Hindawi Publishing Corporation
Journal of Ecosystems
Volume 2014, Article ID 893795, 15 pages
http://dx.doi.org/10.1155/2014/893795

Research Article

Associated Use Attainment Response between Multiple Aquatic Assemblage Indicators for Evaluating Catchment, Habitat, Water Quality, and Contaminants

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Received 30 May 2014; Revised 17 August 2014; Accepted 6 September 2014; Published 15 October 2014

Academic Editor: Wen-Cheng Liu

Use attainability analysis (UAA) at a watershed scale typically relies on the assumption that indicator organisms are responding similarly to the same environmental stressor. Factors explaining variance in fish, crayfish, and macroinvertebrate assemblage structure and function were investigated with emphasis on catchment and reach scale land use, habitat, contaminants, and water quality variables. Habitat quality scores ranged from 25 to 85 (average 61.36 ± 10.08). The substrate score, instream cover, riffle-run score, and channel score were primary factors contributing to declining habitat quality. Factor analysis found that four factors explained 69% of the contributed variance in fish assemblage, two factors accounted for 56% of variance in macroinvertebrate assemblages, and two factors explained 49% of the variance in crayfish assemblages. Overall drivers of assemblage structure were associated with broad scale issues of wastewater treatment, groundwater, and land use. Our results show that fish, macroinvertebrate, and crayfish assemblages respond to similar broad scale stimulus; however, the specific constituents responsible for the stress may vary with the magnitude of the cumulative stress, which may be expressed by each organismal group differently. Our data suggest that varying organismal groups can respond independently and stress reflected in one assemblage may not necessarily be observed in another since each organismal group is measuring different aspects of the environment.

1. Introduction

Regional scale use attainability analysis (UAA) is a structured scientific assessment of the factors affecting the fulfillment of the fishable/swimmable goals of the United States Clean Water Act (Section 101(a)(2)). Watershed use attainment is determined by evaluating multiple indicators occurring within the same reach and exposed to the same stressors. The assumption is that various trophic level biological indicators will respond similarly to stressors [1, 2]. Many state and federal agencies assume that biological assemblages are measuring the same features of the environment [3, 4]. Oftentimes, the response between various indicators is divergent or contradictory, which has been attributed to differing trophic level impact to chemical, land use, and habitat characteristics [2]. When agreement between indicators is not achieved, the UAA created an elaborate interpretation to describe disagreement between biological assemblage indicators. This has resulted in statements of full support, partial support, indeterminate, or not meeting aquatic life designated uses.

The factors considered for determining when UAA does not meet beneficial use include the physical, chemical, biological, and economic use criteria in EPA's water quality standards regulation (40 CFR 131.10(g)(1)-(6)). Under Section 40 CFR 131.10(g), demonstration of nonattainment of designated uses can be attributed to five conditions that prevent stream reaches from not meeting aquatic life uses. Nonattainment can be because of naturally occurring pollutant concentrations that prevent the attainment of the use. These causes can be a result of natural, ephemeral, intermittent, or low flow conditions or water levels that prevent the use attainment. Another reason could be human caused conditions or sources of pollution that prevent use attainment and cannot be remedied or would cause more environmental damage to

correct the condition than to leave in place. Dams, diversions, or other types of hydrologic modifications preclude the attainment of the use, and it is not feasible to restore the water body to its original condition or to operate the hydrologic modification in a way that would result in the use attainment. Physical conditions related to the natural features of the water body, such as the lack of a proper substrate, cover, flow, depth, pools, and riffles, are unrelated to water quality and preclude attainment of aquatic life protection uses. A final control would include more stringent conditions than those required by Sections 301(b) and 306 of the act that would result in substantial and widespread economic and social impact.

Previously, assemblage response to stressors did not include contaminant or water quality models that evaluated synergistic effects or sporadic spikes, which can adversely affect biological assemblage structure [3, 4]. These factors potentially result in a biologic impairment without the occurrence of specific chemical criteria violations. Suter et al. [3] outlined a formal strategy for causal development that begins with the review of existing data, as well as consultation with stakeholders, to develop a list of candidate causes. Morris et al. [4] determined that resource and regional data availability was usually inadequate to account for contributed causes to impaired biologic communities. Morris et al. [4] identified problems with the development of source identifications for impaired biological communities (IBC). These stressors were not always identifiable and were often overlooked when dealing with observational or qualitative data [3, 5].

The need to identify causality for IBC and identify stressor response required evaluation of decision-making processes for determining attainment [6]. The determination of when aquatic life uses were met varies at the individual state level based on a wide variety of measures that only sometimes include biological assemblage data. EPA recommends that two organismal indicator groups be used to determine attainment. Difficulty in tracking whether organisms respond similarly to pervasive nonpoint source impacts, combined with the lack of predetermined signatory relationships with biological assemblage patterns, creates a more complex problem. Morris et al. [4] constructed signatory relationships using multivariate analysis based on definable relationships between aquatic assemblage structure and quantifiable environmental stressors. Simon and Morris [7] used this approach to identify stressor response between contaminants, land use, and crayfish assemblage structure in a mixed use watershed, while Morris and Simon [8] refined the stressor model to predict relative contribution of each stressor type to cumulative explained variance. Norton et al. [9] used multivariate and correlation analysis to demonstrate relationships between ambient chemical, physical, and biological data, while Yoder and Rankin [1], Yoder and DeShon [2], Simon [6], Norton et al. [9], Eagleson et al. [10], and Rive-Murray et al. [11] determined biological response signatures patterns and cause-and-effect relationships. These studies confirm that individual aquatic assemblages respond to different types of stress in ways that are consistent and distinctive. These biological response relationships could be used for future hypothesis testing; however, no studies comparing indicator patterns have previously been conducted using multiple assemblages associated with the same reach scale stressor exposure.

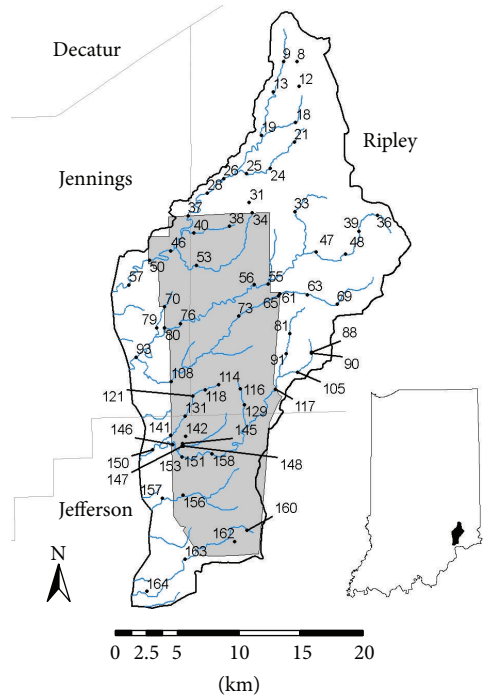

Figure 1: Map showing the distribution of sites sampled both on refuge and upstream during a stressor identification assessment of the Big Oaks National Wildlife Refuge in 2006 and 2007 (site numbers refer to Tables B and C). Black line indicates watershed boundary within the study area. Blue lines represent the lotic stream system, and the gray area is the boundaries of the Big Oaks National Wildlife Refuge.

This project evaluated three aquatic indicator groups and response to varying environmental conditions. Our research question is whether indicators should be expected to respond in a predictable manner across all stressor categories or whether each indicator will respond differentially to response drivers. This question provides the basis for decision-making criteria in UAA. Multiassemblage responses to varying catchment and reach scale land use, habitat, contaminants, and water quality were compared to evaluate patterns in indicator response. The responses of multiple assemblage populations were evaluated using a stressor identification model to determine ecosystem level drivers that provide causal relationships among stressors [4, 8].

2. Methods and Materials

2.1. Study Area. The Big Oaks National Wildlife Refuge encompasses 20,639 ha of the former Jefferson Proving Ground (JPG) (Figure 1). The former JPG was established in 1940 and was operated as a munitions testing facility until 1995. Portions of the study area were primarily used to test ammunition components, ordnance, and propellant ammunition weapons systems. The former military base has documented impairments from exploded ordnances and metal

contamination. Since 2000, the U.S. Fish and Wildlife Service have utilized the northern portion of JPG for ecosystem-based management in conjunction with continued use by the U.S. Department of Army and Indiana Air National Guard for air-to-ground training.

2.2. Study Design. A stratified, random design [12] was used to select 75 reaches in the Big Oaks National Wildlife Refuge based on the Strahler stream order. Each stream reach within the study area was assigned a numeric code to uniquely identify each reach. These reaches were equally weighted so that the fewer higher stream order reaches were equally weighted for selection with respect to the more numerous lower order reaches. A computer generated algorithm randomly selected 75 reaches without replacement from the universe of potential reaches. Reaches included in the study represented streams that drained locations within the refuge and those on the same streams upstream and downstream on private lands. All sites were visited during spring reconnaissance; those that were dry were excluded from further analysis as were sites that had water but had no aquatic life present. Remaining sites were analyzed for biological assemblage structure with the chemical, physical, and land use data following Simon and Morris [7]. Sample distance was 15 times the wetted stream width with the minimum distance 50 m and maximum distance 500 m [13]. Sample distance intervals increased in 50 m increments with increasing stream widths; for example, sample distance for wetted streams widths 0.1 to ≤3.3 m is 50 m linear distance, for widths >3.4 m to 6.6 m is 100 m linear distance, for widths >6.7 m to ≤10 m is 150 m linear distance, and for widths >10 m to ≤13.3 m is 200 m following increasing sequence. Sampling distances of 11 to 15 stream widths are generally adequate to sample a single habitat cycle [13].

2.3. Field Sampling. Fish and macroinvertebrate assemblages are recommended trophic levels for monitoring UAA by the EPA. We selected crayfish since they occupy both aquatic and the terrestrial riparian areas that interface wetland habitats [6–8]. Crayfish are important ecosystem engineers that structure the benthic assemblage structure within streams. Daytime, single-pass fish assemblage inventories were conducted using a Smith-Root 2.5 GPP 150-meter long-line electrofishing system [13]. The long-line system had the capacity of generating 500 v, 3 amps, and 2500 watts. Electrofishing surveys are the only preferred method for sampling the species richness, species composition, and relative abundance of fish assemblages in the Midwestern United States because of the high species richness of warm water streams and the associated habitat complexity. Sampling time was dependent on habitat complexity, but representative samples were collected within 300–1500 s. Fish identified in the field were vouchered for later taxonomic verification, while all other specimens were preserved in 10% formalin for laboratory identification using standard taxonomic references [14–16].

Crayfish sampling included the evaluation of primary, secondary, and tertiary burrowers following Simon [17]. Stream reaches were consistent with macroinvertebrate and fish collection areas and included a variety of survey techniques. Burrowing crayfish were collected by first attempting to coax individuals from the burrows by pouring water down the burrow and agitating the water. If the crayfish failed to emerge, a plunger was used to force the crayfish from the burrow. If that failed to dislodge the crayfish, a hand shovel was used to excavate the burrow and retrieve the individual. Secondary and tertiary burrowers were collected using a backpack electrofishing unit and by turning over large rocks in the stream. Many secondary burrowers are located beneath large stones. By flipping over these rocks, the crayfish can be easily collected by net or hand. All crayfish species collected from each stream reach were composited to estimate relative abundance based on a standardized catch-per-unit effort. Specimens were vouchered for taxonomic verification in 70% ethanol using standard references [18, 19].

Daytime macroinvertebrate assemblages were sampled using a "representative habitat sampling" procedure developed for streams in the Northern Lakes and Forest Ecoregion [20]. A long-handled net shaped like the letter D (D-net) was used to collect 20 efforts within site reach length boundaries and was composited and preserved in 95% ethanol for laboratory sorting. Efforts were established that, would reflect the abundance of predominant habitats. For example, habitats were segregated into rock, fines, overhanging vegetation, woody debris, coarse particulate material, and other categories. If rocky riffle habitat represented 50% of the habitat within the stream reach, then 10 of the 20 efforts would be collected in that particular habitat type. Each single effort was based on a 60-second sample using the D-net.

Samples were brought to the laboratory and placed into a 250 mm × 250 mm gridded sorting pan. A 300-organism count sort was done using a random number generator to reflect the appropriate square to be sorted [2]. Sorting was done until 300 organisms were picked; however, if a square contained the 300th individual, the remainder of that square was sorted until it was fully picked. At the completion of the 300-organism sort, a 15-minute large-rare pick was done. These specimens were identified and used in richness metric calculations but were not included in the trophic or relative abundance metrics. All individuals were identified to the lowest possible taxonomic levels following the state-of-the-art for that particular taxon [21–24].

2.4. Physical Habitat, Land Use, Water Quality, and Contaminant Sample Collection. The Qualitative Habitat Evaluation Index (QHEI) is a habitat assessment procedure developed by the Ohio Environmental Protection Agency to determine the quality of site habitat [25, 26]. The QHEI was used to evaluate habitat condition at each sample location and includes attributes of stream habitat that are typical of Midwestern North American warm water systems. These attributes are divided into a series of five categories and typically three or more subcategories within each category. For example, categories include substrate composition, stream cover habitat, riparian corridor quality, riffle and pool quality, and stream gradient, while subcategories of substrate composition include the variety of sediment particle sizes, embeddedness, siltation, and measures of sedimentation. Each category

has a series of scalar categories with corresponding scores that describe the range of quantitative or qualitative classes for each variable. The five categories each summed to a maximum of 20 points and the cumulative QHEI site score sums to 100 points. Scores above 66 are considered excellent, while scores above 45 are considered habitat meeting and capable of supporting aquatic life designated uses [25, 26]. Obvious point source impacts, that is, field tiles, wastewater treatment plants, or confined feeding operations, occurring along the stream reach are noted for further investigation in the event that site impairment was observed.

Land use was calculated using catchment scale land use obtained from Purdue University's Watershed Delineation Map Interface, which utilizes the United States Geological Surveys' 30-meter resolution National Land Cover Database based on 1992 Landsat imagery. This tool delineates the site-specific catchment and the number of acres associated with each land-use practice.

Grab water samples for laboratory analyses were collected in 1,000 mL certified contaminant-free sample bottles from the visual centroid of flow [4, 7, 8]. Sampling devices were cleaned and then rinsed with deionized water after each use and were placed in clean storage for transport between sites. Once water samples were taken and preservatives added (2 mL sulfuric acid (H_2SO_4) for nutrients and 5 mL nitric acid (HNO_3) for metals), the exteriors of all sample bottles were rinsed with deionized water and placed in ice filled coolers for transport to the State of Indiana Department of Environmental Management laboratory. Duplicate water samples, matrix spike (MS)/matrix spike duplicates (MSDs), and field blanks were collected at a rate of 1 for every 20 samples or 1 sample per week when less than 20 samples were taken. Test methods, reporting limits, and water quality criteria follow standard EPA methods for surface water analyses. Field parameter measurements were taken with a YSI multiparameter water-chemistry analysis unit. Parameters included pH, temperature (°C), specific conductance (micro-S), turbidity (NTU), and dissolved oxygen (ppm).

2.5. Statistical Data Analysis. The biological response gradient is based on the stressor identification methods outlined in Morris et al. [4] and further refined in Morris et al. [8]. Standardized (converted site data to a mean of "0" and standard deviation of "1") assemblage data were analyzed with cluster analysis using Euclidean Distance Similarity Matrix based on Ward's Method to create a two-dimensional dendrogram of the similarity matrix [27, 28].

A basic assumption of the Morris et al. [4] model is that biological assemblage structure is the result of external driving forces. These forces are identifiable and these groupings can be used to evaluate physical and chemical variables relative to the identified groupings [4, 7, 8]. It is expected that the interpretive power of the assemblage data increases as the cluster analysis segregates data into finer resolution groupings; however, at some point groupings cease to be driven by definable stressors and instead become a reflection of increased noise. We use the index of biotic integrity (IBI) scores for fish assemblage [29], which is a multimetric index of fish assemblage condition, and the Shannon Weiner (H') scores [30] for both crayfish and macroinvertebrate assemblages as our calibration tool to determine the point where noise drives the clustering patterns. Summary statistics were generated for each cluster grouping and used to rearrange clusters in order of increasing condition or species diversity, in ascending order, relative to mean IBI [29] or Shannon Weiner (H') score [30]. The lowest tier that elicited a clear increasing dose response that followed an increasing intuitive biological condition or species diversity response was selected for continued analysis [4, 7, 8].

Cluster models were evaluated at varying linkage levels using reach specific physical, chemical, and biological data [4, 7, 8]. Chemical concentrations below the detection limit of the analytical method were maintained in the analysis and given a value consistent with 1/2 of the detection limit. Clusters were treated as grouping variables and tested for significance using the Kruskal-Wallis ANOVA by Ranks test [28]. All variables demonstrating a significant relationship ($\alpha < 0.05$) with the biological condition gradient were retained as stressors explaining biological condition.

The stressor identification methodology [4, 7, 8] identifies a series of variables that collectively explain the variability in biological assemblage structure. Since the model identifies a degree of connectivity between each stressor and biological response, a factor analysis was used to determine each stressors relative contribution to the explanation of cumulative variance. Significant stressors were normalized, standardized, and evaluated for strong correlation using Pearson's correlation [28]. If the correlation coefficient exceeded an r of 0.80, then only one variable was chosen to represent the cumulative relationship [4, 7, 8]. Factor analysis was run using the Principal Components method and interpreted using a Varimax raw rotation.

3. Results

The species richness included 37 fish species (Supplemental Materials Table A, available online at http://dx.doi.org/10.1155/2014/893795), 165 macroinvertebrate taxa (Supplemental Materials Table B), and 7 crayfish species (Supplemental Materials Table C). Analysis of assemblage structure based on cluster analysis showed that each assemblage group explained variance in the data differently (i.e., Figure 2(a) fish, Figure 2(b) macroinvertebrates, and Figure 2(c) crayfish). Each assemblage structure was explained by increasing index of biotic integrity scores for fish or increasing Shannon Weiner species diversity (H') for macroinvertebrates and crayfish (Figure 3). Information content was optimized using the Wards method which focuses on maximizing groupings. These groupings were then arranged to highlight an increasing dose response relationship. Fish assemblage structure was explained with six clusters (Figure 3(a)); macroinvertebrate assemblage structure was explained with five clusters (Figure 3(b)), while crayfish assemblage structure was explained with three clusters (Figure 3(c)). These tier groupings are externally driven by the biological assemblage structure, which can be identified as physical and chemical stress on the respective assemblage [4].

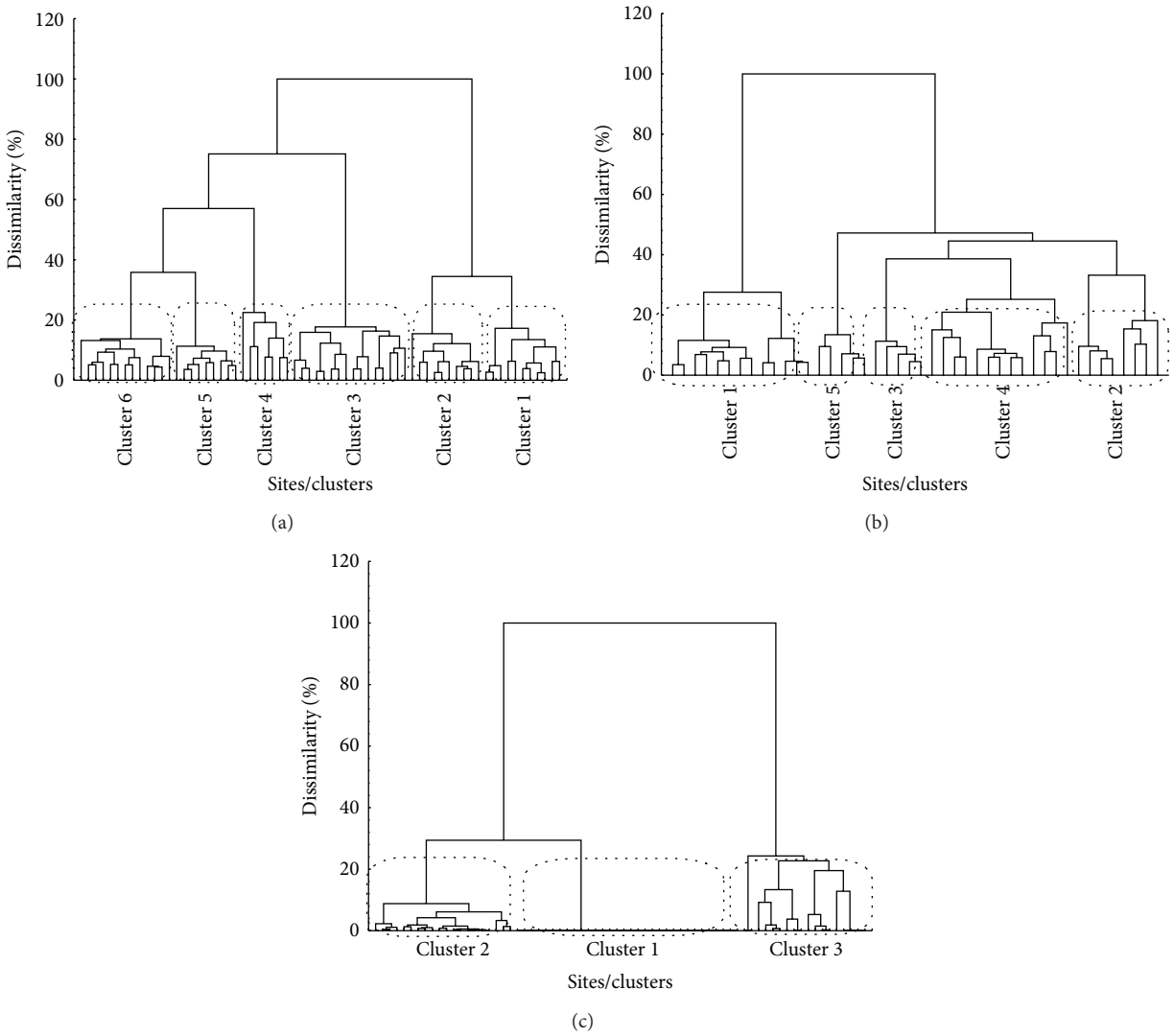

Figure 2: Dendrograms showing the relative similarity of assemblage data collected in the Big Oaks National Wildlife Refuge watersheds during August 2006. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA. (a) Fish, (b) macroinvertebrate, and (c) crayfish assemblages.

3.1. Physical Habitat, Water Quality, and Contaminant Response Patterns. Habitat analysis using the QHEI showed that scores ranged from 25 to 85 (average 61.36 ± 10.08). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life [25, 26]. Sites scoring less than 34 QHEI points are considered not meeting designated uses for aquatic life, while scores above 66 QHEI points are considered reference quality [26]. The substrate score, instream cover, riffle-run score, and channel score were the primary factors contributing to declining QHEI scores in the Big Oaks National Wildlife Refuge study area (Tables 1, 2, and 3).

We selected 75% similarity, determined through cluster analysis using Ward's method [4, 7, 8] as a benchmark for defining assemblage structure clustering (Figure 2). These clusters were used for stressor response measurement. Summary statistics by assemblage for water chemistry results are found in Tables 1–3. Project specific summary statistics and range of scale values for water quality results are presented in Supplemental Materials Table D. In general, the lowest biological integrity and species richness clusters had the highest concentrations of contaminants (Tables 1–3). Several contaminants, such as nitrogen and phosphorus, showed differential distribution with higher concentrations for fish. Barium, sulfate, and manganese showed the highest concentrations in clusters 2 for macroinvertebrates, and fluoride and lead showed the higher concentrations in cluster 3.

3.1.1. Water Quality and Contaminant Response. Kruskal-Wallis ANOVA by Ranks tests was significant showing that ten chemical measures were significantly predictive of the fish assemblage structure (Table 4). Predictive variables chloride, fluoride, sodium, total solids, nitrite + nitrate, and phosphorus demonstrated similar response patterns and are associated with both wastewater effluent and agricultural practice, suggesting an assemblage response signature to

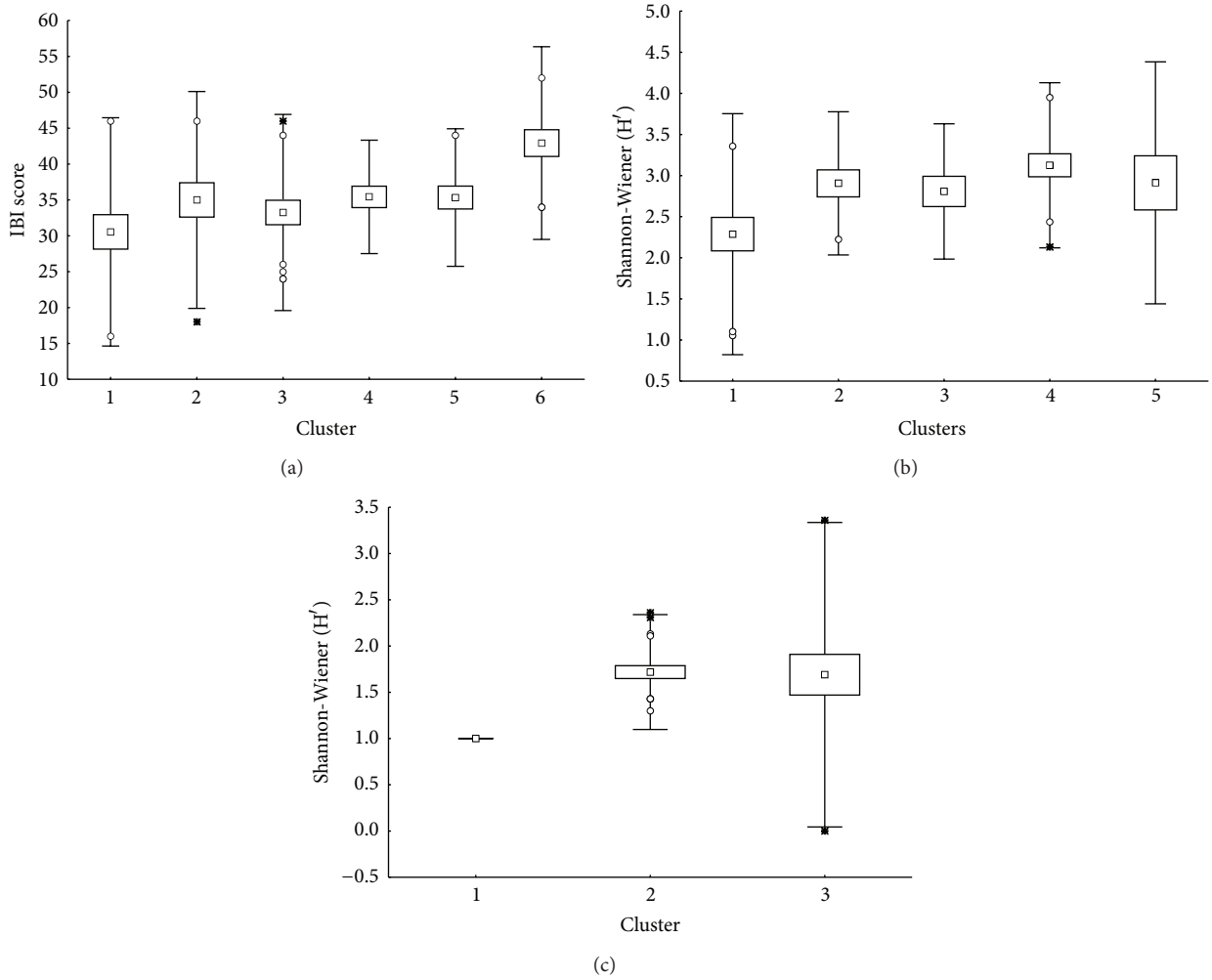

Figure 3: Box-and-whisker plots showing biological assemblage responses for Shannon Weiner (H′) for (a) fish (b) macroinvertebrates and (c) crayfish assemblages based on the biologic response gradient developed from the Big Oaks National Wildlife Refuge watersheds during August 2006.

these practices. Similarly, fish assemblage structure was significantly predicted by the presence of arsenic, calcium, hardness, and conductivity which are common groundwater constituents suggesting a significant ground/surface water interaction response signature as well (Table 4).

Macroinvertebrate assemblages responded to fewer chemical stressors than fish but exhibit similar response patterns. Macroinvertebrate assemblages were significantly explained by both groundwater and wastewater effluent signatures. Common waste water constituents, that is, fluoride, lead, and sulfate, were predictive of macroinvertebrate structure as were groundwater constituents of pH, manganese, and barium. While both fish and macroinvertebrates seemed to react to wastewater and groundwater stimulus, macroinvertebrates responded to only half as many wastewater components suggesting that fish are more responsive to wastewater disturbance (Tables 4 and 5).

In contrast to both fish and macroinvertebrates, crayfish assemblages did not exhibit any relationship with groundwater stimulus; rather, crayfish responded solely to wastewater and agriculture stimulus demonstrating a nutrient dominated response signature. Crayfish assemblages were significantly predicted by nitrogen, phosphorus, chloride, and total solids, all known constituents of wastewater effluent or treatment (Table 4). Crayfish reached higher relative abundance in areas with increased nutrients (i.e., nitrogen and phosphorus), while the highest and the lowest species richness were seen in areas with the lowest nutrient concentrations.

3.1.2. Habitat Response. Fish assemblage structure responded to the most habitat parameters (7) of the three organism groups followed by macroinvertebrates (4) habitat parameters and crayfish (2) habitat attributes (Table 4). Fish assemblage structure showed a significant response to QHEI score, substrate score, instream cover, riparian score, pool-glide score, riffle-run score, and gradient (Table 4).

Macroinvertebrates also responded to QHEI score, riffle-run score, and gradient, but they also responded to channel score (Table 4). These habitat attributes are probably auto-correlated since channel modifications affect the presence of riffles, which is mediated by gradient and affects the total

Table 1: Summary statistics for 22 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Figure 2(a)) in watersheds of the Big Oaks National Wildlife Refuge.

Variable	*N*	Mean	Min	Max	SD
Cluster 1	—	—	—	—	—
Arsenic	11	2.35	0.2	8.15	2.64
Calcium	11	104.45	75	145	21.95
Chloride	11	12.51	0.05	44	13.37
Fluoride	11	0.14	0.05	0.3	0.08
Hardness	11	153.45	118	215	31.27
Nitrogen	11	0.4	0.01	2.8	0.84
Total phosphorus	11	0.11	0.03	0.41	0.11
Sodium	11	6.98	2.27	20.1	4.87
Total solids	11	300.73	180	1010	240.92
Conductivity	11	493.73	21	2600	706.16
QHEI	11	55.77	25	69.5	11.23
QHEI—substrate score	11	11.41	1	15	3.77
QHEI—instream cover score	11	6.64	2	11	3.38
QHEI—riparian score	11	8.82	4	10	1.82
QHEI—pool/glide score	11	4.64	3	8	1.29
QHEI—riffle/run score	11	3.73	0	5	1.62
QHEI—gradient score	11	4.55	2	8	1.81
Water land use	11	1.88	0	14.62	4.38
Agricultural land use	11	26.84	0	53.07	22.26
Grass/pasture land use	11	27.01	0.13	59.75	22.72
Forest land use	11	39.91	0	96.47	36.5
Industrial land use	11	3.92	0	13.19	5.7
Cluster 2	—	—	—	—	—
Arsenic	10	0.47	0.2	1.73	0.58
Calcium	10	81.3	28	156	36.95
Chloride	10	3.85	0.05	12	5.1
Fluoride	10	0.09	0.05	0.1	0.02
Hardness	10	113.2	38	235	54.49
Nitrogen	10	0.08	0.01	0.5	0.16
Total phosphorus	10	0.05	0.03	0.13	0.04
Sodium	10	4.99	2.16	16.9	4.52
Total solids	10	163.6	90	300	55.65
Conductivity	10	237.09	87.2	477	105.49
QHEI	10	60.85	50.5	79	9.51
QHEI—substrate score	10	13.25	10.5	17	2.14
QHEI—instream cover score	10	6.5	2	12	3.44
QHEI—riparian score	10	9.85	8.5	10	0.47
QHEI—pool/glide score	10	5	4	7	1.15
QHEI—riffle/run score	10	4.4	2	7	1.51
QHEI—gradient score	10	5	4	10	2.16
Water land use	10	7.99	0.61	19.34	5.82
Agricultural land use	10	6.8	0.22	20.1	7.56
Grass/pasture land use	10	11.7	0.05	34.57	13.84
Forest land use	10	63.09	40.46	81.59	14.74
Industrial land use	10	9.01	0	17.96	6.12
Cluster 3	—	—	—	—	—
Arsenic	16	1.79	0.2	2.86	0.56
Calcium	16	119.56	85	154	22.47

Table 1: Continued.

Variable	*N*	Mean	Min	Max	SD
Chloride	16	30.63	12	124	26.98
Fluoride	16	0.18	0.1	0.5	0.11
Hardness	16	166.38	108	226	37.37
Nitrogen	16	2.42	0.01	24	5.96
Total phosphorus	16	0.24	0.04	1.82	0.44
Sodium	16	15.75	5.34	71.3	16.35
Total solids	16	279.5	192	575	97
Conductivity	16	438.25	313	812	128.76
QHEI	16	57.78	38	67.5	7.91
QHEI—substrate score	16	13.53	11	17	1.94
QHEI—instream cover score	16	6.25	2	12	2.27
QHEI—riparian score	16	7.28	4	10	2.18
QHEI—pool/glide score	16	5.94	4	9	1.53
QHEI—riffle/run score	16	4.03	1	5.5	1.41
QHEI—gradient score	16	6	4	10	2.42
Water land use	16	1.04	0.17	5.67	1.27
Agricultural land use	16	41.83	7.88	54.27	12.18
Grass/pasture land use	16	37.71	25.43	57.81	10.19
Forest land use	16	17.88	9.35	44.6	8.23
Industrial land use	16	0.49	0	7.9	1.97
Cluster 4	—	—	—	—	—
Arsenic	7	1.86	1.34	3.67	0.81
Calcium	7	90	22	117	32.14
Chloride	7	7.17	0.05	20	9.2
Fluoride	7	0.06	0.05	0.1	0.02
Hardness	7	128.14	35	178	47.11
Nitrogen	7	0.04	0.01	0.2	0.07
Total phosphorus	7	0.07	0.03	0.1	0.02
Sodium	7	5.11	3.04	8.38	2.05
Total solids	7	195.57	88	264	59.26
Conductivity	7	266.84	72.9	370	99.88
QHEI	7	59	41.5	65.5	8.79
QHEI—substrate score	7	12.5	11	15	1.71
QHEI—instream cover score	7	10.57	7	15	2.94
QHEI—riparian score	7	9	3	10	2.65
QHEI—pool/glide score	7	6.43	5	7	0.98
QHEI—riffle/run score	7	2.57	0	5	1.9
QHEI—gradient score	7	3.43	2	4	0.98
Water land use	7	4.79	0.15	14.31	5.35
Agricultural land use	7	18.05	0	49.9	22.16
Grass/pasture land use	7	17.63	0	59.79	23.65
Forest land use	7	51.56	6.5	81.31	33.88
Industrial land use	7	7.45	0	22.43	8.49
Cluster 5	—	—	—	—	—
Arsenic	9	1.45	0.2	2.1	0.52
Calcium	9	111.78	77	178	27.79
Chloride	9	6.67	0.05	12	4.33
Fluoride	9	0.11	0.05	0.2	0.06
Hardness	9	141.33	106	245	43.77
Nitrogen	9	0.41	0.01	1.2	0.36

Table 1: Continued.

Variable	*N*	Mean	Min	Max	SD
Total phosphorus	9	0.1	0.03	0.16	0.05
Sodium	9	4.9	2.34	8.67	1.85
Total solids	9	204.56	155	291	41.22
Conductivity	9	266.08	57.7	431	100.42
QHEI	9	63.72	45	74.5	9.61
QHEI—substrate score	9	12.78	9	16	2.12
QHEI—instream cover score	9	7	5	10	1.87
QHEI—riparian score	9	9.89	9	10	0.33
QHEI—pool/glide score	9	6.67	5	9	1.32
QHEI—riffle/run score	9	4.67	0	7	2.22
QHEI—gradient score	9	6.22	2	10	2.91
Water land use	9	2.12	0.11	5.8	2.33
Agricultural land use	9	18.17	0	36.65	14.18
Grass/pasture land use	9	21.34	0	47.85	16.31
Forest land use	9	53.63	24.15	96.48	26.75
Industrial land use	9	3.99	0	17.65	5.82
Cluster 6	—	—	—	—	—
Arsenic	13	1.54	0.2	2.41	0.66
Calcium	13	116.69	63	180	37.86
Chloride	13	10.64	0.05	40	10.06
Fluoride	13	0.12	0.05	0.3	0.09
Hardness	13	150.85	88	220	48.12
Nitrogen	13	0.69	0.01	5	1.32
Total phosphorus	13	0.11	0.03	0.37	0.09
Sodium	13	6.48	2.85	22.8	5.18
Total solids	13	218.38	129	366	62
Conductivity	13	335.54	195	562	104.56
QHEI	13	70.54	54	85	7.39
QHEI—substrate score	13	14.85	12	18	1.65
QHEI—instream cover score	13	8.54	5	14	2.67
QHEI—riparian score	13	9.27	6	10	1.2
QHEI—pool/glide score	13	7	5	12	2.2
QHEI—riffle/run score	13	5.77	3	7	1.49
QHEI—gradient score	13	7.54	4	10	2.18
Water land use	13	1.88	0.37	6.25	2.03
Agricultural land use	13	24.31	1.13	50.97	14
Grass/pasture land use	13	27.24	5.86	53.14	16.76
Forest land use	13	41.99	17.11	83.95	20.59
Industrial land use	13	3.54	0	20.11	5.76

habitat quality score. While both fish and macroinvertebrate assemblages responded to habitat quality (QHEI), specific response signatures differed by habitat partition and component.

Of the three assemblages, crayfish responded to the fewest habitat variables, significantly responding to only riparian score and the pool-glide score (Table 4). Crayfish preference for slower moving pool/glide habitats in conjunction with the presence of an intact riparian corridor that potentially limits the growth of algae may explain this response. Additionally, lack of algal growth may also limit the invasion of rusty crayfish, which can numerically dominate a system excluding other native species.

3.1.3. Land Use Response. Fish assemblage structure showed the most significant relationship with land use (Table 4) showing a significant response to agriculture, grass-pasture, forest, industrial, and water land uses. Of the three organism groups studied, fish would probably have the greatest dependence on water permanence since desiccation would restrict migration and recolonization following drought conditions. Macroinvertebrate structure was significant for water land

Table 2: Summary statistics for eight chemistry (concentration/ppm), habitat, and land use variables by macroinvertebrate grouping cluster (see Figure 2(b)) in watersheds of the Big Oaks National Wildlife Refuge.

Variable	*N*	Mean	Min	Max	SD
Cluster 1	—	—	—	—	—
Barium	13	52.46	30.5	81.9	12.93
Fluoride	13	0.12	0.05	0.2	0.06
Lead	13	0.19	0.1	1.31	0.34
Manganese	13	124.52	39.7	275	71.42
Sulfate	13	13.99	9.1	23	4.07
pH	13	8.13	7.44	9.5	0.59
QHEI score	13	58.23	50.5	70.5	5.23
Water land use	13	3.53	0.06	14.62	5.01
Cluster 2	—	—	—	—	—
Barium	5	53.3	34.8	67	11.63
Fluoride	5	0.07	0.05	0.1	0.03
Lead	5	0.1	0.1	0.1	0
Manganese	5	67.12	31.7	130	41.7
Sulfate	5	15.6	12	20	3.29
pH	5	8.31	7.45	8.69	0.51
QHEI score	5	64.9	62.5	67.5	2.38
Water land use	5	2.99	0.41	6.25	2.79
Cluster 3	—	—	—	—	—
Barium	5	79.6	56.7	124	27.59
Fluoride	5	0.19	0.05	0.3	0.09
Lead	5	1.27	0.1	3.13	1.29
Manganese	5	672	52	2500	1038.09
Sulfate	5	9.28	7.2	11	1.74
pH	5	7.67	7.14	7.9	0.31
QHEI score	5	56.6	25	67	17.81
Water land use	5	0.23	0	0.5	0.24
Cluster 4	—	—	—	—	—
Barium	13	55.74	31.9	107	18.03
Fluoride	13	0.07	0.05	0.1	0.03
Lead	13	0.1	0.1	0.1	0
Manganese	13	104.31	25.7	265	63.09
Sulfate	13	11.69	5.2	18	3.98
pH	13	8.26	7.66	9.19	0.45
QHEI score	13	68.31	55	79	9.06
Water land use	13	2.84	0.48	5.8	2.07
Cluster 5	—	—	—	—	—
Barium	7	90.43	47.2	158	43.26
Fluoride	7	0.14	0.05	0.2	0.07
Lead	7	0.67	0.1	2.96	1.1
Manganese	7	1352.21	99.5	6360	2322.74
Sulfate	7	22.8	6.9	45	14.67
pH	7	7.68	7.21	8.43	0.39
QHEI score	7	51.93	38	64	9.75
Water land use	7	0.57	0.13	1.23	0.41

use (Table 4), while crayfish showed a significant response to commercial land use (Table 4).

Table 3: Summary statistics for seven chemistry (concentration/ppm), habitat, and land use variables by crayfish grouping cluster (see Figure 2(c)) in watersheds of the Big Oaks National Wildlife Refuge.

Variable	*N*	Mean	Min	Max	SD
Cluster 1	—	—	—	—	—
Chloride	32	9.9	0.05	44	11.14
Nitrogen	32	0.24	0.01	2.8	0.51
Total phosphorus	32	0.07	0.03	0.19	0.05
Total solids	32	202.94	90	301	47.7
QHEI—riparian score	32	9.3	4	10	1.49
QHEI—pool/glide score	32	5.53	3	9	1.46
Commercial land use	32	0.62	0	3.83	1.07
Cluster 2	—	—	—	—	—
Chloride	20	21.91	0.05	124	26.61
Nitrogen	20	2.18	0.01	24	5.41
Total phosphorus	20	0.23	0.03	1.82	0.4
Total solids	20	299.1	105	1010	197.87
QHEI—riparian score	20	8.08	3	10	2.27
QHEI—pool/glide score	20	5.65	4	8	1.27
Commercial land use	20	0.06	0	0.31	0.1
Cluster 3	—	—	—	—	—
Chloride	14	11.4	0.05	46	12.59
Nitrogen	14	0.4	0.01	1.2	0.39
Total phosphorus	14	0.1	0.05	0.16	0.04
Total solids	14	213.5	88	333	60.27
QHEI—riparian score	14	8.96	4	10	1.82
QHEI—pool/glide score	14	7.29	4	12	2.16
Commercial land use	14	1.14	0	7.78	2.4

Fish assemblage structure showed declining dose response patterns for water, agriculture, and industrial land uses (Table 4). Macroinvertebrate assemblage structure was most variable with water land use (Table 4), while crayfish assemblage structure showed the highest species richness in commercial land uses (Table 4).

3.2. Factor Analysis. Further assessment of patterns and relationships between contaminants identified as highly predictive and those significantly correlated with causal patterns in assemblage structure changes was completed using factor analysis. Factor analysis is a statistical method used to explain variability among observed variables in terms of fewer unobserved variables called factors [27, 28]. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used to reduce the set of variables in a dataset [4, 7, 8].

Fish assemblage structure was explained by four factors, accounting for 69% of the cumulative variance (Table 5). This is the proper notation for Factor analysis. Three variables representing groundwater (arsenic), wastewater (phosphorus), and agricultural influences (phosphorus and agriculture land use) explaining 26% of the cumulative variance. Factor 2 loaded significantly on two habitat measures, QHEI score and gradient score, and explained another 23% of the cumulative

Table 4: List of 30 physical and chemical variables significantly predictive ($\alpha = 0.05$) of the fish, macroinvertebrate, and crayfish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Big Oaks National Wildlife Refuge.

Variable	Fish *P*-value	Macroinvertebrate *P*-value	Crayfish *P*-value
General			
Water Temperature	0.0036		
Chemical			
Arsenic	0.0070		
Barium		0.0169	
Calcium	0.0422		
Chloride	0.0000		0.0347
Fluoride	0.0109	0.0140	
Lead		0.0103	
Manganese		0.0075	
Sulfate		0.0477	
Hardness	0.0524		
Nitrogen	0.0013		0.0383
Phosphorus	0.0195		0.0280
Sodium	0.0003		
pH		0.0455	
Total solids	0.0010		0.0332
Conductivity	0.0003		
Habitat			
QHEI score	0.0022	0.0055	
Substrate score	0.0228		
Instream cover Score	0.0354		
Channel score		0.0016	
Riparian score	0.0013		0.0511
Pool/glide score	0.0013		0.0172
Riffle/run score	0.0082	0.0023	
Gradient score	0.0038	0.0346	
Land use			
Water	0.0015	0.0130	
Agriculture	0.0003		
Grass/pasture	0.0140		
Forest	0.0003		
Commercial			0.0076
Industrial	0.0009		

variance. Factor 3 significantly loaded on two additional variables, calcium and conductivity, both of which are associated with ground-water influence, and explained an additional 11% of the cumulative variance. Factor 4 was positively loaded on one habitat variable, in-stream cover, and explained another 9% of the variation. Within our study area, fish assemblages appear to be reacting to a variety of influences expressing distinct response signatures to groundwater, agriculture, and habitat stimulus. Cumulatively, the groundwater and agriculture driven factors (factor 1 and 3) accounted for 53% of the explained variance with the habitat driven factors (factors 2 and 4) explaining the remaining 47%.

Macroinvertebrate assemblage structure was explained by two factors, accounting for 56% of the total variance (Table 5). The primary factor explained 38% of the variance based on a negative association between a wastewater signature (barium and lead) and a groundwater signature (manganese). The second factor was also negatively associated, but with only a single wastewater-driven variable (fluoride) explaining an additional 18% of the variance. Surprisingly, whereas 47% of the explained variance in fish assemblages could be attributed to habitat driven effect, no habitat variables were significant in explaining variation in macroinvertebrate assemblages. Within our study area macroinvertebrates appear to be most susceptible to both wastewater and groundwater stimulus.

Crayfish assemblage structure was explained by two factors that included an increasing relationship with wastewater and agricultural stimulus (chloride, nitrogen, total phosphorus, and total solids) explaining 49% of the cumulative variance (Table 5). The second factor was a positive relationship with habitat stimulus having the pool-glide score explaining an additional 17% of the variance. Of the three organismal groups, crayfish appear to exhibit the most distinct response signature having 49% of the cumulative variance being explained by a single factor dominated by a wastewater stimulus.

4. Discussion

The three biological assemblages elicited clear response patterns to a series of stressor variables. These physical/chemical patterns have been described in terms of broad scale factors such as wastewater treatment, agricultural, ground-water, habitat, and land use. While being intuitive, the direct association between specific source/cause still remains tenuous. However, the spatial interpretation of the significantly predictive variable by our hot spot analysis more clearly delineates these linkages.

We have defined both the wastewater and agriculture responses in terms of nutrient loads (nitrate + nitrite and phosphorus), sodium, chloride, sulfate, fluoride, total solids, and lead. Streams are often nutrient-limited, and addition of nutrients may alter stream community structure [31]. Anthropogenic disturbance of watersheds increases delivery of all forms of nitrogen from impervious surfaces [32–35], lawn-fertilizer runoff [36, 37], pet waste [38], construction sites [37], nutrients in precipitation [39], storm water runoff [39], leakage or overflow of wastewater sewers [40], and sewage effluent (e.g., [41]). Closer examining of the variance associated with these contaminants clearly defines contamination influences based on two patterns. (Table 4). The first, is defined by nitrate + nitrite, phosphorus, sodium, chloride sulfate, and fluoride and a second "hot spot" is defined by both total solids, lead, and to a lesser degree phosphorus and fluoride (Table 4). While all three assemblages responded to these indicator chemicals, crayfish response was the most

Table 5: Results of factor analysis and explained variance observed in fish assemblage biologic structure and 16 significant chemical, habitat, and land use variables at the Big Oaks National Wildlife Refuge. Bold values are considered significant.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Fish assemblage				
Arsenic	**0.783062**	−0.059175	−0.0345	0.31648
Total phosphorus	**0.851726**	−0.017708	0.112252	−0.1624
Agricultural land use	**0.836643**	0.074086	−0.01429	0.063424
QHEI score	−0.08335	**0.929388**	0.033943	0.286131
QHEI—gradient score	0.251651	**0.719733**	0.279072	−0.10598
Conductivity	−0.22656	−0.042178	**0.753321**	−0.04346
Calcium	0.246242	0.219824	**0.739182**	0.033972
QHEI—instream cover score	0.001361	0.146698	0.036967	**0.8519**
Cumulative explained variance	26%	49%	60%	69%
Macroinvertebrate assemblage				
Barium	**−0.80031**	0.002714		
Lead	**−0.74591**	−0.193511		
Manganese	**−0.91379**	0.028777		
Fluoride	−0.06312	**−0.80045**		
Cumulative explained variance	38%	56%		
Crayfish assemblage				
Chloride	**0.821893**	0.220021		
Nitrogen	**0.769671**	0.270388		
Total phosphorus	**0.811131**	0.198719		
Total solids	**0.818145**	−0.221031		
QHEI—pool glide score	0.119627	**0.909592**		
Cumulative explained variance	49%	66%		

distinct having 49% of its variability (Table 5) being explained by factors directly associated with nutrient signatures.

Environmental variables among multiple spatial scales control physicochemical and biological processes in streams [42, 43]. Variables at regional or catchment scales can affect streams directly or indirectly by constraining other environmental variables at lower scales [44–46]. Understanding the relative influence of environmental variables at catchment and local spatial scales is an important step towards improved restoration, management, and assessment of aquatic resources [47, 48]. Patterns observed by groundwater constituents include chemical signatures such as arsenic, manganese, barium, calcium, hardness, conductivity, and pH (Table 5). These contaminants elicited a more spatially diffuse pattern typical of groundwater interaction within the study area. Interestingly, groundwater effect was mostly isolated to macroinvertebrates assemblages explaining 38% of the variation (Table 5).

Knowing which and how different spatial scale factors integratively affect stream communities also increases our ability to detect anthropogenic influences, identify biological response signatures to human-induced stress, and ultimately improve river health [49, 50]. Both habitat and land use factors demonstrated a degree of spatial variation but could not be interpreted in the same context as the chemical variables. Habitat factors, while being significant, lacked the variance demonstrated by most water chemistry constituents. Weigel et al. [51] found that macroinvertebrate assemblage structure was defined along an erosional and depositional gradient. Land-use parameters in the current study demonstrate a wide degree of variation but are expressed in our analysis as finite measure of land coverage.

Although it is evident that stream fishes are influenced by both local and regional factors, the most important spatial scale differs among studies. Reports from agriculture and urban-dominated watersheds indicate that land uses are the main factors influencing stream fish communities and that local scale physical habitat plays a much less important role [52, 53]. Studies from mixed forest-agriculture watersheds indicate that both watershed and reach scale factors are important (e.g., [45, 54]). However, there is only a limited understanding of the role of different spatial scales for relatively undisturbed watersheds [55]. Fish assemblage structure showed the most significant relationship with land use (Table 4). Fish showed a significant response to agriculture, grass-pasture, forest, industrial, and water land uses. Among all of the organismal groups studied, fish would probably have the greatest dependence on water permanence since desiccation would restrict migration and colonization following drought conditions. Macroinvertebrate structure was significant for water land use, while crayfish showed a significant response to commercial land use (Table 4). Significant habitat and land use variables were the product of a dichotomy of habitat qualities ranging from the nearly natural, undisturbed habitat on the refuge compared to the disturbed agricultural land use outside of the refuge. The variety of land uses that were significant to fish assemblage structure were a result of the differences observed between on refuge compared to off refuge land uses (Table 5).

5. Conclusions

Field and toxicology studies often use multiple biological indicators, including fish and macroinvertebrates, to evaluate toxic responses from contaminants. The field linkage of these response patterns has often been less than desirable, since usually these studies are conducted in extremely disturbed environments where either an all or none effect is observed. It has long been recognized that using multiple assemblage indicators in an environmental assessment of causal effects will enable interpretation at multiple trophic levels. Differences caused from toxic effects are common but have not been satisfactorily explained. In addition, it has been almost 40 years since any interpretation of multiple assemblage information has been analyzed for patterns in field assessments. Our results show that fish, macroinvertebrate, and crayfish assemblages respond to similar broad scale stimulus; however, the specific physical/chemical constituents responsible for the stress may vary and the realized magnitude of the overall stress on the system may be expressed differentially by each organismal group. Our data suggest that varying organismal groups can respond independently and stress reflected in one assemblage may not necessarily be observed in another. Assessment of aquatic life beneficial uses should not assume that indicator assemblages will respond similarly; thus, full-, partial-, indeterminate- and nonattainment should not assume that segment scales should be similar.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study was funded by the U.S. Fish and Wildlife Service and although this study may have been funded wholly or in part by that agency, no endorsement should be implied. Special thanks are due to S. Sobat, P. McMurray, and M. McShane for professional courtesies and field support.

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