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A Comparison of Algal, Macroinvertebrate, and Fish Assemblage Indices for Assessing Low-Level Nutrient Enrichment in Wadeable Ozark Streams

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ABSTRACT

Biotic indices for algae, macroinvertebrate, and fish assemblages can be effective for monitoring stream enrichment, but little is known regarding the value of the three assemblages for detecting perturbance as a consequence of low-level nutrient enrichment. In the summer of 2006, we collected nutrient and biotic samples from 30 wadeable Ozark streams that spanned a nutrient-concentration gradient from reference to moderately enriched conditions. Seventy-three algal metrics, 62 macroinvertebrate metrics, and 60 fish metrics were evaluated for each of the three biotic indices. After a group of candidate metrics had been identified with multivariate analysis, correlation procedures and scatter plots were used to identify the four metrics having strongest relations to a nutrient index calculated from log transformed and normalized total nitrogen and total phosphorus concentrations. The four metrics selected for each of the three biotic indices were: algae—the relative abundance of most tolerant diatoms, the combined relative abundance of three species of *Cymbella*, mesosaprobic algae percent taxa richness, and the relative abundance of diatoms that are obligate nitrogen heterotrophs; macroinvertebrate—the relative abundance of intolerant organisms, Baetidae relative abundance, moderately tolerant taxa richness, and insect biomass; fish—herbivore and detritivore taxa richness, pool species relative abundance, fish catch per unit effort, and black bass (*Micropterus* spp.) relative abundance. All three biotic indices were negatively correlated to nutrient concentrations but the algal index had a higher correlation (rho = 0.89) than did the macroinvertebrate and fish indices (rho = 0.63 and 0.58, respectively). Biotic index scores were lowest and nutrient concentrations were highest for streams with basins having the highest poultry and cattle production. Because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity increases with poultry production. Studies are needed that address the synergistic effect of poultry and cattle production on Ozark streams in high production areas before ecological risks can be adequately addressed.

1. Introduction

In 2003, the U.S. Geological Survey (USGS) initiated several studies to evaluate the effects of nutrient enrichment on stream ecosystems in agricultural basins (Munn and Hamilton, 2003). These studies were initiated after the U.S. Environmental Protection Agency (USEPA) reported that nutrient enrichment was the cause of 40% of reported water-quality impairments (USEPA, 1998) and after results from studies conducted in the 1990s by the USGS National Water-Quality Assessment (NAWQA) Program demonstrated that high concentrations of both nitrogen (N) and phosphorus (P) were common in streams draining agricultural areas (Fuhrer et al., 1999). More recent USGS studies have indicated that agricultural streams can transport up to 50% of the N and 20% of the P applied annually to the land (Mueller and Spahr, 2006). USGS models indicate that manure may be a larger source of P to the Gulf of Mexico than are row-crop sources (Alexander et al., 2008), and USGS data indicate that manure sources of total nitrogen (TN) and total phosphorus (TP) are increasing in the Ozarks (Rebich and Demcheck, 2007).

Confined poultry and loosely confined beef cattle are often produced on the same or adjacent farms in the Ozarks and increases in animal production have resulted in increased nutrient runoff to streams. However, nutrient concentrations in most Ozark streams are relatively low compared to concentrations in other regions of the United States. Herlihy and Sifneos (2008) compared nutrient concentrations for wadeable streams across the United States and determined that TP and TN concentrations for reference streams in the nutrient ecoregion containing the Ozarks were typically lowest and second lowest (respectively) of the 11 nutrient ecoregions evaluated.

Interassemblage response to nutrients can vary because of differences related to trophic structure, mobility, and longevity, and the biotic assemblage that is best suited for monitoring nutrients and other forms of ecological disturbance is frequently debated (Griffith et al., 2005; Hering et al., 2006; Resh, 2008). Algal indices have been shown to be effective for monitoring well-established nutrient gradients (Lavoie et al., 2004; Potapova and Charles, 2007; Porter et al., 2008), but indices using macroinvertebrate (King and Richardson, 2007; Haase and Nolte, 2008) or fish assemblages (Wang et al., 2007) have also been successful. Few, if any, studies, however, have compared the value of the three assemblages for detecting perturbance as a consequence of low-level nutrient enrichment.

Conducting biotic assessments when nutrient levels are low can be challenging because effects are often subtle and can appear to be positive in nature (Biggs and Smith, 2002; Stevenson et al., 2008), but also because low-level nutrient enrichment may influence biota less than other water-quality and habitat variables. It is important that relations between nutrient concentrations and biotic assemblages be investigated in this setting to ensure that assessment methods are capable of detecting ecosystem perturbation as a consequence of nutrient enrichment in areas that are relatively undisturbed.

The objectives of this paper are to (1) assess the value of algal, macroinvertebrate, and fish assemblage metrics and indices for assessing low-level nutrient enrichment, and (2) characterize relations between agricultural land use (livestock production) and the three biotic indices.

1.1. Study area

We sampled 30 wadeable streams along a nutrient-concentration gradient in the Ozarks. Sites were divided between the Springfield and Salem Plateau physiographic areas (Fig. 1), which contain most of northern Arkansas, southern Missouri, and extreme eastern Oklahoma, and overlap much of the Ozark Highlands Ecoregion. Topography of the Springfield and Salem Plateaus varies to some degree with gently rolling hills dominating the former and rugged hills dominating the latter; elevation above sea level ranges between 425 and 520 m (Fenneman and Johnson, 1946). The 30 streams generally are clear, with pool, riffle, and run sequences, and have moderate gradients with dominant substrates ranging in size from medium gravel to bedrock. Basin size ranges from 50 to 483 km^2 and streamflow measured at the time of sampling ranged from 0.01 to 0.55 m^3/s (Table S1 in Supplementary Material).

Land use in the 30 basins (Table 1) represented a gradient for pasture; urban land use was usually less than 5%, and no wastewater-treatment plants discharged into the streams. Poultry were produced in 17 of the 30 stream basins and cattle were produced in all basins. Agricultural intensity was greatest in basins of extreme northwestern Arkansas and southwestern Missouri, which have the highest poultry and cattle production of counties within the two states and Oklahoma (NASS, 2008a,b).

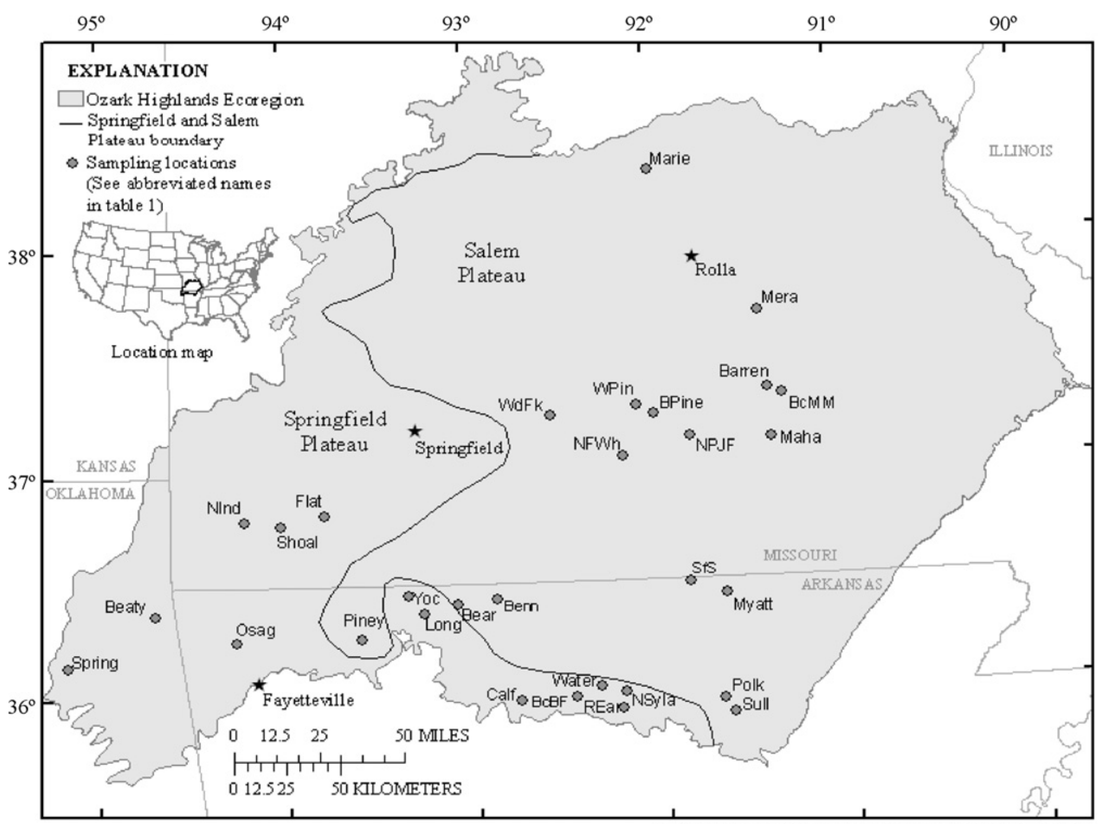

Fig. 1. Locations of 30 wadeable stream sites sampled in the Ozark Highlands in 2006 with a general border for the Springfield and Salem Plateaus.

Table 1. Nutrient and land-use characteristics for 30 wadeable streams sampled in the Ozark Highlands, 2006.

Site name	Abbre-viated name (fig. 1)	Physiographic section	Mean total nitrogen (mg/L)	Mean total phosphorus (mg/L)	Nutrient index score	Pasture (percent)	Cattle produced (number per km²)	Poultry (houses per km²)
Barren Fork near Timber, Missouri	Barren	Salem	0.07	0.003	0.00	7	12	0.0
Big Creek near Big Flat, Arkansas	BcBF	Springfield	0.29	0.027	0.93	33	75	0.2
Big Creek at Mauser Mill, Missouri	BcMM	Salem	0.14	0.002	0.05	4	6	0.0
Bear Creek near Omaha, Arkansas	Bear	Salem	0.14	0.005	0.14	35	86	2.0
Beaty Creek near Sycamore, Oklahoma	Beaty	Springfield	1.56	0.047	2.27	71	259	9.0
Bennetts River near Vidette, Arkansas	Benn	Salem	0.37	0.010	0.47	56	80	0.0
Big Piney River at Simmons, Missouri	BPine	Salem	0.25	0.024	0.78	42	106	0.0
Calf Creek near Silver Hill, Arkansas	Calf	Springfield	0.41	0.029	1.08	32	73	0.0
Little Flat Creek near McDowell, Missouri	Flat	Springfield	2.51	0.031	2.15	58	184	3.3
Long Creek southeast of Denver, Arkansas	Long	Springfield	0.72	0.038	1.55	37	98	1.8
Mahans Creek at West Eminence, Missouri	Maha	Salem	0.39	0.011	0.53	7	11	0.0
Maries River Near Freeburg, Missouri	Marie	Salem	0.56	0.035	1.35	41	104	0.1
Meramec River above Cook Station, Missouri	Mera	Salem	0.10	0.004	0.05	17	29	0.0
Myatt Creek east of Salem, Arkansas	Myatt	Salem	0.39	0.011	0.54	42	52	0.0
North Fork White River near Cabool, Missouri	NFWh	Salem	0.23	0.007	0.27	32	80	0.0
North Indian Creek near Wanda, Missouri	NInd	Springfield	4.71	0.052	3.30	81	265	11.7
North Prong Jacks Fork below Arroll, Missouri	NPJF	Salem	0.22	0.006	0.24	21	52	0.0
North Sylamore Creek near Fifty Six, Arkansas	NSyla	Springfield	0.10	0.005	0.08	2	5	0.2
Little Osage Creek at Healing Springs, Arkansas	Osag	Springfield	3.33	0.051	2.95	76	284	8.5
Piney Creek near Cabanol, Missouri	Piney	Salem	0.56	0.009	0.61	31	94	4.0
Poke Bayou near Sidney, Arkansas	Poke	Salem	0.58	0.025	1.10	47	84	0.0
Roasting Ear Creek near Newnata, Arkansas	REar	Springfield	0.51	0.016	0.77	20	46	0.7
South Fork Spring River north of Moko, Arkansas	SfS	Salem	0.43	0.013	0.63	45	42	0.0
Shoal Creek near Wheaton, Missouri	Shoal	Springfield	2.02	0.062	2.88	81	258	10.9
Spring Creek near Locust Grove, Oklahoma	Spring	Springfield	0.25	0.010	0.38	44	93	2.6
Sullivan Creek near Sandtown, Arkansas	Sull	Salem	0.54	0.018	0.85	31	73	2.2
Water Creek near Evening Shade, Arkansas	Water	Springfield	0.14	0.004	0.10	18	71	0.3
Woods Fork near Hartville, Missouri	WdFk	Salem	0.27	0.035	1.12	55	142	0.2
West Piney Creek at Bado, Missouri	WPin	Salem	0.33	0.015	0.60	48	122	0.0
Yocum Creek near Oak Grove, Arkansas	Yoc	Springfield	2.37	0.047	2.57	71	217	8.4

2. Methods

2.1. Site selection

Geographic information system analysis and field reconnaissance were the primary methods used to select 30 streams that maximized the nutrient gradient across Ozark streams. Potential stream reaches were identified using the Elevation Derivatives for National Applications (USGS, 2005). Field reconnaissance was conducted at 54 candidate stream reaches that were selected from a larger group of reaches that met the basin size criterion (initially 90–300 km^2, however, 5 streams with basins outside this range but with a streamflow characteristic of the remaining streams were included). Nutrient concentrations were measured using a portable nutrient analyzer (Hach™ model DREL/2010) and dissolved oxygen, pH, specific conductance, temperature, and turbidity were measured in the field with water-quality monitors. Field forms were completed that documented observations for habitat quality and flow characteristics. Land use, geographic coverage, and spatial distribution were other factors considered as sites were selected.

2.2. Water-quality sampling

Water-quality samples were collected during base-flow conditions at the 30 sites in late June 2006 and again in July–August 2006 with the following exceptions. Flooding delayed the second round of water-quality sampling until early September at one site and drought conditions in the summer of 2006 resulted in 5 of the original 30 sites sampled in June being replaced for the July–August sampling effort. At the 25 sites sampled twice, nutrient concentrations for the two samples were averaged to indicate nutrient enrichment for the month prior to biotic sampling; at the 5 remaining sites, the concentration from the single sample was used.

Standard USGS methods were used to collect and process water-quality samples. Water-quality samples were grabbed (because water velocities were <0.46 m/s) and were composited from three points that were equally distributed along the stream cross-section. Streamflow and field properties were measured at each site using a current meter (Rantz et al., 1982). Samples were analyzed for nutrient or nutrient-related (e.g. chlorophyll *a* and total organic carbon) constituents and all analyses were performed by the USGS National Water Quality Laboratory (NWQL) in Lakewood, Colorado (Patton and Kryskalla, 2003; Fishman, 1993). Total nitrogen was determined by summing nitrogen species. For purposes of statistical analysis, all nondetect values were assigned one-half of the reporting limit. Quality-control samples were collected to assess bias and variability in the field and laboratory (Brightbill and Munn, 2008). The maximum difference between TP concentrations and TN concentrations in replicate samples was 0.0011 and 0.0260 mg/L, respectively. One of five blank samples had detections of TP (0.0029 mg/L) and TN (0.0350 mg/L).

2.3. Land use

Cattle density on pasture was estimated for each county contained in the stream basin by multiplying the amount of pasture in the county by county-level cattle density (the number of cattle produced in 2005 divided by the area of the county, NASS, 2008a). Cattle density on pasture then was combined for all counties in the stream basin, and that sum was divided by basin area to obtain an estimate of cattle density across the stream basin. Poultry production information was not available for 2005 (NASS, 2008a) and was not available for all counties in other years (NASS, 2008b). Consequently, poultry house density was used as a surrogate for poultry density. Poultry houses in each stream basin were counted using aerial photography (Center for Advanced Spatial Technologies, 2008) and were divided by the stream basin size to estimate the poultry houses per square kilometer of basin (Table 1).

2.4. Biotic sampling

Biotic sampling was conducted concurrently with the second water-quality sampling effort using NAWQA protocols (Moulton et al., 2002). Biotic samples were collected from a reach length that measured approximately 20 times the mean wetted channel width, with a minimum reach length of 150 m and a maximum of 300 m.

Algal assemblages were sampled using a cylinder surface area method. A quantitative algal subsample was collected from five cobbles at each of the five riffle locations (i.e. 25 subsamples were composited). The method involved placing a short cross section of PVC pipe (2.8- or 3.3-cm diameter) on each cobble, dislodging all algae outside of the pipe template with a wire brush or small knife, and rinsing the dislodged algae from the cobble with native water. Algae remaining inside the pipe template was dislodged with a wire brush or (scraped free) with a knife and rinsed into a sample bottle as the subsample. Sample area and total sample volume were recorded, and the sample was preserved with buffered formalin. Taxa were identified and enumerated at the Academy of Natural Sciences of Philadelphia (ANSP) Phycology Section in Philadelphia, Pennsylvania. The ANSP also determined cell density for each algal species using methods described in Charles et al. (2002). Chlorophyll *a* was determined at the USGS NWQL using methods described in Arar and Collins (1997).

A disturbance-removal process was used to collect macroinvertebrate samples from coarse-grained riffle substrates that were adjacent to locations where algal samples were collected. Five discrete samples were collected with a Slack sampler (50-cm 33-cm net frame, 500-mm Nitex™ net, and retrofitted with a 0.25-m^2 template) from riffles located throughout the reach. Macroinvertebrates were sampled from within the template as it was positioned on the stream bottom and immediately upstream from the Slack sampler. Substrate within the template was thoroughly disturbed using a small hand rake (or brushed if large cobble) and dislodged organisms were transported into the net by water current. All sample material was composited into a 20-L container and elutriated to remove sediment and larger particles. The material remaining on a 500-mm sieve after elutriation was preserved in 10% formalin and shipped to the USGS NWQL for identification and enumeration.

Fish were sampled at 29 sites using electrofishing and seining methods (fish were not sampled at Maries River because of potential occurrence of a federally listed threatened species). A backpack unit (Smith-Root model 12B) was used to electrofish all sites, and one pass was made along each bank. Electrofishing passes progressed from the downstream boundary of the sampling reach to the upstream boundary. Riffle habitats also were sampled by kick seining in conjunction with electrofishing. Most fish were identified and counted in the field and then were released. Fish that could not be positively identified in the field were preserved for laboratory identification. Fish were identified using taxonomic keys for Arkansas (Robison and Buchanan, 1988), Missouri (Pflieger, 1997), and Oklahoma (Miller and Robison, 2004), however, nomenclature follows Robins et al. (2004).

2.5. Metric sources

Two USGS software programs—the Macroinvertebrate Data Analysis System (IDAS; Cuffney, 2003) and the Algal Data Analysis System (ADAS; a derivative of the IDAS program)—were the primary means for calculating algal and macroinvertebrate metrics. Both programs process multiple levels of taxonomic resolution, resolve taxonomic ambiguities, and use attribute files to calculate assemblage and tolerance metrics common to the literature (Barbour et al., 1999; Porter, 2008). Also, some macroinvertebrate metrics used by local natural resource agencies were considered as potential metrics, as were all species—order level taxa for the macroinvertebrate and fish assemblages.

ADAS was used to calculate algal metrics using an attribute file of published values (Porter, 2008). A total of 73 algal metrics was calculated for soft algae and diatoms (Table S2 in Supplementary Material). Algal metrics were primarily indicative of trophic preferences (Van Dam et al., 1994) and pollution tolerance (Lange-Bertalot, 1979).

A total of 62 macroinvertebrate metrics was calculated (Table S3 in Supplementary Material) using data specific to the southeastern (Barbour et al., 1999; Lenat, 1993) and midwestern (Hilsenhoff, 1987) United States. Values for richness, percent richness, abundance, and percent relative abundance were evaluated for all but a few metrics where percentages were not beneficial to the analysis (e.g. diversity indices).

A total of 60 fish metrics used by local natural resource agencies or obtained from biotic indices developed for use in the Ozarks or adjacent areas (Dauwalter et al., 2003; Justus, 2003; Dauwalter and Jackson, 2004) were considered as candidates for the fish index (Table S4 in Supplementary Material). Fish metrics were calculated using fish traits from several sources (Robison and Buchanan, 1988; Pflieger, 1997; Petersen et al., 2008; USGS, 2008).

2.6. Statistical analysis

TN and TP were combined into a nutrient index to facilitate comparisons of nutrient enrichment and biotic metrics. TN and TP are commonly used by State monitoring agencies to characterize nutrient enrichment in the Ozarks and typically have close relations to livestock production in the Ozarks (Davis and Bell, 1998) and much of the United States (Alexander et al., 2008). Chlorophyll *a* also is used by State monitoring agencies to characterize nutrient enrichment and also was considered for the nutrient index but relations between chlorophyll *a* and TN and TP were poor (Spearman rho = 0.14 and 0.30, respectively).

A three-step process was used to calculate the nutrient index. First, mean values for TN and TP were normalized to a mean of 0 and a standard deviation of 1. Second, normalized values for TN and TP were averaged, and third, all normalized (average) values were standardized to positive numbers by adding the difference between the minimum value and zero. The resulting nutrient index ranged from 0 to 3.3 (Table 1, Fig. 2).

For each of the biotic indices, four nonredundant metrics were selected from the initial 195 (73 algal, 62 macroinvertebrate, and 60 fish) metrics aggregated for this study. Index robustness may sometimes be associated with increasing metric number, however, a decision was made to limit the number of metrics (to four) after preliminary analyses indicated that, for one or more assemblages, relations between the next best candidate metric(s) and the nutrient index were nonexistent. The decision to select a relatively small number of metrics for each index also reduced the risk that redundant metrics were included in the final indices.

Metrics that were the best candidates for the three biotic indices were identified with a process that included a combination of univariate and nonparametric multivariate methods. Prior to analysis, metrics were separated by guild (e.g. tolerance, behavior, feeding, or nesting traits) and scoring method (e.g. relative abundance, relative density, and richness). Pairs of metrics from respective metric guilds initially were evaluated using Spearman rank correlation to identify and eliminate redundant metrics. When two metrics that had taxa in common had rho > 0.70, the metrics were considered to be redundant and one metric was eliminated to avoid index bias and error. Scatter plot matrices also were used to visually identify outlying values or spurious correlations. Metric relevancy to nutrient enrichment (e.g. increasing biomass, a decrease in organisms intolerant of organic pollution, an increase in organisms tolerant of organic pollution) was the primary consideration that determined which of the redundant metrics was retained for further analysis.

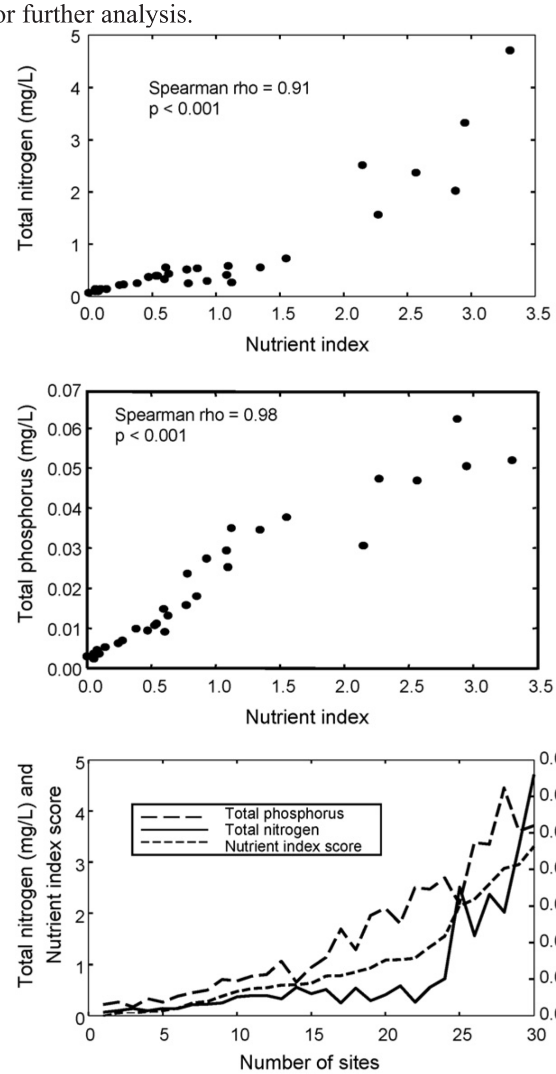

Fig. 2. Scatter plots and a line graph demonstrate relations of a nutrient index to total nitrogen and total phosphorus concentrations at 30 wadeable Ozark streams.

Once redundant metrics had been eliminated, BVSTEP, a nonparametric screening procedure in PRIMER v6 (Clarke and Warwick, 2001), was used to identify candidate metrics that ‘‘best’’ represented each of the three biotic assemblages. First, BVSTEP was used to compare the similarity matrices for an individual metric to the similarity matrix of all metrics in the same guild (group). This step helped identify individual metrics and metric combinations with the highest similarity to the metric guild (i.e. a multivariate sample pattern that matched that of the entire guild) and greatly reduced the number of metrics to be considered in further data reduction steps. The similarity matrix of the metric with the highest correlation to the similarity matrix of the entire guild was retained for further analysis. This step was repeated using an n–1 approach (once identified as an index candidate the metric was removed from the guild) until all metrics having a similarity matrix that was correlated (rho ≥ 0.25) to the similarity matrix of the parent guild had been identified. A rho value of 0.25 was selected because matrix correlations occur over a lower range than simple univariate correlations.

Metrics identified with the analytical step, above, were-combined into a final ‘‘candidate metric subset’’ (generally 10–15 metrics). The BVSTEP process was used again, but on this occasion, similarity matrices of the candidate metrics were compared to the similarity matrix of the nutrient index. The candidate metrics that had similarity matrices with the highest correlations to the similarity matrix of the nutrient index were retained. Spearman rho was used again to evaluate for metric redundancy but this time for the small group of candidate metrics identified with the second round of BVSTEP. When pairs of redundant metrics with similar correlations to the nutrient index were identified, scatter plots were evaluated to determine which of the two redundant metrics had the best relation to nutrients and, ultimately, to identify the four candidate metrics that were selected for the respective assemblage index.

Scores for each of the three biotic indices were calculated by combining values for the four respective metrics using a centering method (Justus, 2003). An advantage of the centering method is that it is more robust than other scoring methods (e.g. scores range from 0 to100 rather than tiered, preassigned metric classes of 1, 3, or 5). A disadvantage of the centering method is that it does not facilitate comparison of sites from independent data sets because metric scores are based on the range of sampling conditions that may not include least- or most-impaired sites.The centering method uses one of two scoring procedures depending if high or low metric values represent least-degraded conditions. If a high metric value indicated least-degraded conditions, the metric value was first divided by the maximum metric value (for all 30 sites), and the resulting quotient was multiplied by 100 to obtain a metric score. To obtain a metric score if low metric values indicated least degraded conditions, the metric value was again divided by the maximum metric value, but the resulting quotient was subtracted from 1 before being multiplied by 100. Scores for the four metrics were averaged to obtain an index score. Sites having the highest biotic index scores had the least-degraded-conditions. Relations between the three biotic indices and the nutrient index and TP and TN also were evaluated with correlation procedures and scatter plots. Scatter plots also were used to determine how poultry (houses) and cattle production varied for the 30 basins and to evaluate relations between the three biotic indices and the two forms of livestock production.

3. Results

3.1. Biotic metric/nutrient relations

Median concentrations of TN and TP were 0.393 mg/L (0.07–4.71 mg/L) and 0.015 mg/L (0.002–0.062 mg/L), respectively. Values for the nutrient index ranged from 0 to 3.3 and were highly correlated to TN and TP concentrations (rho = 0.91 and 0.98, respectively; Fig. 2). The 30 sites were equally divided above and below an index score of 0.75 (because TN and TP concentrations associated with that index score, 0.40 and 0.018 mg/L, respectively, are comparable to median concentrations).

Although, the four metrics selected for each of the three assemblage indices had the strongest relations to the nutrient index of all metrics evaluated for that assemblage, relations between a few of the 12 metrics and the nutrient index were weak (rho ≤ 0.36 and p > 0.05). In most cases, however, metric values above and below the nutrient index score of 0.75 had different distributions. The four biotic metrics selected for each index are reported in the order of the correlation of the metric to the nutrient index, which may also reflect or approximate each metric’s relevance to nutrients (Table 2).

All four metrics selected for the algal index were associated with nutrient tolerance or dependence (Table 2). The four metrics were: relative abundance of most tolerant diatoms, a metric associated with tolerance to elevated nutrient concentrations; the combined relative abundance of *Cymbella delicatula*, C. *affinis*, and C. *hustedtii*, three species of diatoms that respond to low to moderate nutrient concentrations; mesosaprobic algae percent taxa richness, a metric associated with tolerance to moderately elevated nutrients; and lastly, the relative abundance of diatoms that are obligate nitrogen heterotrophs, a metric associated with nitrogen dependence. All but the second metric would be expected to have a positive relation to nutrient concentrations.

The algal index, calculated with the four metrics above, ranged from 20.9 to 94.7 (Table S5 in Supplementary Material) and had a high correlation to the nutrient index (rho = 0.89, Fig. 3). Correlations between the algal index and TP (rho = 0.91) were much higher than between the algal index and TN (rho = 0.72, Fig. 4).

3.3. Macroinvertebrate metric and index performance

The four metrics selected for the macroinvertebrate index included three metrics associated with organisms that are

Table 2. Algae, macroinvertebrate, and fish metrics selected for three indices, their expected response to nutrient exposure, correlation to a nutrient index, and a comparison of values above and below a median concentration.

Assemblage	Metric description	Expected response to nutrients	Rho	Distinction for sites above and below median concentrations
Algae	Most tolerant diatoms, relative abundance (percent)	Positive (Bahls, 1993)	0.80	Percent RA≥3% at 3 of 15 sites; percent RA≥3% at 12 of 15 sites
Algae	*Cymbella affinis*, C. *delicatula*, and C. *hustedtii* relative abundance (percent)	Negative (Potapova and Charles, 2007)	-0.71	Percent RA>10% at 11 of 15 sites; percent RA>10% at 2 of 15 sites
Algae	Mesosaprobic algae taxa richness (percent)	Positive (Lange-Bertalot, 1979)	0.65	Percent TR>10% at 5 of 15 sites; percent TR>10% at 11 of 15 sites
Algae	Obligate nitrogen heterotroph relative abundance (percent)	Positive (Leland, 1995)	0.57	Percent RA>1% at 1 of 15 sites; percent RA>1% at 8 of 15 sites
Macroinvertebrate	Intolerant relative abundance (percent)	Negative (Barbour et al., 1999)	-0.50	Percent RA>85% at 14 of 15 sites; percent RA>85% at 9 of 15 sites
Macroinvertebrate	Baetidae relative abundance (percent)	Positive (USEPA, 2008)	0.48	Percent RA>10% at 2 of 15 sites; percent RA>10% at 9 of 15 sites
Macroinvertebrate	Insect biomass (grams)	Positive (King and Richardson, 2007)	0.47	>2 g at 1 of 15 sites; >2 g at 7 of 15 sites
Macroinvertebrate	Moderately tolerant taxa richness	Positive (Barbour et al., 1999)	0.30	≥20 taxa at 6 of 15 sites; ≥20 taxa at 10 of 15 sites
Fish	Herbivore/detritivore taxa richness	Positive (Rashleigh, 2004)	0.41	≥4 taxa at 7 of 15 sites; ≥4 taxa at 10 of 14 sites
Fish	Pool species relative abundance (percent)	Indirect	-0.38	Percent RA>50% at 11 of 15 sites; percent RA>50% at 7 of 14 sites
Fish	Fish collected per meter	Positive (Pilati et al., 2009)	0.36	>2.5 fish/m at 5 of 15 sites; >2.5 fish/m at 7 of 14 sites
Fish	Black bass relative abundance (percent)	Indirect	-0.35	Percent RA>1% at 8 of 15 sites; percent RA>1% at 4 of 14 sites

intolerant or moderately tolerant of organic pollution, and a fourth metric associated with productivity. The three metrics evaluating tolerance included: the relative abundance of intolerant organisms, Baetidae (a family with several species that are moderately tolerant of nutrients) relative abundance, and moderately tolerant taxa richness. The fourth macroinvertebrate metric, and the metric related to productivity, was insect biomass. All but the first metric would be expected to have a positive relation to nutrient concentrations.

The macroinvertebrate index ranged from 36.3 to 85.7 (Table S6 in Supplementary Material) and decreased in relation to the nutrient index scores (rho = 0.63, Fig. 3). Correlations between the macroinvertebrate index and TN and TP concentrations were similar (0.64 and 0.60, respectively; Fig. 4).

3.4. Fish metric and index performance

The four fish metrics selected for the fish assemblage index were: herbivore and detritivore taxa richness, pool species relative abundance, fish catch per unit effort, and black bass (*Micropterus dolomieu*, *M. punctatus*, and *M. salmoides*) relative abundance. Two of the metrics—herbivore and detritivore taxa richness and fish catch per unit effort would be expected to have a positive relation to nutrient concentrations; however, the two remaining metrics—pool species relative abundance and black bass relative abundance—probably have indirect relations to nutrients.

The fish index ranged from 15.9 to 83.7 (Table S7 in Supplementary Material) and also decreased with increasing nutrient index scores (rho = 0.58, Fig. 3). The fish index had a stronger correlation to TN than to TP (rho = 0.68 and 0.54, respectively; Fig. 4).

3.5. Indices comparison

Of the three biotic indices, the algal index had a much higher correlation to the nutrient index (i.e. a rho of 0.89, compared to 0.63 and 0.58). Correlations to the nutrient index, for the algal, macroinvertebrate, and fish metrics ranged from 0.57 to 0.80, 0.30 to 0.50, and 0.35 to 0.41 (reported as absolute values, Fig. 3), respectively. All relations among the four algal metrics and the nutrient index were statistically significant ($p \leq 0.05$); however, relations for only 3 of 4 macroinvertebrate, and only 2 of 4 fish metrics were statistically significant to the nutrient index. Correlations of the three biotic indices to TN were similar (a range between 0.64 and 0.72, Fig. 4) but the algal index had a much higher correlation to TP (rho = 0.91)

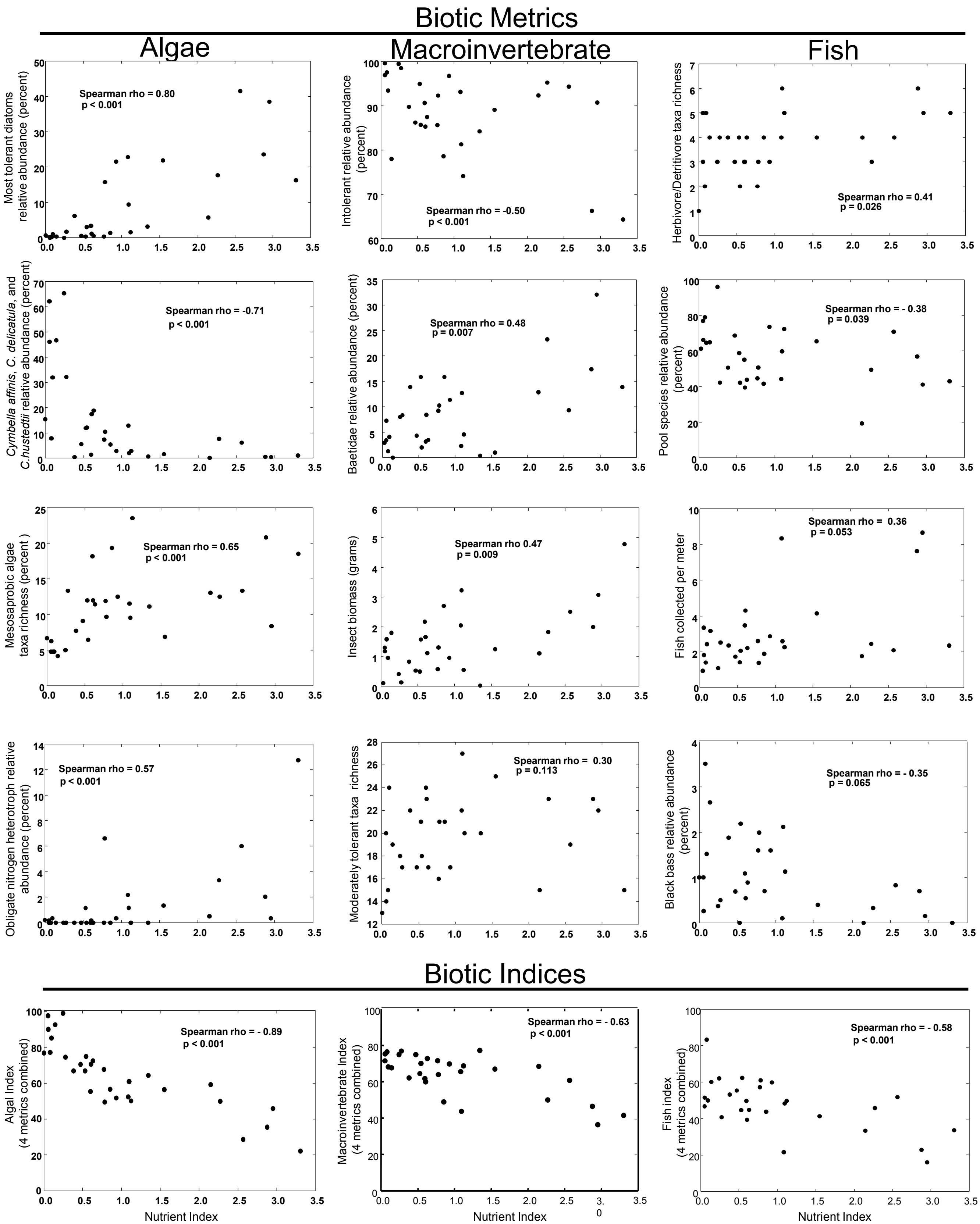

Fig. 3. Scatter plots and correlations comparing 12 biotic metrics and 3 biotic indices to a nutrient index (representing total nitrogen and total phosphorus concentrations) at 30 wadeable Ozark streams.

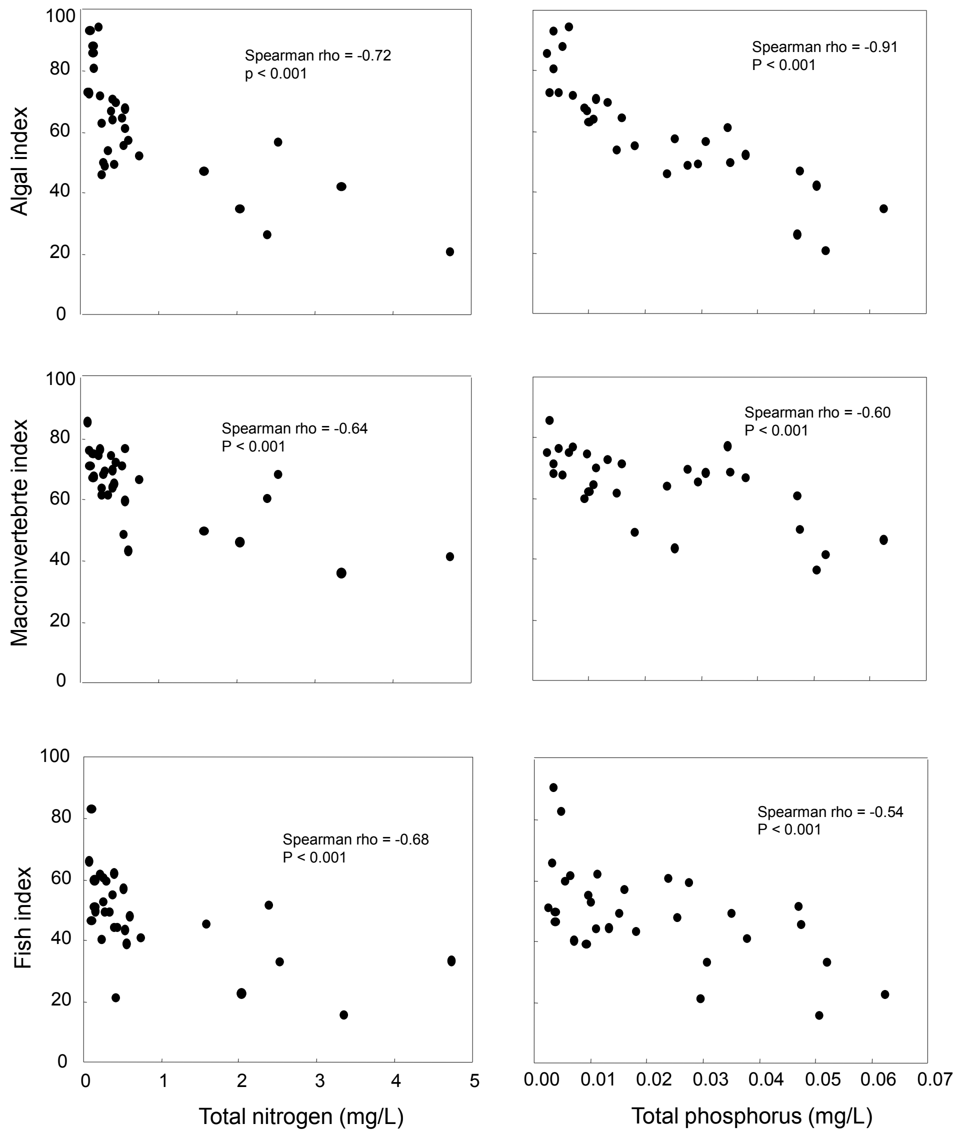

Fig. 4. Scatter plots and correlations comparing relations between three biotic indices and total nitrogen and total phosphorus concentrations at 30 wadeable Ozark streams.

than did the macroinvertebrate and fish indices (rho = 0.60 and 0.54, respectively).

3.6. Land use

Cattle were produced in all basins (a range of 5–284 cattle per km^2 of basin), but poultry were produced in only 17 of the 30 basins (the number of poultry houses ranged from 0 to 11.7 per km^2 of basin, Table 1). Cattle production generally was much higher in basins where poultry were produced than in basins where poultry were not produced, and was highest in basins with the highest poultry production (Fig. 5). The three biotic indices were negatively related to cattle production; correlations ranged from 0.46 to 0.76 (Fig. 6).

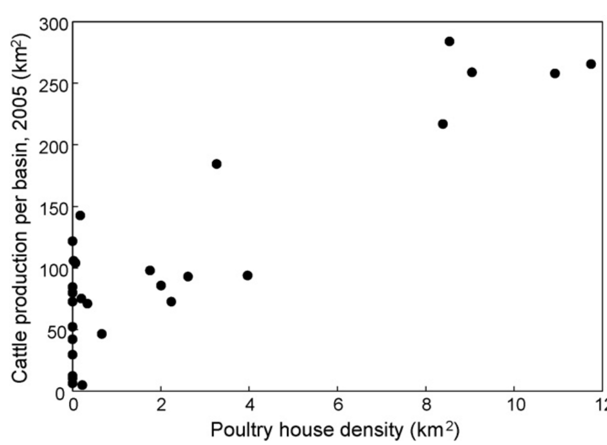

Fig. 5. A scatter plot comparing relations between cattle production and the number of poultry houses in 30 Ozark stream basins. Cattle production in the basins ranged from 5 to 125 cattle/km^2 when no poultry were produced but generally exceeded 75 cattle per km^2 when there was one or more poultry house in the basin.

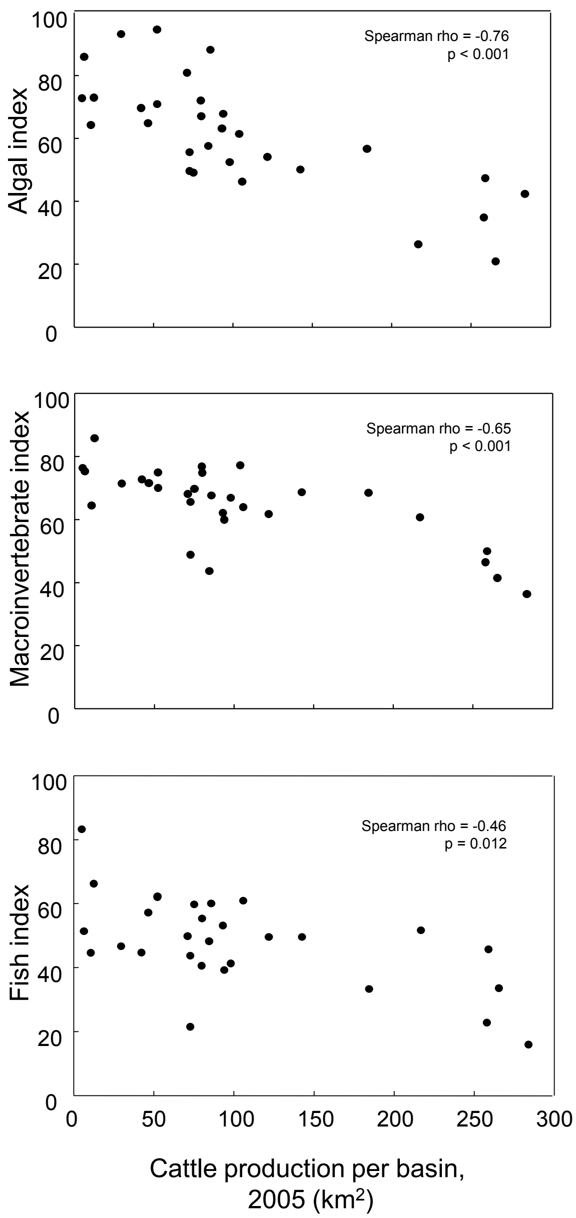

Fig. 6. Relations of three biotic indices to cattle density in 30 Ozark stream basins.

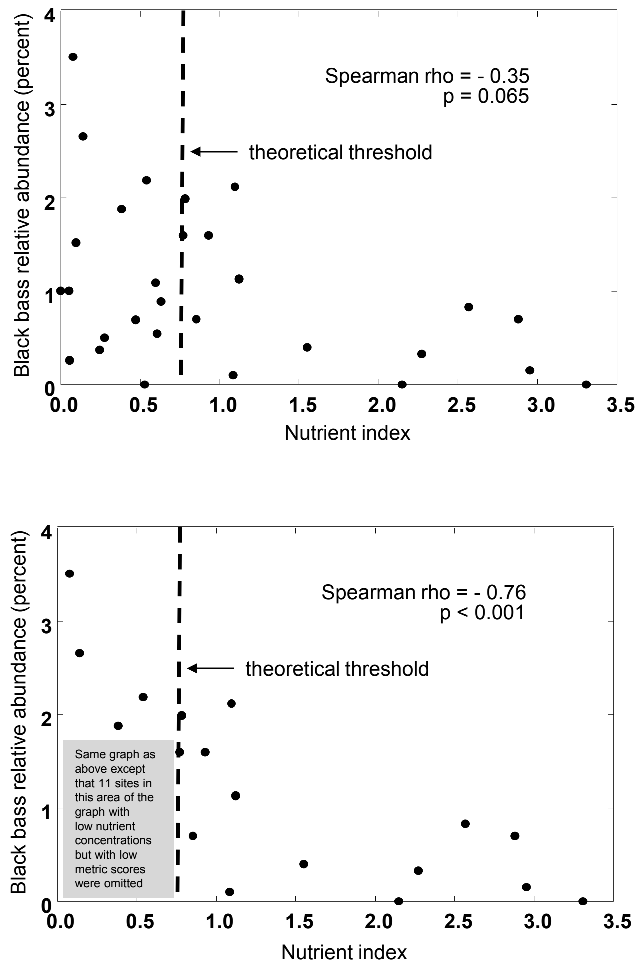

Fig. 7. Relations of black bass relative abundance to the nutrient index emphasize the relevance of the wedge-shaped scatter pattern. The correlations in the second plot doubles that of the previous plot after sites with low nutrient concentrations but with poor metric scores were omitted.

4. Discussion

4.1. Metric performance

Ten of the 12 metrics selected for the three biotic indices were measures of tolerance, biomass, or density that are known to fluctuate in response to stream productivity (e.g. Porter et al., 2008; Ortiz and Puig, 2007), and, thus, have an ecological relevance to nutrients. Correlations between metrics and the nutrient index generally declined across assemblages (from algae to macroinvertebrate to fish)—a probable consequence of the trophic level of the taxa targeted by the metrics and an associated decrease in dependence on inorganic nutrients. For the relative abundances of pool species and black bass, two fish metrics that are comprised of species of Centrarchidae which are known to be moderately tolerant of nutrients (Maceina and Bayne, 2001), relations may have been equal or stronger to variables associated with habitat quality than to nutrients.

Relations between the three biotic indices and the nutrient index were stronger than relations between the biotic metrics and the nutrient index, indication that even metrics that had weak relations to the nutrient index were beneficial to biotic indices. However, weak relations are to be expected between biotic metrics and nutrient enrichment when concentrations at some sites are below a threshold for which a biotic response occurs. Terrel et al. (1996) noted that wedge-shaped scatter plots are characteristic of the relation between a dependent variable and an independent [test] variable when some values for the independent variable are below the threshold for which a response occurs and when other unknown or unmeasured independent variables are influencing the dependent variable (see example in Fig. 7). Of the 12 metrics selected for the three indices, wedge-shaped scatter plots are most apparent for the relative abundance of three *Cymbella* species and black bass relative abundance.

The small size of the data set limits our ability to identify thresholds for TN and TP, however, some literature indicate that TN and TP concentrations near median values for this study are near threshold concentrations that distinguish between reference streams and streams that are slightly enriched (i.e. near background, Table 3). Biotic metric scores were inversely related to nutrients and were generally highest when TN and TP concentrations were less than about 0.40 mg/L and about 0.018 mg/L (respectively), but were generally lowest when concentrations were higher. These TN and TP concentrations are comparable to background concentrations from sites across the United States (Clark et al., 2000; Smith et al., 2003; Herlihy and Sifneos, 2008). Other studies have indicated that substantial changes in macroinvertebrate assemblage structure (Smith et al., 2007) and algal biomass (Stevenson et al., 2006) may occur near these concentrations (Table 3).

Table 3. A comparison of median total nitrogen (TN) and total phosphorus (TP) concentrations at 30 wadeable Ozark streams to TN and TP concentrations that are equivalent to a nutrient index score of 0.75, and to concentrations suspected of distinguishing between reference streams and slightly enriched streams.

Description or data source	Total nitrogen (mg/L)	Total phosphorus (mg/L)
Median concentrations	0.39	0.015
Concentrations equivalent to a nutrient index score of 0.75	0.40	0.018
Dodds et al. (1998)[a]	0.70	0.025
Clark et al. (2000)[b]	0.26	0.022
Smith et al. (2003)[c]	0.26	0.020
Smith et al. (2007)	0.29	0.020
Herlihy and Sifneos (2008)[d]	0.31	0.017

[a] Concentrations are based on differences in chlorophyll *a* for oligotrophic and mesotrophic stream categories.

[b] Flow-weighted concentrations.

[c] Modeled values (not measured).

[d] 75th percentile of least-impaired sites sampled as part of the Environmental Protection Agency Wadeable Stream Assessment.

4.2. Index/nutrient relations

Of the three assemblages evaluated, the algal assemblage seems to be most appropriate for assessing effects of low-level nutrient enrichment in wadeable Ozark streams. These results are consistent with those of Lavoie et al. (2008) who found that algal diatoms were effective for monitoring low-level TN and TP concentrations similar to those observed in this study. Algae are primary producers and nutrient availability may be the most important variable influencing algae (Lowe and Pan, 1996; Borchardt, 1996; Porter, 2008). By contrast, variables other than nutrients may be of equal or greater importance to macroinvertebrates and fish because they are primary and secondary consumers. Other reasons why algae are effective for assessing low-level nutrient enrichment are related to motility and longevity. Most algae are sessile organisms that have a short life cycle that is completed in the sampling area (Lowe and Pan, 1996) and algae may be more resistant to hydrologic disturbance than macroinvertebrates or fish when benthic habitats are armored as they are in Ozark streams (Riseng et al., 2004). Even though algae seem to be well suited for assessing low-level nutrient enrichment, the increased assurance of an accurate assessment (Hering et al., 2006; Griffith et al., 2005) and public perception regarding the economic importance of macroinvertebrates and fish may justify costs associated with sampling multiple assemblages for some monitoring programs.

Algal indices may be an alternative to chlorophyll *a* for assessing the effects of nutrient enrichment in some regions. Relations between chlorophyll *a* and TN and TP were poor for our data set and have been found to be poor in the Midwest United States (Morgan et al., 2006; Lowe et al., 2008), possibly because of confounding factors (i.e. light intensity, degree of nutrient limitation, and habitat quality, Miltner and Rankin, 1998).

4.3. Biotic index/land-use relations

Poultry litter applications are a concern in the Ozarks and elsewhere because N and P application rates are difficult to quantify and because litter application rates may exceed commercial fertilizing rates when an abundance of litter is available (Knowlton et al., 2004). Ozark land-use data also indicate that because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity is increased in areas where poultry are produced.

Although the TN and TP contribution to Ozark streams from manure seems to be increasing in high poultry and cattle production areas (Rebich and Demcheck, 2007), we found no studies that have been designed to address the ecological risks to streams when high poultry and cattle production dominate basin land use. The combined influence of poultry litter and cattle manure on nutrient runoff has been simulated in field experiments (Sauer et al., 1999; Vadas et al., 2007), and several studies have addressed runoff loss from poultry litter (Pierson et al., 2001; Butler et al., 2008; Sistani et al., 2008) or cattle manure (Edwards et al., 2000; Capece et al., 2007; Butler et al., 2008) under various conditions (i.e. different application rates, precipitation rates, soil saturations, and grazing intensities), but the effects of cattle and litter applications are rarely considered in combination.

Cattle production can increase nutrient runoff to streams directly (i.e. fecal deposition) or indirectly (i.e. habitat alteration). Unrestricted cattle generally will spend a large part of the day in the riparian zone regardless of the season or the availability of water elsewhere (Zuo and Miller-Goodman, 2004; Bagshaw et al., 2008), and James et al. (2007) observed that fecal deposition was significantly higher near streams than in other areas of the pasture. Cattle influence habitat variables that have indirect relations to nutrients and can confound relations between biotic integrity and nutrients (Miltner and Rankin, 1998; Maret et al., 2008). Nutrient runoff potential increases when the grass filter in the riparian zone is over grazed (Sistani et al., 2008) and can increase as much as 90% when cattle trample and compact soils (Nguyen et al., 1998). Streambank stability also declines when cattle graze banks and access streams which, in turn, can increase nutrient runoff, particularly for TP (Vidon et al., 2008; Zaimes et al., 2008).

4.4. Conclusions

Biotic assessment methods used to evaluate areas with little or no disturbance should be sensitive to low-level nutrient enrichment because changes in land use and associated effects on water quality and ecological condition often occur slowly and over extended periods. Some biotic metrics selected for the three indices had weak relations to nutrient enrichment probably because TN and TP concentrations were below a threshold to which a biological response occurs. Relations of the three biotic indices to nutrient enrichment, however, were much stronger than relations between the biotic metrics and nutrient enrichment. This observation indicates that metrics selected for the indices were beneficial to index development and provides some validation for the index approach.

The algal index had a much stronger relation to low- to moderate-level nutrient enrichment than did the macroinvertebrate or fish index but all three indices were negatively correlated to nutrient enrichment. Biotic index scores were lowest and nutrient concentrations were highest for streams with basins having the highest poultry and cattle production. Because of the availability of litter for fertilizer and associated increases in grass and hay production, cattle feeding capacity increases with poultry production. The synergistic effect of poultry and cattle production on Ozark streams in high production areas has not been evaluated and additional studies are needed before ecological risks are adequately assessed.

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Supplementary Material

Table S1. General basin and reach characteristics (at the time of sampling) of 30 sites sampled in the Ozark Highlands, 2006.

Site name	USGS station ID	Basin size (km^2)	Mean stream-flow (m^3/s)	Reach length (m)	Lati-tude	Long-itude	Datum
Barren Fork near Timber, Missouri	07064780	132.5	0.46	215	372046	912327	NAD83
Big Creek near Big Flat, Arkansas	07057100	235.8	0.20	253	355843	922853	NAD27
Big Creek at Mauser Mill, Missouri	07065040	108.0	0.11	248	371847	911900	NAD27
Bear Creek near Omaha, Arkansas	07054410	344.6	0.03	262	362650	925600	NAD27
Beaty Creek near Sycamore, Oklahoma	071912219	132.8	0.08	215	362156	944339	NAD83
Bennetts River near Vidette, Arkansas	07058970	155.6	0.06	190	362540	920457	NAD27
Big Piney River at Simmons, Missouri	06928730	275.8	0.55	255	371431	920035	NAD83
Calf Creek near Silver Hill, Arkansas	07055893	116.7	0.03	230	355801	924632	NAD27
Little Flat Creek near McDowell, Missouri	07052790	115.1	0.33	235	364919	934740	NAD83
Long Creek southeast of Denver, Arkansas	07053203	256.0	0.15	230	362151	931614	NAD83
Mahans Creek at West Eminence, Missouri	07065950	140.3	0.29	180	370850	912242	NAD27
Maries River Near Freeburg, Missouri	06926900	483.3	0.01	165	382001	915934	NAD27
Meramec River above Cook Station, Missouri	07010335	243.2	0.10	200	374120	912531	NAD83
Myatt Creek east of Salem, Arkansas	070692655	286.0	0.26	160	362521	913928	NAD83
North Fork White River near Cabool, Missouri	07057280	49.9	0.03	168	370318	921116	NAD83
North Indian Creek near Wanda, Missouri	07188855	113.2	0.19	249	364840	941236	NAD27
North Prong Jacks Fork below Arroll, Missouri	07065160	144.7	0.54	163	370513	914500	NAD83
North Sylamore Creek near Fifty Six, Arkansas	07060710	151.7	0.11	224	355930	921250	NAD27
Little Osage Creek at Healing Springs, Arkansas	07194947	110.9	0.20	300	361513	941612	NAD27
Piney Creek near Cabanol, Missouri	07050228	110.0	0.03	215	361605	933806	NAD27
Poke Bayou near Sidney, Arkansas	07060890	86.0	0.08	200	355715	914155	NAD27
Roasting Ear Creek near Newnata, Arkansas	07060661	162.5	0.10	217	355519	921351	NAD27
South Fork Spring River north of Moko, Arkansas	07069267	242.5	0.09	150	362903	915048	NAD27
Shoal Creek near Wheaton, Missouri	07186670	112.4	0.17	204	364637	940127	NAD83
Spring Creek near Locust Grove, Oklahoma	07192100	297.6	0.08	204	360838	950955	NAD83
Sullivan Creek near Sandtown, Arkansas	07060894	75.0	0.18	227	355315	913830	NAD83
Water Creek near Evening Star, Arkansas	07056695	99.2	0.07	217	360259	923434	NAD27
Woods Fork near Hartville, Missouri	06927590	116.4	0.05	185	371443	923404	NAD27
West Piney Creek at Bado, Missouri	06928750	92.8	0.09	152	371653	920610	NAD83
Yocum Creek near Oak Grove, Arkansas	07053250	136.1	0.19	287	362716	932122	NAD83

Table S2. Algal metrics evaluated for an algal index at 30 wadeable Ozark streams.

Taxonomic metrics[1]	**Tolerance metrics**[2]
Diatom taxa	Benthic algal taxa
Non-diatom taxa	Sestonic algal taxa
Green algal taxa	Nitrogen-fixing algal taxa
Blue-green algal taxa	Non-nitrogen fixing algal taxa
Red algal taxa	Algal taxa in nitrogen uptake category 1: N autotroph (low organic N)
Yellow-green algal taxa	Algal taxa in nitrogen uptake category 2: N autotrophic (high organic N)
Cryptophyte algal taxa	Algal taxa in nitrogen uptake category 3: N heterotroph (high organic N, facultative)
Euglenoid algal taxa	Algal taxa in nitrogen uptake category 4: N heterotroph (high organic N, obligate)[2]
Dinoflagellate algal taxa	Organic N index (diatoms): nitrogen heterotrophs
Total taxa richness (all algae)	Algal taxa in oxygen requirements category 1: high oxygen requirements (~ 100% saturation)
Total number of *Cymbella* sp. (richness only)	Algal taxa in oxygen requirements category 2: fairly high oxygen requirements (> 75% saturation)
Sum of *Cymbella affinis* Kutzing, *Cymbella delicatula* Kutzing, and *Cymbella hustedti*i Krasske (relative abundance only) [2]	Algal taxa in oxygen requirements category 3: moderate oxygen requirements (> 50% saturation)
	Algal taxa in oxygen requirements category 5: very low oxygen requirements (~ 10% saturation)
Motility metrics	Oxygen tolerant: algae with an unknown oxygen tolerance
Benthic-sestonic algae: unknown or not classified	Saprobien index: oligosaprobous (diatoms)
Motile algae (all algae)	Algal taxa in saprobic category 2: b - mesosaprobic
Non-motile algae (all algae)	Algal taxa in saprobic category 3: a - mesosaprobic[2]
Motility: unknown or not classified	Algal taxa in saprobic category 4: a - meso/polysaprobic
	Algal taxa in saprobic category 5: polysaprobic
Biomass metrics	Algal taxa in Bahls (1993) pollution class 1, most tolerant taxa[2]
Ash-free biomass (g/m^2)	Algal taxa in Bahls (1993) pollution class 2, less tolerant taxa
Chlorophyll *a* (mg/m^2)	Algal taxa in Bahls (1993) pollution class 3, most sensitive taxa
Total cells/cm^2 (all algae)	Algal taxa in pollution tolerance category 1: very tolerant (polysaprobic)
Total biovolume/cm^2 (all algae)	Algal taxa in pollution tolerance category 2a: tolerant (a-meso/polysaprobic)
	Algal taxa in pollution tolerance category 2b: tolerant (a-mesosaprobic)
Trophic metrics[1]	Algal taxa in pollution tolerance category 3a: less tolerant (b-mesosaprobic)
Oligotrophic	Algal taxa in pollution tolerance category 3b: less tolerant (oligosaprobic)
Oligo-mesotrophic	Pollution tolerance (Lange-Bertalot, 1979): unknown or not classified
Mesotrophic	Algal taxa that are nuisance benthic bloom producers
Meso-eutrophic	Algal taxa that are nuisance sestonic bloom producers
Eutrophic	Algal taxa not categorized as nuisance algae
Hypereutrophic	Algal taxa categorized as eutrophic soft algal taxa
Trophic: polytrophic (diatoms)	Algal taxa not categorized as eutrophic soft algae
Trophic: eurytrophic (diatoms)	Algal taxa classified as eutrophic soft algae
	Dominant taxa
	Percentage of total abundance represented by the most abundant taxon
	Percentage of total abundance represented by the two most abundant taxa
	Percentage of total abundance represented by the three most abundant taxa
	Percentage of total abundance represented by the four most abundant taxa
	Percentage of total abundance represented by the five most abundant taxa
	Number of taxa in the most abundant class
	Number of taxa in the two most abundant classes
	Number of taxa in the three most abundant classes
	Number of taxa in the four most abundant classes
	Number of taxa in the five most abundant classes

[1]Richness, percent richness, density, and percent density were calculated for diatoms and for all algae unless otherwise specified.

[2]Metrics selected for the algal index

Table S3. Macroinvertebrate metrics evaluated for a macroinvertebrate index at 30 wadeable Ozark streams.

General community[1]	**Tolerance metrics**
Amphipoda	North Carolina biotic index (abundance-weighted)
Baetidae[2, 3]	North Carolina biotic index (tolerant richness)
Bivalvia	
Chironomidae	**Dominant taxa (percent total abundance)**
Coleoptera	Most abundant taxon
Corbicula (abundance and percent abundance)	Two most-abundant taxon
Crustacea and Mollusca	Three most-abundant taxon
Diptera	Four most-abundant taxon
Ephemeroptera	Five most-abundant taxon
Elmidae[2]	
Elmidae and Psephenidae[2]	**Functional feeding group**[1]
Ephemeroptera, Plecoptera, and Trichoptera (EPT)	Collector-gatherer
Gastropoda	Filtering collector
Isonychia and *Leuctra* (abundance and percent abundance)	Omnivore
Isopoda	Parasite
Non-insects	Piercer
Non-midge Diptera	Predator
Non-midge Diptera and non-insects[3]	Scraper
Odonata	Shredder
Oligochaeta	
Orthocladinae	**Diversity**
Plecoptera	Brillouin diversity
Pteronarcys (abundance and percent abundance)	Brillouin evenness
Ratio of EPT to Chironomidae[3]	Margalef diversity
Ratio of Orthocladinae to Chironomidae	Menhinick diversity
Ratio of Tanytarsini to Chironomidae	Shannon diversity
Tanytarsini	Shannon evenness
Total taxa	Simpson diversity
Trichoptera	Simpson dominance
Number of rare taxa	Simpson evenness
Total biomass[2]	
Crayfish	**Other**[4]
Insect[3]	Percent Chironomidae, Naidae, and Tubificidae
Mollusc	Percent of insect taxa
Total abundance	Number of insect taxa

[1]Richness, percent richness, abundance, and percent relative abundance were calculated for all "general community" and "functional feeding group" metrics unless otherwise specified

[2]Metrics calculated manually outside of the IDAS program

[3]Metrics selected for the macroinvertebrate index. Three metrics were calculated using relative abundance; however, "Insect biomass" was a weight calculation

[4]All "Other" metrics originated from the Oklahoma Conservation Commission (Greg Kloxin, Oklahoma Conservation Commission, written communication, September 2008)

Table S4. Fish metrics considered for a fish index at 29 wadeable Ozark streams.

Taxa abundance and taxa richness values were calculated for all metrics except catch per unit effort, which was reported as the number of fish collected per meter.

Tolerance	**Sensitive taxa**
Tolerant	*Ambloplites* and *Lepomis* spp.[6]
Moderately tolerant	*Ambloplites*[6]
Intolerant	Catostomidae
	Catostomidae and Cyprinidae
Feeding habitats	Catostomidae, Cottidae, and Percidae[6]
Grazer	Catostomidae, Cottidae, Cyprinidae, *Noturus*, and Percidae[6]
Herbivore	*Campostoma*[6]
Planktivore	Centrarchidae
Detritivore	Cottidae[6]
Invertivore	Cyprinidae
Carnivore	*Gambusia*
Primary[2]	*Lepomis*
Herbivore and grazer[6]	*Lepomis cyanellus*
Herbivore and detritivore[1,6]	*Lepomis megalotis*[6]
Insectivorous cyprinid[5]	*Gambusia* and *Lepomis*
	Micropterus and *Ambloplites*[6]
Spawning preference	*Micropterus*[1, 4]
Broadcasting	Percidae
Simple-nesting	Key species[2]
Complex-nesting	Sensitive species[2]
Migratory	
Nesting unknown	**Dominance**
	Number of species comprising 75 pecent of the abundance[5]
Distribution	
Endemic[6]	**Species association**
Exotic	Sedentary
	Schooling
Substrate preference	
Cobble or rubble	**Habitat preference**
Gravel	Riffle
Cobble-gravel (combined)[3]	Pool[1]
Sand	Run or main channel
Mud (silt, clay, detritus)	Backwater
Vegetation	Benthic
Substrate generalist	Surface-loving
	Headwater
Density	Habitat generalist
Catch per unit effort[1]	Pool and benthic

[1]Metrics selected for the fish index

[2]Metrics originated from the Arkansas Department of Environmental Quality (Jim Wise, Arkansas Department of Environmental Quality, written communication, August 2008)

[3]Metrics originated from Dauwalter et al., 2003

[4]Metric originated from Justus, 2003

[5]Metric originated from the Oklahoma Conservation Commission (Greg Kloxin, Oklahoma Conservation Commission, written communication, September 2008)

[6]Metric calculated by the authors to characterize taxa considered key to Ozark ecosystems

Table S5. Algal metric values, metric scores, and index scores for 30 wadeable Ozark streams. Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

Site name	Abbreviated name (fig. 1)	Most tolerant diatoms		Sum of *Cymbella affinis*, C. *delicatula*, and C. *hustedtii*		Mesosaprobic algae taxa richness		Diatoms as nitrogen heterotrophs		Algal index score	Nutrient index score
		Relative abundance (%)	Metric score	Relative abundance (%)	Metric score	Taxa richness (%)	Metric score	Relative abundance (%)	Metric score		
Barren Fork near Timber, Missouri	Barren	0.6	98.5	15.3	23.5	6.7	71.7	0.2	98.4	73.0	0.00
Meramec River above Cook Station, Missouri	Mera	0.2	99.6	62.1	95.0	4.8	79.8	0.2	98.7	93.3	0.05
Big Creek at Mauser Mill, Missouri	BcMm	0.0	100.0	46.1	70.4	6.3	73.4	0.0	100.0	86.0	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	0.2	99.6	7.8	11.9	4.8	79.8	0.0	100.0	72.8	0.08
Water Creek near Evening Star, Arkansas	Water	1.0	97.6	31.9	48.8	4.8	79.8	0.3	97.4	80.9	0.10
Bear Creek near Omaha, Arkansas	Bear	0.3	99.2	46.7	71.3	4.2	82.3	0.0	100.0	88.2	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	0.0	100.0	65.4	100.0	5.0	78.8	0.0	100.0	94.7	0.24
North Fork White River near Cabool, Missouri	NFWh	1.7	96.0	32.1	49.1	13.3	43.3	0.0	100.0	72.1	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	6.2	85.1	0.2	0.4	7.7	67.3	0.0	100.0	63.2	0.38
Bennetts River near Vidette, Arkansas	Benn	0.5	98.8	5.4	8.3	9.1	61.4	0.0	100.0	67.1	0.47
Mahans Creek at West Eminence, Missouri	Maha	0.3	99.2	11.8	18.0	12.0	49.0	1.2	90.8	64.3	0.53
Myatt Creek east of Salem, Arkansas	Myatt	3.0	92.8	12.0	18.4	6.5	72.6	0.0	100.0	70.9	0.54
West Piney Creek at Bado, Missouri	Wpin	3.3	92.0	1.2	1.9	18.2	22.7	0.0	100.0	54.1	0.60
Piney Creek near Cabanol, Missouri	Piney	1.2	97.2	17.4	26.5	12.0	49.0	0.2	98.7	67.9	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	0.5	98.8	18.7	28.6	11.4	51.4	0.0	100.0	69.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	0.3	99.2	7.2	11.0	11.9	49.4	0.0	100.0	64.9	0.77
Big Piney River at Simmons, Missouri	Bpine	15.7	62.1	10.4	15.8	9.7	58.9	6.6	48.3	46.3	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	1.3	96.8	5.3	8.2	19.4	17.7	0.0	100.0	55.7	0.85
Big Creek near Big Flat, Arkansas	BcBF	21.5	48.2	2.7	4.2	12.5	46.9	0.3	97.4	49.2	0.93
Calf Creek near Silver Hill, Arkansas	Calf	22.8	45.1	12.8	19.5	11.5	51.0	2.2	83.0	49.6	1.08
Poke Bayou near Sidney, Arkansas	Poke	9.4	77.4	1.9	2.9	9.5	59.5	1.2	90.8	57.7	1.10
Woods Fork near Hartville, Missouri	WdFk	1.5	96.3	2.7	4.2	23.5	0.0	0.0	100.0	50.1	1.12
Maries River Near Freeburg, Missouri	Marie	3.2	92.4	0.5	0.7	11.1	52.8	0.0	100.0	61.5	1.35
Long Creek southeast of Denver, Arkansas	Long	21.8	47.4	1.4	2.2	6.8	71.0	1.3	89.5	52.5	1.55
Little Flat Creek near McDowell, Missouri	Flat	5.7	86.3	0.0	0.0	13.0	44.6	0.5	96.1	56.7	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	17.7	57.4	7.5	11.5	12.5	46.9	3.3	73.8	47.4	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	41.5	0.0	6.0	9.2	13.3	43.3	6.0	52.9	26.3	2.57
Shoal Creek near Wheaton, Missouri	Shoal	23.6	43.2	0.4	0.6	20.8	11.5	2.0	84.1	34.8	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	38.5	7.2	0.2	0.4	8.3	64.6	0.3	97.4	42.4	2.95
North Indian Creek near Wanda, Missouri	NInd	16.2	60.8	0.9	1.4	18.5	21.3	12.7	0.0	20.9	3.30

Table S6. Macroinvertebrate metric values, metric scores, and index scores for 30 wadeable Ozark streams. Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

Site name	Abbreviated name (fig. 1)	Intolerant organisms		Baetidae		Insect biomass		Moderately tolerant taxa		Macroinvertebrate index score	Nutrient index score
		Relative abundance (%)	Metric score	Relative abundance (%)	Metric score	Grams	Metric score	Taxa richness	Metric score		
Barren Fork near Timber, Missouri	Barren	99.66	100.0	2.8	91.4	0.01	99.7	13	51.9	85.7	0.00
Meramec River above Cook Station, Missouri	Mera	96.95	97.3	3.4	89.4	1.29	73.0	20	25.9	71.4	0.05
Big Creek at Mauser Mill, Missouri	BcMm	99.64	100.0	7.2	77.4	1.17	75.6	14	48.1	75.3	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	97.52	97.9	1.3	96.0	1.58	67.0	15	44.4	76.3	0.08
Water Creek near Evening Star, Arkansas	Water	93.46	93.8	4.0	87.4	0.95	80.1	24	11.1	68.1	0.10
Bear Creek near Omaha, Arkansas	Bear	78.01	78.3	0.0	100.0	1.79	62.5	19	29.6	67.6	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	99.49	99.8	8.0	75.0	0.41	91.5	18	33.3	74.9	0.24
North Fork White River near Cabool, Missouri	NFWh	98.52	98.9	8.3	74.0	0.13	97.3	17	37.0	76.8	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	89.78	90.1	13.9	56.8	0.82	82.8	22	18.5	62.1	0.38
Bennetts River near Vidette, Arkansas	Benn	86.24	86.5	4.3	86.6	0.52	89.1	17	37.0	74.8	0.47
Mahans Creek at West Eminence, Missouri	Maha	94.96	95.3	15.9	50.5	0.49	89.7	21	22.2	64.4	0.53
Myatt Creek east of Salem, Arkansas	Myatt	85.69	86.0	2.0	93.7	1.58	67.0	18	33.3	70.0	0.54
West Piney Creek at Bado, Missouri	Wpin	90.65	91.0	3.2	90.1	2.17	54.6	24	11.1	61.7	0.60
Piney Creek near Cabanol, Missouri	Piney	85.32	85.6	8.4	73.8	1.65	65.5	23	14.8	59.9	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	87.50	87.8	3.4	89.3	1.12	76.7	17	37.0	72.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	85.68	86.0	9.2	71.4	0.57	88.0	16	40.7	71.5	0.77
Big Piney River at Simmons, Missouri	Bpine	92.31	92.6	10.2	68.0	1.30	72.9	21	22.2	63.9	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	78.61	78.9	15.9	50.5	2.70	43.6	21	22.2	48.8	0.85
Big Creek near Big Flat, Arkansas	BcBF	96.76	97.1	11.4	64.6	0.94	80.3	17	37.0	69.7	0.93
Calf Creek near Silver Hill, Arkansas	Calf	93.14	93.5	2.2	93.0	2.05	57.2	22	18.5	65.6	1.08
Poke Bayou near Sidney, Arkansas	Poke	81.28	81.6	12.7	60.3	3.23	32.5	27	0.0	43.6	1.10
Woods Fork near Hartville, Missouri	WdFk	74.09	74.3	4.6	85.7	0.55	88.6	20	25.9	68.6	1.12
Maries River Near Freeburg, Missouri	Marie	84.22	84.5	0.4	98.8	0.02	99.5	20	25.9	77.2	1.35
Long Creek southeast of Denver, Arkansas	Long	89.09	89.4	1.0	96.9	1.24	74.0	25	7.4	66.9	1.55
Little Flat Creek near McDowell, Missouri	Flat	92.34	92.6	12.9	59.8	1.11	76.9	15	44.4	68.4	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	95.26	95.6	23.2	27.5	1.82	61.9	23	14.8	49.9	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	94.34	94.7	9.3	70.9	2.50	47.6	19	29.6	60.7	2.57
Shoal Creek near Wheaton, Missouri	Shoal	66.26	66.5	17.3	45.9	1.99	58.4	23	14.8	46.4	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	90.73	91.0	32.0	0.0	3.07	35.8	22	18.5	36.3	2.95
North Indian Creek near Wanda, Missouri	NInd	64.33	64.5	13.9	56.7	4.78	0.0	15	44.4	41.4	3.30

Table S7. Fish metric values, metric scores, and index scores for 29 wadeable Ozark streams (one site was not sampled for fish because of the potential occurrence of a federally-listed threatened species). Metric results are sorted by nutrient index score and rows that are shaded represent sites with a nutrient index score greater than 0.75 and are suspected of being moderately enriched

Site name	Abbreviated name (fig. 1)	Herbivore/Detritivore		Pool species		Catch per unit effort		Black bass		Fish index score	Nutrient index score
		Taxa richness	Metric score	Relative abundance (%)	Metric score	Fish per meter	Metric score	Relative abundance (%)	Metric score		
Barren Fork near Timber, Missouri	Barren	1	83.3	61.2	63.8	0.9	89.2	1.00	28.5	66.2	0.00
Meramec River above Cook Station, Missouri	Mera	5	16.7	76.9	80.1	3.3	61.4	1.00	28.5	46.7	0.05
Big Creek at Mauser Mill, Missouri	BcMm	3	50.0	66.2	68.9	1.8	79.1	0.26	7.4	51.4	0.05
North Sylamore Creek near Fifty Six, Arkansas	Nsyla	2	66.7	79.0	82.3	1.4	83.8	3.51	100.0	83.2	0.08
Water Creek near Evening Star, Arkansas	Water	5	16.7	64.6	67.3	2.4	72.0	1.52	43.3	49.8	0.10
Bear Creek near Omaha, Arkansas	Bear	4	33.3	64.9	67.6	3.2	63.5	2.66	75.8	60.1	0.14
North Prong Jacks Fork below Arroll, Missouri	NPJF	3	50.0	96.0	100.0	1.1	87.4	0.37	10.5	62.0	0.24
North Fork White River near Cabool, Missouri	NFWh	4	33.3	42.1	43.9	2.5	70.9	0.50	14.2	40.6	0.27
Spring Creek near Locust Grove, Oklahoma	Spring	4	33.3	50.6	52.7	2.4	72.8	1.88	53.6	53.1	0.38
Bennetts River near Vidette, Arkansas	Benn	3	50.0	68.7	71.5	1.7	80.1	0.69	19.7	55.3	0.47
Mahans Creek at West Eminence, Missouri	Maha	4	33.3	58.8	61.3	1.4	83.8	0.00	0.0	44.6	0.53
Myatt Creek east of Salem, Arkansas	Myatt	2	66.7	42.0	43.8	2.1	76.2	2.19	62.4	62.3	0.54
West Piney Creek at Bado, Missouri	Wpin	3	50.0	55.1	57.4	3.5	59.9	1.09	31.1	49.6	0.60
Piney Creek near Cabanol, Missouri	Piney	3	50.0	39.4	41.1	4.3	50.4	0.54	15.4	39.2	0.61
South Fork Spring River north of Moko, Arkansas	SFkS	4	33.3	43.7	45.5	2.2	74.6	0.89	25.4	44.7	0.63
Roasting Ear Creek near Newnata, Arkansas	REar	2	66.7	44.6	46.5	2.6	70.0	1.60	45.6	57.2	0.77
Big Piney River at Simmons, Missouri	Bpine	3	50.0	50.7	52.8	1.4	84.1	1.99	56.7	60.9	0.78
Sullivan Creek near Sandtown, Arkansas	Sull	4	33.3	41.6	43.3	1.9	78.2	0.70	19.9	43.7	0.85
Big Creek near Big Flat, Arkansas	BcBF	3	50.0	73.5	76.6	2.9	66.9	1.60	45.6	59.8	0.93
Calf Creek near Silver Hill, Arkansas	Calf	4	33.3	44.1	45.9	8.3	3.8	0.10	2.8	21.5	1.08
Poke Bayou near Sidney, Arkansas	Poke	6	0.0	59.8	62.3	2.6	70.2	2.12	60.4	48.2	1.10
Woods Fork near Hartville, Missouri	WdFk	5	16.7	72.3	75.4	2.3	74.0	1.13	32.2	49.6	1.12
Long Creek southeast of Denver, Arkansas	Long	4	33.3	65.4	68.1	4.1	52.2	0.40	11.4	41.3	1.55
Little Flat Creek near McDowell, Missouri	Flat	4	33.3	19.2	20.0	1.7	79.8	0.00	0.0	33.3	2.15
Beaty Creek near Sycamore, Oklahoma	Beaty	3	50.0	49.4	51.5	2.4	71.9	0.33	9.4	45.7	2.27
Yocum Creek near Oak Grove, Arkansas	Yoc	4	33.3	70.8	73.7	2.1	75.9	0.83	23.6	51.7	2.57
Shoal Creek near Wheaton, Missouri	Shoal	6	0.0	56.9	59.3	7.6	11.9	0.70	19.9	22.8	2.88
Little Osage Creek at Healing Springs, Arkansas	Osag	5	16.7	41.1	42.8	8.7	0.0	0.15	4.3	15.9	2.95
North Indian Creek near Wanda, Missouri	NInd	5	16.7	42.9	44.7	2.3	72.9	0.00	0.0	33.6	3.30