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EVALUATION OF FISH AND MACROINVERTEBRATE INDICES OF BIOTIC INTEGRITY IN THE BIOASSESSMENT OF THE ILLINOIS RIVER BASIN

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Abstract

Evaluating performances of the fish and invertebrate Indices of Biotic Integrity (IBIs) for a region is important to maintain rigorous assessment of the environmental quality of streams, especially with increasing urbanization. Timing of the assessment is considered important, with the critical season (low flow, high temperature) preferred, but the primary season (spring – summer) may be as efficient. I assisted with the collection and analysis of fish and macroinvertebrates using methods developed by the United States Environmental Protection Agency's (USEPA) Rapid Bioassessment Protocols (RBPs) and the Arkansas Department of Environmental Quality (ADEQ), along with obtaining habitat and chemical assessments during primary and critical periods during 2007-2009 at ten sites in the Illinois River Basin up and downstream of two wastewater treatment plants (WWTPs). My objectives were to (1) compare fish and macroinvertebrate IBIs for use in the Illinois River Basin; (2) investigate correlations with each IBI and its metrics to nutrient, habitat, and watershed variables; (3) compare the efficacy of the IBIs during both critical and primary seasons; and (4) determine how two WWTPs in the area affect downstream water quality into Oklahoma. The two IBIs were strongly correlated with each other (Rs of 0.59); however, macroinvertebrates outperformed the fish. More regionally specific fish metrics should allow for better performing fish IBIs, but adequate performance was found. Combining the seasons' data allowed for a more comprehensive and statistically significant assessment; however, the primary season evaluated each site comparably to the combined data and generally outperformed the critical seasons. The combined and primary seasons' macroinvertebrate IBIs revealed sites with lowered environmental quality below the WWTPs but with quick returns to reference conditions. My results indicate that it may be possible to test IBIs during only the primary seasons to get efficient water quality and site comparison assessments.

Introduction

With the increasing quantity and diversity of chemical runoff from industrial, agricultural, and urban areas, along with an array of environmental modifications, the water quality of the United States' surface waters has become an increasingly important issue. For this reason, much legislation has been passed in the U.S. to develop a means for monitoring, assessing, and restoring the nation's environmental quality of wadeable streams. However, biological assessments, which use biological surveys and other measures of the biota in surface waters to evaluate water body conditions, only began to be integrated into state and tribal programs a little over three decades ago (Barbour et al. 1999; Yoder and Barbour 2009). Biological criteria usually are more capable of detecting degradation due to anthropogenic influences than are chemical and toxicological methods (Karr 1991). A study in Ohio found that water quality variables did not recognize the presence of human influence, while bioassessments correctly identified influence 49.8% of the time (Kerans and Karr 1994). An array of natural and anthropogenic influences is detectable by fish and macroinvertebrate assemblages due to their integrative response to stress from habitat, water chemistry, and other environmental factors (Weigel and Robertson 2007).

The Water Pollution Control Act (WPCA) of 1948 was passed for the protection of U.S. waters (Dauwalter et al. 2003). In 1972, amendments were made to the WPCA, which is now known as the Clean Water Act (CWA), to include a fishable and swimmable goal and to restore and preserve the physical, chemical, and biological integrity of the nation's waters (Karr 1991). The U.S. Environmental Protection Agency (USEPA) uses Frey's original definition to define biological integrity as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region" (Hawkins 2006).

In 1981, Karr developed the quick, reliable, and easy Index of Biotic Integrity (IBI), which set a framework for bioassessment with its multimetric index using a biosurvey of the fish community (Dauwalter et al. 2003). The USEPA came to believe that, above tedious individual toxicity measurements, biomonitoring approaches could offer significant advantages (Roop and Hunsaker 1985). The USEPA decided to create Rapid Bioassessment Protocols (RBPs) to fulfill the need for concise, cost-effective biological survey techniques for the application of the CWA (Barbour et al. 1999). Karr's IBI was integrated into the RBPs fish protocols, with various regional modifications, since distinct fish assemblages had been shown to correspond with ecoregions, and soon the Invertebrate Community Index (ICI) was created and modified into a benthic IBI for use in the RBPs (Dauwalter et al. 2003; Barbour et al. 1999). The 1989 RBPs were revised in 1999 as Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition for assessment using three assemblages along with habitat for a more comprehensive approach (Barbour et al. 1999).

The current pressing issue is determining appropriate regional modifications of the biometrics since each region may contain differing species richness and composition, trophic composition, and taxa abundance and conditions. Therefore, one series of biometrics cannot accurately calculate environmental quality for all regions (Barbour et al. 1999).

Effort and cost must be considered for bioassessment. These can be reduced by determining the most effective time(s) to sample in order to minimize samples per year. Seasonality greatly affects streams and their biota; therefore, sample periods usually occur during the critical and/or primary seasons. The critical season usually occurs during the summer of each year and allows for insight into the effects of low flow and high temperatures (> 22°C for northwestern Arkansas), often accompanied by algal blooms (Barbour et al. 1999). The primary season usually entails the less extreme conditions of the spring, with higher flows and cooler temperatures. Sampling both seasons for a combined seasons' dataset is optimal for a more comprehensive assessment; however, past bioassessments have also used only one season, with the critical season usually being the preferred (see ADEQ 2003, Wang et al. 2007, and Dauwalter et al. 2003). If one season's performance is comparable to the combined dataset, effort and cost may be conserved by the use of sampling during only one season for further specificity of the IBI.

With regionally specific IBIs, the individual biometrics can perform optimally; thus, the nation's water quality can be monitored more effectively, and stricter environment protection regulations, especially for industries, can be implemented based on the IBIs' findings in the surrounding surface waters. Therefore, my focus was on evaluating the current IBIs for fish and macroinvertebrate assemblages in the Illinois River Basin on sites around two wastewater treatment plants (WWTPs).

With more understanding of the performance of each metric and the overall IBIs, future studies in this region may use the most advantageous IBI metrics to accurately recognize sources of stream degradation in this rapidly urbanizing region and thus help maintain and restore adequate stream health. Currently, the Arkansas Department of Environmental Quality (ADEQ) has a recommended method for performing a fish IBI for the much larger region of Ozark Highlands Streams, which encompasses the Illinois River Basin. For Arkansas' macroinvertebrate communities, the ADEQ has a list of 25 suggested metrics for individual screening. If the current IBI metrics are not found to perform strongly, there will be a need to develop even more specific and discriminatory metrics for the region.

The objectives of this project were to (1) determine how the fish and macroinvertebrate IBIs compare with each other in their use on the streams of the Illinois River Basin; (2) investigate how effectively each IBI and its individual metrics correlate with nutrient, habitat, and watershed variables; (3) determine if it is necessary for bioassessments to sample surface waters during both the critical and primary seasons; and (4) determine how two wastewater treatment plants (WWTPs) in the area affect downstream water quality into Oklahoma.

Study Site

The headwaters of the Illinois River originate in northwest Arkansas in the Springfield Plateau ecoregion within the Ozark Highlands and then flow into northeastern Oklahoma to confluence with the Arkansas River. The Ozark Highlands contain moderately diverse biota in streams formed predominately of alluvial gravel with distinct riffle-pool geomorphometry (Brown and Matthews 1995).

The study sites were in the Illinois River Basin's northwest Arkansas area, which is influenced by agricultural run-off and effluents from the cities of Fayetteville, Springdale, Rogers, Siloam Springs, and Prairie Grove, Arkansas. The Rogers and Springdale WWTPs were the focus of the current study. Five sites (OSG1, OSG2, OSG3, OSG4, and OSG5) were located on Osage Creek, which contains the Rogers WWTP. Three sites (SPG1, SPG2, and SPG3) were located on Spring Creek, which contains the Springdale WWTP, and the two other sites were reference streams on Little Osage (LOREF) and Camber Springs (CSREF). The layout of the ten sites placed upstream of each plant, two sites downstream of each plant, and two sites on Osage Creek below the confluence with Spring Creek (Figure 1). Watershed sizes of the sites varied greatly from 13.4 square km (CSREF) to 209.2 square km (OSG5).

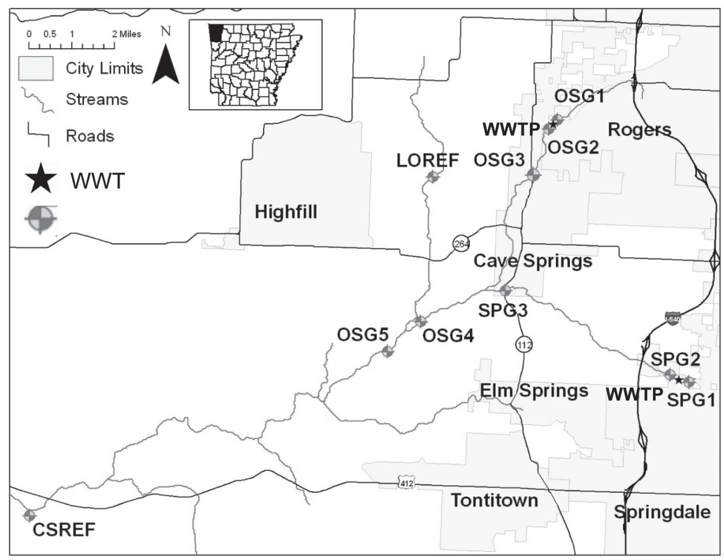

Figure 1. A map of the study area with sample sites and WWTP areas marked. The two reference sites are indicated by their abbreviations: LOREF and CSREF. The sites on Osage Creek EW denoted as OSG; Spring Creek sites, as SPG.

Methods

The methods were adopted from the detailed descriptions in the USEPA's RBPs and ADEQ (Barbour et al. 1999). Fish and macroinvertebrate collections, along with habitat and chemical assessments at all ten sites, occurred in summer 2007, spring and summer 2008, and spring and summer 2009. Summer samples were planned to occur during the critical season of low flow and high temperatures (> 220C) each year; however, there was no critical season during 2008, so the summer 2009 sample was performed to obtain the second critical season sample and replace the summer 2008 data.

Water Chemistry

Another team on which I did not participate collected water samples during base flow conditions a total of 29 times from the summer of 2007 to the summer of 2009, using methods described in the EPA protocols. The nutrient variables used for comparison with biometrics in this study were total phosphorus (TP), total nitrogen (TN), and total organic carbon (TOC). More details are available in the team's final report (see Matlock et al. 2009).

Habitat and Geomorphology

The qualitative RBP Habitat Assessment approach was used to develop a habitat profile for each sample reach. During each habitat assessment, the biotic canopy cover was measured. Geomorphologic assessments were performed once at each site to define the general morphologic characteristics of the reach, including % reach bedrock. Another team was in charge of these methods, but I occasionally helped with measurements. More details are available in the team's final report (see Matlock et al. 2009).

Watershed Areas and Attributes

Through the Center for Advanced Spatial Technology, University of Arkansas, watershed areas and percent dominant land use areas were found in 2006 for select sites on the Illinois River Basin.

Fish Assemblage Analyses

A 350-1000 foot long reach at each site, representing the diverse habitats of each stream, i.e., riffles, runs, and pools, was used for fish collection through single-pass upstream backpack electrofishing with block nets in accordance with the USEPA's RBPs (Barbour et al. 1999). Three persons with long-handled dip nets followed the electrofisher to collect and transfer the fish to livewells for identification of species, which was performed by the same person every time. Hybrids and anomalies, in addition to species, were documented. After enumeration, the fish were released. If field identification of certain specimens was uncertain, preservation in 10% formalin solution and storage for laboratory identification was performed. Stonerollers (Campostoma spp), which are difficult to identify to species, were usually found in large quantities at the sites. Thus, if there were more than 50 individuals at a site, 40-50 individuals were identified to species, and the ratio was applied to the total number collected at the site. ADEQ's Ozark Highlands' fish metrics were summed to obtain an IBI for each collection at a site (Table 1).

Table 1. Fish community biocriteria for Ozark Highland streams established by ADEQ (ADEQ personal communication).

Metric	5	3	1
% Sensitive Individuals	> 31	31 - 20	< 20
% Cyprinidae (Minnows)	48 – 64	39 – 47 or 65 – 73	< 39 or >73
% Ictaluridae (Catfishes)	> 2	1 - 2	< 1 or > 3% bullheads
% Centrarchidae (Sunfishes)	4 - 15	< 4 or 15 - 20	> 20 or > 2% Green sunfish
% Percidae (Darters)	> 11	5 – 11	< 5
% Primary Feeders	< 42	42 – 49	> 49
% "Key" Individuals	> 23	23 – 16	< 16
Diversity	> 2.77	2.77 – 2.37	< 2.37
# Species	>(watershed area**C**0.034)+16.45	(watershed area**C**0.034)+16.45 to (watershed area**C**0.034)+12.26	<(watershed area**C**0.034)+12.26

[1]no more than 3% bullheads

[2]no more than 2% Green sunfish

Macroinvertebrate Assemblage Analyses

Macroinvertebrate kicknet procedures, as described by the RBPs, were followed (Barbour et al. 1999). Collection occurred from ten locations divided evenly between two riffles in each study site using a rectangular dip net and a slight modification of the single habitat approach described by USEPA (riffles only). Net contents were spread in a large tray at streamside. Invertebrates were picked from samples and placed in 75% ethanol for preservation and transport to the laboratory. In the lab, samples were placed in a 6 cm X 6 cm gridded tray for analysis using the 100″ ± 20% organism collection process in accordance with the USEPA protocols (Barbour et al. 1999). Most of the benthic macroinvertebrates were identified to genus using taxonomic keys. An a priori decision was made to identify the Chironomidae only to family to save the time and money required for further taxonomic refinement. Flat worms and leeches, having been preserved using only ethanol in the field, were not relaxed enough to identify past family or order. Instars too young or too badly damaged (missing legs, gills, mouth parts, etc.) were taken to the lowest taxonomic level, generally family, where certainty of identification was not compromised.

The analysis of the macroinvertebrate data is also rather completely prescribed by the USEPA and ADEQ, although ADEQ is still in the process of completing its decisions about the analysis and interpretation of benthic macroinvertebrate IBI data from the different ecoregions across the state. The methods were followed as closely as possible, and conversations were held with ADEQ personnel regarding items of uncertainty. Eleven biometrics were settled upon for the macroinvertebrate IBI (Table 2). With the top score for each biometric assigned as 5, the highest possible IBI score was 55 since the IBI is the sum of the 11 metric scores. It was necessary for scoring criteria (cut-off values) to be established for the biometrics based on our results. All of our data from critical and primary seasons from all ten collecting locations were used to determine these criteria and to have them correspond to the 25% and 75% quartiles.

Data Analyses

Spearman Rank Correlations (Rs) with $p \leq 0.05$ were used for analysis between the fish and macroinvertebrate IBIs, and IBI and metric correlations were investigated to nutrient, habitat, and watershed variables using JMP 8.0 Software (SAS Institute 2008). The three datasets of critical seasons, primary seasons, and combined seasons were used for IBI and metric correlation investigations. The nonparametric Spearman procedure was used to reduce the effects of the assumption of normal data distribution. This statistical method is commonly used for determining correlations between biotic measures and human influence variables (see Wang et al. 2007, Weigel and Robertson 2007, Bramblett et al. 2005, Dauwalter et al. 2003). Since greater numbers of tests cause greater Type I family-wise error rates, the False Discovery Rate (FDR) was performed to adjust the p-values

Table 2. Macroinvertebrate metric scoring ranges established using the 25th and 75th percentile rankings of metric scores from all five collections performed during this study. Invertebrate metric scoring ranges for the Osage and Spring Creek basins of the Illinois River, Arkansas, is shown in A). B) shows percentile ranking of metric used to establish scoring ranges for each of the biometrics. Note that the % Isopoda metric was changed from "0.0%" indicated by the 25th percentile to "< 2" following our best professional judgment. EPT stands for Ephemeroptera, Plecoptera, and Trichoptera Taxa, while HBI is the Hilsenhoff Biotic Index.

A)

Metric	5	3	1
Total Taxa	> 17	17 – 12	< 12
Number EPT Taxa	> 8	8 – 5	< 5
%EPT- %Hydropsychidae	> 55	55 – 28	< 28
% Scrapers	> 33	5 – 33	< 5
% Clingers	> 68	68 – 23	< 23
% Diptera	< 4	4 – 24	> 24
% Chironomidae	< 3	3 – 22	> 22
% Isopoda	< 2	2 – 7	> 7
% Tolerant Organisms (7-10)	< 2	2 – 12	> 12
HBI	< 4.1	4.1 - 5.2	> 5.2
% Intolerant Organisms (1-3)	> 24	24 – 6	< 6

B)

Metric	Min	5th	25th	50th	75th	95th	Max
Total Taxa	8	8.45	12	15	17	19.55	23
Number EPT Taxa	2	2.45	5	6	7.75	10.55	14
%EPT- %Hydropsychidae	4.1%	9.3%	28.0%	44.4%	55.3%	67.1%	73.6%
% Scrapers	0.0%	0.0%	4.5%	17.1%	33.1%	48.4%	60.6%
% Clingers	2.8%	5.8%	23.4%	48.7%	67.7%	84.8%	92.1%
% Diptera	0.0%	0.0%	3.9%	10.6%	23.9%	55.9%	66.7%
% Chironomidae	0.0%	0.0%	2.5%	7.2%	21.6%	44.3%	57.5%
% Isopoda	0.0%	0.0%	0.0%	0.4%	6.8%	55.2%	72.5%
% Tolerant Organisms	0.0%	0.0%	1.7%	3.3%	12.1%	53.9%	67.0%
HBI	2.59	3.11	4.11	4.76	5.15	6.40	6.89
% Intolerant Organisms	0.0%	1.9%	5.7%	12.5%	23.8%	52.8%	64.7%

for each Spearman Rank Correlation set of tests performed (Quinn and Keough 2002). Analyses of Variance (ANOVA) in conjunction with the Tukey-Kramer Honestly Significant Difference test were used for comparisons between sites and are common methods of determining significant differences between means (see Bramblett et al. 2005, Barbour et al. 1999, Kerans and Karr 1994).

Results and Discussion

Fish IBI and Macroinvertebrate IBI Analyses

Fish IBI scores were significantly positively correlated with macroinvertebrate IBI scores (Rs of 0.59, $p < 0.0001$) (Figure 2). The strong correlation may indicate that the assemblages were similarly affected by degradation in their environments and the IBIs were both detecting this degradation. It is pleasing that the assemblages are comparable and do not have conflicting water quality results.

Further investigation of each IBI with nutrient, habitat, and watershed variables allowed for a deeper understanding of the effects of degradation on each assemblage; however, cause-and-effect relations could not be established using this analysis. First, the combined dataset was used to see how the IBIs correlated with the nutrient variables of Total Phosphorous (ranging from 0.029 to 0.643 mg/L, mean of 0.112 mg/L), Total Nitrogen (ranging from 0.47 to 7.37 mg/L, mean of 4.00 mg/L), and Total Organic Carbon (ranging from 0.15 to 4.16 mg/L, mean of 1.15 mg/L). The fish IBI did not have any significant correlations; however, the macroinvertebrate IBI was significantly negatively

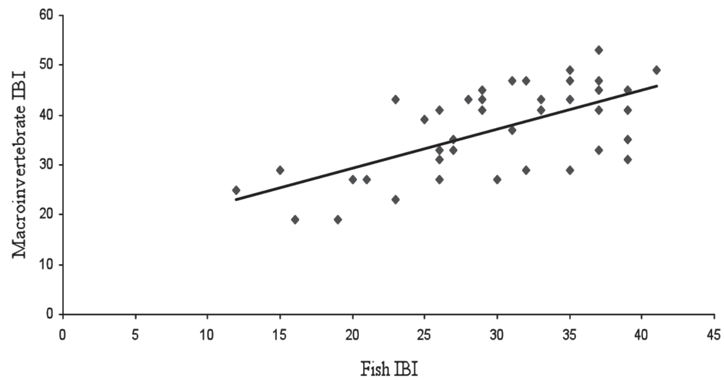

Figure 2. The significant and strong correlation between the fish IBI and the macroinvertebrate IBI is graphically visible, with an Rs of 0.59 and p values much less than even 0.0001.

correlated to Total Phosphorous (Rs = -0.47) and Total Organic Carbon (Rs = -0.50). Nitrogen correlation was completely absent in both assemblages. Next, testing with the habitat variables of the RBP Total (ranging from 120 to 179, mean of 151), % Reach Bedrock (ranging from 0 to 35%, mean of 9%), and Biotic Canopy Cover (ranging from 2.7 to 78, mean of 44) showed that both assemblage IBIs were significantly positively correlated with the RBP Total (fish IBI Rs = 0.58, macroinvertebrate IBI Rs = 0.63). However, the macroinvertebrate IBI also was significantly negatively correlated with % bedrock (Rs = -0.42). With the watershed variables of % urban (ranging from 0 to 60%, mean of 35%), % pasture (ranging from 23 to 79%, mean 43%), and % forest (ranging from 12 to 62%, mean of 19%) tested next, both IBIs correlated significantly to all variables, with % urban being the strongest for both assemblages (fish IBI Rs = -0.73, macroinvertebrate IBI Rs = -0.77) and % forest being the weakest (fish IBI Rs = 0.37, macroinvertebrate IBI Rs = 0.34). Percent pasture showed an Rs of 0.51 for the fish IBI and 0.43 for the macroinvertebrate IBI.

In this study, it appeared that the macroinvertebrate IBI performed better to detect overall degradation since the macroinvertebrate IBI correlated slightly better with the habitat and watershed variables, while at the same time also correlating with nutrients. The fish IBI, however, did not. The macroinvertebrate IBI lacked only the two correlations of Total Nitrogen and canopy, while the fish IBI lacked Total Phosphorous, Total Nitrogen, Total Organic Carbon, % bedrock, and canopy. The better performance of the macroinvertebrate IBI may be due to its being created specifically for the study region through the use of these combined data. The fish IBI was created by ADEQ for the large Ozark Highlands Region. The smaller geographic specificity of the fish IBI may account for the decreased accuracy of detection. In addition, this variety in response may be partially explained by differences in each assemblage's lifespan. Fish indicate more long-term degradation, while invertebrates indicate short-term environmental variations (Barbour et al. 1999). In addition, the different performances might be due to fish being considered reliable indicators of habitat quality and alterations in flow while macroinvertebrates are commonly used for determining the effects of organic pollution and alterations in hydromorphology (Johnson et al. 2006).

The individual seasons' IBI correlations to variables were also investigated to determine if only one season would yield a wide-ranging bioassessment. This investigation involved testing the smaller sample size of 20 for the individual seasons as compared to 40 for the combined. It should be noted that the larger the sample size, the more statistically reliable the correlations are. For the nutrient variables, the only correlation occurred in the critical season with the macroinvertebrate IBI to Total Organic Carbon (Rs = -0.58). For the habitat variables, the only significant correlations occurred during the primary season, with both IBIs to the RBP Total (fish IBI Rs = 0.67; macroinvertebrate IBI Rs = 0.85). For the watershed variables, both IBIs were significantly negatively correlated to % urban in both seasons (fish IBI Rs, -0.74 for critical and -0.76 for primary; macroinvertebrate IBI Rs, -0.70 for critical and -0.84 for primary). However, it was only in the primary season that both IBIs were significantly positively correlated to % pasture (fish IBI Rs = 0.53; macroinvertebrate IBI Rs = 0.50). The primary season had stronger and more significant correlations than the critical season in all areas except for the critical season's single nutrient correlation. Even though the primary season's significant correlations were stronger than the combined data, the season did not compare to the larger number of significant correlations found in the combined data (i.e., three versus seven correlations for the macroinvertebrate IBI). More on individual season contributions will be discussed later.

Fish Metric Analyses

First, the nine metrics of the fish IBI were tested against the nutrient variables. In keeping with the fish IBI results, the metrics never significantly correlated with any nutrient variables in any dataset. The habitat variables showed significant correlations to the metrics. The combined dataset had eight significant correlations within the five metrics of % Sensitive Individuals, % Ictaluridae, % Centrarchidae, % Primary Feeders, and % Key Individuals (Table 3). Although the fish IBI were lacking, there were significant correlations to both % bedrock and canopy among the fish metrics. Half of the significant correlations were to the RBP Total. With the critical dataset, only the three metrics of % Sensitive Individuals, % Primary Feeders, and % Key Individuals were significantly correlated, and of the total of four correlations, none were to % bedrock. The primary season had only a single significant correlation of % Key Individuals to the RBP Total (Rs = 0.76), possibly further presenting this metric as the best indicator of qualitative habitat health at any time during the year. Overall, over half of the fish metrics detected habitat variables with the combined data.

The watershed variables using the combined dataset had seven significant correlations within the four metrics of % Sensitive Individuals, % Ictaluridae, % Primary Feeders, and % Key Individuals (Table 4). Over half of the correlations were to % urban. The loss of sensitive species may be linked to increasing urbanization (Lussier et al. 2008). Indirect nutrient detection may be occurring since increasing urbanization is often linked to increased Total Nitrogen and Total Phosphorous (Campo et al. 2003). With the critical data, there were only four significant correlations within the same metrics as the combined data. The primary season had only one fewer significant correlation; however, only the metrics of % Ictaluridae and % Key Individuals contained the correlations.

Table 3. Correlations among fish IBI metrics from different seasons with habitat variables using A) combined seasonal data, B) critical season data, and C) primary season data. The significant Spearman correlation (p ≤ 0.05 with FDR correction) coefficients are bolded. The fish metrics are numbered with 1. % Sensitive Individuals, 2. % Cyprinidae, 3. % Ictaluridae, 4. % Centrar chidae, 5. % Percidae, 6. % Primary Feeders, 7. % Key Individuals, 8. Diversity, and 9. Total Species.

A)

Habitat Variable	1	2	3	4	5	6	7	8	9
RBP Total	**0.44**	-0.25	**0.47**	0.13	0.19	**-0.61**	**0.69**	-0.12	0.28
% Bedrock	**-0.52**	0.10	-0.13	**0.48**	-0.21	0.27	-0.18	0.20	0.14
Canopy	0.04	-0.37	-0.16	**0.40**	0.35	**-0.45**	0.39	-0.02	0.26

B)

Habitat Variable	1	2	3	4	5	6	7	8	9
RBP Total	**0.61**	-0.32	0.44	0.21	0.29	**-0.67**	**0.69**	-0.14	0.22
% Bedrock	-0.52	0.13	0.04	0.42	-0.30	0.32	-0.09	0.14	0.09
Canopy	0.29	-0.41	0.06	0.53	0.07	**-0.62**	0.54	-0.08	0.22

C)

Habitat Variable	1	2	3	4	5	6	7	8	9
RBP Total	0.40	-0.26	0.47	0.04	0.21	-0.61	**0.76**	-0.07	0.30
% Bedrock	-0.58	0.09	-0.31	0.50	-0.12	0.21	-0.25	0.24	0.13
Canopy	-0.16	-0.36	-0.37	0.27	0.61	-0.26	0.20	0.05	0.31

Table 4. Correlations among fish IBI metrics from different seasons with watershed variables using A) combined seasonal data, B) critical season data, and C) primary season data. The significant Spearman correlation (p ≤ 0.05 with FDR correction) coefficients are bolded. The fish metrics are numbered with 1. % Sensitive Individuals, 2. % Cyprinidae, 3. % Ictaluridae, 4. % Centrar chidae, 5. % Percidae, 6. % Primary Feeders, 7. % Key Individuals, 8. Diversity, and 9. Total Species.

A)

Watershed Variable	1	2	3	4	5	6	7	8	9
% Urban	**-0.66**	0.28	**-0.57**	0.03	-0.34	**0.64**	**-0.73**	-0.13	-0.31
% Pasture	0.30	-0.33	0.34	0.24	0.31	**-0.41**	**0.45**	0.23	0.35
% Forest	0.35	0.03	**0.68**	-0.03	-0.27	-0.22	0.32	-0.02	0.14

B)

Watershed Variable	1	2	3	4	5	6	7	8	9
% Urban	**-0.78**	0.42	-0.40	0.02	-0.54	**0.74**	**-0.71**	-0.27	-0.41
% Pasture	0.39	-0.53	0.12	0.17	0.36	-0.43	0.37	0.43	0.32
% Forest	0.45	-0.05	**0.63**	0.05	-0.10	-0.26	0.30	-0.09	0.20

C)

Watershed Variable	1	2	3	4	5	6	7	8	9
% Urban	-0.58	0.21	**-0.73**	0.03	-0.15	0.56	**-0.74**	-0.03	-0.27
% Pasture	0.25	-0.22	0.53	0.29	0.24	-0.41	0.52	0.06	0.41
% Forest	0.26	0.07	**0.72**	-0.08	-0.41	-0.21	0.34	0.04	0.04

The metrics that performed significantly in both the habitat and watershed variables through at least one dataset correlation were % Sensitive Individuals, % Ictaluridae, % Primary Feeders, and % Key Individuals. However, four out of the nine fish metrics were never significantly correlated to any variable in any dataset. These metrics were % Cyprinidae, % Percidae, Diversity, and Total Species. Furthermore, % Centrarchidae correlated only to geomorphology habitat variables instead of anthropogenic variables; therefore, it should be included as a metric that did not perform well towards degradation detection. Overall, fewer than half of the fish metrics performed well for detecting habitat and watershed degradation; therefore, increasing metric performance towards these forms of degradation along with incorporating nutrient degradation detection would strengthen the fish IBI, especially for the regions around the WWTPs. Neither individual season appeared to perform comparably to the combined data for the fish assemblage.

Macroinvertebrate Metric Analyses

Surprisingly, the 11 macroinvertebrate metrics were not significantly correlated with a single nutrient variable in any dataset. For the habitat variables, the combined data had seven significant correlations within the six macroinvertebrate metrics of Total Taxa; # Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa; % Scrapers; % Diptera; % Chironomidae; and the Hilsenhoff Biotic Index (HBI) (Table 5). Over half of the significant correlations were to the RBP Total. Aligning with the IBI results, the critical season data did not have any significant correlations with habitat variables. Within the primary season data, there were nine significant correlations within the nine metrics of Total Taxa, # EPT, % Scrapers, % Clingers, % Diptera, % Chironomidae, % Isopoda, % Intolerant Organisms, and HBI, each having higher correlations compared to the combined data (e.g., HBI primary data Rs = -0.76 and combined data Rs = -0.46).

Table 5. Correlations among macroinvertebrate IBI metrics from different seasons with habitat variables using the A) combined seasonal data and B) primary season data. The significant Spearman correlation ($p \leq 0.05$ with FDR correction) coefficients are bolded. The macroinvertebrate metrics are number 1. Total Taxa, 2. # EPT, 3. % EPT- % Hypropsychidae, 4. % Scrapers, 5. % Clingers, 6. % Diptera, 7. % Chironomidae, 8. % Isopoda, 9. % Tolerant Organisms, 10. % Intolerant Organisms, and 11. HBI.

A)

Habitat Variable	1	2	3	4	5	6	7	8	9	10	11
RBP Total	**0.67**	**0.53**	0.24	**0.65**	0.39	-0.36	-0.35	-0.38	-0.16	0.37	**-0.46**
% Bedrock	-0.37	-0.31	-0.19	-0.23	-0.02	**0.49**	**0.49**	0.19	-0.09	-0.28	0.24
Canopy	**0.49**	-0.02	-0.09	0.36	0.05	-0.11	-0.14	0.07	0.12	0.16	-0.16

B)

Habitat Variable	1	2	3	4	5	6	7	8	9	10	11
RBP Total	**0.73**	**0.61**	0.47	**0.68**	**0.62**	-0.43	-0.42	**-0.61**	-0.25	**0.68**	**-0.76**
% Bedrock	-0.37	-0.28	-0.15	-0.29	-0.28	**0.64**	**0.61**	0.26	-0.02	-0.27	0.27
Canopy	0.49	0.06	-0.05	0.24	0.22	-0.01	-0.06	-0.04	0.39	0.28	-0.18

The watershed variables had ten significant correlations within the seven metrics of Total Taxa, # EPT, % EPT- % Hypropsychidae, % Scrapers, % Isopoda, % Intolerant Organisms, and HBI through the use of the combined dataset (Table 6). Seven of the correlations were to % urban. With the critical data, there were only four significant correlations to the four metrics of # EPT, % EPT- % Hypropsychidae, % Scrapers, and HBI, with all being only to % urban (i.e., Rs = -0.67, -0.65, -0.63, and 0.69, respectively). The primary season had nine significant correlations within the eight metrics of Total Taxa, # EPT, % Scrapers, % Clingers, % Isopoda, % Tolerant Organisms, % Intolerant Organisms, and HBI. The additional metrics of % Clingers and % Tolerant Organisms were present with the primary data but not present with the combined data; however, the primary data did not show % EPT- % Hypropsychidae performing as it did in the combined data.

Table 6. Correlations among macroinvertebrate IBI metrics from different seasons with watershed variables using A) combined seasonal data, B) critical season data, and C) primary season data. The significant Spearman correlation ($p \leq 0.05$ with FDR correction) coefficients are bolded. The macroinvertebrate metrics are 1. Total Taxa, 2. # EPT, 3. % EPT- % Hypropsychidae, 4. % Scrapers, 5. % Clingers, 6. % Diptera, 7. % Chironomidae, 8. % Isopoda, 9. % Tolerant Organisms, 10. % Intolerant Organisms, and 11. HBI.

A)

Watershed Variable	1	2	3	4	5	6	7	8	9	10	11
% Urban	**-0.59**	**-0.62**	**-0.54**	**-0.61**	-0.34	0.35	0.35	**0.59**	0.34	**-0.60**	**0.66**
% Pasture	0.24	0.26	0.33	**0.39**	0.27	-0.07	-0.08	**-0.52**	-0.34	0.24	-0.30
% Forest	0.20	**0.48**	0.37	0.29	0.15	-0.06	-0.05	-0.23	-0.21	0.27	-0.28

B)

Watershed Variable	1	2	3	4	5	6	7	8	9	10	11
% Urban	-0.54	**-0.67**	**-0.65**	**-0.63**	-0.26	0.50	0.50	0.56	0.20	-0.56	**0.69**
% Pasture	0.17	0.30	0.35	0.47	0.36	-0.18	-0.14	-0.40	-0.26	0.21	-0.35
% Forest	0.25	0.52	0.41	0.40	0.05	-0.10	-0.11	-0.29	0.03	0.25	-0.21

C)

Watershed Variable	1	2	3	4	5	6	7	8	9	10	11
% Urban	**-0.65**	**-0.62**	-0.52	**-0.62**	**-0.57**	0.42	0.38	**0.64**	0.49	**-0.64**	**0.75**
% Pasture	0.31	0.27	0.40	0.37	0.32	-0.01	-0.05	**-0.62**	-0.46	0.28	-0.38
% Forest	0.16	0.44	0.36	0.20	0.23	-0.14	-0.06	-0.22	**-0.62**	0.37	-0.35

Unlike with the fish metrics, every macroinvertebrate metric was significantly correlated to at least one variable category in one of the datasets. Some metrics did, however, have few anthropogenic variable correlations and could be revised. Percent Diptera and % Chironomidae had significant correlations only to the geomorphology habitat variable of % bedrock, based on the combined and primary data. In addition, % Intolerant Organisms significantly correlated only to % forest using the primary dataset (Rs = -0.62); however, it was the only macroinvertebrate metric to ever correlate with % forest, and the metric may be a major contributor to the invertebrate IBI's % forest correlation with the combined data. In addition, % EPT- % Hypropsychidae significantly correlated only to % urban with the combined and critical datasets (Rs = -0.54 and -0.65, respectively). The seven other metrics significantly correlated with both the habitat and watershed variables in at least one dataset, signifying that over half of the metrics performed well towards the IBI for these forms of degradation.

Even though nutrient correlations were not present within the individual metric analysis, the macroinvertebrate metrics' contributions to the macroinvertebrate IBI for the region allowed for degradation detection in all variable categories by the IBI. The macroinvertebrate metrics appeared to perform better overall than the fish metrics. This performance was anticipated due to the use of the data from our study to create the macroinvertebrate IBI. Furthermore, it should be noted that with more data, as mentioned previously, and with more study sites, this study's statistics for detecting nutrient, habitat, and watershed variable correlations to IBIs and metrics would have increased in strength. The individual seasons had dissimilar performances for the invertebrates, which might have been due to the short lifespan affecting sensitivity, especially during the primary season, which appeared to perform comparably to the combined dataset.

Site Comparison Analyses

Due to the finding of the combined macroinvertebrate IBI data for this study having the most significant correlations, best performing metrics, and most statistical reliability due to sample size, the individual sites were first compared for effects along the streams using this assemblage's dataset (see Figure 1 for site map). According to the Tukey-Kramer Test, CSREF had the highest macroinvertebrate IBI mean score (49.5) and was significantly different from all other sites. LOREF had a high IBI score (43.5) and was not significantly different from OSG1 (IBI of 43) and OSG5 (IBI of 43.5); therefore, by this analysis, these sites were at reference condition. After the significant IBI decrease from OSG1 to the effluent of the Roger's WWTP at OSG2 (IBI of 30.5), the means of the IBIs steadily increased back to reference conditions at OSG5, indicating that Osage Creek returned to reference conditions quickly after the WWTPs; thus, the WWTPs did not affect the stream conditions into the Illinois River in Oklahoma. Spring Creek showed that SPG1 was already severely degraded (IBI of 23) and significantly different from all other sites. At SPG2, with the effluent of Springdale's WWTP, there was a significant increase in the IBI; however, this result still signified strong degradation as indicated by an IBI of 27, which showed 49% health. By SPG3, the IBI of 37 indicated a significantly healthier condition; thus, the confluence with Osage Creek by OSG4 (IBI of 37) did not negatively affect the IBI.

To test whether one season could distinguish between sites in a way comparable to the combined data's distinctions, the Tukey-Kramer Test was performed for each season. The critical season data illustrated no significant differences among sites, while the primary season had the same significant differences as the combined test, with two additional ones along the streams. They included a significant decrease from SPG3 (IBI of 33) to OSG4 (IBI of 35) and a significant increase from OSG3 (IBI of 33) to OSG4 (IBI of 35). The primary season data results did not conflict with the combined data results (Figure 3).

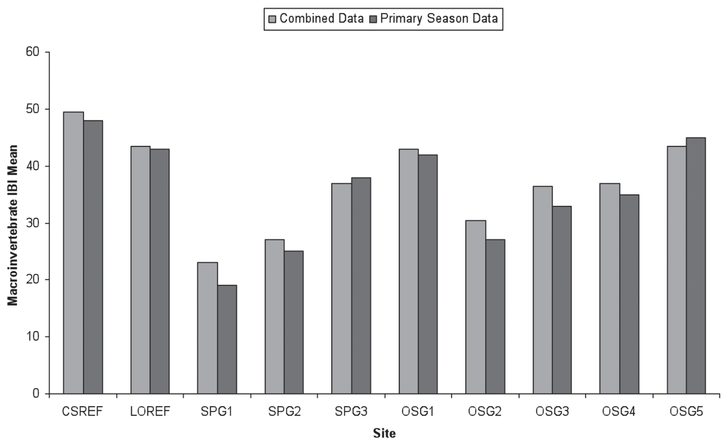

Figure 3. Macroinvertebrate IBI means of the combined seasonal and primary season data for each site.

Investigation of sites using the fish IBI did not show as strong a differentiation among sites as did the macroinvertebrate IBI. With the combined data, the only significant difference along the streams was from SPG1 to SPG3, with increases in means from 15.5 to 23 to 34, and the reference sites were significantly higher than all study sites (CSREF IBI of 38; LOREF IBI of 35). Site comparisons with the individual seasons for fish showed inconsistent results (Figure 4). The current fish IBI seemed to miss many sources of degradation, while the macroinvertebrate IBI was more sensitive to anthropogenic influences. However, revisions to the fish IBI are important, so both fish and macroinvertebrate assemblages can be efficiently used for bioassessment, particularly since together the assemblages complement each other, each having areas of stronger response to stressors (Weigel and Robertson 2007).

Conclusion

For this study on the Illinois River Basin, it appears that even though the fish and macroinvertebrate IBIs correlated strongly with each other and both seemed competent in detecting degradation, the current macroinvertebrate IBI better detected types of degradation, especially when it came to degradation caused by nutrient variables. The individual macroinvertebrate metrics outperformed the fish metrics in their correlations with habitat and watershed variables. For the macroinvertebrate IBI, the suggested metric revisions for the region include less than half its metrics. Due to their lowered number of significant correlations, revisions to % Diptera and % Chironomidae, and possibly % Intolerant Organisms and % EPT- % Hypropsychidae,

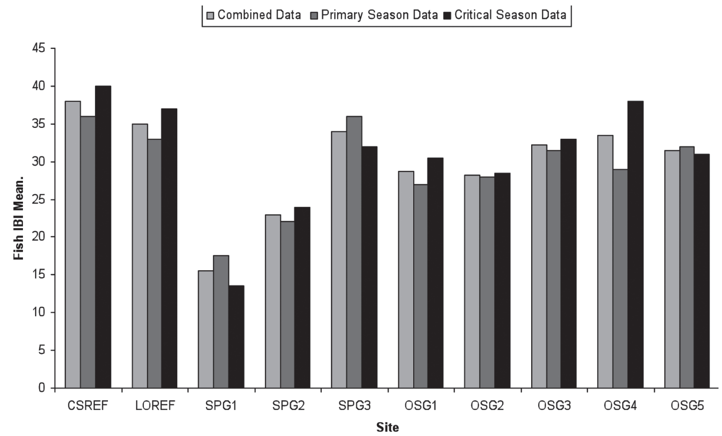

Figure 4. Fish IBI means of the combined, primary season, and critical season data for each site.

could further strengthen the IBI. For the fish IBI, over half of the metrics are in need of revision, either by replacement or slight modifications. These metrics are % Cyprinidae, % Percidae, Diversity, Total Species, and % Centrarchidae.

This study indicated that the most comprehensive bioassessment would use the combined seasonal dataset. However, with the IBIs' variable correlations, the primary season outperformed the critical season, and the macroinvertebrate metrics went on to show this same trend. In contrast, a deeper look into the fish metrics showed the critical season slightly outperforming the primary season. Even so, site comparisons with the macroinvertebrates' combined and primary season datasets were complimentary in indicating that the WWTPs did not affect water quality into Oklahoma. Therefore, for this study, it appeared that the use of only the primary seasons for macroinvertebrate collection was sufficient in bioassessment, particularly for indicating the effects of the WWTPs. For a more thorough bioassessment including all of the possible anthropogenic influences on each site and watershed, the use of the combined data would provide more statistical reliability and a wider range of indications through the distinctive contributions of both seasons. However, the sole use of the primary seasons could be efficient for other studies for a quick bioassessment to determine effects among sites without the need for thorough individual site investigation. More investigation into the use of only the primary seasons for fish and invertebrate bioassessments should be performed in this region and beyond to see if a more cost-efficient and effort-efficient method is possible. Apparently, the use of critical season data is a holdover from times when evaluations were based on only chemical (nutrient, oxygen, etc.) and physical (e.g., temperature) data. Fish and most invertebrate species must endure environmental conditions all year.

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Mentor Comments: When Rebekah originally submitted her manuscript for consideration for the Undergraduate Research Award, Professor Arthur Brown described her tenacity and commitment to working with him on this project. In the following, he provides additional information about her work and its contribution.

Rebekah Hotz tested the performance of the methods being used to evaluate water quality up and downstream of the wastewater treatment plants of Rogers and Springdale, and their potential impact on the Illinois River's water quality as it flows into Oklahoma. The U S Environmental Protection Agency (EPA) and Arkansas Department of Environmental Quality (ADEQ) have been involved in developing methods for biological assessment of stream water quality for nearly 30 years but refinement of the methods is a continuing process and of considerable importance. Water quality of the Illinois River has been the subject of much controversy between Arkansas and Oklahoma for decades.

Drs. Marty Matlock and Brian Haggard, from BAEG, and I comprehensively studied the physical, chemical, and biological aspects the Osage Creek and Spring Creek sub-basins of the Illinois River during 2007-2009 to determine whether the Rogers and Springdale wastewater effluents were degrading water quality in the streams, and, if so, whether the water remained impaired as it left the basins and headed for Oklahoma. We used methods prescribed by EPA and ADEQ that have legal standing. I was curious about just how robust those methods were and encouraged Rebekah to test the biological components of them. She chose four objectives for the study described in her thesis. Of those, the first three were items for which she had primary responsibility. She was less responsible for assessing impacts of the wastewater treatment plants. Additionally, Rebekah assisted with seasonal collections of data and fish. Her focus was on performance of the biological assessment tools we were using. She received some assistance from other members of the team, including Eric Cummins (a technician in BAEG that worked with us as a project leader and data analyst), and a statistician. Sharing of expertise and duties is the primary reason to have a team of collaborative investigators. The EPA, ADEQ and other states' agencies are very interested in our study, mostly because of the interstate socio-political conflict associated with it, but also because we have more actual data. Rebekah became an important member of our team by refining and evaluating the methods we used. She undertook and admirably completed a difficult analysis of the biological assessment methods in use in this ecoregion. This required careful (and difficult) statistical analyses of a large amount of data. She was not familiar with the types of data used, collection methods, the indices being used, or the kinds of statistical analyses necessary to evaluate their performance when she began. She learned all of this with minimal guidance and assistance. I provided some literature citations for her initially then she began to give me important papers to read. This is something that I expect of graduate students, but not of honors undergraduate students.