df = 5, and t (two-tailed, $P = 0.05$) = 2.571; then $t(s^2/6)^{1/2} = 2.571(0.05/6)^{1/2} = 0.235$, which is less than 0.25. Therefore, to be 95% confident that the estimated mean BI is within 0.25 of the true mean BI, at least six samples are needed during a study. Because the BI is affected by seasonal changes in the benthic fauna, the sampling dates should be spaced to account for variations in the life history and seasonal occurrence of the benthic invertebrates within the geographic region. Also, because the spatial distribution of benthic organisms can affect the

TABLE 7.—*Shannon-Weaver diversity values from Missouri Ozark streams, June–December 1978.*

Site	Shannon-Weaver diversity[a] Mean	SE
Spring Creek	2.45	0.03
Bryant Creek	2.29	0.05
North Fork, White River	2.32	0.07
Finley Creek, site 1	2.49	0.03
Finley Creek, site 2	2.53	0.02
Finley Creek, site 3	2.53	0.04
Potters Creek	2.38	0.02
James River, site 1	2.51	0.04
James River, site 2	2.26	0.03
Wilson Creek	1.69	0.11
Big Piney River	0.91	0.09

[a] Based on 15 samples from each site except James River, site 2 and Wilson Creek (11 each).

BI within a site, preliminary samples should be collected to determine the potential for spatial biases.

The criterion used for comparing the BI between two sites was that the 5% Least Significant Difference (LSD) be less than half the smallest range of BI values for any category within the water quality criteria. The smallest range value was 0.75 for the middle two categories; therefore, the critical LSD value would be 0.375. When each N is 5, df are 8, and t ($P = 0.05$) is 2.306; $t[s^2/(2/5)]^{\frac{1}{2}} = 2.306[0.05(2/5)]^{\frac{1}{2}} = 0.326$; therefore, to detect statistically significant differences in the BI values between sites, at least five samples per site should be collected during the study.

Comparison with Other Indices

By the criterion of Wilhm and Dorris (1968) for the Shannon-Weaver diversity index—that unstressed environments have a species diversity greater than 3 and moderately stressed environments less than 1—all the sites sampled in the present study (our data providing an estimate of the relative degree of diversity among streams) would be judged as unstressed environments, except sites on Wilson Creek and Big Piney River, which would be judged as moderately stressed (Table 7). Classification of stream water quality based on the criteria of Wilhm and Dorris did not coincide with our a priori opinions of the environmental condition of these streams, or with the water chemistry data collected during the study. The diversity index was not correlated with any of the water chemistry variables in Table 5 but was related to stream depth (Spearman's rank correlation coefficient $r_s = 0.92$; $P < 0.01$). The criteria based on diversity were not sensitive enough to differentiate between streams with no apparent or only slight enrichment.

We also found more within-site variation over time in the Shannon-Weaver diversity index than in the BI. On the basis of a 95% confidence interval, nine samples would be needed during a study to yield an estimated average diversity index value with the same precision as we obtained from six samples for the BI (for $N = 9$, $\text{df} = 8$, and $t = 2.306$; $t(s^2/9)^{\frac{1}{2}} = 2.306(0.10/9)^{\frac{1}{2}} = 0.243$). Murphy (1978) also found greater temporal variability when assessing river water quality in England with indices based on community diversity than with biotic indices.

Assessment of the Biotic Index

The BI provides a promising approach for assessing the degree of organic enrichment in streams by combining the concepts of species diversity with the ecological sensitivity of the benthic insects into a single numerical expression; it provides reproducible results that lend themselves to statistical analysis. Use of the BI enabled us to discriminate among differences in the water quality of Missouri Ozark streams selected to represent environmental conditions ranging from clean to severely polluted. Differences in the BI among sampling sites were supported by differences in the taxonomic composition of the benthic communities, and were statistically related to stream water chemistry.

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