Biological Contaminants Program & Division of Ecological Services
Bloomington Field Office
Submitted June 2008

Preliminary Diagnosis of Contaminant Patterns in Streams and Rivers of National Wildlife Refuges in Indiana

Fish and Wildlife Service
U.S. Department of the Interior

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DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
REGION # 3

FY06-08 ENVIRONMENTAL CONTAMINANTS PROGRAM
ON-REFUGE INVESTIGATIONS SUB-ACTIVITY

Preliminary Diagnosis of Contaminant Patterns in Streams and Rivers of National Wildlife Refuges in Indiana

U.S. Fish and Wildlife Service
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June 1, 2008

Congressional District # 1, 2, 8, 9

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Project ID: 1261-3N34

by

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Preliminary Diagnosis of Contaminant Patterns in Streams and Rivers of National Wildlife Refuges in Indiana

I. INTRODUCTION

Aquatic assemblages have been used as “canaries in the coal mine” to describe specific patterns that aquatic impacts have caused. These impacts have “signature” effects that change specific attributes of resident members of the aquatic communities (Simon 2003; Karr and Yoder 2004). Fish, benthic macroinvertebrate, crayfish, and mussels have been shown to have varying sensitivity levels that when exposed to different levels and types of contaminants will cause declines in species richness, sensitive species, trophic and reproductive function, abundance, and presence of deformities, eroded fins, lesions, and tumors (DELT) anomalies. These principles have been used by Karr et al (1986) and modified for application in Indiana (Simon 1991, 1992, 1994, 1997, 1998; Simon and Stahl 1998, 2001; Simon and Dufour 1998) to develop indices of biotic integrity (IBI). By evaluating patterns in IBI response, specific biological response signatures can be diagnosed that provide an inexpensive opportunity to evaluate patterns in contaminant and pollution effects (Simon 2003).

The goal of biological integrity, unlike fishable and swimmable goals, encompasses all factors affecting the ecosystem. Karr and Dudley (1981) 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.” That is, a site with high biological integrity will have had little or no influence from human society.

Limited investigations of contaminants in National Wildlife Refuges have been accomplished in Indiana, with the exception of CAP investigations. However, few on-the-ground assessments have been conducted that document the status of aquatic assemblages and how they might be affected by contaminants in these refuges. Documented levels of metals and nutrients have been documented in the vicinity of the National Wildlife Refuges; however, the extent and magnitude of the problem has not been well documented. Simon et al. (1995) conducted a study of the Patoka River National Wildlife Refuge (PNWR) to evaluate patterns caused by acid mine leachate on fish assemblages in areas severely impaired by acid mine drainage influences. Simon (2005) completed an evaluation of oil and brine effects on crayfish in the PNWR. This study found an important link between predictive presence of crayfish species and five inorganic and 23 organic contaminants that were specifically linked to oil and brine effluents including patterns in four metals specific to brine effluents. The Bloomington Field Office conducted a study of 10-12 streams sites in the Muscatatuck National Wildlife Refuge (MNWR) that provides some historical fish assemblage information. However, this lack of information has

caused refuge managers to try and manage and restore habitats without information necessary to avert contaminant problems, which would either jeopardize or diminish the benefits of these actions. Declines in aquatic biodiversity and biological integrity have been observed in the main stem of the Patoka and Muscatatuck Rivers and the tributaries of many of these rivers may no longer be supportive of sensitive rare species (Simon et al. 1995). Anthropogenic environmental stresses and habitat degradation associated with the decline include acid mine leaching, erosion and heavy metal toxicity due to strip mining, waste products related to oil and gas drilling, and agricultural cropland runoff. National Pollution Discharge and Elimination System (NPDES) permited discharges have also caused or influenced the biodiversity of these areas and one refuge occurs on portions of a historic military installation.

Watershed Description

The current study focused on metal contaminants and nutrient impacts in two watersheds that National Wildlife Refuges within Indiana occur. Sampling was conducted over two years in both the Vernon Fork of the Muscatatuck and the Patoka river drainages. National Wildlife Refuges (NWR) occurring in the Vernon Fork of the Muscatatuck River includes the Muscatatuck NWR and Big Oaks NWR, while a single refuge occurs in the Patoka River floodplain.

The Muscatatuck River watershed has a 1,140 mi^2 watershed having a wide range of biological habitats and environmental conditions. The Vernon Fork of the Muscatatuck watershed includes Sloan's crayfish, a species of special interest to Region 3, and has experienced intensive invasion threats from the rusty crayfish (*Orconectes rusticus*). Nutrients impacts are pervasive in the Vernon Fork watershed and the Big Oaks NWR incorporates portions of Jefferson Proving Ground, a former military base that has documented impairments from exploded ordnances and metal contamination. In addition, the Muscatatuck NWR receives runoff drainage through Sandy Branch from the City of Seymour, Indiana, and from high density residential land uses. Sobiech and Sparks (1997) found that metal levels in fish tissue levels exceeded fish consumption advisories. The State of Indiana had determined that several streams entering the Muscatatuck NWR are listed as not meeting designated uses for aquatic life.

The Patoka River watershed has a drainage basin of approximately 838 mi^2 and has a wide range of know conditions that are impairing NWR stream quality including acid mine drainage, oil and gas exploration, and impacts from coal mining (Simon et al., 1995). The area supports the largest known populations of the Indiana crayfish (*Orconectes indianensis*), a former Federal candidate species. Numerous stream segments are listed by the State of Indiana as not meeting aquatic life designed uses because of metal contaminants. Entire lengths of stream within the watershed including the South Fork of the Patoka River are listed because of acid mine drainage contamination.

Study Objectives, Hypotheses, and Threats to Trust Resources

Threats to Resource – Documented or Suspected

Many streams and rivers in Indiana are experiencing declines in the number of individuals and declines in species richness. Declines in fish assemblages are known from the mainstem of the Patoka River as a result of oil exploration and brine effluents (Simon et al. 1994). In addition, Simon (2005) documented impacts from oil and gas exploration from tributaries of the Patoka River in the vicinity of the PRNWR. These impairments showed statistically significant relationships between five metals and brine effluents (Simon 2005). Sparks and Sobiech (1997) found that nutrients, pesticides, military ordnance have impaired portions of the Vernon Fork of the Muscatatuck River. The State of Indiana has listed portions of both of these areas as not meeting aquatic life designated uses on the 303(d) list. The South Fork Patoka River (Patoka River NWR) and portions of Otter Creek (Big Oaks NWR), both which flow through the National Wildlife Refuges are listed as biologically impaired.

Our study concentrated on metals and nutrients, which have been identified by the State of Indiana Department of Environmental Management as the suspected contaminant sources for listing stream segments in the Patoka and Vernon Fork of the Muscatatuck rivers in the 303(d) list. Nutrient runoff is a known cause of lesions and increased levels can eliminate sensitive and rare species, while oil and brine effluents are acutely toxic to crayfish, fish, and mussels and are known to cause developmental deformities. Direct contamination pathways for metals include the surface water and groundwater discharge through permitted discharge effluents and from oil and brine drilling effluents, which cause residual surface water contamination and the accumulation of contaminants in the surface sediments (Simon 2005). Direct pathways for brine effluents are from oil drilling, the residual accumulation of contaminants into the sediments in the vicinity of oil derricks, and the visual staining and smothering of sediments and habitat by oil by-products. Acid mine leachate has affected the Patoka River streams through surface water and groundwater discharge from gob pile recharge areas and areas impaired by historic mining activities. In agricultural areas, direct pathways include surface water runoff from the application of fertilizers in tilled areas and the runoff of nutrient into adjacent streams. Nutrient impacts result from surface runoff from fertilizers, from wastewater treatment plants, and confined animal feedlots. The impact from nutrient impacts has eliminated sensitive species and caused acute toxicity, fish kills, and reduced dissolved oxygen.

There are numerous metal impacts as a result of over 225 oil derrick discharges and acid mine drainage gob piles to the Patoka River. Numerous agricultural drains enter the Muscatatuck River in the vicinity of Big Oaks and the Muscatatuck NWR's and suspected nutrient impacts are documented in the State of Indiana's 305(b) report to Congress. In addition, metal contaminants are known from residual contaminants from military operations at Big Oaks. Population of sensitive species has been decimated (Nature Serve, 2008, http://www.natureserve.org/explorer/servlet/NatureServe?sourceTemplate=tabular_report.wmt&loadTemplate=species_RptComprehensive.wmt&selectedReport=RptComprehensive.wmt&sum

maryView=tabular_report.wmt&elKey=112045&paging=home&save=true&startIndex=1&nextStartIndex=1&reset=false&offPageSelectedElKey=112045&offPageSelectedElType=species&offPageYesNo=true&post_processes=&radiobutton=radiobutton&selectedIndexes=112045), suggests that Sloan's crayfish is critically imperiled in Indiana. Jefferson Proving Grounds has unexploded ordnances and impairments in much of the Vernon Fork Muscatatuck River drainage near Big Oaks and additional impacts occur downstream to the Muscatatuck NWR. The extent and magnitude of the impacts between the refuges are largely unknown; however, levels of metals that are known to cause toxicity and deformities have been observed in the study area.

Populations of the Indiana crayfish are considered imperiled due to impacts associated with contaminants in the Patoka River drainage (Nature Serve, 2006,http://www.natureserve.org/explorer/servlet/NatureServe?sourceTemplate=tabular_report.wmt&loadTemplate=species_RptComprehensive.wmt&selectedReport=RptComprehensive.wmt&summaryView=tabular_report.wmt&elKey=118496&paging=home&save=true&startIndex=1&nextStartIndex=1&reset=false&offPageSelectedElKey=118496&offPageSelectedElType=species&offPageYesNo=true&post_processes=&radiobutton=radiobutton&selectedIndexes=118496). Specific point sources include the Black Beauty Mine, which surrounds the Patoka River NWR and additional threats include the routing of I-69 through the NWR, which may disassociate the refuge in half. Additional threats include the planned or pending construction of an ethanol plant and incinerator, which has been permitted to the northeast of the refuge.

Our understanding of rare species conservation status has benefited significantly from this study. Little was known of Sloan's crayfish and the impacts associated with agriculture and historic operations near the Vernon Fork Muscatatuck River and the two NWR's; however, St. John (1988) conducted an intensive survey of the species in Ohio and Indiana. St. John found that *O. sloanii* was present in the Muscatatuck River near the refuge, but *Orconectes rusticus* was also found to the east in adjacent watersheds. It is unknown what impact the invasion of the rusty crayfish has had on the species; however, the State of Ohio has listed Sloan's crayfish as imperiled, while Indiana has no formal status for the species. No historical data is available for the NWR properties since the State of Indiana has not sampled these refuges during their watershed monitoring surveys over the past two decades. Historic data is available for only the perimeters of the NWR's. Random sampling and limited number of revisits has all but precluded any collection of information from these sites, so the status of the refuge is virtually unknown.

Our need to further define aquatic species declines and threats related to point and non-point sources and determine whether current discharge permits are protective, if best management practices are efficient, and determine the extent of influence acide mine draine, oil and brine effluents, and agricultural nutrient runoff has had on rare or endangered species is paramount to the conservation of rare and imperiled species.

Determination of Impacts to Service Trust Resources

Our understanding of contaminant impacts on trust resources are based on the following hypotheses, which involve the biological response of assemblage structure and function and the specific signatures observed in multivariate cluster analysis of information.

1. Biological Organization: The project included ecosystem, community, and population organization levels. Surveys were conducted at the community level for fish, crayfish, and macroinvertebrate community responses to contaminants. The information collected from previous contaminant investigations, i.e., 1994 was used to direct field ecosystem survey evaluations of oil and brine point source impacts (Sobiech and Sparks 1994) and the 2001-2003 crayfish study (Simon 2005). These sites were included as targeted sites so that specific trend assessment of changes can be evaluated on the Patoka River NWR. The study by Sparks and Sobiech (1997) provides trend information for the Muscatatuck NWR, while the investigation by Litwin will be used to evaluate Big Oaks NWR. The data provides status, magnitude, and extent information that directly diagnose causal relationships between contaminants and aquatic biological assemblages. We differentiate between contaminant impacts and habitat disturbance based on the biological organization of structure and function. Specific water quality impacts can be differentiated from habitat loss. The identification of "hot-spots" using a pleth technique with spline smoothing will generate visual documentation of impacts (Morris *et al*. 2006).

2. Measurement of Contaminant Effects: Measurement of contaminant effects will be evaluated through direct measurement of adverse changes in species richness and diversity. Effects will be determined through changes in assemblage structure and presence of deformities, eroded fins, lesions, and tumors (DELT) anomalies.

3. Contaminant Source(s): Point sources in areas with impaired aquatic assemblages will be further investigated for management issues. Sources in the Patoka NWR sources include identified oil derricks, Black Beauty Coal Mine, NPDES point source discharges, and gob piles. The Muscatatuck NWR and Big Oak NWR sources include metal contamination from former military land use, nutrient runoff from fertilizers, feed lots, identified stream reaches from the State of Indiana 305(b) and 303(d) lists. Water quality analyses will be conducted using Standard Methods and samples were analyzed by the Indiana State Board of Health Laboratory. Contaminants causing structural and functional changes in species assemblages will be identified, including variables that contribute synergistically or additively, and hot-spots will be identified through multivariate data analysis.

4. Contaminant Pathway(s): The major contaminant pathways to trust resources in these systems are through point-source effluents, non-point source runoff, and diffuse point sources (i.e., animal feed lots). Direct pathways include the surface water and groundwater discharge of brine effluents from drilling, the residual accumulation of contaminants in the vicinity of oil derricks, and the visual staining and smothering of sediments and habitat by oil by-products. In addition, acid mine leachate through surface water and groundwater discharge from gob piles and areas impaired by historic mining activities. Also, nutrient impacts result in agricultural areas from surface runoff of fertilizers, acute toxicity from pesticides, herbicides, and fungicides. Crayfish and most fish species have a limited home range and contaminants that affect tributaries and mainstem locations will continue to have long lasting effects on benthic inhabitants, reproductive success, and trophic energy transfer between levels.

B. Scientific Objectives

This study included an intensive investigation of aquatic habitats within the National Wildlife Refuge system of Indiana. This study investigated all aquatic habitats and documented the condition of stream, pond, and lake habitats using a watershed perspective. Each refuge was studied based on a local and watershed scale so that site evaluation and diagnosis of contaminant issues could be evaluated. This information is valuable to refuge managers in diagnosing problems and providing information that will enable restoration of these habitats.

This study documented the presence of specific candidate conservation species and species of Regional importance to the U.S. Fish and Wildlife Service. Several crayfish species including Sloan's crayfish *Orconectes sloanii,* Indiana crayfish *Orconectes indianensis,* and Hoosier cave crayfish *Orconectes inermis testii* are known in the study area. In addition, baseline assessments for the Muscatatuck NWR, Big Oaks NWR, and Patoka NWR will enable a large scale depiction of imminent threats that might be on the threshold of influencing the refuge. The Muscatatuck NWR is conducting their Conservation Assessment Planning for the refuge. This study will provide baseline information for the aquatic fauna occurring in each refuge.

In addition, the Patoka National Wildlife Refuge will have additional information available to evaluate trends in water quality from surveys conducted in 1992 and 2000-2001 investigations. This information will be used to evaluate patterns in biological assemblage using cumulative frequency distributions of index of biotic integrity scores occurring between 1992 and 2007. Additional between year comparisons of biological data will be compared to qualitative habitat (Rankin 1995) and chemistry information (i.e., nutrients, organics, and inorganics) so that impacts predicted by the biological signatures can be determined from 2006-2007 surveys. Source identification studies will be conducted for each watershed using the method developed by Morris et al. (2006) to evaluate "hot-spots" or imminent threats of contaminants.

C. Management Action(s)

Refuge managers will be able to access necessary contaminant information that will enable them to evaluate, manage, and restore habitats on refuge lands. This information will document the impacts and imminent threats associated with land use practices around and within Refuge boundaries. The benefit of the biological integrity diagnostic evaluation is that sites will be scored and quantitatively assessed so that priorities can be developed from the results for restoration and remediation.

This information will enable refuge managers to:

1) Document contaminant impacts and evaluate previously unknown environmental problems on National Wildlife Refuges. The IBI is a powerful tool that will enable documentation of impacts from a variety of indicators. Fish, macroinvertebrate, and crayfish assemblages were used to assess the water body condition. Fish and macroinvertebrates are primary indicators and were correlated with patterns in water chemistry and habitat quality for local level interpretation of refuge contaminant status.

2) The collection of representative samples of biological assemblages would provide an opportunity to document the biodiversity of the aquatic biota of the National Wildlife Refuges. During the past 30 years, numbers of individuals and species diversity of native fish, crayfish, and macroinvertebrates have declined throughout the United States and Canada. The list of state threatened and endangered species continues to increase. This investigation will aid the Service in defining specific causes for the general decline in freshwater aquatic organisms near Indiana National Wildlife Refuges.

3) Differential sensitivities are exhibited by species, especially during different life stages. Abundant data is available for evaluating fish assemblages, while crayfish and macroinvertebrate species are not as well developed for interpreting watershed scale conditions. By establishing baseline local conditions for each Refuge and then increasing scale to watershed levels, patterns in waterbody condition can be determined. The current study provides information on various aquatic fauna of the National Wildlife Refuges and provides an important benchmark in time that will enable future investigators to understand changes in land use, atmospheric changes, and long-term effects that may not be currently understood (i.e., endocrine disruption, reproductive impacts, etc.).

4) The National Wildlife Refuges included in this pilot study is a national model for training contaminant personnel interested in being involved in Phase II aspects of this study. Results from this study should be used as a pilot to evaluate regional or national level patterns. By conducting a systematic evaluation of the National Wildlife Refuges,

we could provide important national scale information in areas that should be among the most important conservation areas remaining in the United States.

5) This information will be reported in the State of Indiana 305(b) report to Congress on the quality of the Nation's Surface Waters and appropriate sites will be included in the 303(d) Total Maximum Daily Load (TMDL) list of impaired waterbodies.

6) The Wildlife Refuges in this study are components of the Ohio River Valley Ecosystem (ORVE). The ORVE encompasses FWS Regions 3, 4, and 5, and State resource agencies. The ORVE Team has ranked 6 resource issues for priority focus in their draft ecosystem plan (USFWS 1994). The #1 resource priority addressed in the ecosystem plan is to reverse the decline of mollusks and biodiversity within the Ohio River Valley Ecosystem with emphasis on endangered, threatened, and candidate species and species of concern. The study area has several rare and former Federal candidate species, including fish and crayfish species of interest to Region 3. The current study provides significant status information for species on National Wildlife Refuges.

7) The invasion of our waters by species that are nonindigenous or exotic species has become a priority of the Service. This study will provide an assessment of rusty crayfish *Orconectes rusticus* and evaluate the impact of nonindigenous species on rare or former candidate species. Records for sloan's crayfish, Indiana crayfish, and Hoosier cave crayfish will be determined as well as bluebreast darter *Etheostoma camarum*, spotted darter *Etheostoma maculatum*, eastern sand darter *Ammocrypta pellucida* and other potential candidate fish species. The presence of exotic and non-indigenous aquatic species will be documented on the NWRs.

Direct Actions

- Supporting information from qualitative habitat assessments assist the manager of each refuge to make habitat management decisions that take into account the presence of rare sensitive species, including the Indiana crayfish, Hoosier cave crayfish, Sloan's crayfish, eastern sand darter, spotted darter, and bluebreast darter. This information provides specific habitat requirements for these species and enables refuge managers to provide specific habitat restoration options. For example, in the Patoka River NWR habitat information will enable the refuge manager to provide notice of violations to well operators. These violations can use habitat restoration options that can be designed to benefit the Indiana crayfish.

- Information gained from this project is essential to future development of an aquatic ecosystem management plan for the protection of all aquatic resources in the Patoka and Vernon Fork of the Muscatatuck rivers and tributaries and their associated Wildlife Refuges.

- This project addresses the Ohio River Valley Ecosystem Plan's #1 and #6 resource priority of defining threats to sensitive species residing in ORVE focus areas. Data collected from this study will aid the ecosystem team in focusing and prioritizing projects within the Ohio River Ecosystem.

Indirect Actions

- Sites that are sampled during this study were reported to the State of Indiana for inclusion in the 305(b) Report to Congress on the status of the nation's surface waters. Currently, none of the surface waters on the NWRs were reported by the state because of lack of information. Stream segments surrounding the NWR's are listed as biologically impaired in the State of Indiana 305(b) report to Congress and the 303(d) list.

- Chemical data will enable the actual measurement of chemical contaminants that are influencing aquatic assemblages. This information provides important datasets that enable listing of streams that are impaired under 303(d) TMDL. If a stream reach is impaired and listed as a 303(d) listed reach, the State of Indiana and the USEPA would be required to determine the level of impact and provide resources necessary for source identification, remediation, and delisting of the site.

II. METHODS

A. Data Collection and Analysis

OBJECTIVES/QUESTIONS/HYPOTHESES

Objectives

This project emphasizes an evaluation of biological integrity and aquatic biodiversity of NWR's using a targeted watershed approach in Indiana. The objectives of this project are to:

1) Determine the current fish, macroinvertebrate, and crayfish assemblage condition of each NWR's using a probability designed watershed approach based on a spatially intensive sampling design.

2) Determine which areas contaminants cause aquatic assemblage degradation and document magnitude and extent of problems as indicated by fish, macroinvertebrate, and crayfish assemblage structure and associated physical and chemical sampling.

Questions/Hypotheses

1) *What is the spatial extent of the aquatic assemblage impairment within each watershed near the NWRs*?

Fish, macroinvertebrate and crayfish community composition, structural and functional metrics, feeding groups, and spatial distributions were interpreted using the Index of Biotic Integrity developed for the appropriate Indiana ecoregion (Simon 1990; Simon and Dufour 1998; Simon, unpublished data). This method will provide a direct measure of impacts to biological assemblages and water quality and habitat data that identifies sources that are impairing biodiversity and biological integrity.

2) *What contaminant stressors are acting upon aquatic communities in the watersheds?*

Physical and chemical variables will be evaluated so that influences on aquatic community expectations can be determined. Aquatic community data will be evaluated using multivariate analysis to determine if specific physical and chemical variables are predictive/ correlative of the degraded biological integrity of the system. Hot spots will be identified that will show the magnitude and extent of the impact from the identified contaminant (Morris et al. 2006).

Assumptions

1) Aquatic community reference condition expectations have not changed.

2) Specific physical, chemical or land-use variables significantly influence aquatic communities in the targeted watersheds.

Site Selection

Due to the density and interspersion of bridge crossings, it is expected that these targeted locations will provide adequate spatial coverage and replication. Sample populations will consist of probability selected bridge crossings. Access points on NWR property will be coordinated with the appropriate Refuge Manager. Prior to final site selection, the watershed upstream of the Refuge and streams on the Refuge will be examined in its entirety as potential sample sites. The watershed then will be broken into a prioritized series of sample sites. The entire population of selected sample sites will be sampled for fish, macroinvertebrates, crayfish, habitat and multiple water quality stressors.

Field Sampling Methodology

Water chemistry procedures

Grab water samples for laboratory analyses were collected by hand from the visual centroid of flow using certified contaminant free sample bottles. Sampling devices were cleaned and then rinsed with de-ionized water after each use and placed in clean storage for transport between sites. Once water samples are taken and appropriate preservatives added the exteriors of all sample bottles were rinsed with de-ionized water and placed in ice filled coolers for transport to the Indiana State Board of Health 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 are taken (Bowren and Ghiasuddin 1999). The initial suite of water chemistry variables to be measured remained constant through out each successive sample year to maintain data integrity over time. Additional chemical variables may be added to individual sampling events depending on any unique characteristics within that target watershed. We met with partners to strategically discuss each watershed independently and to construct a list of chemical variables for evaluation (Tables 1 & 2). All

Table 1. List of general chemistries along with test methods and reporting limits.

Parameters	Test Methods	Test America Reporting Limits	Preservative	Holding Times
Alkalinity as $CaCO_3$	310.1	10 mg/l	None	14 Days
Hardness as $CaCO_3$	SM2340B	1.0 mg/l	HNO_3<pH 2 or H_2SO_4<pH 2	6 Month
Calcium as $CaCO_3$	200.7	200 µg/l		
Ammonia-N	350.1	0.1 mg/l	H_2SO_4 <pH 2	28 Days
Nitrate+Nitrite-N	353.2	0.1 mg/l	H_2SO_4 <pH 2	28 Days
Phosphorus, Total	365.2	0.03 mg/l	H_2SO_4 <pH 2	28 Days
COD	410.4	3.0 mg/l	H_2SO_4 <pH 2	28 Days
TOC	415.1	1.0 mg/l	H_2SO_4 <pH 2	28 Days
TS	160.3	1.0 mg/l	None	7 Days
TSS	160.2	4.0 mg/l	None	7 Days
Fluoride	340.2	0.1 mg/l	None	28 Days
Chloride	325.2	1.0 mg/l	None	28 Days
Sulfate	375.2	1 mg/l	None	28 Days
Cyanide, Total	335.4	0.005 mg/l	NaOH>pH 12	14 Days
Cyanide, Free	SM4500CN-I	0.005 mg/l	NaOH>pH 12	14 Days
Reactive Silica	SM4SOD-SiD6	6.0 mg/l	None	28 Days

chemistry will be evaluated for data quality objectives and exceedences of State of Indiana Water Quality Standards will be determined; as well as, synergistic and additive concentrations that cause biological impairment of the aquatic assemblages based on the multivariate analysis. Data collected from this studywill be used for a variety of purposes depending on the quality assurance/quality control level that the data is assigned. We targeted level 4 QA/QC (see data quality assessment section) so that the data will be relevant for enforcement, listing, and other multi-uses by the State of Indiana and the Service.

Field Parameters

Standard measurements of field parameters will be taken with a Hydrolab multi-parameter water-chemistry analysis unit. Parameters will include pH, temperature, specific conductance, turbidity and dissolved oxygen (Table 3).

Data Quality Assessments (DQAs)

Analytical data will be assessed for Quality Control (QC) and will be assigned to one or more Data Quality Assessment (DQA) Levels listed below:

Level 1 Screening data: Results are usually generated onsite and have no QC checks. Analytical results, which have no QC checks, no precision or accuracy information or no detection limit calculations are included in this category. Primarily, onsite data are used for pre-surveys and for preliminary rapid assessment.

Level 2 Field analysis data: Data are recorded in the field or laboratory on calibrated or standardized equipment. Field duplicates are measured on a regular basis. Calculations may be done in the field or later in the office. Analytical results, which have limited QC checks, are included in this category. Detection limits and ranges have been set for each analysis. The QC check information for field or laboratory results is useable for estimating precision, accuracy, and completeness for the project. Data from this category are used independently for rapid assessment and preliminary decisions.

Level 3 Laboratory analytical data: Analytical results include QC check samples for each batch of samples from which precision, accuracy, and completeness can be determined. Detection limits have been determined using 40 CFR Part 136 Appendix B, Revision 1.11. Raw data, chromatograms, spectrograms, and bench

Table 2. List of metals along with test methods and reporting limits.

Parameters	Test Methods	Test America Reporting Limits	Preservative	Holding Times
Aluminum (Al)	200.7	150 μg/l	HNO_3<pH 2	6 Month
Arsenic (As)	200.8	4.0 μg/l	HNO_3<pH 2	6 Month
Barium (Ba)	200.8	2.5 μg/l	HNO_3<pH 2	6 Month
Cadmium (Cd)	200.8	1.0 μg/l	HNO_3<pH 2	6 Month
Copper (Cu)	200.8	3.0 μg/l	HNO_3<pH 2	6 Month
Iron (Fe)	200.7	20.0 μg/l	HNO_3<pH 2	6 Month
Lead (Pb)	200.8	2.0 μg/l	HNO_3<pH 2	6 Month
Manganese (Mn)	200.8	0.5 μg/l	HNO_3<pH 2	6 Month
Mercury	245.1	0.2 μg/l	HNO_3<pH 2	6 Month
Nickel (Ni)	200.8	2.0 μg/l	HNO_3<pH 2	6 Month
Selenium (Se)	200.8	1.0 μg/l	HNO_3<pH 2	6 Month
Silver	200.8	0.3 μg/l	HNO_3<pH 2	6 Month
Sodium	200.7	200 μg/l	HNO_3<pH 2	6 Month
Zinc (Zn)	200.8	10.0 μg/l	HNO_3<pH 2	6 Month

Table 3. List of general chemistries along with test Methods and reporting limits that will be measured in the field.

Parameters	Test Methods	Reporting Limits
YSI™ Multiparameter Sonde		
Dissolved oxygen	SM 4500-0 G	0.03 mg/L
Turbidity	SM 2130 B (modified)	0.3 NTU
Conductivity	SM 2510 B	3.0 μS/cm
Temperature	SM 2550 B	-5.0º Celsius
pH	SM 4500-H+B	0.03 S.U.
Dissolved oxygen (Winkler titration)	SM 4500-O C	0.03 mg/L
Hach Kits		
Turbidity	SM 2130-B	0.3 NTU
pH	SM 4500-B (Electrode)	0.03 S.U.
Temperature	SM 2550	-5º Celsius

sheets are not included as part of the analytical report, but are maintained by the Contract Laboratory for easy retrieval and review. Data can be elevated from level 3 to level 4 by the inclusion of this information in the report. In addition, level 4 QC data must be reported using CLP forms or CLP format. Data falling under this category are considered as complete, and are used for regulatory decisions.

Level 4 Enforcement data: Analytical results mostly meet the USEPA required Contract Laboratory Program (CLP) data analysis, contract required quantification limits (CRQL), and validation procedures. QC data are reported on CLP forms or CLP format. Raw data, chromatograms, spectrograms, and bench sheets are included as part of the analytical report. Additionally, all reporting information required in the IDEM/BAA and in the Surface Water QAPP is included. Data are legally quantitative in value and are used for regulatory decisions.

Quality Control Samples and Chain-of-Custody

Duplicate water samples were collected at a rate of one (1) for every 20 samples or one (1) sample per week if less than 20 samples. Matrix Spike (MS)/ Matrix Spike Duplicates (MSDs) will be collected at a rate of one (1) for every 20 samples or one sample per week if less than 20 samples. Relative Percent Difference (RPD) will also be calculated. One field blank will be collected at a rate of one (1) for every 20 samples or one (1) sample per week if less than 20 samples are collected. Chain-of-Custody forms for all samples collected will be completed and tracked by designated IDEM and Service staff.

Flow Measurement

Stream flow measurements will be taken once per week at two designated sites following the IDEM Surveys section SOP (Beckman 2002). This method of measurement requires each stream segment to be divided into 20 sections with equal amounts of flow and volume. Average velocity and area will then be measured for each section. The product of each section's width, depth, and velocity will be the "flow" for the section, which is measured in feet per second (fps). The sum of flow for all sections is equal to the flow of the stream in cubic feet per second (cfs). Equipment type, calibration and maintenance procedures follow Beckman (2002).

Biological indicator sampling procedures

Fish collection methods

Daytime fish community inventories were conducted using standard fish community sampling equipment (i.e. Smith-Root backpack electrofisher, Smith-Root 2.5 GPP 150-meter long-line system or a Smith-Root tote barge system). The appropriate sampling gear for each site was

determined by the field crew chief on site. Upstream sample distance are 50 meters unless stream width exceeded 3.4 meters, in which case sample distances will be 15X the wetted width. Leopold *et al*. (1964) documented the hydrologic pattern of repeating riffle-run-pool habitat cycles every 15 times the mean stream width. This pattern has been observed across North America. Fish that can be correctly identified in the field will have 2-3 individuals vouchered for later taxonomic verification, while all other specimens will be preserved in 10 % formalin for laboratory processing using standard taxonomic keys.

Macroinvertebrate collection methods

Daytime macroinvertebrate assemblages will be sampled using a "representative habitat sampling" procedure developed for streams in the Northern Lakes and Forest Ecoregion (Simon and Stewart 1999). D-nets were used to collect 20 efforts within site reach length boundaries and will be composited and preserved in 95% ethanol for laboratory sorting. Efforts will be established that will reflect the abundance of predominant habitats. For example, habitats will be segregated into rock, fines, overhanging vegetation, woody debris, coarse particulate material, and other categories. If rocky riffle habitat represents 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 is based on a 60 second sample using the D-net.

Samples will be brought to the laboratory and placed into a 10 x 10 inch grided sorting pan. A 300 organism count sort will be done using a random number generator to reflect the appropriate square to be sorted. Sorting will be done until 300 organisms are picked; so for example, if a square has the 300^{th} individual, the remainder of the square will be sorted until it is fully picked. At the completion of the 300 organism sort, a 15 minute large-rare pick will be done. These specimens will be identified and used in richness metric calculations, but will not be included in the trophic or relative abundance metrics. All individuals were identified to lowest possible taxonomic levels following the state-of-the-art for that particular taxon.

Crayfish collection methods

Crayfish sampling included the evaluation of primary, secondary, and tertiary burrowers following Simon (2004). Stream reaches include the same stream reach length as the macroinvertebrates and fish collection areas and will include a variety of survey techniques. Burrowing crayfish will be collected using excavation techniques so that individuals will be coaxed from their burrow by pouring water down the burrow and agitating the water. If the crayfish fails to emerge, then a toilet plunger will be used to force the crayfish from the burrow. If that fails to dislodge the crayfish, then a hand shovel will be used to excavate the burrow and retrieve the individual. Secondary and tertiary burrowers will be 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 these rocks over, the crayfish can be easily collected by hand. In addition, the tertiary burrowers can be collected with a dip net or by hand. All crayfish species will be collected from each site and an estimate of relative abundance based on a

standard catch-per-unit-effort of each type will be calculated. Specimens will be vouchered for taxonomic verification.

Physical habitat reach assessment

Instream Habitat Evaluation

The Qualitative Habitat Evaluation Index (QHEI) (Rankin, 1989, 1995) is a habitat assessment procedure developed by the Ohio Environmental Protection Agency to determine the quality of site habitat. The QHEI was used to evaluate habitat condition at each sample locations. The QHEI includes attributes of stream habitat typically of Midwestern North American systems. These attributes include substrate composition, embeddness, siltation, riparian corridor quality, riffle and pool quality, sedimentation, instream cover habitat, and stream gradient. Obvious point source impacts, i.e. 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 characteristics

Land use characteristics were determined for each site location sampled in both the local and watershed scale portions of this study. For each site the upstream drainage land use was determined using Purdue University's online watershed delineation program (Choi and Engle, 2003). All land-use categories were recorded for inclusion in the multivariate analysis.

Data analysis

Statistical analysis was done using STATISTICA © (StatSoft, Inc. 2003). Data transformations were accomplished by converting fish data into a percent relative abundance scale and both habitat, chemistry and land-use data to a percent-of-range scale. Transformed fish community data was sorted by descending species richness and inputted into a Euclidean Distance Similarity Matrix for Cluster analysis using Ward's Method to create dendograms. Sites will be added into the analysis, sorted by descending species richness, in order to impress a degree of intuitive structure and direction to the orientation of the multidimensional alignment of the dendogram. In this application species richness will be used as a surrogate for biological integrity where higher numbers of species are associated with higher biological integrity. Sites within the dendogram from left to right will represent a continuum of decreasing biological integrity as predicted by decreasing species richness.

Clustering was evaluated at varying linkage levels using the transformed physiochemical and biological data by treating clusters as grouping variables and using the Kruskal-Wallis ANOVA

by Ranks test. This test assumes that the variable under consideration is continuous and that it was measured on at least an ordinal (rank order) scale. The test assesses the hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. Thus, the interpretation of the Kruskal-Wallis test is the same as that of the parametric one-way ANOVA, except that it is based on ranks rather than means.

Variables showing significant differences were examined more closely and central tendency visually presented using box and whisker plots. We used ARCGIS v.9.2 © (ESRI Software, Inc. 2007) to generate concentration pleths, using a spline smoothing technique, for chemical variables that significantly predicted fish community structure to aid in spatial interpretation. The visualization of the bathymetric volume of contaminant effects over the local and watershed scale was conducted to evaluate patterns in extent and magnitude of contaminant effects.

REFERENCES

Bowren T, GhiasUddin G. 1999. Quality Assurance Project Plan for Indiana Surface Water Qaulity Monitoring Programs. (Revision 2, June 1999), IDEM/32/01/1442/1999. Indiana Department of Environmental Management, Indianapolis, Indiana.

Choi J., Engel B.A. 2003. Watershed Delineation: Web-GIS tools for Spatial Hydrologic Analysis. http://pasture.ecn.purdue.edu/~jychoi/wd_home/

Clarke KR, Ainsworth M. 1993. A method of linking multivariate community structure to environmental variables. Marine Ecology Progress Series 92:205-219.

ESRI Software, INC. 2007. ArcGIS Version 9.2. Redwood, California.

Karr, J.R. and D.R. Dudley. 1981. Ecological perspective on water quality goals. *Environmental Management,* 5:55-68.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. *Assessing biological integrity in running waters, a method and its rational*. Illinois Natural History Survey, Special Publication 5.

Karr, J.R., Yoder CO. 2004. Biological assessment and criteria improve TMDL planning and decision making. *Journal of Environmental Engineering* 130:594-604.

Leopold LB, Woolman MG, Miller JP. 1964. *Fluvial processes in geomorphology* W.H. Freeman, San Francisco, CA.

Milner AM, Oswood MW. 2000. Urbanization gradients in streams of Anchorage, Alaska: a comparison of multivariate and multimetric approaches to classification. *Hydrobiologia* 422/423: 209-223.

Morris, C.C., Simon T.P., Newhouse, S.A. 2006. A local-scale *in situ* approach for stressor identification for biologically impaired aquatic systems. *Archives of Environmental Contamination and Toxicology* 50: 325-334.

Rankin, E.T. 1989. *The qualitative habitat evaluation index (QHEI), rationale, methods, and application*. Ohio EPA, Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.

Rankin, E.T. 1995. The use of habitat assessments in water resource managementprograms. In, Davis, WS, Simon TP, eds, *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making.* CRC, Boca Raton, Florida, pp 181-208.

Simon, T.P. 1991. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. I. Central Corn Belt Plain*. EPA 905-9-91-025.U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. 1992. *Biological Criteria Development for Large Rivers with an Emphasis on An Assessment of the White River Drainage, Indiana*. EPA 905-R-92-006. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. 1994. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. II. Huron-Erie Lake Plain*. EPA 905-R-92-007. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. 1997. *Biological Characterization of the Middle Fork Anderson River, Perry County, Indiana*. EPA 905-R-96-007. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. 1998. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. III. Northern Indiana Till Plain.* EPA 905-R-96-002. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. 2003. *Biological Response Signatures: Indicator Patterns using Aquatic Communities.* CRC Press, Boca Raton, Florida.

Simon, T.P. 2004. Standard Operating Procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of burrowing crayfish in streams and terrestrial habitats. *Miscellaneous Papers of the Indiana Biological Survey Aquatic Research Center* 2:1-16.

Simon, T.P. 2005. *Defining Brine and Oil Threats in the Patoka River National Wildlife Refuge*. Department of the Interior, U.S. Fish and Wildlife Service, Bloomington, Indiana.

Simon, T.P. and R.Dufour. 1998. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. V. Eastern Corn Belt Plain*. EPA 905-R-96-004. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P., S.A. Sobiech, T.H. Cervone, and N.E. Morales. 1995. Historical and present distribution of fishes in the Patoka River drainage: Pike, Gibson, and Dubois Counties, Indiana. *Proceedings of the Indiana Academy of Science* 104: 193-206.

Simon, T.P. and J.R. Stahl. 1998. *Development of Biotic Integrity Expectations for the Wabash River*. EPA 905-R-96-005. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. and J.R. Stahl. 2001. *Including Clarifying Statement for the Report Entitled, "Development of Index of Biotic Integrity Expectations for the Wabash River*". U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. and P.M. Stewart. 1998. *Standard Operating Procedures for development of watershed indicators in REMAP: Northern Lakes and Forest Streams*. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Biological Assemblages of National Wildlife Refuges

INTRODUCTION

Conservation of imperiled species and the protection of biological integrity of National Wildlife Refuges is an important policy mandate of the Federal Fish and Wildlife Service. The policy 601 FW 3 is a directive for refuge managers to follow while achieving refuge purpose(s) and System mission. The policy provides for the consideration and protection of the broad spectrum of fish, wildlife, and habitat resources found on refuges and associated ecosystems. Further, it provides refuge managers with an evaluation process to analyze their refuge and recommend the best management direction to prevent further degradation of environmental conditions; and where appropriate and in concert with refuge purposes and System mission, restore lost or severely degraded components. National Wildlife Refuge System Administration Act of 1966 as amended by the National Wildlife Refuge System Improvement Act of 1997, 16 U.S.C. 668dd-668ee (Refuge Administration Act), Section 4(a)(4)(B) of this law states that "In administering the System, the Secretary shall . . . ensure that the biological integrity, diversity, and environmental health of the System are maintained for the benefit of present and future generations of Americans . . . ." This is one of 14 directives to the Secretary contained within the Refuge Administration Act.

Biological integrity, diversity, and environmental health can be described at various landscape scales from refuge to ecosystem, national, and international. Each landscape scale has a measure of biological integrity, diversity, and environmental health dependent on how the existing habitats, ecosystem processes, and wildlife populations have been altered in comparison to historic conditions. Levels of biological integrity, diversity, and environmental health vary among refuges, and often within refuges over time. Individual refuges contribute to biological integrity, diversity, and environmental health at larger landscape scales, especially when they support populations and habitats that have been lost at an ecosystem, national, or even international scale. In pursuit of refuge purposes, individual refuges may at times compromise elements of biological integrity, diversity, and environmental health at the refuge scale in support of those components at larger landscape scales. When evaluating the appropriate management direction, refuge managers will consider their refuges' contribution to biological integrity, diversity, and environmental health at multiple landscape scales.

Biological Integrity

(1) Biological integrity is evaluated by examining the extent to which biological composition, structure, and function has been altered from historic conditions. Biological composition refers to biological components such as genes, populations, species, and communities. Biological structure refers to the organization of biological components, such as gene frequencies, social structures of populations, food webs of species, and

niche partitioning within communities. Biological function refers to the processes undergone by biological components, such as genetic recombination, population migration, the evolution of species, and community succession [see 602 FW 3.4C(1)(e), Planning Area and Data Needs].

(2) Biological integrity lies along a continuum from a biological system extensively altered by significant human impacts to the landscape to a completely natural system. No landscape retains absolute biological integrity, diversity, and environmental health. However, we strive to prevent the further loss of natural biological features and processes; i.e., biological integrity.

(3) Maintaining or restoring biological integrity is not the same as maximizing biological diversity. Maintaining biological integrity may entail managing for a single species or community at some refuges and combinations of species or communities at other refuges. For example, a refuge may contain critical habitats for an endangered species. Maintaining that habitat (and, therefore, that species), even though it may reduce biological diversity at the refuge scale, helps maintain biological integrity and diversity at the ecosystem or national landscape scale.

(4) In deciding which management activities to conduct to accomplish refuge purpose(s) while maintaining biological integrity, we consider how the ecosystem functioned under historic conditions. For example, natural frequency and timing of processes is considered such as flooding, fires, and grazing. Where it is not appropriate to restore ecosystem function, refuge management will attempt to duplicate these natural processes including natural frequencies and timing to the extent this can be accomplished.

(5) It may be necessary to modify the frequency and timing of natural processes at the refuge scale to fulfill refuge purpose(s) or to contribute to biological integrity at larger landscape scales. For example, under historic conditions, an area may have flooded only a few times per decade. Migratory birds dependent upon wetlands may have used the area in some years, and used other areas that flooded in other years. However, many wetlands have been converted to agriculture or other land uses, the remaining wetlands must produce more habitat, more consistently, to support wetland-dependent migratory birds. Therefore, to conserve these migratory bird populations at larger landscape scales, we may flood areas more frequently and for longer periods of time than they were flooded historically.

Biological Diversity

(1) Biological diversity is evaluated at various taxonomic levels, including class, order, family, genus, species, subspecies, and--for purposes of Endangered Species Act implementation--distinct population segment. These evaluations of biological diversity begin with population surveys and studies of flora and fauna. The System's focus is on native species and natural communities such as those found under historic conditions [see 602 FW 3.4C(1)(e)].

(2) Biological diversity is evaluated at various landscape scales, including refuge, ecosystem, national, and international. On refuges, evaluations of biological diversity are focused at the refuge scale; however, these refuge evaluations can contribute to assessments at larger landscape scales.

(3) The maintenance of populations of breeding individuals that is genetically viable and functional requires necessary provision for the breeding, migrating, and wintering needs of migratory species. Every effort is made to maximize the size of habitat blocks and maintain connectivity between blocks of habitats, unless such connectivity causes adverse effects on wildlife or habitat (e.g., by facilitating the spread of invasive species).

Environmental Health.

(1) Environmental health is evaluated by examining the extent that environmental composition, structure, and function have been altered from historic conditions. Environmental composition refers to abiotic components such as air, water, and soils, all of which are generally interwoven with biotic components (e.g., decomposers live in soils). Environmental structure refers to the organization of abiotic components, such as atmospheric layering, aquifer structure, and topography. Environmental function refers to the processes undergone by abiotic components, such as wind, tidal regimes, evaporation, and erosion. A diversity of abiotic composition, structure, and function tends to support a diversity of biological composition, structure, and function [see 602 FW 3.4C(1)(e), Planning Area and Data Needs].

(2) Environmental features are an important concern as they affect all living organisms. For example, at the genetic level, environmental health is managed by preventing chemical contamination of air, water, and soils that may interfere with reproductive physiology or stimulate high rates of mutation. Such contamination includes carcinogens and other toxic substances that are released within or outside of refuges.

(3) At the population and community levels, habitat components of food, water, cover, and space is considered. Food and water may become contaminated with chemicals that are not naturally present. Activities such as logging and mining or structures such as buildings and fences may modify security or thermal cover. Unnatural noise and light pollution may also compromise migration and reproduction patterns. Unnatural physical structures, including buildings, communication towers, reservoirs, and other infrastructure, may displace space or may be obstacles to wildlife migration. Refuge facility construction and maintenance projects necessary to accomplish refuge purpose(s) should be designed to minimize their impacts on the environmental health of the refuge.

The following sections document the fish, macroinvertebrate and crayfish assemblages collected from three National Wildlife Refuges in Indiana. Information containing site specific data on biological integrity and diversity can be found in the Appendices by watershed (Appendices 1 -Vernon Fork Muscatatuck River and 2 - Patoka River).

Fish Assemblages of the Patoka River National Wildlife Refuge

The fish assemblages of the Patoka River have been well studied (Simon et al. 1994, 2005) and the condition of the watershed in the vicinity of the National Wildlife Refuge has been assessed (Simon et al. 2005). The current study builds from the previous investigations conducted in the watershed and documents changes in the fish assemblage structure and function around the National Wildlife Refuge during a 14 year period.

Study Sites

Sampling conducted in 2006-2007 included 38 probability based sites in 2006 and 50 probability based sites in 2007. Previous studies of the Patoka River included 66 sites during 1993 and 125 sites during 2001. A portion of the same sites were sampled during both 1993 and 2001 survey events (Simon et al. 1994, 2005).

Sampling was conducted by the same crew leader, using the same techniques, equipment, and locations during all three sampling periods. Relative abundance (catch per unit effort or CPUE is the number of fish/ minute of electrofishing effort) data were gathered by performing surveys at reaches using appropriate electrofishing gear. Sampling gear included a model 6A Smith-Root boat-mounted electrofisher in the main stem river and Smith Root backpack and longline systems in tributaries. Electrofishing surveys included systematic sampling of representative habitat within reaches, including the thalweg or deepedst point in the cross sectional profile, usually for distances of 500 m for a minimum of 1800 s. Captured fish were placed in an onboard holding tank until a sampling event was completed. Data recorded for each survey event included species identification, batch weight, number of fish captured, examination of individuals for external disease and anomalies (DELTs), and sample and habitat conditions.

Species Richness and Composition

The Service and the Department of Environmental Management collected 9,658 individuals representing 82 fish species from streams and rivers on the Patoka River National Wildlife Refuge and tributaries (Table 1 and 2).

Dominant families collected during 2006-2007 included the Cyprinidae (20 species), Centrarchidae (15 species) and Catostomidae (12 species). Dominant families during 1993 and 2001 included Cyprinidae (20 species), Centrarchidae (14 species), and Catostomidae (13 species)(Simon et al. 1994, 2005).

During 1993-2001, *Erimyzon sucetta* and *Polyodon spathula* (verification of species presence by Doug Carnahan, Indiana DNR, personal communication) was found in oxbow lakes from the floodplain wetlands. Other new discoveries from Carnahan (1997a) include *Hiodon alosoides*, *Macrhybopsis aestivalis*, *Notropis wickliffi*, *Moxostoma anisurum*, and *Noturus flavus*.

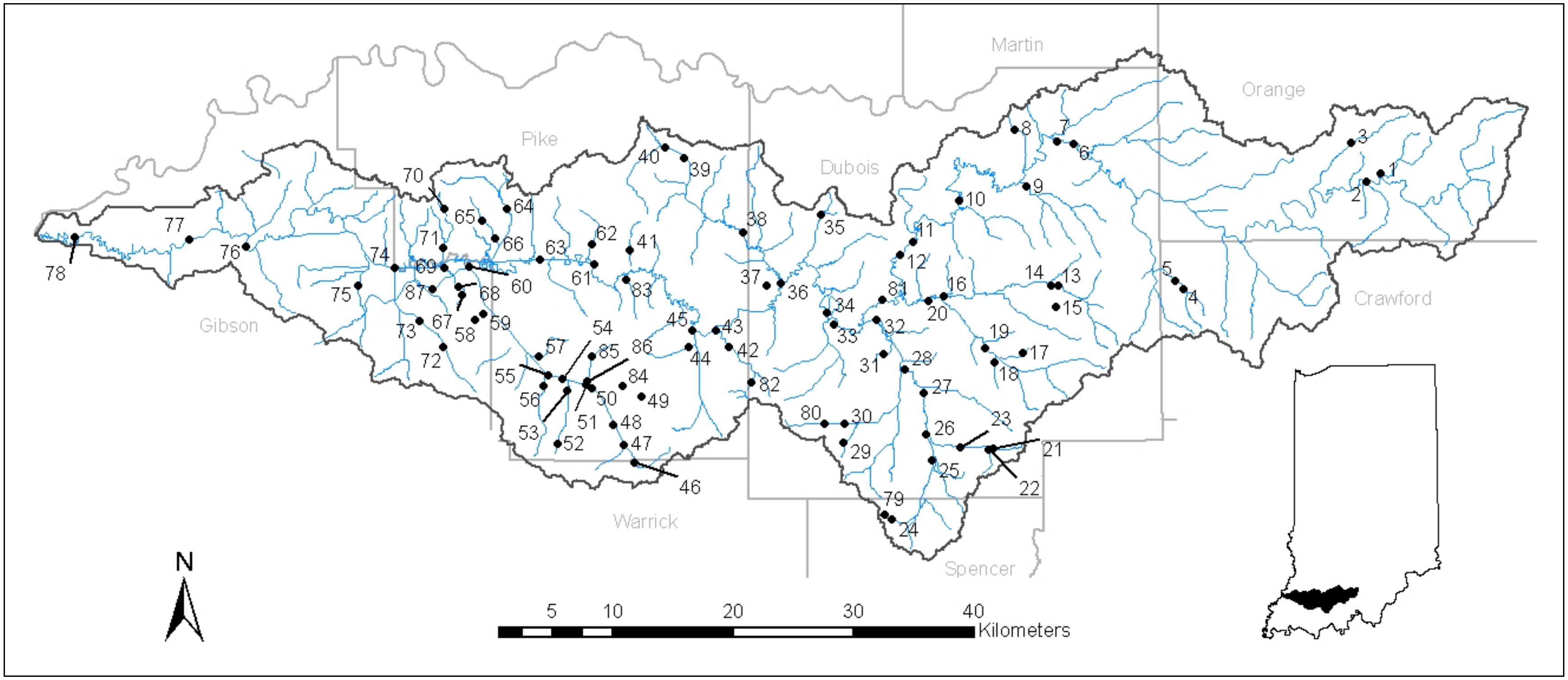


Figure 1. Distribution of sites sampled during 2006-2007 in the Patoka River drainage. Numbers refer to site location in Table 1.

Table 1. Site locations sampled during 2006-2007 in the Patoka River drainage. Site numbers correspond to Table 2 and are shown in Figure 1. Source codes are as follows: a - Indiana Department of Environmental Management (2006), b - Fish and Wildlife Service (2007).

Site No	Source	County	Locality	Latitude	Longitude
1	a	Orange	Patoka River @ CR 50 W	38º 26.84'	-86º 28.19'
2	a	Orange	Patoka River @ CR 175 W	38º 26.45'	-86º 29'
3	a	Orange	Youngs Creek @ CR 350 W	38º 28.24'	-86º 29.87'
4	a	Crawford	Ritter Creek @ Dillard Rd	38º 21.7'	-86º 39.48'
5	a	Crawford	Ritter Creek @ SR 164	38º 22.08'	-86º 39.92'
6	a	Dubois	Patoka River @ NE Dubois Rd	38º 28.23'	-86º 45.71'
7	a	Dubois	Patoka River @ NE Dubois Rd	38º 28.33'	-86º 46.64'
8	a	Dubois	Leistner Creek @ SR 56	38º 28.88'	-86º 49.07'
9	a	Dubois	Polson Creek @ SR 545	38º 26.31'	-86º 48.4'
10	a	Dubois	Patoka River @ CR 175 E	38º 25.69'	-86º 52.22'
11	a	Dubois	Patoka River @ Patoka River Walk	38º 23.81'	-86º 54.87'
12	b	Dubois	Patoka River @ SR 164/162 Bridge	38º 23.26'	-86º 55.62'
13	a	Dubois	Hall Creek @ CR 100 S	38º 21.87'	-86º 46.6'
14	b	Dubois	Hall Creek Trib @ Celestine Rd S	38º 21.85'	-86º 46.99'
15	b	Dubois	Hall Creek Trib @ Celestine Rd	38º 20.89'	-86º 46.74'
16	a	Dubois	Hall Creek @ SR 162	38º 21.39'	-86º 53.15'
17	a	Dubois	Unnamed Trib of Flat Creek @ CR 450 S	38º 18.82'	-86º 48.63'
18	b	Dubois	Flat Creek Trib @ St. Anthony Rd Bridge	38º 18.43'	-86º 50.22'
19	a	Dubois	Flat Creek @ CR 450 S	38º 19.05'	-86º 50.79'
20	a	Dubois	Hall Creek @ SR 162	38º 21.21'	-86º 54'
21	b	Dubois	Trib Green Creek @ U/S CR 350 E Bridge	38º 14.54'	-86º 50.28'
22	b	Dubois	Green Creek @ U/S SR 264 Bridge	38º 14.49'	-86º 50.58'
23	a	Dubois	Green Creek @ Holiday Lake Rd	38º 14.61'	-86º 52.17'
24	a	Spencer	Unnamed Trib of Hunley Creek @ E of CR 600 E	38º 11.38'	-86º 56.09'
25	b	Dubois	Hunley Creek @ U/S CR 1000 S Bridge	38º 14.02'	-86º 53.81'
26	a	Dubois	Hunley Creek @ CR 850 S	38º 15.18'	-86º 54.15'
27	b	Dubois	Hunley Creek @ U/S CR E 660 S Bridge	38º 17.03'	-86º 54.24'
28	b	Dubois	Indian Creek @ U/S W Old Road 64 Bridge	38º 18.09'	-86º 55.35'
29	b	Dubois	Short Creek Trib @ U/S CR 900 S	38º 14.82'	-86º 58.81'
30	b	Dubois	Short Creek @ U/S CR 400 W Bridge	38º 15.67'	-86º 58.77'
31	b	Dubois	Hunley Creek Trib @ U/S Cherry St Bridge	38º 18.81'	-86º 56.54'
32	b	Dubois	Hunley Creek @ U/S SR 231 Bridge	38º 20.35'	-86º 56.95'
33	a	Dubois	Patoka River @ Ell Creek Rd	38º 20.13'	-86º 59.41'
34	a	Dubois	Patoka River @ Ell Creek Rd	38º 20.65'	-86º 59.8'
35	a	Dubois	Altar Creek @ SR 56	38º 25.07'	-87º 0.13'
36	a	Dubois	Patoka River @ CR 100 S	38º 21.97'	-87º 2.41'
37	b	Dubois	Patoka River Trib @ U/S CR 100 S Bridge	38º 21.85'	-87º 3.25'
38	a	Pike	Flat Creek @ CR 900 N	38º 24.24'	-87º 4.59'
39	a	Pike	Flat Creek @ CR 250 N	38º 27.6'	-87º 7.92'
40	a	Pike	Flat Creek @ CR 700 E	38º 28.08'	-87º 9.05'
41	b	Pike	Unnamed Trib of Patoka River @ U/S CR 200 S	38º 23.44'	-87º 11.03'
42	a	Pike	Rock Creek @ CR 1025 E	38º 19.12'	-87º 5.34'
43	a	Pike	Rock Creek @ CR 925 E	38º 19.84'	-87º 6.14'
44	a	Pike	Beadens Creek @ CR 775 E	38º 19.11'	-87º 7.67'
45	b	Pike	Cup Creek @ U/S CR 625 S Bridge	38º 19.86'	-87º 7.46'

Site No	Source	County	Locality	Latitude	Longitude
46	b	Warrick	South Fork Patoka River @ U/S CR 1300 S Bridge	38º 13.89'	-87º 10.77'
47	b	Pike	South Fork Patoka River @ U/S CR 1200 S Bridge	38º 14.74'	-87º 11.37'
48	b	Pike	South Fork Patoka River @ U/S CR 1100 S Bridge	38º 15.6'	-87º 11.93'
49	b	Pike	South Fork Patoka River Trib @ U/S CR 550 E	38º 16.91'	-87º 10.32'
50	b	Pike	South Fork Patoka River @ U/S CR 300 E	38º 17.23'	-87º 13.16'
51	a	Pike	South Fork Patoka River @ CR 875 S	38º 17.38'	-87º 13.47'
52	b	Pike	Rough Creek @ U/S CR 1200 S	38º 14.74'	-87º 15.12'
53	b	Pike	Rough Creek @ U/S CR 925 S	38º 17.14'	-87º 14.56'
54	a	Pike	South Fork Patoka River @ CR 900 S	38º 17.68'	-87º 14.87'
55	b	Pike	South Fork Patoka River @ U/S State Hwy 61	38º 17.83'	-87º 15.64'
56	b	Pike	Honey Creek @ U/S CR 900 S	38º 17.37'	-87º 15.94'
57	b	Pike	Hat Creek @ U/S CR 50 E	38º 18.69'	-87º 16.19'
58	b	Gibson	Turkey Creek @ CR 1275 E	38º 20.3'	-87º 19.83'
59	b	Gibson	Unnamed Trib of Turkey Creek @ CR 75 S	38º 20.57'	-87º 19.37'
60	b	Gibson	South Fork Patoka River @ SR 57 Bridge	38º 22.68'	-87º 20.21'
61	b	Pike	Patoka River @ U/S SR 61 Bridge	38º 22.8'	-87º 13.03'
62	b	Pike	Stone Coe Creek @ U/S SR 61 Bridge	38º 23.69'	-87º 13.19'
63	a	Pike	Patoka River @ Meridian Rd	38º 23.01'	-87º 16.14'
64	b	Pike	Flat Creek @ Chandler Rd Bridge	38º 25.29'	-87º 18.06'
65	b	Pike	Flat Creek Trib @ U/S CR 250 S Bridge	38º 24.77'	-87º 19.46'
66	b	Pike	Flat Creek Trib @ U/S State Hwy 57	38º 23.94'	-87º 18.73'
67	a	Gibson	Hurricane Creek @ CR 00	38º 21.42'	-87º 20.59'
68	b	Gibson	Hurricane Creek @ U/S CR 50 N	38º 21.79'	-87º 20.77'
69	b	Gibson	Hurricane Creek @ U/S CR 150 N	38º 22.66'	-87º 21.58'
70	b	Pike	Robinson Creek @ U/S Chandler Rd Bridge	38º 25.31'	-87º 21.64'
71	b	Pike	Robinson Creek @ U/S CR 200	38º 23.53'	-87º 21.69'
72	a	Gibson	Keg Creek @ CR 250 S	38º 19.09'	-87º 21.63'
73	b	Gibson	East Fork Keg Creek @ U/S CR 125 S Bridge	38º 20.24'	-87º 22.99'
74	b	Gibson	Patoka River @ CR 850 E	38º 22.65'	-87º 24.44'
75	b	Gibson	Lost Creek @ U/S CR 50 N	38º 21.8'	-87º 26.47'
76	a	Gibson	Patoka River @ Old Petersburg Rd	38º 23.56'	-87º 32.89'
77	a	Gibson	Patoka River @ CR 350 N	38º 23.85'	-87º 36.12'
78	a	Gibson	Patoka River @ S of 350 N	38º 23.93'	-87º 42.7'
79	b	Spencer	Hunley Creek @ U/S CR 600 E Bridge	38º 11.6'	-86º 56.51'
80	b	Dubois	Short Creek @ CR 500 W	38º 15.67'	-86º 59.9'
81	b	Dubois	Patoka River @ US 231	38º 21.25'	-86º 56.61'
82	b	Dubois	Rock Creek @ U/S CR 900 S Bridge	38º 17.53'	-87º 4.11'
83	b	Pike	Mill Creek @ U/S CR 450 E Bridge	38º 22.16'	-87º 11.24'
84	b	Pike	Houchin Ditch Trib @ U/S CR 900	38º 17.39'	-87º 11.45'
85	b	Pike	South Fork Patoka River Trib @ U/S CR 300 E	38º 18.68'	-87º 13.17'
86	b	Pike	South Fork Patoka River Trib @ U/S CR 875 S	38º 17.57'	-87º 13.44'
87	b	Gibson	Wabash-Erie Canal @ U/S CR 1050 E	38º 21.7'	-87º 22.24'

Table 2. List of fish species collected during 2006-2007 from the Patoka River drainage. Numbers indicate the sites at which each species has been collected based on information in Table 1 and Figure 1.

Species	2006	2007
Sites with no fish captured.		79, 83-86
Lepisosteidae		
Lepisosteus oculatus	6	
Lepisosteus osseus	10	
Lepisosteus platostomus	20, 34, 76, 78	61, 74
Amiidae		
Amia calva	6, 20, 33, 34, 36	55, 68, 69
Hiodontidae		
Hiodon alosoides		61
Clupeidae		
Alosa chrysochloris	63	
Dorosoma cepedianum	1, 2, 6, 7, 9, 11, 13, 16, 20, 33, 34, 63, 78	12, 61, 74
Dorosoma petenense		61
Cyprinidae		
Campostoma anomalum	1-5, 8, 9, 13, 16, 17, 19, 20, 24, 26, 35, 38, 51, 72	14, 15, 18, 21, 22, 25, 27, 29, 30, 45, 47, 48, 50, 64, 65, 73
Cyprinella spiloptera	2, 6, 7, 9-11, 13, 16, 19, 20, 26, 34, 42, 78	12, 25, 27, 32, 74
Cyprinella whipplei	9, 16, 19, 20, 44, 63,	12
Cyprinus carpio	2, 6, 7, 33, 34, 36, 43, 63, 67, 76-78	12, 74
Ericymba buccata	13, 16, 19, 20, 26, 35, 44	12, 14, 18, 21, 25, 27, 29, 30, 45, 47, 48, 50, 73
Hybognathus nuchalis	20, 63	65, 66
Hybopsis amblops		12
Hypophthalmichthys molitrix	36	61, 74
Hypophthalmichthys nobilis		74
Luxilus chrysocephalus	1-3, 7-9, 13, 19,	12, 14, 18, 21, 22, 45, 61, 66
Lythrurus fumeus	6, 7, 10, 42, 51	
Lythrurus umbratilis	1-3, 5, 6, 8, 9, 13, 19, 20, 26, 34, 42	25, 27, 31, 61
Notemigonus crysoleucus	40	28, 31, 45
Notropis atherinoides	54, 63, 77	74
Phenacobius mirabilis	13, 16, 26, 34, 72	12, 27, 45, 73, 74
Phoxinus erythrogaster	4	
Pimephales notatus	1-9, 13, 16, 19, 20, 23, 26, 34, 38, 42, 63	12, 14, 18, 21, 22, 25, 27-32, 45, 47, 61, 66, 74, 75
Pimephales promelas	4, 8	75
Pimephales vigilax		12, 61
Semotilus atromaculatus	1, 3-5, 8, 9, 13, 17, 19, 24, 35, 39, 40, 42, 44, 51, 54	14, 15, 18, 21, 22, 25, 27-31, 45-50, 56-58, 60, 64-66, 70, 71, 73, 74
Catostomidae		
Carpiodes carpio	10, 33	74
Carpiodes cyprinus		45, 61

Species	2006	2007
Catostomus commersonii	1-4, 8, 13, 17, 19	
	3, 8, 13, 19, 20, 23, 26, 35, 40, 42	14, 22
Erimyzon oblongus		
Erimyzon sucetta		18, 21, 25, 27-32, 45
Ictiobus bubalus	6, 10, 11, 33, 63, 76	61, 74
Ictiobus cyprinellus	6, 10, 33	74
Ictiobus niger	6, 7, 10, 11, 33, 34, 36, 63, 78	
Minytrema melanops	2, 6, 9, 13, 16, 19	
Moxostoma duquesnei	11	
Moxostoma erythrurum	1, 2, 6, 7, 9, 16, 19	12
Moxostoma macrolepidotum		61
Ictaluridae		
Ameiurus catus		74
Ameiurus melas	38	62, 68, 69, 71
Ameiurus natalis	4, 5, 8, 9, 13, 16, 19, 20, 26, 38-40, 42, 44, 51, 54, 72	14, 18, 21, 22, 27, 28, 30, 32, 45-48, 50, 52, 57, 62, 64-66, 75
Ameiurus nebulosus	6, 13, 19, 26, 39, 40, 42	46
Ictalurus punctatus	11, 20, 23, 34, 54, 77	12, 61, 74
Noturus flavus	78	
Noturus gyrinus		25, 45
Noturus miurus		12
Noturus nocturnus		32
Pylodictus olivaris	6, 33, 34, 54, 78	12, 74
Esocidae		
Esox americanus	20, 35, 38-40, 51, 54	55, 59, 64-66, 74
Umbridae		
Umbra limi		65, 66
Apherododeridae		
Aphredoderus sayanus	2, 7, 8, 11, 20, 23, 26, 34, 38-40, 42, 43, 51	31, 32, 48, 59, 65
Fundulidae		
Fundulus notatus	1-3, 5, 9, 13, 16, 19, 20, 23, 26, 38-40, 51, 54, 67, 72	12, 14, 18, 21, 22, 25, 27-31, 41, 45-48, 50, 52, 53, 55, 56, 58, 59, 61, 64-66, 68, 71, 73, 75
Poeciliidae		
Gambusia affinis	16, 20, 26, 33-35, 44, 51, 67, 77, 78	12, 14, 18, 21, 25, 27, 28, 30-32, 37, 45-47, 53, 56, 58, 59, 64, 66, 69-71, 73, 75
Atherinidae		
Labidesthes sicculus	6, 13, 16, 20	12, 61, 74
Cottidae		
Cottus carolinae	1, 3	
Centrarchidae		
Ambloplites rupestris	1, 2	
Centrarchus macropterus		62, 68
Lepomis cyanellus	1-9, 13, 16, 17, 19, 20, 26, 35, 38-40, 42-44, 51, 54, 67, 72	12, 14, 18, 21, 22, 25, 27, 28, 30, 31, 37, 45-50, 52, 53, 55, 56, 58, 59, 62, 64-66, 68-71, 73-75
Lepomis gulosus	1, 2, 5	12, 59, 62, 70

Species	2006	2007
Lepomis humilis		12
Lepomis macrochirus	1-5, 8-11, 13, 16, 17, 19, 20, 23, 26, 33-36, 38-40, 42-44, 51, 54, 63, 76	12, 14, 21, 22, 25, 37, 46-48, 50, 52, 55, 60-62, 64-66, 68, 70, 74
Lepomis megalotis	1-11, 13, 16, 19, 20, 23, 26, 33-35, 38-40, 43, 44, 51, 54, 63	12, 14, 18, 21, 22, 25, 27, 28, 32, 47, 48, 50, 52, 53, 55, 59-62, 64, 66, 74
Lepomis microlophus	1, 9, 20	59, 62, 65
Lepomis miniatus		62
Lepomis symmetricus		52
Micropterus dolomieu	1, 2, 7	
Micropterus punctulatus	5-8, 17, 19, 20, 40, 77	12, 28, 32, 47, 61, 64, 66, 74
Micropterus salmoides	1, 2, 6, 7, 9, 13, 16, 23, 26, 38, 54, 67, 72	22, 46, 69, 75
Pomoxis annularis	1, 2, 11, 20, 36, 63	
Pomoxis nigromaculatus	33, 36	
Percidae		
Etheostoma asprigene	38	32, 74
Etheostoma gracile	34	31, 32, 37, 65
Etheostoma histrio	78	
Etheostoma nigrum	1-3, 42	14, 45
Etheostoma spectabile	1, 3-5, 8, 9, 19	14, 18, 21, 22, 27
Percina caprodes	7, 13	12
Percina maculata	7, 19, 39, 42	48
Percina phoxocephala	11, 16	
Percina sciera	7, 10, 11, 39	12, 74
Sciaenidae		
Aplodinotus grunniens	7, 11, 20, 33, 34, 36, 63, 76, 78	12

Species that were rediscovered between 1993 and 2001 include river shiner *Notropis blennius*, sand shiner *N. stramineus*, bullhead minnow *Pimephales vigilax*, shorthead redhorse *Moxostoma macrolepidotum*, brook silverside *Labidesthes sicculus*, yellow bass *Morone mississippiensis*, and smallmouth bass *M. dolomeiu*. The increase in the number of new species records was probably due to increased sampling during this period. The rediscovery of harlequin darter *Etheostoma histrio* and dusky darter *Percina sciera* was also documented upstream to the mouth of South Fork Patoka River. Lake chubsucker *Erimyzon sucetta*, paddlefish *Polyodon spathula*, rock bass *Ambloplites rupestris*, slough darter *Etheostoma gracile*, *E. histrio,* blackside darter *Percina maculata, P. sciera*, and banded sculpin *Cottus carolinae* are all species sensitive to siltation and acidity.

During 2006, the dominant species include longear sunfish (1,314 individuals), central stoneroller minnow (827 individuals), bluegill (461 individuals) and striped shiner (378 individuals). These species were dominant in pool habitats (longear sunfish and bluegill), headwater streams (central stoneroller minnow), and in wadeable stream pool habitats (striped shiner) draining the refuge (Table 3).

During 2007, the four dominant species included creek chub (671 individuals), western mosquitofish (568 individuals), bluntnose minnow (524 individuals), and blackstripe

Table 3. Comparison of data collected from two periods in the Patoka River drainage, 2006 to 2007.

	2006		2007		Total	
Species	Count	%	Count	%	Count	%
Lepisosteidae						
Lepisosteus oculatus	1	<1%			1	<1%
Lepisosteus osseus	1	<1%			1	<1%
Lepisosteus platostomus	11	<1%	5	<1%	16	<1%
Amiidae						
Amia calva	9	<1%	3	<1%	12	<1%
Hiodontidae						
Hiodon alosoides			1	<1%	1	<1%
Clupeidae						
Alosa chrysochloris	1	<1%			1	<1%
Dorosoma cepedianum	90	2%	72	2%	162	2%
Dorosoma petenense			2	<1%	2	<1%
Cyprinidae						
Campostoma anomalum	827	16%	323	7%	1150	12%
Cyprinella spiloptera	142	3%	89	2%	231	2%
Cyprinella whipplei	70	1%	2	<1%	72	1%
Cyprinus carpio	47	1%	39	1%	86	1%
Ericymba buccata	21	<1%	203	4%	224	2%
Hybognathus nuchalis	12	<1%	70	2%	82	1%
Hybopsis amblops			1	<1%	1	<1%
Hypophthalmichthys molitrix	1	<1%	6	<1%	7	<1%
Hypophthalmichthys nobilis			3	<1%	3	<1%
Luxilus chrysocephalus	378	7%	71	2%	449	5%
Lythrurus fumeus	5	<1%			5	<1%
Lythrurus umbratilis	75	1%	10	<1%	85	1%
Notemigonus crysoleucus	1	<1%	50	1%	51	1%
Notropis atherinoides	6	<1%	8	<1%	14	<1%
Phenacobius mirabilis	7	<1%	81	2%	88	1%
Phoxinus erythrogaster	3	<1%			3	<1%
Pimephales notatus	283	6%	524	12%	807	8%
Pimephales promelas	2	<1%	2	<1%	4	<1%
Pimephales vigilax			27	1%	27	<1%
Semotilus atromaculatus	210	4%	671	15%	881	9%
Catostomidae						
Carpiodes carpio	2	<1%	1	<1%	3	<1%
Carpiodes cyprinus			2	<1%	2	<1%
Catostomus commersoni	89	2%			89	1%
Erimyzon oblongus	114	2%	7	<1%	121	1%
Erimyzon sucetta			209	5%	209	2%
Ictiobus bubalus	18	<1%	8	<1%	26	<1%
Ictiobus cyprinellus	4	<1%	4	<1%	8	<1%
Ictiobus niger	28	1%			28	<1%
Minytrema melanops	21	<1%			21	<1%
Moxostoma duquesnei	1	<1%			1	<1%

	2006		2007		Total	
Species	Count	%	Count	%	Count	%
Moxostoma erythrurum	31	1%	1	<1%	32	<1%
Moxostoma macrolepidotum			1	<1%	1	<1%
Ictaluridae						
Ameiurus catus			1	<1%	1	<1%
Ameiurus melas	1	<1%	22	<1%	23	<1%
Ameiurus natalis	141	3%	69	2%	210	2%
Ameiurus nebulosus	17	<1%	1	<1%	18	<1%
Ictalurus punctatus	8	<1%	11	<1%	19	<1%
Noturus flavus	4	<1%			4	<1%
Noturus gyrinus			2	<1%	2	<1%
Noturus miurus			1	<1%	1	<1%
Noturus nocturnus			2	<1%	2	<1%
Pylodictus olivaris	5	<1%	5	<1%	10	<1%
Esocidae						
Esox americanus	17	<1%	13	<1%	30	<1%
Umbridae						
Umbra limi			7	<1%	7	<1%
Apherododeridae						
Aphredoderus sayanus	19	<1%	24	1%	43	<1%
Fundulidae						
Fundulus notatus	58	1%	423	9%	481	5%
Poeciliidae						
Gambusia affinis	33	1%	568	12%	601	6%
Atherinidae						
Labidesthes sicculus	15	<1%	5	<1%	20	<1%
Cottidae						
Cottus carolinae	24	<1%			24	<1%
Centrarchidae						
Ambloplites rupestris	9	<1%			9	<1%
Centrarchus macropterus			2	<1%	2	<1%
Lepomis cyanellus	260	5%	288	6%	548	6%
Lepomis gulosus	5	<1%	5	<1%	10	<1%
Lepomis humilis			1	<1%	1	<1%
Lepomis macrochirus	461	9%	178	4%	639	7%
Lepomis megalotis	1314	26%	264	6%	1578	16%
Lepomis microlophus	8	<1%	7	<1%	15	<1%
Lepomis miniatus			11	<1%	11	<1%
Lepomis symmetricus			1	<1%	1	<1%
Micropterus dolomieu	7	<1%			7	<1%
Micropterus punctulatus	26	1%	57	1%	83	1%
Micropterus salmoides	25	<1%	11	<1%	36	<1%
Pomoxis annularis	22	<1%			22	<1%
Pomoxis nigromaculatus	2	<1%			2	<1%
Percidae						
Etheostoma asprigene	1	<1%	2	<1%	3	<1%

Species	2006 Count	2006 %	2007 Count	2007 %	Total Count	Total %
Etheostoma gracile	2	<1%	41	1%	43	<1%
Etheostoma histrio	1	<1%			1	<1%
Etheostoma nigrum	8	<1%	3	<1%	11	<1%
Etheostoma spectabile	74	1%	17	<1%	91	1%
Percina caprodes	2	<1%	1	<1%	3	<1%
Percina maculata	5	<1%	1	<1%	6	<1%
Percina phoxocephala	2	<1%			2	<1%
Percina sciera	5	<1%	3	<1%	8	<1%
Sciaenidae						
Aplodinotus grunniens	18	<1%	5	<1%	23	<1%
Total Number of Individuals	5110		4548		9658	

topminnow (423 individuals). These species are tolerant forms that can occur in acid mine drainage streams.

Biological Diversity Changes

Assemblage records from 1992 to 2002. – Five times as much collection effort has been expended in the watershed since 1992 than had previously occurred over the last two centuries. Simon et al. (1994) documented the increase in species diversity as a result of increased sampling intensity. They documented the increase in species diversity as a result of increased sampling intensity. They documented nine new species in the watershed, including threadfin shad *Dorosoma petenense*, cypress minnow *Hybognathus hayi*, ribbon shiner *Lythrurus fumeus*, pallid shiner *Hybopsis amnis*, southern redbelly dace *Phoxinus erythrogaster*, fathead minnow *Pimephales promelas*, blacknose dace *Rhinichthys obtusus*, and starhead topminnow *Fundulus dispar*.

The range extension of *L. fumeus* may have been a result of misidentification since *L. umbratilis* does occur in the upper portion of the watershed. The ribbon shiner, *L. fumeus,* was previously known from only a few small streams in southwestern Indiana (Gerking 1945).

Bait-bucket release of *Pimephales promelas* into the watershed was speculated by Simon et al. (1994), while the presence of *Dorosoma petenense* was probably a result of immigration from upstream reservoir habitats. *Notropis amnis* and *F. dispar* suggest that water quality conditions were improving. All of these species are considered sensitive to acidity and turbidity (Simon 1991).

Additional sampling since 1993, added the discovery of *Erimyzon sucetta* and *Polyodon spathula* (verification of species presence by Doug Carnahan, Indiana DNR, personal communication) in oxbow lakes from the floodplain wetlands. Other new discoveries from Carnahan (1997a) include goldeye *Hiodon alosoides*, shoal chub *Macrhybopsis hyostoma*, channel shiner *Notropis wickliffi*, silver redhorse *Moxostoma anisurum*, and

stonecat *Noturus flavus*. Species that were rediscovered include *Notropis blennius*, sand shiner *N. stramineus*, *Pimephales vigilax*, *Moxostoma macrolepidotum*, *Labidesthes sicculus*, *Morone mississippiensis*, and *M. dolomeiu*.

The increase in the number of new species records was probably due to increased sampling. The rediscovery of *Etheostoma histrio* and *Percina sciera* was also documented upstream to the mouth of South Fork Patoka River. *Erimyzon sucetta*, *Polyodon spathula*, *Ambloplites rupestris*, *Etheostoma gracile*, *E. histrio, Percina maculata, P. sciera*, and *Cottus carolinae* are all species sensitive to siltation and acidity. The increased occurrence of these species in the watershed may be an environmental indicator of recovery.

Assemblage records during 2006. During 2006, spotted gar (*Lepisosteus oculatus*), longnose gar (*L. osseus*), skipjack herring (*Alosa chrysochloris*), ribbon shiner (*Lythrurus fumeus*), southern redbelly dace (*Phoxinus erythrogaster*), white sucker (*Catostomus commersonii*), black buffalo (*Ictiobus niger*), spotted sucker (*Minytrema melanops*), black redhorse (*Moxostoma duquesnei*), stonecat (*Noturus flavus*), banded sculpin (*Cottus carolinae*), rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*), white crappie (*Pomoxis annularis*), black crappie (*P. nigromaculatus*), harlequin darter (*Etheostoma histrio*), and slenderhead darter (*Percina phoxocephala*) were collected. Many of these species, such as rock bass, southern redbelly dace, banded sculpin, smallmouth bass, and slenderhead darter, were collected in areas upstream of the refuge in high gradient tributaries that drain the Hoosier National Forest. While larger floodplain species such as spotted and longnose gar, black buffalo, spotted sucker, harlequin darter were collected from the Patoka River downstream of the refuge.

Assemblage records during 2007. – Forty-eight of the 82 fish species (58.5%) collected during 2006 were also collected during 2007. During 2007, the following species were collected including goldeye (*Hiodon alosoides*), threadfin shad (*Dorosoma petenense*), bigeye chub (*Hybopsis amblops*), bighead carp (*Hypophthalmichthys nobilis*), bullhead minnow (*Pimephales vigilax*), quillback (*Carpiodes cyprinus*), lake chubsucker (*Erimyzon sucetta*), shorthead redhorse (*Moxostoma macrolepidotum*), white catfish (*Ameiurus catus*), tadpole madtom (*Noturus gyrinus*), brindled madtom (*Noturus miurus*), freckled madtom (*Noturus nocturnus*), central mudminnow (*Umbra limi*), flier (*Centrarchus macropterus*), orangespotted sunfish (*Lepomis humilis*), redspotted sunfish (*Lepomis miurus*), and bantam sunfish (*Lepomis symmetricus*).

New Drainage records during 2006-2007 -- During 2006-2007 sampling, six new species records were collected from Patoka River National Wildlife Refuge that were not previously known from the Patoka River (Table 3 and 4). These species included skipjack herring (*Alosa chrysochloris*), bigeye shiner (*Hybopsis amblops*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Hypophthalmichthys nobilis*), white catfish (*Ameiurus catus*), and freckled madtom (*Noturus nocturnus*).

Skipjack herring is a large river species that is common in the Wabash River. The species was collected from the Patoka River near Meridian Road. The skipjack herring is a pelagic species that is capable of feeding as a predator as an adult.

Bigeye chub was collected from a single site on the Patoka River at SR 164/162 bridge. This species has experienced significant decline over its range in Illinois and Ohio, but has maintained large populations in Indiana portions of its range. The species is a benthic insectivore that is usually associated with expansive sand bars and coarse gravel and sand substrates.

Freckled madtom was collected from Hunley Creek at US 231 bridge. The freckled madtom is a nocturnal species that spends most of its time hiding beneath instream habitat. It is possible that this species may have been misidentified in the past since it is similar to several other *Noturus* species that were previously found in the watershed.

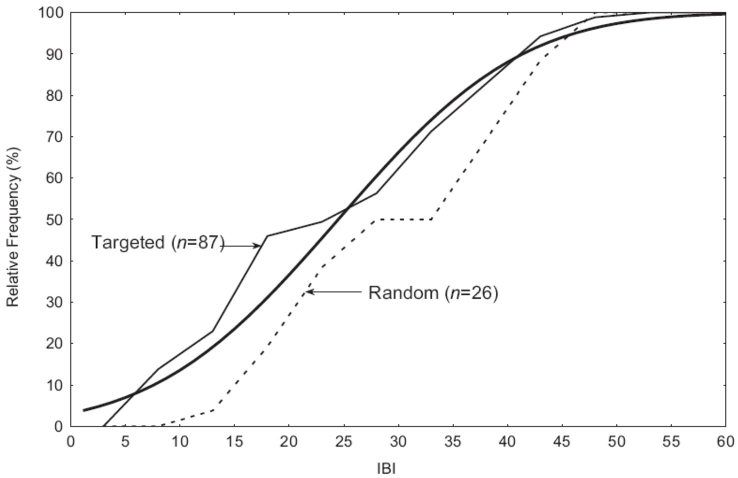
Alien species presence

Silver carp and bighead carp are exotic species, while white catfish is a non-indigenous species from the Atlantic Slope. These species were all collected from the Patoka River at Oatsville Bottom and the Asian carps were also collected from the Patoka River at Winslow. These records represent the first record for this species in the Patoka River. The white catfish was stocked into Patoka Lake and into several other large reservoirs in Indiana near Indianapolis. The species has a forked tail similar to other members of genus *Ictalurus,* but has white chin barbels and a head shape like other *Ameiurus.* White catfish were only collected from the Patoka River at Oatsville Bottom (Table 5). These species were collected from large main stem river habitats over degraded substrates.

Changes in Biological Integrity

Biological integrity classification scores, based on targeted least-impacted sampling at 34 sites between 1992 and 2001, showed that stream biological integrity of the Patoka River National Wildlife Refuge and associated watersheds had declined slightly over this period (Figure 2A). Simon et al. (2005) reported that watershed integrity declined reaching the lowest levels recorded during the 2001 survey.

In order to determine the biological integrity and ecological health of the Patoka River National Wildlife Refuge, we chose an unbiased approach to verify our understanding of overall biological integrity. We sampled 37 sites during 2006 and another 46 sites during 2006. We did not sample lake or pond locations during the current study. The probability distributions of biological integrity, based on index of biotic integrity (IBI) score for the watershed (Figure 2B) showed that the two years had similar results. The results from 2006 were slightly higher than scores from 2007, but this is to be expected because of the drought conditions that occurred in 2007. The two years showed that site mean cumulative frequency distribution (CFD_{50}) had higher biological integrity during 2006 with IBI scores of 35, which approximates the statewide average for Indiana, while



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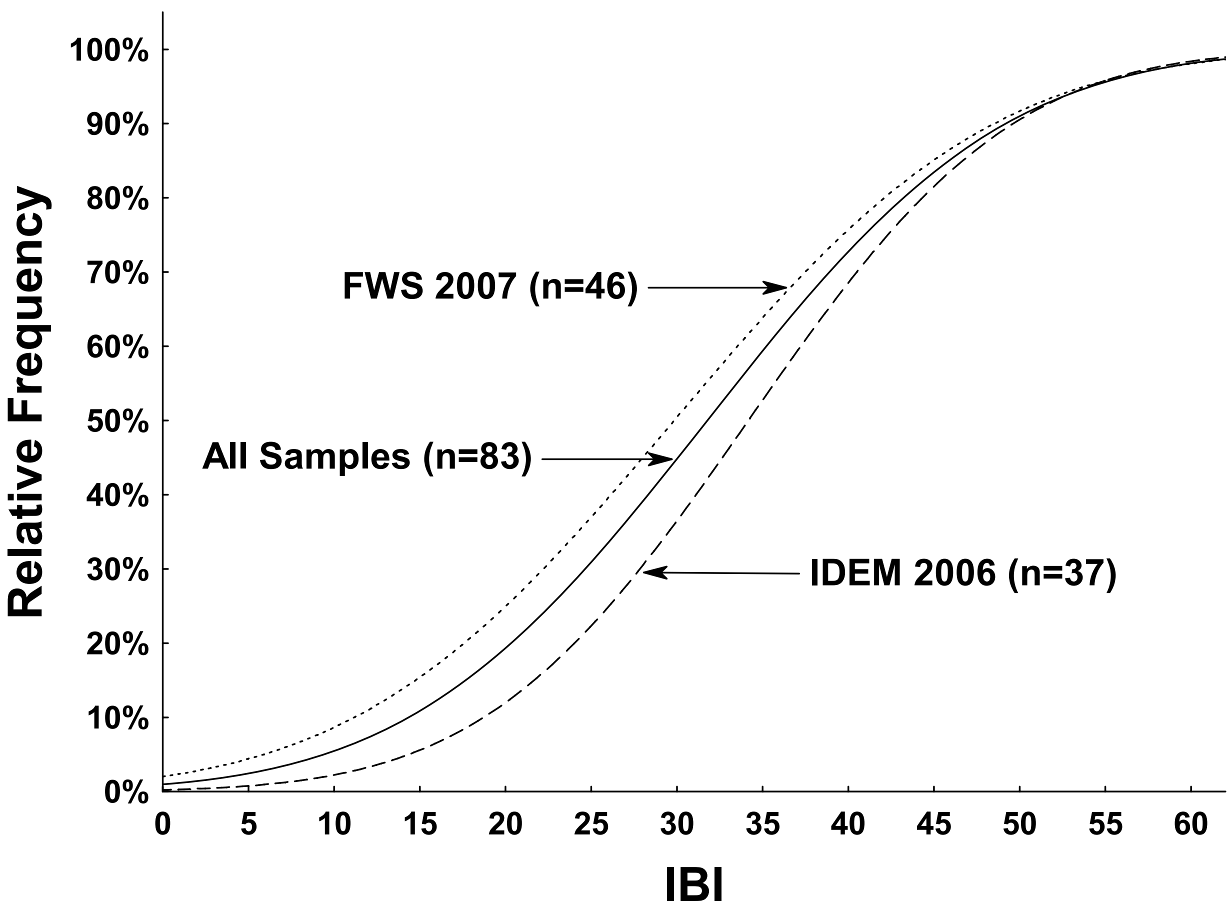

B

Figure 2. Comparison of Relative IBI Cumulative Distribution Frequency for sites sampled within the Patoka River drainage including: A) Fish & Wildlife Service 2001 and B) Fish & Wildlife Service 2007 (n=46) and the Indiana Department of Environmental Management in 2006 (n=37) and a combination of all sites (n=83).

the CFD_{50} for 2007 had mean biological integrity scores of 31. Both integrity categories would have scored between "Poor-Fair" based on index classification assessments (Karr et al. 1981; Simon and Dufour 1998). Based on the assessment of all 83 sample events collected at Patoka River, the mean CFD_{50} would have scored 32 using the IBI (Simon and Dufour 1998).

From study periods 2001 to 2006-2007, conditions favored a slight increase in biological integrity of streams in the Patoka River watershed. Over this time period, drought conditions possibly reduced nonpoint source runoff of nutrients and toxic materials into streams, while groundwater infiltration has potentially enabled some species to recolonize areas that had been in past decline. Unfortunately, the lack of water in 2007 caused some loss of biological integrity gain with declines in species richness and changes in trophic dynamics.

The trend for biological integrity in the Patoka River National Wildlife Refuge is not significantly different during the surveys conducted from 1993 to 2001. IBI scores from 1993 surveys averaged 21 (range: 0-48) and represented "very poor" IBI biological integrity class. During 2001, the mean IBI score was 17 (range: 0-42). The trend in IBI score has had a positive slope and has slightly improved since the original watershed survey in 1993. Survey results based on 2006 and 2007 sampling showed that the watershed has improved enough to meet the statewide average.

A variety of sites still do not possess any fish species in the South Fork Patoka River, which is an area that is impacted from acid mine drainage. Another site on Rough Creek was also without aquatic life.

REFERENCES

Simon, T.P., S.A. Sobiech, T.H. Cervone, and N.E. Morales. 1994. Historical and present distribution of fishes in the Patoka River basin: Pike, Gibson, and Dubois counties. *Proceedings of the Indiana Academy of Science* 104: 193-206.

Simon, T.P., R.L. Dufour, and B.E. Fisher. 2005. Changes in the biological integrity of fish assemblages in the Patoka River drainage as a result of anthropogenic disturbance from 1888 to 2001. In. J.N. Rinne, R.M. Hughes, and B. Calamusso (eds). *Historical Changes in Large River Fish Assemblages of the Americas*. American Fisheries Society Symposium 45, Bethesda, MD.

Fish Assemblages of the Big Oaks National Wildlife Refuge

Only a single study has been conducted on the Big Oaks National Wildlife Refuge (Pruitt et al. 1994). Mike Litwin, U.S. Fish and Wildlife Service, Bloomington Field Office surveyed 17 sites on the refuge and collected 41 fish species during 1993.

Study Sites

Thirty four sites were sampled on the Big Oaks National Wildlife Refuge in Jennings, Jefferson, and Ripley counties (Figure 3). These sites represent a variety of habitat types from lentic lakes and ponds to lotic streams and rivers (Table 4).

Sites were sampled with appropriate field equipment including Smith-Root generator powered backpack electrofishing units and long-line units capable of 300 v DC output and 5-6 amps in streams, while lake and pond habitats were sampled using boat mounted electrofishing gear. Sampling during 2006 was under normal hydrologic conditions, while sampling during 2007 was during drought conditions. Streams during 2007 were intermittent pools causing fish to be confined to shallows and making gear efficiency much greater. We anticipate that Index of Biotic Integrity scores may be slightly higher during 2007 than during 2006.

Fish were collected from areas that included distances from 50-500 m linear distance. Headwater streams (<20 sq. mi drainage area) that were less than 3.4 m wetted width were sampled for 50 m, while Otter Creek was sampled for 300 m. These distances are based on 15 times the wetted stream width.

Lake sites included a minimum of a single site and increased with increasing lake size to a maximum of two sites on Old Timbers Lake using boat mounted Smith-Root electrofishing gear. Sampling distance occurred along 500 m of shoreline. The U.S. Fish and Wildlife Service collected all sampled during 2006 and during 2007 samples were collected by the Service and Indiana Department of Environmental Management biologists.

Species Richness and Composition

The Service and the Department of Environmental Management collected 9,747 individuals representing 37 fish species from streams, lakes, and ponds on the Big Oaks

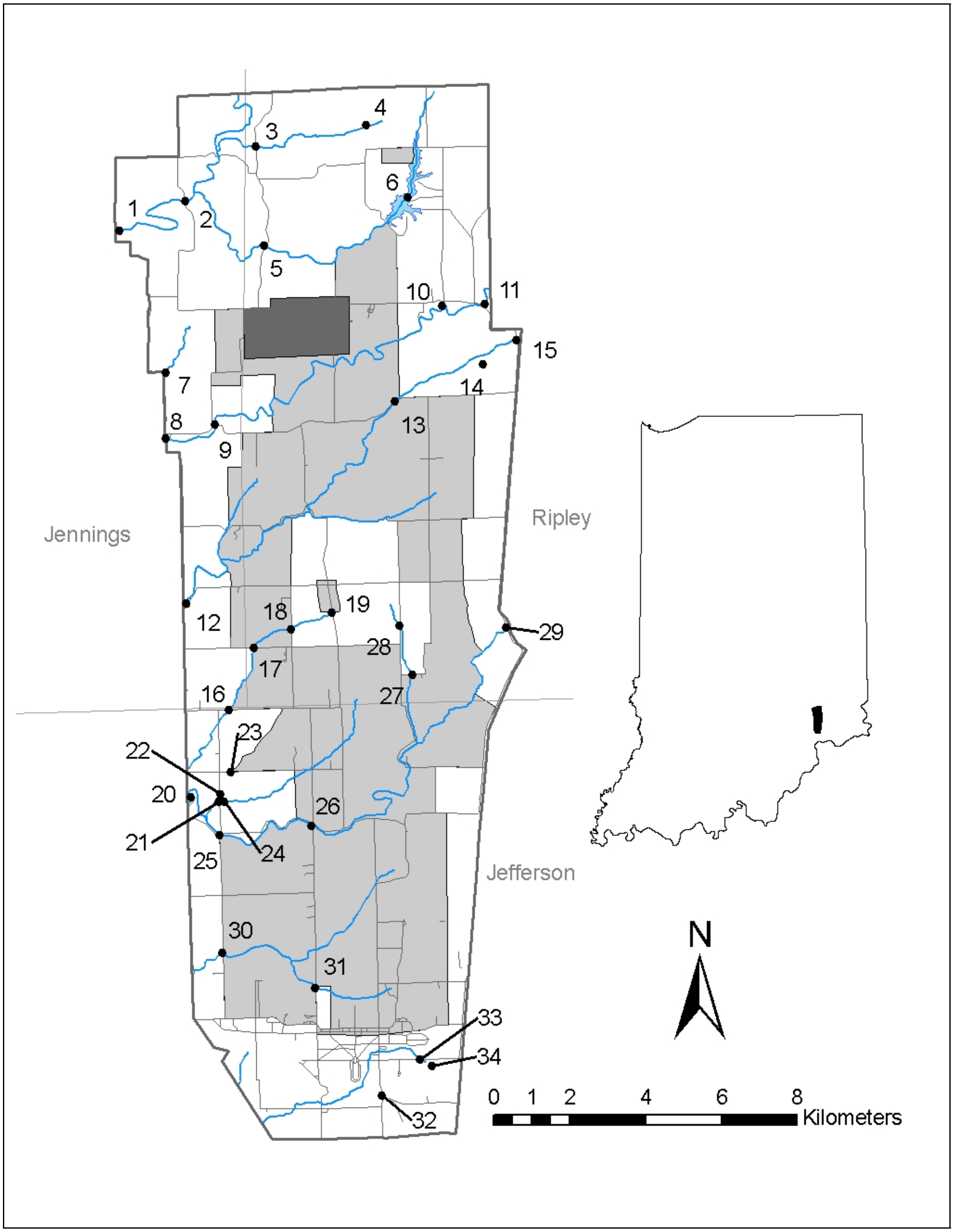

Figure 3. Distribution of sites during 2006-2007 in the Big Oaks National Wildlife Refuge. Numbers refer to site location in Table 1.

Table 4. Site locations sampled during 2006-2007 in the Big Oaks National Wildlife Refuge for Fish Assemblage. Site numbers correspond to Table 2 and are shown in Figure 3. Source codes are as follows: a - Fish and Wildlife Service (2006), b - Indiana Department of Environmental Management (2007).

Site No.	Source	County	Locality	Latitude	Longitude
1	a	Jennings	Otter Creek @ W Perimeter Rd	39º 1.61'	-85º 28.86'
2	a	Jennings	Otter Creek @ Northwest Exit Rd	39º 2.02'	-85º 27.64'
3	a	Ripley	Falling Timbers Branch @ Shaped Charge Rd	39º 2.78'	-85º 26.34'
4	a	Ripley	Fallen Timbers Creek @ L Rd	39º 3.06'	-85º 24.33'
5	a, b	Ripley	Little Otter Creek @ Shaped Charge Rd	39º 1.37'	-85º 26.21'
6	a	Ripley	Old Timbers Lake @ Unnamed Rd off NE exit Rd	39º 2.01'	-85º 23.59'
7	a, b	Jennings	Rush Branch @ W Perimeter Rd	38º 59.58'	-85º 28.04'
8	a, b	Jennings	Graham Creek @ W Perimeter Rd	38º 58.65'	-85º 28.06'
9	a, b	Jennings	Graham Creek @ J Rd	38º 58.83'	-85º 27.16'
10	a, b	Ripley	Big Graham Creek @ K Rd and NE Exit Rd	39º 0.47'	-85º 22.99'
11	a, b	Ripley	Graham Creek @ East Outlet Rd	39º 0.49'	-85º 22.22'
12	a, b	Jennings	Little Graham Creek @ W Perimeter Rd Area 33	38º 56.30'	-85º 27.75'
13	a, b	Ripley	Little Graham Creek @ J Rd Ford	38º 59.12'	-85º 23.89'
14	a	Ripley	Gate 8 Pond @ Michigan Rd	38º 59.63'	-85º 22.26'
15	a, b	Ripley	Little Graham Creek @ East Outlet Rd	38º 59.97'	-85º 21.65'
16	a	Jefferson	Marble Creek @ F Rd	38º 54.78'	-85º 27.00'
17	a	Ripley	Marble Creek @ G Rd	38º 55.65'	-85º 26.53'
18	a	Ripley	Marble Creek @ Morgan Rd	38º 55.91'	-85º 25.85'
19	a	Ripley	Marble Creek @ Center Recovery Rd	38º 56.13'	-85º 25.10'
20	a	Jefferson	Big Creek @ W Perimeter Rd	38º 53.55'	-85º 27.72'
21	a	Jefferson	Unnamed Trib of Big Creek @ U/S Jinestown Rd	38º 53.48'	-85º 27.21'
22	a	Jefferson	Unnamed Trib of Big Creek @ U/S Jinestown Rd	38º 53.58'	-85º 27.17'
23	a	Jefferson	Unnamed Trib of Big Creek @ E Rd	38º 53.90'	-85º 26.99'
24	a	Jefferson	Unnamed Trib of Big Creek @ D/S Jinestown Rd	38º 53.47'	-85º 27.11'
25	a	Jefferson	Big Creek @ Jinestown Rd	38º 53.00'	-85º 27.22'
26	a	Jefferson	Big Creek @ Morgan Rd	38º 53.11'	-85º 25.53'
27	a	Ripley	Unnamed Trib of Big Creek @ Cottrell Rd	38º 55.23'	-85º 23.66'
28	a	Ripley	Unnamed Trib of Big Creek @ Wonju Rd	38º 55.93'	-85º 23.87'
29	a	Ripley	Big Creek @ E Perimeter Rd	38º 55.88'	-85º 21.92'
30	a	Jefferson	Middle Fk Creek @ Jinestown Rd	38º 51.33'	-85º 27.19'
31	a	Jefferson	Trib of Middle Fork Creek @ Morgan Rd Bridge	38º 50.79'	-85º 25.52'
32	a	Jefferson	Harberts Creek @ CR 100 W	38º 49.26'	-85º 24.34'
33	a	Jefferson	Harberts Creek @ Main Entrance Rd	38º 49.76'	-85º 23.64'
34	a	Jefferson	Kruegers Lake @ Main Entrance Rd	38º 49.67'	-85º 23.43'

Table 5. List of fish species collected during 2006-2007 from the Big Oaks National Wildlife Refuge. Numbers indicate sites from which species were collected based on information in Table 1 and Figure 3.

Species	2006	2007
Cyprinidae		
Campostoma anomalum	1-5, 7, 9-12, 15, 16, 20-27, 29-32	5, 7-13
Cyprinella spiloptera	5, 12, 20	9
Ericymba buccata	1, 2, 5, 10, 11, 26	7-10, 12, 13
Hybopsis amblops	1-3, 5, 8-12	8-12
Luxilus chrysocephalus	1, 5, 7-11, 13, 15, 20, 21, 24-27, 29, 30, 32	5, 7-13
Lythrurus umbratilis	2, 11, 17, 19-22, 24, 30	8-12
Notemigonus crysoleucus	28	
Notropis ariommus	1, 11, 20	
Notropis boops	1, 2, 8-10, 12, 20, 29, 30	8-10, 12
Notropis photogenis		8, 10
Pimephales notatus	1-5, 7-12, 16, 20-24, 26, 28-33	5, 7-13
Semotilus atromaculatus	2-5, 7, 9-12, 15-33	5, 7-13
Esocidae		
Esox americanus	13, 16, 27, 30	
Catostomidae		
Catostomus commersoni	3, 5, 10, 13, 28, 32, 33	5, 8-13
Erimyzon oblongus	18-21, 24-27, 29	7
Hypentelium nigricans	1, 2, 9-11, 23, 30	8-12
Moxostoma duquesnei	1, 2, 20	8-12
Moxostoma erythrurum	1, 2, 5	8, 10, 11
Ictaluridae		
Ameiurus natalis	1, 5, 10-13, 15, 28, 29, 33	9, 11-13
Ameiurus nebulosus	1	
Noturus miurus	10, 20	9, 10
Fundulidae		
Fundulus notatus	20	
Poeciliidae		
Gambusia affinis	15	12, 13
Atherinidae		
Labidesthes sicculus	13	
Centrarchidae		
Ambloplites rupestris	1, 2, 11, 12	11, 12
Lepomis cyanellus	1-5, 7, 10-13, 15-22, 24-30, 32, 33	7, 9-13
Lepomis macrochirus	1, 2, 5, 6, 8-15, 17, 19, 20, 28, 29, 32, 34	10
Lepomis megalotis	1-7, 9-17, 26, 27, 29, 30	8-13
Lepomis microlophus	5, 6, 14, 34	
Micropterus dolomieu	1	
Micropterus salmoides	2, 6, 14, 15, 34	11
Pomoxis nigromaculatus	6, 14, 34	
Percidae		
Etheostoma blennioides	1, 2, 5, 8-12, 20, 29, 30	5, 9-12
Etheostoma caeruleum	1-3, 5, 8-12, 20, 25, 26, 29, 30	5, 8-13
Etheostoma flabellare	1-5, 7-13, 16, 20, 23-25, 29, 30	5, 7-13
Etheostoma nigrum	1-5, 7-13, 15, 16, 20-26, 28-30	5, 7-13
Etheostoma spectabile	2-5, 7-9, 10-13, 15-17, 21-25, 27, 29, 30, 32, 33	5, 7-13

Table 6. Comparison of fish assemblage data collected from two periods in the Big Oaks National Wildlife Refuge, 2006 to 2007.

	2006		2007		Total	
Species	Count	%	Count	%	Count	%
Cyprinidae						
Campostoma anomalum	426	8%	751	17%	1177	12%
Cyprinella spiloptera	10	<1%	18	<1%	28	<1%
Ericymba buccata	40	1%	171	4%	211	2%
Hybopsis amblops	72	1%	223	5%	295	3%
Luxilus chrysocephalus	182	3%	461	10%	643	7%
Lythrurus umbratilis	29	1%	16	<1%	45	<1%
Notemigonus crysoleucus	18	<1%			18	<1%
Notropis ariommus	10	<1%			10	<1%
Notropis boops	38	1%	28	1%	66	1%
Notropis photogenis			15	<1%	15	<1%
Pimephales notatus	964	18%	857	19%	1821	19%
Semotilus atromaculatus	523	10%	485	11%	1008	10%
Esocidae						
Esox americanus	4	<1%			4	<1%
Catostomidae						
Catostomus commersoni	26	<1%	235	5%	261	3%
Erimyzon oblongus	30	1%	1	<1%	31	<1%
Hypentelium nigricans	39	1%	93	2%	132	1%
Moxostoma duquesnei	10	<1%	75	2%	85	1%
Moxostoma erythrurum	21	<1%	7	<1%	28	<1%
Ictaluridae						
Ameiurus natalis	18	<1%	8	<1%	26	<1%
Ameiurus nebulosus	1	<1%			1	<1%
Noturus miurus	6	<1%	3	<1%	9	<1%
Fundulidae						
Fundulus notatus	1	<1%			1	<1%
Poeciliidae						
Gambusia affinis	123	2%	52	1%	175	2%
Atherinidae						
Labidesthes sicculus	2	<1%			2	<1%
Centrarchidae						
Ambloplites rupestris	12	<1%	6	<1%	18	<1%
Lepomis cyanellus	285	5%	78	2%	363	4%
Lepomis macrochirus	1159	22%	3	<1%	1162	12%
Lepomis megalotis	147	3%	148	3%	295	3%
Lepomis microlophus	161	3%			161	2%
Micropterus dolomieu	1	<1%			1	<1%
Micropterus salmoides	23	<1%	2	<1%	25	<1%
Pomoxis nigromaculatus	21	<1%			21	<1%
Percidae						
Etheostoma blennioides	72	1%	32	1%	104	1%
Etheostoma caeruleum	286	5%	137	3%	423	4%
Etheostoma flabellare	86	2%	66	1%	152	2%
Etheostoma nigrum	177	3%	375	8%	552	6%
Etheostoma spectabile	269	5%	109	2%	378	4%
Total Number of Individuals	5292		4455		9747	

National Wildlife Refuge (Table 5). Dominant families include the Cyprinidae (12 species), Centrarchidae (8 species) and Percidae (5 species). Litwin (1994) collected 6,703 individuals and dominant families included Cyprinidae (12 species), Centrarchidae (7 species), and Percidae (7 species) during 1993.

During 1993, bluntnose minnow (1,512 individuals), striped shiner (1,146 individuals), and creek chub (778 individuals) were the three dominant species on the Big Oaks National Wildlife Refuge (Pruitt et al. 1994).

During 2006, the dominant species include bluegill (1,159 individuals), bluntnose minnow (964 individuals), creek chub (523 individuals) and central stoneroller minnow (426 individuals). These species were dominant in lakes (bluegill), headwater streams (bluntnose minnow and creek chub), and in large wadeable streams (central stoneroller minnow) draining the refuge, respectively (Table 6).

During 2007, the dominant species included bluntnose minnow (857 individuals), central stoneroller minnow (751 individuals), creek chub (485 individuals), and striped shiner (461 individuals). These four minnow species were dominant at Little Otter Creek (site 5), Rush Creek, and a variety of sites on Graham Creek ((Big and Little Graham Creeks) sites 7-13).

<u>Biological Diversity Changes</u>

Assemblage records during 1993. -- Litwin collected eleven fish species from the refuge during this time. Longnose gar (*Lepisosteus osseus*), bowfin (*Amia calva*), gizzard shad (*Dorosoma cepedianum*), carp (*Cyprinus carpio*), mimic shiner (*Notropis volucellus*), suckermouth minnow (*Phenacobius mirabilis*), spotted sucker (*Minytrema melanops*), channel catfish (*Ictalurus punctatus*), spotted bass (*Micropterus punctulatus*), logperch (*Percina caprodes*), and blackside darter (*Percina maculata*) were only found during the 1993 surveys.

Longnose gar, bowfin, and gizzard shad were collected as single individuals and were found at Blue Hole on Otter Creek. This site was not surveyed during the 2006-2007 collections due to access restrictions. Carp, an exotic species, was not collected during 2006-2007 surveys but was found during 1993 as a single individual at Otter Creek and at Graham Creek. Suckermouth minnow, mimic shiner, logperch, and blackside darter were collected from Otter Creek from locations not sampled during the 2006-2007 surveys. These species were either represented by single individuals or were collected from single locations.

Assemblage records during 2006 -- During 2006 sampling nine species were collected from Big Oaks National Wildlife Refuge that were not collected during 2007 (Table 3). These species included golden shiner (*Notemigonus crysoleucas*), popeye shiner (*Notropis ariommus*), grass pickerel (*Esox americanus*), brown bullhead (*Ameiurus nebulosus*), blackstripe topminnow (*Fundulus notatus*), brook silverside (*Labidesthes*

sicculus), redear sunfish (*Lepomis microlophus*), smallmouth bass (*Micropterus dolomieu*), and black crappie (*Pomoxis nigromaculatus*).

Golden shiner was collected from Big Creek from pool habitat over sand and gravel substrates. This area was associated with a beaver dam that created lentic conditions on Big Creek.

The collection of popeye shiner is important since these records represent the first record for this species in Indiana since the species was originally described from the White River near Indianapolis in the late 1800's. The species has been considered extirpated, but during this study specimens was collected from Otter Creek, Big Graham Creek, and Big Creek (Table 5). The species was collected from moderate sized flowing rivers over cobble and gravel substrates.

Grass pickerel was collected from Little Graham Creek, Marble Creek, unnamed tributary of Big Creek, and Middle Fork Creek. This species is a pelagic predator that usually is associated with submerged aquatic vegetation, woody debris, and leaf debris.

Brown bullhead was collected from Otter Creek from a deep pool along an outside channel bend. The species was associated with large collapsed clay bank habitat that had recently been severed from the bank.

Blackstripe topminnow is a surface dwelling species that is commonly associated with overhanging grasses or submerged aquatic vegetation. The species was only collected from Big Creek. Brook silverside is also a pelagic species that usually occurs in lakes; however, the species was collected from Little Graham Creek.

Redear sunfish is typically a lake inhabitant that is not native to southeastern Indiana. It has been stocked throughout the state into lentic systems. The species was collected from Old Timbers Lake, Gate 8 pond, and Kruegers Lake, as well as, Little Otter Creek. The species grows to large sizes and is a desireable sport fish among anglers. Likewise, black crappie is also a lake species occurring around woody debris and submerged tree trunks. The species was also collected from Old Timbers Lake, Gate 8 pond, and Kruegers Lake.

Smallmouth bass was collected from Otter Creek. This species is a native predator that is an important indicator of water quality because of the temperature sensitivity to cool water temperatures.

Assemblage records during 2007. – Twenty-seven of the 36 fish species collected during 2006 were also collected during 2007. The only species that was unique to the 2007 season included silver shiner, which was collected from Graham Creek. The silver shiner is a large insectivorous minnow species that is an indicator of high quality habitat and water conditions.

Alien species presence

The only exotic or non-indigenous fish species collected on the refuge during either 2006-2007 surveys was the western mosquitofish. This species was collected from three sites on Little Graham Creek (sites 12, 13, and 15). This species is widely stocked into ponds and slow moving waters for mosquito control; however, diet studies in Indiana streams has shown that the species consumes snails and other aquatic insects and not mosquitoes (Clem and Whitaker 1998).

Changes in Biological Integrity

Biological integrity classification scores, based on targeted least-impacted sampling at 34 sites between 1992 and 2001, showed that stream biological integrity of the Big Oaks National Wildlife Refuge and associated watersheds had declined slightly over this period (Figure 4). Over this time period, prolonged drought conditions possibly reduced nonpoint source runoff of nutrients and toxic materials into streams, while groundwater infiltration has potentially enabled some species to recolonize areas that had been decimated in the past (U.S. Geological Survey Water Resources data, 2004-2006). Unfortunately, the lack of water also caused declines in species richness and changes in trophic dynamics.

We chose an unbiased approach to verify our understanding of overall biological integrity during at the Big Oaks National Wildlife Refuge. We sampled 14 sites during 2007 that was the same sites sampled during 2006. We did not sample lake or pond locations that had been sampled during 2006. The probability distributions of biological integrity, based on index of biotic integrity (IBI) score for the watershed (Figure 4) showed that the two years had similar results. The results from 2007 had several sites that had higher integrity scores than the 2006 random sites, but this is to be expected because of the drought situation. The two years showed that site mean site cumulative frequency distribution (CFD_{50}) had higher biological integrity with IBI scores of 41, while the CFD_{50} for 2006 had biological integrity scores of 35, which approximates the statewide average for Indiana. Both integrity categories would have scored between "Poor-Fair" based on index classification assessments (Karr et al. 1981; Simon and Dufour 1998). Based on the assessment of all 104 sample events collected at Big Oaks, the mean CFD_{50} would have scored 37 using the IBI (Simon and Dufour 1998).

The trend for biological integrity in the Big Oaks National Wildlife Refuge is not significantly different during the surveys conducted between 1993 to 2007. IBI scores from 1993 sampling averaged 46 (range: 32-58) and represented "good-fair" integrity classes of biological integrity. Although the trend has slightly declined since the original surveys in 1993, this is most likely a result of including the lake sites in the 2006 IBI statistics and the greater number of higher order streams in the 1993 sampling events. Larger streams such as Otter Creek represented 23.5% of the 1993 collections compared to 5.9% of the 2006 and none of the 2007 collections. Since the 1993 stream sites were not randomly selected, there was a greater opportunity to target highest quality habitats.

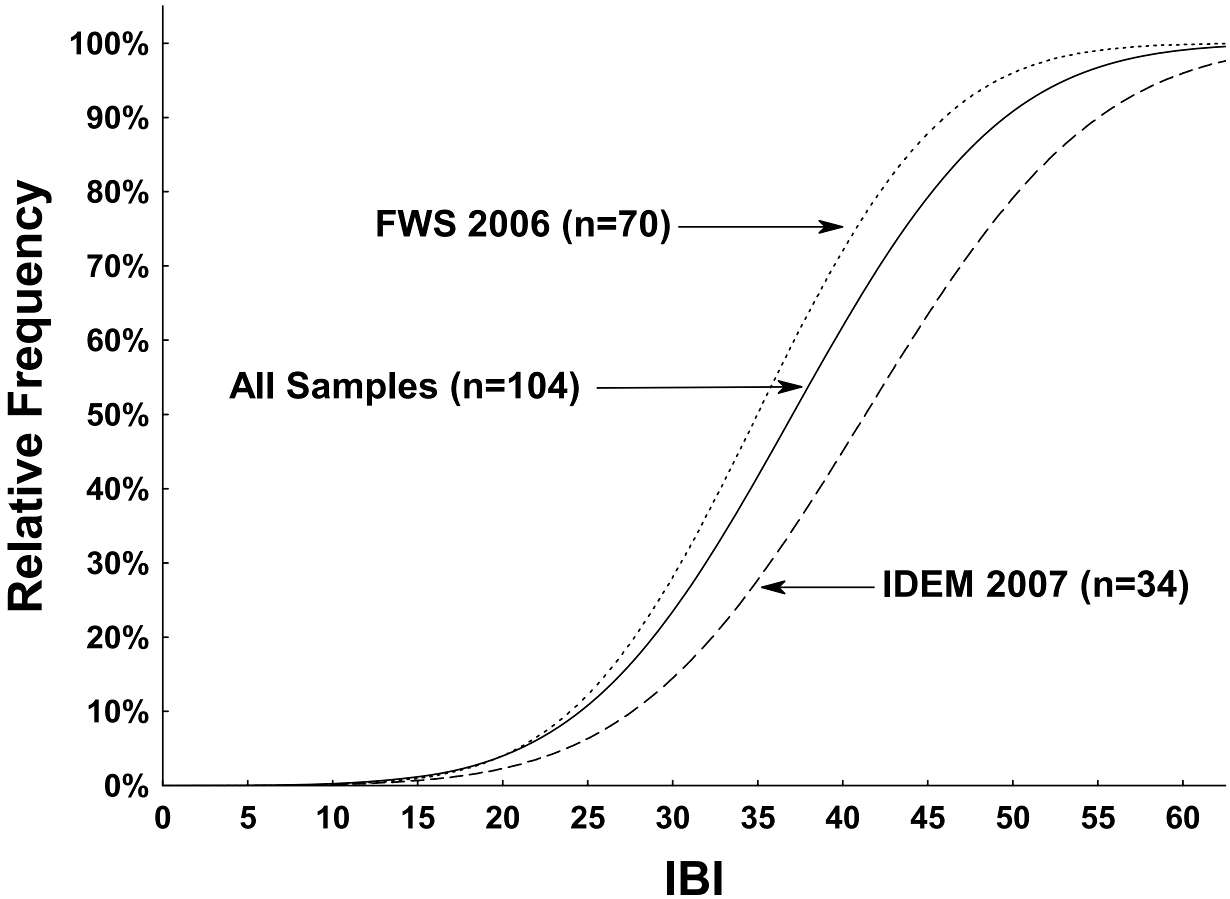

Figure 4. Comparison of Relative IBI Frequency for sites sampled by Fish & Wildlife Service in 2006 (n=70), the Indiana Department of Environmental Management in 2007 (n=34), and a combination of all sites (n=104) within and around the Big Oaks National Wildlife Refuge.

The survey results based on 2006 and 2007 sampling is probably most representative of the variety of aquatic habitat conditions found at Big Oaks.

REFERENCES

Clem, P.D., and J.O. Whitaker, Jr. 1996. Distribution of the mosquito fish, *Gambusia affinis* (Baird & Girard), in Indiana, with comments on resource competition. *Proceedings of the Indiana Academy of Science* 104: 249-258.

Pruitt, L., S. Pruitt, and M. Litwin. 1994. *Jefferson Proving Ground fish and wildlife management plan September 1994*. U.S. Department of the Interior, U.S. Fish and Wildlife Service, Bloomington Field Office, Bloomington, Indiana.

Fish Assemblages of the Muscatatuck National Wildlife Refuge

Fish assemblage inventory surveys have not been previously conducted at the Muscatatuck National Wildlife Refuge. An evaluation of fish tissue contaminants has been conducted by the U.S. Fish and Wildlife Service, Bloomington Field Office (Sparks, unpublished data).

Study Sites

Thirteen sites were sampled within the boundaries of the Muscatatuck National Wildlife Refuge along with one site downstream and one upstream of the refuge on the Vernon Fork Muscatatuck River to assess fish communities (Table 7 and Figure 5). Sampling was done during June 2007. Fish assemblages were assessed using electrofishing equipment. Lakes and wetland areas were sampled using a boat mounted Smith Root 2500 watt DC generator unit. Large to medium size streams (> 8 m wetted width) were assessed using a long-line and backpack electrofishing units. Small steams (< 8 m wetted width) were assessed using a Smith-Root DC generator backpack unit. Sampling of streams was conducted along a linear reach based on 15 times the wetted width bounded by 50 m increments. Sample reach length was a minimum of 50 m (wetted width <3.3) and maximum of 500 m. Lakes and moist soil units were sampled based on 500 m reaches. Lake reaches were selected based on natural shoreline features, which included intact riparian vegetation and bank condition. Lake Linda, Stansfield Lake, and the moist soil unit south of Moss Lake (MSU) had two 500 m sample reaches on separate shores. Approximately 500 m of accessible water at a single site was sampled on Moss Lake.

Species Richness and Composition

Fifty one species of fish representing 14 families were collected from the 15 sample sites (Table 8). Overall, minnows (Cyprinidae), suckers (Catostomidae), sunfish (Centarchidae), and darters were the most dominate families. Fish assemblage structure differed according to stream size and hydrologic characteristics of each environment.

We sampled four lakes including a moist soil unit (lentic waters) on refuge property (Table 7). All four sites are artificial impoundments and three (Lake Linda, Stansfield Lake, and MSU) have been stocked for sport fishing. Sixteen species belonging to eight families were collected from these sites. The most numerically dominate group at Lake Linda, Stansfield Lake, and MSU was Centarchidae. Bluegill (*Lepomis macrochirus*), redear sunfish (*Lepomis microlophus*), and largemouth bass (*Micropterus salmoides*) constituted over three-fourths of the catch with 42.3, 32.9, and 13.6 % of catch respectively. Largemouth bass (56.1%), bowfin (*Amia calva*) (19.7%), and bluegill (13.2%) were the most dominate fish by weight. These three waterbodies remain level year round and are mostly dominated by stocked fish. The water level in Moss Lake is managed according to season and its fish assemblage differed from the other lentic sites. Moss Lake was sampled at low flow conditions and was heavily vegetated with aquatic

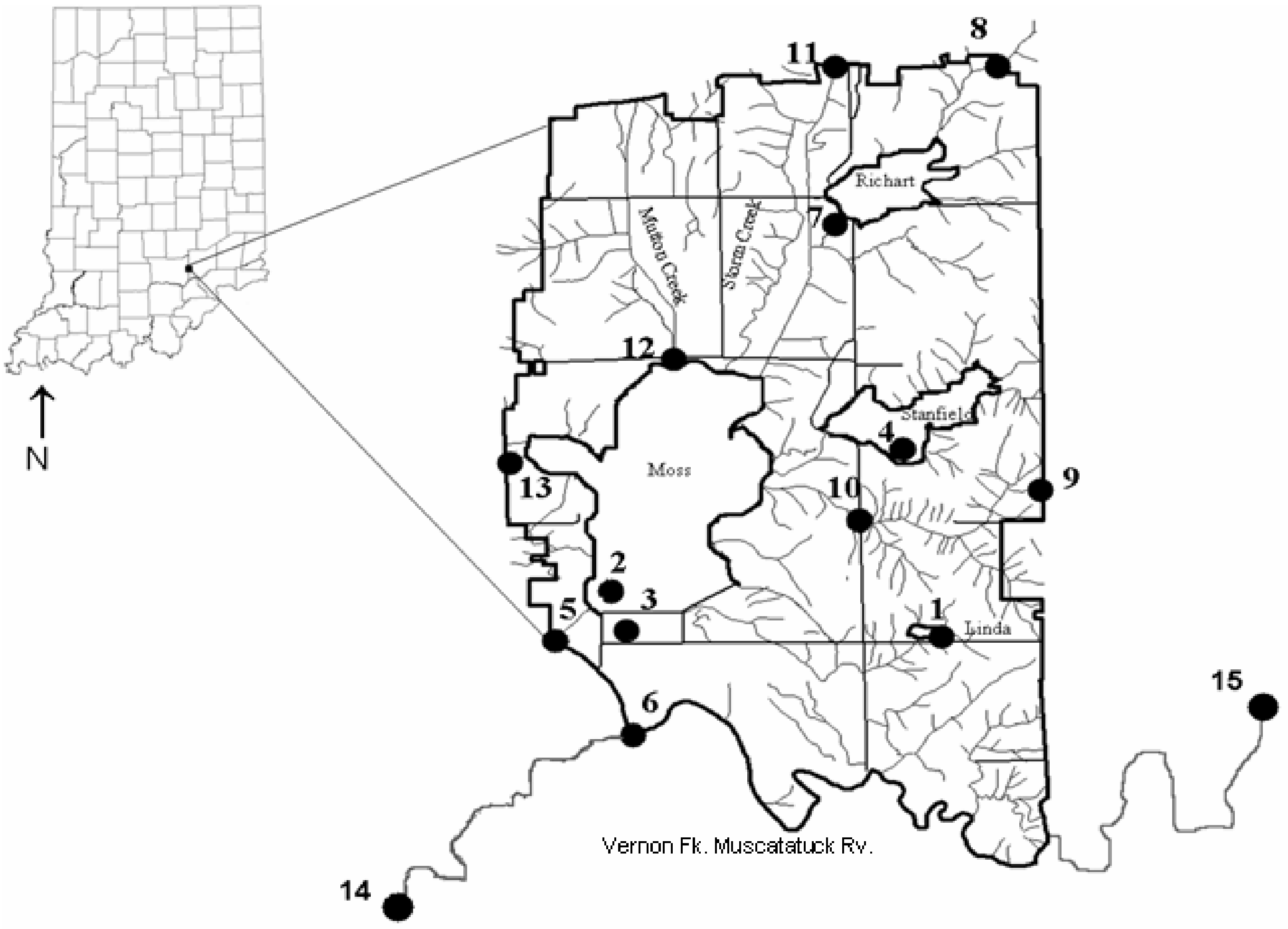

Figure 5. The Muscatatuck NWR consists of 7,724 acres and lies within Jennings and Jackson Counties in southeastern Indiana. Black dots denote sample locations during 2007 and numbers correspond to site numbers in Table 7.

Table 7. List of collection locations during 2007 corresponding to Figure 5 along with Index of Biotic Integrity (IBI) score and classification for each site.

Site	Waterbody	IBI	Classification
Lakes/Moist Soil Units			
1	Lake Linda	32	Fair
2	Moss Lake	33	Fair
3	MSU South of Moss Lake	35	Fair-Good
4	Stansfield Lake	36	Fair-Good
Large-Medium Streams			
5	Mutton Creek	48	Very Good
6	Vernon Fork Muscatatuck River	46	Very Good
14	Vernon Fork Muscatatuck River	52	Exceptional
15	Vernon Fork Muscatatuck River	56	Exceptional
Small Streams			
7	Richart Lake Outlet	26	Poor
8	Richart Lake Tributary	28	Fair
9	Tributary	32	Fair
10	Mutton Creek Tributary	34	Fair
11	Storm Ditch	30	Fair
12	Mutton Creek	28	Fair
13	Sandy Branch	32	Fair

Table 8. Fish species collected from Muscatatuck National Wildlife Refuge during June 2007. Numbers represent site locations corresponding to Table 1 and Figure 1. Numbers in parentheses represent number of individuals collected followed by weight (g) collectively for each species.

Scientific Name	Lake/Moist Soil Impoundments	Large-Medium Streams	Small Streams
Petromyzontidae			
Lampetra appendix		6 (2, -)	11 (1, 10.6)
Amiidae			
Amia calva	2 (17, 21484), 3 (8, 9829)	5 (1, 1500)	
Lepisosteidae			
Lepisosteus osseus		14 (1, 0.5)	
Umbridae			
Umbra limi	2 (1, 0.3)		7 (1, 4.1), 9 (21, 158.3), 11 (3, 27.6), 13 (42, 31)
Esocidae			
Esox americanus	2 (3, 6.4)	6 (4, 16.3)	7 (8, 228.1), 10 (1, 62.3), 11 (3, 149.7), 12 (8, 375), 13 (3, 36.4)
Cyprinidae			
Campostoma anomalum		6 (8, 6.8), 14 (16, 13.7)	8 (2, 6.5)
Cyprinella spiloptera		6 (9, 38.6), 14 (24, 81) 15 (10, 23)	
Cyprinella whippleii		6 (7, 18.4), 14 (24, 73) 15 (8, 18)	
Ericymba buccata		15 (7, 179)	13 (24, 53.5)

Hybopsis amblops		15 (7, 15)	
Lythrurus umbratilis		6 (1, 0.3), 14(1, 0.8) 15 (6, 0.2)	11 (1, 3)
Notemigonus crysoleucas	1 (4, 1.4), 2 (35, 100.6), 4 (4, 84.7)		10 (5, 51.9), 12 (3, 71.5)
Notropis atherinoides		15 (1, 0.1)	
Pimephales notatus		6 (9, 15.4), 14 (11, 33.9) 15 (9, 21.4)	8 (13, 13.7), 11 (3, 1.1), 13(24, 53.5)
Pimephales vigilax		6 (106, 123), 14 (56, 140) 15 (26, 42)	
Semotilus atromaculatus		14 (7, 4.8), 15 (5, 1.9)	8 (258, 357.3), 9 (6, 150.8), 10 (69, 599), 11 (8, 1.6)
Catostomidae			
Catostomus commersonii		5 (7, 2343)	8 (2, 54.2), 11 (3, 1.0)
Hypentelium nigricans		5 (2, 281), 14 (14, 285.5) 15 (14, 540)	
Minytrema melanops	2 (2, 372), 3 (5, 401.5)	5 (38, 12511), 15 (1, 99)	
Moxostoma anisurum		5 (1, 136), 6 (1, 148) 15 (5, 69.4)	
Moxostoma duquesnei	3 (1, 89.2)	5 (4, 1482), 6 (5, 1.2), 14 (1, 44.2), 15 (17, 1172)	
Moxostoma erythrurum		5 (1, 139), 14 (1, 69) 15 (8, 1475)	
Ictaluridae			
Ameiurus melas			9 (2, 132.2)
Ameiurus natalis		5 (1, 69)	7 (1, 82.7), 9 (3, 104.8), 10 (2, 23.1), 12 (1, 20.2)
Ameiurus nebulosus	3 (3, 1284)	15 (1, 188)	

Noturus miurus		6 (2, 7.4), 14 (5, 26.5) 15 (4, 44)	
Aphredoderidae			
Aphredoderus sayanus	2 (1, 0.8)		10 (2, 27.0)
Fundulidae			
Fundulus notatus			13 (1, 1.8)
Poeciliidae			
Gambusia affinis affinis	1 (1, 0.7), 2 (37, 56.6)	14 (1, 1.8)	12 (8, 5.5), 13 (6, 1.5)
Atherinidae			
Labidesthes sicculus			12 (1, 1.9)
Centrarchidae			
Ambloplites rupestris		15 (3, 270)	
Centarchus macropterus	2 (8, 459)	5 (2, 35)	12 (1, 8.2)
Lepomis cyanellus	2	5 (4, 34), 6 (2, 74.2), 14 (6, 72), 15 (2, 15.1)	7 (3, 62.6), 8 (16, 125.6), 9 (7, 125.8), 10 (15, 231), 11 (9, 112.8), 12 (8, 158), 13 (1, 1.9)
Lepomis gulosus	1 (7, 75.1), 2 (3, 132.1), 3 (6, 113.9), 4 (3, 74.6)	5 (18, 394.3), 14 (1, 70.8)	7 (4, 30.1), 9 (6, 136.9), 10 (6, 85.7), 11 (4, 97.7), 12 (4, 173)
Lepomis macrochirus	1 (30, 735.5), 2 (8,459), 3 (127, 4511), 4 (135, 1313)	5 (7, 171), 14 (7, 69.5), 15 (1, 5)	7 (5, 56.4), 9 (6, 110.6), 10 (34, 265.8), 12 (2, 39.7)
Lepomis megalotis		5 (46, 705), 6 (18, 348), 14 (42, 51.6), 15 (139, 1594)	9, 11 (7, 132.1), 13 (1, 9.5)

Lepomis microlophus	1 (159, 878), 2 (1, 3.2), 3 (34, 1012), 4 (62, 777.5)		
Lepomis miniatus	2 (1, 40.1)		
Micropterus punctulatus		6 (16, 4.9), 15 (2, 0.2)	
Micropterus salmoides	1 (23, 3136.6), 2 (4, 956), 3 (19, 15890), 4 (52, 8968.2)	5 (1, 340)	9 (7, 124.6), 10 (1, 26.2), 12 (3, 144.6)
Pomoxis nigromaculatus	3 (6, 646.9), 4 (2, 52.9)	5 (2, 226.9)	
Percidae			
Ammocrypta pellucida		15 (3, 3.9)	
Etheostoma asprigene		5 (1, 5), 6 (1, 5.2)	
Etheostoma blennioides		14 (1, 3.8), 15 (1, 0.4)	
Etheostoma caeruleum		14 (2, 3.8)	
Etheostoma histrio		6 (1, 2.9), 14 (1, 1.8)	
Etheostoma nigrum		6 (1, 0.1), 14 (3, 0.8), 15 (1, 0.3)	11 (1, 0.1), 13 (4, 1.2)
Percina caprodes		5 (6, 92.1), 15 (5, 66)	
Percina maculata		15 (1, 3.5)	
Percina phoxocephala		6 (1, 2.8), 14 (8, 29), 15 (6, 22)	
Percina sciera		6 (3, 17), 14 (1, 6.0), 15 (2, 8)	

macrophytes. In Moss Lake, mosquito fish (*Gambusia affinis affinis*), golden shiner (*Notomigonus crysoleucus*), and bowfin were the most numerically dominate fish; 32.1, 30.4, and 14.8% of catch respectively. Bowfin also constituted 91% of the catch by relative biomass followed by largemouth bass (4.1%) and bluegill (2%).

Four medium-large wadable streams (>8 m wetted width) were sampled. Mutton Creek was sampled upstream of US 31 bridge where it is a channelized and slow flowing stream. Mutton Creek was dominated by centarchid and catostomid species. Longear sunfish (*Lepomis megalotis*) (31.7%), spotted sucker (*Minytrema melanops*) (26.2%), and warmouth (*Lepomis gulosus*) (12.4%) were the most numerically dominate species. spotted sucker (60.7%), white sucker (*Catostomus commersonii*) (11.4%), and bowfin (7.28%) were the most common fish by relative biomass at the Mutton Creek site. In addition to Mutton Creek, the Vernon Fork Muscatatuck River was sampled at three locations; one upstream of the refuge, one on refuge, and one downstream of the refuge. These three sites had similar and diverse fish assemblages (Table 2). Thirty nine species from nine families were collected from the Vernon Fork. The most dominate species by number were longear sunfish (27.9%), bullhead minnow (*Pimephales vigilax*) (26.4%), and spotfin shiner (*Cyprinella spiloptera*) (6%). Longear sunfish (23.7%), golden redhorse (*Moxostoma erythrurum*) (18.4%), black redhorse (*M. duquesnei*) (14.5%), silver redhorse (*M. anisurum*) (10%), and northern hogsucker (*Hypentelium nigricans*) (9.8%) were the dominant species by relative biomass at the three Vernon Fork sites.

Seven small streams (<8 m wetted width) were sampled on refuge property. Many of these streams were channelized or effected by impoundments and were dominated mostly by cyprinid and centarchid species. Twenty three species were collected at these stream sites. Creek chub (*Semotilis atromaculatus*) (50%), central mudminnow (*Umbra limi*) (9.8%), green sunfish (*Lepomis cyanellus*) (8.6%), and bluegill (6%) were the most numerically dominate species. Creek chub was also the most dominate species by mass (21.5%) followed by grass pickerel (*Esox americanus*) (16.5%), and green sunfish (16%).

Rare Species Records.- Several species uncommon within the state were found during this study. The harlequin darter (*Etheostoma histrio*) was thought to be extripated from Indiana until its rediscovery in 1991 within the White River Drainage (Simon and Kiley 1993) and has since been collected from other subwatersheds within the White River including the Patoka River (Simon et al. 1995). The harlequin darter was collected at two sites on the Vernon Fork Muscatatuck River (Table 8). Two individuals were collected over gravel/sand riffles with swift current. These records constitute the furthest removed records for the harlequin darter from the main stem of either fork of the White River.

The eastern sand darter (*Ammocrypta pellucida*) was collected from one site on the Vernon Fork Muscatatuck River (Table 8). The eastern sand darter was also once recognized as state threatened based on limited presence in the state (Simon et al. 1992), but has since been removed from threatened status. The eastern sand darter is still considered rare and is susceptible to impacts of habitat degradation (Simon 1993). Three individuals were collected from one site on the Vernon Fork over shallow, sandy-riffle habitat.

Flier (*Centarchus macropterus*) is a centarchid species largely associated with the southeastern and eastern United States. Its distribution is restricted to the Coastal Plain from the Chesapeake Bay to Eastern Texas and north through the Mississippi Embayment to southern Illinois and Indiana (Smith 1979; Lee et al. 1980). Records for Indiana depict its distribution to be limited to the south western and central portions of the state (Gerking 1945). The Flier was collected from three sites in this study; in the Vernon Fork, from Mutton Creek, and from Moss Lake (Table 8). A total of 11 individuals were collected. These records constitute the furthest north and east collections for the species (Gerking 1945; Lee et al. 1980).

Alien species presence. -- The only non-indigenous species collected on the Muscatatuck National Wildlife Refuge was the western mosquitofish (*Gambusia affinis affinis*). It was collected from Linda (site 1) and Moss lakes (site 2), Mutton Creek, Sandy Branch, and the Vernon Fork Muscatatuck River downstream of the refuge. The western mosquitofish is intentionally stocked for mosquito control. The species is not effective for control mosquitoes in flowing waters and may be only marginally successful in lakes and ponds (Clem and Whitaker 1998).

Assessment of Muscatatuck NWR Streams.- The seven small, wadable streams sampled on refuge ranged from "poor" to "fair" (Table 7) when compared to reference conditions for the Eastern Corn Belt Plain ecoregion. Index of Biotic Integrity scores ranged from 26 to 34 for these stream sites. The low IBI scores are largely a result of hydrologic modifications to the aquatic habitat on refuge to benefit migratory waterfowl and sport fishery. These streams are dominated by sunfish and bass species and lack sensitive sucker and darter species resulting from habitat modification and stocking of lakes for sport fishing.

The larger streams showed higher quality biological conditions. The four larger stream sites ranged from "very good" to "exceptional" (Table 7). Scores ranged from 46 to 56 with two of the Vernon Fork sites scoring "exceptional." These sites supported populations of sensitive minnow species, such as bigeye chub (*Hybopsis amblops*), sucker species including golden redhorse, black redhorse, northern hogsucker; and several sensitive darter species including greenside (*Etheostoma blennioides*), rainbow (*E. caeruleum*), harlequin, logperch (*Percina caprodes*), dusky (*P. sciera*), and eastern sand darters. Hydrologic modifications on refuge have had little impact on the Vernon Fork and the river continues to support a high quality assemblage of native species.

The four lakes ranged from “fair” to “fair-good” (Table 7). Both Lake Linda and Moss Lake were considered “fair”, while the MSU and Lake Stansfield were both considered “fair-good”. The lack of benthic species was the primary reason for the lower sustainability score in Lake Linda and Moss Lake.

The cumulative frequency distributions did not include the lake or pond sites that had been sampled during 2006. Based on probability distributions of biological integrity, index of biotic integrity (IBI) score for the watershed (Figure 6) showed that the two

sampling periods had a wide range of results. The earlier sampling (N=49) showed lower integrity than sites sampled later in the summer (N=30). This was expected as the drought conditions concentrated fish into isolated pools. The two sampling periods showed that site mean site cumulative frequency distribution (CFD_{50}) in the early summer (June) had lower biological integrity with IBI scores of 31, while the CFD_{50} for late summer (August) had biological integrity scores of 40, which was above the statewide average for Indiana. The early summer integrity category would have considered "Poor", while the later summer integrity class would have been considered "fair-good" based on index classification assessments (Karr et al. 1981; Simon and Dufour 1998). Based on the assessment of all 79 sample events collected at Big Oaks, the mean CFD_{50} would have scored 35 using the IBI (Simon and Dufour 1998).

Although the trend has slightly improved between the two surveys periods in 2007, this is most likely a result of not including the lake sites in the IBI statistics and the greater number of higher order streams in the later summer sampling events. Larger streams

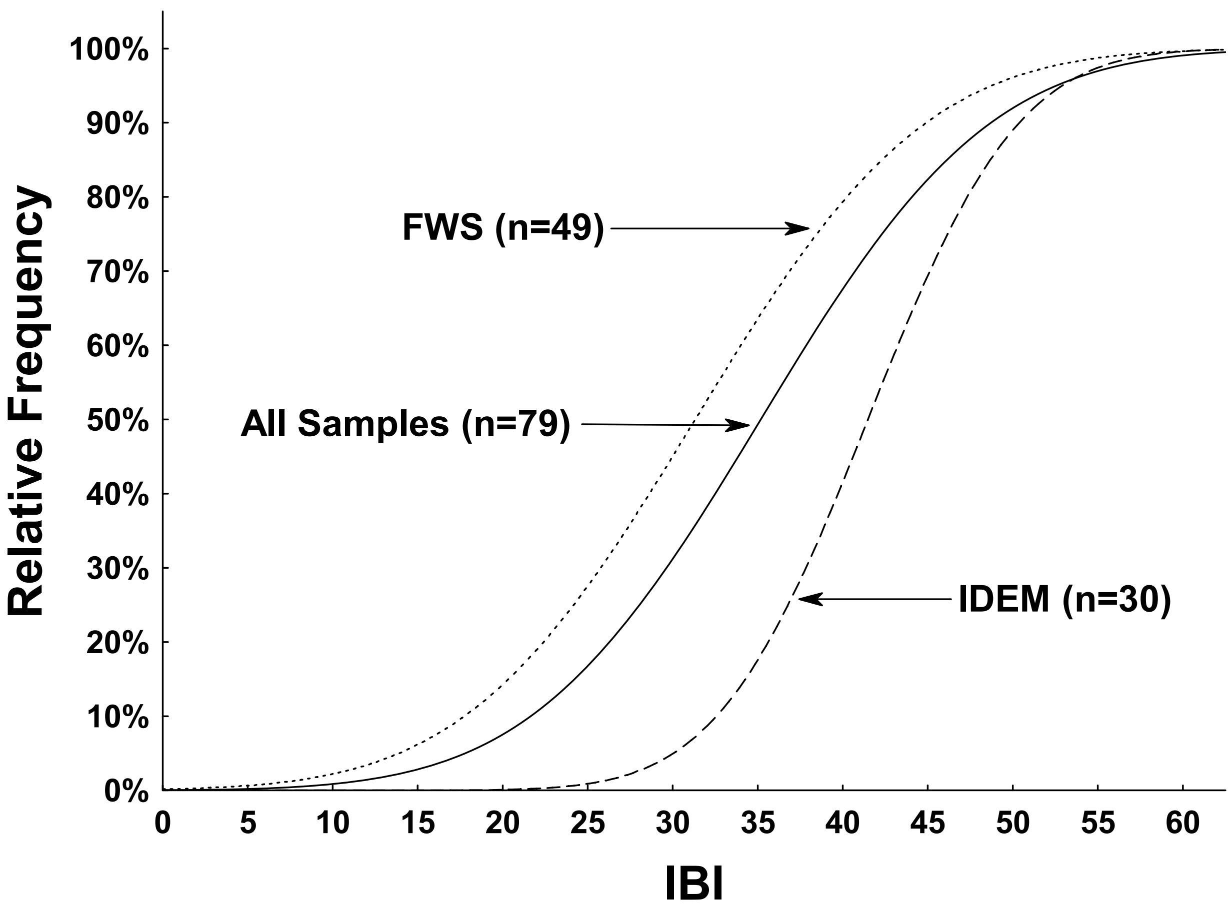

Figure 6. Comparison of Relative IBI Cumulative Distribution Frequency for sites sampled by Fish & Wildlife Service (n=49), the Indiana Department of Environmental Management (n=30), and a combination of all sites (n=79) within and around the Muscatatuck National Wildlife Refuge during 2007.

such as Vernon Fork represented a higher percentage of the 2007 collections compared to 4.08% of the early summer 2007 collections. A different random draw was selected between the two periods so that only a few of the samples included the same sites. The survey results based on 2007 sampling is probably most representative of the variety of aquatic habitat conditions found at Muscatatuck National Wildlife Refuge.

REFERENCES

Clem, P.D., and J.O. Whitaker, Jr. 1996. Distribution of the mosquito fish, *Gambusia affinis* (Baird & Girard), in Indiana, with comments on resource competition. *Proceedings of the Indiana Academy of Science* 104: 249-258.

Gerking, S.D. 1945. Distribution of fishes of Indiana. *Investigations of Indiana Lakes and Streams* 3:1-137.

Karr, J.R. 1981. Assessment of biological integrity using fish communities. *Fisheries* 6: 21-27.

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr. 1980. *Atlas of North American Freshwater Fishes*. North Carolina State Museum of Natural History, Raleigh, NC. Special Publication 1980-12.

Simon, T.P. 1993. Assessment of the range of the threatened darter, *Ammocrypta pellucida* (Putman), from the Maumee River basin, Indiana. *Proceedings of the Indiana Academy of Science* 102:139-145.

Simon, T.P. and R.L. Dufour. 1998. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. V. Eastern Corn Belt Plain*. EPA905/R-96/003. U.S. Environmental Protection Agency, Chicago, IL.

Simon, T.P. and A.L. Kiley. 1993. Rediscovery of the harlequin darter, *Etheostoma histrio* Jordan and Gilbert, in the White River drainage, Indiana. *Proceedings of the Indiana Academy of Science* 102:279-281.

Simon, T.P., S.A. Sobiech, T.H. Cervone, and N.E. Morales. 1995. Historical and present distribution of fishes in the Patoka River basin: Pike, Gibson, and Dubois counties, Indiana. *Proceedings of the Indiana Academy of Science* 104:193-206.

Simon, T.P., J.O. Whitaker, Jr., J.S. Castrale, and S.A. Minton. 1992. Checklist of vertebrate species of Indiana. *Proceedings of the Indiana Academy of Science*. 101:95-126.

Smith, P.W. 1979. *The Fishes of Illinois*. University of Illinois Press. Champaign.

U.S. Fish and Wildlife Service. 2003. *Annual narrative report. Fiscal year 2003*. U.S. Department of the Interior, U.S. Fish and Wildlife Service, National Refuge System, Seymour, IN.

Macroinvertebrate Assemblages of the Patoka River National Wildlife Refuge

No previous survey of macroinvertebrate assemblages has been done near the National Wildlife Refuge or in the Patoka River watershed. The current study is the first comprehensive evaluation of the Patoka River that included taxonomic identifications to lowest possible levels.

Study Sites

Sites sampled during the current study are the same as sampled for fish assemblages (Table 1). These sites include a variety of qualities ranging from high biological integrity to the lowest integrity sites affected by acid mine drainage.

Macroinvertebrates were collected using a single pass d-net method based on stream assessment approaches (Simon and Stewart 1998). The method is based on 20- one (1) minute samples that are referred to as efforts. These twenty efforts are collected from a variety of habitats that represent the variety of physical structures. For example, if cobble riffles represent 20% of the habitat at a site then four efforts would be collected from that habitat type. No more than a single sample is collected from fine substrates. The operator uses jabs, scraping, and swirling techniques while shuffling their feet to dislodge macroinvertebrates. In addition, sampling can include using downstream kicking and dislodging of organisms from cobble and interstitial pore spaces or may include the rubbing of stones from the stream bottom to remove larger invertebrates. Each effort is compiled into a 500 micron mesh bucket and at the completion of the sampling the entire sample is preserved in ethanol with 5% formalin mixed.

In the laboratory, samples are rinsed using mesh screens and placed into a large white pan containing 100 equal sized boxes. Each box measures 50 x 50 mm. The sample is equally distributed across the pan and then using a random number table, numbers are drawn representing individual squares. Each square is sorted and picked until all macroinvertebrates are removed. Squares are picked until a minimum of 300 organisms are removed from the sample. The final square is picked in its entirety once 300 organisms are obtained. At the completion of the 300 organism pick a five minute pick is made for unique large or rare taxa that would not have been collected during the intitial sorting. These individuals are placed into a separate container from the 300 organisms sample.

Individuals are identified to the lowest possible taxonomic level practical, usually genus or species, based on Merrit et al. (2007), Smith (2001), and other appropriate regional texts for state-of-the-art taxonomic references.

Since no macroinvertebrate index has been currently developed for the State of Indiana, no multimetric index could be calculated. The State of Indiana is currently working on

Table 9. List of macroinvertebrate taxa collected during 2006-2007 from the Patoka River drainage. Numbers indicate the sites at which each species has been collected based on information in Table 1 and Figure 1.

Taxa List	2006	2007
Ephemeroptera	67	
Ameletidae		
Ameletus spp.		15, 18, 27
Baetidae	23, 26, 38, 43, 44, 72	
Acerpenna pygmaea		14, 15, 18, 25, 27, 30
Baetis flavistriga	4	14
Baetis intercalaris	3, 19, 26, 34, 36	
Callibaetis spp.	23, 24, 26, 35, 44, 72	30
Centroptilum spp.	5, 8, 16, 17, 20, 42, 43	15, 27, 47, 48, 50
Plauditus dubius		14
Plauditus spp.		15, 18, 22, 27, 29
Procloeon spp.	19	
Pseudocloeon spp.	26, 35, 78	
Caenidae		
Caenis spp.	2-4, 7, 9, 16, 17, 20, 23, 26, 33, 38, 40, 42-44, 51, 63, 72	14, 15, 18, 22, 25, 27, 29-31, 41, 47, 48, 50, 52, 55, 65, 66, 73
Ephemeridae		
Hexagenia limbata	26	
Heptageniidae	5, 34, 76, 77	
Nixe spp.		14, 15, 18, 22, 29, 37, 49, 82
Stenacron interpunctatum	7, 36, 42, 63, 76	
Stenonema femoratum	1-5, 9, 23, 26, 42, 43	14, 15, 18, 22, 25, 27, 29, 30
Leptohyphidae		
Tricorythodes spp.	6, 33-35, 63, 76-78	
Leptophlebiidae		
Paraleptophlebia spp.	1, 2, 10	14, 15, 18, 22, 82, 83
Choroterpes spp.	5	
Siphlonuridae		
Siphlonurus spp.		83
Odonata	9, 20, 23, 38, 40, 43, 44, 67	
Calopterygidae	54	
Calopteryx maculata	39, 40, 44, 51, 63, 77	
Calopteryx spp.		15, 18, 22, 30, 46, 48-50, 56, 59, 64, 66
Hetaerina americana	13, 16, 26	
Hetaerina spp.	40	
Coenagrionidae	1, 9, 19, 20, 23, 26, 33-35, 38, 40, 44, 67, 72, 77	14, 15, 22, 25, 27, 29-31, 41, 47, 48, 50, 52, 55, 56, 59, 62, 66, 73, 82
Argia apicalis-tibialis	6, 7, 9-11, 20, 33, 34, 40, 54, 63, 76-78	
Argia fumipennis	9, 19, 23, 39, 40, 44, 51, 72	
Argia moesta	76	
Argia sedula	26, 40	

Taxa List	2006	2007
Argia spp.	6, 7, 11, 16, 20, 26, 33, 40, 42-44, 76-78	14, 25, 29, 30, 41, 46-48, 50, 52, 53, 56, 62, 64, 66, 73
Enallagma basidens	9, 13, 16, 19, 23, 26, 38, 40, 72	
Enallagma divagans	6, 13, 16, 20, 26, 33, 34, 38-40, 44, 72, 77	
Enallagma exsulans	9, 13, 16, 19, 20, 23, 38, 40, 72	
Enallagma signatum	67	
Enallagma spp.	2, 7, 13, 16, 19, 20, 23, 26, 38-40, 72	
Ischnura posita	9, 33, 63	
Ischnura posita-verticalis	1, 38	
Ischnura spp.	9, 17, 23, 24, 33, 35, 38, 40, 63, 67, 72	25, 27
Aeshnidae		
Aeshna umbrosa	35	
Basiaeschna janata	1-3, 13, 16, 20, 38-40, 42-44, 54, 77	
Boyeria vinosa	1, 40, 44, 51	
Boyeria spp.		41, 46
Nasiaeschna pentacantha	34, 77	
Cordulegastridae		
Cordulegaster spp.		49, 83
Corduliidae		
Epitheca princeps	11, 16, 26, 33, 38, 43	
Epitheca spp.	40, 43	
Somatochlora ensigera	43	
Somatochlora spp.	42, 67	
Libellulidae		
Libellula spp.		14, 29, 56, 59, 62, 73,
Pantala hymenaea	23	
Perithemis tenera	72	
Plathemis lydia	26, 38, 42, 72	
Sympetrum spp.	40	
Macromiidae		
Macromia taeniolata	78	
Macromia spp.	7, 34, 39, 42, 43, 51	55
Gomphidae	54	62
Dromogomphus spinosus	38-40, 44	
Dromogomphus spoliatus	1, 6, 42	
Dromogomphus spp.	11	
Gomphus spp.		64, 66
Progomphus obscurus	44, 51	64
Plecoptera		
Leuctridae		
Leuctra spp.	3	14, 15, 37, 48, 82
Nemouridae		
Amphinemura spp.		14, 18, 22, 29, 37, 41, 46-50, 82, 83, 86
Perlidae		

Taxa List	2006	2007
Acroneuria spp.	3	
Neoperla spp.	3	
Perlodidae	56	
Isoperla spp.		14, 15, 18, 22, 27, 29, 49, 82
Hemiptera		
Belostomatidae		
Belostoma flumineum	23, 26, 35, 38	
Belostoma lutarium		30, 41, 62
Belostoma spp.	1, 34, 72	
Corixidae	1, 11, 23, 36, 38, 43, 67	
Palmacorixa nana	11	
Sigara modesta		15
Sigara spp.	17	
Trichocorixa calva	1, 20, 33, 36, 43, 67	55, 59
Trichocorixa kanza	77, 78	
Trichocorixa spp.	35, 76	
Gerridae	1, 2, 9, 23, 38	
Gerridae larvae		41
Aquarius spp.	3, 8, 17	
Gerris spp.	8, 17, 35, 38-40, 42, 67, 72	
Limnoporus spp.	72, 77	
Rheumatobates palosi	38	
Rheumatobates rileyi	3	
Rheumatobates tenuipes	6	
Rheumatobates spp.	3, 6, 11, 13, 16, 20, 26, 33, 34, 36, 38, 39, 42, 43, 77, 78	
Trepobates pictus	4, 5, 42	
Trepobates subnitidus	6, 9, 13, 16, 19, 20, 38, 72	
Trepobates spp.	3, 5, 7, 9, 13, 16, 19, 20, 26, 42, 72	
Hebridae		
Hebrus spp.	23	
Hydrometridae		
Hydrometra martini	1, 44	
Mesoveliidae		
Mesovelia mulsanti	1, 6, 11, 13, 16, 20, 38, 72	
Naucoridae		
Pelocoris femoratus	38, 67	
Nepidae		
Ranatra buenoi	11, 39	
Notonectidae		
Notonecta irrorata	43, 77	83
Notonecta spp.	24, 36	
Pleidae		
Neoplea striola	9, 11, 13, 20, 33, 38, 67, 72	
Saldidae		
Micracanthia spp.	19	
Veliidae	23	

Taxa List	2006	2007
Microvelia americana	1, 3-5, 8, 13, 17, 35, 39, 40, 42-44, 51, 77	
Microvelia spp.	19, 23, 35, 43, 54, 67, 72	46, 47, 83-86
Rhagovelia obesa	39, 40	
Rhagovelia spp.	76	
Megaloptera		
Corydalidae		
Chauliodes pectinicornis	36	
Chauliodes spp.		70
Nigronia serricornis	3	
Sialidae		
Sialis spp.	1-4, 38, 39, 42-44, 51, 54	53, 62, 83
Trichoptera		
Hydropsychidae		
Cheumatopsyche spp.	3-5, 13, 19, 20, 26, 33, 34, 36, 39, 44, 51, 54, 63, 77, 78	14, 15, 25, 27, 29, 30, 41, 47-50, 52, 55, 64, 65
Hydropsyche betteni		25, 29, 50
Hydropsyche betteni-depravata	19, 51	
Hydropsyche cuanis	36	
Hydropsyche hageni	63	
Hydropsyche simulans	4, 7, 33, 34, 54, 76, 77, 78	
Hydropsyche spp. pupae		22
Hydroptilidae		
Hydroptila spp.	38, 40	25, 27, 47, 48, 50, 53, 55, 59, 64
Oxyethira spp.	44	
Leptoceridae	20, 39	
Nectopsyche candida	77	
Nectopsyche exquisita	34, 78	
Nectopsyche spp.	26, 76, 78	
Oecetis cinerascens	40	
Oecetis spp.	20, 38, 40	14, 41, 56, 64
Limnephilidae		
Ironoquia spp.		18, 22, 27, 29, 41, 46, 49, 82, 84
Philopotamidae		
Chimarra aterrima	4, 19	
Chimarra obscura	19, 51	
Chimarra spp.		41, 47, 48, 50
Phyrganeidae		
Ptilostomis spp.		83, 85
Polycentropodidae		
Cernotina spicata	2, 4	
Neureclipsis crepuscularis	5, 7, 34, 63, 76-78	
Polycentropus spp.		83
Rhyacophilidae		
Rhyacophila spp.		27, 41
Uenoidae		
Neophylax spp.		15

Taxa List	2006	2007
Lepidoptera		
Crambidae		
Acentria spp.	26	
Noctuidae		
Bellura spp.	8	
Coleoptera		
Curculionidae	26, 34	
Dryopidae		
Helichus basalis	39	
Helichus fastigiatus		41
Helichus lithophilus	2, 9, 16, 19, 20, 40, 44, 77	18
Dytiscidae	44	
Acilius fraternus	43	
Agabus semivittatus		84
Agabus spp.		15, 18, 22, 29, 30, 47, 52
Brachyvatus apicatus	72	
Copelatus chevrolati	67	
Copelatus glyphicus	35	
Heterosternuta laetus		82
Heterosternuta pulcher		22, 29
Hydroporus spp.	17, 44	
Laccophilus fasciatus	24, 35, 67	
Laccophilus spp.	23, 24, 35	
Liodessus spp.	23	
(Hydroporinae)		18, 22, 29, 62, 66
Neoporus dimidiatus		55, 65, 66
Neoporus spp.	1-3, 11, 16, 23, 26, 34, 38, 40, 42, 43, 78	
Neoporus undulatus		25, 73, 83
Elmidae		
Ancyronyx variegatus	1, 2, 7, 33, 34, 38, 76-78	
Dubiraphia minima	1-3, 6, 7, 9, 16, 20, 23, 26, 33, 34, 38-40, 43, 44, 54, 63, 76-78	
Dubiraphia quadrinotata	77	
Dubiraphia spp.	23, 26, 38-40, 43, 44	
Dubiraphia spp. larvae and adults		14, 47, 48, 52, 46, 59, 64-66, 70, 73
Macronychus glabratus	7, 11, 33, 34, 38-40, 63, 76-78	
Stenelmis crenata	2-4, 6, 11, 16, 20, 26, 33, 34, 36, 38-40, 51, 63, 76-78	
Stenelmis decorata	51	
Stenelmis quadrimaculata	44	
Stenelmis spp.	3-5, 19, 26, 34, 40, 44, 76	
Stenelmis spp. larvae and adults		14, 18, 22, 25, 27, 29, 30, 41, 46-50, 55, 56, 64
Gyrinidae	44	
Dineutus serrulatus	10, 43	
Dineutus spp.	20	
Dineutus spp. adults		73

Taxa List	2006	2007
Gyretes sinuatus	6, 77	
Gyrinus spp.	35, 42-44	
Gyrinus spp. adults		47, 55
Haliplidae		
Haliplus deceptus		62
Peltodytes dunavani	38, 40	86
Peltodytes duodecimpunctatus	1, 9, 11, 13, 16, 17, 20, 23, 26, 35, 38, 40, 43, 44, 67, 72	15, 18, 29-31, 37, 47, 48, 50, 55, 59, 66, 73
Peltodytes edentulus	23	
Peltodytes lengi	1, 2, 5, 16, 20, 23, 26, 33, 35, 39, 40, 44, 67	
Peltodytes litoralis	44	31
Peltodytes muticus	1, 67	48, 62, 70
Peltodytes pedunculatus	1	
Peltodytes sexmaculatus	16, 20, 35, 67	59, 65
Peltodytes spp.	20, 35, 44, 67	
Peltodytes spp. larvae		59, 62, 66
Heteroceridae	13, 23, 35, 39, 40, 72	
Hydrochidae		
Hydrochus spp.	1, 43, 67	
Hydrophilidae	35, 72	
Berosus infuscatus	2, 67	
Berosus peregrinus	9, 13, 16, 20, 23, 26, 35, 38, 40, 44, 51, 72	
Berosus spp.	9, 16, 20, 23, 26, 34, 38, 40, 44, 51, 72	
Berosus spp. larvae		14, 18, 25, 29, 46, 48, 50, 53, 55, 56, 59, 62, 64, 73
Enochrus pygmaeus nebulosus		22
Enochrus spp.	1, 13, 24, 26, 35, 40, 44	
Hydrobius spp.	35	
Paracymus spp.	8, 17, 35	
Tropisternus glaber	23, 35, 40, 44, 67	
Tropisternus lateralis nimbatus	67	
Tropisternus spp.	16, 17, 23, 24, 26, 39, 44	
Tropisternus spp. larvae		27
Psephenidae		
Psephenus herricki	3-5, 13	
Scirtidae		
Cyphon spp.	1, 3, 5, 6, 9, 10, 23, 26, 33	
Scirtes spp.		46, 70
Diptera		
Chironomidae	1, 3, 4, 11, 13, 23, 26, 33-35, 38, 40, 43, 44, 72	
(Diamesinae)		
Diamesa spp.		14, 15, 18
Potthastia longimana group		29
(Orthocladiinae)	7	
Acricotopus spp.		62

Taxa List	2006	2007
Chaetocladius spp.		15, 49, 55, 82, 84-86
Limnophyes spp.		15, 18, 22, 46, 47, 50
Orthocladius spp.	23, 26, 35, 40	
Parametriocnemus spp.		18, 41
Paraphaenocladius spp.	44	
Pseudorthocladius spp.		85
Smittia spp.		31
(Corynoneurini)		
Corynoneura spp.		15, 18, 22, 49, 59
Thienemanniella spp.		14, 22, 30, 48, 49, 52, 56, 73
(Orthocladiini/Metriocnemini)		
Cricotopus spp.		14, 15, 18, 22, 25, 27, 29, 30, 31, 37, 41, 46-50, 52, 53, 55, 56, 59, 62, 64-66, 70, 73, 82, 84
Cricotopus sylvestris	38	
Cricotopus/Orthocladius spp.	26, 35, 44	
Eukiefferiella spp.		49
Hydrobaenus spp.		15, 18, 22, 47-50, 52, 55, 56, 59, 64, 66, 73
Nanocladius spp.	72	52
Parakiefferiella spp.		47, 62
Psectrocladius spp.		62
Rheocricotopus spp.		82
(Chironominae)	5, 6, 8, 13, 17, 20, 26, 33, 35, 43, 72, 77	
(Chironomini)	5, 8, 19, 23, 33, 35, 38-40, 43	14, 15, 25, 27, 56, 62, 64, 70
Chironomus spp.	1, 8, 24, 34, 35, 39, 43, 44	15, 22, 29, 52, 53, 56, 58, 59, 62, 64, 66, 70, 73, 86
Cladopelma spp.		62
Cryptochironomus spp.	6, 23, 26, 35, 38, 40, 42, 43	25, 50, 56, 64, 66, 73
Cryptotendipes spp.	26, 38, 40, 43	56, 66
Dicrotendipes spp.	5, 9, 13, 16, 19, 20, 23, 26, 35, 38, 40, 42-44, 72	15, 25, 27, 29, 30, 56, 62, 66, 73
Endochironomus spp.	7, 16, 35	52, 62, 73
Glyptotendipes spp.	42, 43, 63	70, 86
Microtendipes spp.	4, 5, 8, 44	22
Parachironomus spp.	6, 7, 16	52
Paralauterborniella spp.	39, 40, 43, 76	70
Paratendipes spp.	42	15, 18, 29, 30, 47, 70
Phaenopsectra spp.	5, 11, 13, 19, 35, 39, 42	56
Polypedilum fallax	13	
Polypedilum spp.	1, 2, 4-7, 9, 13, 16, 19, 20, 23, 26, 33-36, 38-40, 42-44, 51, 63, 67, 72, 77, 78	14, 15, 18, 22, 25, 27, 29-31, 41, 46-49, 52, 55, 56, 64, 66, 73, 83
Stenochironomus spp.		50
Stictochironomus spp.	3, 4, 26, 42, 43	
Tribelos spp.	77	
(Pseudochironomini)		

Taxa List	2006	2007
Pseudochironomus spp.	23, 26	22, 25, 29, 30
(Tanytarsini)	9, 33, 35, 43, 44, 63	
Cladotanytarsus spp.	9, 20, 23, 26, 43, 44, 67	18, 22, 48, 50, 64
Micropsectra spp.		15, 18, 22, 29, 48, 49, 52, 53, 59, 62, 66
Micropsectra/Tanytarsus spp.	3, 8, 13, 20, 23, 26, 34, 35, 38, 40, 43, 44,	
Paratanytarsus spp.	8, 26, 35, 38, 40, 44	14, 27, 29, 59, 62, 64, 66, 70
Rheotanytarsus spp.	26, 33, 34, 40, 43	14, 25, 27, 30, 46, 49, 50, 52, 70
Tanytarsus spp.	16, 20, 26, 35, 38, 40, 43, 44, 51	14, 15, 18, 22, 25, 27, 30, 41, 47, 48, 50, 55, 56, 59, 62, 64, 66, 70, 83
(Tanypodinae)	4, 35	18
(Coelotanypodini)		
Clinotanypus spp.	20, 23, 40, 72	41
(Natarsiini)		
Natarsia baltimoreus	1, 4, 17, 42, 44	
Natarsia spp.		22, 29, 79
(Procladiini)		
Procladius spp.	16, 20, 35, 38-40, 42, 43, 72	52, 55, 59, 62
(Pentanuerini)		
Ablabesmyia janta	2, 6, 7, 11, 33, 34, 63, 76-78	
Ablabesmyia mallochi	3, 5, 7, 17, 26, 33, 35, 38-40, 42-44, 72	
Ablabesmyia spp.		15, 48, 50, 53
Labrundinia becki	1	
Labrundinia pilosella	33, 34, 43, 63	
Labrundinia spp.	40	
Larsia spp.	5, 40	18, 22, 31, 46, 52, 62, 64, 70, 73, 79, 82
Paramerina spp.	35	
Pentaneura spp.		66
Thienemannimyia group	1, 5, 13, 17, 19, 26, 33, 35, 40, 51	14, 15, 18, 22, 25, 27, 29-31, 46-48, 50, 53, 55, 56, 64, 66
Zavrelimyia spp.		15, 37, 48, 79, 82
(Tanypodini)		
Tanypus neopunctipennis	67, 72	
Tanypus spp.		59, 62
Ceratopogonidae	54	47, 50, 53, 55, 56, 59, 62, 64, 66, 70, 85
Atrichopogon spp.	13, 24	
Bezzia-Palpomyia group	2, 3, 40	46, 70
Ceratopogon spp.	33, 35, 38, 40, 43, 54	
Dasyhelea spp.	44	53, 59, 62
Forcipomyia spp.	23	
Probezzia spp.	26	
Serromyia spp.	40	
Chaoboridae		

Taxa List	2006	2007
Chaoborus spp.		18
Culicidae		
Aedes / Ochlerotatus		86
Aedes spp.	24	
Anopheles punctipennis	4, 6, 8, 9, 17, 36	
Anopheles quadrimaculatus	67	
Anopheles spp.	24, 42, 44	
Dixidae		
Dixella spp.	3, 4, 6, 7	
Dolichopodidae		31
Empididae		
Clinocera spp.		14
Hemerodromia spp.	3, 4, 44	14, 47, 48, 50, 56, 64, 66
Ephydridae		27, 59
Simuliidae		
Simulium jenningsi	4	
Simulium tuberosum	3	
Simulium vittatum	51	
Simulium spp.		25, 27, 29, 46, 52, 53, 64-66, 70, 73
Stratiomyidae		
Stratiomys spp.	2, 67	
Tabanidae		29, 37, 41, 50, 53, 62, 83
Chlorotabanus crepuscularis	1, 42	
Chrysops spp.	1, 6, 23, 35, 40, 43, 44	
Tabanus spp.	72	
Tipulidae	26	
Pilaria spp.		49
Pseudolimnophila spp.	4	
Tipula spp.	19, 38	14, 18, 29, 30, 41, 56, 64, 82
Tipulidae pupae		47
Collembola		
Isotomidae	4, 10, 20, 24, 26, 35, 38, 67, 72	
Poduridae	63	
Sminthuridae	23	
Decapoda		
Cambaridae		
Cambarus species "A"	24, 35, 44, 54, 67	48
Cambarus polychromatus	35, 39, 44	
Orconectes immunis	11, 24, 34, 35	14, 15, 29, 30, 37, 56, 65, 70
Orconectes indianensis	1-3, 6, 7, 17, 19, 26, 34, 38-40	22, 25, 29
Orconectes propinquus	63	
Orconectes virilis	5	
Procambarus acutus		59
Procambarus spp.		62
(Cambarinae)		14, 15, 22, 25, 27, 29-31, 37, 41, 47-50, 55, 56, 65, 66, 73, 79, 83, 84
Amphipoda		

Taxa List	2006	2007
Hyalellidae		
Hyalella azteca	1, 2, 26, 38, 40, 63	18, 25, 27, 46-48, 50, 52, 55, 59, 62,
Crangonyctidae		
Crangonyx spp.	11, 40, 63	18, 22, 27, 31, 37, 41, 46-50, 55, 70, 73, 79, 82-84
Synurella dentata		22, 27, 29, 41, 64-66, 82, 83
Isopoda		
Asellidae		
Caecidotea spp.	5, 17, 35, 36, 39, 40, 42, 43, 63, 76	14, 15, 18, 22, 25, 27, 29, 30, 37, 41, 46-50, 52, 53, 55, 56, 58, 59, 62, 64-66, 70, 73, 82-84, 86
Lirceus fontinalis.	1, 3, 35, 36	22, 25, 27, 29-31, 73, 79
Acariformes	4, 11, 35, 67	
Veneroida		
Corbiculidae		
Corbicula fluminea	26, 33, 34, 38-40, 44, 78	
Corbicula spp.		27, 56, 59, 64, 66, 73
Pisidiidae		65, 66
Pisidium spp.	67	15, 25, 27, 29-31, 37, 58, 64, 65, 70
Sphaerium spp.	1, 6, 11, 33, 34, 38, 40, 42, 43, 67, 72, 76-78	56, 58, 59, 65
Gastropoda		
Ancylidae		
Ferrissia spp.	35	
Laevapex spp.	43	
Lymnaeidae	35	22, 30, 31, 37, 47, 48, 56, 59, 62, 73
Fossaria spp.	35, 44, 67	
Planorbidae	35, 38, 40	
Gyraulus spp.		70
Helisoma spp.	1	25, 31, 59, 64, 70
Planorbella spp.	26	
Physidae		
Physa/Physella spp.	1, 8, 9, 13, 16, 17, 19, 20, 23, 24, 26, 33, 35, 38-40, 42-44, 67, 72, 76-78	15, 22, 25, 27, 30, 31, 37, 41, 53, 56, 58, 59, 62, 65, 70, 73
Pleuroceridae		
Elimia spp.	1	
Viviparidae	40	
Campeloma spp.	1	
Oligochaeta		
Branchiobdellida	35	
Enchytraeidae		14, 31, 55, 62
Lumbricidae		15, 25, 30, 37, 47, 58, 73, 79, 83, 84, 86
Lumbriculidae	1, 10, 76	79

Taxa List	2006	2007
Naididae	16, 23, 24, 26, 34, 35, 38-40, 42, 43, 54, 67, 72, 76	
Dero spp.		58, 62, 66
Nais communis		14, 15, 30, 31, 58, 70, 83
Nais pardalis		14, 22, 27, 29-31, 64, 66
Nais variabilis		14, 30, 52, 53, 56, 58, 64, 73
Pristina leidyi		62
Slavina appendiculata		22
Tubificidae	34, 42, 51, 78	
Branchiura sowerbyi	7, 11, 43, 76	22, 29, 30
Limnodrilus spp.		30, 73
Potamothrix bavaricus		50
Tubifex tubifex		62
Tubificidae with hair chaetae		22, 27, 29, 31, 37, 47, 56, 58, 59, 62, 64-66, 70
Tubificidae without hair chaetae		14, 15, 18, 22, 25, 27, 29-31, 37, 41, 49, 50, 52, 55, 56, 58, 59, 62, 64-66, 70, 73, 83
Hirudinea		
Erpobdellidae	35, 38, 43	
Erpobdella punctata		14, 31
Mooreobdella fervida		58
Glossiphoniidae	33, 38, 43, 67	
Nematomorpha		
Paragordius varius	11, 67	
Nemertea		
Prostoma spp.		52, 59
Neuroptera		
Climacia areolaris	33, 77, 78	
Turbellaria	6, 19, 72	15, 82
Dugesia spp.		29, 52

the completion of an index of biotic integrity for macroinvertebrates; however, for the purposes of this study no current index is available.

Species Richness and Composition

During this 2006-2007 investigation of the Patoka River watershed, 355 taxa representing 93 families were collected (Table 9). Dominant families included the Hemiptera and Diptera (12 families), Coleoptera (11 families), Ephemeroptera (8 families).

Among the most diverse taxa was the Diptera or flies and midges (103 taxa), Hemiptera or true bugs (67 taxa), and the Odonata or dragonflies (47 taxa) (Table 9).

Tolerance and Sensitivity

Among the most sensitive taxa in North America are the mayflies, stoneflies, and caddisflies (Merritt et al. 2007). These groups of aquatic macroinvertebrates comprise a sensitive species metric known as the EPT index.

Comparing differences between 2006 and 2007 macroinvertebrate sampling, 2006 sampling found 16 mayfly taxa compared to 11 mayfly taxa in 2007. Unique taxa collected during 2006 included, *Baetis intercalaris, Procloeon* spp., *Psuedocloeon* spp., *Hexagenia limbata, Tricorythodes* spp., and *Choroterpes* spp., while during 2007 *Ameletus* spp., *Acerpenna pygmaea, Plauditus dubius, Plauditus* spp., and *Nixes* spp. were collected (Table 9).

Three stonefly taxa were collected during the inventory including *Acroneuria* spp. and *Neoperla* spp during 2006 and *Isoperla* spp. during 2007 (Table 9).

Caddisfly taxa collected during 2006 and 2007 included 25 taxa (Table 9). During 2006, the following 13 caddisfly taxa were unique including, *Hydrophyche betteni-depravata, Hydrophyche cuanis, H. hageni, H. simulans, Oxyethira* spp., *Nectopsyche candida, N. exquisite, Nectopsyche* spp., *Oecetis cinerascens, Chimarra aterrima, C. obscura, Cernatina spicata,* and *Neurclipsis crepuscularis.* During 2007 the following seven taxa were unique including, *Hydrophysche betteni, Ironoquia* spp., *Chimarra* spp., *Ptilostomis* spp., *Polycentropus* spp., *Rhacophila* spp., and *Neophylax* spp.

REFERENCES

Merritt, R.W., K.W. Cummins, and M.B. Berg (eds). 2007. An Introduction to the Aquatic Insects of North America, 3rd edition. Kendall/ Hunt Publishing Company, Dubuque, Iowa.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th edition. John Wiley and Sons, New York, New York.

Macroinvertebrate Assemblages of the Big Oaks National Wildlife Refuge

No previous surface water survey of macroinvertebrate assemblages has been completed near the National Wildlife Refuge or in the Vernon Fork of the Muscatatuck River watershed in the vicinity of the refuge. The current study is the first comprehensive evaluation of the Big Oaks National Wildlife Refuge that included taxonomic identifications to lowest possible levels.

A subterranean faunal study was completed by Lewis and Rafail (2002). This study documented the cave and spring invertebrate faunas of Big Oaks National Wildlife Refuge. Several cave and spring invertebrate taxa were collected during the surface water surveys.

Study Sites

Sites sampled on the refuge included 19 sites (Figure 7). These sites are the same locations sampled for fish assemblages; however, because of the effort required fewer sites were sampled (Table 10). These sites generally include sites with high biological integrity, which is representative of the region.

Species Richness and Composition

During this 2007 investigation of the Big Oaks National Wildlife Refuge watersheds, 163 taxa representing 66 families were collected (Table 11). Dominant families included the Hemiptera, Diptera, and Odonata (8 families), and Coleoptera and Ephemeroptera (7 families). Among the most diverse taxa was the Diptera or flies and midges (35 taxa), Hemiptera or true bugs (25 taxa), Odonata or dragonflies (24 taxa), and Ephemeroptera or mayflies (18 taxa)(Table 11).

Lewis and Rafail (2002) reported that bluegrass spring isopod (*Lirceus fontinalis*) was collected from caves in the Middle Fork, Big Creek, and Graham Creek watersheds. This species rank is S3/G4. The species ranges from southern Indiana, Kentucky, southwestern Ohio, and northern Tennessee.

The toothed spring amphipod (*Synurella dentata*) was collected by Lewis and Rafail (2002) from Big Creek from Three Raiders Monument. This species rank is S4/G5 and is ubiquitous in springs and some caves.

Table 10. Site locations sampled during 2006-2007 in the Big Oaks National Wildlife Refuge for Macroinvertebrate Assemblage. Site numbers correspond to Table 2 and are shown in Figure 1. Source codes are as follows: a - Fish and Wildlife Service (2006), b - Indiana Department of Environmental Management (2007).

Site No.	Source	County	Locality	Latitude	Longitude
5	a	Ripley	Little Otter Creek @ Shaped Charge Rd	39º 1.37'	-85º 26.21'
7	a, b	Jennings	Rush Branch @ W Perimeter Rd	38º 59.58'	-85º 28.04'
8	b	Jennings	Graham Creek @ W Perimeter Rd	38º 58.65'	-85º 28.06'
9	b	Jennings	Graham Creek @ J Rd	38º 58.83'	-85º 27.16'
10	a, b	Ripley	Big Graham Creek @ K Rd and NE Exit Rd	39º 0.47'	-85º 22.99'
11	a, b	Ripley	Graham Creek @ East Outlet Rd	39º 0.49'	-85º 22.22'
12	a, b	Jennings	Little Graham Creek @ W Perimeter Rd Area 33	38º 56.3'	-85º 27.75'
13	a, b	Ripley	Little Graham Creek @ J Rd Ford	38º 59.12'	-85º 23.89'
15	a, b	Ripley	Little Graham Creek @ East Outlet Rd	38º 59.97'	-85º 21.65'
16	a	Jefferson	Marble Creek @ F Rd	38º 54.78'	-85º 27'
18	a	Ripley	Marble Creek @ Morgan Rd	38º 55.91'	-85º 25.85'
19	a	Ripley	Marble Creek @ Center Recovery Rd	38º 56.13'	-85º 25.1'
20	a	Jefferson	Big Creek @ W Perimeter Rd	38º 53.55'	-85º 27.72'
23	a	Jefferson	Unnamed Trib of Big Creek @ E Rd	38º 53.9'	-85º 26.99'
25	a	Jefferson	Big Creek @ Jinestown Rd	38º 53'	-85º 27.22'
27	a	Ripley	Unnamed Trib of Big Creek @ Cottrell Rd	38º 55.23'	-85º 23.66'
29	a	Ripley	Big Creek @ E Perimeter Rd	38º 55.88'	-85º 21.92'
30	a	Jefferson	Middle Fork Creek @ Jinestown Rd	38º 51.33'	-85º 27.19'
32	a	Jefferson	Harberts Creek @ CR 100 W	38º 49.26'	-85º 24.34'

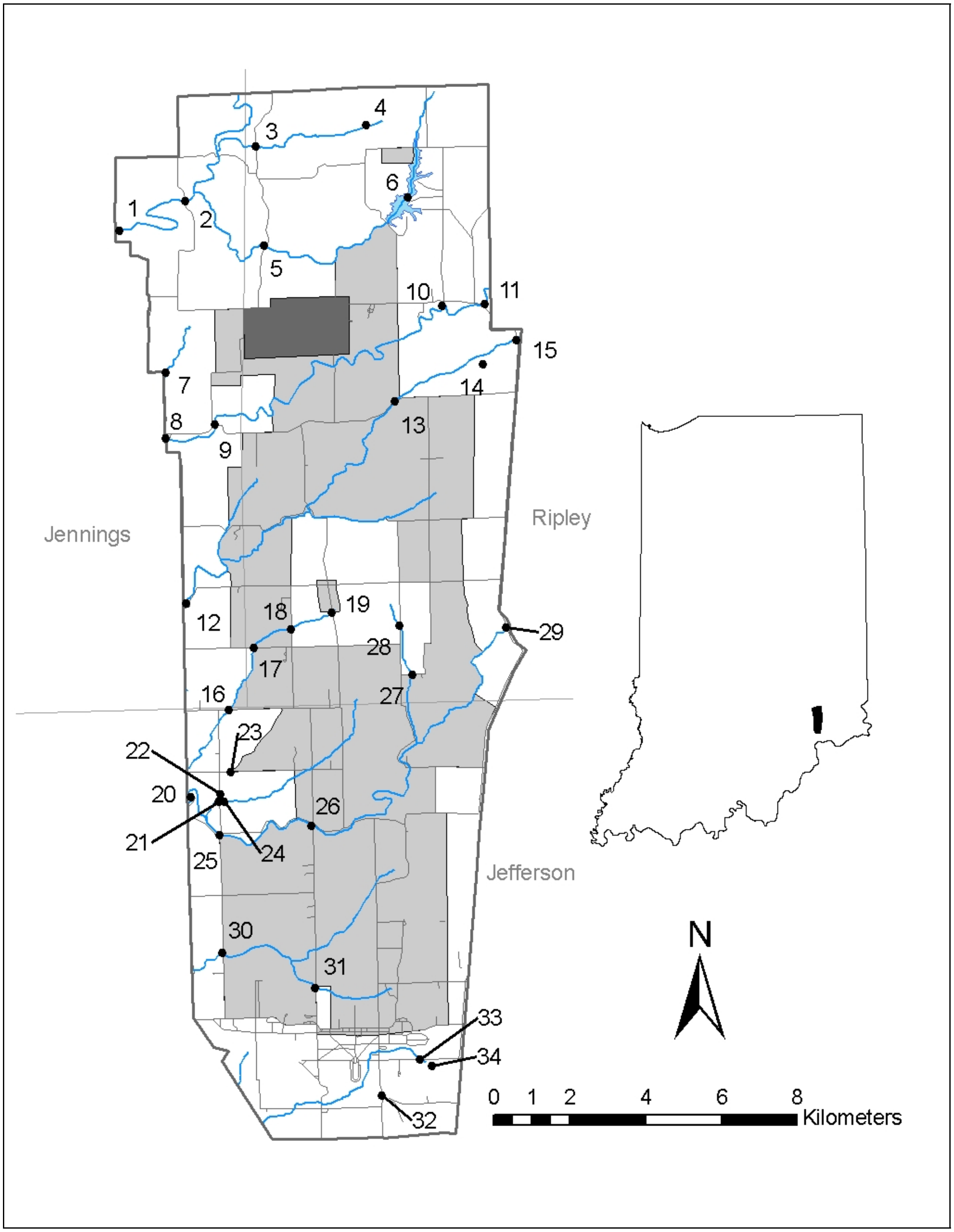

Figure 7. Distribution of sites during 2006-2007 in the Big Oaks National Wildlife Refuge. Numbers refer to site location in Table 10.

Table 11. List of macroinvertebrate taxa collected during 2006-2007 from the Big Oaks National Wildlife Refuge. Numbers indicate the sites at which each species has been collected based on information in Table 10 and Figure 7.

Taxa List		2006	2007
Ephemeroptera			
Baetidae			13, 15
	Baetis flavistriga	5	
	Baetis intercalaris	20	
	Centroptilum spp.	29	15
Caenidae			
	Caenis spp.	10, 12, 13, 15, 16, 19, 20, 25, 29, 30	8-13, 15
Ephemeridae			
	Ephemera spp.	7, 10-12, 20, 25, 27, 30	
	Ephemera simulans		7-10, 12
	Hexagenia spp.	30	11
Ephemererellidae			
	Attenella attenuata		8
Heptageniidae			8, 9, 12
	Leucrocuta spp.	11, 20, 25	
	Maccaffertium pulchellum	5, 11, 25	
	Stenacron spp.	5, 11, 20, 27, 30	
	Stenacron interpunctatum		9, 11
	Stenonema femoratum	7, 10-13, 15, 16, 20, 25, 29, 30	7-13, 15
Isonychiidae			
	Isonychia spp.	12, 20, 25, 30	
Leptophlebiidae			
	Paraleptophlebia spp.		7-12, 15
	Choroterpes basalis	20, 29	
Odonata			
Calopterygidae			
	Calopteryx maculata		12
Coenagrionidae		7, 15	15
	Argia spp.	11, 12, 20	9, 12
	Argia fumipennis		9
	Argia moesta		9
	Enallagma basidens		13
	Enallagma divagans		8, 10, 12, 13
	Ischnura spp.		10
Aeshnidae			
	Basiaeschna janata	13, 15	15
	Boyeria spp.	12	
Cordulegastridae			
	Cordulegaster spp.	12, 32	
Corduliidae / Libellulidae		15	
Corduliidae			

Taxa List	2006	2007
Somatochlora spp.	7, 18, 27, 32	15
Libellulidae		
Libellula spp.	13, 19	13
Libellula luctuosa		15
Pachydiplax longipennis		13
Perithemis tenera	13	
Plathemis lydia		13, 15
Macromiidae		
Didymops transversa		13
Gomphidae	13, 30	
Dromogomphus spinosus		7, 9, 12, 15
Gomphus spp.	5, 11, 30	
Hagenius brevistylus	11, 30	
Progomphus obscurus	10	
Plecoptera		
Perlidae		
Acroneuria spp.	5, 12, 30	
Acroneuria evoluta		9
Leuctridae		
Leuctra spp.	5	
Hemiptera		
Belostomatidae		
Belostoma flumineum		13
Corixidae larvae		15
Sigara spp.		15
Sigara modesta	16	
Trichocorixa calva		15
Gerridae larvae	15	
Aquarius spp.		7
Aquarius remigis	23	
Trepobates spp.	29	
Trepobates subnitidus		13
Mesoveliidae		
Mesovelia mulsanti		8
Nepidae		
Ranatra buenoi		12
Notonectidae		
Notonecta irrorata		13
Pleidae		
Neoplea striola		10
Veliidae		
Microvelia americana		7, 8, 12
Megaloptera		
Corydalidae		
Corydalus cornutus	5, 11-13, 20, 25	8
Chauliodes pectinicornis		15

Taxa List	2006	2007
Nigronia spp.	5, 7, 11-13, 15, 20, 25, 30	
Sialidae		
Sialis spp.	7, 10, 11, 13, 15, 16, 27, 30	7, 8, 10, 12, 13
Trichoptera		
Hydropsychidae		
Cheumatopsyche spp.	5, 11, 12, 20	8
Hydropsyche betteni	5	
Helicopsychidae		
Helicopsyche borealis	7, 25	7, 15
Leptoceridae		
Ceraclea flava		8, 9
Philopotamidae		
Chimarra spp.	5, 11, 20, 30	
Polycentropodidae		
Cernotina spicata		8, 10
Coleoptera		
Dryopidae		
Helichus basalis	10, 12, 20, 25, 27, 30	7, 8, 12
Helichus lithophilus	12, 20, 25	9, 12
Dytiscidae		
Acilius fraternus	18	
Agabus gagates	18	
Laccophilus fasciatus rufus	27	
Laccophilus maculosus		13
Neoporus spp.		7, 9, 11-13, 15
Elmidae		
Dubiraphia spp. larvae		8, 9, 13, 15
Dubiraphia minima		8-10, 13, 15
Macronychus glabratus	10	
Stenelmis spp.	5, 13, 20, 25, 29	8-10
Stenelmis sexlineata		8-10
Haliplidae		
Peltodytes duodecimpunctatus	13, 15, 16, 23, 27, 29	8, 12, 13, 15
Peltodytes lengi		8, 13, 15
Peltodytes muticus		15
Peltodytes pedunculatus		13
Peltodytes sexmaculatus		13
Hydrophilidae		
Berosus spp.		13, 15
Berosus peregrinus		15
Tropisternus glaber		8, 15
Tropisternus natator	27	
Psephenidae		
Ectopria spp. larvae	11, 23	

Taxa List	2006	2007
Psephenus herricki larvae	5, 7, 10-12, 18, 20, 23, 25, 29, 30	7-9, 11, 12
Scirtidae		
Cyphon spp.		10, 12, 13
Curculionidae		7
Diptera		
Chironomidae		7, 15
(Chironominae)		15
(Chironomini)		7, 10-12
Chironomus spp.	18	10, 11
Dicrotendipes spp.	29	
Glyptotendipes spp.	30	9, 10, 12, 15
Microtendipes spp.	5, 7, 12, 20	7, 9, 10
Paratendipes spp.	11, 23	
Polypedilum spp.	5, 13, 20, 30	7, 10, 11
Stictochironomus spp.	7, 11, 12, 16, 27, 29, 32	7, 8, 10, 12, 15
(Tanytarsini)		15
Cladotanytarsus spp.	13	10, 11
Rheotanytarsus spp.	5	
Stempellinella spp.		9
Tanytarsus spp.	11, 13, 29, 30	13, 15
(Orthocladiinae)		
Corynoneura spp.		7, 8, 15
Nanocladius spp.	20	
(Tanypodinae)		15
Natarsia baltimoreus		7
Procladius spp.		11
Thienemannimyia group		8
Ceratopogonidae	19	
Dixidae		12
Ptychopteridae		
Bittacomorpha spp.	18	
Sciomyzidae		7, 9
Tabanidae	5, 20	
Chrysops spp.		13
Tabanus spp.		8
Tipulidae		7
Hexatoma spp.	5, 7, 12, 20	8
Limnophila spp.		9
Pilaria spp.	11	12
Tipula spp.		8
Stratiomyidae		
Stratiomys spp.		7, 13
Lepidoptera		
Crambidae		8
Pyralidae	19	
Orthoptera		

Taxa List	2006	2007
Acrididae		
Metaleptea brevicornis		13
Collembola		
Isotomidae		9
Decapoda		
Cambaridae		
Cambarus species "A"	18	
Orconectes juvenilis		9-12
Orconectes juvenilis x virilis		12
Orconectes rusticus	11	9
Orconectes sloanii	5, 7, 10-13, 15, 16, 20, 23, 25, 27, 29, 30, 32	7-10, 12
Orconectes virilis		13
Amphipoda		
Hyalellidae		
Hyalella azteca	15	8, 9, 12, 13, 15
Crangonyctidae		
Synurella dentata	29	
Isopoda		
Asellidae		
Lirceus fontinalis	13, 20	15
Acariformes		13, 15
Bivalvia		
Unionidae	10	
Veneroida		
Corbicula fluminea		8, 11
Corbicula spp.	12	
Pisidium spp.	10, 18, 19, 27, 29	
Sphaerium spp.	5, 7, 11-13, 15, 20, 25, 30, 32	7-11, 13
Gastropoda		
Ancylidae	7	
Ferrissia spp.		10
Planorbiidae		
Helisoma spp.	13, 15, 27, 29, 30	13, 15
Planorbella spp.		13
Physidae		
Physa/Physella spp.	7, 13, 15, 16, 18, 20, 23, 27, 32	13, 15
Pleuroceridae		
Elimia spp.	5, 7, 10-13, 15, 20, 25, 30	7-13, 15
Branchiobdellida		7, 10, 11, 13
Oligochaeta		8-13, 15
Tubificidae without hair chaetae	11, 13, 19	
Lumbricidae	18	
Naididae	13	

Taxa List	2006	2007
Chaetogaster limnaei	13	
Nais variabilis	25	
Pristina spp.	16	
Slavina appendiculata	16	

Sensitivity and tolerance

Comparisons between 2006 and 2007 macroinvertebrate sampling showed that 12 mayfly taxa were found at the Big Oaks National Wildlife Refuge (Table 11). The 2006 sampling found 12 taxa including *Baetis flavistriga, B. intercalaris, Ephemera* spp., *Leucrocuta* spp.. *Maccaffertium pulchellum, Stenacron* spp., *Isonychia* spp., and *Choroterpes basalis*, which were unique. During 2007 *Ephemera simulans, Attenella attenuate, Stenacron interpunctatum, Paraleptophlebia* spp. were unique mayfly taxa collected.

Three stonefly taxa were collected during 2006-2007 surveys including *Acroneuria* spp., and *Leuctra* spp. during 2006 and *Acroneuria evoluta* during 2007.

Six caddisfly species were collected during surveys at Big Oaks National Wildlife Refuge during 2006-2007 (Table 11). Four taxa were unique with two collected in 2006 (*Hydrophyche betteni* and *Chimarra* spp.) and two in 2007 (*Cerclea flava* and *Cernotina spicata*).

REFERENCES

Lewis, J.J. and S.T. Rafail. 2002. *The subterranean fauna of the Big Oaks National Wildlife Refuge.* Department of the Interior, U.S. Fish and Wildlife Service, Big Oaks National Wildlife Refuge, Madison, Indiana.

Macroinvertebrate Assemblages of the Muscatatuck National Wildlife Refuge

No previous survey of macroinvertebrate assemblages has been done near the National Wildlife Refuge or in the Vernon Fork of the Muscatatuck River watershed. The current study is the first comprehensive evaluation of the Muscatatuck National Wildlife Refuge that included taxonomic identifications to lowest possible levels.

Study Sites

Eleven sites were sampled during the current study (Figure 8), which are the same as sampled for fish assemblages (Table 12). These sites generally include sites with high biological integrity, which is representative of the region.

Species Richness and Composition

During this 2007 investigation of the Muscatatuck National Wildlife Refuge watersheds, 2,505 individuals representing 96 taxa and 45 families were collected (Table 12 and 13). Dominant families included the Ephemeroptera (6 families), Odonata and Coleoptera (5 families), and Diptera (4 families). Among the most diverse taxa was the Diptera or flies and midges (26 taxa), Coleoptera (10 taxa), and Ephemeroptera and Odonata (9 taxa) (Table 13).

The three most dominant taxa in the refuge included isopods and amphipods (Table 14). The dominant taxa included *Lirceus fontinalis* (24%), *Synurella dentata* (16%), and *Hyalella azteca* (14%). These species are considered to have a wide habitat tolerance. Both *Lirceus fontinalis* and *Synurella dentata* are cave spring species that are associated with karst habitats (Lewis and Rafail 2002). *Hyallela azteca* is an epibenthic detritivore that occurs in a wide range of habitats (Hargrave 1970).

Sensitivity and Tolerance

The Ephemeropter, Plecoptera, and Tricoptera (EPT) taxa are considered among the most sensitive groups of aquatic macroinvertebrates in North American streams (Merritt et al. 2007).

Nine mayfly taxa were collected from the Muscatatuck National Wildlife Refuge (Table 14). Taxa sensitive to water quality degradation included *Acerpenna macdunnoughi, Eurylophella.* Intermediate mayfly taxa sensitive to degradation includes *Plauditus* and *Leptophlebia. Caenis, Callibaetis, Stenacron, Siphlonurus,* and *Stenonema femoratum* are considered tolerant members of the mayfly group (Barbour et al. 1999). These species are capable of tolerating warm water and lower dissolved oxygen levels.

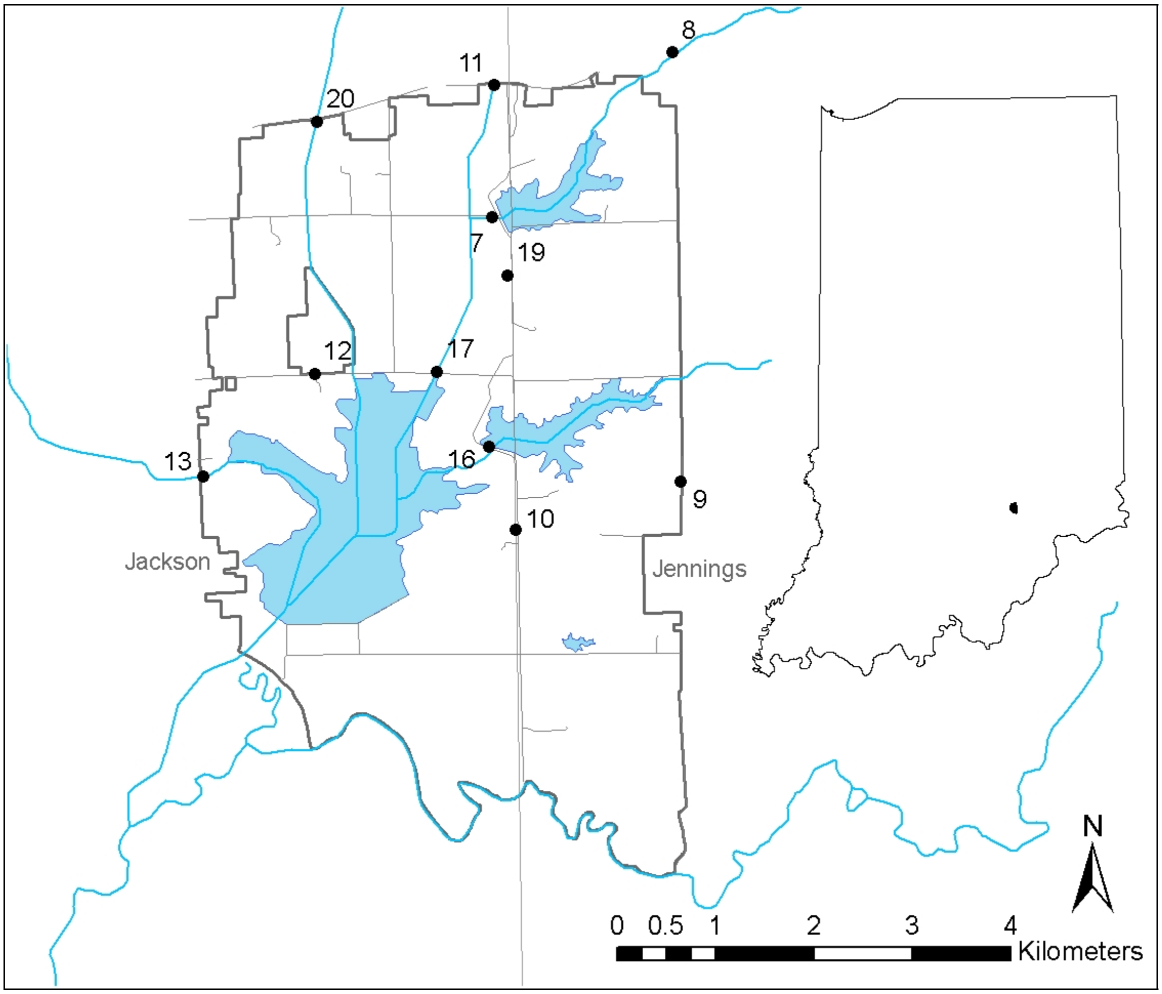

Figure 8. Distribution of macroinvertebrate sites during 2007 in the Muscatatuck National Wildlife Refuge. Numbers refer to site location in Table 12.

Table 12. Site locations sampled for macroinvertebrates during 2007 in the Muscatatuck National Wildlife Refuge. Site numbers correspond to Table 2 and are shown in Figure 1.

Site No.	County	Locality	Latitude	Longitude
7	Jackson	Richart Lake Outlet @ D/S CR 1300 E	38º 57.29'	-85º 47.95'
8	Jennings	Richart Lake Trib @ D/S CR 900 W	38º 58.18'	-85º 46.66'
9	Jennings	Unnamed Trib @ D/S CR 900 W	38º 55.82'	-85º 46.64'
10	Jennings	Unnamed Trib Storm Creek Ditch @ CR 1300 E	38º 55.57'	-85º 47.81'
11	Jackson	Storm Creek Ditch @ U/S US 50 Bridge	38º 58.01'	-85º 47.92'
12	Jackson	Mutton Creek Original Channel @ CR 400 N	38º 56.44'	-85º 49.21'
13	Jackson	Sandy Branch @ D/S US 31	38º 55.88'	-85º 50'
16	Jackson	Stanfield Lake Outlet @ CR 1300 E	38º 56.02'	-85º 47.99'
17	Jackson	Storm Creek Ditch @ CR 400 N	38º 56.44'	-85º 48.35'
19	Jackson	Unnamed Trib of Storm Creek @ CR 1300 E	38º 56.96'	-85º 47.84'
20	Jackson	Mutton Creek Ditch @ D/S US 50 Bridge	38º 57.82'	-85º 49.17'

Table 13. List of macroinvertebrate taxa collected during 2007 from the Muscatatuck National Wildlife Refuge. Numbers indicate the sites at which each species has been collected based on information in Table 1 and Figure 1.

Taxa List		Site
Ephemeroptera		
Baetidae		
	Acerpenna macdunnoughi	8, 11
	Callibaetis spp.	16, 17
	Plauditus spp.	11
Caenidae		
	Caenis spp.	7, 8, 16
Ephemerellidae		
	Eurylophella spp.	11, 12
Heptageniidae		
	Stenacron spp.	11
	Stenonema femoratum	11
Leptophlebiidae		
	Leptophlebia spp.	12, 17, 20
Siphlonuridae		
	Siphlonurus spp.	20
Odonata		
Calopterygidae		
	Calopteryx spp.	8, 13, 19
Coenagrionidae		13, 16, 17
	Argia spp.	13
Aeshnidae		
	Basiaeschna janata	13
	Boyeria spp.	13
Libellulidae		17
	Erythemis simplicicollis	17
	Pachydiplax longipennis	7, 12
Corduliidae		
	Tetragoneuria spp.	16
Plecoptera		
Perlodidae		
	Isoperla spp.	11
Nemouridae		
	Amphinemura spp.	11, 19
Hemiptera		
Belostomatidae		
	Belostoma lutarium	17
Nepidae		
	Ranatra nigra	16
Naucoridae		
	Pelocoris femoratus	17
Trichoptera		
Hydropsychidae		

Taxa List	Site
Cheumatopsyche spp.	9, 11, 13
Hydropsyche spp.	9
Limnephilidae	
Ironoquia spp.	8-10, 19, 20
Pycnopsyche spp.	12
Phyrganeidae	
Ptilostomis spp.	17
Rhyacophilidae	
Rhyacophila spp.	11, 19
Coleoptera	
Dytiscidae	
Acilius spp. larvae	20
Neoporus undulatus	12, 16
(Hydroporinae)	16
Gyrinidae	
Dineutus spp. adults	13
Haliplidae	
Peltodytes dunavani	9
Peltodytes duodecimpunctatus	13, 16
Peltodytes muticus	12, 17, 20
Peltodytes spp. larvae	17
Hydrophilidae	
Hydrochara spp. larvae	10
Hydrochara soror	20
Scirtidae	
Scirtes spp.	20
Diptera	
Chironomidae	
(Chironominae)	
(Chironomini)	
Chironomus spp.	9, 17, 20
Dicrotendipes spp.	7, 11
Endochironomus spp.	16, 17
Glyptotendipes spp.	7, 11, 16, 17
Parachironomus spp.	16
Paratendipes spp.	11
Polypedilum spp.	7-9, 11, 13, 16, 17
Saetheria tylus	8
(Tanytarsini)	
Cladotanytarsus spp.	11
Micropsectra spp.	9
Paratanytarsus spp.	7
Tanytarsus spp.	7, 10
(Orthocladiinae)	
Corynoneura spp.	9
Cricotopus spp.	7-9, 11, 13, 16

Taxa List	Site
Hydrobaenus spp.	8, 9, 11, 13
Nanocladius spp.	7
(Tanypodinae)	
Ablabesmyia spp.	16, 17
Clinotanypus spp.	12
Labrundinia spp.	17
Larsia spp.	16
Procladius spp.	16
Tanypus spp.	17
Thienemannimyia group	8, 9
Simuliidae	
Simulium spp.	7, 11
Tabanidae	9
Tipulidae	
Tipula spp.	11, 13
Lepidoptera	
Pyralidae	17
Decapoda	
Cambaridae	
Cambarus species “A”	7, 19, 20
Amphipoda	
Hyalellidae	
Hyalella azteca	7, 12, 16, 17, 19
Crangonyctidae	
Crangonyx spp.	8-10, 12, 13, 16, 19, 20
Synurella dentata	8-11, 13, 19, 20
Isopoda	
Asellidae	
Caecidotea spp.	9, 10, 12, 13, 20
Lirceus spp.	7-13, 16, 19, 20
Veneroida	
Corbicula spp.	13
Pisidium spp.	12
Sphaerium spp.	7, 12, 16, 17, 19
Gastropoda	
Planorbiidae	
Helisoma spp.	11
Physidae	
Physa spp.	7, 9-13, 16, 20
Gyraulus spp.	7, 12, 16
Lymnaeidae	9, 11-13, 20
Oligochaeta	
Enchytraeidae	7, 11
Lumbriculidae	12
Naididae	
Chaetogaster diaphanus	9, 16

Taxa List	Site
Dero spp.	7, 12, 16
Nais communis	9, 12, 13, 16, 19, 20
Nais pardalis	13, 16
Ophidonais serpentina	7
Slavina appendiculata	9, 16
Stephensoniana tandyi	7
Tubificidae	
Tubificidae without hair chaetae	12, 16, 19
Tubificidae with hair chaetae	12
Quistradrilus multisetosus	16
Hirudinea	
Erpobdellidae	
Erpobdella punctata	9
Mooreobdella microstoma	20
Glossiphoniidae	12
Turbellaria	
Planariidae	
Dugesia spp.	7, 16
Cnidaria	
Hydra spp.	7, 16

Table 14. Summary of macroinvertebrate data collected during 2007 from the Muscatatuck National Wildlife Refuge.

Taxa List	Count	%
Ephemeroptera		
Baetidae		
Acerpenna macdunnoughi	4	<1%
Callibaetis spp.	2	<1%
Plauditus spp.	2	<1%
Caenidae		
Caenis spp.	11	<1%
Ephemerellidae		
Eurylophella spp.	4	<1%
Heptageniidae		
Stenacron spp.	1	<1%
Stenonema femoratum	5	<1%
Leptophlebiidae		
Leptophlebia spp.	50	2%
Siphlonuridae		
Siphlonurus spp.	2	<1%
Odonata		
Calopterygidae		
Calopteryx spp.	21	1%
Coenagrionidae	10	<1%
Argia spp.	5	<1%
Aeshnidae		
Basiaeschna janata	1	<1%
Boyeria spp.	1	<1%
Libellulidae	1	<1%
Erythemis simplicicollis	11	<1%
Pachydiplax longipennis	2	<1%
Corduliidae		
Tetragoneuria spp.	1	<1%
Plecoptera		
Perlodidae		
Isoperla spp.	1	<1%
Nemouridae		
Amphinemura spp.	2	<1%
Hemiptera		
Belostomatidae		
Belostoma lutarium	2	<1%
Nepidae		
Ranatra nigra	2	<1%
Naucoridae		
Pelocoris femoratus	10	<1%
Trichoptera		
Hydropsychidae		
Cheumatopsyche spp.	3	<1%

Taxa List	Count	%
Hydropsyche spp.	1	<1%
Limnephilidae		
Ironoquia spp.	9	<1%
Pycnopsyche spp.	1	<1%
Phyrganeidae		
Ptilostomis spp.	1	<1%
Rhyacophilidae		
Rhyacophila spp.	2	<1%
Coleoptera		
Dytiscidae		
Acilius spp. larvae	1	<1%
Neoporus undulatus	2	<1%
(Hydroporinae)	1	<1%
Gyrinidae		
Dineutus spp. adults	1	<1%
Haliplidae		
Peltodytes dunavani	1	<1%
Peltodytes duodecimpunctatus	3	<1%
Peltodytes muticus	4	<1%
Peltodytes spp. larvae	3	<1%
Hydrophilidae		
Hydrochara spp. larvae	1	<1%
Hydrochara soror	1	<1%
Scirtidae		
Scirtes spp.	1	<1%
Diptera		
Chironomidae		
(Chironominae)		
(Chironomini)		
Chironomus spp.	5	<1%
Dicrotendipes spp.	3	<1%
Endochironomus spp.	12	<1%
Glyptotendipes spp.	11	<1%
Parachironomus spp.	3	<1%
Paratendipes spp.	1	<1%
Polypedilum spp.	33	1%
Saetheria tylus	1	<1%
(Tanytarsini)		
Cladotanytarsus spp.	1	<1%
Micropsectra spp.	3	<1%
Paratanytarsus spp.	1	<1%
Tanytarsus spp.	2	<1%
(Orthocladiinae)		
Corynoneura spp.	1	<1%
Cricotopus spp.	55	2%
Hydrobaenus spp.	18	1%

Taxa List	Count	%
Nanocladius spp.	1	<1%
(Tanypodinae)		
Ablabesmyia spp.	6	<1%
Clinotanypus spp.	1	<1%
Labrundinia spp.	1	<1%
Larsia spp.	1	<1%
Procladius spp.	1	<1%
Tanypus spp.	1	<1%
Thienemannimyia group	6	<1%
Simuliidae		
Simulium spp.	6	<1%
Tabanidae	1	<1%
Tipulidae		
Tipula spp.	3	<1%
Lepidoptera		
Pyralidae	1	<1%
Decapoda		
Cambaridae		
Cambarus species *"A"*	40	2%
Amphipoda		
Hyalellidae		
Hyalella azteca	345	14%
Crangonyctidae		
Crangonyx spp.	173	7%
Synurella dentata	397	16%
Isopoda		
Asellidae		
Caecidotea spp.	100	4%
Lirceus spp.	592	24%
Veneroida		
Corbicula spp.	17	1%
Pisidium spp.	4	<1%
Sphaerium spp.	102	4%
Gastropoda		
Planorbiidae		
Helisoma spp.	4	<1%
Physidae		
Physa spp.	167	7%
Gyraulus spp.	25	1%
Lymnaeidae	19	1%
Oligochaeta		
Enchytraeidae	2	<1%
Lumbriculidae	1	<1%
Naididae		
Chaetogaster diaphanus	3	<1%
Dero spp.	17	1%

Taxa List	Count	%
Nais communis	20	1%
Nais pardalis	3	0%
Ophidonais serpentina	1	<1%
Slavina appendiculata	57	2%
Stephensoniana tandyi	1	<1%
Tubificidae		
Tubificidae without hair chaetae	5	<1%
Tubificidae with hair chaetae	1	<1%
Quistradrilus multisetosus	3	<1%
Hirudinea		
Erpobdellidae		
Erpobdella punctata	2	<1%
Mooreobdella microstoma	1	<1%
Glossiphoniidae	1	<1%
Turbellaria		
Planariidae		
Dugesia spp.	10	<1%
Cnidaria		
Hydra spp.	26	1%
Total Number of Individuals	2505	

Two stonefly taxa include the very sensitive *Isoperla* and *Amphinemura.*

Six members of the order Tricoptera were collected including the sensitive *Pycnopsyche* and *Rhyacophila*; and intermediate tolerant *Cheumatopsyche, Hydropsyche, Ironoquia, Ptilostomis* (Barbour et al. 1999).

REFERENCES

Barbour, M.T., J. Gerritsen, B.D. Snyder, J.B. Stribling. 1999. *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish, Second Edition.* EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.

Hargrave, B.T. 1970. The utilization of benthic microflora by *Hyalella azteca*. *J. Animal Ecology* 39:427-437.

Lewis, J.J. and S.T. Rafail. 2002. *The subterranean fauna of the Big Oaks National Wildlife Refuge*. Department of the Interior, U.S. Fish and Wildlife Service, Big Oaks National Wildlife Refuge, Madison, Indiana.

Merritt, R.W., K.W. Cummins, and M.B. Berg (eds). 2007. *An Introduction to the Aquatic Insects of North America, 3rd edition.* Kendall/ Hunt Publishing Company, Dubuque, Iowa.

Crayfish Assemblages of the Patoka River National Wildlife Refuge

Simon and Thoma (2003) described the crayfish assemblages of the Patoka River watershed including species occurring around the National Wildlife Refuge. Thoma et al. (2005) described a new species of crayfish, the painted-hand mudbug *Cambarus* (*Tubericambarus*) *polychromatus* from Flat Creek on the Patoka River National Wildlife Refuge. Simon and Thoma (2006) described the conservation of the Indiana crayfish (*Orconectes indianensis*), while Simon et al. (2005) described the reproductive biology, distribution, and habitat needs of species occurring in the Patoka River drainage. Simon and Morris (2008) described the effects of oil brine and acid mine leachate on the crayfish fauna of the Patoka River watershed.

Study Sites

The current study evaluated 88 sites during 2006 and 2007 (Table 1). These sites include the same locations that were sampled for fish and macroinvertebrate assemblages (Table 15).

Sample collection used three basic methods and included all available habitats in the vicinity of the site. Electrofishing, rock flipping, and excavation followed the procedures described by Simon (2004).

Species Richness and Composition

Species collected by Simon and Thoma (2003) from the Patoka River were found during the 2006 and 2007 investigation. The dominant species was the calico crayfish *Orconectes immunis,* which was found throughout the refuge and areas surrounding the Patoka River National Wildlife Refuge. Both *Orconectes virilis* and *Procambarus acutus* were collected from a single location.

Two species of primary burrowing crayfish were collected from the refuge (Table 15). The painted hand mudbug *Cambarus polychromatus* was more common than the Great Plains mudbug *Cambarus* species “A” from the area surrounding the refuge.

Additional sixteen records of *Orconectes indianensis* were found from areas surrounding the refuge. These sites included solid rock substrate habitats as described by Simon et al. (2005) and Simon and Thoma (2006).

No records of any invasive species were found during 2006-2007 sampling in the Patoka River watershed.

Table 15. List of macroinvertebrate taxa collected during 2006-2007 from the Patoka River drainage. Numbers indicate the sites at which each species has been collected based on information in Table 1 and Figure 1.

Taxa List	2006	2007
Cambarus species "A'	24, 35, 44, 54, 67	48
Cambarus polychromatus	35, 39, 44	
Orconectes immunis	11, 24, 34, 35	14, 15, 22, 25, 27, 29-31, 37, 41, 47-50, 55, 56, 65, 66, 70, 73, 79, 83, 84
Orconectes indianensis	1-3, 6, 7, 17, 19, 26, 34, 38-40	22, 25, 29, 63
Orconectes virilis	5	
Procambarus acutus		59
Procambarus spp.		62

REFERENCES

Simon, T.P. 2004. Standard operating procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of burrowing crayfish in streams and terrestrial habitats. *Misc Pap Ind Biol Surv Aquatic Res Center* 2:1–16

Simon, T.P. and C.C. Morris. 2008. Biological response signature of oil brine threats, sediment contaminants, and crayfish assemblages in an Indiana watershed, USA. *Archives Environmental Contaminants and Toxicology* (online first).

Simon, T.P. and R.F. Thoma. 2003. Distribution patterns of freshwater shrimp and crayfish (Decapoda: Cambaridae) in the Patoka River basin of Indiana. *Proceedings of the Indiana Academy of Science* 112: 175-185.

Simon, T.P. and R.F. Thoma. 2006. Conservation of imperiled crayfish – *Orconectes* (*Faxonius*) *indianensis* Hay (Decapoda: Cambaridae). *Journal of Crustacean Biology* 26: 436-440.

Simon, T.P., M. Weisheit, E. Seabrook, L. Freeman, S. Johnson, L. Englum, K.W. Jorck, M. Abernathy, and T.P. Simon, IV. 2005. Notes on Indiana crayfish (Decapoda: Cambaridae) with comments on distribution, taxonomy, life history, and habitat. *Proceedings of the Indiana Academy of Science* 114: 55-61.

Thoma, R.F., R.F. Jezerinac, and T.P. Simon. 2005. *Cambarus* (*Tubericambarus*) *polychromatus* (Decapoda: Cambaridae), a new species of crayfish from the United States. *Proceedings of the Biological Society of Washington* 118(2):326-336.

Crayfish Assemblages of the Big Oaks National Wildlife Refuge

Limited information about crayfish species is available from the Big Oaks National Wildlife Refuge. Lewis and Rafail (2002) documented the presence of the karst crayfish *Cambarus* (*Erebicambarus*) *laevis* from the springs and caves occurring on the refuge. St. John (1988) evaluated the distribution of Sloan's crayfish (*Orconectes* (*Rhoadesius*) *sloanii*) from areas around the refuge and did a repeat sample of select sites in southwestern Ohio (St. John 1991). The results of St. John's survey resulted in Sloan's crayfish being considered vulnerable by the Heritage data base.

<u>Study Sites</u>

Thirty four sites were sampled on the Big Oaks National Wildlife Refuge (Table 16). These sites were part of an inventory of macroinvertebrate and crayfish assemblages on the Big Oaks National Wildlife Refuge. These sites represented a wide range of stream sizes from headwater creeks to moderate sized rivers, ponds, and impounded lakes.

During the survey sampling protocols followed Simon (2004), which included specific targeting of primary, secondary, and tertiary burrowers. This procedure uses aquatic and terrestrial procedures to collect representative samples of crayfish from burrows and from stream habitats. A Smith-Root backpack electrofishing DC unit capable of 300 v and 2-3 amp output was used to capture tertiary and secondary burrowing crayfish species, while secondary burrowing species were captured by turning over boulders and slab rocks within the stream and the bank margins. Primary burrowers were collected by using either mist nets, Norrocky traps, or excavation techniques.

All crayfish were identified to species, enumerated, sexed, and females were further evaluated to determine whether ova or sperm plugs were present following reproduction. Individuals were identified to species using Page (1985), Hobbs (1989), or Thoma and Jezerinac (2000).

<u>Species Richness and Composition</u>

Seven crayfish species were collected from the Big Oaks National Wildlife Refuge (Table 17). Three primary burrowing species were collected including the painted-hand mudbug (*Cambarus polychromatus*), Ortmann's mudbug (*Cambarus ortmannii*), and the Great Plains mudbug (*Cambarus* species "A").

Four species of tertiary burrowing crayfish were collected including Sloan's crayfish (*Orconectes sloanii*), northern crayfish (*O. virilis*), Mud River crayfish (*O. juvenilis*), and calico crayfish (*O. immunis*). The Mud River crayfish is a native species and is known

Table 16. Site locations sampled during 2006-2007 in the Big Oaks National Wildlife Refuge for Crayfish. Site numbers correspond to Table 2 and are shown in Figure 1.

Site No.	County	Locality	Latitude	Longitude
1	Jennings	Otter Creek @ W Perimeter Rd	39º 1.61'	-85º 28.86'
2	Jennings	Otter Creek @ Northwest Exit Rd	39º 2.02'	-85º 27.64'
3	Ripley	Falling Timbers Branch @ Shaped Charge Rd	39º 2.78'	-85º 26.34'
4	Ripley	Fallen Timbers Creek @ L Rd	39º 3.06'	-85º 24.33'
5	Ripley	Little Otter Creek @ Shaped Charge Rd	39º 1.37'	-85º 26.21'
6	Ripley	Old Timbers Lake @ Unnamed Rd off NE exit Rd	39º 2.01'	-85º 23.59'
7	Jennings	Rush Branch @ W Perimeter Rd	38º 59.58'	-85º 28.04'
8	Jennings	Graham Creek @ W Perimeter Rd	38º 58.65'	-85º 28.06'
9	Jennings	Graham Creek @ J Rd	38º 58.83'	-85º 27.16'
10	Ripley	Big Graham Creek @ K Rd and NE Exit Rd	39º 0.47'	-85º 22.99'
11	Ripley	Graham Creek @ East Outlet Rd	39º 0.49'	-85º 22.22'
12	Jennings	Little Graham Creek @ W Perimeter Rd Area 33	38º 56.30'	-85º 27.75'
13	Ripley	Little Graham Creek @ J Rd Ford	38º 59.12'	-85º 23.89'
14	Ripley	Gate 8 Pond @ Michigan Rd	38º 59.63'	-85º 22.26'
15	Ripley	Little Graham Creek @ East Outlet Rd	38º 59.97'	-85º 21.65'
16	Jefferson	Marble Creek @ F Rd	38º 54.78'	-85º 27.00'
17	Ripley	Marble Creek @ G Rd	38º 55.65'	-85º 26.53'
18	Ripley	Marble Creek @ Morgan Rd	38º 55.91'	-85º 25.85'
19	Ripley	Marble Creek @ Center Recovery Rd	38º 56.13'	-85º 25.10'
20	Jefferson	Big Creek @ W Perimeter Rd	38º 53.55'	-85º 27.72'
21	Jefferson	Unnamed Trib of Big Creek @ U/S Jinestown Rd	38º 53.48'	-85º 27.21'
22	Jefferson	Unnamed Trib of Big Creek @ U/S Jinestown Rd	38º 53.58'	-85º 27.17'
23	Jefferson	Unnamed Trib of Big Creek @ E Rd	38º 53.90'	-85º 26.99'
24	Jefferson	Unnamed Trib of Big Creek @ D/S Jinestown Rd	38º 53.47'	-85º 27.11'
25	Jefferson	Big Creek @ Jinestown Rd	38º 53.00'	-85º 27.22'
26	Jefferson	Big Creek @ Morgan Rd	38º 53.11'	-85º 25.53'
27	Ripley	Unnamed Trib of Big Creek @ Cottrell Rd	38º 55.23'	-85º 23.66'
28	Ripley	Unnamed Trib of Big Creek @ Wonju Rd	38º 55.93'	-85º 23.87'
29	Ripley	Big Creek @ E Perimeter Rd	38º 55.88'	-85º 21.92'
30	Jefferson	Middle Fk Creek @ Jinestown Rd	38º 51.33'	-85º 27.19'
31	Jefferson	Trib of Middle Fork Creek @ Morgan Rd Bridge	38º 50.79'	-85º 25.52'
32	Jefferson	Harberts Creek @ CR 100 W	38º 49.26'	-85º 24.34'
33	Jefferson	Harberts Creek @ Main Entrance Rd	38º 49.76'	-85º 23.64'
34	Jefferson	Kruegers Lake @ Main Entrance Rd	38º 49.67'	-85º 23.43'

Table 17. List of crayfish species collected during 2006-2007 from the Big Oaks National Wildlife Refuge. Numbers indicate the sites at which each species has been collected based on information in Table 1 and Figure 1. Numbers in parentheses refer to the number of individuals collected.

Species	Site No.
No crayfish present at site	6, 14, 28, 34
Family Cambaridae (crayfish)	
Orconectes (Gremicambarus) virilis	1(7), 2(1)
O. (Procericambarus) juvenilis	8(13), 9(9), 10(4), 11(1), 15(2)
O. (Rhoadesius) sloanii	3(15), 4(1), 5(38), 7(12), 8(17), 9(13), 10(17), 11(29), 12(8), 13(6), 15(42), 16(9), 17(1), 20(22), 21(3), 22(1), 23(42), 24(11), 25(15), 26(2), 27(9), 29(14), 30(14), 31(2), 32(3), 33(8)
O. (Trisellescens) immunis	5(3)
Cambarus (Cambarus) ortmanni	10(2), 15(6), 19(5), 32(2)
C. (Lacunicambarus) species "A"	7(1), 10(21), 11(1), 18(7)
C. (Tubericambarus) polychromatus	3(2), 7(2), 10(2), 11(1), 25(2), 33(6)

from the study area (Simon 2001). The species resembles the rusty crayfish, but differs in the shape of the mandibles and the first form male gonopod (Taylor 2000). The rusty crayfish is native to the Whitewater River drainage, which is just to the east of the National Wildlife Refuge. No specimens of the rusty crayfish were observed on the refuge; however, specimens were collected from streams draining the refuge in areas upstream.

Two orconectid species were collected from only a few sites on the refuge (Table 17). The northern crayfish (*O. virilis*) was collected from two sites on Otter Creek, while the calico crayfish (*O. immunis*) was collected from Little Otter Creek (Table 16 and 17). Both species appear superficially similar; however, the calico crayfish has a deeply incised (notched) dactyl while the northern crayfish does not. There are also differences in the shape and curvature of the first form male gonopod. The northern crayfish reaches much larger sizes and is known to inhabit firm substrates including gravel and cobble substrates, while the calico crayfish inhabits sand and other fine substrates.

Crayfish were not collected from the lentic habitats on the refuge. No crayfish were collected from Old Timbers Lake (site 6), Gate 8 pond (site 14), or Kruegers Lake (site 34). In addition, no crayfish were collected from Big Creek (site 28). The Big Creek site was impounded by a beaver dam and was more lentic than lotic during the time period when sampled. Two attempts to collect crayfish from this site both resulted in no crayfish being collected.

Historic and Present Status of *Orconectes sloanii* at Big Oaks National Wildlife Refuge

St. John (1988) did an extensive survey of southwestern Ohio and southeastern Indiana in order to document the distribution and status of Sloan's crayfish (*Orconectes sloanii*). Based on the work of St. John the species was believed to be imperiled throughout it's range (Figure 10). Closer inspection of the distribution maps show that areas included within the Big Oaks National Wildlife Refuge were represented by only Sloan's crayfish and did not possess the invasive rusty crayfish (*Orconectes rusticus*).

Sampling conducted during 2006-2007 evaluations of crayfish assemblages found similar results as St. John (1988). Sloan's crayfish is stable and has a relatively high relative

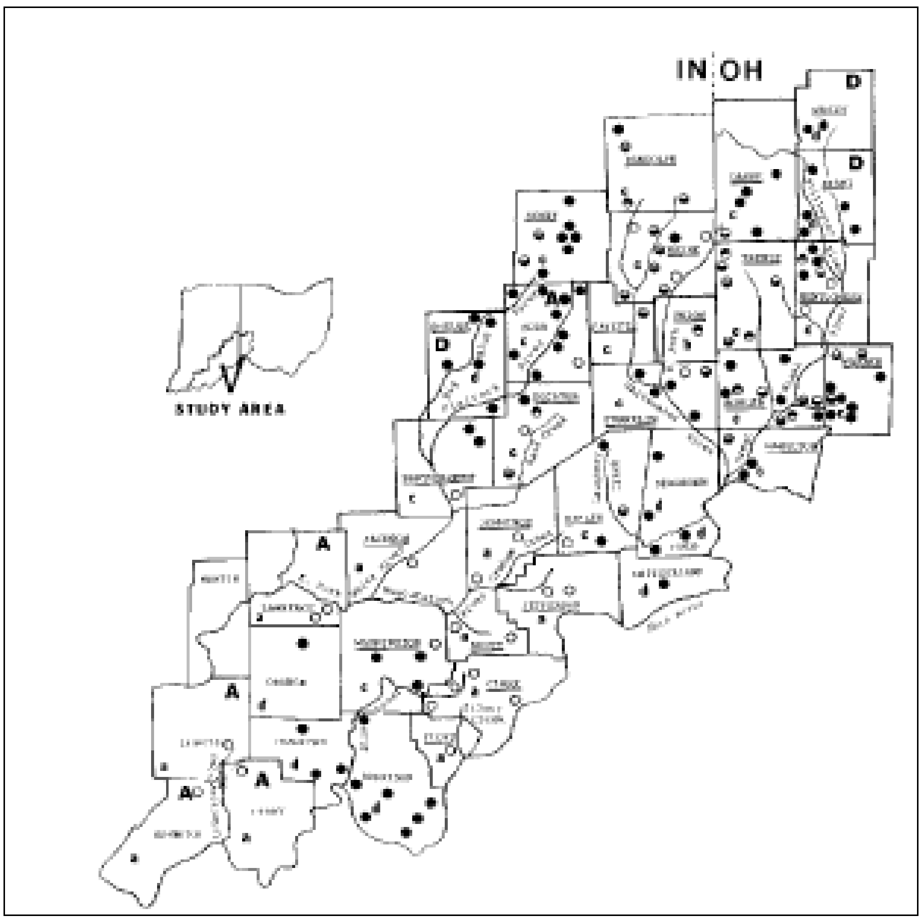

Figure 10. Distribution of *Orconectes sloanii* in Ohio and Indiana (St. John 1988). A. additions to Rhoades' (1962) records; D. deletions from Rhoades' (1962) records; a, *O. sloanii* and not *O. rusticus* present in the county; b. *O. sloanii* >50% and *O. rusticus* <50% of the specimens in the county; c. O. rusticus > 50% and O. sloanii < 50%; d. *O. rusticus* and not *O. sloanii* present. Underlined counties are Rhoades' 1962 records. Circles represent one or more collections at a site and indicate: open circle – *O. sloanii* without *O. rusticus*; darkened upper half of circle – *O. sloanii* sympatric with *O. rusticus* (*O. sloanii* >50% in collection); darkened lower half of circle – *O. sloanii* sympatric with *O. rusticus* (*O. rusticus* > 50%); and darkened circle – *O. rusticus* without *O. sloanii*.

abundance on the refuge. No instances of rusty crayfish were observed on the refuge (Table 17).

Sloan's crayfish was collected at 76.5% of the sites on the refuge. Relative abundance averaged 13.6 individuals per site. Mean density of Sloan's crayfish was 0.272 individuals per square meter.

REFERENCES

Hobbs, H. H. Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology*, No. 480.

Lewis, J.J. and S.T. Rafail. 2002. *The subterranean fauna of the Big Oaks National Wildlife Refuge*. Department of the Interior, U.S. Fish and Wildlife Service, Big Oaks National Wildlife Refuge, Madison, Indiana.

Page, L.M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. *Illinois Natural History Survey Bulletin* 33(4): 335-448.

Simon, T.P. 2001. Checklist of the crayfish and freshwater shrimp (Decapoda) of Indiana. *Proceedings of the Indiana Academy of Science* 110: 104-110.

Simon, T.P. 2004. Standard Operating Procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of of burrowing crayfish in streams and terrestrial habitats. *Miscellaneous Papers of the Indiana Biological Survey Aquatic Research Center* 2: 1-16.

St. John, F.L. 1988. Distribution and status of *Orconectes* (*Rhoadesius*) *sloanii* (Bundy) (Crustacea: Decapoda: Cambaridae). *Ohio Journal of Science* 88(5): 202-204.

St. John, F.L. 1991. Changes in mixed populations of *Orconectes* (*R.*) *sloanii* and *O.* (*P.*) *rusticus* (Crustacea: Decapoda: Cambaridae) in southwestern Ohio. *Ohio Journal of Science* 91(4): 172-173.

Taylor, C.A. 2000. Systematic studies of the *Orconectes juvenilis* complex (Decapoda: Cambaridae), with descriptions of two new species. *Journal of Crustacean Biology* 20: 132-152.

Thoma, R.F. and R.F. Jezerinac. 2000. Ohio crayfish and shrimp atlas. *Ohio Biological Survey Miscellaneous Publication,* 7.

Crayfish Assemblages of the Muscatatuck National Wildlife Refuge

Limited crayfish species information is available from the vicinity of the Muscatatuck National Wildlife Refuge. St. John (1988) evaluated the distribution of Sloan's crayfish (*Orconectes* (*Rhoadesius*) *sloanii*) from areas around the refuge and did a repeat sample of select sites in southwestern Ohio (St. John 1991). The results of St. John's survey resulted in Sloan's crayfish being considered vulnerable by the Heritage data base.

Study Sites

Nineteen sites on the Muscatatuck National Wildlife Refuge were surveyed for crayfish species during the 2007 inventory (Table 18). These sites were part of an inventory of macroinvertebrate and crayfish assemblages on the National Wildlife Refuges of Indiana. These sites represented a wide range of stream sizes from headwater creeks to moderate sized rivers, ponds, and impounded lakes.

Methods followed those of Simon (2004) and taxonomic identifications were based on Page (1985), Hobbs (1989), or Thoma and Jezerinac (2000).

Table 18. Site locations sampled during 2007 for crayfish in the Muscatatuck National Wildlife Refuge. Site numbers correspond to Table 2 and are shown in Figure 1.

Site No.	County	Locality	Latitude	Longitude
1	Jennings	Linda Lake @ CR 475 S	38º 54.95'	-85º 47.47'
2	Jackson	Moss Lake @ CR 475 S at Dam	38º 55.11'	-85º 49.49'
5	Jackson	Mutton Creek @ US 31 Bridge	38º 54.83'	-85º 49.92'
6	Jackson	Vernon Fork @ US 31	38º 54.38'	-85º 49.29'
7	Jackson	Richart Lake Outlet @ D/S CR 1300 E	38º 57.29'	-85º 47.95'
8	Jennings	Richart Lake Trib @ D/S CR 900 W	38º 58.18'	-85º 46.66'
9	Jennings	Unnamed Trib @ D/S CR 900 W	38º 55.82'	-85º 46.64'
10	Jennings	Unnamed Trib Storm Creek Ditch @ CR 1300 E	38º 55.57'	-85º 47.81'
11	Jackson	Storm Creek Ditch @ U/S US 50 Bridge	38º 58.01'	-85º 47.92'
12	Jackson	Mutton Creek Original Channel @ CR 400 N	38º 56.44'	-85º 49.21'
13	Jackson	Sandy Branch @ D/S US 31	38º 55.88'	-85º 50'
14	Jackson	Vernon Fk Muscatatuck River @ CR 50 N	38º 53.32'	-85º 51.1'
15	Jennings	Vernon Fk Muscatatuck River @ CR 500 S	38º 54.66'	-85º 43.82'
16	Jackson	Stanfield Lake Outlet @ CR 1300 E	38º 56.02'	-85º 47.99'
17	Jackson	Storm Creek Ditch @ CR 400 N	38º 56.44'	-85º 48.35'
18	Jackson	Unnamed Trib Moss Lake @ E CR 400 N	38º 56.42'	-85º 49.76'
19	Jackson	Unnamed Trib of Storm Creek @ CR 1300 E	38º 56.96'	-85º 47.84'
20	Jackson	Mutton Creek Ditch @ D/S US 50 Bridge	38º 57.82'	-85º 49.17'
21	Jackson	Pond @ Muscatatuck NWR	38º 57.52'	-85º 47.9'

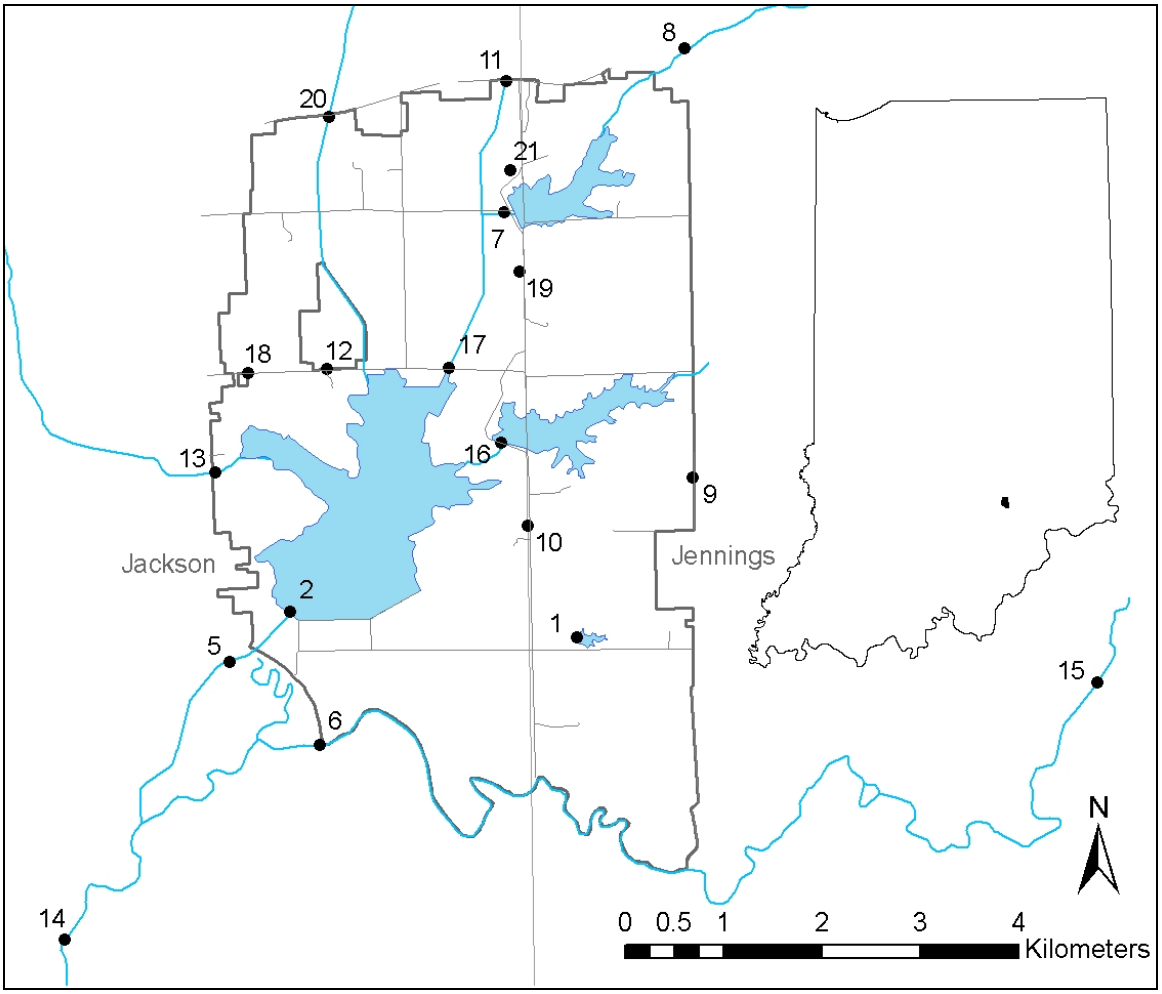

Figure 9. Distribution of crayfish sites during 2007 in the Muscatatuck National Wildlife Refuge. Numbers refer to site location in Table 18.

Table 19. List of crayfish species collected during 2007 from the Muscatatuck National Wildlife Refuge. Numbers indicate the sites at which each species has been collected based on information in Table 16 and Figure 9. Numbers in parentheses refer to the number of individuals collected.

Species	Site No.
No crayfish present at site	2, 17
Family Cambaridae (crayfish)	
Procambarus (Ortmannicus) acutus	13(3)
Orconectes (Rhoadesius) sloanii	5(2), 6(18), 10(1), 13(3), 14(24), 15(13), 20(3)
O. (Trisellescens) immunis	6(2), 8(10), 9(4), 10(1), 11(38), 13(266), 21(2)
Cambarus (Erebicambarus) laevis	19(1)
C. (Lacunicambarus) species "A"	1(1), 5(1), 7(3), 8(2), 9(1), 10(1), 11(4), 12(4), 13(4), 15(1), 16(5), 18(7), 19(2), 20(2)
C. (Tubericambarus) polychromatus	8(1), 13(1), 15(1)

Species Richness and Composition

Six crayfish species were collected from the Muscatatuck National Wildlife Refuge (Table 19). Two primary burrowing species were collected including the painted-hand mudbug (*Cambarus polychromatus*) and the Great Plains mudbug (*Cambarus* species "A"). The Great Plains mudbug was most common on the refuge occurring at 14 sites (73.7% sites). A blue form of the Great Plains mudbug was collected downstream of the Stanfield Lake outlet (site 16). The habitat had a large number of burrows and the soil was grey in color. The blue form crayfish when left in the sun returned to the typical olive green and brown coloration.

Two secondary burrowing species were collected from the refuge. The karst crayfish (*Cambarus laevis*) is typical of springs and cave streams. The species was collected from the unnamed tributary of Storm Creek (site 19). The other species is the White River crayfish (*Procambarus acutus*), which was collected from Sandy Branch (site 13).

Two tertiary burrowing crayfish species were collected including Sloan's crayfish (*Orconectes sloanii*) and the calico crayfish (*O. immunis*). Sloan's crayfish was considered imperiled by the Heritage National database.

Historic and Present Status of *Orconectes sloanii* near Muscatatuck National Wildlife Refuge

St. John (1988) did an extensive survey of southwestern Ohio and southeastern Indiana in order to document the distribution and status of Sloan's crayfish (*Orconectes sloanii*). Based on the work of St. John the species was believed to be imperiled throughout it's range (Figure 10). Closer inspection of the distribution maps show that areas surrounding the Muscatatuck National Wildlife Refuge included both species, with usually the rusty

crayfish representing more than 50% of the specimens to the east, while only Sloan's crayfish was present to the west. In areas where Sloan's crayfish was stable the invasive rusty crayfish (*Orconectes rusticus*) was not present.

Our sampling conducted during 2007 evaluations of crayfish assemblages found different results than St. John (1988). Sloan's crayfish is stable and was present in the absence of rusty crayfish. Sloan's crayfish had relatively high relative abundance on the refuge. No instances of rusty crayfish were observed on the refuge (Table 19).

Sloan's crayfish was collected at 36.8% of the sites on the refuge. Relative abundance averaged 9.14 individuals per site. Mean density of Sloan's crayfish was 0.182 individuals per square meter.

REFERENCES

Hobbs, H. H. Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology*, No. 480.

Page, L.M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. *Illinois Natural History Survey Bulletin* 33(4): 335-448.

Simon, T.P. 2004. Standard Operating Procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of of burrowing crayfish in streams and terrestrial habitats. *Miscellaneous Papers of the Indiana Biological Survey Aquatic Research Center* 2: 1-16.

St. John, F.L. 1988. Distribution and status of *Orconectes* (*Rhoadesius*) *sloanii* (Bundy) (Crustacea: Decapoda: Cambaridae). *Ohio Journal of Science* 88(5): 202-204.

St. John, F.L. 1991. Changes in mixed populations of *Orconectes* (*R.*) *sloanii* and *O.* (*P.*) *rusticus* (Crustacea: Decapoda: Cambaridae) in southwestern Ohio. *Ohio Journal of Science* 91(4): 172-173.

Thoma, R.F. and R.F. Jezerinac. 2000. Ohio crayfish and shrimp atlas. *Ohio Biological Survey Miscellaneous Publication,* 7.

Identification of Stressors Affecting Refuge Scale Indicators at Indiana National Wildlife Refuges

The importance of managing environmental health throughout the National Wildlife Refuge system requires evaluation of the extent that environmental composition, structure, and function have been altered from historic conditions. The change in environmental composition over time is interwoven with biotic components so that processes that affect the abiotic diversity in turn supports biological condition diversity [see 602 FW 3.4C(1)(e), Planning Area and Data Needs]. Since environmental conditions affect all living organisms, environmental health is a measurement of the chemical contamination of air, water, and soils that may interfere with reproduction or cause increased mutation rates. Such contamination includes carcinogens and other toxic substances that are released internal or external to refuges or may migrate onto the refuge.

Indirect effects at the population and community levels include the loss of vital resources such as habitat, food, water, cover, and space. For example, food and water may become contaminated with chemicals that are not naturally present. Refuge construction and maintenance projects needed to support refuge purpose should be designed to lessen their impacts on the environmental health of the refuge. In addition, contaminants coming from sources external to the refuge through air or water transport can affect resources without being able to identify sources.

Stressor identification models need to account for slightly increased contaminant concentrations, synergistic effects, or sporadic spikes that could adversely affect biological assemblage structure. As a result, these factors can potentially result in a biologic impairment without the occurrence of specific chemical criteria violations. Suter et al. (2002) 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. (2005) tested the application of this approach; however, resources and regional availability of data is usually inadequate to account for causes contributing to impaired biologic communities. Morris et al. (2005) identified the problem with the development of source identifications for impaired biological communities (IBC). These stressors are not always easily identifiable and are often overlooked when dealing with observational or qualitative data (EPA 2000, Suter et al. 2002).

The need to identify causality for biologically impaired systems and identify a stressor response has shifted from point-source influences to more diffuse non–point-source influences. Difficulty in tracking these pervasive non–point-source impacts, combined with the lack of predetermined signatory relationships with biologic assemblage patterns, creates a more complex problem. Morris et al. (2005) increasing our knowledge of signatory relationships by using multivariate analysis based on definable relationships between aquatic assemblage structure and quantifiable environmental stressors. Simon

and Morris (in press) used this approach to identify stressor responses between crayfish assemblage and contaminant and land use in a mixed use watershed in Indiana. Norton et al. (2000) used multivariate and correlation analysis to demonstrate the relationships between ambient chemical, physical, and biologic data. While others including Eagelson et al. (1990), Yoder and Rankin (1998), Norton et al. (2000), Rive-Murray et al. (2002), Yoder and DeShon (2003), and Simon (2003) have contributed towards increasing our knowledge biological response signatures and cause and effect relationships. These studies reported that aquatic communities respond to different types of stress in ways that are consistent and distinctive and that these relationships could be used for future hypothesis testing. Yoder and DeShon (2003) indicated that an important signature in the Ohio data (deformities, eroded fins, lesions, and tumors [DELT] anomalies on fish) had been missed that drove the analytic results. Thus, chemical concentration data is subject to the unintentional commission of type II errors not only in the assessment process but also in the diagnosis of impairments and the delineation of biologic response signatures.

In order to manage individual refuges at multiple spatial scales for biological integrity, biological diversity, and environmental health it is necessary to understand contaminant patterns across various local, regional, and national scales. This chapter evaluates the effects of contaminants in surface waters of Indiana National Wildlife Refuges at local or refuge level scales. This project evaluates three biological indicators that respond to environmental condition and compares changes resulting from contaminant and toxic substance release. This chapter evaluates the response of refuge populations and communities using a stressor identification model (Morris et al. 2005).

MATERIALS AND METHODS

Many state and federal agencies assume that biological assemblages are measuring the same features of the environment and are thus perplexed when agreement between indicators is not consistent. An elaborate interpretation has been devised to describe disagreement between biological assemblage indicators. This has resulted in statements of meeting, partially meeting, or not meeting aquatic life designated uses.

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 and toxic environments where either an all or none effect was 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.

We used multiple biological assemblage indicators including fish, macroinvertebrates and crayfish assemblages to assess contaminant and toxic effects on three National Wildlife Refuges in Indiana.

Data Transformation

Natural environmental stream conditions exist within each of the three National Wildlife Refuge watersheds, including some dry and/or naturally intermittent streams, which could potentially be underestimated using our existing protocols, thus resulting in a type I error. In addition, the collapse of biological structure as a result of contaminants and toxic situations may result in a type II error when the entire landscape is similarly affected. This causes an unresolved problem in the stressor identification approach that requires further tool development.

The Index of Biotic Integrity (IBI) has been regionally calibrated for the Eastern Corn Belt Plain (ECBP) to accommodate for low fish abundance and headwater stream conditions by providing special scoring criteria and alternative metrics (Simon and Dufour 1998). In this particular study, it was necessary to determine whether low IBI scores, associated with the lower fish abundance, truly represented impaired conditions. To control the type I error rate associated with low fish abundance in intermittent headwater streams, additional site-rejection criteria were established so that sites with <5 individual fish collected were removed from the multivariate analysis unless those 5 fish represented ≥ 3 species.

Statistical analysis was performed using STATISTICA© software (StatSoft 2002). Data were transformed by converting fish data into a percent relative abundance scale and both habitat and chemistry data to a percent-of-range scale.

Data Analysis

Transformed fish assemblage data were sorted by ascending species richness, and cluster analysis was used by developing a Euclidean distance similarity matrix based on Ward's method to create a two dimensional dendogram of the similarity matrix (StatSoft 2002). This similarity matrix model groups data, or in this case sites, in ''clusters'' that represent relative similarity within the group and a corresponding dissimilarity with adjacent groups. The vertical distance that separates each model cluster represents the degree of dissimilarity between the groups. In this case, the degree of dissimilarity represented the varying structural characteristics of the fish assemblage causing sites to be placed in each of the corresponding clusters.

Assuming biological assemblage structure is the result of external driving forces and that those forces are identifiable, these groupings can be used to evaluate physical and chemical variables relative to the identified groupings. To impress a degree of intuitive structure and direction to the orientation of the multidimensional alignment of the

dendogram, sites were placed into the analysis sorted by descending species richness. In this application, species richness was used to arrange clusters into a relative scale of quality by placing both high and low-diversity sites at respective ends of the gradient while still maintaining the similarity matrix-driven pattern. Sites within the dendogram from left to right represent a continuum of decreasing biologic integrity as predicted by the multidimensional clustering of biological assemblage data.

Cluster models were evaluated at varying linkage levels using the transformed physical, chemical, and biologic data. Clusters were treated as grouping variables and tested for significance using the Kruskal-Wallis analysis of variance (ANOVA) by ranks test. This test assumes that the variables are both continuous and/or measured on an ordinal scale and that comparison of samples were drawn from the same distribution or distributions having the same median. Thus, the interpretation of the Kruskal-Wallis test is virtually identical to that of the one-way ANOVA except that it is based on ranks rather than means (Siegel and Castellan 1988).

Graphic Presentation of Hot Spots

Variables showing significant interactions were examined more closely, and central tendency was visually presented using box-and-whisker plots (Morris et al. 2005; Simon and Morris 2008). Central tendencies of group mean structure of statistically significant physical and chemical parameters in the box-and-whisker data presentation demonstrated the significance of the relationship identified by the numeric classification analysis in the dendogram.

In order to show the extent and magnitude of impairments, watershed scale concentration pleths were generated for variables found to be significant in the multivariate analysis (Simon and Morris in press). These concentration pleths or “hot spots” were generated using the Kriging method and spline smoothed by inserting five columns and rows between each data point (ArcGIS Version 9.2, ESRI, Redwood, CA.).

RESULTS AND DISCUSSION

Stressor Identification of Contaminant Affects at the Patoka River National Wildlife Refuge

Study Design

Stressor identification on the refuge scale was accomplished using a random design to select 50 sites in the Patoka River National Wildlife Refuge. These sites represented locations on the refuge and those immediately upstream. Sites were visited and those sites that were dry were excluded from the 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 (Figure 1).

Analysis of assemblage structure based on Cluster Analysis showed that each assemblage group explained variance in the data differently (Figure 2). Assemblage structure was explained by increasing index of biotic integrity scores for fish and increasing species richness for macroinvertebrates and crayfish (Figure 3). Fish and macroinvertebrate assemblage structure was explained with eight clusters representing tier 4 and tier 5 linkage structure (Figure 2), while crayfish assemblage structure was explained with tier 4 linkage. The basis for these groupings is that the biological community structure results are externally driven and can be identified to evaluate physical and chemical stress on the respective assemblage (Morris et al. 2006).

Habitat Quality

Habitat analysis using the QHEI showed that scores ranged from 0 to 67 (average 42.2 $\pm$ 12.58). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life. Sites scoring less than 34 are considered not meeting designated uses for aquatic life. The instream cover, riffle-run score, and pool-glide score varied with the amount of channelization in the study area.

Chemical Quality

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 Appendices 1-3. In general, the lowest biological integrity and species richness clusters (cluster 1) had the highest concentrations of contaminants (Tables 1-3). Several contaminants showed differential distribution with higher concentrations in clusters 2, 3, 5, or 6 (Figures 4-6). Zinc and chloride had higher concentrations in clusters 2 and 3 than in cluster 1 for fish. Macroinvertebrates showed the highest concentrations for metals and pH in cluster 5, while alkalinity was highest in cluster 6 (Figure 5). Crayfish had the highest response for conductivity in cluster 3 (Figure 6).

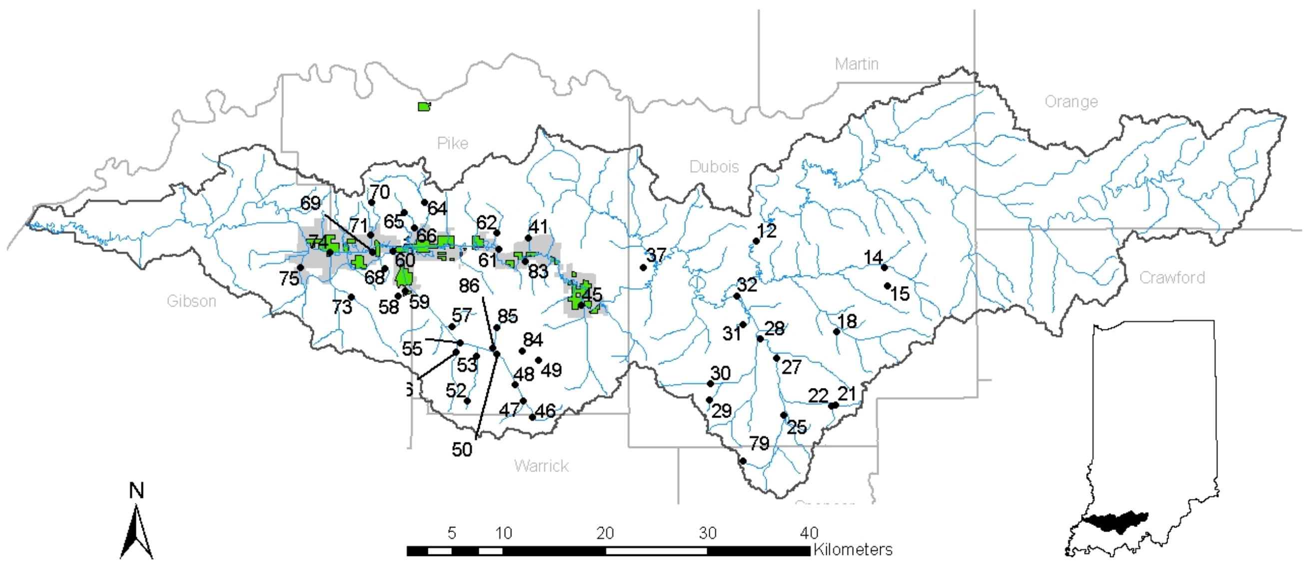

Figure 1. Map showing the distribution of sites both on refuge and upstream of the Patoka River National Wildlife Refuge that were used for the stressor identification project during 2007.

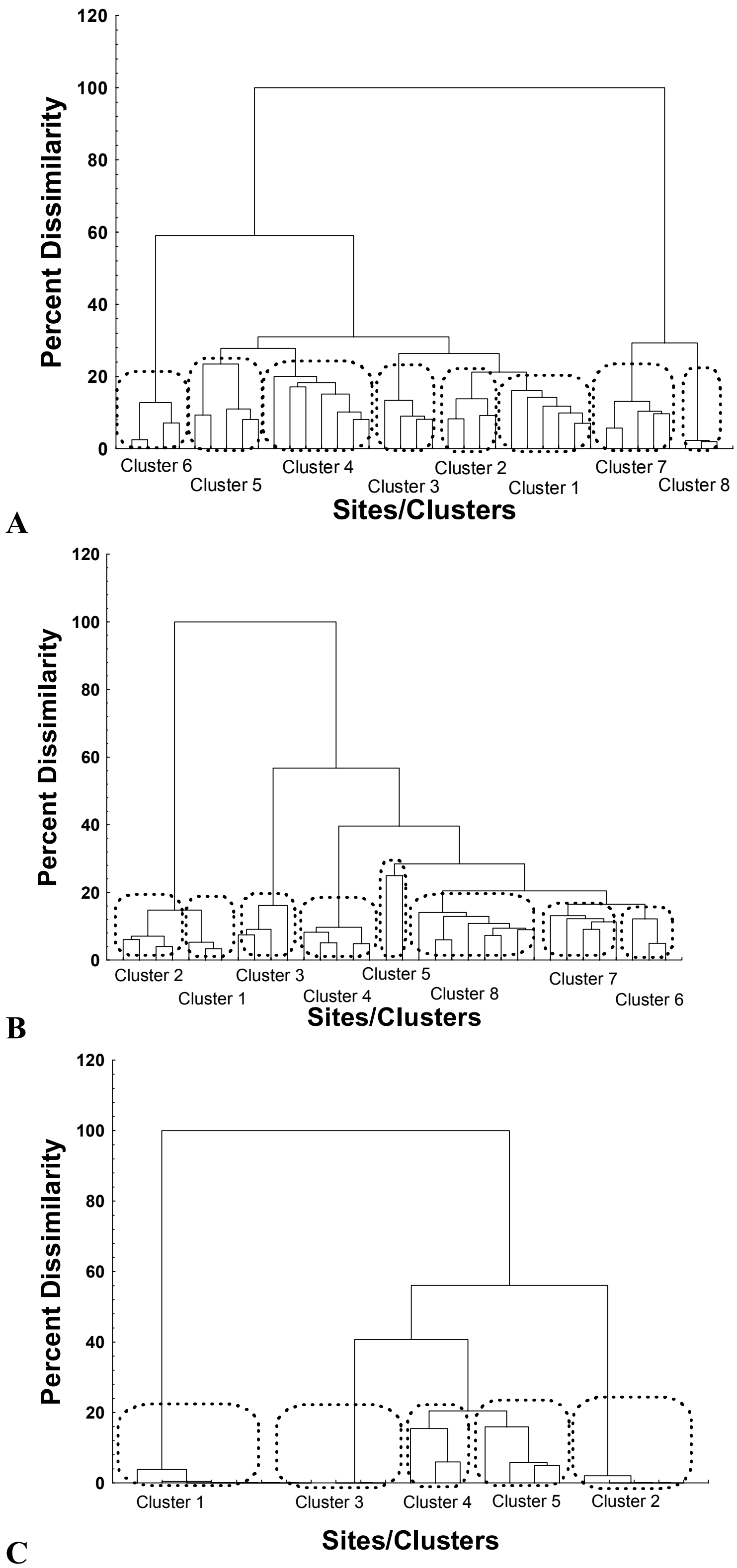

Figure 2. Dendograms showing the relative similarity of assemblage data collected in the Patoka River National Wildlife Refuge watersheds during August 2007. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA. (a) fish, (b) macroinvertebrate, and (c) crayfish assemblages.

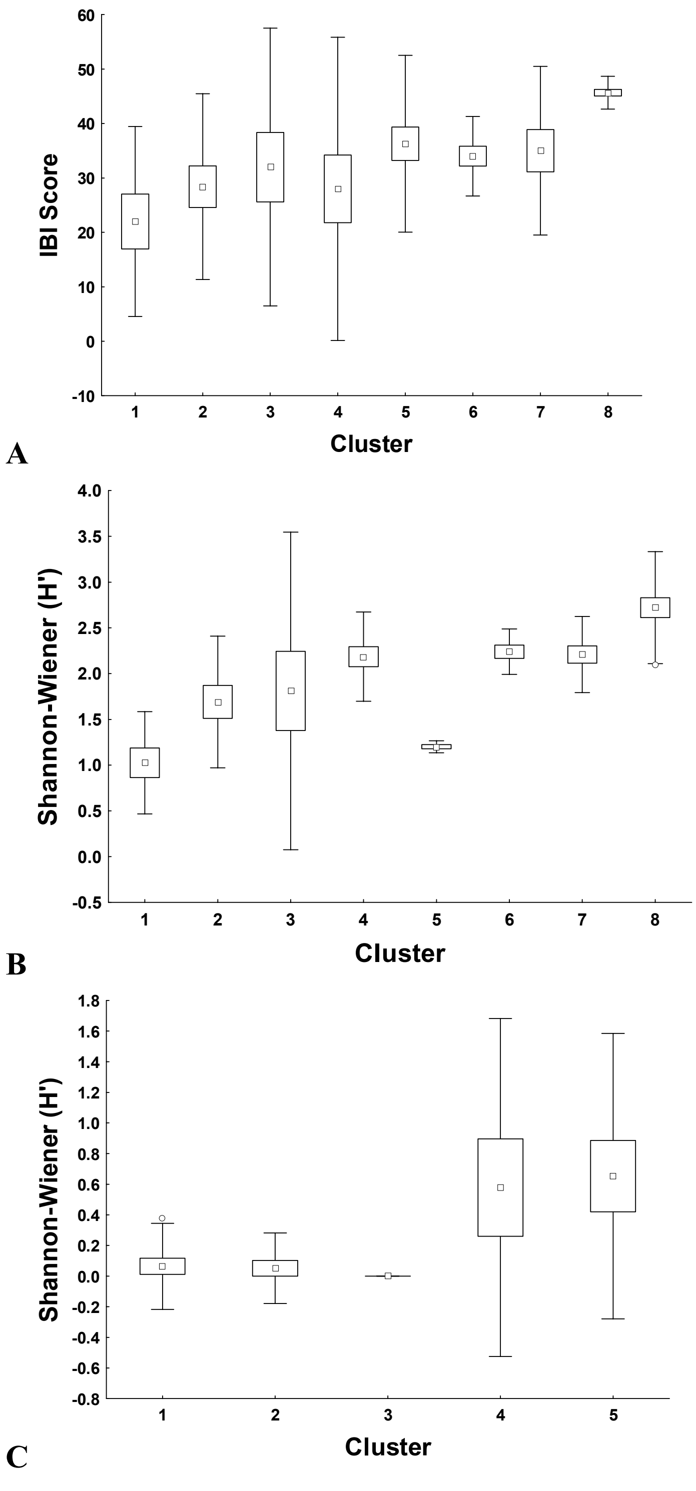

Figure 3. Box-and-whisker plots showing biological assemblage responses for index of biotic integrity (IBI) for (a) fish, or Shannon-Wiener (H') (b) macroinvertebrates and (c) crayfish assemblages had to the biologic response gradient developed from the Patoka River National Wildlife Refuge watersheds during August 2006.

Table 1. Summary statistics for 50 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Patoka River National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Alkalinity (as CaCO3)	3	205.00	170.00	229.00	31.00	5	229.40	25.00	680.00	259.87
Aluminum	3	331.83	61.70	840.00	440.38	5	236.20	10.00	479.00	204.61
Arsenic	3	1.16	0.65	1.53	0.46	5	1.25	0.65	2.22	0.66
Barium	3	29.37	18.40	43.60	12.91	5	30.82	23.70	51.50	11.78
Cadmium	3	1.00	1.00	1.00	0.00	5	1.17	1.00	1.87	0.39
Calcium (as CaCO3)	3	516.00	196.00	1010.00	434.00	5	739.60	100.00	1110.00	383.95
Chloride	3	7.20	5.10	9.70	2.33	5	16.40	0.50	62.00	25.89
COD	3	15.47	8.80	22.00	6.60	5	11.86	5.60	23.40	6.96
Copper	3	3.42	1.06	4.81	2.06	5	6.75	3.03	11.90	3.32
Cyanide	3	0.00	0.00	0.00	0.00	5	0.00	0.00	0.00	0.00
Fluoride	3	0.50	0.40	0.60	0.10	5	0.50	0.20	0.80	0.25
Hardness (as CaCO3)	3	994.00	324.00	1700.00	688.71	5	1629.40	127.00	2820.00	980.05
Iron	3	360.90	63.70	875.00	447.03	5	311.98	93.90	671.00	227.56
Lead	3	0.52	0.52	0.52	0.00	5	1.05	0.52	3.19	1.19
Manganese	3	1319.00	536.00	2680.00	1183.11	5	818.62	83.10	1620.00	692.93
Nickel	3	8.31	4.28	12.70	4.22	5	13.09	3.19	30.60	11.01
Nitrogen, Ammonia	3	0.05	0.05	0.05	0.00	5	0.10	0.05	0.30	0.11
Nitrogen, Nitrate+Nitrite	3	0.07	0.01	0.20	0.11	5	0.16	0.01	0.60	0.25
Phosphorus, Total	3	0.06	0.03	0.08	0.03	5	0.47	0.03	2.22	0.98
Selenium	3	1.74	1.18	2.86	0.97	5	2.50	1.18	3.25	0.83
Silica (Reactive)	3	7.37	6.40	7.90	0.84	5	7.94	2.90	14.00	4.12
Sodium	3	35.17	17.30	52.00	17.37	5	49.24	11.30	109.00	39.06
Sulfate	3	907.00	110.00	1850.00	879.14	5	1321.60	48.00	2140.00	794.67
TOC	3	4.40	2.40	6.30	1.95	5	4.12	2.10	7.10	1.89
TS	3	1596.00	428.00	2940.00	1265.21	5	2446.40	332.00	3960.00	1374.32
TSS	3	9.00	4.00	18.00	7.81	5	8.90	0.50	25.00	9.46
Zinc	3	6.11	3.00	8.11	2.73	5	28.44	6.26	59.30	27.89
Dissolved Oxygen	3	5.82	4.33	6.74	1.30	5	5.66	2.75	6.91	1.74
pH	3	7.41	6.80	8.01	0.61	5	7.39	6.99	7.84	0.38

Water Temperature	3	22.83	20.66	24.45	1.96	5	24.73	21.76	29.68	3.00
Specific Conductivity	3	1419.03	539.10	2375.00	920.31	5	2019.68	431.40	2902.00	992.13
Turbidity	3	48.60	35.90	69.90	18.56	4	46.45	11.10	103.60	41.31
Water	3	18.00	0.00	44.40	23.36	5	491.10	2.20	2183.90	948.32
Commercial	3	1.13	0.00	3.40	1.96	5	53.02	0.00	223.20	96.85
Agriculture	3	147.60	0.00	437.90	251.42	5	3042.54	222.30	12615.50	5374.35
High Density Residential	3	3.53	0.00	10.60	6.12	5	116.78	0.00	450.70	194.50
Low Density Residential	3	35.27	4.10	94.80	51.58	5	471.92	20.20	1826.00	760.56
Residential	3	38.80	4.10	105.40	57.69	5	588.70	20.20	2276.70	950.00
Grass Pasture	3	110.50	61.90	183.70	64.52	5	924.84	33.30	3802.50	1615.58
Forest	3	452.37	3.90	1067.00	550.68	5	6995.18	138.30	27191.90	11503.99
Industrial	3	1.13	0.00	3.40	1.96	5	53.68	0.00	256.60	113.55
Other	3	2.30	0.10	3.60	1.92	5	54.62	0.20	263.60	116.85
QHEI Score	3	49.00	28.00	67.00	19.67	5	34.25	0.00	51.00	22.79
QHEI Substrate Score	3	8.33	0.00	14.00	7.37	5	6.20	0.00	11.00	5.26
QHEI Instream Cover Score	3	4.33	3.00	7.00	2.31	5	7.20	0.00	14.00	5.17
QHEI Channel Score	3	15.33	10.00	19.00	4.73	5	8.00	0.00	12.00	5.34
QHEI Riparian Score	3	8.00	6.00	10.00	2.00	5	3.25	0.00	7.00	2.83
QHEI Pool Glide Score	3	5.00	4.00	7.00	1.73	5	5.20	0.00	9.00	3.63
Riffle Run Score	3	2.67	1.00	4.00	1.53	5	0.40	0.00	2.00	0.89
Gradient Score	3	5.33	4.00	8.00	2.31	5	4.00	0.00	8.00	2.83

Variable	Cluster 3 N	Mean	Minimum	Maximum	SD	Cluster 4 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	4	288.25	103.00	794.00	337.94	5	256.20	130.00	478.00	145.362
Aluminum	4	2507.50	635.00	7880.00	3582.15	5	544.20	10.00	1700.00	671.094
Arsenic	4	3.65	2.15	5.66	1.63	5	2.03	0.65	5.06	1.830
Barium	4	68.83	31.60	136.00	46.30	5	34.20	10.50	73.10	28.227
Cadmium	4	1.00	1.00	1.00	0.00	5	1.00	1.00	1.00	0.000
Calcium (as CaCO3)	4	119.00	98.00	136.00	16.21	5	707.60	94.00	1050.00	420.246
Chloride	4	44.25	25.00	55.00	13.50	5	8.68	0.50	15.00	6.054
COD	4	37.20	15.80	85.70	32.58	5	13.78	6.00	31.50	10.123
Copper	4	4.02	2.25	7.19	2.17	5	4.87	1.82	6.76	1.867
Cyanide	4	0.00	0.00	0.00	0.00	5	0.00	0.00	0.00	0.000
Fluoride	4	0.50	0.30	0.60	0.14	5	0.42	0.20	0.70	0.179
Hardness (as CaCO3)	4	239.00	159.00	378.00	95.72	5	1570.80	134.00	2490.00	928.102
Iron	4	2795.50	823.00	8480.00	3790.95	5	934.24	48.20	1960.00	956.960
Lead	4	2.60	0.52	7.79	3.50	5	0.68	0.52	1.31	0.353
Manganese	4	862.45	81.80	2500.00	1116.26	5	996.80	178.00	2670.00	997.224
Nickel	4	5.97	2.66	14.10	5.44	5	17.54	2.57	66.40	27.444
Nitrogen, Ammonia	4	0.10	0.05	0.20	0.07	5	0.16	0.05	0.60	0.246
Nitrogen, Nitrate+Nitrite	4	1.28	0.01	5.00	2.48	5	0.18	0.01	0.30	0.127
Phosphorus, Total	4	0.52	0.07	1.41	0.62	5	0.08	0.03	0.25	0.098
Selenium	4	1.18	1.18	1.18	0.00	5	2.34	1.18	3.71	1.121
Silica (Reactive)	4	8.73	1.80	22.00	9.34	5	6.98	2.10	12.00	4.393
Sodium	4	187.85	18.40	665.00	318.27	5	114.54	31.60	387.00	152.784
Sulfate	4	264.00	46.00	912.00	432.01	5	1462.20	91.00	2590.00	1003.511
TOC	4	8.28	5.70	11.80	2.58	5	4.14	2.00	7.10	1.933
TS	4	889.25	326.00	2310.00	953.41	5	2572.00	370.00	4060.00	1507.670
TSS	4	70.25	18.00	216.00	97.22	5	16.50	0.50	56.00	22.644
Zinc	4	21.11	6.72	38.10	16.34	5	33.48	3.00	126.00	52.087
Dissolved Oxygen	4	6.55	4.37	10.38	2.73	5	5.88	1.49	7.97	2.620
pH	4	8.62	7.74	9.35	0.69	5	7.84	7.27	8.38	0.409
Water Temperature	4	27.60	22.50	33.90	4.73	5	24.98	23.82	26.74	1.165
Specific Conductivity	4	1132.48	417.90	3140.00	1338.86	5	2176.42	422.10	3389.00	1129.466

Turbidity	1	397.30	397.30	397.30		4	24.15	10.80	39.10	11.791
Water	4	54.35	0.00	127.40	53.13	5	225.78	0.00	707.90	279.688
Commercial	4	29.35	0.00	108.60	53.00	5	26.92	0.00	72.80	35.921
Agriculture	4	4654.15	225.70	12533.00	5588.38	5	2151.04	135.30	5732.80	2182.051
High Density Residential	4	113.23	0.00	336.90	157.68	5	46.02	6.60	106.20	39.103
Low Density Residential	4	645.68	19.00	2025.10	930.67	5	310.22	68.90	762.40	275.873
Residential	4	758.90	19.00	2362.00	1085.69	5	356.24	85.60	817.40	283.044
Grass Pasture	4	1833.43	0.00	6892.70	3374.80	5	625.38	73.80	1486.10	557.200
Forest	4	1969.73	0.00	6373.30	2966.93	5	4609.84	50.80	17890.70	7459.302
Industrial	4	89.80	0.00	204.90	85.01	5	43.94	0.00	131.80	59.846
Other	4	4.88	0.20	15.50	7.25	5	24.98	0.00	98.10	42.265
QHEI Score	4	39.00	28.00	43.50	7.38	5	43.35	35.00	53.50	8.317
QHEI Substrate Score	4	10.25	7.00	14.00	3.30	5	5.80	0.00	12.00	4.494
QHEI Instream Cover Score	4	6.00	4.00	8.00	1.63	5	8.40	2.00	16.00	5.320
QHEI Channel Score	4	9.13	5.00	11.00	2.78	5	10.20	7.50	13.50	2.465
QHEI Riparian Score	4	4.75	3.00	9.50	3.18	5	6.95	3.75	10.00	2.896
QHEI Pool Glide Score	4	4.50	4.00	6.00	1.00	5	7.00	4.00	10.00	2.236
Riffle Run Score	4	0.38	0.00	1.50	0.75	5	1.00	0.00	4.00	1.732
Gradient Score	4	4.00	4.00	4.00	0.00	5	4.00	4.00	4.00	0.000

Variable	Cluster 5 N	Mean	Minimum	Maximum	SD	Cluster 6 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	7	273.00	85.000	958.0	309.29	4	111.75	86.00	150.00	27.256
Aluminum	7	808.23	87.600	2070.0	700.69	4	716.75	213.00	1590.00	613.459
Arsenic	7	2.87	0.650	6.3	1.98	4	1.70	0.65	2.67	0.897
Barium	7	34.94	13.800	48.1	13.78	4	43.85	31.20	59.10	12.474
Cadmium	7	1.00	1.000	1.0	0.00	4	1.00	1.00	1.00	0.000
Calcium (as CaCO3)	7	358.86	90.000	1060.0	410.97	4	143.00	96.00	270.00	84.766
Chloride	7	11.07	0.500	19.0	6.50	4	14.68	5.30	24.00	9.206
COD	7	23.77	1.500	52.5	15.98	4	20.58	6.80	29.30	9.924
Copper	7	3.65	1.960	6.0	1.29	4	2.52	2.32	2.67	0.145
Cyanide	7	0.00	0.003	0.0	0.00	4	0.00	0.00	0.00	0.000
Fluoride	7	0.39	0.100	0.8	0.24	4	0.28	0.20	0.40	0.096
Hardness (as CaCO3)	7	733.71	121.000	2370.0	889.02	4	264.00	131.00	605.00	227.991
Iron	7	1162.20	60.400	3440.0	1214.19	4	767.00	391.00	1180.00	356.235
Lead	7	0.90	0.520	1.4	0.37	4	0.52	0.52	0.52	0.000
Manganese	7	654.71	118.000	1760.0	749.83	4	568.25	97.00	1750.00	789.993
Nickel	7	6.04	2.250	16.2	4.73	4	3.83	2.72	5.39	1.225
Nitrogen, Ammonia	7	0.21	0.050	1.1	0.39	4	0.05	0.05	0.05	0.000
Nitrogen, Nitrate+Nitrite	7	0.96	0.010	3.0	1.20	4	0.66	0.01	1.90	0.891
Phosphorus, Total	7	0.13	0.025	0.2	0.10	4	0.06	0.03	0.12	0.046
Selenium	7	2.60	1.180	7.5	2.32	4	1.18	1.18	1.18	0.000
Silica (Reactive)	7	5.90	2.800	9.7	2.86	4	6.18	5.20	7.00	0.826
Sodium	7	198.73	13.000	1180.0	433.38	4	32.78	10.80	47.80	17.729
Sulfate	7	852.43	18.000	2080.0	973.76	4	200.75	55.00	549.00	234.271
TOC	7	7.17	2.000	18.8	5.63	4	6.30	2.70	8.60	2.531
TS	7	1651.86	199.000	4080.0	1672.57	4	479.25	230.00	1040.00	377.945
TSS	7	30.29	0.500	98.0	37.15	4	15.00	7.00	27.00	8.641
Zinc	7	11.40	6.060	24.5	6.68	4	3.00	3.00	3.00	0.000
Dissolved Oxygen	7	6.35	0.530	13.9	4.09	4	5.32	4.26	7.49	1.477
pH	7	8.21	6.270	9.1	0.94	4	8.24	7.13	8.94	0.779
Water Temperature	7	26.32	19.100	30.0	3.83	4	25.44	22.40	30.00	3.231
Specific Conductivity	7	1627.13	272.000	4658.0	1656.21	4	567.75	300.00	1042.00	329.641

Turbidity	5	257.88	22.200	1000.0	416.55	4	45.20	21.50	97.10	35.746
Water	7	1528.73	0.000	10069.1	3767.41	4	129.93	3.40	347.70	151.340
Commercial	7	270.73	0.000	1478.9	541.15	4	25.50	0.00	91.30	44.029
Agriculture	7	23102.46	28.600	130240.3	48127.66	4	4130.10	698.00	9637.40	3970.396
High Density Residential	7	643.07	0.000	3410.5	1252.02	4	82.40	0.00	255.60	118.213
Low Density Residential	7	4045.37	3.400	23619.5	8737.91	4	657.95	58.70	1509.40	615.286
Residential	7	4688.44	3.400	27030.0	9987.16	4	740.35	58.70	1765.00	731.031
Grass Pasture	7	11951.84	0.000	72849.8	27107.49	4	2213.50	31.80	5135.10	2134.013
Forest	7	34605.73	17.000	226490.5	84705.77	4	2916.00	123.70	7014.80	3054.685
Industrial	7	261.47	0.000	1586.2	586.22	4	17.58	0.00	60.50	28.987
Other	7	1153.19	0.100	7949.2	2996.83	4	8.10	0.20	31.60	15.667
QHEI Score	6	36.58	25.000	45.0	7.14	4	38.00	29.50	45.00	7.927
QHEI Substrate Score	6	6.83	1.000	15.0	5.78	4	8.50	4.00	13.00	3.697
QHEI Instream Cover Score	6	6.67	4.000	8.0	1.51	4	6.50	3.00	8.00	2.380
QHEI Channel Score	6	8.67	8.000	10.0	1.03	4	9.63	7.00	12.00	2.136
QHEI Riparian Score	6	5.08	2.000	10.0	2.85	4	3.75	2.00	5.00	1.258
QHEI Pool Glide Score	6	4.33	3.000	7.0	1.51	4	4.25	3.00	5.00	0.957
Riffle Run Score	6	1.00	0.000	2.0	1.10	4	1.38	0.00	2.00	0.946
Gradient Score	6	4.00	4.000	4.0	0.00	4	4.00	4.00	4.00	0.000

Variable	Cluster 7 N	Mean	Minimum	Maximum	SD	Cluster 8 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	4	188.00	100.00	426.00	158.78	6	225.3333	86.0000	606.000	216.268
Aluminum	4	165.00	10.00	410.00	178.92	6	369.5667	87.2000	1030.000	359.583
Arsenic	4	1.95	0.65	3.90	1.39	6	1.1750	0.6500	1.760	0.576
Barium	4	30.58	17.20	58.80	19.03	6	48.4500	28.6000	63.300	12.067
Cadmium	4	1.00	1.00	1.00	0.00	6	1.0000	1.0000	1.000	0.000
Calcium (as CaCO3)	4	613.00	72.00	944.00	376.87	6	266.6667	76.0000	672.000	259.043
Chloride	4	7.08	0.50	22.00	10.20	6	16.5333	9.2000	25.000	7.218
COD	4	18.35	6.90	42.00	16.11	6	12.0333	6.1000	16.400	4.054
Copper	4	4.33	1.96	6.37	1.81	6	2.9150	1.1400	6.020	1.974
Cyanide	4	0.00	0.00	0.00	0.00	6	0.0025	0.0025	0.003	0.000
Fluoride	4	0.50	0.20	0.70	0.24	6	0.2000	0.1000	0.300	0.063
Hardness (as CaCO3)	4	1315.50	112.00	2140.00	859.86	6	644.8333	109.0000	1830.000	780.616
Iron	4	361.90	28.60	673.00	263.60	6	657.8333	143.0000	1440.000	465.414
Lead	4	0.52	0.52	0.52	0.00	6	0.5200	0.5200	0.520	0.000
Manganese	4	557.25	129.00	861.00	311.70	6	769.1667	143.0000	2010.000	659.239
Nickel	4	5.96	3.45	9.26	2.66	6	3.3867	2.1700	5.720	1.298
Nitrogen, Ammonia	4	0.05	0.05	0.05	0.00	6	0.0500	0.0500	0.050	0.000
Nitrogen, Nitrate+Nitrite	4	0.03	0.01	0.10	0.05	6	1.0017	0.0100	3.300	1.221
Phosphorus, Total	4	0.04	0.03	0.07	0.02	6	0.0417	0.0250	0.070	0.019
Selenium	4	2.58	1.18	4.16	1.24	6	1.6900	1.1800	2.970	0.807
Silica (Reactive)	4	5.93	4.10	8.20	1.70	6	8.6500	5.3000	15.000	3.494
Sodium	4	36.73	17.30	86.20	33.15	6	31.8500	11.0000	60.100	18.753
Sulfate	4	1205.50	32.00	2110.00	864.91	6	416.3333	27.0000	1200.000	541.087
TOC	4	6.15	2.50	13.00	4.78	6	3.8167	2.5000	5.000	1.011
TS	4	2043.75	235.00	3150.00	1258.06	6	947.6667	181.0000	2590.000	1055.093
TSS	4	10.88	0.50	22.00	10.27	6	12.8333	4.0000	24.000	8.796
Zinc	4	5.89	3.00	7.92	2.07	6	3.5650	3.0000	6.390	1.384
Dissolved Oxygen	4	5.88	4.11	8.85	2.07	6	6.5617	4.1300	8.880	2.017
pH	4	7.87	7.34	8.83	0.66	6	7.9217	6.9200	9.130	0.762
Water Temperature	4	27.89	24.40	32.27	3.30	6	25.6883	22.8000	27.900	1.807
Specific Conductivity	4	1707.75	325.00	2429.00	946.52	6	975.7000	234.4000	2500.000	1004.417

Turbidity	4	16.75	1.00	37.00	14.93	4	45.7500	25.7000	90.200	30.046
Water	4	114.80	12.30	275.60	114.12	6	23.9167	1.9000	79.200	28.613
Commercial	4	0.23	0.00	0.90	0.45	6	0.5667	0.0000	3.200	1.293
Agriculture	4	1708.58	471.70	2972.10	1191.00	6	700.6000	56.8000	2646.300	996.736
High Density Residential	4	5.80	1.40	11.80	4.54	6	3.4833	0.0000	14.800	5.757
Low Density Residential	4	218.05	111.30	396.10	133.67	6	128.5167	0.2000	412.700	151.211
Residential	4	223.85	112.70	402.70	136.25	6	132.0000	0.2000	427.500	156.731
Grass Pasture	4	435.23	134.80	908.70	341.33	6	611.7500	36.8000	2140.500	788.427
Forest	4	3886.43	361.80	10214.60	4485.57	6	611.0167	53.3000	2280.500	862.633
Industrial	4	0.00	0.00	0.00	0.00	6	0.5667	0.0000	3.400	1.388
Other	4	7.83	0.20	24.20	11.14	6	0.2000	0.1000	0.300	0.063
QHEI Score	4	42.38	35.50	49.00	6.86	6	45.5000	36.0000	62.000	10.559
QHEI Substrate Score	4	9.13	8.00	10.00	1.03	6	8.7500	0.0000	16.500	5.981
QHEI Instream Cover Score	4	6.50	4.00	8.00	1.91	6	8.6667	3.0000	16.000	4.502
QHEI Channel Score	4	10.00	8.00	11.00	1.41	6	9.6667	5.0000	15.000	3.777
QHEI Riparian Score	4	5.75	3.50	10.00	2.96	6	5.5833	3.0000	10.000	3.073
QHEI Pool Glide Score	4	4.75	3.00	6.00	1.26	6	5.3333	3.0000	7.000	1.862
Riffle Run Score	4	1.25	0.00	2.00	0.96	6	1.8333	0.0000	3.500	1.211
Gradient Score	4	5.00	4.00	8.00	2.00	6	5.6667	4.0000	10.000	2.658

Table 2. Summary statistics for 50 chemistry (concentration/ppm), habitat, and land use variables by macroinvertebrate grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Patoka River National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Alkalinity (as CaCO3)	3	289.67	86.00	680.00	338.15	4	340.50	159.00	606.00	197.16
Aluminum	3	2998.33	240.00	7880.00	4239.55	4	270.60	57.00	840.00	380.00
Arsenic	3	2.97	1.49	5.66	2.33	4	1.40	0.65	1.76	0.51
Barium	3	63.93	24.30	136.00	62.52	4	38.38	25.10	56.20	14.34
Cadmium	3	1.00	1.00	1.00	0.00	4	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	3	450.00	136.00	724.00	296.03	4	660.00	342.00	1110.00	328.89
Chloride	3	18.47	0.50	45.00	23.45	4	6.20	0.50	10.00	4.36
COD	3	37.73	7.30	85.70	42.04	4	10.85	6.10	15.60	3.99
Copper	3	5.99	4.74	7.19	1.23	4	5.69	4.40	7.63	1.48
Cyanide	3	0.00	0.00	0.00	0.00	4	0.00	0.00	0.00	0.00
Fluoride	3	0.40	0.20	0.50	0.17	4	0.45	0.20	0.80	0.26
Hardness (as CaCO3)	3	1074.33	213.00	1960.00	873.75	4	1764.50	958.00	2820.00	789.03
Iron	3	3544.67	384.00	8480.00	4329.94	4	447.23	93.90	875.00	388.66
Lead	3	3.14	0.52	7.79	4.04	4	0.52	0.52	0.52	0.00
Manganese	3	1684.67	934.00	2500.00	785.00	4	884.00	197.00	2010.00	784.84
Nickel	3	10.07	7.37	14.10	3.55	4	5.25	3.63	7.38	1.67
Nitrogen, Ammonia	3	0.05	0.05	0.05	0.00	4	0.05	0.05	0.05	0.00
Nitrogen, Nitrate+Nitrite	3	0.04	0.01	0.10	0.05	4	0.15	0.01	0.40	0.17
Phosphorus, Total	3	0.18	0.03	0.45	0.24	4	0.04	0.03	0.06	0.02
Selenium	3	2.31	1.18	3.25	1.05	4	2.45	1.18	3.18	0.90
Silica (Reactive)	3	11.70	6.40	22.00	8.92	4	7.15	5.30	8.90	1.55
Sodium	3	32.23	11.10	60.40	25.39	4	39.83	18.20	60.10	17.48
Sulfate	3	768.67	46.00	1260.00	639.21	4	1280.25	761.00	2140.00	600.82
TOC	3	6.87	3.00	11.80	4.50	4	3.75	2.50	4.50	0.90
TS	3	1643.00	569.00	2660.00	1046.66	4	2487.50	1420.00	3960.00	1091.77
TSS	3	92.00	8.00	216.00	109.62	4	8.75	4.00	18.00	6.29
Zinc	3	17.42	6.26	32.10	13.27	4	5.22	3.00	7.65	2.57
Dissolved Oxygen	3	7.67	5.81	10.38	2.40	4	5.06	4.13	6.45	1.06
pH	3	7.47	7.17	7.74	0.29	4	8.19	7.41	9.13	0.74

Water Temperature	3	26.04	25.00	27.59	1.37	4	23.16	20.66	25.50	2.31
Specific Conductivity	3	1430.97	417.90	2430.00	1006.12	4	2188.75	1343.00	2902.00	671.53
Turbidity	2	225.70	54.10	397.30	242.68	2	45.95	22.00	69.90	33.87
Water	3	53.23	0.00	83.20	46.22	4	63.80	1.90	164.50	75.60
Commercial	3	0.57	0.00	1.70	0.98	4	0.00	0.00	0.00	0.00
Agriculture	3	214.00	7.60	408.70	200.81	4	602.65	0.00	1492.80	711.86
High Density Residential	3	0.93	0.00	1.90	0.95	4	1.78	0.00	3.70	2.05
Low Density Residential	3	45.67	2.20	115.80	61.32	4	97.50	0.20	209.40	110.94
Residential	3	46.60	3.10	117.70	62.09	4	99.28	0.20	212.80	112.96
Grass Pasture	3	120.00	0.00	325.70	178.97	4	229.18	36.80	414.70	195.36
Forest	3	641.03	0.00	1456.30	743.62	4	1692.88	53.30	5666.10	2665.38
Industrial	3	0.00	0.00	0.00	0.00	4	0.00	0.00	0.00	0.00
Other	3	0.47	0.20	1.00	0.46	4	2.40	0.10	6.10	2.86
QHEI Score	3	49.75	41.50	57.00	7.80	4	46.75	37.00	52.00	6.70
QHEI Substrate Score	3	11.00	9.00	12.00	1.73	4	8.75	5.00	11.00	2.63
QHEI Instream Cover Score	3	10.67	8.00	14.00	3.06	4	7.75	3.00	16.00	6.18
QHEI Channel Score	3	11.50	10.00	12.50	1.32	4	10.75	7.00	17.00	4.50
QHEI Riparian Score	3	5.58	3.50	8.50	2.60	4	9.00	7.00	10.00	1.41
QHEI Pool Glide Score	3	6.33	4.00	8.00	2.08	4	5.50	4.00	7.00	1.73
Riffle Run Score	3	0.67	0.00	2.00	1.15	4	1.00	0.00	3.00	1.41
Gradient Score	3	4.00	4.00	4.00	0.00	4	4.00	4.00	4.00	0.00

Variable	N	Mean	Cluster 3 Minimum	Maximum	SD	N	Mean	Cluster 4 Minimum	Maximum	SD
Alkalinity (as CaCO3)	4	125.75	86.00	206.00	54.36	5	142.40	95.00	216.00	55.69
Aluminum	4	643.25	383.00	1030.00	286.19	5	138.06	10.00	474.00	189.87
Arsenic	4	1.93	0.65	2.67	0.88	5	1.20	0.65	1.70	0.51
Barium	4	44.98	36.60	53.00	7.24	5	30.74	18.00	63.30	18.44
Cadmium	4	1.00	1.00	1.00	0.00	5	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	4	117.50	100.00	134.00	17.00	5	549.20	76.00	944.00	390.36
Chloride	4	26.75	21.00	39.00	8.26	5	5.40	0.50	11.00	4.95
COD	4	19.70	15.60	26.00	4.62	5	15.00	9.60	22.00	4.45
Copper	4	2.45	2.32	2.62	0.15	5	3.57	1.06	6.37	2.22
Cyanide	4	0.00	0.00	0.00	0.00	5	0.00	0.00	0.00	0.00
Fluoride	4	0.20	0.20	0.20	0.00	5	0.48	0.20	0.70	0.22
Hardness (as CaCO3)	4	234.00	147.00	432.00	132.91	5	1116.60	109.00	2140.00	867.55
Iron	4	750.50	391.00	1440.00	469.05	5	299.46	28.60	854.00	336.11
Lead	4	0.52	0.52	0.52	0.00	5	0.52	0.52	0.52	0.00
Manganese	4	236.50	97.00	518.00	191.31	5	877.00	118.00	2680.00	1052.90
Nickel	4	2.79	2.65	3.00	0.15	5	6.24	2.17	9.26	2.78
Nitrogen, Ammonia	4	0.05	0.05	0.05	0.00	5	0.05	0.05	0.05	0.00
Nitrogen, Nitrate+Nitrite	4	0.93	0.01	1.90	0.79	5	0.03	0.01	0.10	0.04
Phosphorus, Total	4	0.07	0.03	0.12	0.04	5	0.04	0.03	0.08	0.02
Selenium	4	1.18	1.18	1.18	0.00	5	2.39	1.18	4.16	1.30
Silica (Reactive)	4	6.30	5.00	8.30	1.53	5	5.18	2.80	7.90	1.90
Sodium	4	38.53	20.20	60.10	18.66	5	22.86	11.00	50.20	15.56
Sulfate	4	151.50	71.00	295.00	98.63	5	1033.40	27.00	2110.00	920.84
TOC	4	6.13	4.20	8.60	1.95	5	4.80	3.30	6.30	1.32
TS	4	423.75	282.00	707.00	192.03	5	1713.80	181.00	3150.00	1325.65
TSS	4	16.75	7.00	23.00	7.41	5	4.40	0.50	13.00	5.12
Zinc	4	3.85	3.00	6.39	1.70	5	5.45	3.00	7.92	2.30
Dissolved Oxygen	4	6.02	4.55	8.43	1.74	5	6.36	4.91	8.85	1.54
pH	4	8.34	7.91	8.94	0.44	5	7.44	6.80	8.17	0.57
Water Temperature	4	25.32	22.40	27.80	2.28	5	28.55	24.45	32.27	2.89
Specific Conductivity	4	568.98	389.90	957.00	266.16	5	1440.50	234.40	2429.00	986.06

Turbidity	3	50.80	21.60	90.20	35.42	5	39.88	14.40	100.90	35.78
Water	4	46.10	0.00	107.90	48.57	5	84.88	0.00	275.60	114.65
Commercial	4	25.08	0.00	91.30	44.34	5	0.82	0.00	3.20	1.39
Agriculture	4	3581.45	35.30	9637.40	4486.00	5	1319.18	4.90	2972.10	1416.61
High Density Residential	4	80.40	2.40	255.60	119.77	5	4.96	0.00	14.80	6.15
Low Density Residential	4	549.08	16.70	1509.40	700.70	5	187.68	3.40	412.70	203.34
Residential	4	629.48	20.80	1765.00	818.51	5	192.64	3.40	427.50	208.86
Grass Pasture	4	1843.68	6.60	5135.10	2383.42	5	649.18	0.00	2140.50	911.36
Forest	4	1146.05	0.00	3390.30	1584.56	5	3281.98	3.90	10214.60	4208.07
Industrial	4	17.58	0.00	60.50	28.99	5	0.68	0.00	3.40	1.52
Other	4	0.20	0.00	0.30	0.14	5	6.62	0.10	24.20	10.07
QHEI Score	4	41.63	29.50	56.00	11.50	5	43.20	25.00	67.00	16.20
QHEI Substrate Score	4	6.38	0.00	12.50	5.50	5	8.30	1.00	14.00	4.71
QHEI Instream Cover Score	4	7.75	7.00	9.00	0.96	5	6.80	4.00	10.00	2.17
QHEI Channel Score	4	12.50	7.00	18.00	4.51	5	11.00	8.00	19.00	4.58
QHEI Riparian Score	4	4.13	2.00	6.00	1.75	5	4.30	2.00	8.00	2.22
QHEI Pool Glide Score	4	4.50	4.00	5.00	0.58	5	5.00	3.00	7.00	2.00
Riffle Run Score	4	2.38	1.50	3.50	0.85	5	1.80	0.00	4.00	1.48
Gradient Score	4	4.00	4.00	4.00	0.00	5	6.00	4.00	10.00	2.83

Variable	Cluster 5 N	Mean	Minimum	Maximum	SD	Cluster 6 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	2	48.50	5.00	92.00	61.52	3	620.67	426.00	958.00	293.29
Aluminum	2	38089.50	479.00	75700.00	53189.28	3	342.67	183.00	447.00	140.43
Arsenic	2	1.44	0.65	2.22	1.11	3	2.93	1.76	4.72	1.57
Barium	2	20.65	11.80	29.50	12.52	3	36.10	17.20	55.40	19.10
Cadmium	2	9.50	1.00	18.00	12.02	3	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	2	630.00	100.00	1160.00	749.53	3	434.00	106.00	748.00	321.23
Chloride	2	31.25	0.50	62.00	43.49	3	8.17	0.50	14.00	6.93
COD	2	12.45	1.50	23.40	15.49	3	14.70	6.90	27.10	10.86
Copper	2	11.35	10.80	11.90	0.78	3	4.34	3.75	4.76	0.53
Cyanide	2	0.00	0.00	0.00	0.00	3	0.00	0.00	0.00	0.00
Fluoride	2	0.75	0.50	1.00	0.35	3	0.47	0.20	0.80	0.31
Hardness (as CaCO3)	2	1358.50	127.00	2590.00	1741.60	3	1048.33	345.00	1600.00	641.10
Iron	2	9335.50	671.00	18000.00	12253.45	3	446.00	344.00	608.00	141.86
Lead	2	5.25	3.19	7.30	2.91	3	0.52	0.52	0.52	0.00
Manganese	2	17791.55	83.10	35500.00	25043.53	3	353.67	126.00	556.00	216.12
Nickel	2	511.60	3.19	1020.00	718.99	3	3.64	3.08	4.20	0.56
Nitrogen, Ammonia	2	0.60	0.30	0.90	0.42	3	0.05	0.05	0.05	0.00
Nitrogen, Nitrate+Nitrite	2	0.31	0.01	0.60	0.42	3	0.70	0.10	1.80	0.95
Phosphorus, Total	2	1.12	0.03	2.22	1.55	3	0.11	0.03	0.23	0.11
Selenium	2	1.18	1.18	1.18	0.00	3	3.81	1.18	7.48	3.28
Silica (Reactive)	2	31.45	2.90	60.00	40.38	3	5.80	3.00	8.20	2.62
Sodium	2	31.00	14.70	47.30	23.05	3	432.60	31.60	1180.00	647.84
Sulfate	2	1559.00	48.00	3070.00	2136.88	3	1376.67	790.00	2080.00	652.87
TOC	2	3.80	0.50	7.10	4.67	3	4.07	2.50	6.10	1.84
TS	2	2516.00	332.00	4700.00	3088.64	3	2743.33	1700.00	4080.00	1216.81
TSS	2	12.75	0.50	25.00	17.32	3	19.33	14.00	22.00	4.62
Zinc	2	2014.65	59.30	3970.00	2765.28	3	6.69	6.15	7.23	0.54
Dissolved Oxygen	2	4.48	2.75	6.21	2.45	3	8.15	5.00	13.90	4.99
pH	2	5.28	2.80	7.75	3.50	3	8.07	7.34	8.50	0.64
Water Temperature	2	28.17	23.91	32.42	6.02	3	26.58	24.30	28.65	2.18
Specific Conductivity	2	1957.70	431.40	3484.00	2158.51	3	2880.33	1809.00	4658.00	1550.28

Turbidity	2	26.70	4.30	49.10	31.68	1	37.00	37.00	37.00	
Water	2	26.90	2.20	51.60	34.93	3	94.47	63.20	150.10	48.30
Commercial	2	37.35	32.80	41.90	6.43	3	45.60	0.00	136.80	78.98
Agriculture	2	447.60	421.80	473.40	36.49	3	2026.23	471.70	3796.10	1672.63
High Density Residential	2	66.90	5.90	127.90	86.27	3	52.70	1.40	150.10	84.39
Low Density Residential	2	118.90	49.60	188.20	98.00	3	308.00	111.30	455.40	177.27
Residential	2	185.80	55.50	316.10	184.27	3	360.70	112.70	605.50	246.42
Grass Pasture	2	98.85	83.40	114.30	21.85	3	407.13	226.90	746.10	293.75
Forest	2	1244.35	138.30	2350.40	1564.19	3	1295.60	1077.10	1643.90	304.88
Industrial	2	17.10	11.80	22.40	7.50	3	25.33	0.00	76.00	43.88
Other	2	12.15	0.20	24.10	16.90	3	2.53	0.00	6.10	3.18
QHEI Score	2	29.25	21.50	37.00	10.96	3	47.00	38.50	53.50	7.70
QHEI Substrate Score	2	5.50	1.00	10.00	6.36	3	10.00	8.00	12.00	2.00
QHEI Instream Cover Score	2	5.00	5.00	5.00	0.00	3	8.33	6.00	11.00	2.52
QHEI Channel Score	2	7.50	5.00	10.00	3.54	3	10.83	8.00	13.50	2.75
QHEI Riparian Score	2	3.75	3.50	4.00	0.35	3	6.50	4.50	10.00	3.04
QHEI Pool Glide Score	2	3.50	3.00	4.00	0.71	3	6.00	4.00	8.00	2.00
Riffle Run Score	2	0.00	0.00	0.00	0.00	3	1.33	0.00	4.00	2.31
Gradient Score	2	4.00	4.00	4.00	0.00	3	4.00	4.00	4.00	0.00

Variable	Cluster 7 N	Mean	Minimum	Maximum	SD	Cluster 8 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	5	38.00	5.00	150.00	63.21	8	172.13	86.00	325.00	88.68
Aluminum	5	8571.60	213.00	23400.00	10094.09	8	435.13	10.00	1590.00	491.78
Arsenic	5	0.65	0.65	0.65	0.00	8	1.45	0.65	3.90	1.16
Barium	5	37.08	10.90	59.10	19.69	8	29.25	10.50	58.80	18.16
Cadmium	5	3.64	1.00	6.66	2.69	8	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	5	738.40	270.00	1200.00	336.66	8	550.75	72.00	1060.00	496.90
Chloride	5	1.46	0.50	5.30	2.15	8	11.10	0.50	25.00	8.46
COD	5	5.52	1.50	6.90	2.31	8	17.06	1.50	42.00	13.93
Copper	5	5.37	2.54	8.54	2.77	8	3.94	1.14	6.76	2.23
Cyanide	5	0.00	0.00	0.01	0.00	8	0.00	0.00	0.00	0.00
Fluoride	5	0.61	0.05	1.40	0.54	8	0.34	0.10	0.70	0.21
Hardness (as CaCO3)	5	1455.00	605.00	2510.00	687.75	8	1173.00	112.00	2490.00	1133.60
Iron	5	1603.20	243.00	3340.00	1200.40	8	641.70	48.20	1950.00	673.61
Lead	5	1.40	0.52	2.45	0.85	8	0.60	0.52	1.12	0.21
Manganese	5	15586.60	783.00	41300.00	16516.55	8	992.75	178.00	2670.00	846.14
Nickel	5	194.76	4.21	469.00	193.92	8	14.06	2.25	66.40	21.62
Nitrogen, Ammonia	5	0.22	0.05	0.40	0.16	8	0.05	0.05	0.05	0.00
Nitrogen, Nitrate+Nitrite	5	0.30	0.01	0.70	0.36	8	0.52	0.01	3.30	1.13
Phosphorus, Total	5	0.03	0.03	0.04	0.01	8	0.03	0.03	0.07	0.02
Selenium	5	1.90	1.18	2.44	0.66	8	2.10	1.18	3.71	1.03
Silica (Reactive)	5	19.26	7.00	40.00	14.38	8	8.51	3.60	15.00	3.97
Sodium	5	20.97	9.17	46.50	14.94	8	82.08	10.80	387.00	125.57
Sulfate	5	1429.80	549.00	2730.00	796.05	8	1077.25	18.00	2590.00	1129.49
TOC	5	2.14	1.20	2.70	0.57	8	5.38	2.00	13.00	3.83
TS	5	2226.00	1040.00	3950.00	1063.08	8	1908.00	199.00	4060.00	1800.98
TSS	5	9.50	0.50	20.00	7.58	8	10.25	0.50	27.00	9.49
Zinc	5	499.32	3.00	1410.00	584.14	8	23.48	3.00	126.00	42.27
Dissolved Oxygen	5	6.60	5.37	7.54	0.93	8	6.60	4.11	8.88	1.98
pH	5	4.96	2.91	7.13	1.99	8	7.87	6.27	8.83	0.81
Water Temperature	5	23.14	20.52	24.46	1.61	8	25.49	22.80	30.00	2.26
Specific Conductivity	5	1840.40	1042.00	2845.00	655.92	8	1591.14	272.00	3389.00	1386.39

Turbidity	5	52.86	11.10	97.10	36.53	8	22.35	1.00	50.90	14.30
Water	5	100.44	10.10	347.70	139.73	8	166.88	0.00	707.90	239.58
Commercial	5	7.12	0.00	32.80	14.38	8	19.85	0.00	72.80	29.67
Agriculture	5	644.28	164.00	1861.80	731.68	8	1555.19	109.90	5732.80	1931.70
High Density Residential	5	4.60	0.00	14.50	5.98	8	34.91	0.00	106.20	37.48
Low Density Residential	5	119.32	10.60	432.20	177.32	8	215.93	58.70	762.40	233.66
Residential	5	123.92	12.30	446.70	183.09	8	250.84	58.70	817.40	248.93
Grass Pasture	5	403.26	19.70	1628.20	689.25	8	424.13	31.80	1486.10	470.21
Forest	5	2631.36	610.30	7014.80	2566.88	8	3200.61	17.00	17890.70	6044.15
Industrial	5	4.48	0.00	22.40	10.02	8	31.71	0.00	131.80	48.78
Other	5	12.82	1.30	31.60	14.73	8	19.59	0.10	98.10	34.16
QHEI Score	5	44.20	33.00	64.00	13.29	8	41.66	35.00	51.00	5.59
QHEI Substrate Score	5	8.80	4.00	12.00	3.11	8	7.75	0.00	14.00	5.15
QHEI Instream Cover Score	5	6.40	3.00	13.00	4.22	8	7.63	2.00	16.00	4.00
QHEI Channel Score	5	11.40	9.00	13.00	1.52	8	9.13	5.00	12.00	2.25
QHEI Riparian Score	5	4.00	1.00	6.00	1.87	8	5.53	3.75	10.00	2.07
QHEI Pool Glide Score	5	6.20	3.00	11.00	3.56	8	6.00	3.00	10.00	2.07
Riffle Run Score	5	1.80	1.00	2.00	0.45	8	1.13	0.00	2.00	0.99
Gradient Score	5	5.60	4.00	8.00	2.19	8	4.50	4.00	8.00	1.41

Table 3. Summary statistics for 50 chemistry (concentration/ppm), habitat, and land use variables by crayfish grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Patoka River National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Alkalinity (as CaCO3)	4	78.00	5.00	109.00	49.00	3	281.33	86.00	606.00	283.10
Aluminum	4	3296.75	383.00	7880.00	3596.02	3	532.40	87.20	875.00	403.80
Arsenic	4	2.67	0.65	5.66	2.16	3	1.85	1.64	2.15	0.27
Barium	4	68.90	36.60	136.00	46.30	3	35.83	28.60	47.40	10.12
Cadmium	4	1.31	1.00	2.24	0.62	3	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	4	238.50	76.00	642.00	270.13	3	429.33	126.00	672.00	278.01
Chloride	4	20.13	0.50	45.00	19.17	3	11.57	0.50	25.00	12.42
COD	4	33.25	6.90	85.70	35.84	3	17.27	6.10	25.50	10.03
Copper	4	3.95	1.54	7.19	2.56	3	4.34	2.25	6.02	1.92
Cyanide	4	0.00	0.00	0.00	0.00	3	0.00	0.00	0.00	0.00
Fluoride	4	0.24	0.05	0.50	0.19	3	0.33	0.20	0.50	0.15
Hardness (as CaCO3)	4	428.75	109.00	1220.00	529.24	3	1028.67	206.00	1830.00	812.21
Iron	4	2756.25	391.00	8480.00	3833.84	3	984.33	143.00	1770.00	814.93
Lead	4	2.66	0.52	7.79	3.47	3	0.72	0.52	1.11	0.34
Manganese	4	4418.00	97.00	14300.00	6665.21	3	718.67	588.00	934.00	187.90
Nickel	4	47.07	2.17	169.00	81.47	3	5.71	2.66	8.75	3.05
Nitrogen, Ammonia	4	0.11	0.05	0.30	0.13	3	0.07	0.05	0.10	0.03
Nitrogen, Nitrate+Nitrite	4	0.36	0.01	0.70	0.40	3	0.07	0.01	0.10	0.05
Phosphorus, Total	4	0.15	0.04	0.45	0.20	3	0.05	0.03	0.07	0.02
Selenium	4	1.47	1.18	2.35	0.59	3	2.22	1.18	2.97	0.93
Silica (Reactive)	4	10.03	5.20	22.00	8.04	3	4.77	2.60	6.40	1.96
Sodium	4	23.29	9.17	47.80	17.84	3	29.87	11.10	60.10	26.44
Sulfate	4	355.25	27.00	1220.00	578.16	3	750.67	52.00	1200.00	613.27
TOC	4	6.95	2.40	11.80	4.11	3	5.53	2.50	8.30	2.91
TS	4	751.25	181.00	1890.00	775.53	3	1538.67	326.00	2590.00	1140.59
TSS	4	64.00	12.00	216.00	101.34	3	27.33	4.00	52.00	24.03
Zinc	4	83.28	3.00	295.00	141.82	3	7.87	3.00	13.90	5.54
Dissolved Oxygen	4	6.70	4.91	10.38	2.57	3	5.33	4.86	5.81	0.48
pH	4	6.75	3.41	8.94	2.38	3	8.11	7.51	8.46	0.52

Water Temperature	4	26.21	24.46	27.90	1.78	3	25.81	25.50	26.40	0.51
Specific Conductivity	4	705.08	234.40	1640.00	634.94	3	1469.67	464.00	2500.00	1018.22
Turbidity	4	117.53	21.60	397.30	186.55	1	54.10	54.10	54.10	
Water	4	37.20	0.00	60.70	28.98	3	69.60	46.40	83.20	20.19
Commercial	4	3.00	0.00	8.80	4.15	3	0.57	0.00	1.70	0.98
Agriculture	4	1841.28	169.90	4323.20	2017.56	3	686.10	7.60	1189.70	610.15
High Density Residential	4	18.80	0.00	59.50	27.97	3	3.17	0.90	4.90	2.05
Low Density Residential	4	277.78	19.00	631.50	296.16	3	116.30	2.20	176.30	98.86
Residential	4	296.58	19.00	691.00	322.05	3	119.47	3.10	180.00	100.80
Grass Pasture	4	1103.80	0.00	2140.50	1153.82	3	242.93	34.30	414.70	192.86
Forest	4	1176.75	0.00	2280.50	934.14	3	1090.23	765.90	1456.30	347.09
Industrial	4	3.30	0.00	9.80	4.62	3	27.33	0.00	82.00	47.34
Other	4	0.73	0.20	2.20	0.98	3	5.57	0.20	15.50	8.61
QHEI Score	4	46.50	29.50	64.00	14.61	3	50.17	43.50	57.00	6.75
QHEI Substrate Score	4	9.00	4.00	12.00	3.83	3	8.00	5.00	12.00	3.61
QHEI Instream Cover Score	4	9.50	7.00	13.00	2.65	3	10.67	6.00	16.00	5.03
QHEI Channel Score	4	9.75	7.00	12.00	2.06	3	10.50	8.00	12.50	2.29
QHEI Riparian Score	4	3.88	2.00	6.00	1.65	3	9.00	8.50	9.50	0.50
QHEI Pool Glide Score	4	6.50	4.00	11.00	3.32	3	7.00	6.00	8.00	1.00
Riffle Run Score	4	1.38	0.00	2.00	0.95	3	1.00	0.00	2.00	1.00
Gradient Score	4	6.50	4.00	10.00	3.00	3	4.00	4.00	4.00	0.00

Variable	Cluster 3 N	Mean	Minimum	Maximum	SD	Cluster 4 N	Mean	Minimum	Maximum	SD
Alkalinity (as CaCO3)	3	364.00	100.00	794.00	375.60	5	131.60	85.00	216.00	54.37
Aluminum	3	413.00	57.00	771.00	357.00	5	601.74	61.70	1250.00	516.78
Arsenic	3	2.12	0.65	4.25	1.89	5	1.38	0.65	2.58	1.00
Barium	3	32.87	15.60	60.30	24.02	5	42.30	26.10	59.10	12.02
Cadmium	3	1.00	1.00	1.00	0.00	5	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	3	697.33	98.00	1050.00	521.74	5	156.00	90.00	270.00	76.60
Chloride	3	20.87	5.30	52.00	26.96	5	14.00	5.30	23.00	7.09
COD	3	12.23	6.00	15.80	5.42	5	20.02	6.80	29.30	9.01
Copper	3	5.14	3.19	6.37	1.71	5	2.25	1.06	3.34	0.83
Cyanide	3	0.00	0.00	0.00	0.00	5	0.00	0.00	0.00	0.00
Fluoride	3	0.67	0.60	0.70	0.06	5	0.26	0.10	0.40	0.13
Hardness (as CaCO3)	3	1669.33	378.00	2490.00	1131.94	5	271.20	121.00	605.00	204.10
Iron	3	415.73	48.20	839.00	398.34	5	903.34	63.70	1440.00	523.46
Lead	3	0.52	0.52	0.52	0.00	5	0.75	0.52	1.12	0.31
Manganese	3	1144.93	81.80	2670.00	1354.52	5	1282.60	120.00	2680.00	1119.47
Nickel	3	26.37	3.46	66.40	34.79	5	4.11	2.25	7.94	2.27
Nitrogen, Ammonia	3	0.05	0.05	0.05	0.00	5	0.06	0.05	0.10	0.02
Nitrogen, Nitrate+Nitrite	3	0.04	0.01	0.10	0.05	5	0.83	0.01	3.00	1.30
Phosphorus, Total	3	0.07	0.03	0.15	0.07	5	0.09	0.03	0.24	0.09
Selenium	3	2.75	1.18	4.16	1.50	5	1.18	1.18	1.18	0.00
Silica (Reactive)	3	5.63	1.80	11.00	4.79	5	7.64	5.30	9.70	1.63
Sodium	3	248.30	17.30	665.00	361.58	5	22.94	13.00	46.50	13.42
Sulfate	3	1664.00	912.00	2110.00	655.00	5	167.00	18.00	549.00	217.39
TOC	3	4.50	2.00	5.80	2.17	5	5.86	2.70	8.10	2.37
TS	3	2966.67	2310.00	3440.00	586.88	5	450.60	199.00	1040.00	341.13
TSS	3	9.67	4.00	21.00	9.81	5	12.80	4.00	23.00	7.66
Zinc	3	46.67	6.49	126.00	68.70	5	5.19	3.00	7.49	2.07
Dissolved Oxygen	3	7.45	6.58	8.85	1.22	5	6.54	4.61	8.43	1.54
pH	3	8.32	7.73	9.35	0.90	5	7.85	6.80	8.97	0.89
Water Temperature	3	30.00	23.82	33.90	5.41	5	23.84	19.10	26.19	2.74
Specific Conductivity	3	2752.33	2429.00	3140.00	359.84	5	515.00	272.00	1042.00	310.84

Turbidity	2	20.30	14.60	26.00	8.06	4	62.38	22.20	97.10	36.94
Water	3	286.53	43.60	707.90	366.34	5	116.80	0.00	347.70	159.34
Commercial	3	22.60	0.00	59.00	31.83	5	55.48	0.00	252.40	110.50
Agriculture	3	3781.67	944.00	5732.80	2514.48	5	5578.84	4.90	25587.70	11210.49
High Density Residential	3	56.50	3.40	111.10	53.87	5	176.26	0.00	799.00	349.13
Low Density Residential	3	416.63	119.30	762.40	324.27	5	874.04	6.90	3808.70	1649.38
Residential	3	473.13	122.70	817.40	347.39	5	1050.30	6.90	4607.70	1996.17
Grass Pasture	3	594.03	134.80	1486.10	772.67	5	2409.34	61.90	10077.30	4336.58
Forest	3	7413.33	457.10	17890.70	9234.79	5	3634.50	3.90	11078.10	5145.08
Industrial	3	95.30	0.00	204.90	103.20	5	28.54	0.00	134.10	59.13
Other	3	34.60	0.30	98.10	55.05	5	17.80	0.10	57.00	25.80
QHEI Score	3	34.25	28.00	39.25	5.73	5	44.50	33.00	67.00	13.42
QHEI Substrate Score	3	9.50	8.00	12.00	2.18	5	9.00	0.00	15.00	6.00
QHEI Instream Cover Score	3	4.67	4.00	6.00	1.15	5	6.80	3.00	9.00	2.28
QHEI Channel Score	3	6.83	5.00	8.00	1.61	5	12.20	9.00	19.00	4.09
QHEI Riparian Score	3	3.58	3.00	4.00	0.52	5	4.60	3.00	8.00	1.98
QHEI Pool Glide Score	3	5.00	4.00	6.00	1.00	5	4.60	3.00	7.00	1.52
Riffle Run Score	3	0.67	0.00	2.00	1.15	5	2.50	2.00	4.00	0.87
Gradient Score	3	4.00	4.00	4.00	0.00	5	4.80	4.00	8.00	1.79

Variable	N	Mean	Cluster 5 Minimum	Maximum	SD
Alkalinity (as CaCO3)	7	262.57	86.00	680.00	228.69
Aluminum	7	490.71	10.00	1590.00	555.73
Arsenic	7	1.23	0.65	2.32	0.63
Barium	7	34.01	10.50	55.40	16.09
Cadmium	7	1.00	1.00	1.00	0.00
Calcium (as CaCO3)	7	473.14	88.00	926.00	322.12
Chloride	7	9.06	0.50	25.00	8.58
COD	7	13.37	7.30	29.30	7.47
Copper	7	4.32	1.14	6.76	1.91
Cyanide	7	0.00	0.00	0.00	0.00
Fluoride	7	0.34	0.10	0.70	0.21
Hardness (as CaCO3)	7	1121.14	129.00	2060.00	781.68
Iron	7	754.66	28.60	1950.00	652.94
Lead	7	0.52	0.52	0.52	0.00
Manganese	7	713.29	129.00	1620.00	525.48
Nickel	7	5.84	3.64	9.60	2.23
Nitrogen, Ammonia	7	0.05	0.05	0.05	0.00
Nitrogen, Nitrate+Nitrite	7	0.56	0.01	3.30	1.21
Phosphorus, Total	7	0.04	0.03	0.06	0.02
Selenium	7	1.99	1.18	3.71	1.10
Silica (Reactive)	7	7.23	3.60	15.00	3.66
Sodium	7	33.34	10.80	60.40	15.89
Sulfate	7	887.00	53.00	1870.00	683.44
TOC	7	4.17	2.80	7.20	1.53
TS	7	1702.86	230.00	3290.00	1162.97
TSS	7	11.79	0.50	27.00	8.70
Zinc	7	7.63	3.00	19.30	5.51
Dissolved Oxygen	7	6.13	4.26	8.88	1.70
pH	7	7.73	7.17	8.54	0.54
Water Temperature	7	25.37	20.66	30.00	3.19
Specific Conductivity	7	1530.87	300.00	2574.00	916.56

Turbidity	5	30.44	14.40	69.90	22.43
Water	7	90.43	3.40	275.60	103.26
Commercial	7	0.33	0.00	1.40	0.58
Agriculture	7	892.00	0.00	2972.10	1100.27
High Density Residential	7	4.54	0.00	16.70	6.13
Low Density Residential	7	154.87	4.10	396.10	155.57
Residential	7	159.41	4.10	402.70	157.20
Grass Pasture	7	352.40	31.80	908.70	340.57
Forest	7	2142.13	123.70	10214.60	3625.74
Industrial	7	0.99	0.00	6.90	2.61
Other	7	7.63	0.00	25.30	11.75
QHEI Score	7	44.32	35.00	53.50	7.87
QHEI Substrate Score	7	9.29	0.00	14.00	4.61
QHEI Instream Cover Score	7	7.00	2.00	14.00	4.32
QHEI Channel Score	7	11.00	5.00	17.00	3.75
QHEI Riparian Score	7	5.89	3.50	10.00	2.85
QHEI Pool Glide Score	7	5.71	3.00	10.00	2.69
Riffle Run Score	7	1.43	0.00	4.00	1.62
Gradient Score	7	4.00	4.00	4.00	0.00

Table 4. List of three physical and chemical variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Patoka River National Wildlife Refuge during August 2007.

Variable	P Value
Chemical	
Chloride	p = 0.0413
Zinc	p = 0.0039
Land-use	
Grass/Pasture	p = 0.049

<u>Multivariate Results</u>

For this analysis we chose 75-80% similarity as a benchmark for defining assemblage structure clustering (Figure 2). These clusters were used for stressor response measurement. Kruskal-Wallis ANOVA by ranks tests were significant for eight clusters for fish, showing that two inorganic and a single land use measure was significantly predictive of the fish assemblage structure (Table 4). Macroinvertebrate assemblages were significant for eight clusters, showing that eight physical-chemical and a single land use type were predictive of the structure (Table 5), while crayfish structure was significant for five clusters and only a single variable was significant. Simon and Morris (in press) showed that crayfish structure was highly correlated with sediment chemistry in the Patoka River; however, the current study was a measure of broad contaminant issues and did not focus on chemical specific responses.

Fish assemblage structure was significantly predicted by the presence of chloride, zinc, and grass/pasture land use types (Table 4). Chloride was significantly different in clusters 2 and 3 from all of the other clusters, while zinc was significant in clusters 1-3. All three parameters showed a slight dose response curve associated with the biological integrity response of the fish assemblage (Figure 4).

Box and whisker plots for significant variable responses for fish (Figure 4), macroinvertebrates (Figure 5), and crayfish (Figure 6) show the greatest response was observed among macroinvertebrate assemblage structure. Macroinvertebrate assemblage structure was predicted by metals (i.e., aluminum, cadmium, lead, manganese, nickel), nutrients (i.e., nitrogen, ammonia), pH, alkalinity, and high density residential land use (Table 5). All metals and nitrogen (Ammonia) was highly predictive with cluster 5 (Figure 5). These parameters were associated with the mixing of the urban populations in the vicinity of the acid mine drainage leaching of contaminants.

Crayfish assemblage structure was highly predictive with specific conductivity (Kruskal-Wallis ANOVA, p= 0.0438). Crayfish were absent from acid pH streams in the South Fork Patoka River and also from areas surrounding the Sugar Ridge Fish and Wildlife Area. Crayfish presence was an important indicator of stressor response to acid mine drainage and oil brines in the vicinity of the refuges (Figure 6) and was associated with cluster 3.

Table 5. List of three physical and chemical variables significantly predictive (a = 0.05) of the macroinvertebrate assemblage biologic gradient using the Kruskal-Wallis ANOVA b

Variable	P Value
Chemical	
Alkalinity	p = 0.0073
Aluminum	p = 0.0440
Cadmium	p = 0.0023
Lead	p = 0.0063
Manganese	p = 0.0477
Nickel	p = 0.0323
Nitrogen, Ammonia	p = 0.0012
pH	p = 0.0224
Land-use	
High Density Residential	p = 0.0322

Land Use Response

Both fish (Table 4) and macroinvertebrate (Table 5) organism groups showed significant results with land use. Grass/Pasture land use was predictive of fish assemblages structure, while high density residential land use was significant for macroinvertebrates.

The significant relationship between grass/pasture land use and fish assemblage integrity at first glance may seem puzzling; however, in light of the study by Simon and Morris (in press) we know that this land use type has the predominance of oil derricks found in the Patoka River watershed. The current study did not measure polycyclic aromatic hydrocarbons (PAHs) in the water column, since this contaminant group is best measured in sediment media, but the previous study by Simon and Morris (in press) showed that PAHs were a dominant contaminant in the Patoka River that drove the response of the biological assemblages.

Macroinvertebrate assemblage structure was predictive by high density residential development (Table 5). This relationship was significant for clusters 3, 5, and 6 patterns (Figure 5). The largest variation and greatest response was observed in cluster 5. These areas are associated with Oakland City and the City of Huntington (Figure 7).

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. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used later to reduce the set of variables in a dataset.

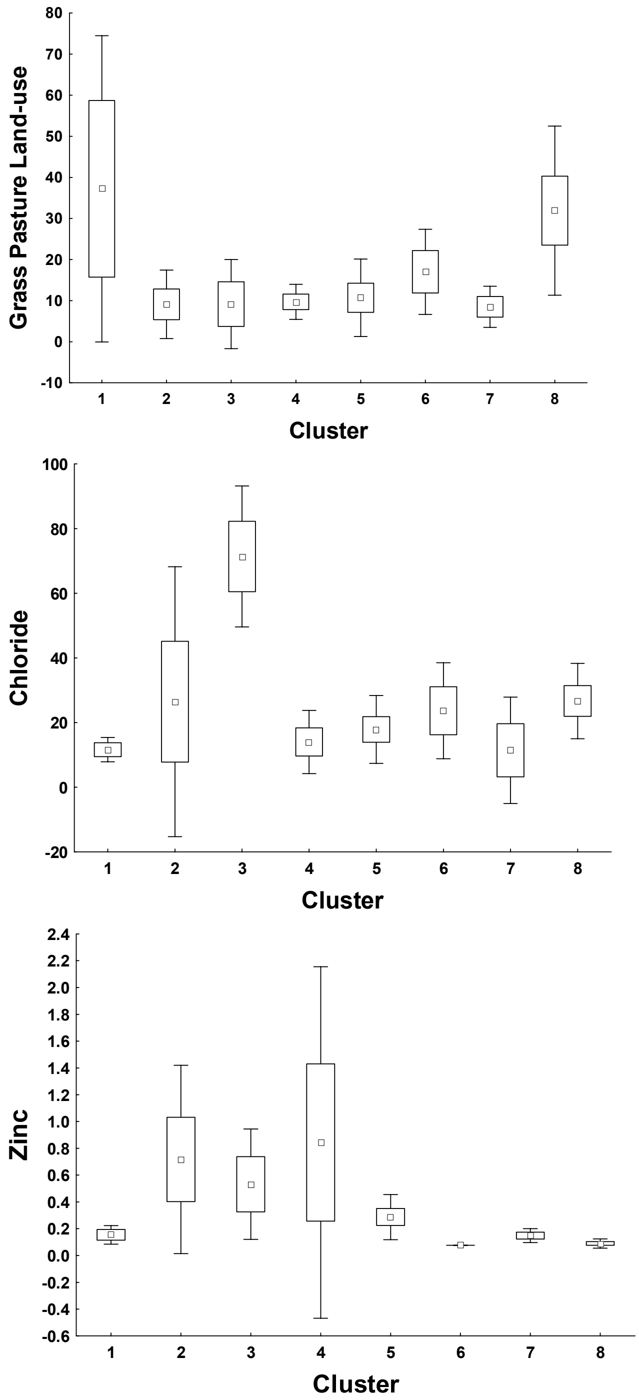

Figure 4. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the fish assemblage clustering.

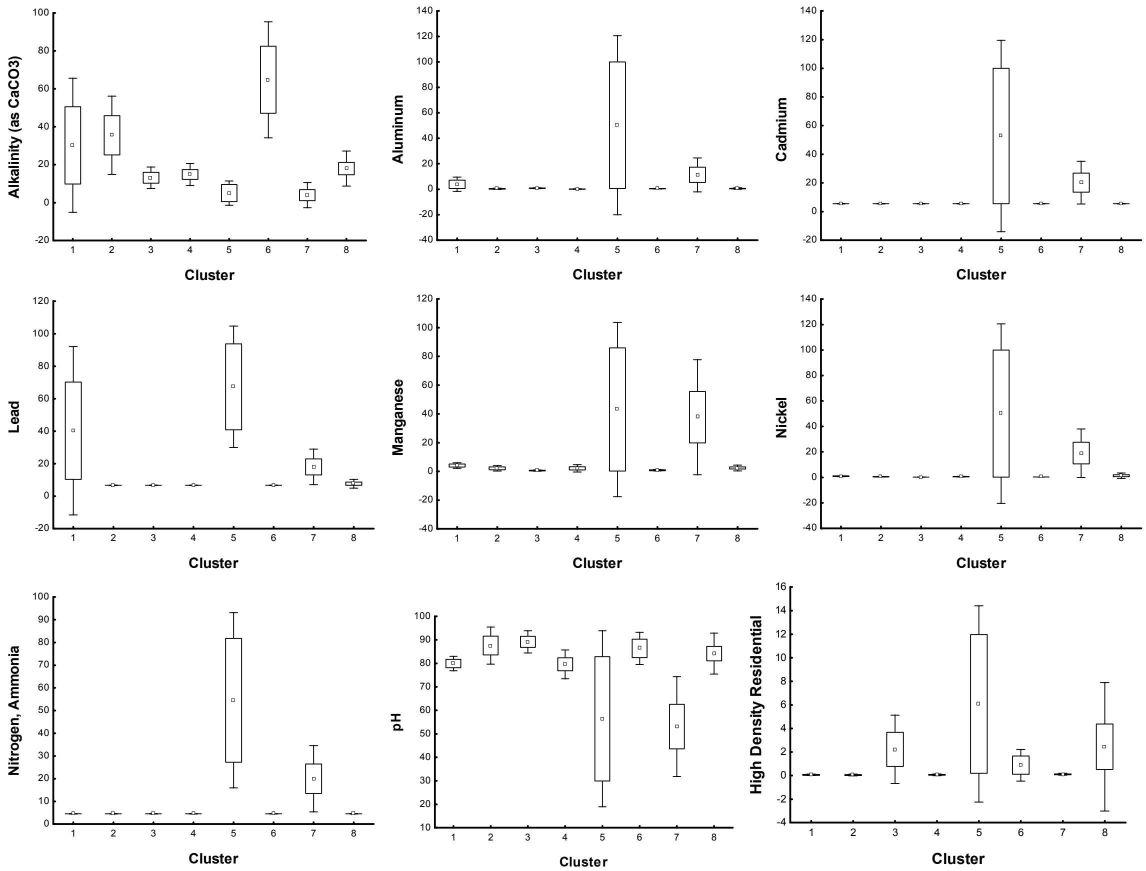

Figure 5. Box-and-whisker plots of the significant water chemistry and land use variables that were predictive of the macroinvertebrate assemblage clustering.

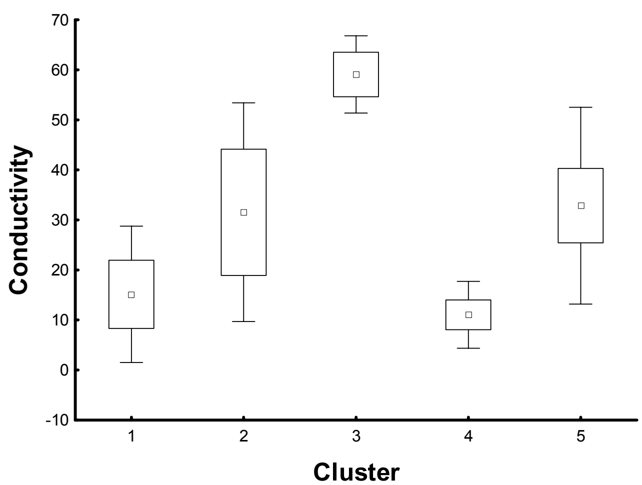

Figure 6. Box-and-whisker plot of the only significant water chemistry Variable that was predictive of the crayfish assemblage clustering.

Table 6. Results of factor analysis and explained variance observed in fish assemblage biologic structure and three significant chemical and land use variables at the Patoka River National Wildlife Refuge during 2007.

Variable	Factor 1
Grass Pasture	**-0.849481**
Chloride	**-0.620058**
Zinc	**0.742997**
Proportion Total Variance	55.2711

Table 7. Results of factor analysis and explained variance observed in macroinvertebrate assemblage biologic structure and nine significant chemical and land use variables at the Patoka River National Wildlife Refuge during 2007.

Variable	Factor 1	Factor 2
High Density Residential	-0.039712	**0.963681**
Alkalinity (as CaCO3)	**-0.885465**	0.159796
Aluminum	**0.878243**	0.041797
Cadmium	**0.942505**	-0.152446
Lead	**0.916089**	0.189047
Manganese	**0.812220**	-0.364556
Nickel	**0.862434**	-0.386097
Nitrogen, Ammonia	**0.953747**	0.052572
pH	**-0.917012**	0.287055
Proportion Total Variance	71.538996	15.356423

Fish assemblage structure explained observed variance on a single factor, which explained 55.3% of the variance in the fish assemblage information (Table 6). Grass/Pasture land use and chloride were negatively associated with fish assemblages, while zinc was positively associated with fish assemblage integrity.

Macroinvertebrate assemblage structure was explained by two factors, which explained a total of 86.9% of the variance (Table 7). The primary factor included the metals and nitrogen (Ammonia), while alkalinity and pH were negatively correlated with the explained variance. The secondary factor included high density residential land use.

Since only a single contaminant was explained by the crayfish assemblage, no further analysis of that information could be conducted.

Associations between Contaminants and Hot Spot Analysis

Contaminant distribution, based on percentage-of-range concentrations, showed variable responses between organism groups. Fish assemblage structure was predicted by negative associations with chlorides, which are typical of sewage treatement waste effluents. The primary distributions of chlorides in study area are from the urban centers

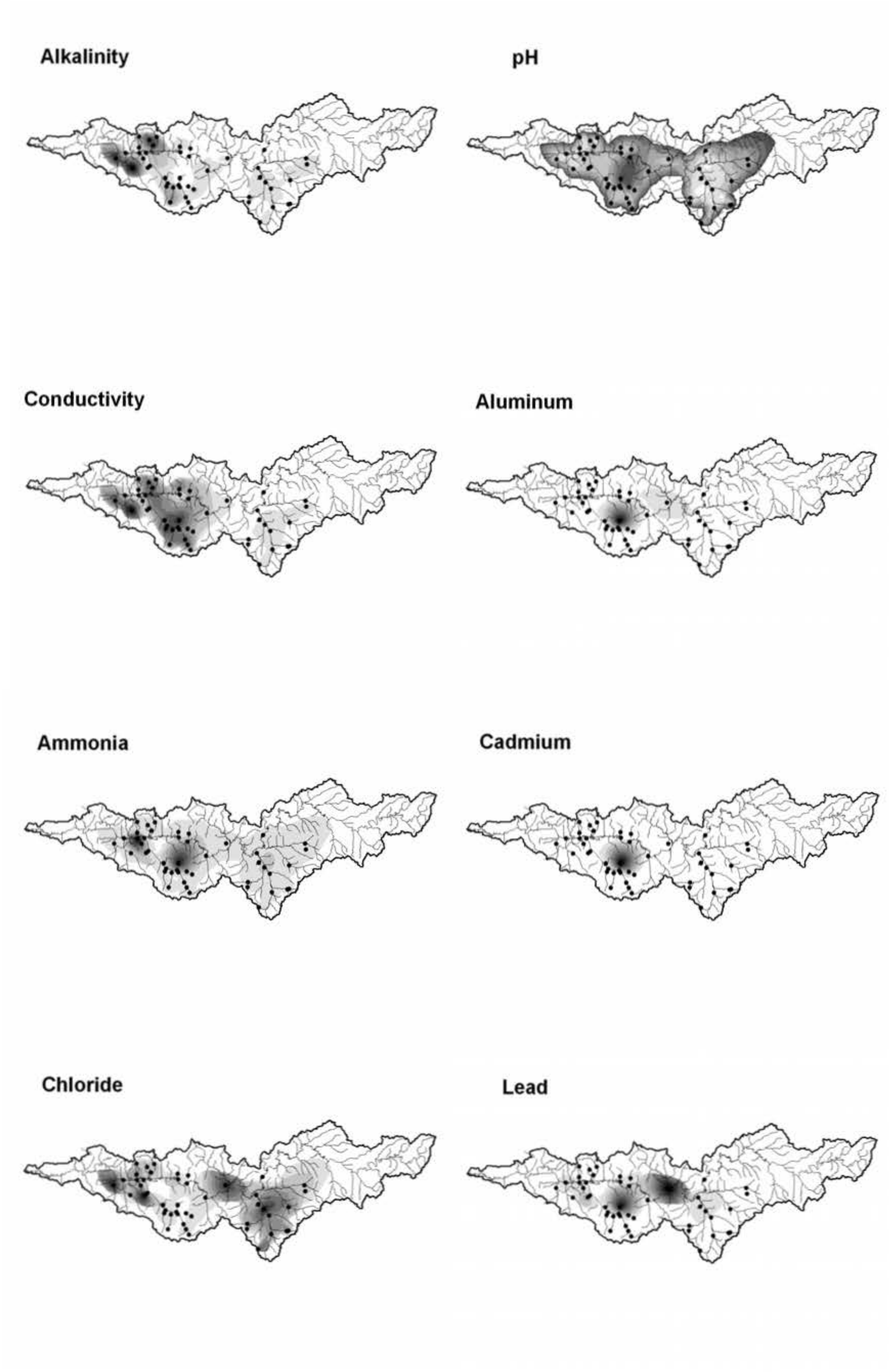
Alkalinity
pH
Conductivity
Aluminum
Ammonia
Cadmium
Chloride
Lead

Figure 7. Relative concentration pleths of significant water quality, habitat, and land use chemistry variables that were predictive of fish, macroinvertebrate and crayfish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area. All concentrations are presented on a relative (percentage of range) scale.

of Oakland City, Winslow, Jasper, and Huntington (Figure 7). Zinc contamination was localized and emanated from the vicinity of the South Fork Patoka River (Figure 7), while grass pasture land use associations were clearly associated with both the oil derricks and brine effluent discharge and from areas that drained into the refuge (Figure 7).

Macroinvertebrates showed a significant relationship with low pH and low alkalinity, which resulted from acid mine leachate (Figure 7). These parameters are widespread issues in the area surrounding the refuge and can only be mitigated through large scale changes in land reclamation. All of the heavy metals were linked to a single location in the South Fork Patoka River (Figure 7). This area is currently being remediated by the Office of Surface Mines and the Indiana Department of Natural Resources. Based on the macroinvertebrate factor analysis, the metals all emanate from a single area and react as a single contaminant class.

Appendix 1. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use for fish assemblage structure in the Patoka River National Wildlife Refuge.

Variable	N	Mean	Minimum	Maximum	SD
Contaminant					
Alkalinity (as CaCO3)	46	198.17	5.00	958.0	205.83
Aluminum	46	3202.16	10.00	75700.0	11658.56
Arsenic	46	1.87	0.65	6.3	1.41
Barium	46	38.92	10.50	136.0	21.83
Cadmium	46	1.66	1.00	18.0	2.72
Calcium (as CaCO3)	46	454.70	64.00	1200.0	393.69
Chloride	46	13.95	0.50	62.0	15.24
COD	46	17.40	1.50	85.7	14.63
Copper	46	4.30	1.06	11.9	2.46
Cyanide	46	0.00	0.00	0.0	0.00
Fluoride	46	0.42	0.05	1.4	0.28
Hardness (as CaCO3)	46	973.37	81.00	2820.0	883.74
Iron	46	1404.80	28.60	18000.0	2853.81
Lead	46	1.10	0.52	7.8	1.50
Manganese	46	3110.72	81.80	41300.0	8385.69
Nickel	46	49.56	1.61	1020.0	168.24
Nitrogen, Ammonia	46	0.13	0.05	1.1	0.22
Nitrogen, Nitrate+Nitrite	46	0.58	0.01	5.0	1.05
Phosphorus, Total	46	0.15	0.03	2.2	0.38
Selenium	46	1.97	1.18	7.5	1.21
Silica (Reactive)	46	9.39	1.80	60.0	10.18
Sodium	46	80.20	4.41	1180.0	197.81
Sulfate	46	901.41	18.00	3070.0	897.35
TOC	46	5.08	0.50	18.8	3.34
TS	46	1659.46	140.00	4700.0	1405.53
TSS	46	21.88	0.50	216.0	35.00
Zinc	46	151.89	3.00	3970.0	620.68
General Chemistry					
Dissolved Oxygen	46	6.08	0.53	13.9	2.19
pH	46	7.56	2.80	9.4	1.48
Water Temperature	46	25.84	19.10	33.9	3.16
Specific Conductivity	46	1498.11	157.60	4658.0	1152.34
Turbidity	36	81.44	1.00	1000.0	171.08
Land Use					
Water	46	588.30	0.00	10069.1	1858.31
Commercial	46	88.79	0.00	1478.9	279.10
Agriculture	46	7761.60	0.00	130240.3	24213.23
High Density Residential	46	206.88	0.00	3410.5	644.88
Low Density Residential	46	1437.38	0.20	23619.5	4534.51
Residential	46	1644.27	0.20	27030.0	5174.36
Grass Pasture	46	4602.22	0.00	72849.8	14798.69
Forest	46	13756.96	0.00	226490.5	43989.63
Industrial	46	87.45	0.00	1586.2	267.71
Other	46	482.40	0.00	7949.2	1794.78

Habitat					
QHEI Score	44	42.22	0.00	67.0	12.58
QHEI-Substrate Score	44	8.28	0.00	16.5	4.63
QHEI-Instream Cover Score	44	7.05	0.00	16.0	3.42
QHEI-Channel Score	44	10.22	0.00	19.0	3.43
QHEI-Riparian Score	44	5.31	0.00	10.0	2.68
QHEI-Pool Glide Score	44	5.36	0.00	12.0	2.34
QHEI-Riffle Run Score	44	1.36	0.00	5.5	1.38
QHEI-Gradient Score	44	4.64	0.00	10.0	1.87

Appendix 2. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use for macroinvertebrate assemblage structure in the Patoka River National Wildlife Refuge.

Variable	N	Mean	Minimum	Maximum	SD
Contaminant					
Alkalinity (as CaCO3)	34	205.059	5.0000	958.00	210.95
Aluminum	34	4026.079	10.0000	75700.00	13510.84
Arsenic	34	1.609	0.6500	5.66	1.20
Barium	34	36.703	10.5000	136.00	23.58
Cadmium	34	1.888	1.0000	18.00	3.14
Calcium (as CaCO3)	34	525.471	72.0000	1200.00	393.00
Chloride	34	11.685	0.5000	62.00	14.20
COD	34	15.985	1.5000	85.70	15.33
Copper	34	4.777	1.0600	11.90	2.62
Cyanide	34	0.003	0.0025	0.01	0.00
Fluoride	34	0.437	0.0500	1.40	0.30
Hardness (as CaCO3)	34	1156.500	109.0000	2820.00	899.09
Iron	34	1472.965	28.6000	18000.00	3287.55
Lead	34	1.176	0.5200	7.79	1.73
Manganese	34	4012.944	83.1000	41300.00	9622.14
Nickel	34	65.117	2.1700	1020.00	193.99
Nitrogen, Ammonia	34	0.107	0.0500	0.90	0.17
Nitrogen, Nitrate+Nitrite	34	0.381	0.0100	3.30	0.70
Phosphorus, Total	34	0.121	0.0250	2.22	0.38
Selenium	34	2.161	1.1800	7.48	1.31
Silica (Reactive)	34	10.574	2.8000	60.00	11.53
Sodium	34	77.814	9.1700	1180.00	205.01
Sulfate	34	1065.147	18.0000	3070.00	911.92
TOC	34	4.635	0.5000	13.00	2.80
TS	34	1905.853	181.0000	4700.00	1419.83
TSS	34	18.029	0.5000	216.00	36.61
Zinc	34	201.459	3.0000	3970.00	718.05
General Chemistry					
Dissolved Oxygen	34	6.422	2.7500	13.90	2.13
pH	34	7.301	2.8000	9.13	1.60
Water Temperature	34	25.602	20.5200	32.42	2.89
Specific Conductivity	34	1676.876	234.4000	4658.00	1144.61
Turbidity	28	51.021	1.0000	397.30	73.31
Land Use					
Water	34	94.062	0.0000	707.90	138.91
Commercial	34	15.059	0.0000	136.80	31.46
Agriculture	34	1370.915	0.0000	9637.40	2027.83
High Density Residential	34	27.956	0.0000	255.60	55.44
Low Density Residential	34	210.221	0.2000	1509.40	298.68
Residential	34	238.176	0.2000	1765.00	342.93
Grass Pasture	34	550.756	0.0000	5135.10	993.56
Forest	34	2200.762	0.0000	17890.70	3556.17
Industrial	34	13.529	0.0000	131.80	29.73
Other	34	8.753	0.0000	98.10	18.76

Habitat					
QHEI Score	34	43.309	21.5000	67.00	10.32
QHEI Substrate Score	34	8.294	0.0000	14.00	4.06
QHEI Instream Cover Score	34	7.529	2.0000	16.00	3.50
QHEI Channel Score	34	10.588	5.0000	19.00	3.21
QHEI Riparian Score	34	5.353	1.0000	10.00	2.43
QHEI Pool Glide Score	34	5.529	3.0000	11.00	2.09
QHEI Riffle Run Score	34	1.368	0.0000	4.00	1.23
QHEI Gradient Score	34	4.647	4.0000	10.00	1.61

Appendix 3. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use for crayfish assemblage structure in the Patoka River National Wildlife Refuge.

Variable	N	Mean	Minimum	Maximum	SD
Contaminant					
Alkalinity (as CaCO3)	22	215.64	5.00	794.00	216.11
Aluminum	22	1021.22	10.00	7880.00	1795.30
Arsenic	22	1.73	0.65	5.66	1.27
Barium	22	42.33	10.50	136.00	25.47
Cadmium	22	1.06	1.00	2.24	0.26
Calcium (as CaCO3)	22	383.00	76.00	1050.00	328.41
Chloride	22	14.15	0.50	52.00	13.78
COD	22	18.87	6.00	85.70	16.84
Copper	22	3.90	1.06	7.19	1.92
Cyanide	22	0.00	0.00	0.00	0.00
Fluoride	22	0.35	0.05	0.70	0.20
Hardness (as CaCO3)	22	864.23	109.00	2490.00	803.55
Iron	22	1137.48	28.60	8480.00	1729.43
Lead	22	0.99	0.52	7.79	1.55
Manganese	22	1575.85	81.80	14300.00	2963.23
Nickel	22	15.73	2.17	169.00	36.73
Nitrogen, Ammonia	22	0.07	0.05	0.30	0.05
Nitrogen, Nitrate+Nitrite	22	0.45	0.01	3.30	0.92
Phosphorus, Total	22	0.07	0.03	0.45	0.10
Selenium	22	1.85	1.18	4.16	0.98
Silica (Reactive)	22	7.28	1.80	22.00	4.34
Sodium	22	57.99	9.17	665.00	136.68
Sulfate	22	714.05	18.00	2110.00	708.34
TOC	22	5.29	2.00	11.80	2.55
TS	22	1395.18	181.00	3440.00	1153.22
TSS	22	23.34	0.50	216.00	44.49
Zinc	22	26.19	3.00	295.00	65.45
General Chemistry					
Dissolved Oxygen	22	6.40	4.26	10.38	1.66
pH	22	7.71	3.41	9.35	1.18
Water Temperature	22	25.87	19.10	33.90	3.34
Specific Conductivity	22	1308.06	234.40	3140.00	982.02
Turbidity	16	60.41	14.40	397.30	93.59
Land Use					
Water	22	110.65	0.00	707.90	163.26
Commercial	22	16.42	0.00	252.40	54.32
Agriculture	22	2495.75	0.00	25587.70	5423.03
High Density Residential	22	53.06	0.00	799.00	169.10
Low Density Residential	22	371.10	2.20	3808.70	797.25
Residential	22	424.16	3.10	4607.70	962.67
Grass Pasture	22	974.53	0.00	10077.30	2141.33
Forest	22	2881.14	0.00	17890.70	4617.31
Industrial	22	24.12	0.00	204.90	53.75
Other	22	12.08	0.00	98.10	24.07

Habitat					
QHEI Score	22	44.18	28.00	67.00	10.50
QHEI Substrate Score	22	9.02	0.00	15.00	4.11
QHEI Instream Cover Score	22	7.59	2.00	16.00	3.66
QHEI Channel Score	22	10.41	5.00	19.00	3.37
QHEI Riparian Score	22	5.34	2.00	10.00	2.55
QHEI Pool Glide Score	22	5.68	3.00	11.00	2.23
Riffle Run Score	22	1.50	0.00	4.00	1.27
Gradient Score	22	4.64	4.00	10.00	1.68

Stressor Identification of Contaminant Affects at the Big Oaks National Wildlife Refuge

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Study Design

Stressor identification on the refuge scale was accomplished using a random design to select 75 sites in the Big Oaks National Wildlife Refuge. These sites represented locations on the refuge and those immediately upstream. Sites were visited and those sites that were dry were excluded from the 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 (Figure 8).

Analysis of assemblage structure based on Cluster Analysis showed that each assemblage group explained variance in the data differently (Figure 9). Assemblage structure was explained by increasing index of biotic integrity scores for fish and increasing species richness for macroinvertebrates and crayfish (Figure 10). Fish assemblage structure was explained with six clusters representing tier 4, macroinvertebrate assemblage structure was explained with five clusters representing tier 5 linkage structure (Figure 10), while crayfish assemblage structure was explained with tier 2 linkage and three clusters. The basis for these groupings is that the biological community structure results are externally driven and can be identified to evaluate physical and chemical stress on the respective assemblage (Morris et al. 2006).

Habitat Quality

Habitat analysis using the QHEI showed that scores ranged from 25 to 85 (average 61.36 $\pm$ 10.08). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life. 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. 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.

Chemical Quality

Summary statistics by assemblage for water chemistry results are found in Tables 8-10. Project specific summary statistics and range of scale values for water quality results are presented in Appendix 4. In general, the lowest biological integrity and species richness clusters (cluster 1) had the highest concentrations of contaminants (Tables 8-10). Several contaminants, such as nitrogen and phosphorus, showed differential distribution with higher concentrations in cluster 3 for fish (Figures 11). Barium, sulfate, and manganese showed the highest concentrations in clusters 2 for macroinvertebrates, and fluoride and lead showed the higher concentrations in cluster 3 (Figure 12). Crayfish showed a normal distribution with the highest contaminant concentrations in cluster 2 (Figure 13).

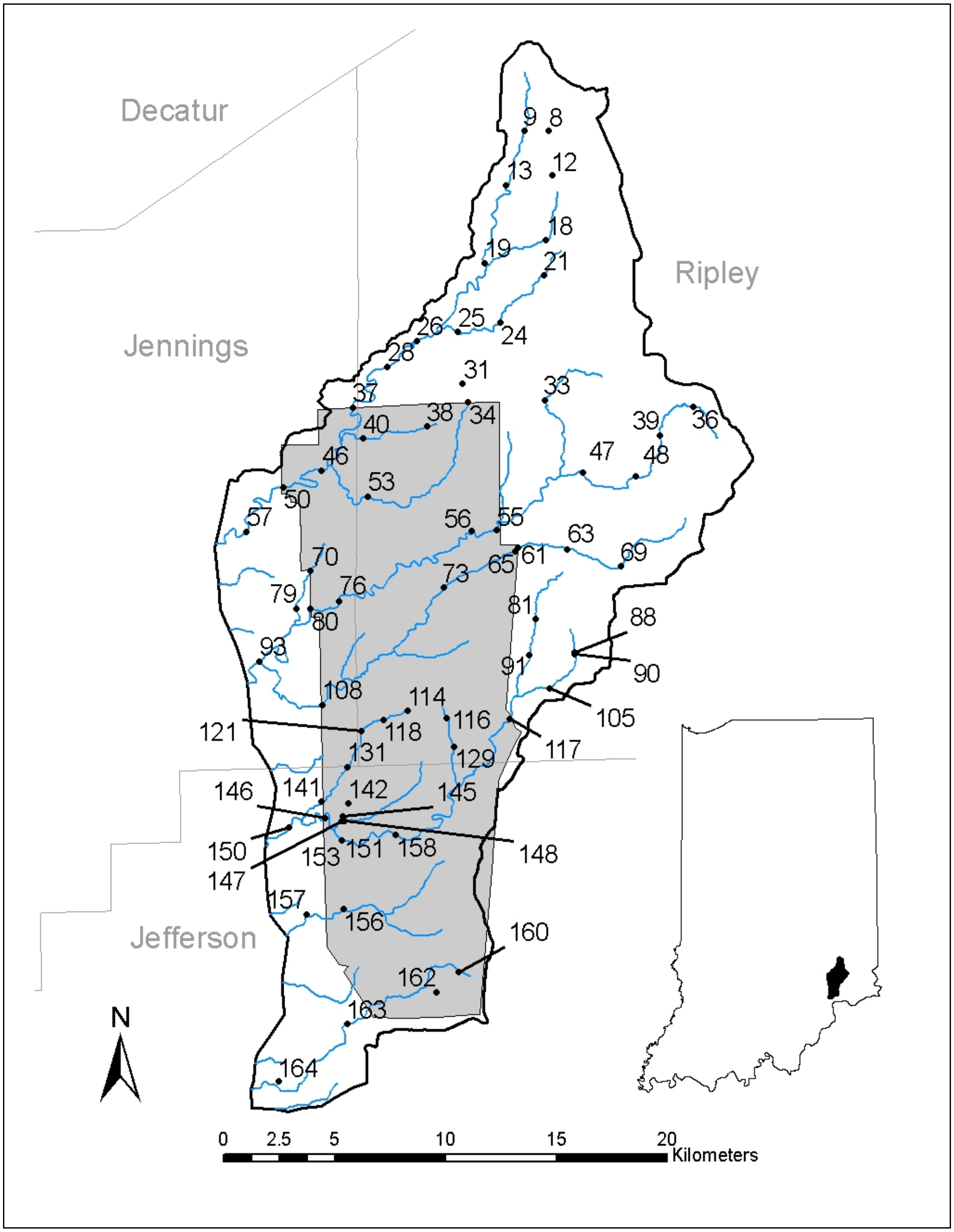


Figure 8. Map showing the distribution of sites sampled both on refuge and upstream during a stressor identification assessment of the Big Oaks National Wildlife Refuge in 2006 and 2007 (site numbers refer to Appendix).

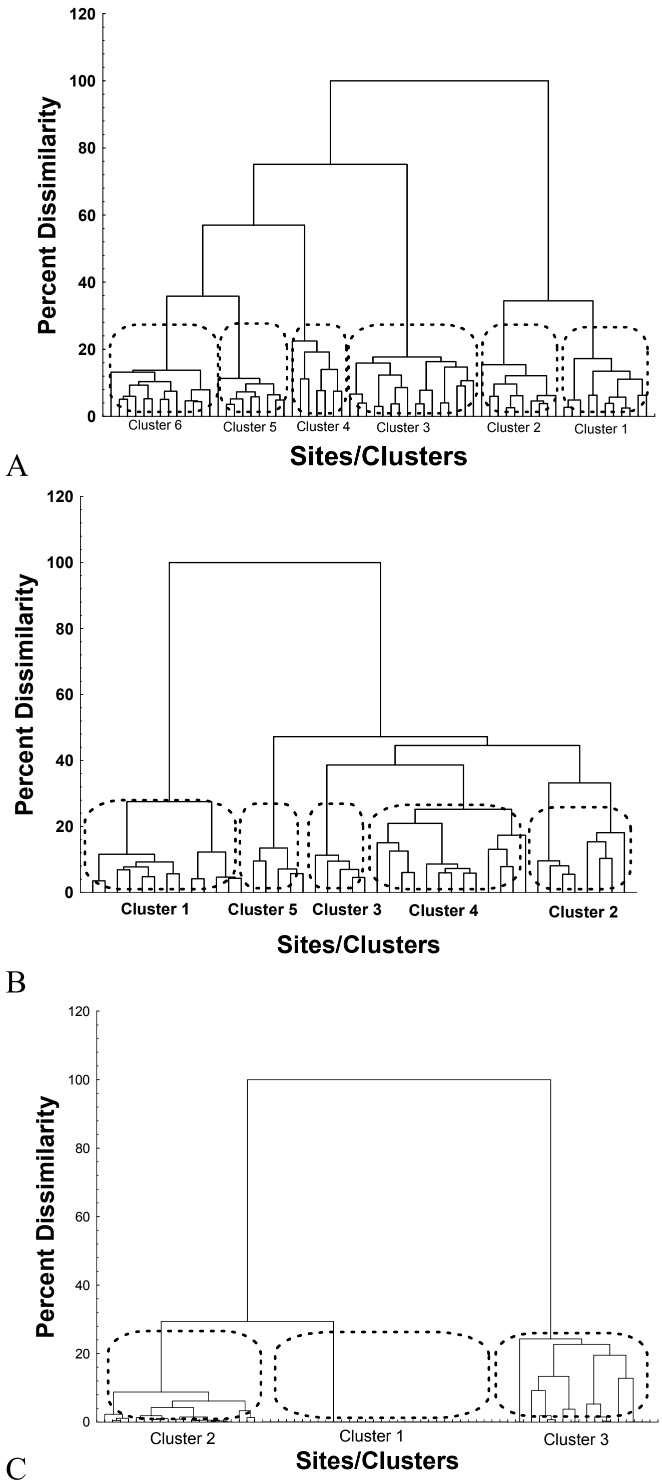


Figure 9. Dendograms showing the relative similarity of assemblage data collected in the Big Oaks National Wildlife Refuge watersheds during August 2006. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA. (a) fish, (b) macroinvertebrate, and (c) crayfish assemblages.

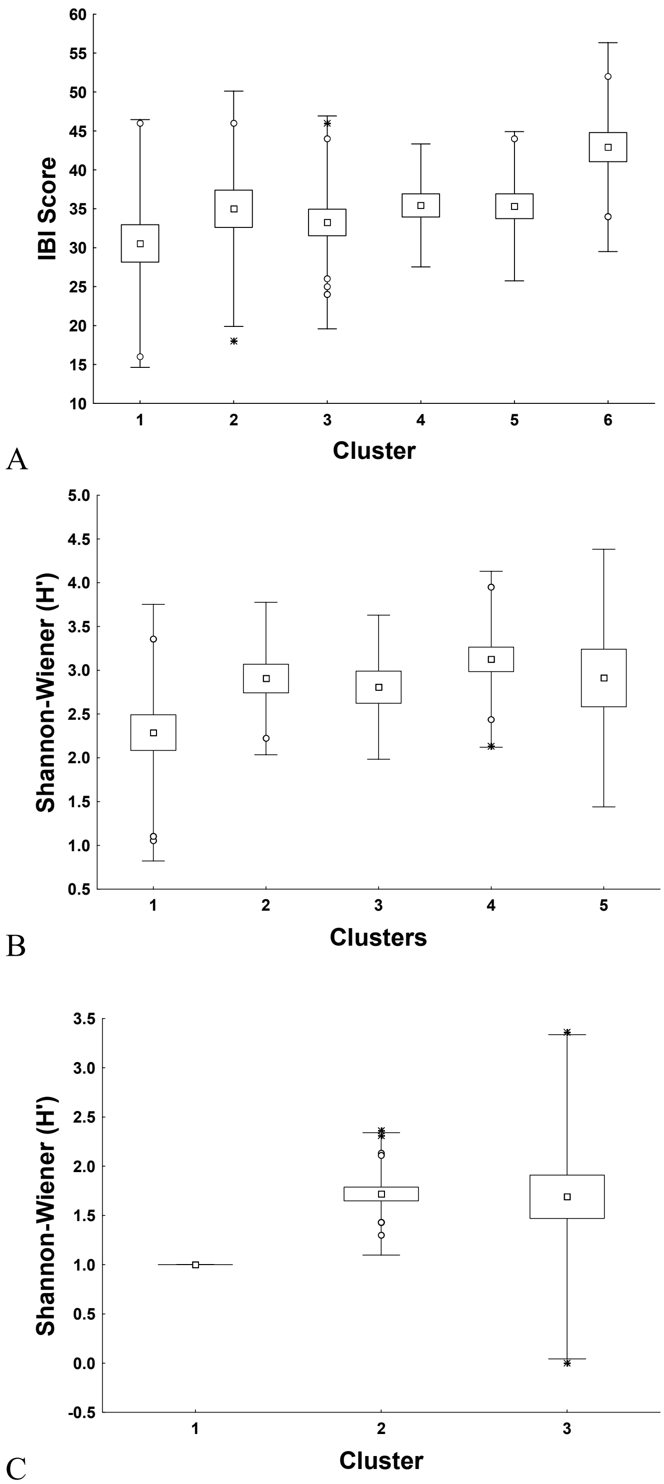


Figure 10. Box-and-whisker plots showing biological assemblage responses for index of biotic integrity (IBI) for (a) fish, or Shannon-Wiener (H') (b) macroinvertebrates and (c) crayfish assemblages had to the biologic response gradient developed from the Big Oaks National Wildlife Refuge watersheds during August 2006.

Table 8. Summary statistics for 22 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Big Oaks National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Arsenic	11	2.35	0.20	8.15	2.64	10	0.47	0.20	1.73	0.58
Calcium	11	104.45	75.00	145.00	21.95	10	81.30	28.00	156.00	36.95
Chloride	11	12.51	0.05	44.00	13.37	10	3.85	0.05	12.00	5.10
Fluoride	11	0.14	0.05	0.30	0.08	10	0.09	0.05	0.10	0.02
Hardness	11	153.45	118.00	215.00	31.27	10	113.20	38.00	235.00	54.49
Nitrogen	11	0.40	0.01	2.80	0.84	10	0.08	0.01	0.50	0.16
Total Phosphorus	11	0.11	0.03	0.41	0.11	10	0.05	0.03	0.13	0.04
Sodium	11	6.98	2.27	20.10	4.87	10	4.99	2.16	16.90	4.52
TS	11	300.73	180.00	1010.00	240.92	10	163.60	90.00	300.00	55.65
Conductivity	11	493.73	21.00	2600.00	706.16	10	237.09	87.20	477.00	105.49
QHEI	11	55.77	25.00	69.50	11.23	10	60.85	50.50	79.00	9.51
QHEI - Substrate Score	11	11.41	1.00	15.00	3.77	10	13.25	10.50	17.00	2.14
QHEI - Instream Cover Score	11	6.64	2.00	11.00	3.38	10	6.50	2.00	12.00	3.44
QHEI - Riparian Score	11	8.82	4.00	10.00	1.82	10	9.85	8.50	10.00	0.47
QHEI - Pool/Glide Score	11	4.64	3.00	8.00	1.29	10	5.00	4.00	7.00	1.15
QHEI - Riffle/Run Score	11	3.73	0.00	5.00	1.62	10	4.40	2.00	7.00	1.51
QHEI - Gradient Score	11	4.55	2.00	8.00	1.81	10	5.00	4.00	10.00	2.16
Water Land-use	11	1.88	0.00	14.62	4.38	10	7.99	0.61	19.34	5.82
Agricultural Land-use	11	26.84	0.00	53.07	22.26	10	6.80	0.22	20.10	7.56
Grass/Pasture Land-use	11	27.01	0.13	59.75	22.72	10	11.70	0.05	34.57	13.84
Forest Land-use	11	39.91	0.00	96.47	36.50	10	63.09	40.46	81.59	14.74
Industrial Land-use	11	3.92	0.00	13.19	5.70	10	9.01	0.00	17.96	6.12

Variable	Cluster 3					Cluster 4				
	N	Mean	Min	Max	SD	N	Mean	Min	Max	SD
Arsenic	16	1.79	0.20	2.86	0.56	7	1.86	1.34	3.67	0.81
Calcium	16	119.56	85.00	154.00	22.47	7	90.00	22.00	117.00	32.14
Chloride	16	30.63	12.00	124.00	26.98	7	7.17	0.05	20.00	9.20
Fluoride	16	0.18	0.10	0.50	0.11	7	0.06	0.05	0.10	0.02
Hardness	16	166.38	108.00	226.00	37.37	7	128.14	35.00	178.00	47.11
Nitrogen	16	2.42	0.01	24.00	5.96	7	0.04	0.01	0.20	0.07
Total Phosphorus	16	0.24	0.04	1.82	0.44	7	0.07	0.03	0.10	0.02
Sodium	16	15.75	5.34	71.30	16.35	7	5.11	3.04	8.38	2.05
TS	16	279.50	192.00	575.00	97.00	7	195.57	88.00	264.00	59.26
Conductivity	16	438.25	313.00	812.00	128.76	7	266.84	72.90	370.00	99.88
QHEI	16	57.78	38.00	67.50	7.91	7	59.00	41.50	65.50	8.79
QHEI - Substrate Score	16	13.53	11.00	17.00	1.94	7	12.50	11.00	15.00	1.71
QHEI - Instream Cover Score	16	6.25	2.00	12.00	2.27	7	10.57	7.00	15.00	2.94
QHEI - Riparian Score	16	7.28	4.00	10.00	2.18	7	9.00	3.00	10.00	2.65
QHEI - Pool/Glide Score	16	5.94	4.00	9.00	1.53	7	6.43	5.00	7.00	0.98
QHEI - Riffle/Run Score	16	4.03	1.00	5.50	1.41	7	2.57	0.00	5.00	1.90
QHEI - Gradient Score	16	6.00	4.00	10.00	2.42	7	3.43	2.00	4.00	0.98
Water Land-use	16	1.04	0.17	5.67	1.27	7	4.79	0.15	14.31	5.35
Agricultural Land-use	16	41.83	7.88	54.27	12.18	7	18.05	0.00	49.90	22.16
Grass/Pasture Land-use	16	37.71	25.43	57.81	10.19	7	17.63	0.00	59.79	23.65
Forest Land-use	16	17.88	9.35	44.60	8.23	7	51.56	6.50	81.31	33.88
Industrial Land-use	16	0.49	0.00	7.90	1.97	7	7.45	0.00	22.43	8.49

Variable	Cluster 5 N	Mean	Min	Max	SD	Cluster 6 N	Mean	Min	Max	SD
Arsenic	9	1.45	0.20	2.10	0.52	13	1.54	0.20	2.41	0.66
Calcium	9	111.78	77.00	178.00	27.79	13	116.69	63.00	180.00	37.86
Chloride	9	6.67	0.05	12.00	4.33	13	10.64	0.05	40.00	10.06
Fluoride	9	0.11	0.05	0.20	0.06	13	0.12	0.05	0.30	0.09
Hardness	9	141.33	106.00	245.00	43.77	13	150.85	88.00	220.00	48.12
Nitrogen	9	0.41	0.01	1.20	0.36	13	0.69	0.01	5.00	1.32
Total Phosphorus	9	0.10	0.03	0.16	0.05	13	0.11	0.03	0.37	0.09
Sodium	9	4.90	2.34	8.67	1.85	13	6.48	2.85	22.80	5.18
TS	9	204.56	155.00	291.00	41.22	13	218.38	129.00	366.00	62.00
Conductivity	9	266.08	57.70	431.00	100.42	13	335.54	195.00	562.00	104.56
QHEI	9	63.72	45.00	74.50	9.61	13	70.54	54.00	85.00	7.39
QHEI - Substrate Score	9	12.78	9.00	16.00	2.12	13	14.85	12.00	18.00	1.65
QHEI - Instream Cover Score	9	7.00	5.00	10.00	1.87	13	8.54	5.00	14.00	2.67
QHEI - Riparian Score	9	9.89	9.00	10.00	0.33	13	9.27	6.00	10.00	1.20
QHEI - Pool/Glide Score	9	6.67	5.00	9.00	1.32	13	7.00	5.00	12.00	2.20
QHEI - Riffle/Run Score	9	4.67	0.00	7.00	2.22	13	5.77	3.00	7.00	1.49
QHEI - Gradient Score	9	6.22	2.00	10.00	2.91	13	7.54	4.00	10.00	2.18
Water Land-use	9	2.12	0.11	5.80	2.33	13	1.88	0.37	6.25	2.03
Agricultural Land-use	9	18.17	0.00	36.65	14.18	13	24.31	1.13	50.97	14.00
Grass/Pasture Land-use	9	21.34	0.00	47.85	16.31	13	27.24	5.86	53.14	16.76
Forest Land-use	9	53.63	24.15	96.48	26.75	13	41.99	17.11	83.95	20.59
Industrial Land-use	9	3.99	0.00	17.65	5.82	13	3.54	0.00	20.11	5.76

Table 9. Summary statistics for eight chemistry (concentration/ppm), habitat, and land use variables by macroinvertebrate grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Big Oaks National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Barium	13	52.46	30.50	81.90	12.93	5	53.30	34.80	67.00	11.63
Fluoride	13	0.12	0.05	0.20	0.06	5	0.07	0.05	0.10	0.03
Lead	13	0.19	0.10	1.31	0.34	5	0.10	0.10	0.10	0.00
Manganese	13	124.52	39.70	275.00	71.42	5	67.12	31.70	130.00	41.70
Sulfate	13	13.99	9.10	23.00	4.07	5	15.60	12.00	20.00	3.29
pH	13	8.13	7.44	9.50	0.59	5	8.31	7.45	8.69	0.51
QHEI Score	13	58.23	50.50	70.50	5.23	5	64.90	62.50	67.50	2.38
Water Land-use	13	3.53	0.06	14.62	5.01	5	2.99	0.41	6.25	2.79

	Cluster 3					Cluster 4				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Barium	5	79.60	56.70	124.00	27.59	13	55.74	31.90	107.00	18.03
Fluoride	5	0.19	0.05	0.30	0.09	13	0.07	0.05	0.10	0.03
Lead	5	1.27	0.10	3.13	1.29	13	0.10	0.10	0.10	0.00
Manganese	5	672.00	52.00	2500.00	1038.09	13	104.31	25.70	265.00	63.09
Sulfate	5	9.28	7.20	11.00	1.74	13	11.69	5.20	18.00	3.98
pH	5	7.67	7.14	7.90	0.31	13	8.26	7.66	9.19	0.45
QHEI Score	5	56.60	25.00	67.00	17.81	13	68.31	55.00	79.00	9.06
Water Land-use	5	0.23	0.00	0.50	0.24	13	2.84	0.48	5.80	2.07

	Cluster 5				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**
Barium	7	90.43	47.20	158.00	43.26
Fluoride	7	0.14	0.05	0.20	0.07
Lead	7	0.67	0.10	2.96	1.10
Manganese	7	1352.21	99.50	6360.00	2322.74
Sulfate	7	22.80	6.90	45.00	14.67
pH	7	7.68	7.21	8.43	0.39
QHEI Score	7	51.93	38.00	64.00	9.75
Water Land-use	7	0.57	0.13	1.23	0.41

Table 10. Summary statistics for seven chemistry (concentration/ppm), habitat, and land use variables by crayfish grouping cluster (see Fig. 1A) sampled in August 2006 in watersheds of the Big Oaks National Wildlife Refuge.

Cluster Summary Stats

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**	**N**	**Mean**	**Min**	**Max**	**SD**
Chloride	32	9.90	0.05	44.00	11.14	20	21.91	0.05	124.00	26.61
Nitrogen	32	0.24	0.01	2.80	0.51	20	2.18	0.01	24.00	5.41
Total Phosphorus	32	0.07	0.03	0.19	0.05	20	0.23	0.03	1.82	0.40
TS	32	202.94	90.00	301.00	47.70	20	299.10	105.00	1010.00	197.87
QHEI - Riparian Score	32	9.30	4.00	10.00	1.49	20	8.08	3.00	10.00	2.27
QHEI - Pool/Glide Score	32	5.53	3.00	9.00	1.46	20	5.65	4.00	8.00	1.27
Commercial Land-use	32	0.62	0.00	3.83	1.07	20	0.06	0.00	0.31	0.10

	Cluster 3				
Variable	**N**	**Mean**	**Min**	**Max**	**SD**
Chloride	14	11.40	0.05	46.00	12.59
Nitrogen	14	0.40	0.01	1.20	0.39
Total Phosphorus	14	0.10	0.05	0.16	0.04
TS	14	213.50	88.00	333.00	60.27
QHEI - Riparian Score	14	8.96	4.00	10.00	1.82
QHEI - Pool/Glide Score	14	7.29	4.00	12.00	2.16
Commercial Land-use	14	1.14	0.00	7.78	2.40

Table 11. List of 22 physical and chemical variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Big Oaks National Wildlife Refuge during August 2006.

Variable	P Value
Water Temperature	p = 0.0036
Chemical	
Arsenic	p = 0.007
Calcium	p = 0.0422
Chloride	p = < 0.0000
Fluoride	p = 0.0109
Hardness	p = 0.0524
Nitrogen	p = 0.0013
Phosphorus	p = 0.0195
Sodium	p = 0.0003
TS	p = 0.001
Conductivity	p = 0.0003
Habitat	
QHEI	p = 0.0022
Substrate Score	p = 0.0228
Instream Cover Score	p = 0.0354
Riparian Score	p = 0.0013
Pool/Glide Score	p = 0.0013
Riffle/Run Score	p = 0.0082
Gradient Score	p = 0.0038
Land-use	
Water	p = 0.0015
Agriculture	p = 0.0003
Grass/Pasture	p = 0.014
Forest	p = 0.0003
Industrial	p = 0.0009

Multivariate Results

For this analysis we chose 75% similarity as a benchmark for defining assemblage structure clustering (Figure 9). These clusters were used for stressor response measurement. Kruskal-Wallis ANOVA by ranks tests were significant for six clusters for fish, showing that 10 chemical, seven habitat, and five land use measures were significantly predictive of the fish assemblage structure (Table 11). Macroinvertebrate assemblages were significant for five clusters, showing that six physical-chemical, four habitat, and a single land use type were predictive of the structure (Table 12), while crayfish structure was significant for three clusters and four chemical, two habitat, and a single land use variable.

Fish assemblage structure was significantly predicted by the presence of chloride, fluoride, arsenic, calcium, hardness, sodium, total solids, and nutrients (Table 11). Chloride was significantly different in cluster 3, which is a signature of wastewater treatmen effluents, while calcium and hardness are autocorrelated showing similar

Table 12. List of 11 physical and chemical variables significantly predictive (a = 0.05) of the macroinvertebrate assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Big Oaks National Wildlife Refuge during August 2006.

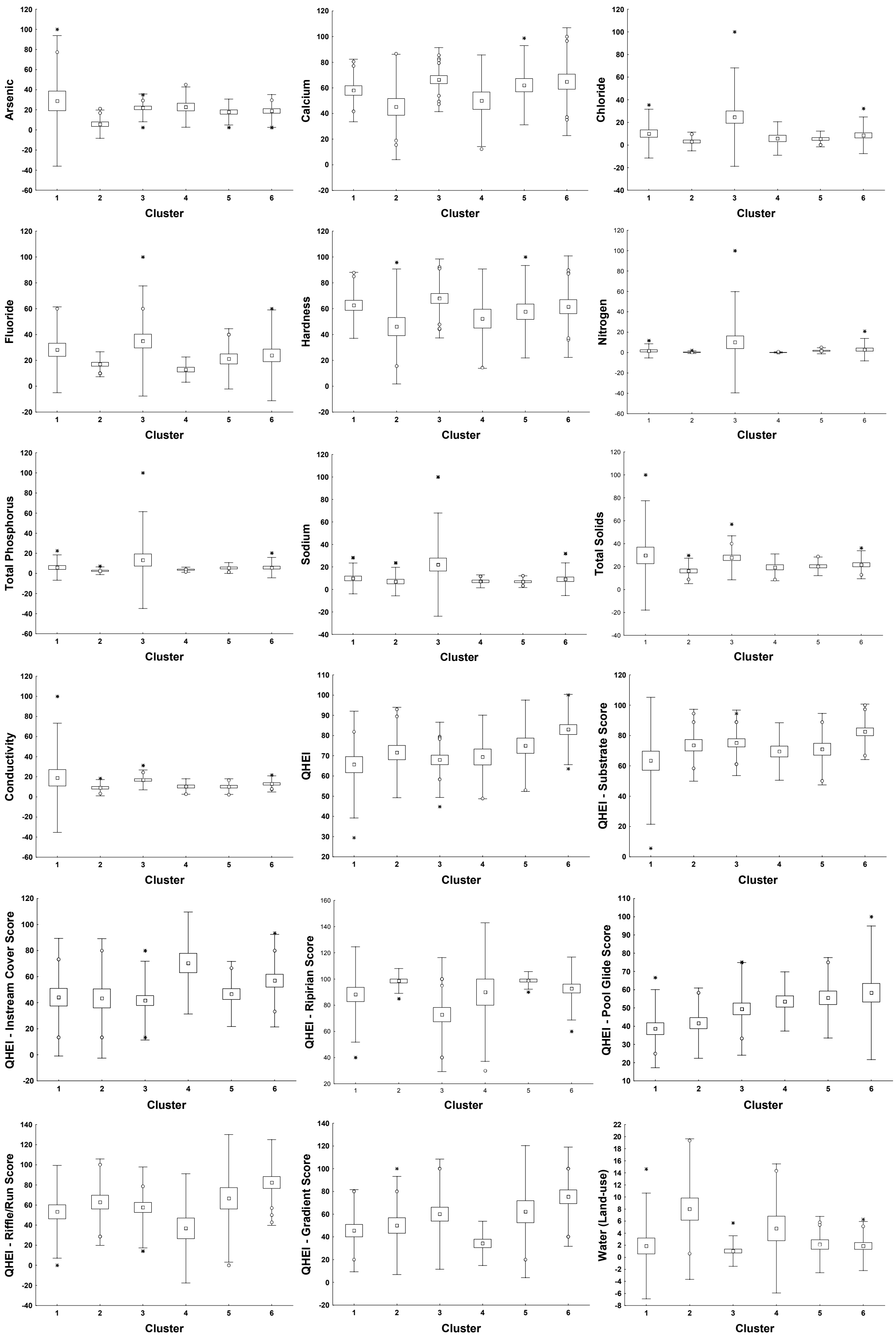
Variable	p Value
Chemical	
Barium	p = 0.0169
Fluoride	p = 0.0140
Lead	p = 0.0103
Manganese	p = 0.0075
Sulfate	p = 0.0477
pH	p = 0.0455
Habitat	
QHEI Score	p = 0.0055
QHEI Channel Score	p = 0.0016
QHEI Riffle Score	p = 0.0023
QHEI Gradient Score	p = 0.0346
Land-use	
Water	p = 0.0130

Table 13. List of 7 physical and chemical variables significantly predictive (a = 0.05) of the crayfish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Big Oaks National Wildlife Refuge during August 2006.

Variable	P Value
Chemical	
Chloride	p = 0.0347
Nitrogen	p = 0.0383
Phosphorus	p = 0.028
TS	p = 0.0332
Habitat	
Riparian Score	p = 0.0511
Pool/Glide Score	p = 0.0172
Land-use	
Commercial	p = 0.0076

relationship patterns (Figure 11). Nutrients also showed an autocorrelated pattern with highest response in cluster 3. Macroinvertebrates showed a significant pattern with groundwater and wastewater treatment (Table 12). Crayfish showed a significant response with nutrients and wastewater treatment (Table 13).

Box and whisker plots for significant variable responses for fish (Figure 11), macroinvertebrates (Figure 12), and crayfish (Figure 13) show the greatest response was observed among fish assemblage structure. Fish assemblage structure was predicted by groundwater (i.e., arsenic, hardness, water temperature), nutrients (i.e., nitrogen and phosphorus), habitat, and land use (Table 11). All groundwater associated parameters


Arsenic
Calcium
Chloride
Fluoride
Hardness
Nitrogen
Total Phosphorus
Sodium
Total Solids
Conductivity
QHEI
QHEI - Substrate Score
QHEI - Instream Cover Score
QHEI - Riprian Score
QHEI - Pool Glide Score
QHEI - Riffle/Run Score
QHEI - Gradient Score
Water (Land-use)
Cluster

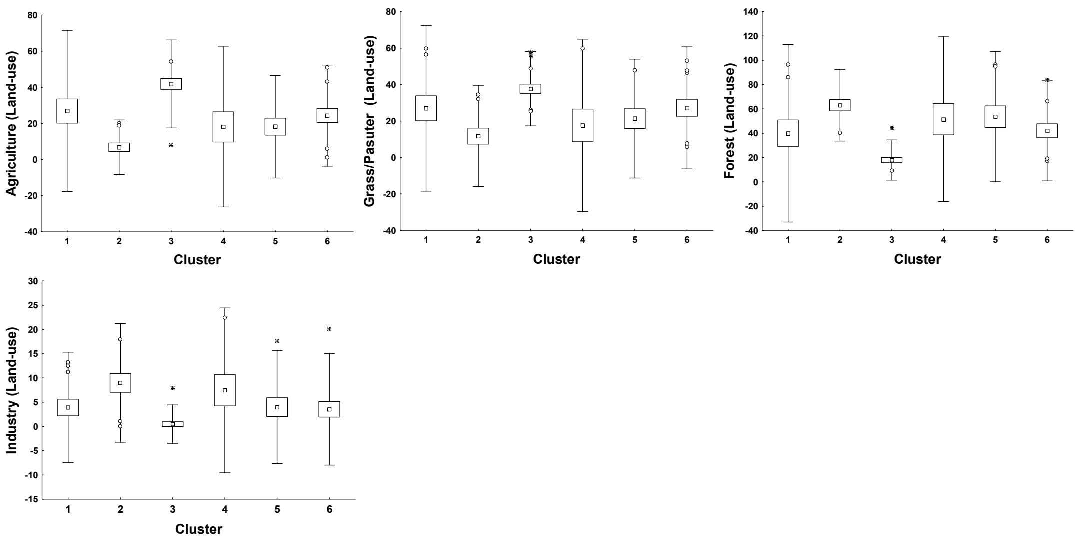


Figure 11. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the fish assemblage clustering.

was highly predictive with cluster 1 (Figure 11), while nutrient signatures were associated with cluster 3.

Macroinvertebrate assemblage structure was predicted by barium, sulfate, and manganese in cluster 2 and fluoride and lead in cluster 3 (Table 12). Cluster 2 may be a signature of groundwater parameters, while cluster 3 appears to be related to wastewater effluent.

Crayfish assemblage structure was highly predictive with chloride (Table 13). Crayfish showed a similar response pattern with a normally distributed contaminant pattern consistent with stimulation (Figure 13). Crayfish reached higher relative abundance in areas with increased nutrients (i.e., nitrogen and phosphorus), while the highest and lowest species richness was seen in areas with the lowest nutrient concentrations (Figure 13). Chloride and total solids also showed a similar pattern as the nutrients with the highest concentration observed in the middle cluster.

<u>Habitat Response</u>

Fish assemblage structure had the most habitat parameters showing a significant response among the three organism groups followed by macroinvertebrates and crayfish, respectively (Table 11-13). Fish assemblage structure showed a significant response to substrate score, instream cover, riparian score, pool-glide score, riffle-run score, and gradient (Table 11).

Macroinvertebrates responded to habitat condition (based on QHEI score), channel score, riffle-run score, and gradient score (Table 12). These habitat attributes are probably autocorrelated since channel modifications affect the presence of riffles, which is mediated by gradient and affects the total habitat quality score.

Crayfish were significantly responding to riparian score and the pool-glide score (Table 13). The presence of an intact riparian corridor affected the growth of algae and potentially eliminated the invasion of rusty crayfish from the refuge.

Land Use Response

Fish assemblage structure showed the most significant relationship with land use (Table 10). Fish showed a significant response to agriculture, grass-pasture, forest, industrial, and water land uses. Of the entire 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 use (Table 12), while crayfish showed a significant response to commercial land use (Table 13).

Fish assemblage structure showed declining dose response patterns for water, agriculture, and industrial land uses (Figure 11). The increased percentage of agriculture land use in cluster 1 and 3 and increased industrial land use in cluster 4 suggests that industrial land use may not be causing environmental harm in the watershed since it was correlated with higher biological integrity.

Macroinvertebrate assemblage structure was most variable with water land use in cluster 1 (Figure 12). While crayfish assemblage structure showed the highest species richness in commercial land uses (Figure 13).

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. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used later to reduce the set of variables in a dataset.

Fish assemblage structure explained observed variance on four factors, which explained 69% of the variance in the fish assemblage information (Table 14). Arsenic, total phosphorus, and agricultural land use explained 26% of the variance on the primary factor. Habitat explained another 23% of the variance on factor 2 based on QHEI score and gradient. Calcium and conductivity explained 11% of the variance on factor 3, while instream cover explained another 9% of the variation on factor 4. All of the relationships were positively correlated and explained 69% of the variance.

Macroinvertebrate assemblage structure was explained by two factors, which explained a total of 56% of the variance (Table 15). The primary factor explained 38% of the variance based on a negative association between barium, lead, and manganese. The

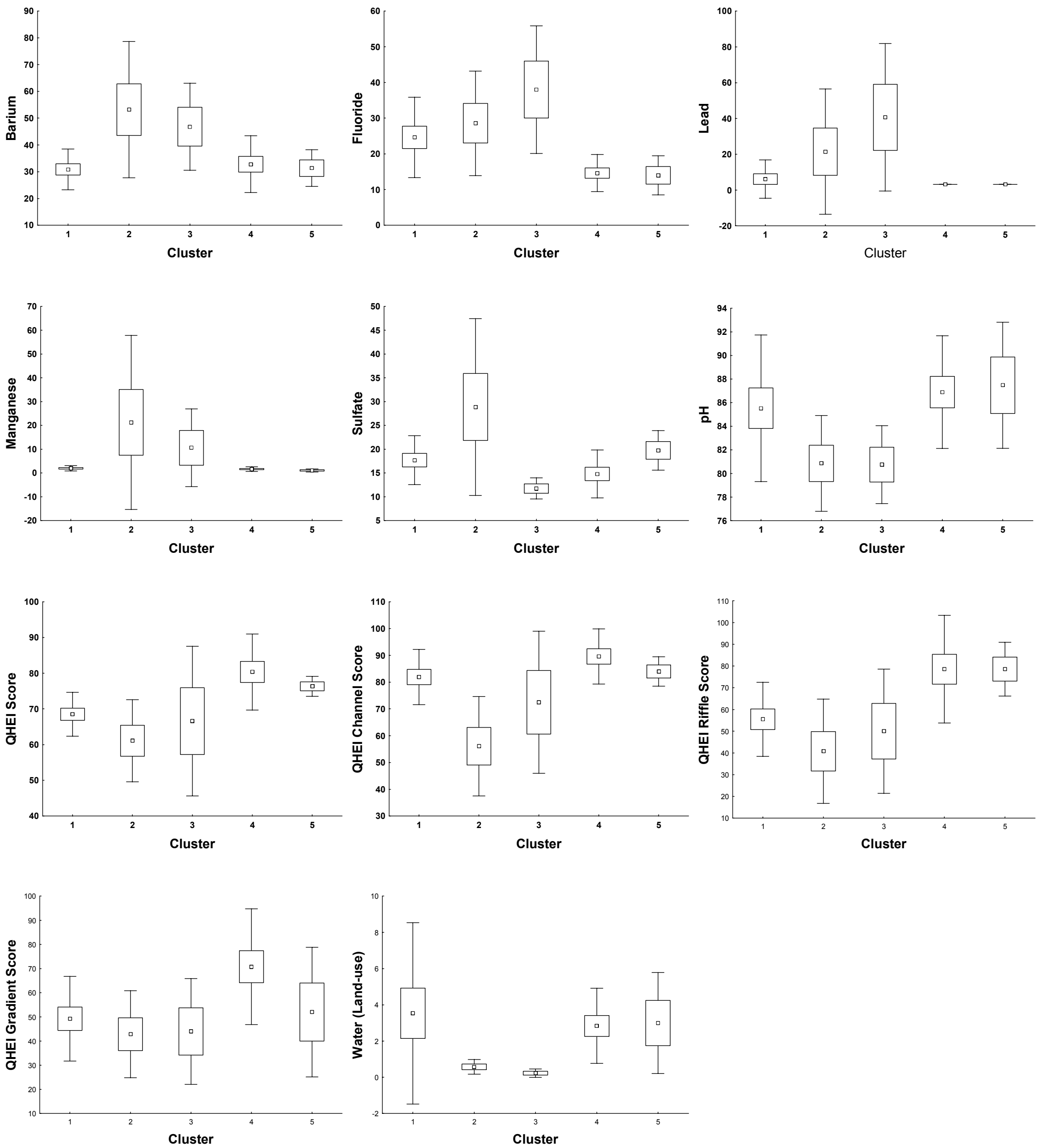


Figure 12. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the macroinvertebrate assemblage clustering.

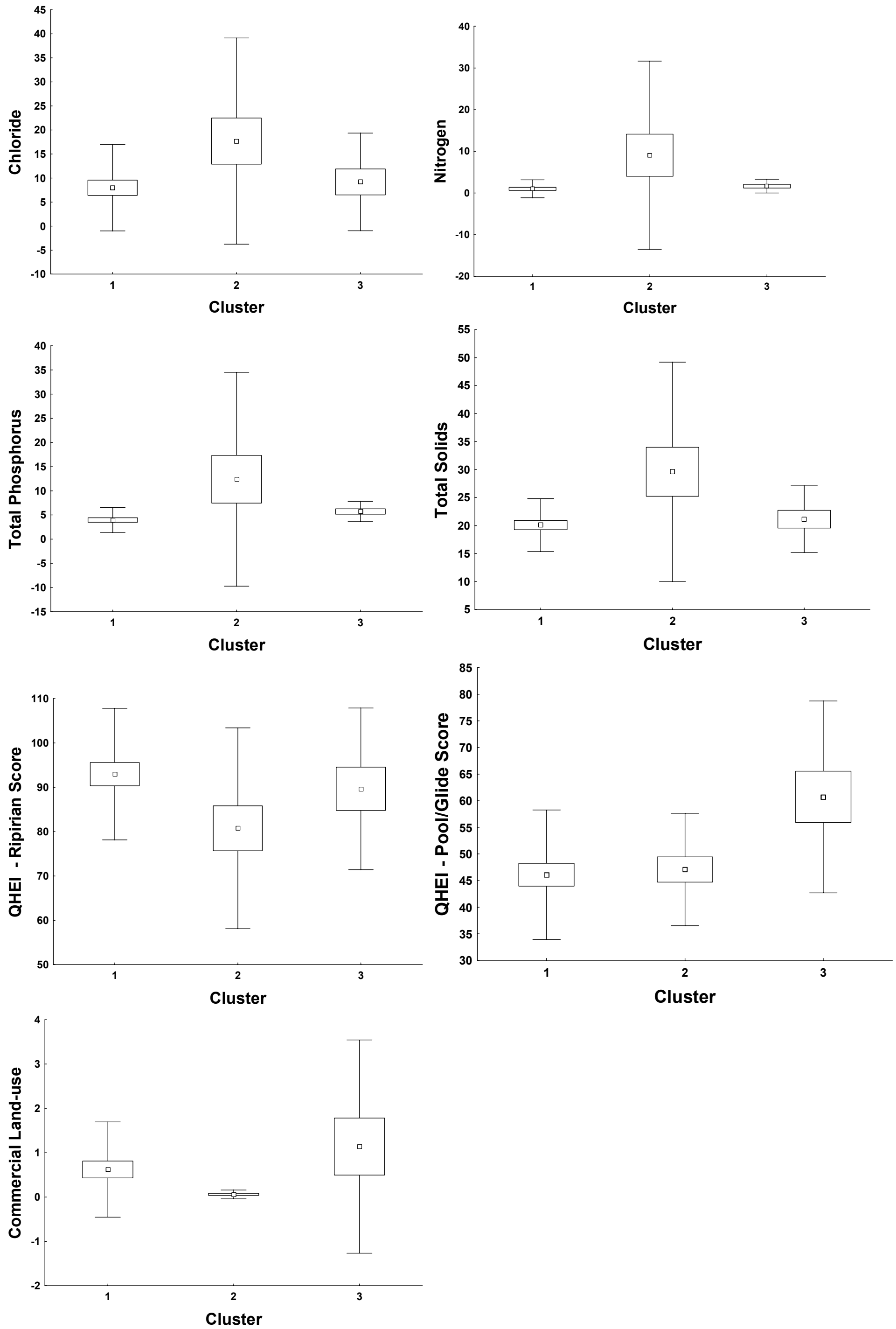


Figure 13. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the crayfish assemblage clustering.

Table 14. Results of factor analysis and explained variance observed in fish assemblage biologic structure and 14 significant chemical, habitat, and land use variables at the Big Oaks National Wildlife Refuge during August 2006.

Factor Analysis Results

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Arsenic	**0.783062**	-0.059175	-0.0345	0.31648
Calcium	0.246242	0.219824	**0.739182**	0.033972
Nitrogen	0.592974	0.441209	0.239038	-0.29702
Total Phosphorus	**0.851726**	-0.017708	0.112252	-0.1624
Sodium	0.479958	-0.05162	0.490525	-0.41225
Total Solids	0.499786	-0.504704	0.532454	0.06942
Conductivity	-0.22656	-0.042178	**0.753321**	-0.04346
QHEI Score	-0.08335	**0.929388**	0.033943	0.286131
QHEI - Substrate Score	0.114931	0.694877	-0.13642	-0.11779
QHEI - Instream Cover Score	0.001361	0.146698	0.036967	**0.8519**
QHEI - Riparian Score	-0.47554	0.590502	-0.13299	0.146314
QHEI - Pool/Glide Score	0.302307	0.478238	-0.20979	0.494537
QHEI - Gradient Score	0.251651	**0.719733**	0.279072	-0.10598
Agricultural Land-use	**0.836643**	0.074086	-0.01429	0.063424
Expl. Variance	26%	49%	60%	69%

second factor was also a negative association with fluoride that explained an additional 18% of the variance.

Crayfish assemblage structure was explained by two factors that included an increasing relationship with chloride, nitrogen, total phosphorus, and total solids (Table 16). The second factor was a positive relationship with pool-glide habitat.

Associations between Contaminants and Hot Spot Analysis

Contaminant distribution, based on percentage-of-range concentrations, showed variable responses between organism groups. The majority of the significant contaminant responses were diagnosed from a single location upstream of the refuge on Little Graham Creek (Figure 14). The contaminants that showed a significant relationship included nitrate + nitrite, total phosphorus, sodium, chloride, fluoride, and sulfate. These contaminants were linked to the City of Versailles land application of sludge in the headwaters of Little Graham Creek.

Fish assemblage structure was predicted by negative associations with the application of the sludge. In addition, additional 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 upstream 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 (Figure 14).

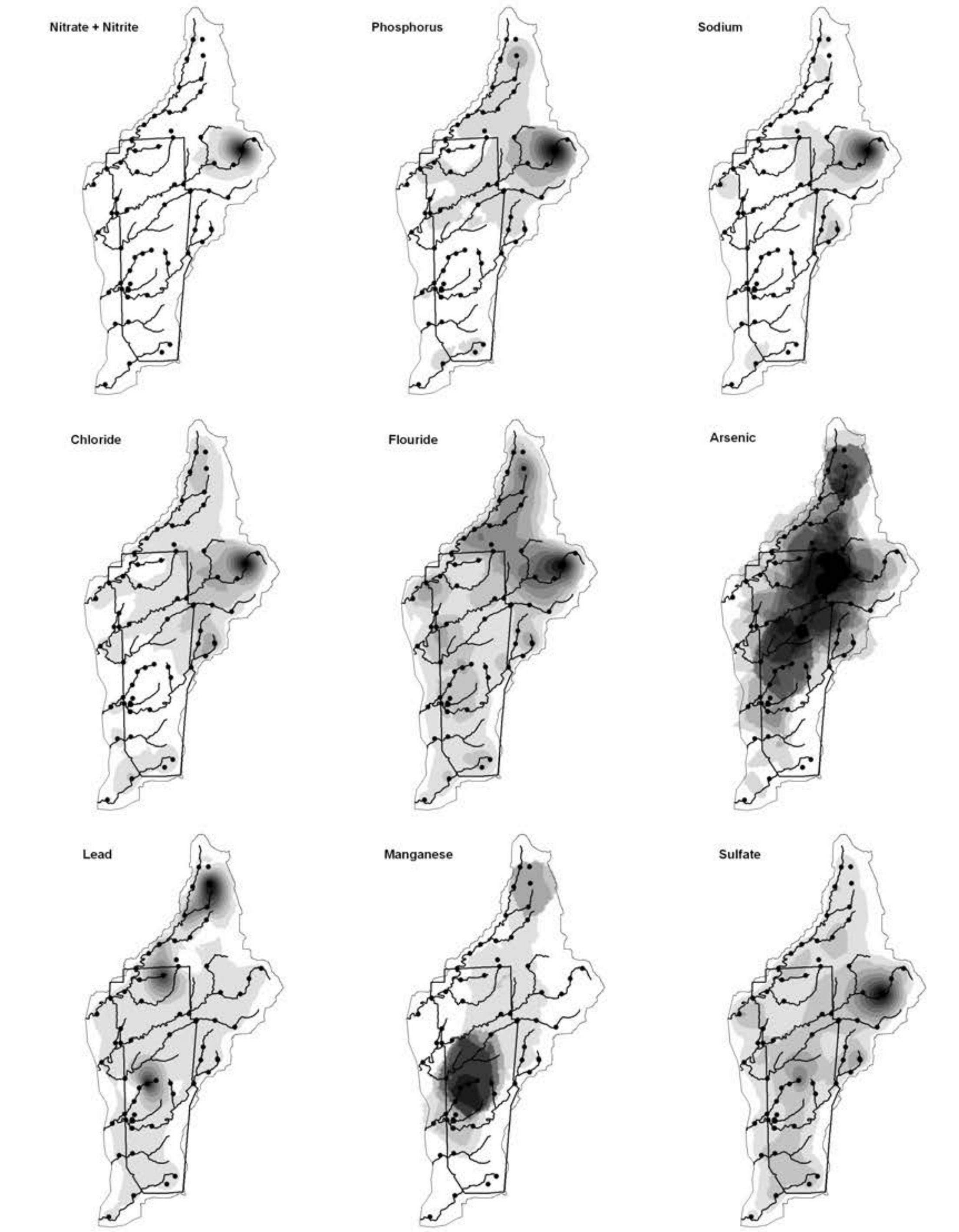
Table 15. Results of factor analysis and explained variance observed in macroinvertebrate assemblage biologic structure and eight significant chemical, habitat, and land use variables at the Big Oaks National Wildlife Refuge during August 2006.

Variable	Factor 1	Factor 2
Barium	**-0.800307**	0.002714
Fluoride	-0.063120	**-0.800452**
Lead	**-0.745909**	-0.193511
Manganese	**-0.913786**	0.028777
Sulfate	0.256497	-0.639508
pH	0.606534	0.334115
QHEI Score	0.386893	0.407818
Water	0.423283	0.589365
Expl. Var.	38%	56%

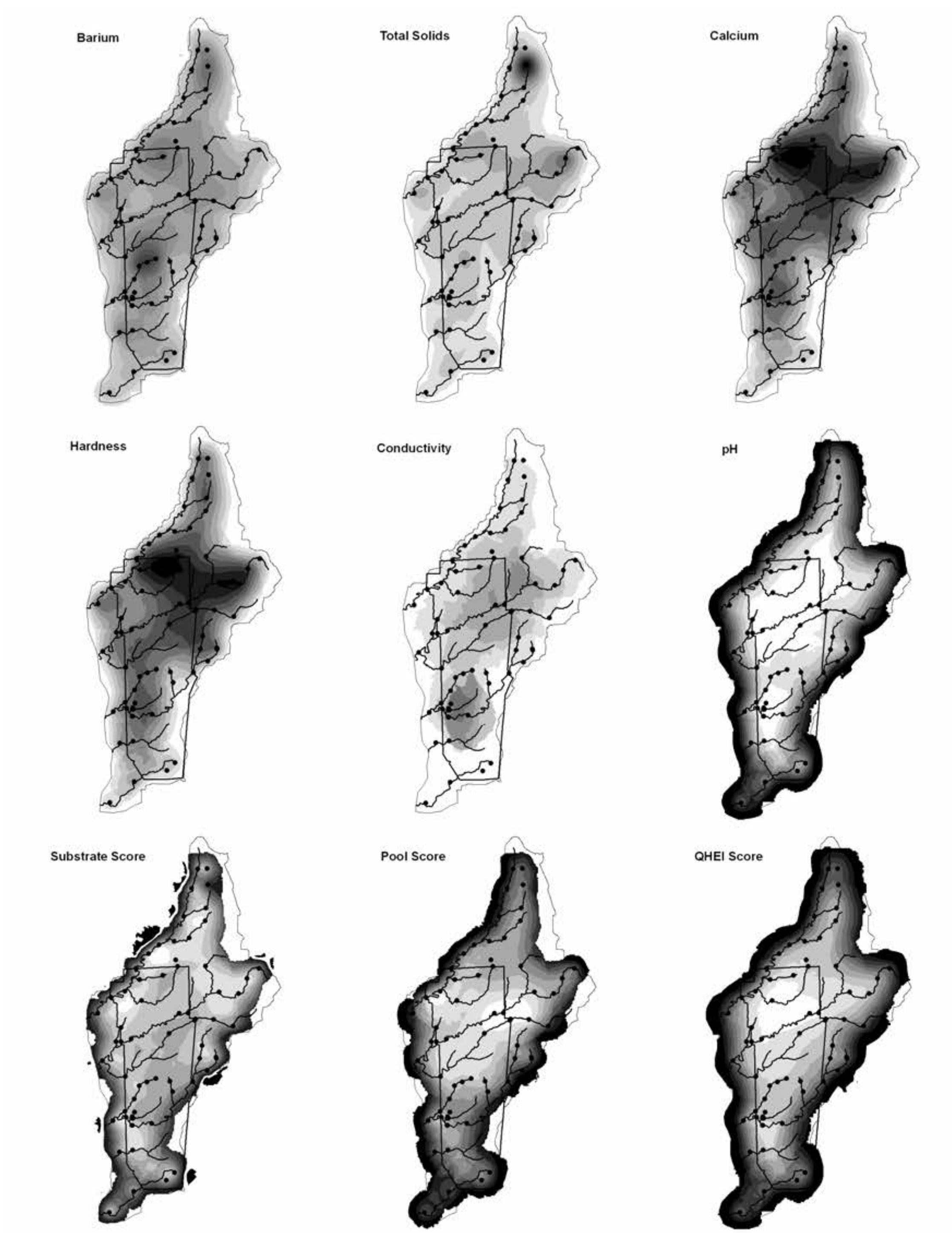
Table 16. Results of factor analysis and explained variance observed in crayfish assemblage biologic structure and seven chemical, habitat, and land use variables at the Big Oaks National Wildlife Refuge during August 2006.

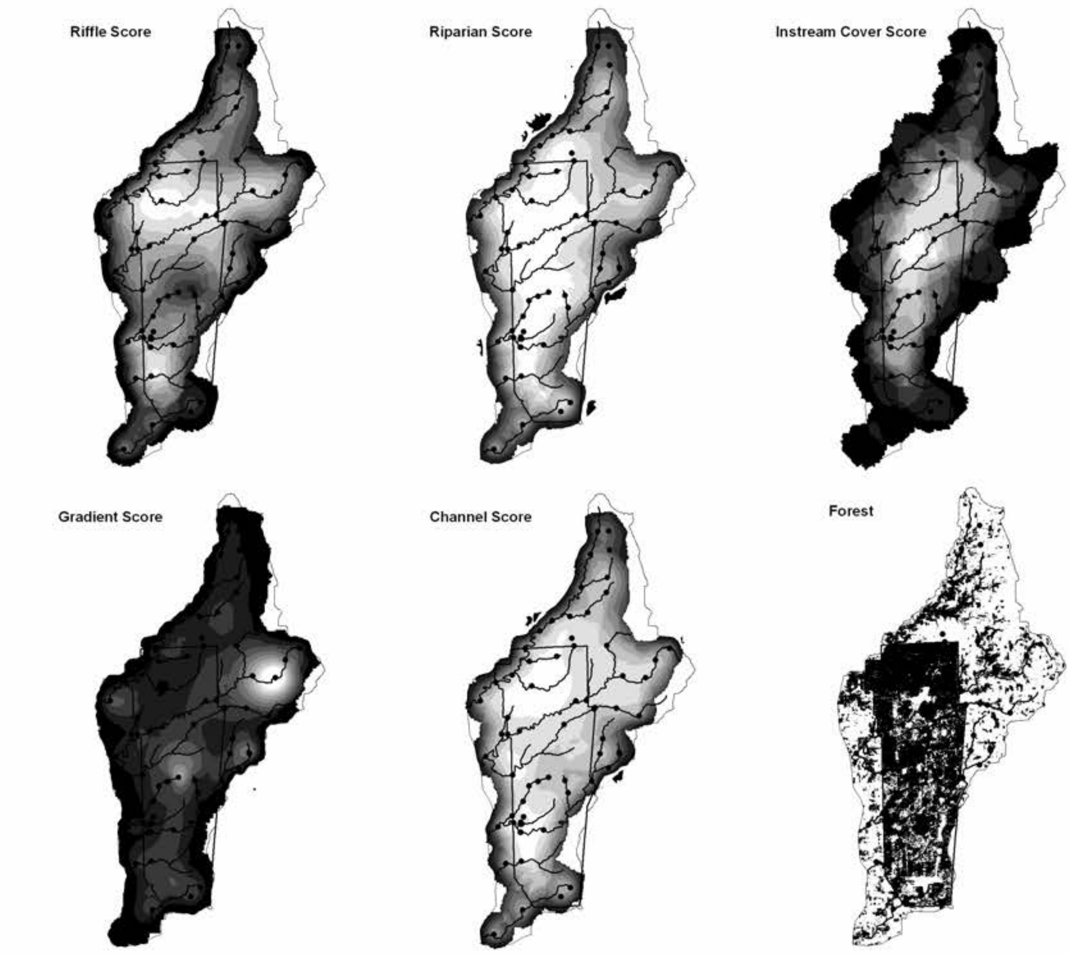
Factor Analysis Results

Variable	Factor 1	Factor 2
Chloride	**0.821893**	0.220021
Nitrogen	**0.769671**	0.270388
Total Phosphorus	**0.811131**	0.198719
Total Solids	**0.818145**	-0.221031
QHEI - Riparian Score	-0.61667	0.395294
QHEI - Pool Glide Score	0.119627	**0.909592**
Commercial Land-use	-0.63112	-0.18345
Expl. Variance	49%	66%



Nitrate + Nitrite
Phosphorus
Sodium
Chloride
Flouride
Arsenic
Lead
Manganese
Sulfate


Barium
Total Solids
Calcium
Hardness
Conductivity
pH
Substrate Score
Pool Score
QHEI Score



Riffle Score
Riparian Score
Instream Cover Score
Gradient Score
Channel Score
Forest

Figure 14. Relative concentration pleths of significant water quality, habitat, and land use chemistry variables that were predictive of fish, macroinvertebrate and crayfish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area or lower habitat quality. All concentrations are presented on a relative (percentage of range) scale (see Appendix for contaminant concentration, habitat, and land use range).

Macroinvertebrate assemblage structure was determined by barium, lead, and manganese. These contaminants were generally found on the refuge, but lead was also found in upstream areas of Otter Creek (Figure 14). Fluoride was similarly affecting the macroinvertebrate assemblage in the headwaters of Little Graham Creek.

Crayfish assemblage structure showed the strongest response signature to nutrient contaminants associated with the headwaters of Little Graham Creek (Figure 14). Crayfish assemblage structure showed that the relative abundance of crayfish increased in deep pools that were unaffected by the nutrient gradient.

Appendix 4. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use at the Big Oaks National Wildlife Refuge.

Variable	Mean	Minimum	Maximum	SD
Contaminant				
Alkalinity	120.68	20.00	218.00	39.03
Aluminum	490.16	10.00	2740.00	581.89
Arsenic	1.59	0.20	8.15	1.30
Barium	63.21	24.20	170.00	27.67
Cadmium	0.09	0.05	1.55	0.23
Calcium	106.48	22.00	180.00	32.08
Chloride	13.86	0.05	124.00	18.08
Chemical Oxygen Demand	22.97	6.20	57.30	8.57
Copper	1.72	0.10	11.50	1.45
Fluoride	0.12	0.05	0.50	0.08
Hardness	145.64	35.00	245.00	45.51
Iron	976.78	43.60	14900.00	2000.97
Lead	0.30	0.10	3.13	0.65
Manganese	327.74	25.70	6360.00	876.27
Nickel	0.68	0.25	3.92	0.90
Ammonia	0.06	0.05	0.60	0.08
Nitrogen	0.86	0.01	24.00	3.08
Total Phosphorus	0.13	0.03	1.82	0.23
Silica	8.12	4.30	15.00	1.92
Sodium	8.22	2.16	71.30	9.64
Sulfate	15.19	5.20	79.00	11.02
Total Organic Carbon	6.54	2.00	14.40	2.42
Total Solids	234.32	88.00	1010.00	122.99
Total Suspended Solids	26.48	0.50	846.00	104.84
Zinc	5.99	3.00	24.90	4.03
General Chemistry				
Dissolved Oxygen	5.37	0.98	12.79	3.16
pH	8.06	7.14	9.50	0.50
Water Temperature	22.21	18.84	26.81	1.74
Conductivity	355.13	21.00	2600.00	308.83
Turbidity	8.16	0.00	56.80	11.01
Habitat				
QHEI - Score	61.36	25.00	85.00	10.08
QHEI - Substrate Score	13.18	1.00	18.00	2.49
QHEI - Instream Cover Score	7.36	2.00	15.00	3.01
QHEI - Channel Score	15.97	5.00	20.00	3.22
QHEI - Riparian Score	8.86	3.00	10.00	1.87
QHEI - Pool/Glide Score	5.94	3.00	12.00	1.71
QHEI - Riffle/Run Score	4.31	0.00	7.00	1.84
QHEI - Gradient Score	5.67	2.00	10.00	2.47
Land-use				
Water	2.94	0.00	19.34	4.22
Commercial	0.56	0.00	7.78	1.36
Agricultural	24.83	0.00	54.27	18.91
Residential	0.33	0.00	4.81	0.67

Grass/Pasture	25.56	0.00	59.79	18.50
Forest	41.60	0.00	96.48	27.64
Industrial	4.17	0.00	22.43	6.02
Other	0.02	0.00	0.19	0.04

Stressor Identification of Contaminant Affects at the Muscatatuck National Wildlife Refuge

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Study Design

Stressor identification on the refuge scale was accomplished using a random design to select 50 sites in the Muscatatuck National Wildlife Refuge. These sites represented locations on the refuge and those immediately upstream. Sites were visited and those sites that were dry were excluded from the 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 (Figure 8).

Analysis of assemblage structure based on Cluster Analysis showed that each assemblage group explained variance in the data differently (Figure 9). Assemblage structure was explained by increasing index of biotic integrity scores for fish and increasing species richness for macroinvertebrates and crayfish (Figure 10). Fish assemblage structure was explained with seven clusters representing tier 4, macroinvertebrate assemblage structure was explained with three clusters representing tier 2 linkage structure (Figure 10), while crayfish assemblage structure was explained with 9 clusters and tier 4 linkage. The basis for these groupings is that the biological community structure results are externally driven and can be identified to evaluate physical and chemical stress on the respective assemblage (Morris et al. 2006).

Habitat Quality

Habitat analysis using the QHEI showed that scores ranged from 34 to 81 (average 54.77 $\pm$ 12.38). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life. 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. The substrate score, instream cover, riparian channel, pool-glide, and riffle-run score were the primary factors contributing to declining QHEI scores in the Muscatatuck National Wildlife Refuge study area.

Chemical Quality

Summary statistics by assemblage for water chemistry results are found in Tables 17-19. Project specific summary statistics and range of scale values for water quality results are presented in Appendix 5. In general, the lowest biological integrity and species richness clusters (highest numerical cluster) had the highest concentrations of contaminants (Tables 8-10). Several variables, such as dissolved oxygen, showed differential distribution with higher concentrations in clusters 1 and 2 for fish (Figures 18). All of the contaminants identified as significant showed the dose response range with increasing concentrations for macroinvertebrates (Figure 19). Crayfish showed an inverted normal distribution for specific conductivity and total solids, otherwise observed distributions were as expected with increasing concentrations with increasing cluster (Figure 20).

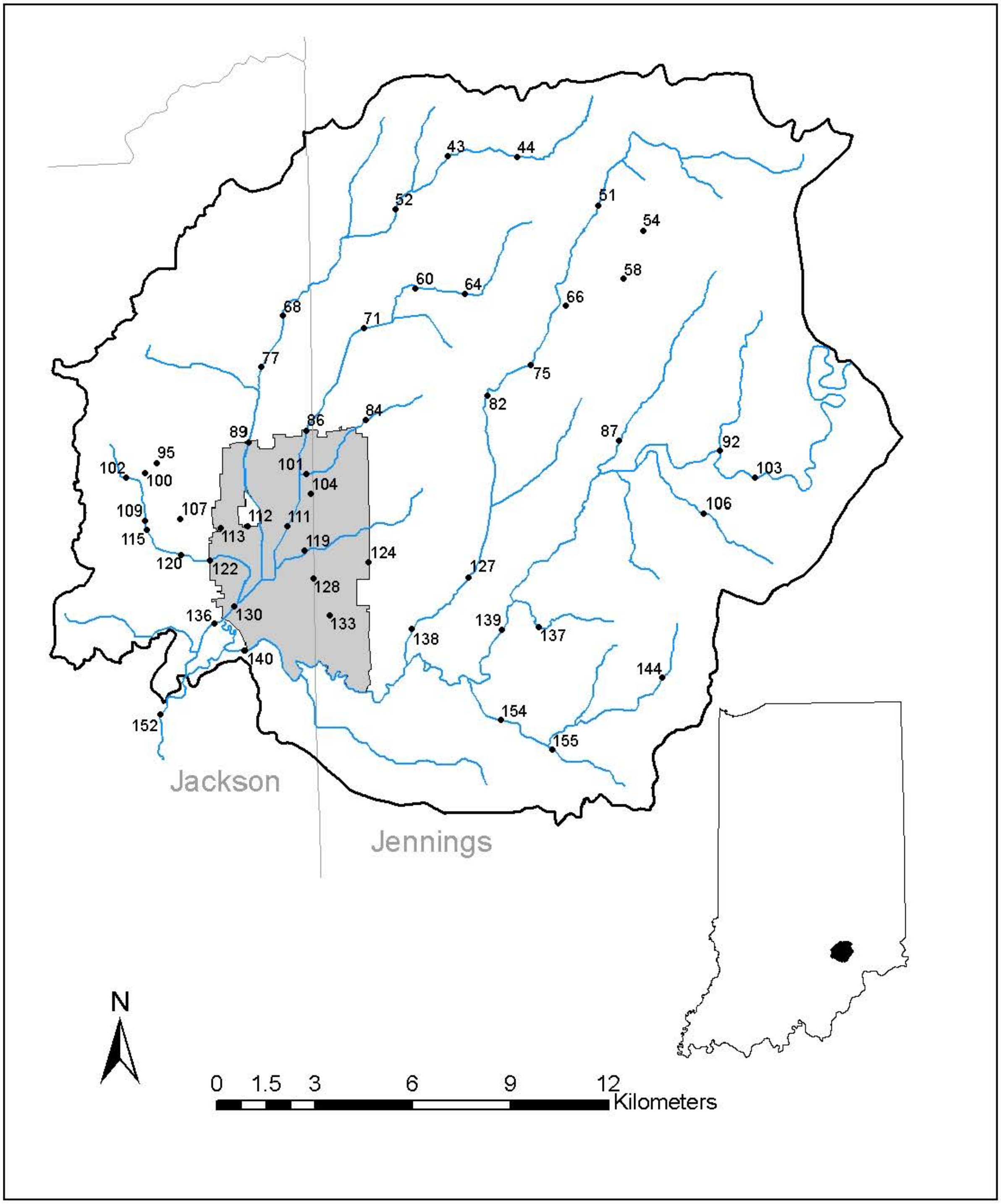


Figure 15. Map showing sites sampled on refuge and upstream of the Muscatatuck National Wildlife Refuge during stressor sampling in 2007 (site numbers refer to Appendix).

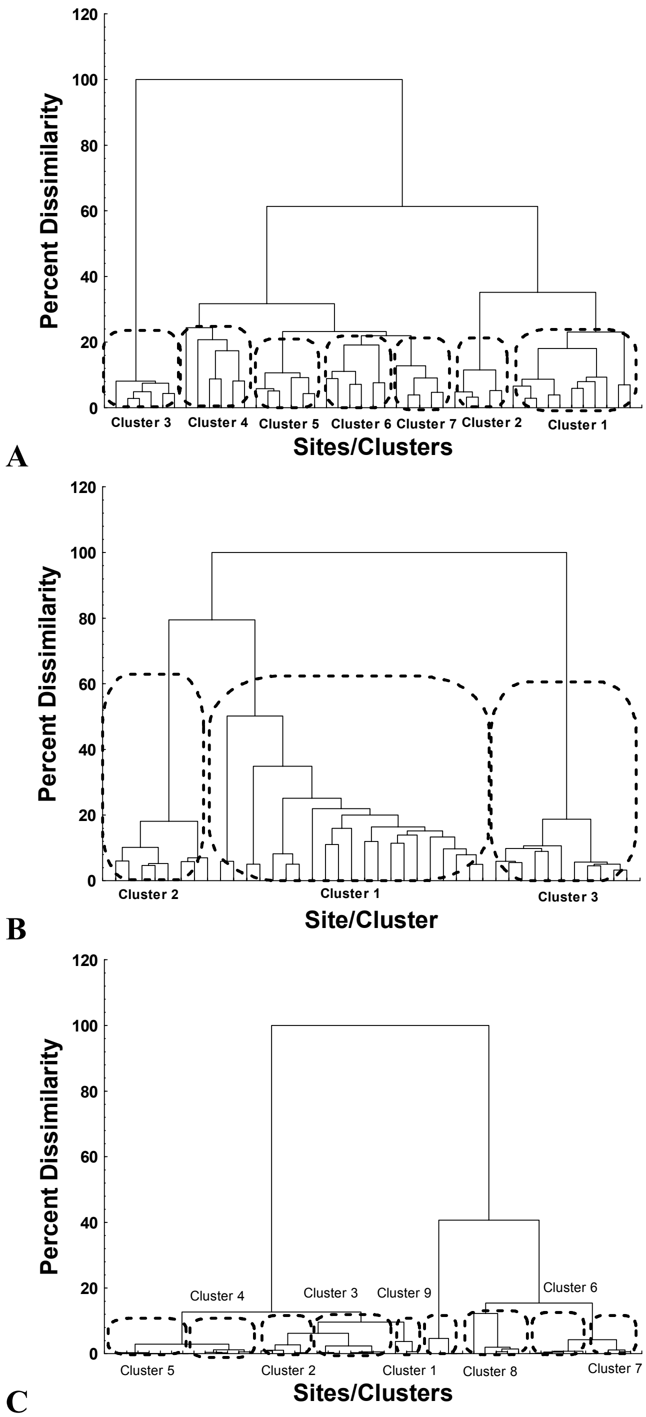


Figure 16. Dendograms showing the relative similarity of assemblage data collected in the Muscatatuck National Wildlife Refuge watersheds during August 2006. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA. (a) fish, (b) macroinvertebrate, and (c) crayfish assemblages.

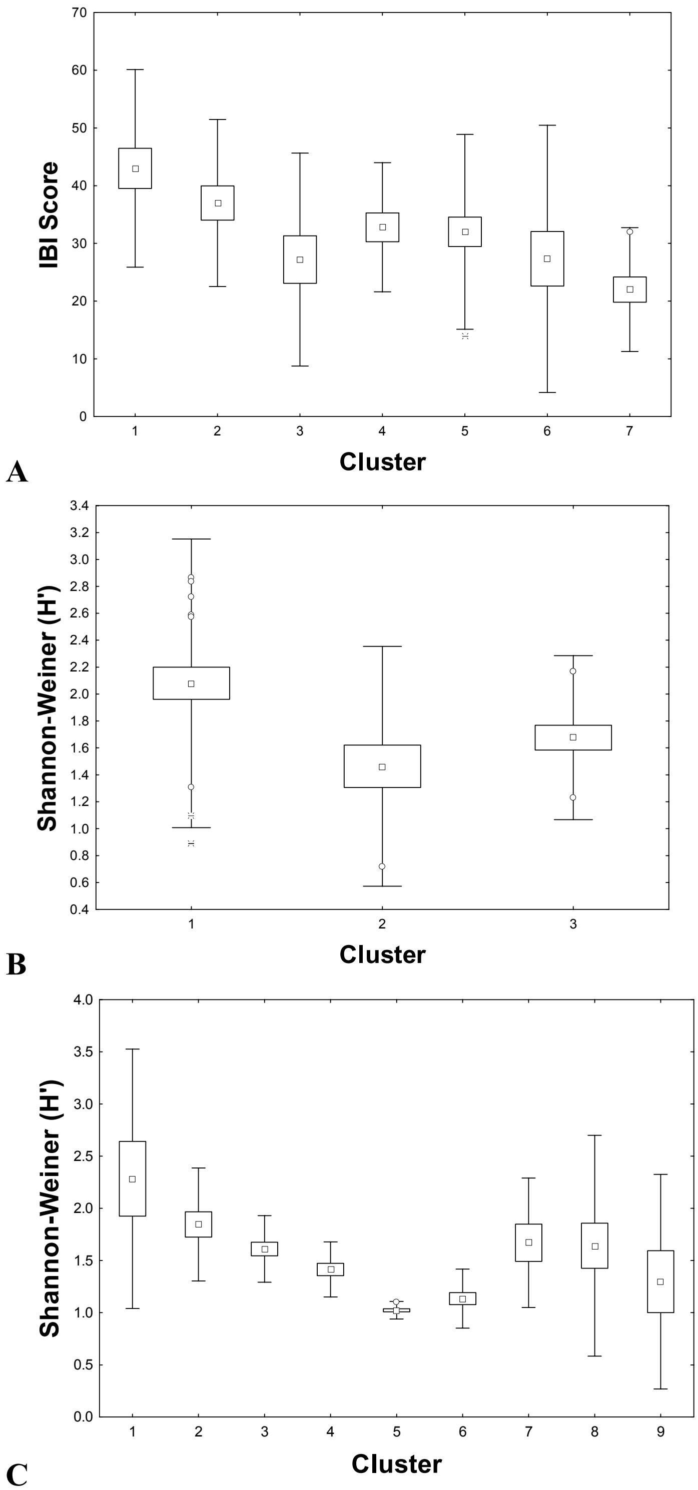


Figure 17. Box-and-whisker plots showing biological assemblage responses for index of biotic integrity (IBI) for (a) fish, or Shannon-Wiener (H’) (b) macroinvertebrates and (c) crayfish assemblages had to the biologic response gradient developed from the Muscatatuck National Wildlife Refuge watersheds during August 2007.

Table 17. Summary statistics for 11 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Fig. 1A) sampled in June-August 2007 in watersheds of the Muscatatuck National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Commercial	6	0.42	0.01	1.35	0.54	6	0.57	0.12	1.14	0.44
Agriculture	6	24.66	19.07	39.06	7.69	6	26.54	17.99	41.21	9.56
Residential	6	2.59	0.02	4.86	1.91	6	4.29	0.13	10.68	4.21
Grass Pasture	6	37.61	8.33	48.10	14.63	6	28.88	8.42	39.42	14.45
Forest	6	33.30	23.97	47.09	8.59	6	38.29	33.64	43.81	4.24
Industrial	6	0.10	0.00	0.48	0.19	6	0.31	0.00	0.87	0.30
Other	6	0.00	0.00	0.01	0.00	6	0.00	0.00	0.01	0.00
Chloride	6	18.07	8.40	31.00	9.12	6	23.33	12.00	34.00	7.26
Silica (Reactive)	6	5.27	1.30	7.50	2.25	6	4.27	3.10	8.20	1.95
Sulfate	6	20.88	1.30	46.00	14.36	6	33.67	16.00	46.00	12.34
Dissolved Oxygen	6	5.78	4.32	8.82	1.86	6	8.37	5.95	11.08	1.77

	Cluster 3					Cluster 4				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Commercial	5	0.12	0.00	0.32	0.13	5	0.86	0.05	2.50	0.99
Agriculture	5	20.06	12.71	27.07	5.69	5	18.86	6.55	39.58	12.78
Residential	5	0.60	0.00	1.48	0.73	5	6.73	0.05	16.44	6.35
Grass Pasture	5	47.35	37.51	54.61	6.35	5	27.38	8.15	43.54	17.48
Forest	5	31.09	24.15	49.01	10.14	5	45.18	32.68	65.91	13.11
Industrial	5	0.00	0.00	0.00	0.00	5	0.18	0.00	0.66	0.29
Other	5	0.00	0.00	0.01	0.01	5	0.01	0.00	0.02	0.01
Chloride	5	15.12	6.60	25.00	7.44	5	17.40	11.00	29.00	8.14
Silica (Reactive)	5	9.84	5.30	15.00	4.01	5	5.30	2.60	9.70	2.98
Sulfate	5	23.80	18.00	32.00	5.67	5	36.00	24.00	47.00	8.46
Dissolved Oxygen	5	4.20	2.10	6.79	1.79	5	5.62	3.76	7.36	1.49

Variable	N	Mean	Cluster 5 Minimum	Maximum	SD	N	Mean	Cluster 6 Minimum	Maximum	SD
Commercial	11	0.23	0.00	1.89	0.56	6	5.15	0.09	20.62	8.43
Agriculture	11	24.50	15.04	52.43	12.32	6	44.80	16.14	72.60	18.09
Residential	11	2.43	0.00	11.48	4.29	6	10.39	5.86	25.29	7.36
Grass Pasture	11	38.29	6.78	61.35	17.53	6	21.88	9.67	34.34	11.65
Forest	11	33.50	14.23	73.96	15.92	6	16.47	2.35	34.22	15.48
Industrial	11	0.00	0.00	0.00	0.00	6	0.23	0.00	1.11	0.44
Other	11	0.03	0.00	0.20	0.06	6	0.04	0.00	0.16	0.06
Chloride	11	17.28	6.00	58.00	14.98	6	36.67	13.00	80.00	23.72
Silica (Reactive)	11	8.04	2.50	14.00	3.33	6	9.90	4.10	14.00	3.26
Sulfate	11	31.32	6.50	62.00	15.10	6	22.27	1.30	52.00	19.99
Dissolved Oxygen	11	5.64	3.45	7.85	1.39	6	6.31	1.32	10.21	2.91

Variable	N	Mean	Cluster 7 Minimum	Maximum	SD
Commercial	6	10.88	6.53	19.15	4.36
Agriculture	6	47.76	26.36	62.62	12.42
Residential	6	20.04	8.07	44.76	13.97
Grass Pasture	6	14.64	0.00	34.53	15.62
Forest	6	3.08	0.80	5.59	1.95
Industrial	6	3.49	0.00	8.83	3.54
Other	6	0.01	0.01	0.02	0.00
Chloride	6	33.50	18.00	51.00	13.58
Silica (Reactive)	6	10.50	5.40	17.00	4.11
Sulfate	6	48.00	31.00	62.00	11.73
Dissolved Oxygen	6	5.37	3.36	6.31	1.04

Table 18. Summary statistics for 11 chemistry (concentration/ppm), habitat, and land use variables by macroinvertebrate grouping cluster (see Fig. 1A) sampled in March-August 2007 in watersheds of the Muscatatuck National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Water	21	1.19	0.04	6.17	1.35	8	1.36	0.00	3.39	0.99
Commercial	21	1.32	0.00	19.15	4.14	8	0.08	0.00	0.21	0.09
Agriculture	21	22.75	6.55	47.90	10.06	8	28.10	15.48	72.60	19.46
HD-Residential	21	1.57	0.00	23.30	5.21	8	0.06	0.00	0.26	0.10
Forest	21	34.21	0.80	65.91	12.55	8	36.64	3.31	73.96	19.55
Alkalinity (as CaCO3)	21	171.14	66.00	242.00	46.28	8	137.75	59.00	252.00	54.41
Calcium (as CaCO3)	21	143.24	48.00	224.00	38.45	8	131.75	96.00	204.00	32.24
Chloride	21	17.46	6.60	45.00	9.18	8	16.19	6.00	41.00	12.14
Hardness (as CaCO3)	21	206.81	78.00	283.00	50.74	8	196.00	143.00	292.00	50.18
Sulfate	21	26.35	1.30	59.00	14.76	8	34.38	20.00	52.00	10.53
Total Solids	21	279.52	138.00	372.00	55.69	8	998.25	190.00	6140.00	2078.42
Specific Conductivity	20	401.35	256.00	538.00	56.55	8	348.89	236.00	483.10	80.78

	Cluster 3				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Water	11	0.30	0.04	1.37	0.41
Commercial	11	7.05	0.00	20.62	6.39
Agriculture	11	42.28	16.04	62.62	13.62
HD-Residential	11	3.02	0.00	14.59	4.54
Forest	11	10.83	1.75	38.20	12.30
Alkalinity (as CaCO3)	11	194.64	140.00	262.00	39.33
Calcium (as CaCO3)	11	180.00	120.00	232.00	32.74
Chloride	11	35.00	10.00	80.00	20.70
Hardness (as CaCO3)	11	270.18	179.00	373.00	61.06
Sulfate	11	42.36	28.00	62.00	11.83
Total Solids	11	363.09	258.00	466.00	76.97
Specific Conductivity	11	504.56	383.00	639.50	97.10

Table 19. Summary statistics for 11 chemistry (concentration/ppm), habitat, and land use variables by crayfish grouping cluster (see Fig. 1A) sampled in March-August 2007 in watersheds of the Muscatatuck National Wildlife Refuge.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Forest	3	25.88	23.97	29.52	3.15	5	40.598	15.676	65.912	19.299
Arsenic	3	7.13	1.94	17.00	8.55	5	0.950	0.800	1.550	0.335
Chloride	3	24.67	22.00	27.00	2.52	5	14.720	7.600	23.000	7.217
Iron	3	3705.00	795.00	8080.00	3857.13	5	210.000	130.000	389.000	102.135
Phosphorus, Total	3	0.36	0.11	0.84	0.42	5	0.040	0.020	0.070	0.019
Sodium	3	14.33	12.10	16.10	2.04	5	10.808	7.840	15.300	3.508
Sulfate	3	18.43	1.30	34.00	16.41	5	39.400	21.000	62.000	15.662
TS	3	2256.33	265.00	6140.00	3363.72	5	302.400	258.000	374.000	43.598
Specific Conductivity	3	367.73	321.00	393.00	40.52	5	418.960	383.000	464.000	34.663
QHEI-Channel Score	3	7.67	6.00	9.00	1.53	5	17.100	15.500	19.000	1.432
QHEI-Riparian Score	3	9.33	9.00	10.00	0.58	5	6.100	5.000	7.500	1.140
QHEI-Riffle Run Score	3	0.67	0.00	2.00	1.15	5	4.800	3.000	7.000	1.483

	Cluster 3					Cluster 4				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Forest	6	37.35	29.15	49.01	7.24	5	28.61	3.12	47.09	16.03
Arsenic	6	1.46	0.80	2.15	0.58	5	1.50	0.80	3.40	1.08
Chloride	6	15.43	6.60	31.00	8.99	5	15.04	6.20	23.00	6.53
Iron	6	522.67	232.00	819.00	229.38	5	276.80	102.00	485.00	161.11
Phosphorus, Total	6	0.06	0.02	0.11	0.03	5	0.04	0.02	0.10	0.03
Sodium	6	12.17	5.25	25.60	7.89	5	9.36	5.74	16.30	4.44
Sulfate	6	27.83	20.00	46.00	10.25	5	25.70	6.50	40.00	12.67
TS	6	271.50	221.00	314.00	33.71	5	239.60	175.00	304.00	58.17
Specific Conductivity	6	387.03	293.00	454.00	58.56	5	377.40	236.00	597.00	148.27
QHEI-Channel Score	6	11.92	9.00	18.00	3.14	5	15.20	11.00	19.00	4.02
QHEI-Riparian Score	6	4.50	3.00	7.00	1.38	5	7.05	4.50	9.75	1.99
QHEI-Riffle Run Score	6	3.92	2.00	6.00	1.43	5	4.00	2.00	7.00	2.00

Variable	N	Cluster 5 Mean	Minimum	Maximum	SD	N	Cluster 6 Mean	Minimum	Maximum	SD
Forest	8	27.13	2.52	43.81	15.47	6	8.65	0.80	39.75	15.31
Arsenic	8	2.21	1.30	2.93	0.64	6	1.49	1.28	1.97	0.26
Chloride	8	28.75	15.00	80.00	21.35	6	33.07	8.40	51.00	15.11
Iron	8	585.00	126.00	2280.00	718.02	6	577.00	256.00	896.00	246.90
Phosphorus, Total	8	0.11	0.03	0.22	0.07	6	0.07	0.03	0.10	0.03
Sodium	8	18.21	6.74	36.10	9.36	6	15.24	9.06	24.40	5.65
Sulfate	8	30.13	16.00	40.00	9.83	6	44.83	17.00	62.00	16.76
TS	8	299.13	238.00	466.00	76.36	6	363.67	282.00	461.00	66.37
Specific Conductivity	8	429.69	349.00	639.50	96.41	6	517.30	399.00	603.80	82.47
QHEI-Channel Score	8	13.69	8.00	19.00	4.46	6	10.00	7.00	12.00	2.17
QHEI-Riparian Score	8	4.91	1.00	8.75	2.54	6	5.50	3.50	9.50	2.24
QHEI-Riffle Run Score	8	4.38	0.00	7.00	2.33	6	2.00	1.00	4.00	1.14

Variable	N	Cluster 7 Mean	Minimum	Maximum	SD	N	Cluster 8 Mean	Minimum	Maximum	SD
Forest	3	14.62	3.31	26.33	11.52	6	34.29	27.55	38.20	3.61
Arsenic	3	1.98	1.59	2.56	0.51	6	2.02	0.80	5.46	1.73
Chloride	3	39.67	20.00	58.00	19.04	6	15.00	6.00	34.00	9.72
Iron	3	625.33	462.00	952.00	282.90	6	442.00	183.00	1340.00	443.46
Phosphorus, Total	3	0.07	0.04	0.12	0.05	6	0.07	0.03	0.17	0.05
Sodium	3	28.13	23.30	35.90	6.79	6	13.00	4.54	27.90	7.88
Sulfate	3	35.33	18.00	52.00	17.01	6	27.72	1.30	46.00	17.05
TS	3	377.33	328.00	456.00	68.86	6	253.50	234.00	303.00	28.93
Specific Conductivity	3	509.10	449.00	614.10	91.25	6	363.38	318.00	399.50	28.91
QHEI-Channel Score	3	14.17	11.00	18.00	3.55	6	13.00	8.00	18.00	4.20
QHEI-Riparian Score	3	7.75	5.00	10.00	2.54	6	4.75	2.00	8.00	2.40
QHEI-Riffle Run Score	3	2.50	1.50	4.00	1.32	6	3.75	1.00	7.00	2.14

Variable	N	Mean	Cluster 9 Minimum	Maximum	SD
Forest	3	43.46	23.76	73.96	26.79
Arsenic	3	9.74	0.80	26.50	14.52
Chloride	3	23.10	8.30	39.00	15.38
Iron	3	3328.67	268.00	8920.00	4849.48
Phosphorus, Total	3	0.39	0.04	0.96	0.50
Sodium	3	20.07	17.30	25.20	4.45
Sulfate	3	20.77	1.30	45.00	22.24
TS	3	397.33	309.00	527.00	114.73
Specific Conductivity	3	343.53	168.40	483.10	160.34
QHEI-Channel Score	3	16.50	15.50	17.00	0.87
QHEI-Riparian Score	3	10.00	10.00	10.00	0.00
QHEI-Riffle Run Score	3	1.50	0.00	2.50	1.32

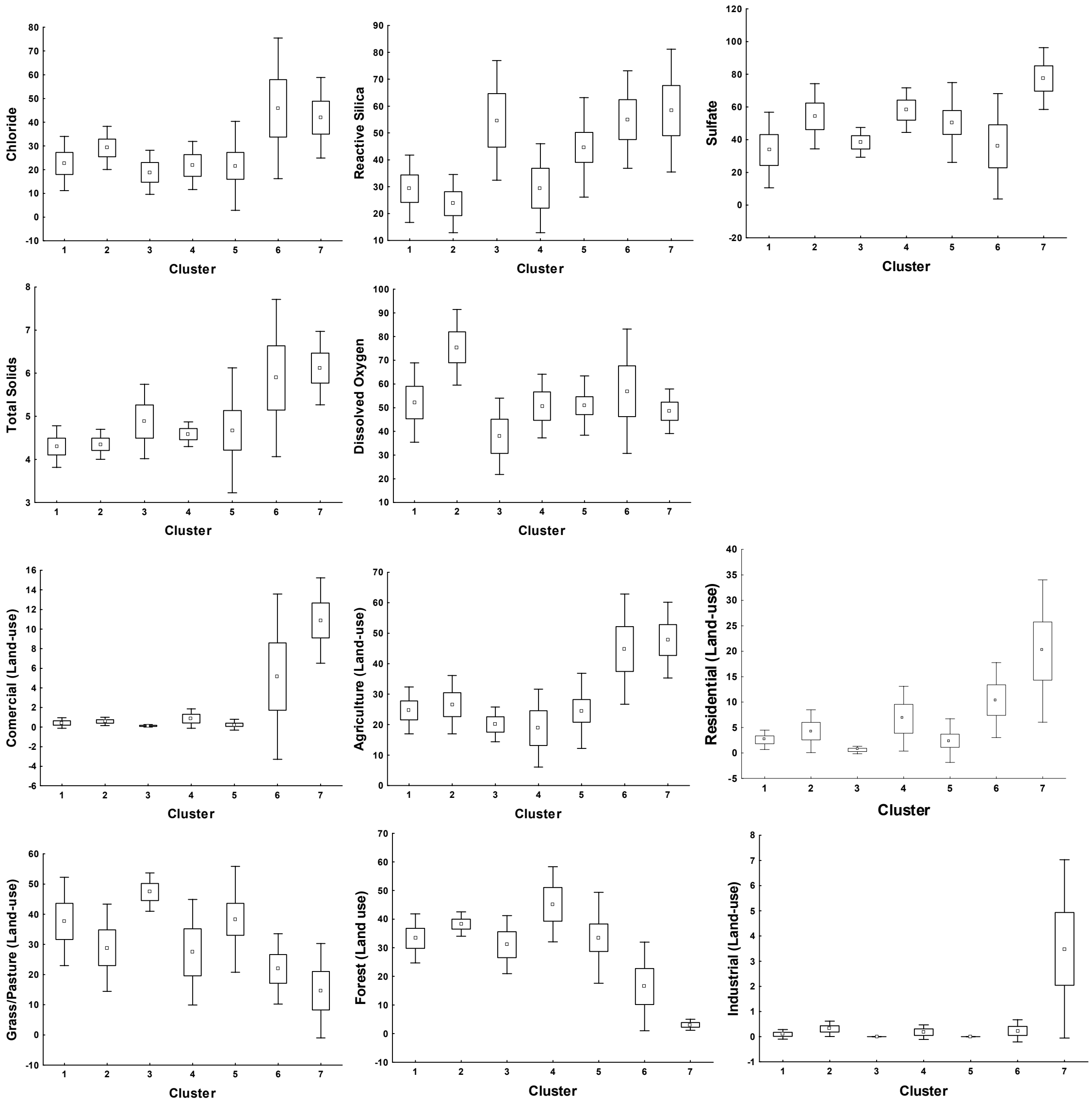


Figure 18. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the fish assemblage clustering.

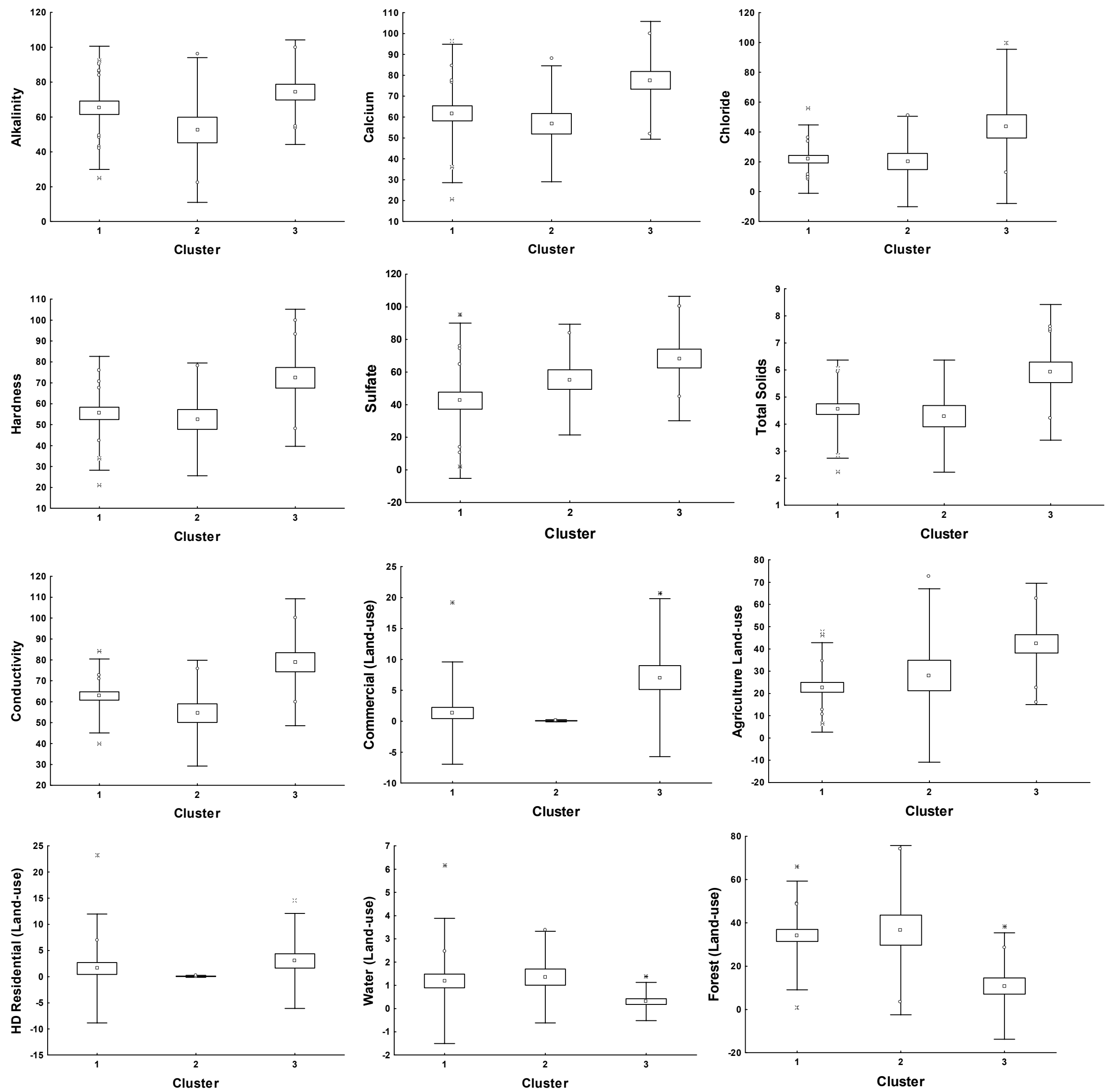


Figure 19. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the macroinvertebrate assemblage clustering.

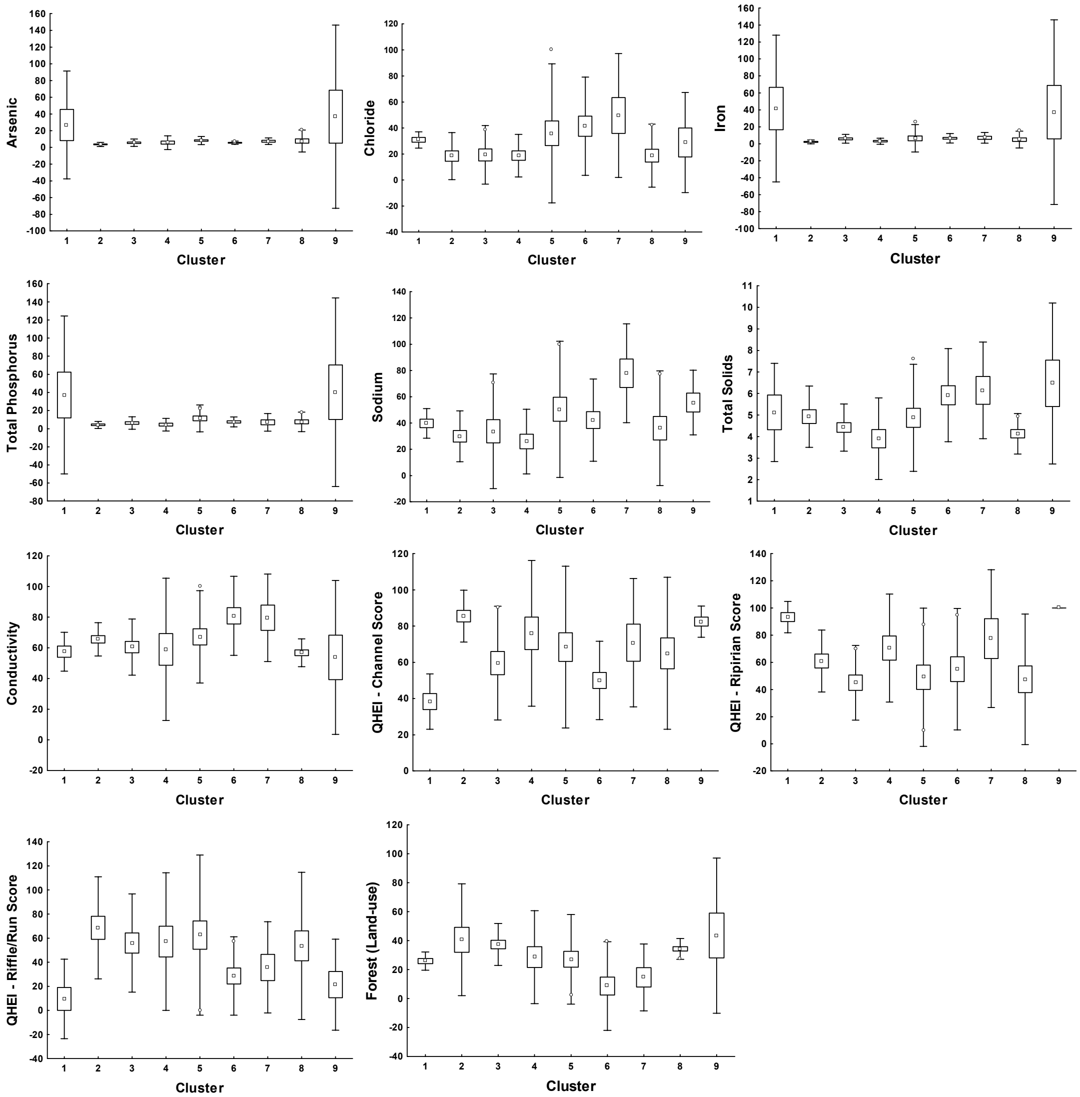


Figure 20. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the crayfish assemblage clustering.

Table 20. List of 13 physical and chemical variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Muscatatuck National Wildlife Refuge during June-August 2007.

Variable	P Value
Contaminant	
Chloride	p = 0.0357
Silica (Reactive)	p = 0.0087
Sulfate	p = 0.0363
General Chemistry	
Dissolved Oxygen	p = 0.0482
Total Solids	p = 0.0365
Land-use	
Commercial	p = 0.0008
Agriculture	p = 0.0065
High Density Residential	p = 0.0008
Low Density Residential	p = 0.0016
Residential	p = 0.0007
Grass Pasture	p = 0.0048
Forest	p = 0.0004
Industrial	p = 0.0058

Multivariate Results

For this analysis we chose 70-80% similarity as a benchmark for defining assemblage structure clustering (Figure 9). These clusters were used for stressor response measurement. Kruskal-Wallis ANOVA by ranks tests were significant for seven clusters for fish, showing that three chemical, two general chemistry, and eight land use measures were significantly predictive of the fish assemblage structure (Table 20). Macroinvertebrate assemblages were significant for three clusters, showing that four chemical, three general chemistry, and five land use types were predictive of the structure (Table 21). Crayfish structure was significant for nine clusters and was explained by two chemical, two general chemistry, and three habitat variables (Table 22).

Fish assemblage structure was significantly predicted by the presence of chloride, reactive silica, sulfate, dissolved oxygen, total solids, and seven land use variables (Table 20). Chloride, sulfate, and total solids were significantly different in cluster 6 and 7, which is a signature of wastewater treatmen effluents, while dissolved oxygen was significant in cluster 1 and 2 (Figure 18). Macroinvertebrates assemblage structure showed expected distribution patterns with an increasing dose response for all of the significant chemical and general chemistry parameters. Crayfish assemblage structure was significant for arsenic, chloride, iron, phosphorus, sodium, specific conductivity, and total solids (Table 22). Arsenic, iron, and phosphorus showed increasing concentrations in clusters 1 and 9, while chloride, sodium, conductivity, and total solids showed significant concentration increases for clusters 5, 6, and 7. Only crayfish assemblage structure was significantly predicted by habitat, while fish were significantly affected by all surrounding land uses and macroinvertebrates were significantly affected by commercial, agriculture, high density residential, water, and forest.

Table 21. List of 12 physical and chemical variables significantly predictive (a = 0.05) of the macroinvertebrate assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Muscatatuck National Wildlife Refuge during March-August 2007.

Variable	P Value
Contaminant	
Alkalinity	p = 0.0379
Calcium	p = 0.0079
Chloride	p = 0.0117
Sulfate	p = 0.0167
General Chemistry	
Hardness	p = 0.0080
Specific Conductivity	p = 0.0018
Total Solids	p = 0.0281
Land-use	
Commercial	p = 0.0051
Agriculture	p = 0.0072
High Density Residential	p = 0.0222
Water	p = 0.0093
Forest	p = 0.0014

Table 22. List of 12 physical and chemical variables significantly predictive (a = 0.05) of the crayfish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in watersheds of the Muscatatuck National Wildlife Refuge during March-August 2007.

Variable	P Value
Contaminant	
Arsenic	p = 0.0447
Iron	p = 0.0225
General Chemistry	
Specific Conductivity	p = 0.0465
Total Solids	p = 0.0068
Habitat	
QHEI-Channel Score	p = 0.0328
QHEI-Riparian Score	p = 0.0081
QHEI-Riffle Run Score	p = 0.0267

Box and whisker plots for significant variable responses for fish (Figure 18), macroinvertebrates (Figure 19), and crayfish (Figure 20) show the greatest response was observed among fish and macroinvertebrate assemblage structures. Fish and macroinvertebrate assemblage structure was predicted by wastewater treatment (i.e., chloride and sulfate), and alkalinity and calcium, and land use (Table 20 and 21). Crayfish was associated with groundwater associated parameters such as arsenic and iron, which may be an indirect signature of cool water seeps on the refuge (Figure 21).

General Chemistry

All three organism indicators showed a significant relationship with total solids (Tables 20-22). Both macroinvertebrates and crayfish showed a significant relationship with specific conductivity, while hardness was significant for macroinvertebrates, and dissolved oxygen was significant for fish. These chemistries show differential responses from multiple locations both on the refuge and surrounding the refuge. Total solids was highest from an area near the Mutton Creek tributary, while increased concentrations of conductivity, alkalinity, and calcium are emanating from four to five areas surrounding the refuge (Figure 21).

Habitat Response

Only crayfish showed a significant response relationship with habitat parameters from among the three organism groups (Table 22). Crayfish assemblage structure showed a significant response to channel score, riparian score, and riffle-run score (Table 22). The presence of an intact riparian corridor affected the growth of algae and potentially eliminated the invasion of rusty crayfish from the refuge.

Land Use Response

Fish assemblage structure showed the most significant relationship with land use (Table 20). Fish showed a significant response to commercial, agriculture, grass-pasture, forest, industrial, and all three residential classes (high density, low density, and combined) land uses. Macroinvertebrate structure was significant for commercial, agriculture, high density residential, water, and forest land uses (Table 21), while crayfish showed no significant response to any land use type (Table 22).

Fish assemblage structure showed increasing biological integrity with increasing percentages of grass-pasture and forest land uses (Figure 18). The fish assemblage showed decreasing biological integrity associated with increased percentage of commercial, agriculture, high density, low density, and residential land use, which shows a direct relationship with increased disturbance. The only land use type that did not show a dose response pattern was the industrial land use type. Industrial land use showed a presence/ absence distribution with all of the occurrences occurring in cluster 7. This cluster corresponds to the highest contamination gradient cluster.

Macroinvertebrate assemblage structure showed increasing species richness with increasing relationships for water and forest land use types (Figure 19). Macroinvertebrates showed decreasing species richness with increasing dose response relationships with commercial, agriculture, and high density residential land uses.

Table 23. Results of factor analysis and explained variance observed in fish assemblage biologic structure and seven significant chemical, habitat, and land use variables at the Muscatatuck National Wildlife Refuge during August 2007.

Variable	Factor 1	Factor 2
Total Solids	**0.910303**	-0.041431
Commercial	0.649027	0.431428
Agriculture	0.316884	0.586255
Residential	0.434609	0.687711
Grass Pasture	-0.151246	**-0.845083**
Chloride	**0.833052**	0.252372
Dissolved Oxygen	-0.282109	0.659589
Expl.Var	2.335625	2.217402
Proportion Total Variance	33.366066	31.677173

Table 24. Results of factor analysis and explained variance observed in macroinvertebrate assemblage biologic structure and six significant chemical, habitat, and land use variables at the Muscatatuck National Wildlife Refuge during August 2007.

Variable	Factor 1	Factor 2
Commercial	0.305941	**0.818837**
Agriculture	**0.934068**	0.044473
Forest	**-0.849301**	-0.341784
Chloride	0.510598	0.641492
Hardness (as CaCO3)	0.535762	0.482661
Sulfate	0.042198	**0.890791**
Explained Variance	2.236928	2.227270
Proportion of Total Variance	0.372821	0.371212

Table 25. Results of factor analysis and explained variance observed in crayfish assemblage biologic structure and nine significant chemical, habitat, and land use variables at the Muscatatuck National Wildlife Refuge during August 2007.

Variable	Factor 1	Factor 2	Factor 3
Forest (Land-use)	-0.569543	0.106680	-0.418260
Chloride	**0.915652**	0.133818	0.129175
Iron	-0.096570	**0.823644**	0.323296
Phosphorus, Total	0.274800	**0.851771**	-0.059838
Sodium	**0.831054**	0.179535	0.036732
Specific Conductivity	0.697880	-0.440596	0.265555
QHEI-Channel Score	-0.117242	0.075302	**-0.930761**
QHEI-Riparian Score	-0.071527	0.628160	-0.439573
QHEI-Riffle Run Score	-0.228523	-0.344929	**-0.748656**
Explained Variance	2.496410	2.178779	1.991624
Proportion of Total	27.7379	24.2087	22.1292

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. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used later to reduce the set of variables in a dataset.

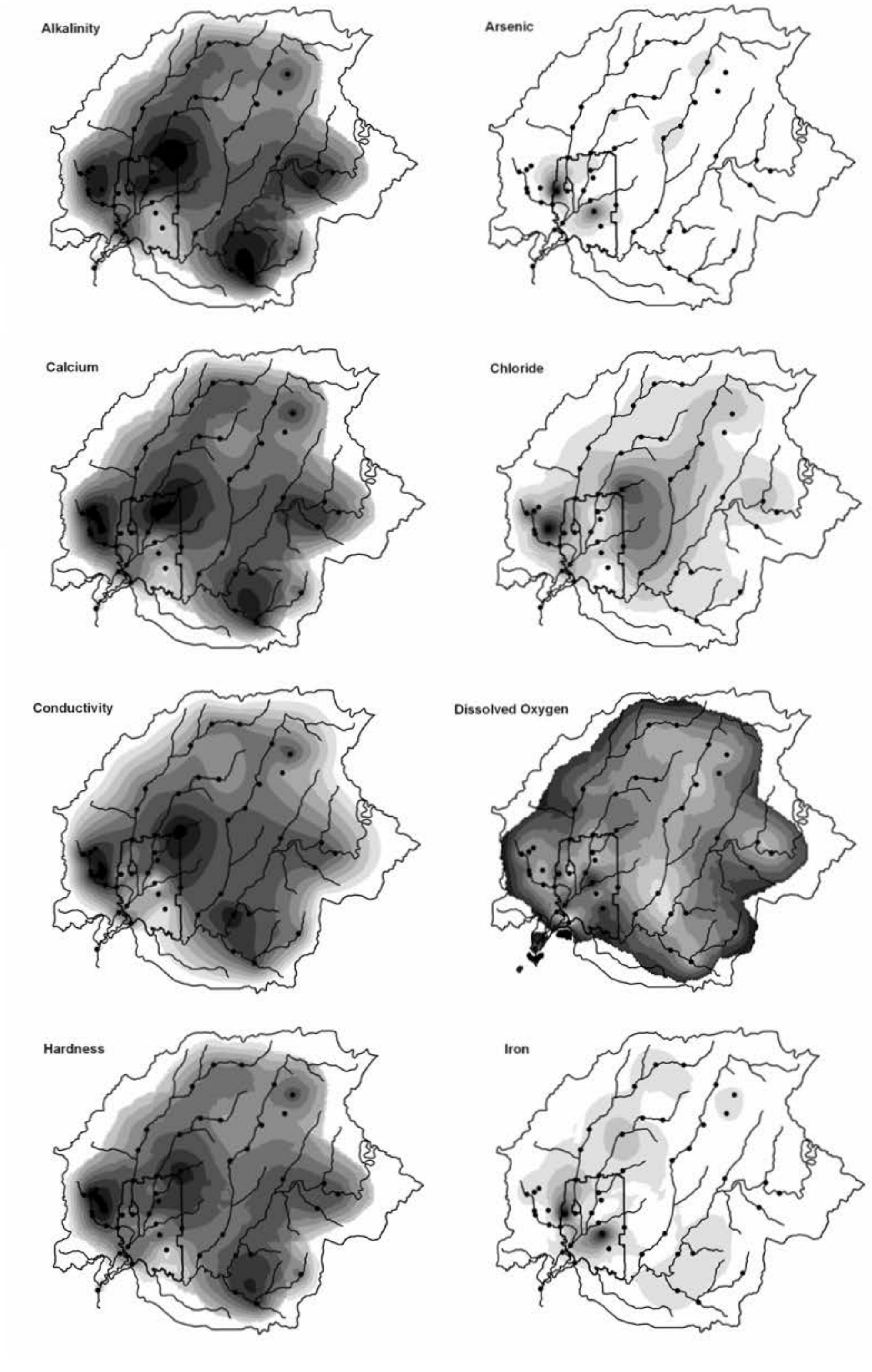
Fish assemblage structure was explained by two factors, which explained about 65% of the variance (Table 23). The primary factor explained 33.4% of the variance based on associations between chloride and total solids. The second factor explained 31.7% of the variance based on a negative association with grass-pasture land use.

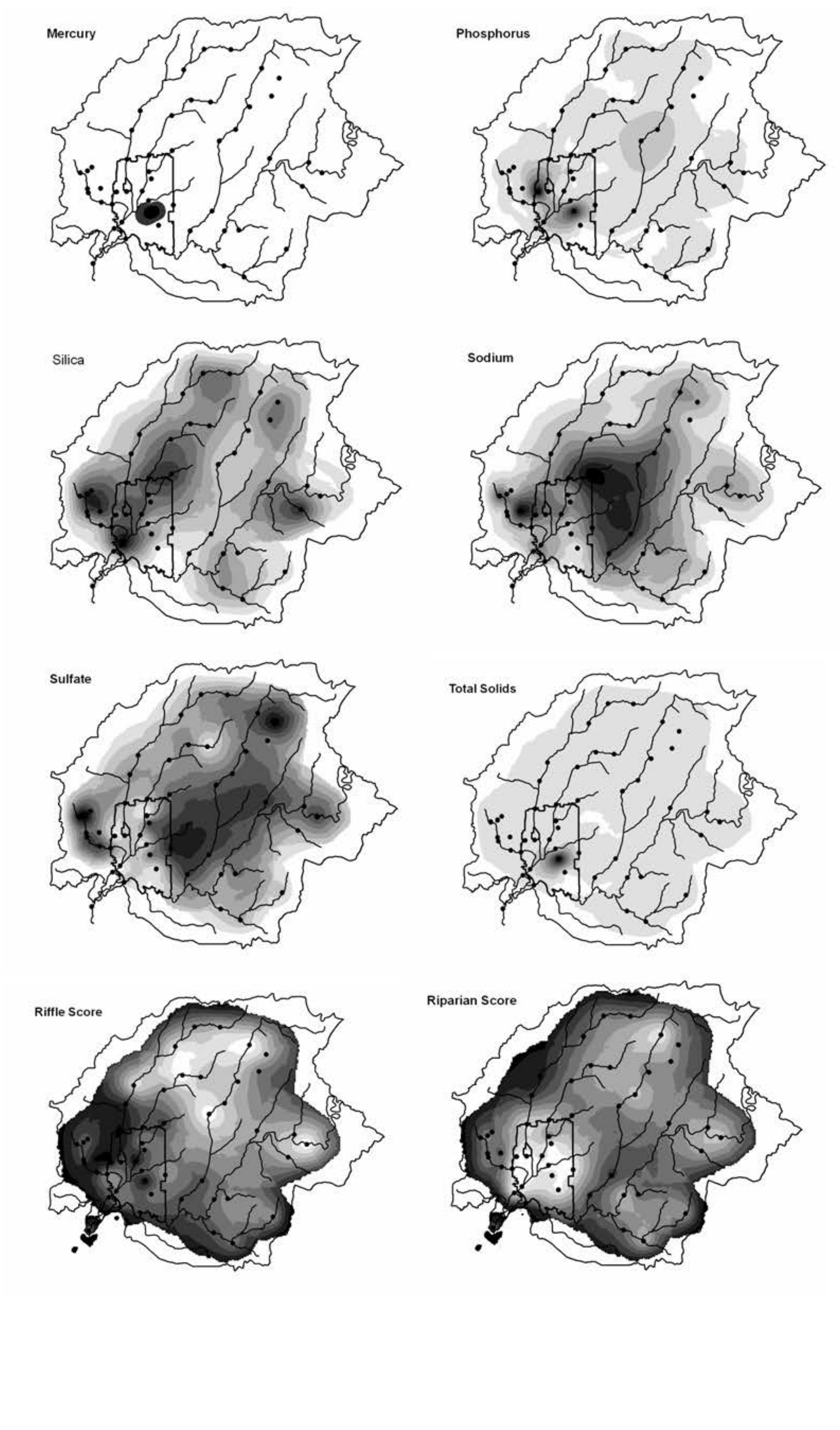
Macroinvertebrate assemblage structure was explained by two factors, which explained a total of 74% of the variance (Table 24). The primary factor explained 37.3% of the variance based on an association between agriculture and a negative relationship with forest land use. The second factor explained 37.1% of the variance based on sulfate and commercial land use.

Crayfish assemblage structure explained observed variance on three factors, which explained about 74% of the variance in the fish assemblage information (Table 25). Chloride and sodium explained 27.7% of the variance on the primary factor. Iron and total phosphorus explained another 24.2% of the variance on factor 2. Habitat factors such as channel score and riffle-run score was negatively associated and explained another 22.1% of the variation on factor 3. All of the relationships for factors 1 and 2 were positively correlated (Table 25).

Associations between Contaminants and Hot Spot Analysis

Contaminant distribution, based on percentage-of-range concentrations, showed variable responses between organism groups. The majority of the significant contaminant responses were diagnosed from a four locations including several surrounding the refuge on Sandy Branch, Richart Lake tributary, Vernon Fork Muscatatuck River, and a location to the east of Moss Lake (Figure 21). The contaminants that showed a significant relationship included mercury, arsenic, calcium, total phosphorus, sodium, chloride, silica, sodium, and sulfate. These contaminants were linked to the industrial area in the headwaters of Sandy Branch, an animal processing facility on Richart Lake tributary, and a legacy train derailment on Storm Ditch. Locations on the refuge that showed disturbance gradients emanating from them include Moss Lake for both dissolved oxygen and mercury, and two locations that have hot spots of arsenic, total phosphorus, and iron (Figure 21).


Alkalinity
Arsenic
Calcium
Chloride
Conductivity
Dissolved Oxygen
Hardness
Iron


Mercury
Phosphorus
Silica
Sodium
Sulfate
Total Solids
Riffle Score
Riparian Score

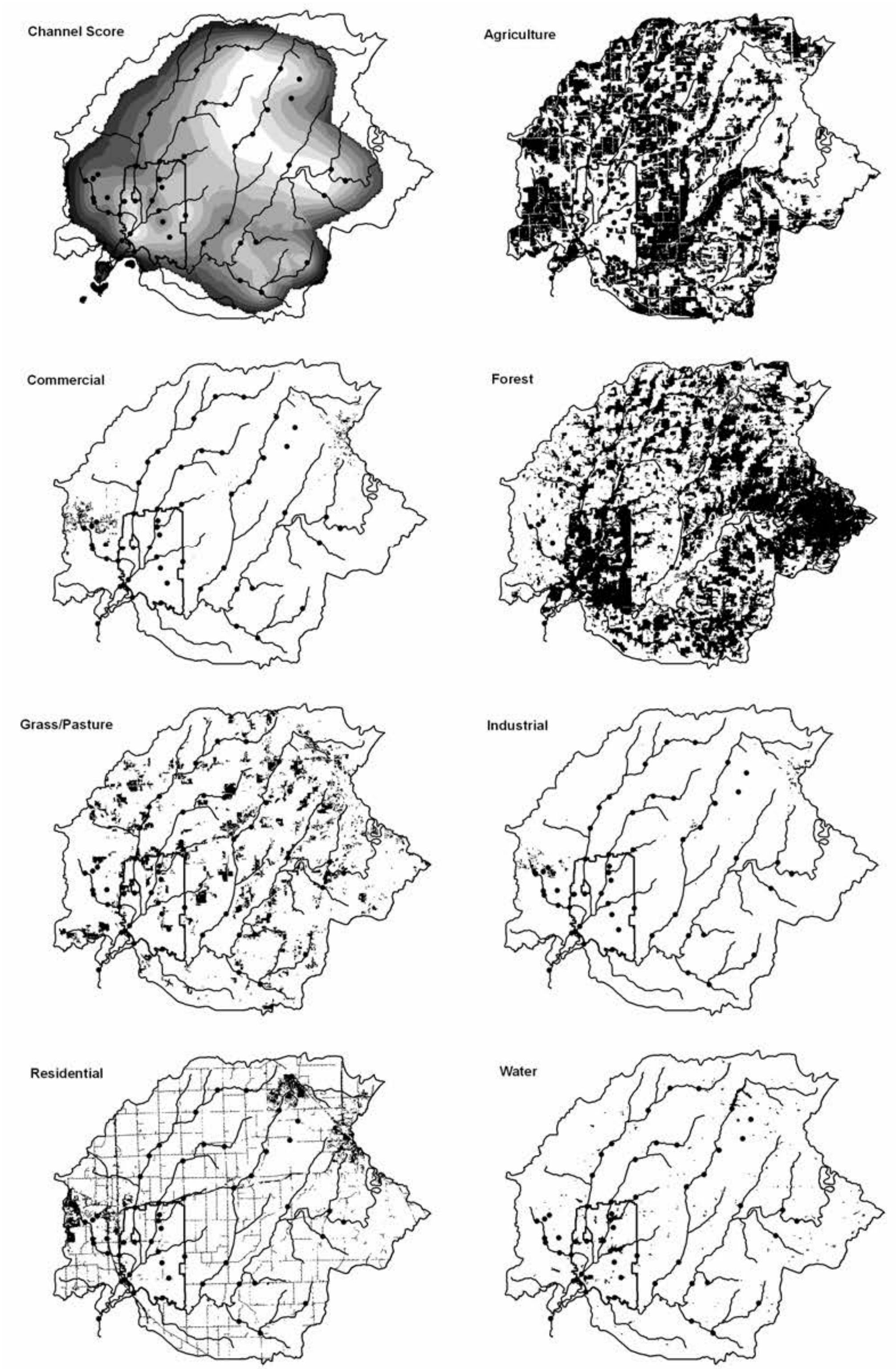


Figure 21. Relative concentration pleths of significant water quality, habitat, and land use chemistry variables that were predictive of fish, macroinvertebrate and crayfish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area. All concentrations are presented on a relative (percentage of range) scale (see Appendix for contaminant concentration, habitat, and land use range).

Fish and crayfish assemblage structure was predicted by associations with chlorides and total solids emanating from Sandy Branch and from Richart Lake tributary. The response signature from these two locations may represent either failed septic systems or problems form wastewater effluent. 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 use types (Figure 21). High density of forested land use is found on the refuge compared to the high density of agricultural land use outside of the refuge.

Macroinvertebrate assemblage structure was determined by sodium. This contaminant was generally found outside of the refuge on Sandy Branch and also the area upstream of the eastern boundary near Mutton Creek tributary (Figure 21).

Crayfish assemblage structure showed the strongest response signature to chloride and sodium, which was associated with Sandy Branch and the Richart Lake tributary (Figure 21). Crayfish also showed significant relationships with iron and total phosphorus, which emanated from the refuge in the area near Mutton Creek and Lake Linda. Habitat was also an important factor with channel degradation and riffle-run degradation being significant.

Appendix 5. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use at the Muscatatuck National Wildlife Refuge.

Variable	N	Mean	Minimum	Maximum	SD
Contaminant					
Alkalinity (as CaCO3)	49	167.00	27.00	262.00	49.70
Aluminum	49	457.24	10.00	7360.00	1200.81
Arsenic	49	2.54	0.80	26.50	4.21
Barium	49	85.91	5.32	805.00	108.46
Calcium (as CaCO3)	49	144.73	22.00	232.00	41.41
Chloride	49	21.91	0.50	80.00	14.84
COD	49	21.07	6.50	103.00	18.13
Copper	49	2.89	0.51	45.20	6.33
Fluoride	49	0.10	0.05	0.30	0.06
Hardness (as CaCO3)	49	214.33	38.00	373.00	61.44
Iron	49	878.14	102.00	8920.00	1679.42
Lead	49	2.71	1.00	66.90	9.48
Manganese	49	695.45	27.40	5840.00	1112.04
Mercury	49	0.05	0.05	0.24	0.03
Nickel	49	3.63	0.25	60.60	8.61
Nitrogen, Ammonia	49	0.18	0.05	1.60	0.31
Nitrogen, Nitrate+Nitrite	49	0.33	0.01	2.80	0.51
Phosphorus, Total	49	0.11	0.02	0.96	0.17
Silica (Reactive)	49	7.72	1.10	18.00	4.12
Sodium	49	14.74	3.01	36.10	7.78
Sulfate	49	29.05	1.30	62.00	16.13
TOC	49	7.11	1.90	63.40	8.65
TS	49	414.24	69.00	6140.00	839.01
TSS	49	138.71	0.50	5860.00	835.47
Zinc	49	10.75	3.00	224.00	31.81
General Chemistry					
Dissolved Oxygen	48	5.65	1.06	11.08	2.20
pH	48	8.54	7.49	10.38	0.58
Water Temperature	48	22.61	17.62	30.00	3.25
Specific Conductivity	48	403.23	68.50	639.50	113.00
Turbidity	18	34.24	4.00	84.70	21.30
Habitat					
QHEI Score	49	54.77	34.00	81.00	12.38
QHEI-Substrate Score	49	11.60	1.00	20.00	4.11
QHEI-Instream Cover Score	49	8.82	2.00	18.00	3.40
QHEI-Channel Score	49	13.32	6.00	20.00	4.04
QHEI-Riparian Score	49	6.40	1.00	10.00	2.59
QHEI-Pool Glide Score	49	6.26	2.00	12.00	2.16
QHEI-Riffle Run Score	49	3.27	0.00	7.00	2.00
QHEI-Gradient Score	49	5.10	4.00	10.00	1.96
Land-use					
Water	49	1.17	0.00	6.17	1.30
Commercial	49	2.26	0.00	20.62	4.76
Agriculture	49	29.52	6.55	72.60	14.73
HD-Residential	49	1.62	0.00	23.30	4.29
LD-Residential	49	4.37	0.00	21.46	4.75

Residential	49	5.99	0.00	44.76	8.50
Grass Pasture	49	31.08	0.00	61.35	16.97
Forest	49	29.44	0.80	73.96	16.53
Industrial	49	0.52	0.00	8.83	1.62
Other	49	0.02	0.00	0.20	0.04

REFERENCES

Eagleson K.W., D.L. Lenat, I.W. Ausley, and E.B. Winterborne. 1990. Comparison Of measured instream biological responses with responses predicted using the *Ceriodaphnia dubia* chronic toxicity test. *Environmental Toxicology Chemistry* 9:1091–1028

Morris, C.C., T.P. Simon, and S.A. Newhouse. 2005 A local-scale in situ approach for stressor identification of biologically impaired aquatic systems. *Archives Environmental Contamination Toxicology* 49: 1–11.

Norton S.B., S.M. Cormier, M. Smith M, and C. Jones. 2000. Can biological assessments discriminate among types of stress? A case study from the Eastern Corn Belt Plains Ecoregion. *Environmental Toxicology Chemistry* 19:1113–1119.

Norton S.B., S.M. Cormier, G.W. Suter II, B. Subramanian, E. Lin, D. Altfater. 2002. Determining probable causes of ecological impairment in the Little Scioto River, Ohio, USA: Part I. Listing candidate causes and analyzing evidence. *Environmental Toxicology Chemistry* 21:1112–1124.

Rive-Murray K., R.W. Bode RW, P.J. Phillips, and G.L. Wall. 2002. Impact source determinations with biomonitoring data in New York state: Concondance with environmental data. *Northeast Naturalist* 9: 127–162

Siegel, S. and N.J. Castellan. 1988. *Nonparametric statistics for the behavioral sciences, 2 nd ed*. McGraw-Hill, New York, NY.

Simon, T.P. 2003. *Biological response signatures: Indicator patterns using aquatic communities* CRC, Boca Raton, FL.

Simon T.P. and R.L. Dufour. 1998. Development of index of biotic integrity expectations for the ecoregions of Indiana. V. Eastern Corn Belt Plain. EPA 905/R-96/002. United States Environmental Protection Agency, Region V. Water Division. Watershed and Non-Point Source Branch, Chicago, IL.

Simon, T.P. and C.C. Morris. in press. Biological response signature of oil brine threats, sediment contaminants, and crayfish assemblages in an Indiana Watershed, USA. *Archives Environmental Contamination Toxicology*. DOI 10.1007/s00244-008-9155-0.

StatSoft. 2002. STATISTICA for Windows. StatSoft, Tulsa, OK

Suter G.W., II, S.B. Norton, and S.M. Cormier. 2002. A methodology for inferring the causes of observed impairments in aquatic systems. *Environmental Toxicology Chemistry* 21: 1101–1111.

United States Environmental Protection Agency (2000*) Stressor identification guidance document*. EPA/822/B-00/025. Washington, DC.

Yoder C.O. and J.E. DeShon. 2003. Using biological response signatures within a framework of multiple indicators to assess and diagnosis causes and sources of impairment to aquatic assemblages in selected Ohio rivers and streams. In: Simon TP (ed) *Biological response signatures: Indicator patterns using aquatic communities*. CRC, Boca Raton, FL, pp 23–81.

Yoder C.O. and E.T. Rankin. 1995. Biological response signatures and the area of degradation value: New tools for interpreting multimetric data. In: Davis WS, Simon TP (eds) *Biological assessment and criteria: Tools for water resource planning and decision making*. CRC, Boca Raton, FL, pp 263–286

Yoder C.O. and E.T. Rankin. 1998. The role of biological indicators in a state water quality management process. *Environmental Monitoring Assessment* 51:61–88.

Identification of Stressors Affecting Watershed Level Scale Indicators at Areas Surrounding Indiana National Wildlife Refuges

The issue of managing landscapes at local compared to regional scales has significant implications for the refuge manager. In order to protect biological diversity and promote biological integrity and ecological health, the manager must sometimes make choices to facilitate integrity at the expense of ecological health. Biological integrity lies along a continuum or gradient from a biological system extensively altered by significant human impacts to a landscape scale that may include natural systems. No landscape retains absolute biological integrity, diversity, and environmental health; however, the refuge manager attempts to prevent the further loss of natural biological features and processes.

The refuge manager sometimes must choose between maintaining or restoring biological integrity, which is not the same as maximizing biological diversity, and maintaining a single species. The refuge manager may need to manage for a single species or community at some refuges and combinations of species or communities at another refuge within the same landscape scale. For example, a refuge may contain critical habitats for an endangered species and maintaining that habitat (and, therefore, that species) could reduce biological diversity at the refuge scale. However, at the landscape scale the refuge is maintaining regional biological integrity and diversity at an ecosystem or national landscape scale. This balancing of activities is something that the refuge manager must do in concert with other refuges nationally. In deciding which management activities to accomplish refuge purpose(s), the manager may need to consider how the ecosystem functioned under historic conditions so that biological integrity can be maintained. For example, natural frequency and timing of processes such as flooding, fires, and grazing are important natural disturbance features. Where it is not appropriate to restore ecosystem function, refuge management will attempt to duplicate these natural processes including natural frequencies and timing to the extent this can be accomplished.

It may be necessary to modify the frequency and timing of natural processes at the refuge scale to fulfill refuge purpose(s) or to contribute to biological integrity at larger landscape scales. For example, under historic conditions, an area may have flooded only a few times per decade. Migratory birds dependent upon wetlands may have used the area in some years, and used other areas that flooded in other years. However, many wetlands have been converted to agriculture or other land uses, the remaining wetlands must produce more habitat, more consistently, to support wetland-dependent migratory birds. Therefore, to conserve these migratory bird populations at larger landscape scales, we may flood areas more frequently and for longer periods of time than they were flooded historically. The refuge manager must balance the needs of some species, which may conflict with the management of other species in order to maintain ecosystem health.

The current study is an evaluation of contaminant, land use, and habitat factors at a larger scale than usually considered by individual refuge managers. The information that is presented in this chapter will provide the tools and information needed by the refuge manager to make decisions concerning management implications for biological integrity, biological diversity, and ecological health issues outside of the refuge boundaries. The application of this information will enable important planning for linking landscape corridors and promoting species migration routes. All of the procedures including data analysis, data collection, and reporting follow the same procedures as found in Morris et al. (2005) and Simon and Morris (in press).

The information presented in this chapter is based on data collected by the State of Indiana Department of Environmental Management as an added benefit to this study (IDEM unpublished data). The State of Indiana collected information outside of the refuge boundaries at the watershed scale so that the stressor identification model could be evaluated for the entire Patoka River and Vernon Fork Muscatatuck River watersheds. The information used for this aspect of the data assessment was collected either simultaneously in 2006 or 2007 so that similar hydrologic conditions would have been present for comparison and combination with the U.S. Fish and Wildlife Service information. The State of Indiana monitoring strategy uses a probability based design strategy within a rotating basin framework so that the entire waters of the state are monitored every five years (IDEM 1996). The current effort was part of the stressor identification program, which is part of the second year studies of the Department to identify areas not meeting designated uses.

This study stressor model is based on only the fish assemblage indicator. The fish assemblage indicator is the only one that currently has an index of biological integrity (IBI) available to assist in scaling response directionality. Herricks and Schafer (1985) indicate that fish are important for a variety of reasons including they are long-lived, well studied, have important identity to anglers and the public, and have a gradient of response to environmental degradation. Fish are also much easier to identify in the field and as a result data information can be collected in the field and much more quickly analyzed than can macroinvertebrates. Fish can also be identified to low taxonomic resolution (i.e., species) and abundant life history and ecological information exists for North American species (Simon and Lyons 1995).

RESULTS AND DISCUSSION

Identification of Stressors Affecting Watershed Level Scale Indicators in the Patoka River

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Study Design

Stressor identification on the watershed scale was accomplished using a random design to select 88 sites in the Patoka River National Wildlife Refuge. These sites represented locations throughout the Patoka River watershed. Sites were visited and those sites that were dry were excluded from the 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 (Figure 1).

Analysis of assemblage structure based on Cluster Analysis showed that six similarity clusters represented the biological structure (Figure 2). Assemblage structure was explained by increasing index of biotic integrity scores for fish (Figure 3). Fish assemblage structure was explained with six clusters representing tier 3 and tier 4 linkage structure (Figure 2). The linkage structure was modified so that clusters 4 through 6 could be interpreted. The basis for these groupings is that the biological community structure results are externally driven and can be identified to evaluate physical and chemical stress on the respective assemblage (Morris et al. 2006).

Habitat Quality

Habitat analysis using the QHEI showed that scores ranged from 21.5 to 77 (average 45.29 $\pm$ 12.2). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life. Sites scoring less than 34 are considered not meeting designated uses for aquatic life. The substrate score, instream cover, riparian score, riffle-run score, and pool-glide score varied with the amount of channelization in the study area and were the primary reasons for sites not meeting aquatic life use.

Chemical Quality

Summary statistics by assemblage for water chemistry results are found in Table 1. Project specific summary statistics and range of scale values for water quality results are presented in Appendix 1. In general, the lowest biological integrity and species richness clusters (cluster 1) had the highest concentrations of contaminants (Table 1). Several contaminants show distributions with higher concentrations in clusters 2 or 4 (Figure 4). Chemical Oxygen Demand (COD) and Total Organic Carbon (TOC) had higher concentrations in cluster 2 than in cluster 1. Calcium, hardness, manganese, nickel, total solids, and zinc showed the highest concentration in cluster 4 (Figure 4).

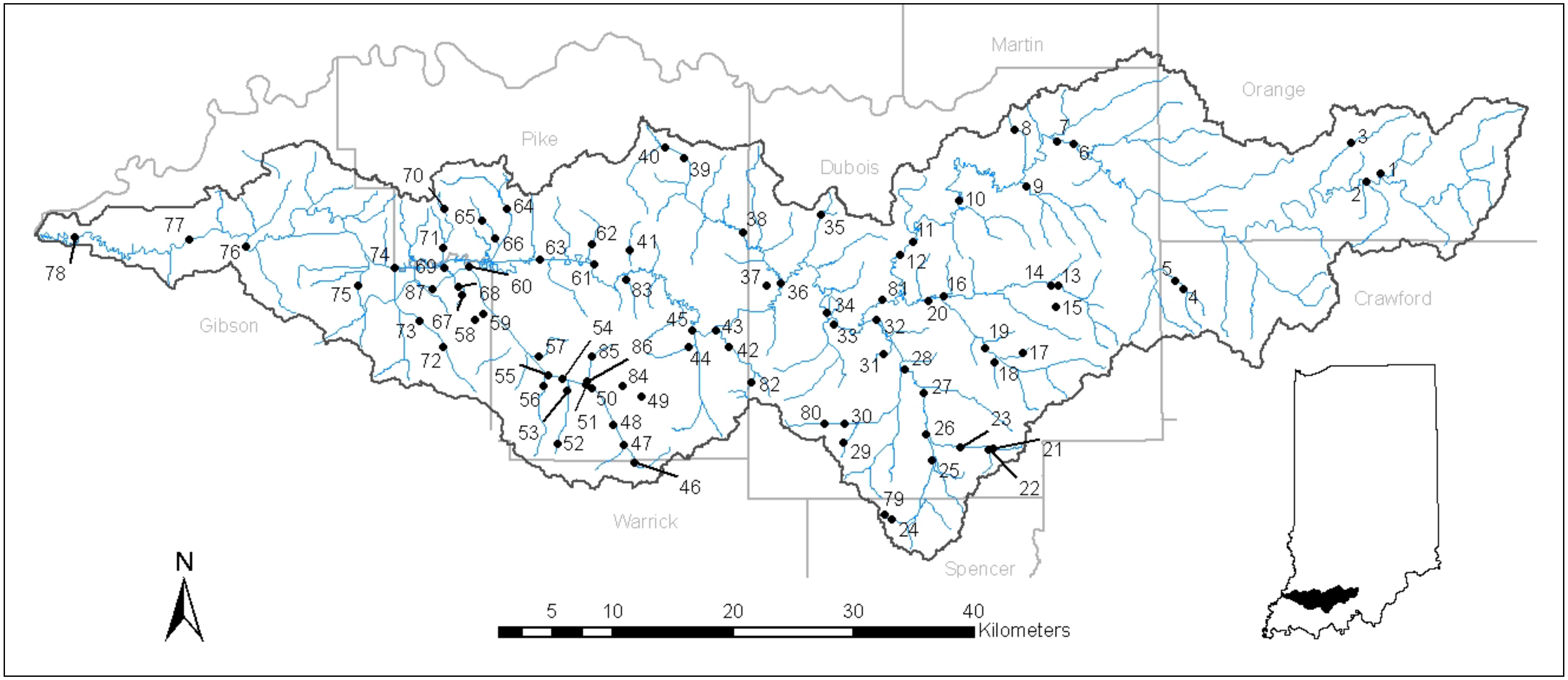


Figure 1. Distribution of sites sampled during 2006-2007 in the Patoka River drainage. Numbers refer to site location in Appendix A.

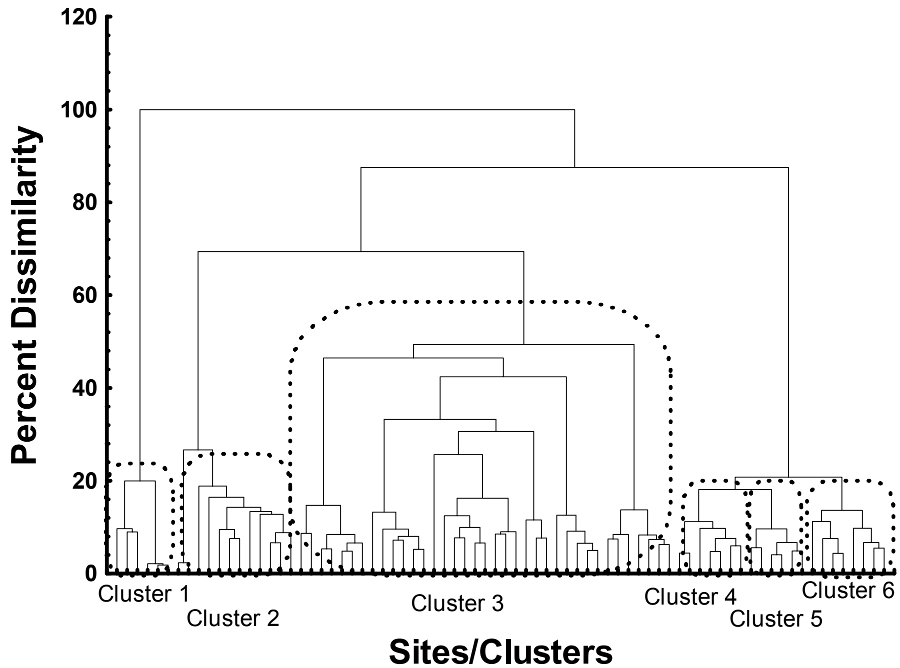


Figure 2. Dendograms showing the relative similarity of assemblage data collected in the Patoka River watershed during 2006-2007. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA for the fish assemblage.

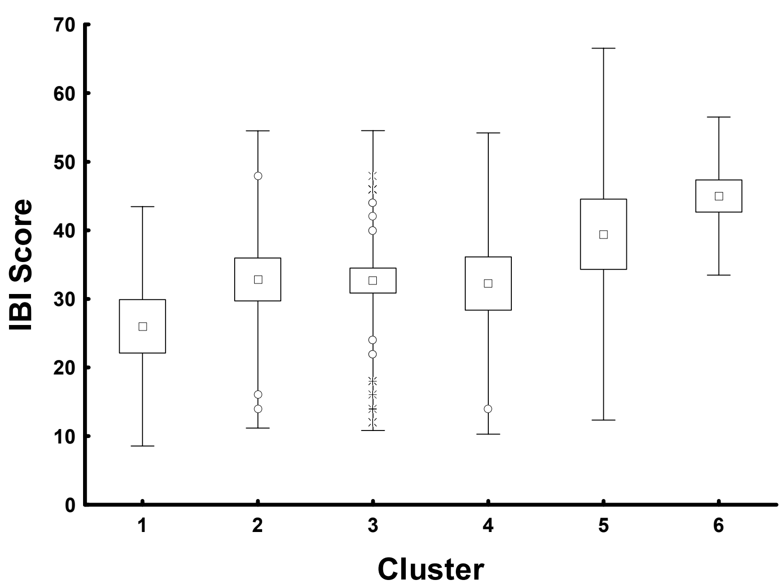


Figure 3. Box-and-whisker plots showing biological assemblage responses for index of biotic integrity (IBI) for fish assemblages had to the biologic response gradient developed from the Patoka River watershed during August 2006-2007.

Table 1. Summary statistics for 42 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Fig. 1A) sampled in August 2006-2007 in the Patoka River watershed.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Dissolved Oxygen	5	5.41	2.75	6.83	1.80	12	6.31	0.53	13.9	3.4
pH	5	7.43	6.80	8.01	0.48	12	8.17	6.27	9.4	0.9
Water Temperature	5	23.48	20.66	25.00	1.69	12	27.04	19.10	35.3	4.5
Specific Conductivity	5	1423.70	431.40	2430.00	960.64	12	1386.54	272.00	4658.0	1444.3
Turbidity	4	48.73	35.90	69.90	15.16	7	315.00	22.20	1000.0	361.9
Alkalinity (as CaCO3)	5	277.40	92.00	680.00	231.36	12	263.67	77.00	958.0	293.5
Aluminum	5	342.90	61.70	840.00	323.01	12	2492.30	87.60	11200.0	3614.2
Arsenic	5	2.50	2.50	2.50	0.00	12	3.39	2.50	6.3	1.6
Cadmium	5	0.50	0.50	0.50	0.00	12	0.50	0.50	0.5	0.0
Calcium (as CaCO3)	5	474.40	100.00	1010.00	382.22	12	328.67	90.00	1060.0	345.5
Chloride	5	18.70	5.10	62.00	24.29	12	23.52	2.50	55.0	18.0
Chemical Oxygen Demand	5	15.42	7.30	23.40	7.36	12	29.57	2.50	85.7	21.2
Copper	5	5.63	1.00	11.90	3.97	12	4.59	1.00	10.0	2.6
Hardness (as CaCO3)	5	1013.80	127.00	1960.00	811.10	12	517.58	121.00	2370.0	709.4
Lead	5	1.44	1.00	3.19	0.98	12	2.90	1.00	15.2	4.3
Manganese	5	1132.02	83.10	2680.00	1029.90	12	683.73	81.80	2500.0	825.9
Nickel	5	7.10	3.19	12.70	3.72	12	6.36	2.25	16.2	4.6
Nitrogen, Ammonia	5	0.10	0.05	0.30	0.11	12	0.17	0.05	1.1	0.3
Nitrogen, Nitrate+Nitrite	5	0.20	0.05	0.60	0.23	12	2.25	0.05	14.1	4.1
Phosphorus, Total	5	0.47	0.01	2.22	0.98	12	0.29	0.01	1.4	0.4
Selenium	5	2.00	2.00	2.00	0.00	12	2.46	2.00	7.5	1.6
Sulfate	5	805.80	48.00	1850.00	767.64	12	614.80	18.00	2080.0	816.9
Total Organic Carbon	5	4.66	2.40	7.10	2.03	12	8.53	2.00	18.8	4.9
Total Solids	5	1556.00	332.00	2940.00	1216.89	12	1303.00	199.00	4080.0	1403.3
Total Suspended Solids	5	12.00	4.00	25.00	9.14	12	80.08	2.00	374.0	115.1
Zinc	5	16.78	3.00	59.30	23.85	12	17.88	6.06	48.4	14.5
QHEI Score	5	43.85	21.50	67.00	18.71	12	36.96	25.00	45.0	6.4
QHEI-Substrate Score	5	7.00	0.00	14.00	6.20	12	7.33	0.00	15.0	5.1
QHEI-Instream Cover Score	5	6.40	3.00	14.00	4.56	12	6.58	4.00	9.0	1.6

QHEI-Channel Score	5	12.60	5.00	19.00	5.59	12	8.71	5.00	11.0	1.7
QHEI-Riparian Score	5	6.45	3.50	10.00	2.59	12	4.71	2.00	10.0	2.6
QHEI-Pool Glide Score	5	5.00	3.00	7.00	1.87	12	4.67	3.00	9.0	1.8
QHEI-Riffle Run Score	5	1.60	0.00	4.00	1.82	12	0.63	0.00	2.0	0.9
QHEI-Gradient Score	5	4.80	4.00	8.00	1.79	12	4.33	4.00	8.0	1.2
QHEI-Drainage Area	5	1.49	0.12	2.89	1.13	12	82.74	0.10	831.9	237.2
Water	5	26.54	0.00	76.50	33.16	12	3590.35	0.00	42234.8	12170.2
Commercial	5	9.06	0.00	41.90	18.42	12	594.40	0.00	6599.2	1892.7
Agriculture	5	264.98	0.00	473.40	240.75	12	59579.39	28.60	664349.6	190600.9
Residential	5	110.04	4.10	316.10	126.89	12	11128.73	3.40	124491.2	35725.6
Grass/Pasture	5	148.12	61.90	325.70	109.88	12	26696.20	0.00	302025.2	86768.4
Forest	5	392.44	3.90	1067.00	414.55	12	81849.18	0.00	958444.4	276076.7
Industrial	5	3.04	0.00	11.80	5.11	12	796.01	0.00	8948.8	2568.3

Variable	Cluster 3 N	Mean	Minimum	Maximum	SD	Cluster 4 N	Mean	Minimum	Maximum	SD
Dissolved Oxygen	36	5.6	1.49	9.9	1.9	8	5.30	1.43	7.49	2.04
pH	36	7.6	6.76	9.1	0.6	8	7.35	6.59	7.88	0.36
Water Temperature	36	26.3	21.76	36.8	3.4	8	26.25	23.82	28.82	1.62
Specific Conductivity	36	1094.8	222.00	5430.0	1151.8	8	1786.13	285.00	2740.00	999.48
Turbidity	34	44.1	1.00	125.1	35.7	8	46.74	19.20	95.90	30.89
Alkalinity (as CaCO3)	36	153.3	25.00	775.0	157.9	8	141.25	64.00	198.00	47.32
Aluminum	36	1141.4	25.00	7800.0	1759.6	8	613.13	153.00	2270.00	679.38
Arsenic	36	2.6	2.50	5.1	0.4	8	2.50	2.50	2.50	0.00
Cadmium	36	0.5	0.50	1.9	0.2	8	0.66	0.50	1.80	0.46
Calcium (as CaCO3)	36	510.9	72.00	2330.0	444.9	8	1717.63	246.00	3970.00	1340.78
Chloride	36	12.8	2.50	41.8	9.5	8	10.22	2.50	19.00	5.02
Chemical Oxygen Demand	36	15.0	2.50	42.0	8.9	8	10.63	2.50	15.60	4.41
Copper	36	2.6	1.00	7.6	1.9	8	2.33	1.00	6.76	2.47
Hardness (as CaCO3)	36	614.2	97.20	2820.0	736.6	8	1251.50	108.00	2490.00	900.17
Lead	36	1.2	1.00	3.3	0.6	8	1.00	1.00	1.00	0.00
Manganese	36	461.2	56.40	2010.0	483.5	8	1240.73	93.80	2670.00	971.60
Nickel	36	5.4	0.75	30.6	5.6	8	21.84	2.70	73.70	29.90
Nitrogen, Ammonia	36	0.1	0.05	0.6	0.1	8	0.11	0.05	0.27	0.09
Nitrogen, Nitrate+Nitrite	36	1.8	0.05	7.9	2.1	8	2.29	0.05	11.20	4.01
Phosphorus, Total	36	0.1	0.01	0.4	0.1	8	0.20	0.01	0.70	0.23
Selenium	36	2.3	2.00	9.1	1.2	8	2.00	2.00	2.00	0.00
Sulfate	36	652.5	13.90	3890.0	912.2	8	1173.80	40.80	3170.00	1091.90
Total Organic Carbon	36	4.6	1.56	13.0	2.3	8	4.12	2.00	5.50	1.19
Total Solids	36	1095.3	181.00	4140.0	1232.5	8	1885.25	186.00	3440.00	1266.96
Total Suspended Solids	36	30.0	2.00	147.0	39.3	8	16.50	4.00	26.00	7.63
Zinc	36	8.4	3.00	58.6	10.0	8	46.26	3.00	199.00	74.60
QHEI Score	36	46.3	28.00	75.0	12.2	8	42.28	32.00	51.00	6.46
QHEI-Substrate Score	36	8.8	0.00	18.0	5.3	8	5.88	0.00	12.00	4.32
QHEI-Instream Cover Score	36	8.5	3.00	16.0	3.6	8	6.75	2.00	13.00	3.28
QHEI-Channel Score	36	10.6	5.00	18.0	3.3	8	11.31	7.50	14.00	2.66
QHEI-Riparian Score	36	5.4	1.00	10.0	2.7	8	5.34	2.00	10.00	2.52

QHEI-Pool Glide Score	36	6.2	0.00	11.0	2.8	8	6.75	5.00	10.00	1.83
QHEI-Riffle Run Score	36	1.6	0.00	6.0	1.7	8	0.50	0.00	2.00	0.76
QHEI-Gradient Score	36	5.3	4.00	10.0	2.0	8	5.75	4.00	10.00	1.98
QHEI-Drainage Area	36	108.1	0.23	852.9	238.3	8	25.40	3.92	41.99	15.58
Water	36	4988.1	0.00	42446.8	11703.2	8	1135.95	185.00	2753.20	840.37
Commercial	36	878.8	0.00	6666.8	1975.2	8	122.43	0.00	260.50	97.31
Agriculture	36	73315.8	7.60	696209.6	176112.7	8	24450.40	135.30	71181.50	22668.66
Residential	36	15752.9	0.20	126723.6	35758.2	8	3284.05	85.60	7543.00	2625.63
Grass/Pasture	36	44319.8	31.80	302574.4	97568.6	8	7080.24	198.50	33874.50	11015.28
Forest	36	123774.9	27.00	965515.6	280898.4	8	26458.73	1867.50	70493.60	21653.74
Industrial	36	876.6	0.00	8985.6	2241.0	8	132.41	0.00	259.00	115.91

Variable	Cluster 5					Cluster 6				
	N	Mean	Minimum	Maximum	SD	N	Mean	Minimum	Maximum	SD
Dissolved Oxygen	7	6.0	3.82	8.0	1.4	6	6.32	4.550	8.65	1.65
pH	7	7.2	6.85	7.4	0.2	6	7.50	7.070	8.38	0.46
Water Temperature	7	25.2	22.22	31.3	3.1	6	27.29	22.540	31.59	3.78
Specific Conductivity	7	272.9	167.00	462.0	132.3	6	567.33	285.000	1809.00	608.68
Turbidity	7	42.7	11.60	101.0	30.3	5	33.42	12.000	57.30	17.87
Alkalinity (as CaCO3)	7	93.0	57.00	178.0	51.8	6	145.33	62.000	478.00	163.47
Aluminum	7	523.0	77.00	1250.0	442.8	6	385.33	25.000	962.00	321.32
Arsenic	7	2.5	2.50	2.5	0.0	6	2.50	2.500	2.50	0.00
Cadmium	7	0.5	0.50	0.5	0.0	6	0.50	0.500	0.50	0.00
Calcium (as CaCO3)	7	377.1	246.00	713.0	197.8	6	347.00	295.000	448.00	53.62
Chloride	7	3.6	2.50	10.2	2.9	6	12.29	7.640	17.80	3.84
Chemical Oxygen Demand	7	7.7	2.50	10.9	2.8	6	7.27	2.500	11.40	4.02
Copper	7	1.0	1.00	1.0	0.0	6	1.63	1.000	4.76	1.54
Hardness (as CaCO3)	7	118.0	78.50	206.0	52.0	6	301.33	110.000	1200.00	440.32
Lead	7	1.0	1.00	1.0	0.0	6	1.00	1.000	1.00	0.00
Manganese	7	59.1	42.10	99.0	20.1	6	144.57	89.000	379.00	115.04
Nickel	7	1.0	0.75	2.4	0.6	6	2.36	0.750	4.09	1.41
Nitrogen, Ammonia	7	0.1	0.05	0.1	0.0	6	0.07	0.050	0.15	0.04
Nitrogen, Nitrate+Nitrite	7	0.9	0.29	2.7	0.9	6	6.03	0.200	19.60	7.09
Phosphorus, Total	7	0.0	0.01	0.1	0.1	6	0.06	0.005	0.09	0.04
Selenium	7	2.0	2.00	2.0	0.0	6	2.00	2.000	2.00	0.00
Sulfate	7	22.5	18.40	37.2	6.6	6	165.05	17.700	790.00	306.38
Total Organic Carbon	7	2.8	2.10	3.3	0.5	6	2.95	1.500	4.50	1.23
Total Solids	7	175.4	131.00	242.0	44.4	6	477.17	204.000	1700.00	599.94
Total Suspended Solids	7	29.3	2.00	60.0	21.2	6	14.17	2.000	39.00	13.75
Zinc	7	4.3	3.00	12.1	3.4	6	7.07	3.000	15.30	5.22
QHEI Score	7	49.6	36.00	77.0	15.1	6	51.92	40.000	65.00	10.36
QHEI-Substrate Score	7	6.6	0.00	15.0	5.9	6	11.00	8.000	14.00	2.37
QHEI-Instream Cover Score	7	8.7	4.00	12.0	3.1	6	9.17	4.000	15.00	4.17
QHEI-Channel Score	7	11.4	8.00	15.0	2.1	6	11.08	7.000	15.00	3.17

QHEI-Riparian Score	7	5.9	3.00	9.0	2.5	6	5.83	1.000	10.00	2.99
QHEI-Pool Glide Score	7	9.6	7.00	12.0	2.0	6	6.50	4.000	8.00	1.76
QHEI-Riffle Run Score	7	1.7	0.00	6.0	2.4	6	2.00	0.000	4.00	2.19
QHEI-Gradient Score	7	5.7	2.00	10.0	2.9	6	6.33	4.000	8.00	1.51
QHEI-Drainage Area	7	144.0	11.45	273.1	110.0	6	17.70	1.420	62.20	22.58
Water	7	9736.5	44.00	17824.4	9035.0	6	181.68	0.000	821.00	317.76
Commercial	7	231.8	0.00	789.2	284.8	6	72.75	0.000	322.00	125.40
Agriculture	7	29122.5	3428.00	70919.6	25836.5	6	13456.95	1810.900	55804.50	20872.24
Residential	7	14948.6	2024.00	29723.6	11606.0	6	3210.27	363.900	12400.00	4617.93
Grass/Pasture	7	68096.2	10294.50	130543.2	50913.2	6	14732.38	4.500	55115.50	20724.42
Forest	7	239315.8	11140.50	421020.0	163885.9	6	20915.62	304.500	77880.00	29280.12
Industrial	7	482.4	0.00	1180.4	479.1	6	26.52	0.000	149.00	60.06

Table 2. List of 18 physical, chemical, habitat, and land use variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in the Patoka River watershed during August 2006-2007.

Variable	P Value
Chemical	
Alkalinity	p = 0.0158
Arsenic	p = 0.0480
Chloride	p = 0.0101
Chemical Oxygen Demand	p = 0.0006
Copper	p = 0.0015
Hardness	p = 0.0074
Manganese	p = 0.0002
Nickel	p = 0.0001
Sulfate	p = 0.0010
Total Organic Carbon	p = 0.0013
Total Solids	p = 0.0008
Zinc	p = 0.0229
Habitat	
QHEI-Pool Glide Score	p = 0.0047
QHEI-Drainage Area	p = 0.0041
Land-use	
Agriculture	p = 0.0062
Residential	p = 0.0068
Forest	p = 0.0219
Industrial	p = 0.0334

Multivariate Results

For this analysis we chose 50% similarity as a benchmark for defining assemblage structure clustering (Figure 2). These clusters were used for stressor response measurement. Kruskal-Wallis ANOVA by ranks tests were significant for six clusters for fish, showing that 12 chemical, two habitat, and four land use measures were significantly predictive of the fish assemblage structure (Table 2). Fish assemblage structure was significantly predicted by the presence of alkalinity, arsenic, chloride, COD, copper, hardness, manganese, nickel, sulfate, TOC, total solids (TS), and zinc (Table 4). Hardness, manganese, nickel, sulfate, total solids, and zinc was significantly higher in concentration in cluster 4, while all of the other significant parameters showed a decreasing dose response in clusters 1-6 (Figure 4).

Box and whisker plots for significant variable responses for fish (Figure 4) show the greatest response was observed among metals (i.e., arsenic, copper, manganese, nickel, and zinc), wastewater treatment (i.e., alkalinity, chloride, hardness, total solids, and TOC), and COD (Table 4). All significant metals were associated with cluster 4 (Figure 4), which was associated with the acid mine drainage leaching of contaminants.

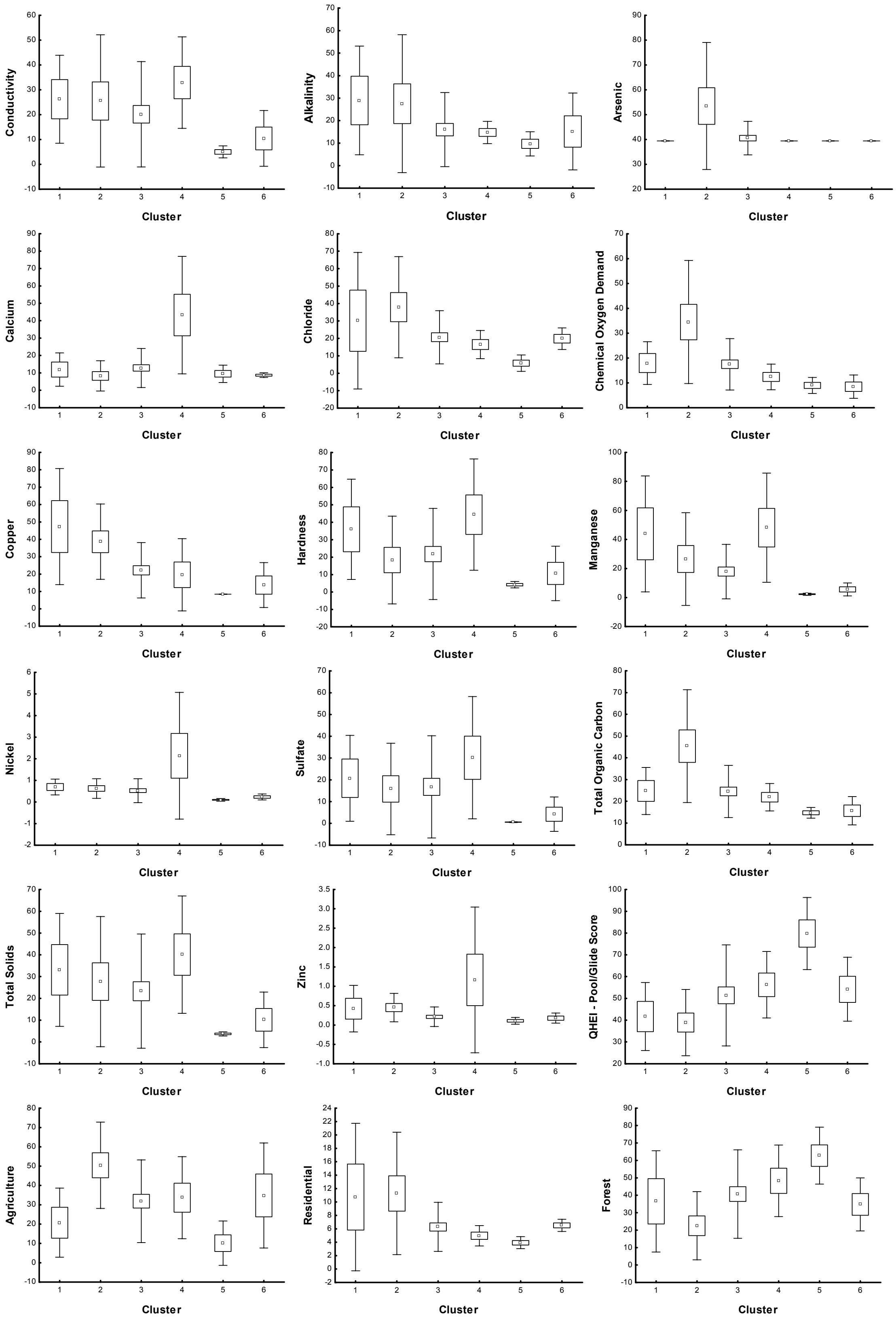


Figure 4. Box-and-whisker plots of the significant water chemistry, habitat, and land use variables that were predictive of the fish assemblage clustering.

Table 3. Results of factor analysis and explained variance observed in fish assemblage biologic structure and 15 significant chemical, habitat, and land use variables at the Patoka River watershed during 2006-2007.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Specific Conductivity	**0.984474**	-0.012447	0.048717	-0.044090
Alkalinity (as CaCO3)	0.311855	-0.205350	**-0.852694**	0.117439
Arsenic	-0.055990	-0.173332	0.106757	**0.846274**
Calcium (as CaCO3)	0.627190	0.408877	0.097471	-0.241869
Chloride	-0.180896	**-0.834866**	-0.204340	0.107574
Copper	0.670575	0.023948	0.258956	0.350498
Manganese	0.535842	-0.075616	0.642924	0.027608
Nickel	0.617428	0.023341	**0.735425**	0.029206
Sulfate	**0.958623**	0.046260	0.116740	-0.110348
Total Organic Carbon	-0.182297	-0.334777	-0.439210	0.597986
Total Solids	**0.981907**	0.036002	0.106031	-0.020186
Zinc	0.372441	0.099641	**0.832710**	0.110466
QHEI-Pool Glide Score	-0.268511	**0.744190**	-0.096426	0.005669
Agriculture	0.105298	**-0.799903**	-0.050977	0.112148
Forest	0.233926	**0.812592**	0.157132	-0.210231
Expl.Var	4.806634	2.922795	2.761142	1.365633
Prp.Totl	32.0442	19.4853	18.4076	9.1042

Habitat

Fish assemblage integrity was predicted by an increasing amount of pool-glide habitat (Figure 4). This relationship was a direct increase in integrity with increasing amounts of pool habitat. Since some fish species are dependent on deeper pool habitats for survival, the assemblage integrity would likewise increase with more pool habitat.

Land Use Response

Fish assemblages showed significant results with land use including agriculture, residential, forest, and industrial uses (Table 4). Fish assemblage structure declined with increasing density of both agriculture, industrial, and residential land use, while fish assemblage biological integrity increased with increasing forest land use.

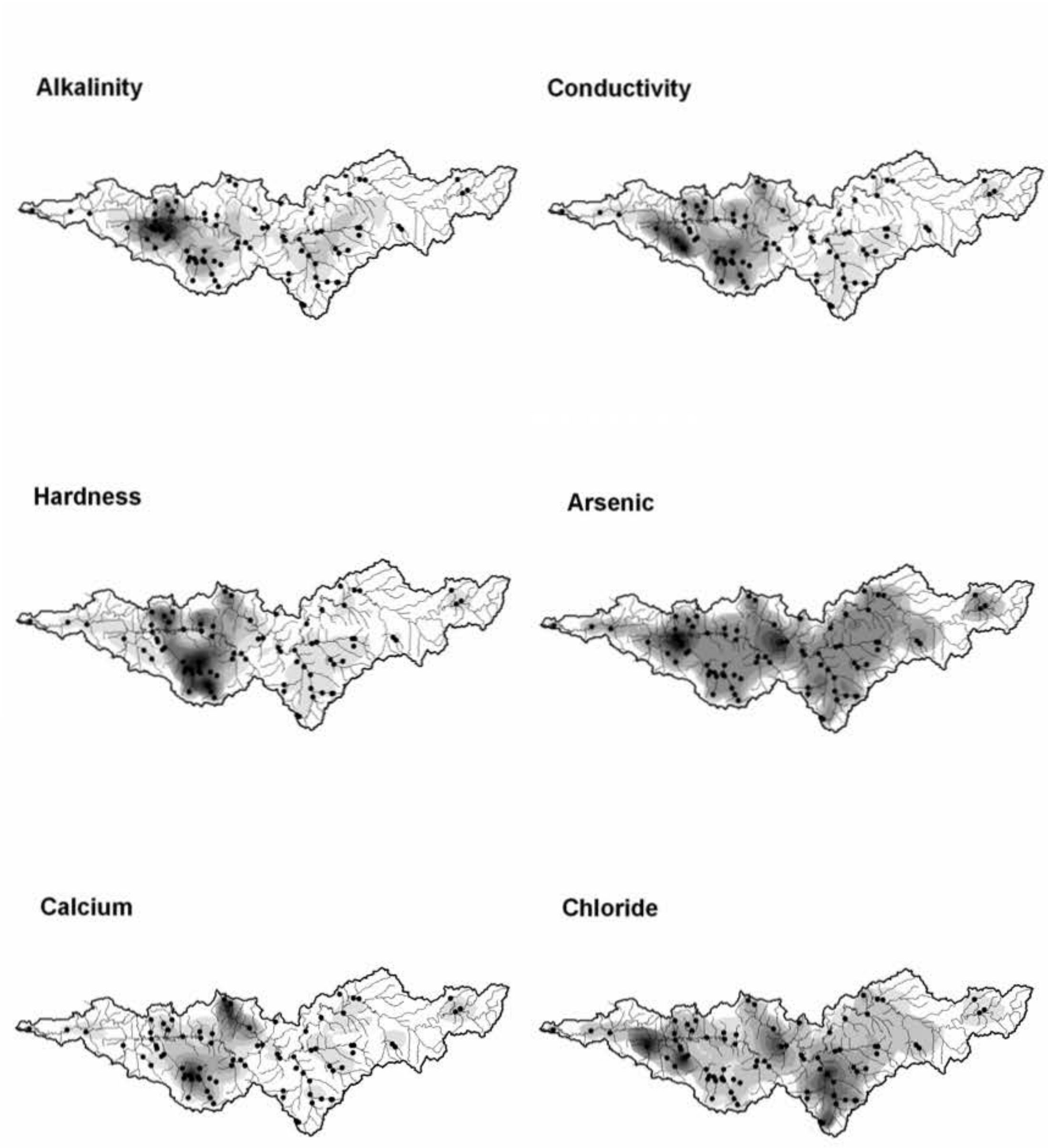
Factor Analysis

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. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used later to reduce the set of variables in a dataset.

Fish assemblage structure explained observed variance on four factors, which explained 79.0% of the variance in the fish assemblage information (Table 3). The primary factor includes specific conductance, sulfate, and total solids, which was correlated with the explained variance of 32%. The secondary factor included negative associations between chlorides and agricultural land uses and positive relationships between forested land use and the amount of pool-glide habitat. The third factor explains 18.4% of the variance with the increase of zinc and nickel and a negative association with alkalinity. The fourth factor is based on the increase of arsenic, which explains 9.1% of the variance.

Associations between Contaminants and Hot Spot Analysis

Contaminant distribution, based on percentage-of-range concentrations, showed variable responses between organism groups. Fish assemblage structure was predicted by negative associations with chlorides, sulfates, and total solids, which are typical of sewage treatement waste effluents. The primary distributions of these variables in study area are from the urban centers of Oakland City, Winslow, Jasper, and Huntington (Figure 5). Zinc, nickel, manganese, arsenic, and copper contamination was localized and emanated from the vicinity of the South Fork Patoka River (Figure 5). The land use associations were clearly differentiated between agriculture and forest and represented the extremes of area quality within the watershed (Figure 5).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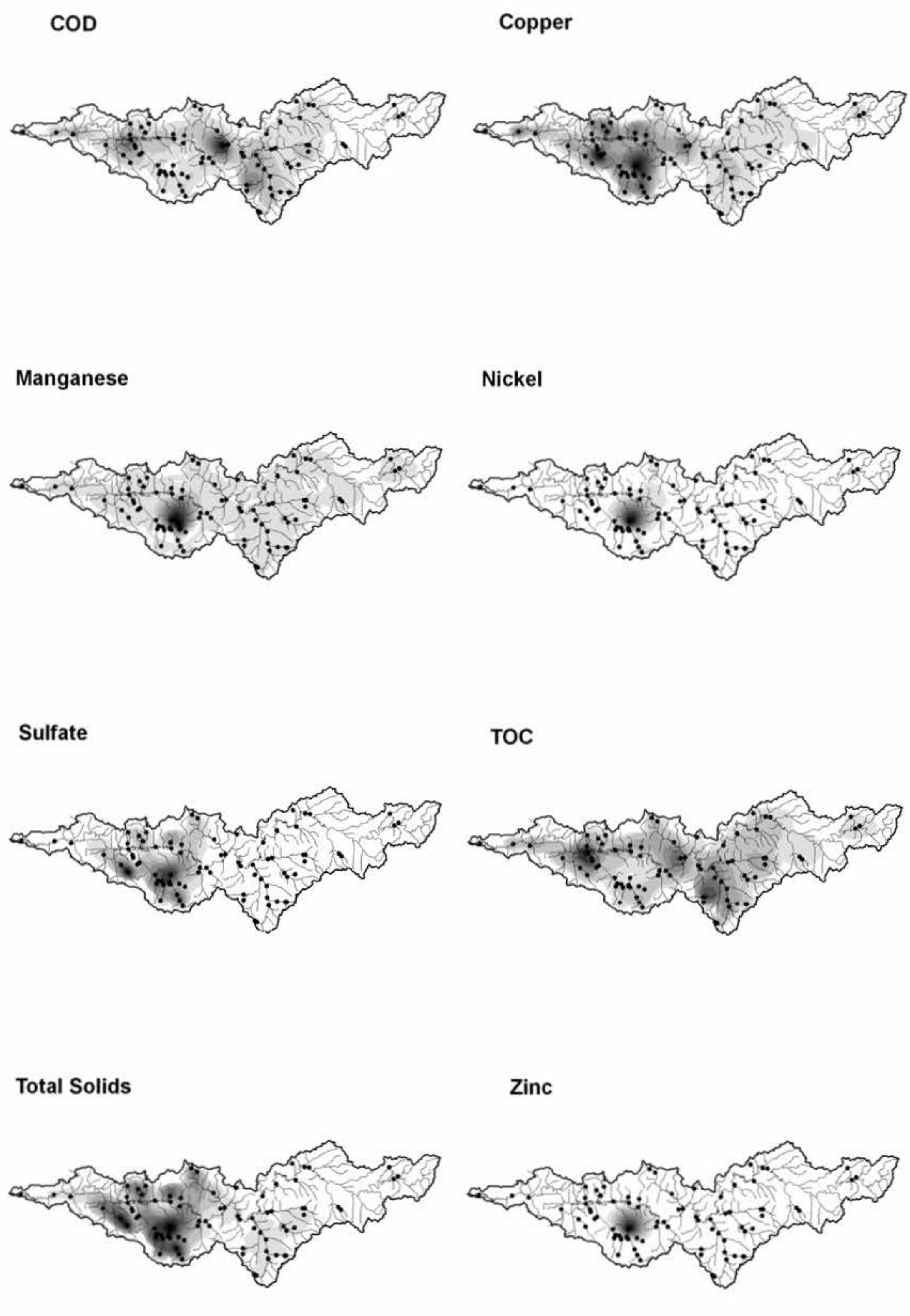
Conductivity
Hardness
Arsenic
Calcium
Chloride


COD
Copper
Manganese
Nickel
Sulfate
TOC
Total Solids
Zinc

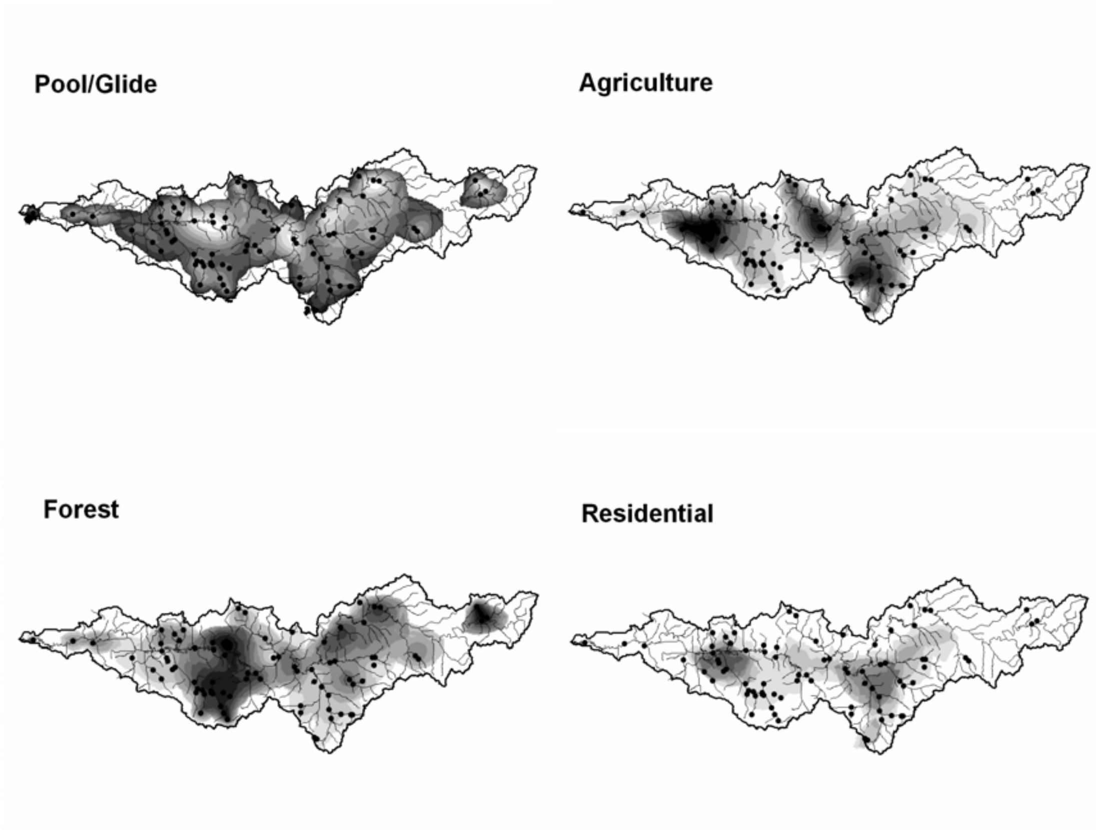


Figure 5. Relative concentration pleths of significant water quality, habitat, and land use chemistry variables that were predictive of fish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area. All concentrations are presented on a relative (percentage of range) scale.

Appendix 1. Data summary statistics for percent of range values for chemical (concentration in ppm), habitat, and land use for fish assemblage structure in the Patoka River watershed.

Variable	N	Mean	Minimum	Maximum	SD
Dissolved Oxygen	80	5.84	0.5300	13.9	2.1
pH	80	7.40	2.8000	9.4	1.1
Water Temperature	80	26.18	19.1000	36.8	3.5
Specific Conductivity	80	1174.60	167.0000	5430.0	1146.1
Turbidity	70	71.68	1.0000	1000.0	137.9
Alkalinity (as CaCO3)	80	162.86	5.0000	958.0	179.2
Aluminum	80	2538.48	25.0000	75700.0	8984.0
Arsenic	80	2.67	2.5000	6.3	0.7
Cadmium	80	0.92	0.5000	18.0	2.2
Calcium (as CaCO3)	80	589.23	72.0000	3970.0	663.6
Chloride	80	13.48	2.5000	62.0	12.6
COD	80	15.09	2.5000	85.7	12.4
Copper	80	3.13	1.0000	11.9	2.6
Hardness (as CaCO3)	80	680.23	78.5000	2820.0	786.2
Lead	80	1.51	1.0000	15.2	1.9
Manganese	80	1917.05	42.1000	41300.0	6490.1
Nickel	80	30.83	0.7500	1020.0	129.1
Nitrogen, Ammonia	80	0.11	0.0500	1.1	0.2
Nitrogen, Nitrate+Nitrite	80	1.95	0.0500	19.6	3.3
Phosphorus, Total	80	0.15	0.0050	2.2	0.3
Selenium	80	2.18	2.0000	9.1	1.0
Sulfate	80	676.18	13.9000	3890.0	910.4
Total Organic Carbon	80	4.71	0.0500	18.8	3.1
Total Solids	80	1196.69	131.0000	4700.0	1290.4
Total Suspended Solids	80	32.86	2.0000	374.0	55.2
Zinc	80	93.27	3.0000	3970.0	473.9
QHEI Score	80	45.29	21.5000	77.0	12.2
QHEI-Substrate Score	80	8.27	0.0000	18.0	5.1
QHEI-Instream Cover Score	80	7.85	2.0000	16.0	3.4
QHEI-Channel Score	80	10.78	5.0000	19.0	3.2
QHEI-Riparian Score	80	5.38	1.0000	10.0	2.5
QHEI-Pool Glide Score	80	6.29	0.0000	12.0	2.7
QHEI-Riffle Run Score	80	1.43	0.0000	6.0	1.7
QHEI-Gradient Score	80	5.30	2.0000	10.0	2.0
IBI Score	80	31.70	0.0000	56.0	13.6
QHEI-Drainage Area	80	85.33	0.1000	852.9	198.6
Water	80	3849.17	0.0000	42446.8	9744.5
Commercial	80	538.91	0.0000	6666.8	1539.9
Agriculture	80	49259.76	0.0000	696209.6	140497.0
Residential	80	10925.01	0.2000	126723.6	28203.8
Grass/Pasture	80	32549.58	0.0000	302574.4	76924.4
Forest	80	95474.22	0.0000	965515.6	228159.2
Industrial	80	582.99	0.0000	8985.6	1815.6
Other	80	3996.10	0.0000	34330.0	9884.3

Identification of Stressors Affecting Watershed Level Scale Indicators in the Vernon Fork Muscatatuck River

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Study Design

Stressor identification on the watershed scale was accomplished using a random design to select 63 sites in the Vernon Fork Muscatatuck River watershed. These sites represented locations throughout the Vernon Fork watershed and included random sites from both the Muscatatuck and Big Oaks National Wildlife Refuges. Sites were visited and those sites that were dry were excluded from the analysis. Remaining sites were analyzed for biological assemblage structure with the chemical, physical, and land use data (Figure 6). In addition, we evaluated pesticide distribution in the watershed; however, this assessment is a preliminary assessment that has several problems with watershed level interpretation. This information is presented to show patterns with the fish assemblage indicator; however, the level of detail is not equivalent to the remainder of the chemical, habitat, and land use variables assessed for the remainder of the dataset.

Analysis of assemblage structure based on Cluster Analysis showed that four similarity clusters represented the biological structure (Figure 7). Assemblage structure was explained by increasing index of biotic integrity scores for fish (Figure 8). Fish assemblage structure was explained with tier 3 linkage structure (Figure 7). The basis for these groupings is that the biological community structure results are externally driven and can be identified to evaluate physical and chemical stress on the respective assemblage (Morris et al. 2006).

Habitat Quality

Habitat analysis using the QHEI showed that scores ranged from 41 to 86 (average 60.87 $\pm$ 9.78). The QHEI scores reflected habitat quality that is meeting designated uses for aquatic life. Sites scoring less than between 34 and 67 are considered meeting designated uses for aquatic life, while scores above 67 are considered reference quality habitat. The average site quality score was near the reference quality classification. The instream cover, riparian score, riffle-run score, and pool-glide score varied within the Vernon Fork based on the amount of channelization in the study area. These attributes were the primary reasons for sites not being considered reference quality streams.

Chemical Quality

Summary statistics by assemblage for water chemistry results are found in Table 1. Project specific summary statistics and range of scale values for water quality results are presented in Appendix 2. In general, the lowest biological integrity and species richness clusters (cluster 1) had the highest concentrations of contaminants (Table 1). Most of the contaminants show a dose response distributions, with the exception of iron which had a high concentration in cluster 3 (Figure 9). The pesticides Acetochlor and Clomazone had the highest concentration in cluster 4, while simazine was highest in cluster 2 (Figure 10).

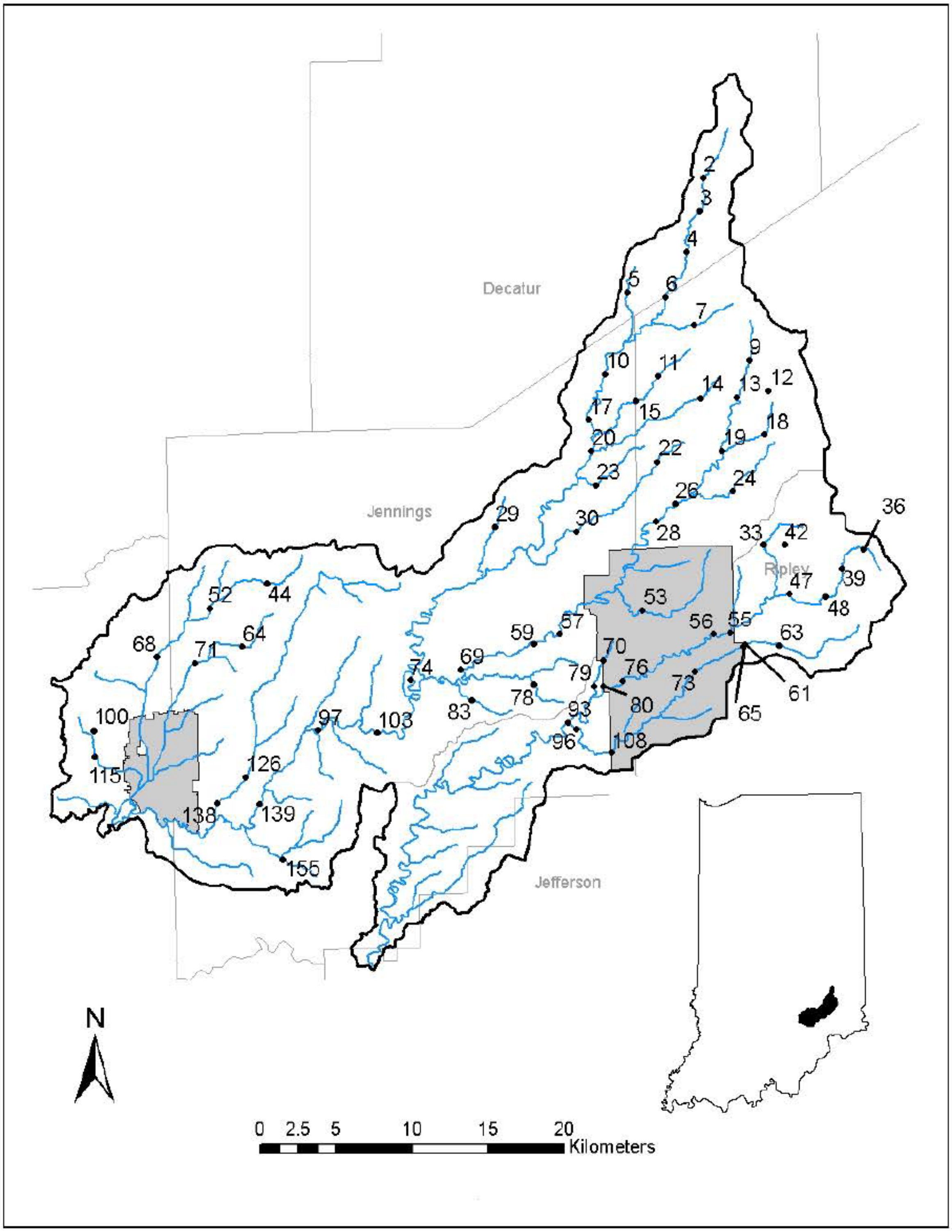


Figure 6. Locations of sites sampled in the Vernon Fork Muscatatuck River during a watershed scale assessment conducted during 2006-2007 (numbers refer to Appendix B).

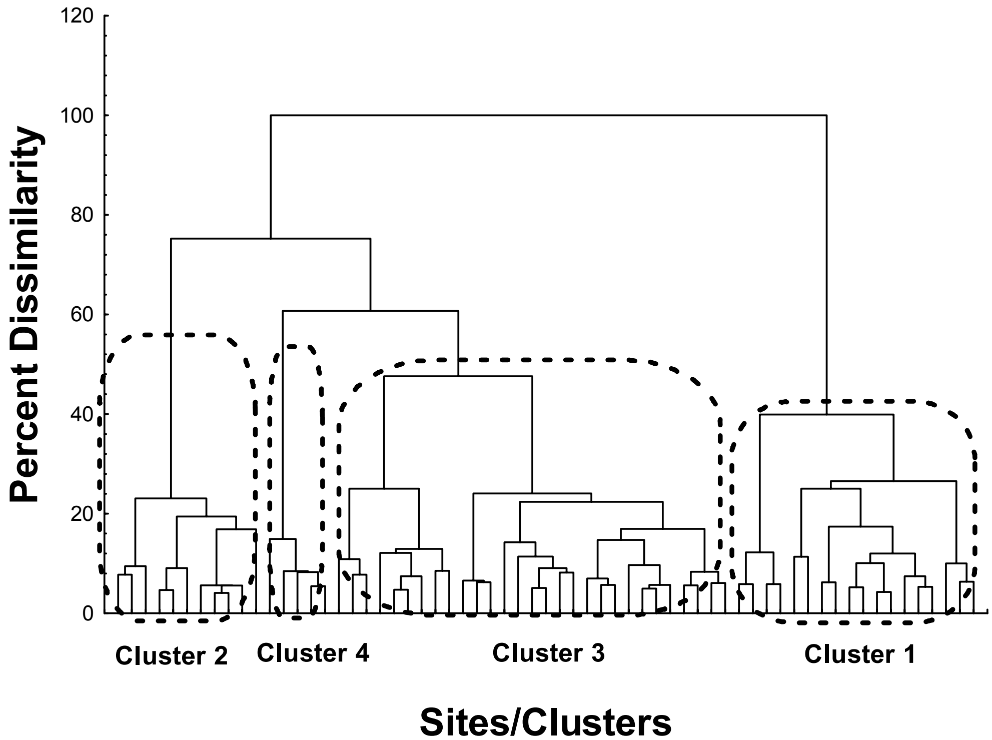


Figure 7. Dendograms showing the relative similarity of assemblage data collected in the Vernon Fork Muscatatuck River watershed during 2007. Boxes show the clustering at each of the similarity tiers evaluated with ANOVA for the fish assemblage.

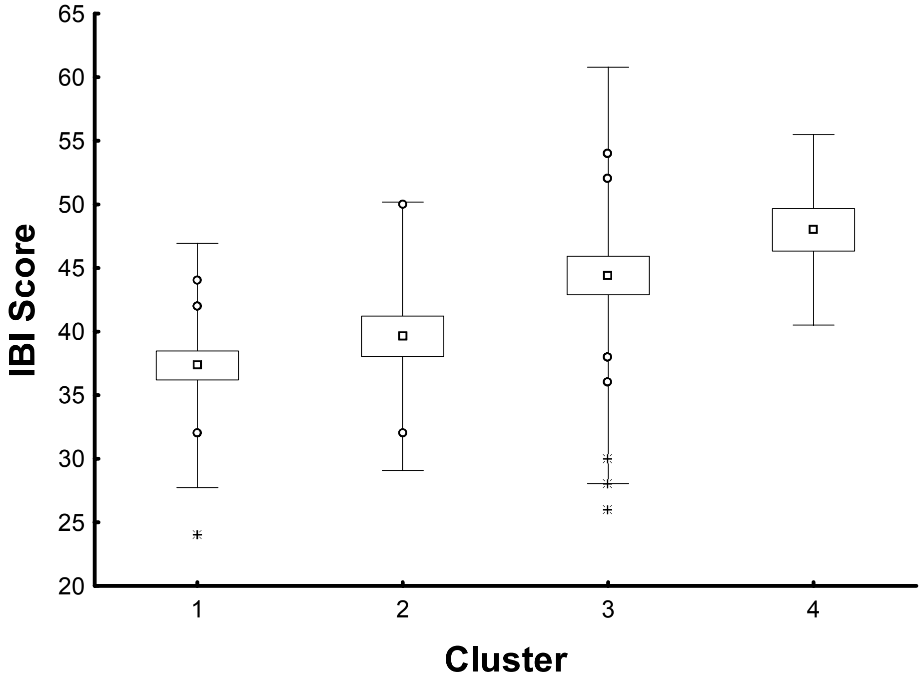


Figure 8. Box-and-whisker plots showing biological assemblage responses for index of biotic integrity (IBI) for fish assemblages had to the biologic response gradient developed from the Vernon Fork Muscatatuck River watershed during 2007.

Table 4. Summary statistics for 18 chemistry (concentration/ppm), habitat, and land use variables by fish grouping cluster (see Fig. 1A) sampled in August 2006 in the Vernon Fork Muscatatuck River watershed.

	Cluster 1					Cluster 2				
Variable	**N**	**Mean**	**Minimum**	**Maximum**	**SD**	**N**	**Mean**	**Minimum**	**Maximum**	**SD**
Water Temperature	18	22.50	18.87	28.74	2.44	11	25.25	20.90	34.35	4.02
Acetochlor	18	0.05	0.05	0.10	0.01	11	0.05	0.05	0.05	0.00
Atrazine (Aatrex)	18	0.54	0.05	2.50	0.67	11	1.78	0.30	3.60	1.11
Chloride	18	22.19	2.10	120.00	25.82	11	45.45	17.00	130.00	40.22
Chromium (Total)	18	2.57	2.50	3.20	0.20	11	2.50	2.50	2.50	0.00
Clomazone	18	0.05	0.05	0.05	0.00	11	0.05	0.05	0.05	0.00
Di(2-ethylhexyl)phthalate	18	0.30	0.30	0.30	0.00	11	0.30	0.30	0.30	0.00
Iron	18	58.54	0.18	350.00	111.73	11	27.54	0.08	160.00	60.71
Simazine	18	0.09	0.04	0.32	0.08	11	0.21	0.04	0.53	0.15
Sodium	18	15.12	4.70	79.00	16.88	11	25.67	7.80	82.00	26.64
IBI Score	18	37.33	24.00	44.00	4.80	11	9.09	6.00	10.00	1.38
QHEI Score	18	57.67	41.00	69.00	7.34	11	58.91	51.00	66.00	4.89
QHEI-Instream Cover Score	18	6.89	2.00	14.00	3.23	11	7.82	4.00	13.00	2.68
QHEI-Channel Score	18	14.50	12.00	20.00	2.64	11	15.09	12.00	19.00	2.26
QHEI-Riparian Score	18	7.83	3.00	10.00	2.26	11	6.36	3.00	10.00	2.38
QHEI-Pool Glide Score	18	2.72	0.00	6.00	1.90	11	3.64	0.00	7.00	1.86
QHEI-Gradient Score	18	8.78	6.00	10.00	1.22	11	9.09	6.00	10.00	1.38
QHEI-Drainage Area	18	5.26	1.62	24.15	5.71	11	12.78	2.30	59.34	16.01
Commercial Land Use	18	0.05	0.00	0.26	0.08	11	0.16	0.00	0.58	0.21

Variable	N	Cluster 3 Mean	Cluster 3 Minimum	Cluster 3 Maximum	Cluster 3 SD	N	Cluster 4 Mean	Cluster 4 Minimum	Cluster 4 Maximum	Cluster 4 SD
Water Temperature	29	25.48	19.45	32.13	2.81	5	25.51	24.49	27.28	1.05
Acetochlor	29	0.05	0.05	0.05	0.00	5	0.09	0.05	0.20	0.07
Atrazine (Aatrex)	29	2.84	0.05	17.00	4.22	5	1.96	1.40	2.50	0.42
Chloride	29	27.44	6.30	75.00	15.82	5	31.80	18.00	51.00	12.68
Chromium (Total)	29	2.80	2.50	10.00	1.39	5	2.98	2.50	3.70	0.50
Clomazone	29	0.05	0.05	0.05	0.00	5	0.06	0.05	0.10	0.02
Di(2-ethylhexyl)phthalate	29	0.32	0.30	1.00	0.13	5	0.46	0.30	0.70	0.22
Iron	29	272.55	0.10	2200.00	441.60	5	0.56	0.16	0.98	0.29
Simazine	29	0.16	0.04	0.67	0.16	5	0.14	0.10	0.22	0.05
Sodium	29	14.84	5.50	46.00	8.91	5	27.20	13.00	51.00	14.34
IBI Score	29	44.41	26.00	54.00	8.18	5	48.00	44.00	54.00	3.74
QHEI Score	29	64.55	41.00	86.00	11.18	5	55.40	41.00	72.00	11.19
QHEI-Instream Cover Score	29	9.17	1.00	14.00	3.45	5	11.40	7.00	14.00	2.70
QHEI-Channel Score	29	15.34	10.00	20.00	2.88	5	11.40	8.00	13.00	2.07
QHEI-Riparian Score	29	8.17	3.00	10.00	2.02	5	5.80	4.00	10.00	2.39
QHEI-Pool Glide Score	29	4.83	0.00	9.00	2.11	5	7.00	4.00	9.00	2.12
QHEI-Gradient Score	29	8.48	4.00	10.00	1.82	5	5.20	4.00	6.00	1.10
QHEI-Drainage Area	29	36.25	0.09	219.11	51.77	5	103.91	18.19	233.46	107.22
Commercial Land Use	29	0.08	0.00	0.28	0.09	5	0.50	0.03	1.11	0.44

Table 5. List of 13 physical and chemical variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in the Vernon Fork Muscatatuck River watershed during 2007.

Variable	p Value
Chemical	
Water Temperature	p = 0.0036
Chloride	p = 0.0124
Chromium	p = 0.0073
Iron	p = 0.0015
Sodium	p = 0.0477
Habitat	
QHEI Score	p = 0.0482
QHEI Instream Cover Score	p = 0.0157
QHEI Channel Score	p = 0.0260
QHEI Riparian Score	p = 0.0403
QHEI Gradient Score	p = 0.0346
QHEI Drainage Area	p = 0.0001
Biotic Integrity	
IBI Score	p = 0.0011
Land-use	
Commercial	p = 0.0203

Table 6. List of 5 pesticide variables significantly predictive (a = 0.05) of the fish assemblage biologic gradient using the Kruskal-Wallis ANOVA by ranks test in the Vernon Fork Muscatatuck River during 2007.

Variable	p Value
Pesticide	
Acetochlor	p = 0.0013
Atrazine (Aatrex)	p = 0.0004
Clomazome	p = 0.0089
Di(2-ethylhexyl)phthalate	p = 0.0075
Simazine	p = 0.0232

Multivariate Results

For this analysis we chose 50% similarity as a benchmark for defining assemblage structure clustering (Figure 7). These clusters were used for stressor response measurement. Kruskal-Wallis ANOVA by ranks tests were significant for four clusters for fish, showing that five chemical, six habitat, and a single land use measure were significantly predictive of the fish assemblage structure (Table 5). Fish assemblage structure was significantly predicted by the presence of water temperature, chloride, chromium, iron, and sodium (Table 5). Five pesticides was also able to significantly predict fish assemblage structure; however, these results may not be representative. Different pesticides are applied in the spring during different times. The presence of

select pesticides in different parts of the Vernon Fork watershed does not constitute an anthropogenic impact because these pesticides are applied for different crops, on different soil types, and application is done when soil temperature reach select ranges. As a result, despite the significant relationships between pesticides and fish assemblage structure it is not intuitive that these relationships would favor pesticide application to enhance fish biological integrity (Table 6).

Hardness, manganese, nickel, sulfate, total solids, and zinc was significantly higher in concentration in cluster 4, while all of the other significant parameters showed a decreasing dose response in clusters 1-6 (Figure 4). Low water temperature was associated with lower fish integrity; however, this is most likely a result of the index, which is calibrated to favor warm water fish assemblage structure in the assessment classification.

Box and whisker plots for significant variable responses for fish (Figure 4) show the greatest response was observed among metals (i.e., chromium and iron), wastewater treatment (i.e., chloride and sodium), and water temperature (Table 4). All significant metals were associated with cluster 3, while chloride and sodium were highest in cluster 2 (Figure 9). Pesticide box and whisker plots showed that acetochlor, clomazone, and di(2-ethyl)hexylphthalate were present in cluster 4 (Figure 10). This is associated with the highest biological integrity observed in the Vernon Fork watershed. It is counterintuitive that the application of pesticides would be beneficial to fish assemblage integrity. We reject this conclusion recognizing the limitations of our pesticide sampling procedure which was done during a single month one time from each location during the spring. Representative sampling should have been conducted weekly and estimates of pesticide presence at each site should have been based on multiple sampling events so that the maximum pesticide concentration would have been representative of the farms that had late season or different crop rotation patterns.

Habitat

Fish assemblage integrity was predicted by an increasing amount of instream cover, pool-glide habitat, and increasing drainage area (Figure 9). These variable relationship was a direct increase in integrity with increasing amounts of habitat. Channel score increased along the first three clusters and then declined in the highest integrity cluster, while riparian score oscillated between high and low with increasing cluster. Gradient score decreased with increasing fish integrity.

Land Use Response

Fish assemblages showed significant results with only commercial land use (Table 5). Fish assemblage integrity increased with increasing density of commercial land use.

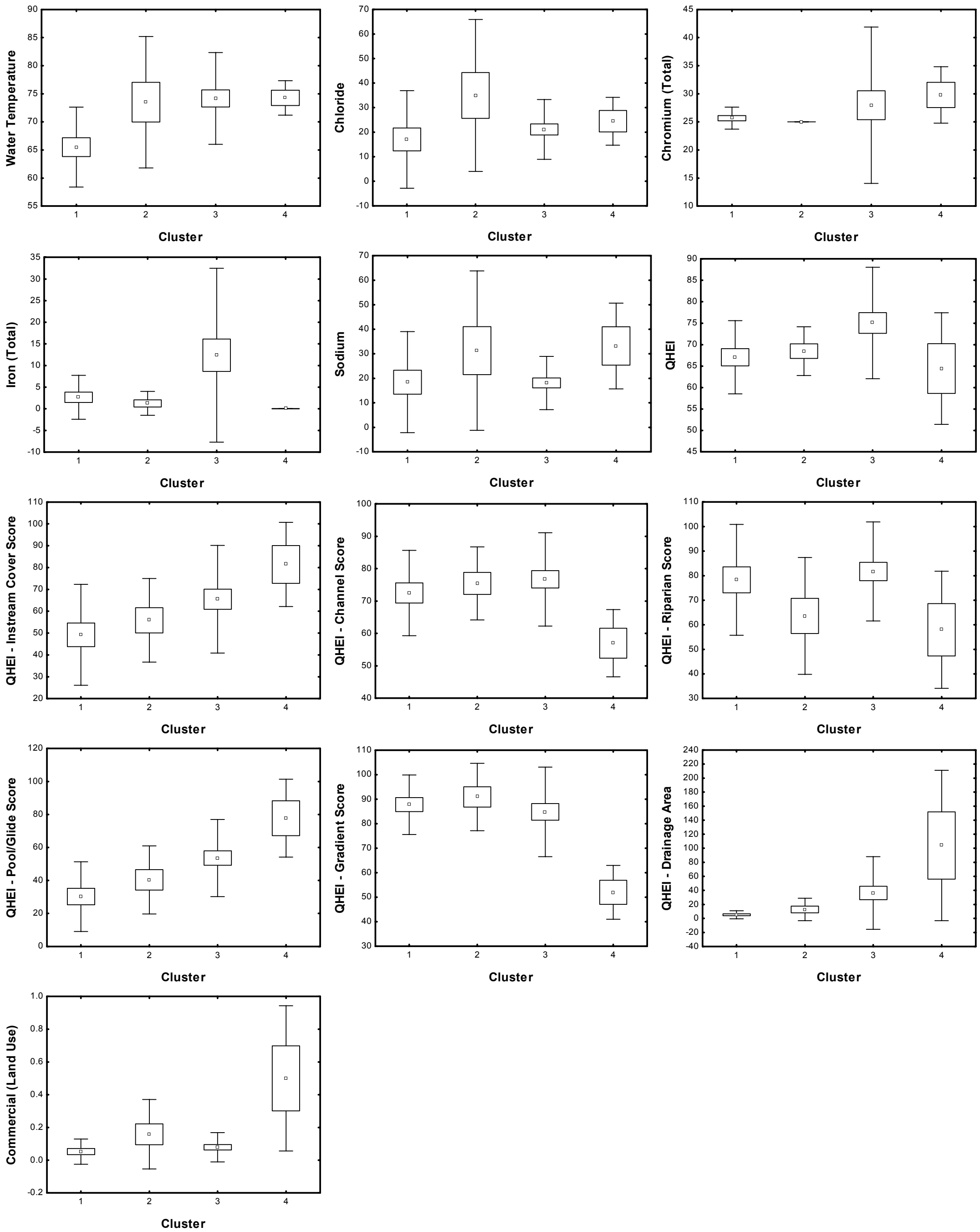


Figure 9. Box-and-whisker plots of significant water chemistry, habitat, and land use variables that were predictive of the fish assemblage clustering for the Vernon Fork Muscatatuck River watershed during 2007.

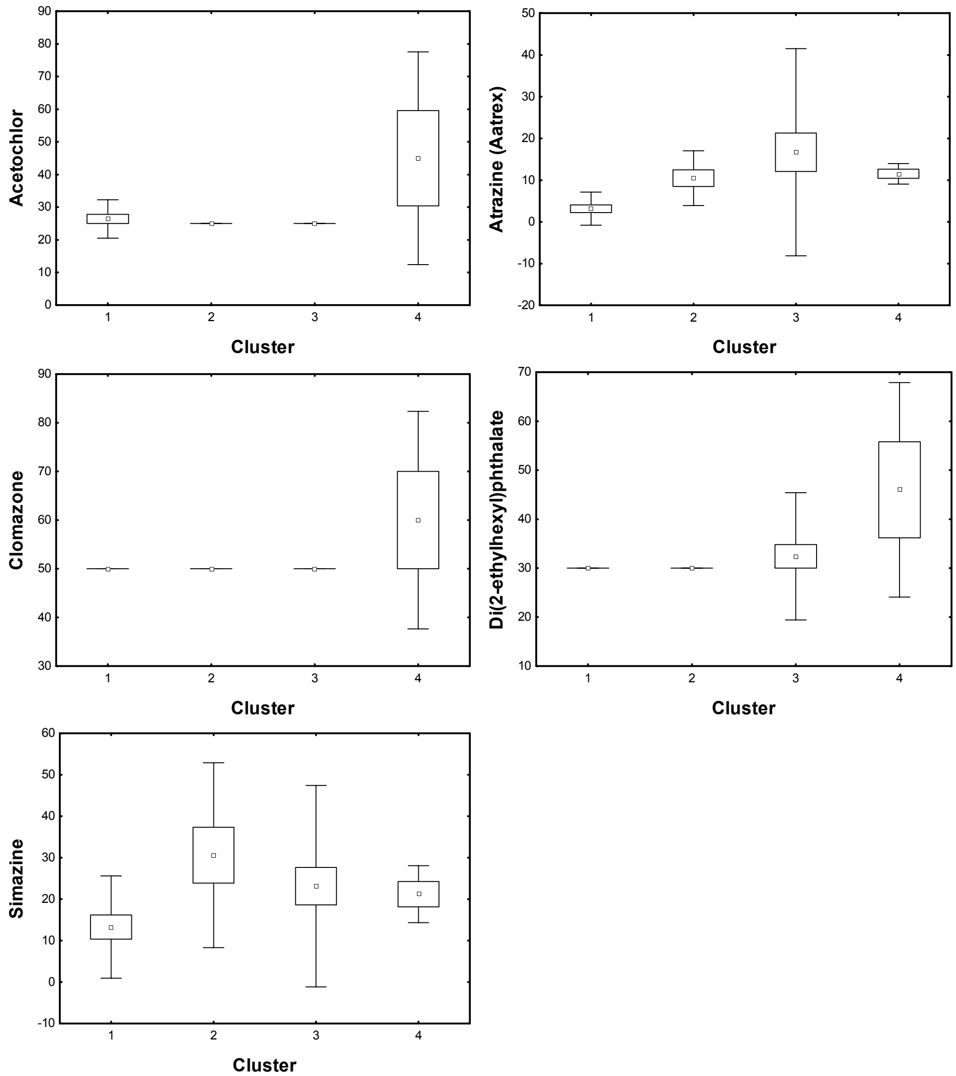


Figure 10. Box-and-whisker plots of five significant pesticide variables that were predictive of the fish assemblage clustering in the Vernon Fork Muscatatuck River watershed during 2007.

Table 7. Results of factor analysis and explained variance observed in fish assemblage biologic structure and 12 chemical, habitat, and land use variables in the Vernon Fork Muscatatuck River watershed during 2007.

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Water Temperature	-0.090158	-0.033025	-0.162656	**-0.842080**
Chloride	-0.309133	0.363376	-0.582710	0.147900
Chromium (Total)	**-0.703141**	0.116236	0.219721	-0.175198
Iron	0.423922	-0.040776	-0.684603	-0.196208
QHEI Score	**0.727610**	0.642896	-0.026672	0.104301
QHEI-Instream Cover Score	0.035992	**0.839237**	0.009760	0.010150
QHEI-Channel Score	**0.743172**	0.103361	0.081829	0.093616
QHEI-Riparian Score	**0.779540**	-0.059234	-0.185672	-0.292750
QHEI-Pool Glide Score	-0.134499	**0.864677**	0.125100	0.013787
QHEI-Gradient Score	0.676333	-0.154553	-0.106961	0.456359
QHEI-Drainage Area	0.209481	0.598605	0.267094	-0.364653
Commercial Land Use	-0.108339	0.203133	**0.891560**	0.081412
Expl. Variance	25.00	45.43	60.58	71.03

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. The observed variables are modeled as linear combinations of the factors, plus "error" terms. The information gained about the interdependencies can be used later to reduce the set of variables in a dataset.

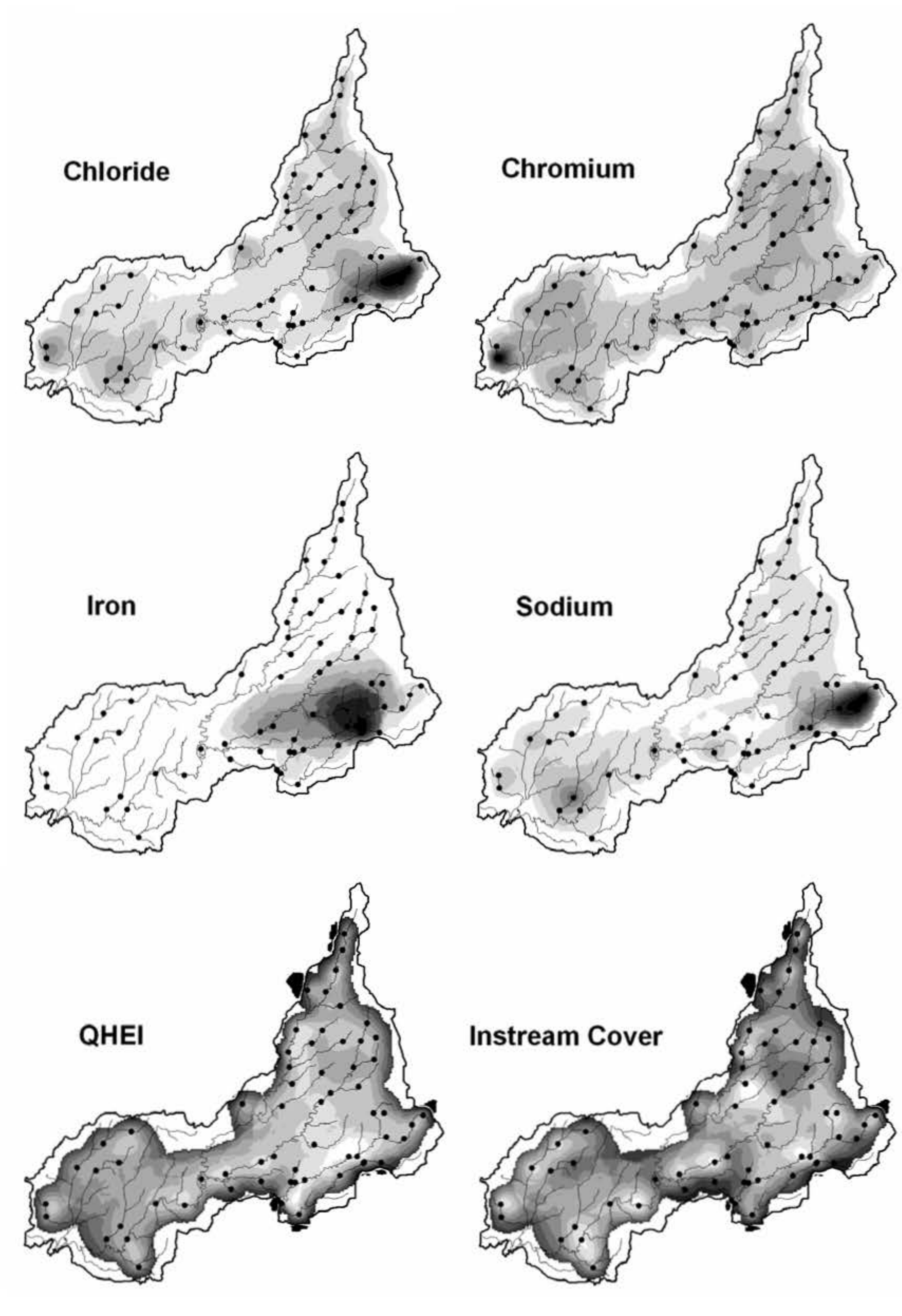
Fish assemblage structure explained observed variance on four factors, which explained 71.0% of the variance in the fish assemblage information (Table 7). The primary factor includes a negative association with chromium and positive association with three habitat variables (i.e., QHEI score, channel score, and riparian score), which explained 25% of the variance. The secondary factor included a positive relationship between instream cover and pool glide score and explained 20.43% of the variance. The third factor was associated with commercial land use and explained 15.2% of the variance. The fourth factor is based on a negative association between fish assemblage structure and the decrease in water temperature that explains 10.45% of the variance. The presence of springs and groundwater dominated streams in the headwaters of the Vernon Fork watershed and the warm water ditches of the lower watershed provided a stark dichotomy in water temperatures.

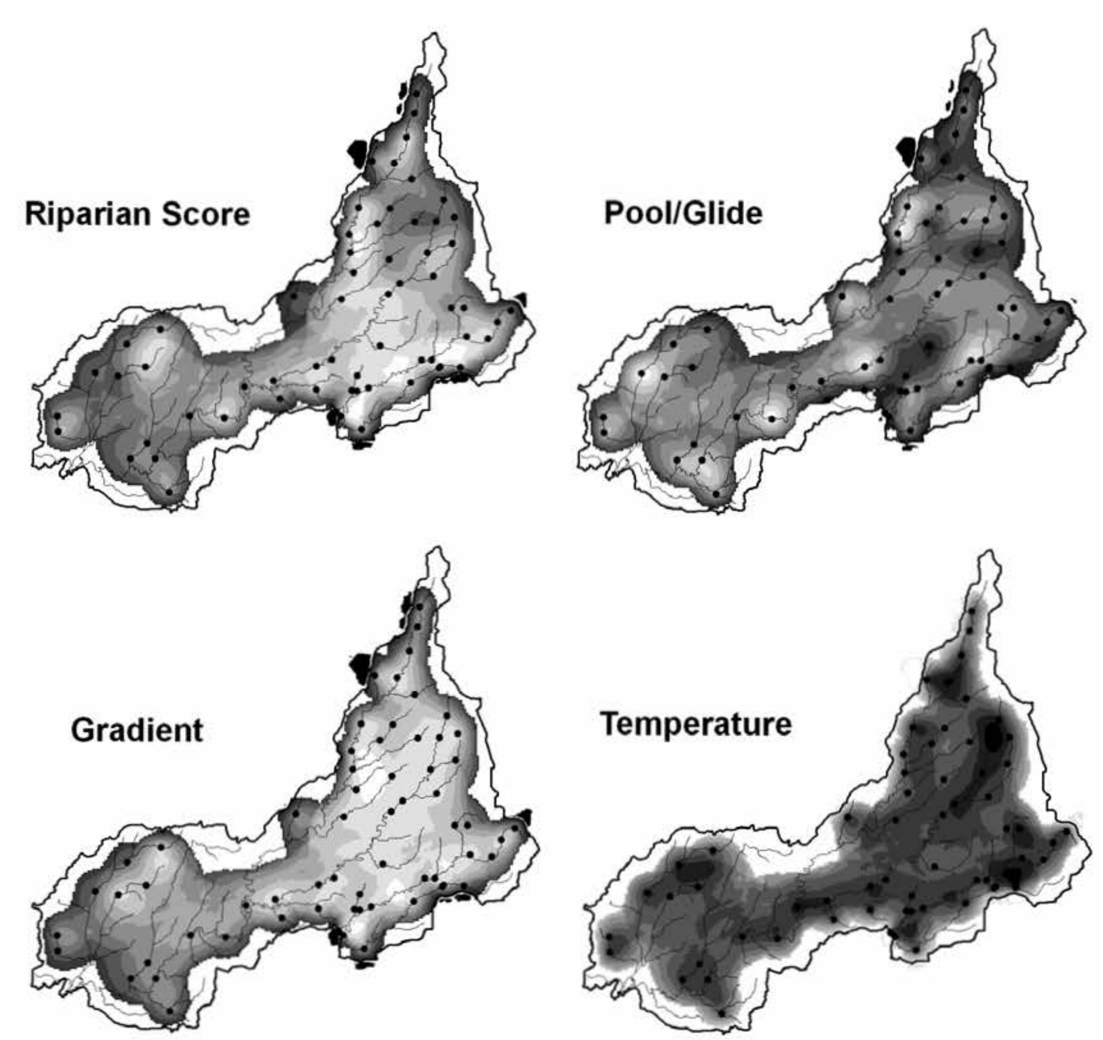
Associations between Contaminants and Hot Spot Analysis

Contaminant distribution, based on percentage-of-range concentrations, showed that fish assemblage structure was predicted by associations with chlorides and sodium (Figure 11). This contaminant emanated from the Little Graham Creek headwaters near the City of Versailles land application sludge site.

All of the other contaminants in the Vernon Fork watershed were found in either a single or a few locations. Chromium was found in the Sandy Branch near the western edge of the Muscatatuck National Wildlife Refuge. Commercial land use impacts were found on the Jefferson Proving Ground and near the Vernon Fork upstream of Muscatatuck National Wildlife Refuge (Figure 11).

Pesticides were clearly reflecting the crop rotation within the watershed and did not capture the full extent of pesticide useage with the single grab water sample design. Clearly future pesticide monitoring will need to be conducted weekly during the application period and cumulative loadings of pesticides at each site would need to be measured to estimate a reliable stressor gradient among the random sites sampled in the watershed. As a result, we do not know the extent or magnitude that pesticides may be having in the Vernon Fork watershed on aquatic life (Figure 12).


Chloride
Chromium
Iron
Sodium
QHEI
Instream Cover


Riparian Score
Pool/Glide
Gradient
Temperature

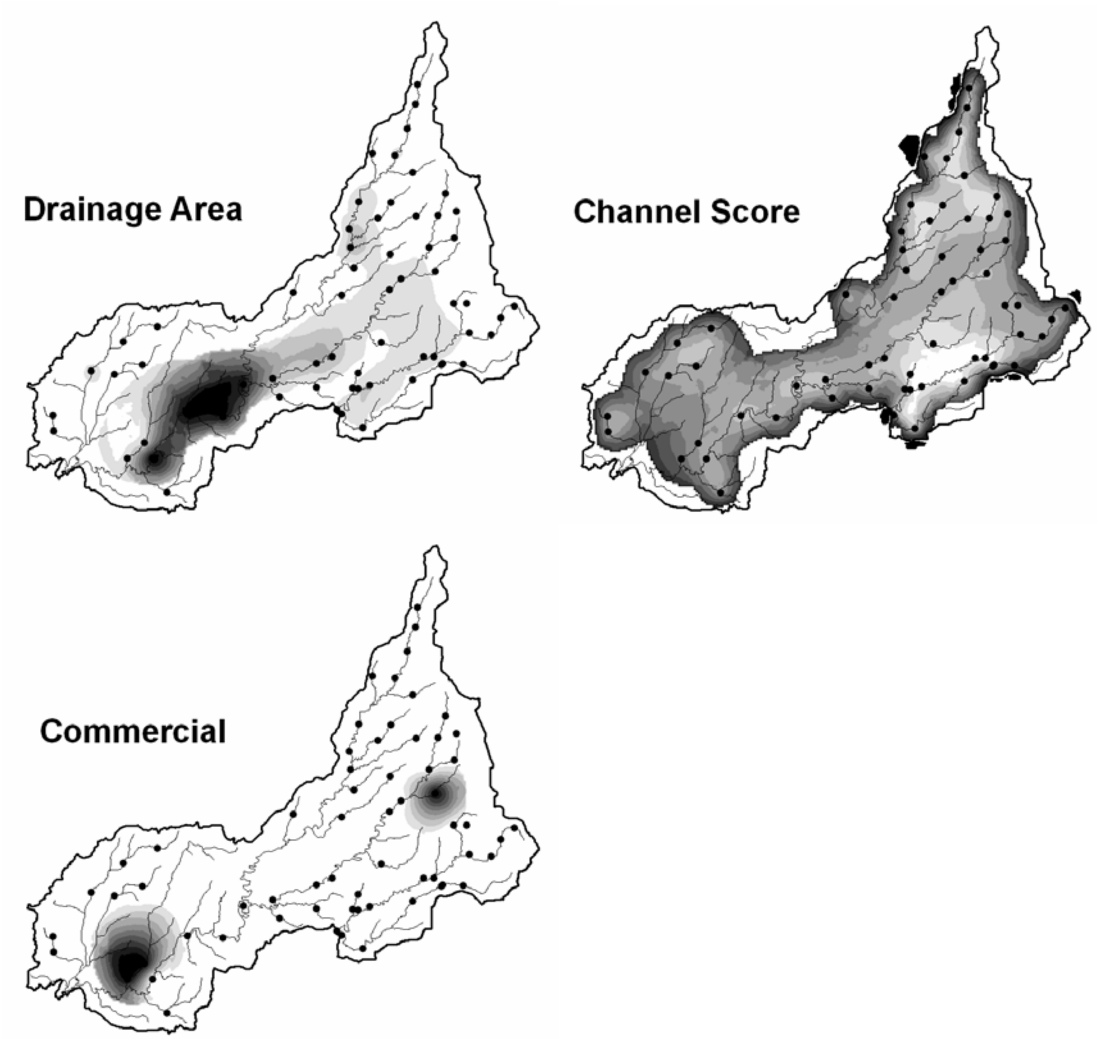


Figure 11. Relative concentration pleths of significant water quality, habitat, and land use chemistry variables that were predictive of fish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area. All concentrations are presented on a relative (percentage of range) scale (see Appendix for range of concentrations).

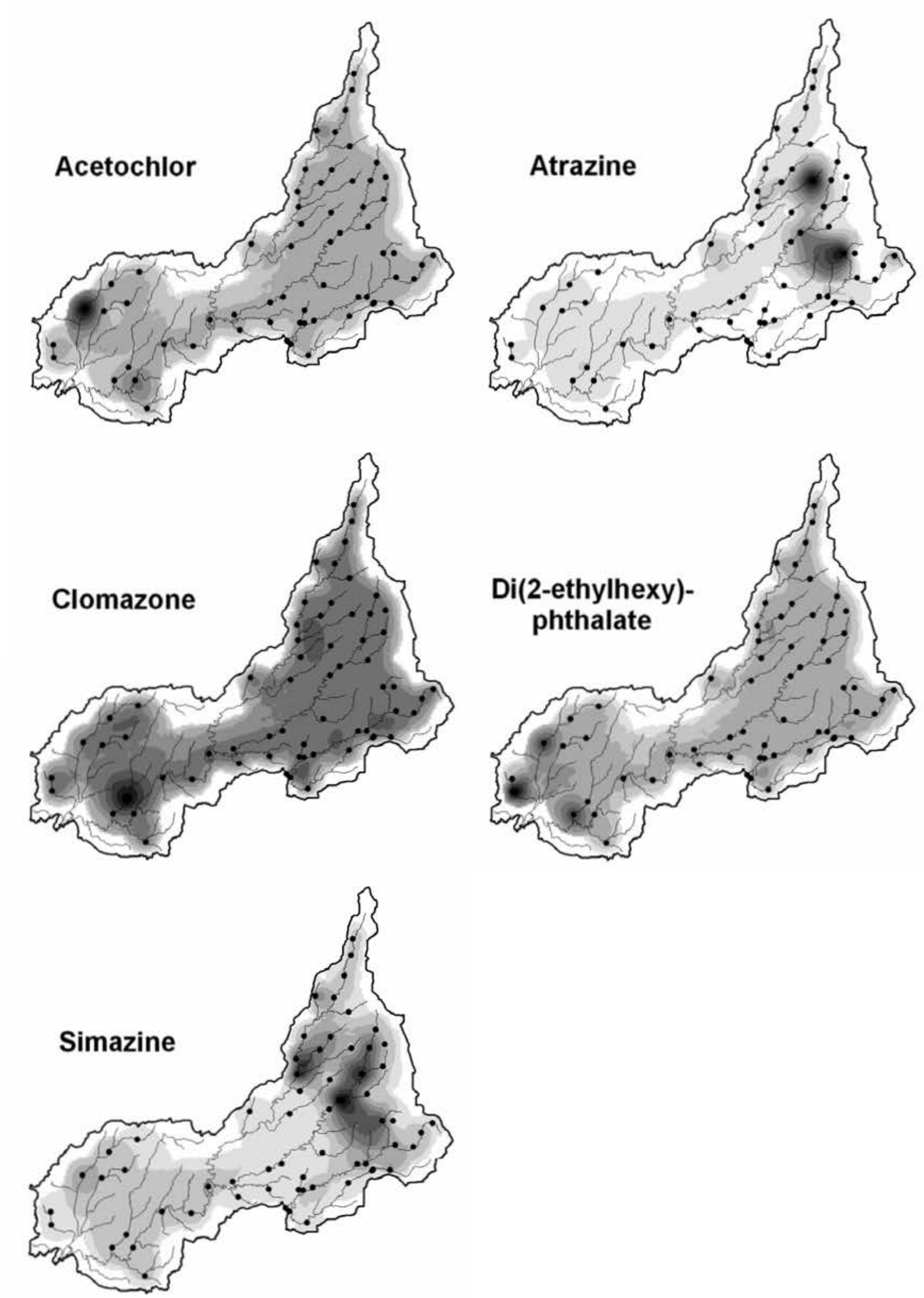


Figure 12. Relative concentration pleths of significant pesticide variables that were predictive of fish assemblage clustering using kriging and spline smoothed bathymetric methods. Darker regions on each pleth represent relatively higher concentrations of the variable associated with that area. All concentrations are presented on a relative (percentage of range) scale (see Appendix for range of concentrations).

Appendix 2. Data summary statistics for percent of range values for maximum chemical (concentration in ppm), habitat, and land use for Vernon Fork Muscatatuck River watershed.

Variable	N	Mean	Minimum	Maximum	SD
Contaminant					
Acetochlor	63	0.05	0.05	0.20	0.02
Alkalinity (as CaCO3)	63	190.16	100.00	300.00	34.01
Aluminum	63	318.97	39.00	4700.00	609.56
Antimony	63	0.51	0.50	1.10	0.08
Arsenic	63	2.97	2.50	9.70	1.55
Atrazine (Aatrex)	63	1.93	0.05	17.00	3.05
Barium	63	75.13	30.00	230.00	26.83
Benzo-a-pyrene	63	0.03	0.01	1.30	0.16
Calcium	63	660.08	31.00	38000.00	4780.28
Chloride	63	29.43	2.10	130.00	25.10
Chromium	63	2.69	2.50	10.00	0.96
Clomazone	63	0.05	0.05	0.10	0.01
Cobalt	63	0.81	0.26	4.60	0.71
Chemical Oxygen Demand	63	26.01	6.00	98.00	19.49
Copper	63	3.01	1.00	22.00	4.12
Cyanide	63	0.00	0.00	0.01	0.00
Desethylatrazine	63	1.03	0.50	6.00	1.21
Di(2-ethylhexyl)phthalate	63	0.32	0.30	1.00	0.11
Fluoride	63	0.20	0.12	0.65	0.10
Hardness (as CaCO3)	63	211.75	120.00	310.00	38.04
Iron	63	147.04	0.08	2200.00	325.56
Lead	63	1.32	1.00	19.00	2.27
Magnesium	63	16.67	8.20	28.00	3.50
Manganese	63	474.76	31.00	3200.00	607.14
Metolachlor	63	0.65	0.05	8.00	1.47
Nickel	63	2.87	1.50	17.00	2.24
Nitrogen (Ammonia)	63	0.11	0.05	1.10	0.16
Nitrogen (Nitrate+Nitrite)	63	18.92	0.01	1100.00	138.47
Phosphorus (Total)	63	-0.04	-1.00	1.80	0.57
Potassium	63	7.10	2.10	25.00	4.85
Silicon	63	5747.70	5.00	14000.00	2551.34
Simazine	63	0.14	0.04	0.67	0.14
Sodium	63	17.79	4.70	82.00	16.31
Sulfate	63	33.33	5.80	160.00	30.72
Total Dissolved Solids	63	262.86	170.00	590.00	84.29
Total Keldahl Nitrogen	62	1.03	0.41	5.40	0.76
Total Organic Carbon	63	5.75	1.90	17.00	3.05
Total Solids	63	299.52	180.00	610.00	93.64
Total Suspended Solids	63	22.81	1.00	310.00	39.31
Zinc	63	7.89	3.00	150.00	18.70
General Chemistry					
Dissolved Oxygen	63	4.94	0.20	13.70	2.38
pH	63	7.74	7.06	8.83	0.30
Water Temperature	63	24.59	18.87	34.35	3.11
Specific Conductivity	63	455.56	280.00	862.00	118.43

Turbidity	63	44.48	8.20	311.00	40.92
Habitat					
QHEI-Score	63	60.87	41.00	86.00	9.78
QHEI - Substrate Score	63	14.87	8.00	20.00	2.84
QHEI - Instream Cover Score	63	8.46	1.00	14.00	3.41
QHEI - Channel Score	63	14.75	8.00	20.00	2.81
QHEI - Riparian Score	63	7.57	3.00	10.00	2.28
QHEI - Pool Glide Score	63	4.19	0.00	9.00	2.31
QHEI - Riffle Run Score	63	2.63	0.00	7.00	1.90
QHEI - Gradient Score	63	8.41	4.00	10.00	1.80
IBI Score	63	41.84	24.00	54.00	7.41
QHEI - Drainage Area	63	28.67	0.09	233.46	51.86
Land Use					
Water	63	0.63	0.00	4.05	0.71
Commercial	63	0.12	0.00	1.11	0.20
Agriculture	63	38.93	0.00	80.60	19.96
Residential	63	2.51	0.00	10.06	2.69
Grass - Pasture	63	24.32	0.00	65.55	18.67
Forest	63	29.37	0.00	81.59	17.76
Industrial	63	0.93	0.00	15.71	2.72
Other	63	3.18	0.00	100.00	17.67

REFERENCES

Herricks, E.E. and D.J. Schaeffer. 1985. Can we optimize biomonitoring? *Environmental Management* 9: 487-492.

Indiana Department of Environmental Management. 1996. *Surface Water Probability Monitoring Strategy*. State of Indiana, Department of Environmental Management, Biological Studies Section, Indianapolis, IN.

Morris, C.C., T.P. Simon, and S.A. Newhouse. 2005 A local-scale in situ approach for stressor identification of biologically impaired aquatic systems. *Archives Environmental Contamination Toxicology* 49: 1–11.

Simon, T.P. and J. Lyons. 1995. Application of the index of biotic integrity to evaluate water resource integrity in freshwater ecosystems. pp 245-262. In: Davis, W.S. & T.P. Simon (eds.). *Biological Assessment and Criteria: Tools for water resources planning and decision making.* Lewis Publishers, Boca Raton, Florida.

Simon, T.P. and C.C. Morris. In press. in press. Biological response signature of oil brine threats, sediment contaminants, and crayfish assemblages in an Indiana Watershed, USA. *Archives Environmental Contamination Toxicology.* DOI 10.1007/s00244-008-9155-0.

Management Actions

This purpose of this project is to provide the individual refuge manager the information needed to manage biological integrity, biological diversity, and ecological health at both the local (or refuge) and watershed scale (Karr and Dudley 1981). This is an important responsibility since managers are responsible for ensuring that they protect biological integrity at multiple scales (Karr et al. 1986). In addition, the refuge manager is required to protect biological diversity, especially for multiple uses that can be conflicting at times (Karr and Dudley 1981). The following information is a summary of the information contained in this report meant to assist the refuge manager in making decisions concerning direct and indirect effects from contaminant impacts.

Determination of Impacts to Service Trust Resources

Our understanding of contaminant impacts on trust resources are based on the following hypotheses, which involve the biological response of assemblage structure and function and the specific signatures observed in multivariate cluster analysis of information.

1. Biological Organization: The project included ecosystem, community, and population organization levels. Surveys were conducted at the community level for fish, crayfish, and macroinvertebrate community responses to contaminants. The information collected from previous contaminant investigations, i.e., 1994 was used to direct field ecosystem survey evaluations of oil and brine point source impacts (Sobiech and Sparks 1994; Simon et al. 1995) and the 2001-2003 crayfish study (Simon 2004, 2005). These sites were used to assess trends of changes on the Patoka River National Wildlife Refuge. The study by Sparks and Sobiech (1997) provides trend information for the Muscatatuck National Wildlife Refuge, while the investigation by Litwin was used to evaluate Big Oaks National Wildlife Refuge. The data provides status, magnitude, and extent information that directly diagnose causal relationships between contaminants and aquatic biological assemblages. We differentiate between contaminant impacts and habitat disturbance based on the biological organization of structure and function. Specific water quality impacts can be differentiated from habitat loss. The identification of "hot-spots" using a pleth technique with spline smoothing generated visual documentation of impacts (Morris *et al*. 2006).

2. Measurement of Contaminant Effects: Measurement of contaminant effects was evaluated through direct measurement of adverse changes in species richness and diversity. Effects will be determined through changes in assemblage structure and presence of deformities, eroded fins, lesions, and tumors (DELT) anomalies.

3. Contaminant Source(s): Point sources in areas with impaired aquatic assemblages will be further investigated for management issues. Sources in the Patoka NWR sources include 225 identified oil derricks (Simon 2005), Black Beauty Coal Mine, NPDES point source discharges, and gob piles. The Muscatatuck NWR and Big Oak NWR sources include metal contamination from former military land use, nutrient runoff from fertilizers, feed lots, identified stream reaches from the State of Indiana 305(b) and 303(d) lists. Water quality analyses will be conducted using Standard Methods and samples were analyzed by the Indiana State Department of Health Chemistry Laboratory Division (Bowren and GhiasUddin 1999). Contaminants causing structural and functional changes in species assemblages will be identified, including variables that contribute synergistically or additively, and hot-spots will be identified through multivariate and GIS data analysis (Morris et al. 2006).

4. Contaminant Pathway(s): The major contaminant pathways to trust resources in these systems are through point-source effluents, non-point source runoff, and diffuse point sources (i.e., animal feed lots). Direct pathways include the surface water and groundwater discharge of brine effluents from drilling, the residual accumulation of contaminants in the vicinity of oil derricks, and the visual staining and smothering of sediments and habitat by oil by-products. In addition, acid mine leachate through surface water and groundwater discharge from gob piles causes impairment of areas by historic mining activities. Also, nutrient impacts result in agricultural areas from surface runoff of fertilizers, acute toxicity from pesticides, herbicides, and fungicides. Crayfish and most fish species have a limited home range and contaminants that affect tributaries and mainstem locations will continue to have long lasting effects on benthic inhabitants, reproductive success, and trophic energy transfer between levels.

C. Management Action(s)

Refuge managers will be able to access necessary contaminant information that will enable them to evaluate, manage, and restore habitats on refuge lands. This information will document the impacts and imminent threats associated with land use practices around and within Refuge boundaries. The benefit of the biological integrity diagnostic evaluation is that sites are scored and quantitatively assessed so that priorities can be developed from the results for restoration and remediation.

The following information is the list of management actions that were planned and accomplished during this project. These actions are written in italics, with specific notations to those actions beneath each action.

The current project will enable refuge managers to:

1) *Document contaminant impacts and evaluate previously unknown environmental problems on National Wildlife Refuges. The IBI is a powerful tool that will enable documentation of impacts from a variety of indicators. Fish, macroinvertebrate, and crayfish assemblages were used to assess the water body condition. Fish and macroinvertebrates are primary indicators and were correlated with patterns in water chemistry and habitat quality for local level interpretation of refuge contaminant status.*

The current study documents the extent and magnitude of impairments for each of the three National Wildlife Refuges in Indiana. This study found that measurement scale is an important consideration in study design. Results of this study found that differential response was observed by each biological indicator and that differences in response were not a measure of environmental sensitivity, but instead that each indicator measured different attributes of the environment. The refuge scale indicators measured different stressors than the watershed level scale for the same indicator. The results found that species respond to contaminants at the refuge or local scale and to habitat and land use stressors at the watershed scale. The reason for these differences is due to the reference or orientation of the stressor. It is apparent that stressors at the refuge scale may not influence areas immediately beyond the refuge boundaries and in some cases contaminants may be contained within the refuge and would not be of risk since no exposure was found.

2) *The collection of representative samples of biological assemblages would provide an opportunity to document the biodiversity of the aquatic biota of the National Wildlife Refuges. During the past 30 years, numbers of individuals and species diversity of native fish, crayfish, and macroinvertebrates have declined throughout the United States and Canada. The list of state threatened and endangered species continues to increase. This investigation will aid the Service in defining specific causes for the general decline in freshwater aquatic organisms near Indiana National Wildlife Refuges.*

This study provides important information for the three National Wildlife Refuges and documents the fish, crayfish, and aquatic macroinvertebrate assemblages found on each refuge. Standard operating procedures were used for sampling fish (Karr et al. 1986), macroinvertebrates (Simon and Stewart 1998), and crayfish (Simon 2004). The presence and absence of state and federal species of interest were documented, as were species such as the popeye shiner, which were found in the Big Oaks and had not been seen in the State of Indiana for over 100 years. New distribution records for fish on the Muscatatuck National Wildlife Refuge found the northernmost occurrence of the redspotted sunfish and flier, new records for harlequin and eastern sand darter (both state species of interest), and the presence of Sloan's crayfish at the majority of locations in the Vernon Fork Muscatatuck River. In addition, the Indiana crayfish, a former Federal candidate species, was observed at new locations in the vicinity of the Patoka River National Wildlife Refuge.

3) *Differential sensitivities are exhibited by species, especially during different life stages. Abundant data is available for evaluating fish assemblages, while crayfish and macroinvertebrate species are not as well developed for interpreting watershed scale conditions. By establishing baseline local conditions for each Refuge and then increasing scale to watershed levels, patterns in waterbody condition can be determined. The current study provides information on various aquatic fauna of the National Wildlife Refuges and provides an important benchmark in time that will enable future investigators to understand changes in land use, atmospheric changes, and long-term effects that may not be currently understood (i.e., endocrine disruption, reproductive impacts, etc.).*

This study provides benchmarks for biological integrity, biological diversity, and ecological health of three National Wildlife Refuges in Indiana. This snapshot in time provides two years of intensive investigation during a normal water year in 2006 and a drought year in 2007. The influence of low water levels, high temperatures, and stressful conditions can be an important source of information for modeling global temperature effects in the future. In addition, the documentation of land use (Choi and Engle 2003), habitat (Rankin 1989, 1995), and water quality conditions (Bowren and GhiasUddin 1999) provide important ecological health information for each refuge and the surrounding areas.

4) *The National Wildlife Refuges included in this pilot study is a national model for training contaminant personnel interested in being involved in Phase II aspects of this study. Results from this study should be used as a pilot to evaluate regional or national level patterns. By conducting a systematic evaluation of the National Wildlife Refuges, we could provide important national scale information in areas that should be among the most important conservation areas remaining in the United States.*

National Wildlife Refuges are important conservation areas and could be used to evaluate the status of wild areas in North America. These areas will become important migration corridors during future climate change and with sufficient planning, phenology studies can link these areas with other natural areas so that organisms can move north. The success of this project enables other contaminant biologists to monitor their systems and make important choices for their management areas so that the most up-to-date and relevant information is included in decision making processes, especially as contaminants and stressors are considered. Habitat restoration and monitoring success is dependent on knowing where "hot spots" are located so that bottlenecks and problems do not cause blocks to species use of these conservation oases and migration corridors (Morris et al. 2006).

5) *This information will be reported in the State of Indiana 305(b) report to Congress on the quality of the Nation's Surface Waters and appropriate sites will be included in the 303(d) Total Maximum Daily Load (TMDL) list of impaired waterbodies.*

The State of Indiana Department of Environmental Management Biological Studies Section, Office of Water Management and the Indiana State Department of Health Chemistry Laboratory Division provided important support of this project. Their investment in the chemical analyses and provision of enforcement sensitive information will enable the assessment of this information for reporting on the status of the Nation's surface waters. The information collected during this study is being used by the State of Indiana Department of Environmental Management and will appear in the 305(b) report in 2010 to Congress.

6) *The Wildlife Refuges in this study are components of the Ohio River Valley Ecosystem (ORVE). The ORVE encompasses FWS Regions 3, 4, and 5, and State resource agencies. The ORVE Team has ranked 6 resource issues for priority focus in their draft ecosystem plan (USFWS 1994). The #1 resource priority addressed in the ecosystem plan is to reverse the decline of mollusks and biodiversity within the Ohio River Valley Ecosystem with emphasis on endangered, threatened, and candidate species and species of concern. The study area has several rare and former Federal candidate species, including fish and crayfish species of interest to Region 3. The current study provides significant status information for species on National Wildlife Refuges.*

This study provided important biodiversity information that can be used for determining status of specific aquatic organism groups (see chapter entitled "Biological Assemblages of National Wildlife Refuges"). Species such as the Indiana crayfish, Sloan's crayfish, eastern sand darter, and harlequin darter were found to have stable populations on refuges and concerns about species decline was reduced. In addition, status of streams, rivers, ponds, wetlands, and lakes on the National Wildlife Refuge properties were assessed for biological integrity (Simon and Dufour 1998). Understanding of these areas, in light of biological diversity protection, is improved by the current study. The current study documented the fish, crayfish, and macroinvertbrate assemblages in both the Patoka and Vernon Fork Muscatatuck rivers. As a result, a strong baseline is established for future monitoring and assessment initiatives.

7) *The invasion of our waters by species that are nonindigenous or exotic species has become a priority of the Service. This study will provide an assessment of rusty crayfish Orconectes rusticus and evaluate the impact of nonindigenous species on rare or former candidate species. Records for sloan's crayfish, Indiana crayfish, and Hoosier cave crayfish will be determined as well as bluebreast darter Etheostoma camarum, spotted darter Etheostoma maculatum, eastern sand darter Ammocrypta pellucida and other potential candidate fish species. The presence of exotic and non-indigenous aquatic species will be documented on the NWRs.*

This study found that rusty crayfish has not invaded any of the National Wildlife Refuges, while instead finding sufficiently large populations of imperiled species such as Indiana crayfish and Sloan's crayfish on the refuge properties (see chapter entitled "Biological Assemblages of National Wildlife Refuges"). This study found no alien fish species present on either the Big Oaks or Muscatatuck National Wildlife Refuges. Presence of bighead carp, silver carp, and other asian carps were observed on Patoka River National Wildlife Refuge and important management decisions must be made to inhibit the stabilization of these alien populations in the refuge (see chapter entitled "Biological Assemblages of National Wildlife Refuges"). These species reach large sizes and can out compete native species causing collapse of important biodiversity areas on the refuges. Efforts to reduce and restore habitats that are not conducive to these species increase should be explored as habitat and restoration management decisions are made on the Patoka River. Little is known about macroinvertebrate populations; however, the current study provides important baseline information for monitoring future changes.

<u>Direct Actions</u>

- *Supporting information from qualitative habitat assessments assist the manager of each refuge to make habitat management decisions that take into account the presence of rare sensitive species, including the Indiana crayfish, Hoosier cave crayfish, Sloan's crayfish, eastern sand darter, spotted darter, and bluebreast darter. This information provides specific habitat requirements for these species and enables refuge managers to provide specific habitat restoration options. For example, in the Patoka River NWR habitat information will enable the refuge manager to provide notice of violations to well operators. These violations can use habitat restoration options that can be designed to benefit the Indiana crayfish.*

Qualitative habitat information was collected from each site sampled during this study (Rankin 1989, 1995). The primary use of this information was to evaluate stressor gradients on the refuges based on contaminant, general chemistry, land use, and habitat information. A secondary use of this habitat information can be compiled for areas where important species are known and a factor analysis of this information can be done to evaluate habitat attributes that can determine important factors for the presence of these rare and sensitive species. For example, analysis of site information where Indiana crayfish were found determined that it requires cobble or firm rock cover, stable riparian corridors, and natural riffle habitats. Analysis of sites where Sloan's crayfish were found was correlated with high quality pool-glide habitats and a lack of nutrients. The acquisition of land use, habitat, contaminant, and chemical information can provide a complete picture of species needs.

- *Information gained from this project is essential to future development of an aquatic ecosystem management plan for the protection of all aquatic resources in*

the Patoka and Vernon Fork of the Muscatatuck rivers and tributaries and their associated Wildlife Refuges.

This study is the most complete probability based design study of the Patoka and Vernon Fork Muscatatuck rivers ever completed and used 50 sites to assess the Patoka River National Wildlife Refuge and 125 sites to assess the Muscatuck and Big Oaks National Wildlife Refuges (Simon et al. 1995). In addition, the State of Indiana provided watershed scale information for another 38 sites in the Patoka River and 63 sites in the Vernon Fork Muscatatuck River so that assessments at the watershed scale could be made to assist the refuge in assessing biological integrity, biological diversity, and ecological health of the areas surrounding the refuges.

- *This project addresses the Ohio River Valley Ecosystem Plan's #1 and #6 resource priorities of defining threats to sensitive species residing in ORVE focus areas. Data collected from this study will aid the ecosystem team in focusing and prioritizing projects within the Ohio River Ecosystem.*

The current study has assessed the three National Wildlife Refuges in Indiana and has identified areas that are in need of remediation. These areas if managed properly, can be isolated so that reduced environmental risk associated with the stressors will affect aquatic assemblages occurring in these areas. This study has identified the biological integrity of each river, stream, pond, and lake occurring on the refuge (see Appendices A and B) and threats to the aquatic wildlife of these areas has been identified through the stressor identification model (Morris et al. 2006).

<u>Indirect Actions</u>

- *Sites that are sampled during this study were reported to the State of Indiana for inclusion in the 305(b) Report to Congress on the status of the nation's surface waters. Currently, none of the surface waters on the NWRs were reported by the state because of lack of information. Stream segments surrounding the NWR's are listed as biologically impaired in the State of Indiana 305(b) report to Congress and the 303(d) list.*

This study has provided a comprehensive and complete assessment of the three National Wildlife Refuges in Indiana. This project has assessed 100% of the surface water stream miles occurring on Indiana refuges. By using probability sample design these areas equally represent areas that were not sampled. Thus, the stratified assessment provides an important linkage to larger scale refuge issues. The hot spot pleths provide information on the scale and magnitude of stressors and specific stressors are identified for each refuge (Morris et al. 2006).

- *Chemical data will enable the actual measurement of chemical contaminants that are influencing aquatic assemblages. This information provides important datasets that enable listing of streams that are impaired under 303(d) TMDL. If a*

stream reach is impaired and listed as a 303(d) listed reach, the State of Indiana and the USEPA would be required to determine the level of impact and provide resources necessary for source identification, remediation, and delisting of the site.

Karr and Yoder (2004) found that use of biological assemblage indicators increased the assessment capability of watershed monitoring. As a result of the high level of contaminant information obtained from this study the following measures are being investigated to determine the impairment of surface waters beneath Clean Water Act Section 303(d). These waters are impaired and will require remediation in order to meet aquatic life designated uses as defined by the State of Indiana in the Water Quality Standards Code.

Patoka River National Wildlife Refuge

Several large scale disturbance gradients were found in the Patoka River in the vicinity of the National Wildlife Refuge. The legacy acid mine drainage leachate and oil brine effluents have had significant impact on the area surrounding the refuge (Simon et al. 1995; Simon 2005). Alkalinity, conductivity, and pH were associated with this stressor signature. Another signature was associated with the presence of grass-pasture land use, which was one of a few variables that explained fish assemblage structure. Simon (2005) and Simon and Morris (in press) found that these same identified areas were associated with oil derricks and brine effluent discharge. Even though this study did not measure polyaromatic hydrocarbons (PAHs), these volatile organics would not be expected to be in high quantities in the surface water, but rather would be found in high amounts in the sediments (Simon 2005; Simon and Morris in press). This response signature is associated with oil brine effluents.

The second stressor pattern identified in the Patoka River National Wildlife Refuge study included chloride, which was associated with municipal wastewater treatment facilities found throughout the study area (Miltner and Oswood 2000).

Macroinvertebrates are considered a better indicator organism than fish at identifying stressors at the refuge scale. Macroinvertebrates identified more stressors at the refuge scale than any other biological indicator. Aluminum, cadmium, lead, manganese, nickel, and zinc were all associated with the South Fork Patoka River and explained the macroinvertebrate structure (Figure 7, pp. 36-37). Ammonia response was directly related to the treatment facility in Oakland City and the South Fork Patoka River (Figure 7, pp. 36-37). Crayfish were responsive to elevated conductivity levels (Figure 7, pp. 36).

At the watershed scale, heavy metals, waste water treatment, and chemical oxygen demand explained the variance observed in fish assemblage structure in the Patoka River (Figure 5, pp. 16-18). These variables were associated with similar stressor response found at the refuge scale for all three biological indicators, but substantially improved the scale response observed by the fish assemblage indicator.

Big Oaks National Wildlife Refuge

Three contaminant response signatures were identified on the Big Oaks National Wildlife Refuge (See Figure 14, pp. 63-66). The first area was a groundwater signature that explained fish assemblage structure based on arsenic distribution. The second response signature was identified in 2006 from a nutrient and wastewater treatment response signature that explained crayfish assemblage structure from the headwaters of Little Graham Creek (see chapter entitled "Identification of stressors affecting refuge scale indicators at Indiana National Wildlife Refuges"). Phosphorus, nitrate + nitrite, sodium, chloride, fluoride, and sulfate emanated from the uppermost site on Little Graham Creek (site 10). This contaminant load impaired the Little Graham Creek watershed downstream to the East Perimeter Road at the refuge border. Further investigation during 2007 found that the source of the problem was land application of sludge from the City of Versailles that apparently was running off the fields into the adjacent creek. This single source of nutrient pollution accounted for the majority of the refuge scale response and half of the watershed response in the vicinity of the Big Oaks National Wildlife Refuge (see chapter entitled, "Identification of stressors affecting watershed level scale indicators at areas surrounding Indiana National Wildlife Refuges").

The third response signature explained macroinvertebrate assemblage structure based on the distribution of lead, manganese, and barium, which originated on the refuge in the vicinity of the unnamed tributary of Big Creek. This signature may be a result of heavy metal exposure from artillery ordnance on the refuge.

Muscatatuck National Wildlife Refuge

Stressors responsible for assessing the aquatic assemblage structure included chlorides, which are a response signature of wastewater treatment effluents. Alkalinity, calcium, hardness, and conductivity are most likely related to wastewater treatment and occur in areas surrounding the refuge (Figure 21, pp. 87-88). Sandy Branch, the unnamed tributary of Richart Lake, and Vernon Fork Muscatatuck River all show a similar response from these contaminants. These areas do not possess high amounts of karst habitats. Possible explanations include failed septic systems or discharge of groundwater through field tiles. Arsenic, iron, phosphorus, and total solids are groundwater signatures that were observed on the refuge in the area near Linda Lake and Mutton Creek (Figure 21, pp. 87-88). High levels of these contaminants show agricultural affects from field tile discharge.

Perhaps the most surprising stressor identified at Muscatatuck National Wildlife Refuge is the presence of a mercury hot spot on the eastern edge of Moss Lake downstream of Stansfield Lake outlet. This is the first mercury spike observed in surface water chemical monitoring anywhere in the State of Indiana. A further effort to identify the source of the

contamination is needed to ensure that bioaccumulation of sport fishes is not occurring in Stansfield or Moss lakes (Figure 21, pp. 88).

The low dissolved oxygen level throughout the Muscatatuck National Wildlife Refuge is a concern. Levels below 4.0 mg/L is considered insufficient for supporting aquatic life. Low levels were measured (i.e., 1.06 mg/L) and mean dissolved oxygen was well below saturation (mean = 5.65 mg/L). It is important to recognize that stagnate conditions and the lack of stream flow exaggerated this situation during 2007; however, drought conditions had not aggravated the situation when the survey was conducted in June. This situation is a directly related to Moss Lake, which is an artificial retention basin that stabilizes water levels on the agricultural landscape. Fish species occurring in this lake include those capable of breathing atmospheric oxygen, such as bowfin and central mudminnow. Management consideration should be made to balance the need of the shallow surface water for migrating waterfowl and the aquatic resources of the refuge including Storm ditch and Mutton Creek watersheds.

In conclusion, the current study identified stressors to aquatic resources occurring on National Wildlife Refuges in Indiana. Causal relationships between biological assemblages structure were measured by the index of biotic integrity (IBI) for fish and the Shannon-Weiner index (H') for macroinvertebrates and crayfish. These three biological assemblage indicators showed distinct and differential patterns when exposed to contaminant, land use, habitat, and general chemistry stressors. The extent and magnitude of response was graphically shown using the "hot spot" GIS plething technique, which is based on spline-smoothed graphing of the information. It is apparent that correspondence between biological indicators should not be expected with each indicator being related to specific contaminants. For example, fish were most sensitive to land use and refuge scale contaminants in the surface water. Macroinvertebrates were responding to groundwater infiltration of contaminants and was the only indicator that reflected habitat stressors. Crayfish structure was associated with nutrient and conductivity stressors and should be further studied as an environmental indicator. The lack of correspondence of information between indicators at the refuge scale also showed a lack of correspondence at watershed level scales among indicators. Fish assemblage structure was causally related to contaminants at the refuge assessment scale, but was primarily related to land use at the watershed scale.

REFERENCES

Bowren T and G. GhiasUddin. 1999. Quality Assurance Project Plan for Indiana Surface Water Quality Monitoring Programs. (Revision 2, June 1999), IDEM/32/01/1442/1999. Indiana Department of Environmental Management, Indianapolis, Indiana.

Choi J. and B.A. Engel. 2003. Watershed Delineation: Web-GIS tools for Spatial Hydrologic Analysis. http://pasture.ecn.purdue.edu/~jychoi/wd_home/

Karr, J.R. and D.R. Dudley. 1981. Ecological perspective on water quality goals. *Environmental Management,* 5:55-68.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant, and I.J. Schlosser. 1986. *Assessing biological integrity in running waters, a method and its rational*. Illinois Natural History Survey, Special Publication 5.

Karr, J.R., Yoder CO. 2004. Biological assessment and criteria improve TMDL planning and decision making. *Journal of Environmental Engineering* 130:594-604.

Leopold LB, Woolman MG, Miller JP. 1964. *Fluvial processes in geomorphology* W.H. Freeman, San Francisco, CA.

Milner AM, Oswood MW. 2000. Urbanization gradients in streams of Anchorage, Alaska: a comparison of multivariate and multimetric approaches to classification. *Hydrobiologia* 422/423: 209-223.

Morris, C.C., Simon T.P., Newhouse, S.A. 2006. A local-scale *in situ* approach for stressor identification for biologically impaired aquatic systems. *Archives of Environmental Contamination and Toxicology* 50: 325-334.

Rankin, E.T. 1989. *The qualitative habitat evaluation index (QHEI), rationale, methods, and application*. Ohio EPA, Division of Water Quality Planning and Assessment, Ecological Assessment Section, Columbus, Ohio.

Rankin, E.T. 1995. The use of habitat assessments in water resource managementprograms. In, Davis, WS, Simon TP, eds, *Biological Assessment and Criteria: Tools for Water Resource Planning and Decision Making.* CRC, Boca Raton, Florida, pp 181-208.

Simon, T.P. 2003. *Biological Response Signatures: Indicator Patterns using Aquatic Communities.* CRC Press, Boca Raton, Florida.

Simon, T.P. 2004. Standard Operating Procedures for the collection and study of burrowing crayfish in Indiana. I. Methods for the collection of burrowing crayfish in streams and terrestrial habitats. *Miscellaneous Papers of the Indiana Biological Survey Aquatic Research Center* 2:1-16.

Simon, T.P. 2005. *Defining Brine and Oil Threats in the Patoka River National Wildlife Refuge*. Department of the Interior, U.S. Fish and Wildlife Service, Bloomington, Indiana.

Simon, T.P. and R.Dufour. 1998. *Development of Index of Biotic Integrity Expectations for the Ecoregions of Indiana. V. Eastern Corn Belt Plain*. EPA 905-R-96-004. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.

Simon, T.P. and C.C. Morris. In press. Biological response signature of oil brine threats, sediment contaminants, and crayfish assemblages in an Indiana watershed, USA. *Archives Environmental Contamination and Toxicology*.

Simon, T.P., S.A. Sobiech, T.H. Cervone, and N.E. Morales. 1995. Historical and present distribution of fishes in the Patoka River drainage: Pike, Gibson, and Dubois Counties, Indiana. *Proceedings of the Indiana Academy of Science* 104: 193-206.

Simon, T.P. and P.M. Stewart. 1998. *Standard Operating Procedures for development of watershed indicators in REMAP: Northern Lakes and Forest Streams*. U.S. Environmental Protection Agency, Region 5, Chicago, Illinois.