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Breakpoint Analysis and Relations of Nutrient and Turbidity Stressor Variables to Macroinvertebrate Integrity in Streams in the Crawford-Mammoth Cave Uplands Ecoregion, Kentucky, for the Development of Nutrient Criteria

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Cover photograph: Hardins Creek at Stone Hill Fork near Hardinsburg, Kentucky (USGS STAID 03303202), August 27, 2007. (Photograph by Kentucky Division of Water.)

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Conversion Factors

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
kilometer (km)	0.6214	inch (in)
mile (mi)	1.609	kilometer (km)
	Area	
square kilometer (km^2)	247.1	acre
square kilometer (km^2)	0.3861	square mile (mi^2)
	Volume	
liter (L)	0.2642	gallon (gal)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of nutrients in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L). Concentrations of turbidity are given in Formazin nephelometric units.

Abbreviations/Acronyms

CHL*a*	chlorophyll a
EPT	Ephemeroptera, Plecoptera, and Trichoptera
FNU	Formazin nephelometric units
HBI	Hilsenhoff's Biotic Index
KDOW	Kentucky Division of Water
N	nitrogen
NO_2+NO_3-N	nitrite plus nitrate, as nitrogen
P	phosphorus
r_2	coefficient of determination
τ	Kendall tau statistics
TKN	Total Kjeldahl nitrogen
TN	total nitrogen
TNI	Total Number of Individuals
TP	total phosphorus
TR	Taxa Richness
TV	Tolerance Value
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey

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Breakpoint Analysis and Relations of Nutrient and Turbidity Stressor Variables to Macroinvertebrate Integrity in Streams in the Crawford-Mammoth Cave Uplands Ecoregion, Kentucky, for the Development of Nutrient Criteria

By Angela S. Crain and Brian J. Caskey

Abstract

To assist Kentucky in refining numeric nutrient criteria in the Pennyroyal Bioregion, the U.S. Geological Survey and the Kentucky Division of Water collected and analyzed water chemistry, turbidity, and biological-community data from 22 streams throughout the Crawford-Mammoth Cave Upland ecoregion (U.S. Environmental Protection Agency Level IV Ecoregion, 71a) within the Pennyroyal Bioregion from September 2007 to May 2008. Statistically significant and ecologically relevant relations among the stressor (total phosphorus, total nitrogen, and turbidity) variables and response (macroinvertebrate-community attributes) variables and the breakpoint values of biological-community attributes and metrics in response to changes in stressor variables were determined.

Thirteen of 18 macroinvertebrate attributes were significantly and ecologically correlated (p-value < 0.10) with at least one nutrient measure. Total number of individuals, Ephemeroptera-Plecoptera-Trichoptera richness, and average tolerance value were macroinvertebrate measures that most strongly correlated with the concentrations of nutrients. Comparison of the average macroinvertebrate-breakpoint value for the median concentration of total phosphorus (TP, 0.033 mg/L) and for median concentration of total nitrogen (TN, 1.1 mg/L) to Dodds' trophic classification for TP and TN indicates streams in the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion would be classified as mesotrophic-eutrophic. The biological breakpoint relations with median concentrations of TP in this study were similar to the U.S. Environmental Protection Agency proposed numeric TP criteria (0.037 mg/L), but were 1.5 times higher than the proposed numeric criteria for concentrations of TN (0.69 mg/L). No sites were impacted adversely using median turbidity values based on a 25 Formazin nephelometric turbidity unit biological threshold. The breakpoints determined in this study, in addition to Dodds' trophic classifications, were used as multiple lines of evidence to show changes in macroinvertebrate community and attributes based on exposure to nutrients.

Introduction

In 1996, the U.S. Environmental Protection Agency's (USEPA) National Water Quality Inventory identified excess amounts of nutrients as the second leading cause of impairment in rivers and streams and as the primary cause of impairments in lakes and reservoirs (U.S. Environmental Protection Agency, 1997). The excess amounts of nutrients, primarily nitrogen and phosphorus, are commonly cited as principal reasons why water bodies in Kentucky do not fully support their designated uses (Kentucky Energy and Environment Cabinet, 2008a). Many rivers and streams in Kentucky have been listed as impaired streams for nutrients in the state's 2008 Integrated Report to Congress on the Condition of Water Resources in Kentucky (Kentucky Energy and Environment Cabinet, 2008a). Recently, a model of the Mississippi River Basin found Kentucky was one of nine states that contributes the majority of nitrogen (N) and phosphorus (P) to the Gulf of Mexico, resulting in hypoxia of those waters (Alexander and others, 2008). Although nutrients are necessary for the growth of plants and animals, excessive amounts can be detrimental to aquatic ecosystems and to the health of organisms living in and using water and can limit human uses of a water body. For example, elevated concentrations of nutrients can lead to excessive aquatic plant growth that can reduce the amount of dissolved oxygen in the water and alter the stream habitat, both of which are critical for fish and other aquatic life. Excessive aquatic plant growth also can interfere with recreational activities, such as fishing, swimming, and boating, and can cause unpleasant taste and odors in drinking water.

In 1998, a new initiative to the Clean Water Act called the Clean Water Action Plan was launched to direct states, in conjunction with the USEPA, to develop numeric criteria for total phosphorus and total nitrogen (causal variables) along with chlorophyll *a* (CHL*a*) and turbidity (response variables) (U.S. Environmental Protection Agency, 1998). Although individual states are responsible for establishing their own water-quality criteria, the USEPA recommended ecoregional nutrient water-quality criteria as a baseline and has provided guidance to states for establishing more precise numeric water-quality criteria for nutrients to protect aquatic life and recreational and other uses of rivers and streams on a site-specific or subregion-specific basis (U.S. Environmental Protection Agency, 2000a and b). The USEPA-recommended ecoregional nutrient criteria are based on USEPA Aggregated Nutrient Ecoregions that are areas with similar geographic features including topography, soils, geology, land use, and biogeography (U.S. Environmental Protection Agency, 2000c and d). The USEPA established recommended nutrient criteria for nitrate as nitrogen (NO_3-N), total Kjeldahl nitrogen as nitrogen (TKN-N), total nitrogen (TN), and total phosphorus (TP) based on either the 25^{th} percentile of median concentrations at all sampled sites or the 75^{th} percentile when only reference-site concentration data are used. Discussions of the USEPA numeric nutrient criteria relevant to this study are limited to TP, TN, and turbidity.

Kentucky is subdivided into seven Level III Ecoregions: Southwestern Appalachians, Central Appalachians, Western Allegheny Plateau, Interior Plateau, Interior River Valley and Hills, Mississippi Alluvial Plain, and Mississippi Loess Plain (Woods and others, 2002). Because Kentucky has a diverse topography, an empirical regional classification scheme based on river basins, physiographic regions, and ecoregions was developed by the Kentucky Division of Water *(http://www.epa.gov/wed/pages/ecoregions/ky_eco.htm#Principal%20 Authors*, Woods and others, 2002) to help clarify inherent biological differences in the state (Pond and others, 2003; Pond and McMurray, 2002; and Pond and others, 2000). For macroinvertebrates, four bioregions have been established: Bluegrass, Mountains, Mississippi Valley-Interior River, and Pennyroyal. Only the Crawford-Mammoth Cave Uplands (USEPA Level IV Ecoregion, 71a) within the Pennyroyal Bioregion was examined in this study.

Although nutrients are nontoxic to biological organisms at most ambient concentrations (Miltner and Rankin, 1998), relations between nutrient concentrations (causal variables) and biological attributes (response variables) are important for evaluating the effects of eutrophication on river and stream ecosystems. In a typical stressor-response relation, a change in a stressor (causal) variable (TP and TN, in the case of nutrients) results in a corresponding change in a response variable. Significant relations between nutrients and algal biomass have been found in streams (Biggs, 2000; Dodds and others, 2002; Stevenson and others, 2006). Robertson and others (2006) found significant relations between nutrients and periphyton CHL*a* in Wisconsin. However, other recent studies in nutrient-rich streams have found no significant relations or weak relations between nutrient concentrations and algal biomass (Figueroa-Nieves and others, 2006; Caskey and others, 2007; Frey and others, 2007; Leer and others, 2007; Lowe and others, 2008; Royer and others, 2008). The mixed results of these studies suggest that relations between nutrient concentrations and CHL*a* may not be applicable elsewhere and that developing nutrient-criteria for rivers and streams in nutrient-rich areas may be problematic. In addition, nutrient thresholds based on relations between nutrients and algae may not be the same as those between nutrients and macroinvertebrates, because macroinvertebrates are indirect consumers of nutrients and generally are the direct indicators of stream health (Wang and others, 2007). Thus, using other biological community attributes and metrics (that is, macroinvertebrate communities) could be helpful in developing nutrient criteria for rivers and streams.

Statistical analyses that directly relate eutrophication (stressor) to biological indicators or attributes can be used to develop ecologically meaningful nutrient criteria. Previous nutrient and algal biomass studies have shown mixed results because linear statistical techniques were applied to nonlinear data. Instead, nonlinear statistical techniques (breakpoint analysis) may be better at discerning the relations between nontoxic stressor and response variables and identifying breakpoints (Qian and others, 2003). Development of defensible nutrient criteria for rivers and streams in nutrient-rich areas will be complex and require intensive data analysis.

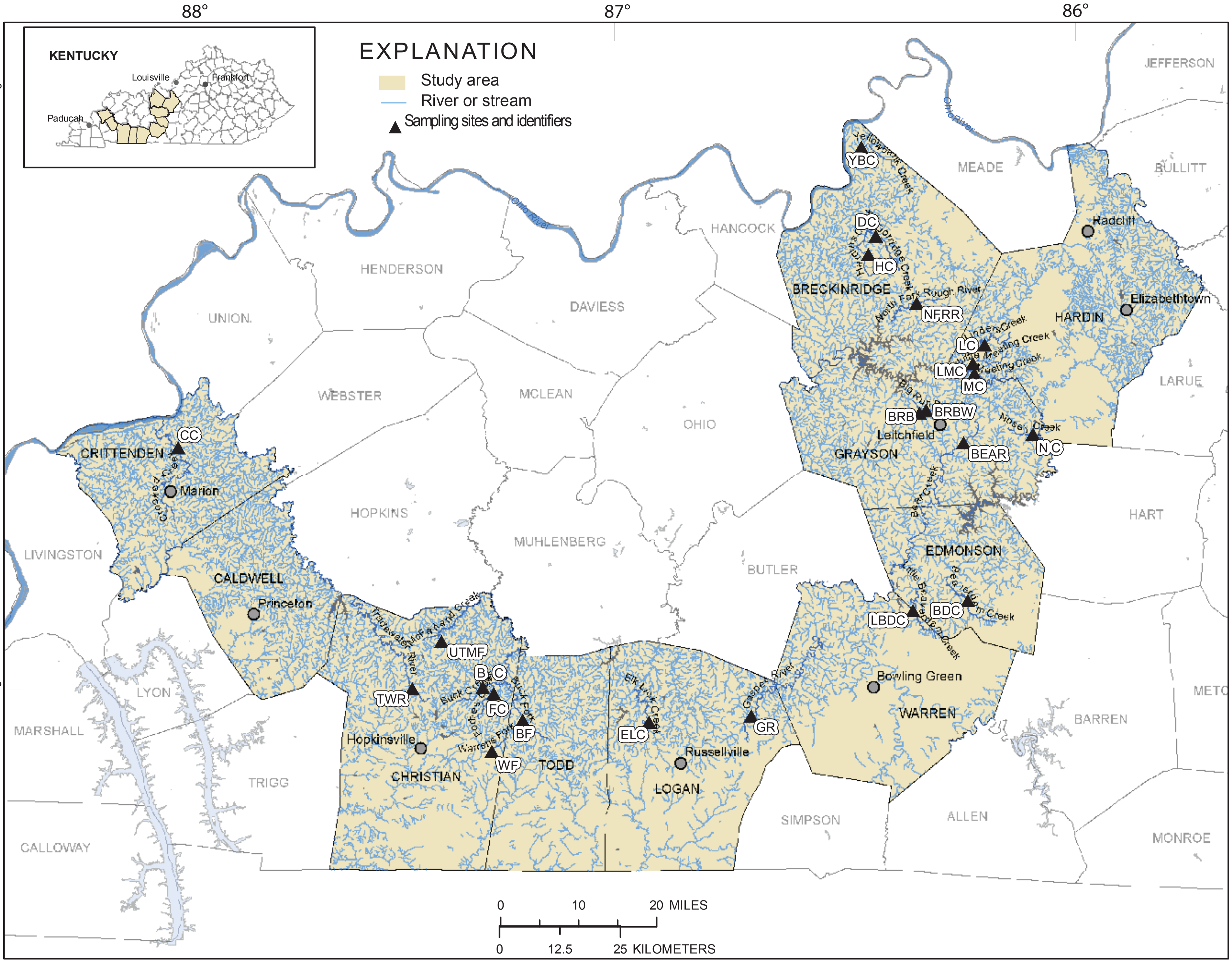
In 2007, the U.S. Geological Survey (USGS), the USEPA, and the Kentucky Energy and Environment Cabinet–Kentucky Division of Water began a cooperative study to identify statistically significant and ecologically relevant relations between casual variables (TP, TN, and turbidity) and response variables (macroinvertebrate-community attributes) at 22 sampling sites in the Crawford-Mammoth Cave Uplands ecoregion (USEPA Level IV Ecoregion, 71a) within the Pennyroyal Bioregion as defined by the Kentucky Division of Water (Kentucky Energy and Environment Cabinet, 2003).

Purpose and Scope

This report summarizes the statistically significant and ecologically relevant relations between the causal variables (TP, TN, and turbidity) and the biological response variables (macroinvertebrate-community attributes) in the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion. Breakpoint analysis was used to detect the concentration of the stressor variables where there was a significant change in the biological response variables. Results of the breakpoint analysis will provide the Kentucky Division of Water with relevant information that could be useful for evaluating which potential macroinvertebrates indicators are most appropriate for the development of nutrient criteria for rivers and streams in the Pennyroyal Bioregion.

Description of Study Area

The 22 sampling sites for this study were selected from the Kentucky Division of Water's Reference Reach Program and are located within the Crawford-Mammoth Cave Uplands ecoregion (part of the Pennyroyal Bioregion). Thirteen sampling sites were located northeast of Bowling Green, Ky., and nine sites were located west and northwest of Bowling Green, Ky (fig.1). Sampling-site drainage areas ranged from 6.99 km^2 to 101 km^2 (table 1).



Base from U.S. Geological Survey digital data, 2004, NAD83, Lambert Conformal Conic

Figure 1. Location of the sampling sites used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study in Kentucky, 2007–08.

Table 1. Names and locations of sampling sites used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study in Kentucky, 2007–08.

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; STAID, station identification; KDOW, Kentucky Division of Water; dd.mm.ss, degrees minutes seconds; KY, Kentucky; UT, unnamed tributary; km^2, square kilometers]

Sampling site name (refer to figure 1 for location)	USGS STAID	KDOW stream name	County	Latitude (dd.mm.ss)	Longitude (dd.mm.ss)	Site type[2]	Drainage area (km^2)
BC1	03320300	Buck Creek 0.3 mi below KY 189 below 2nd UT	Christian	36 58 57	-87 20 56	Wadeable	16.1
BDC	03311600	Beaverdam Creek at KY 101 and KY 259	Edmonson	37 09 14	-86 13 34	Wadeable	28
BEAR	03311800	Bear Creek at St. Augustine Church Road near Clarkson	Grayson	37 26 52	-86 14 18	Wadeable	27.5
BF	03320100	Buck Fork Pond River above KY 507 off Flat Rock Road	Todd	36 55 20	-87 15 18	Wadeable	41.2
BRB	03317940	Big Run Branch at Sunbeam Road near Leitchfield	Grayson	37 30 13	-86 20 27	headwater	11.9
BRBW	03317395	Big Run Branch at Watson School Road near Leitchfield	Grayson	37 30 28	-86 19 35	headwater	6.99
CC	03384330	Crooked Creek below Turkey Knob Road	Crittenden	37 24 58	-88 04 03	Wadeable	101
DC	03303203	Dorridge Creek at KY 1385 near Hardinsburg	Breckinridge	37 49 50	-86 26 49	Wadeable	42.3
ELC	03316110	Elk Lick Creek 0.2 miles below Epleys-Stuart Church Road bridge	Todd	36 55 21	-86 57 45	Wadeable	59.3
FC	03320200	Forbes Creek 0.1 miles above SR 189 bridge	Christian	36 58 15	-87 19 23	Wadeable	25.9
GR	03315180	Gasper River 0.2 miles above Bucksville Road bridge	Logan	36 56 10	-86 43 33	Wadeable	68.1
HC	03303202	Hardins Creek at Stone Hill Fork near Hardinsburg	Breckinridge	37 47 46	-86 27 49	Wadeable	18.4
LBDC	03311700	Little Beaverdam Creek on Pleasant Grove Road	Warren	37 08 04	-86 21 13	Wadeable	34.4
LC	03316870	Linders Creek at KY 920 near White Mills	Hardin	37 37 47	-86 11 19	Wadeable	72.3
LMC	03316900	Little Meeting Creek at Limp Road near Leitchfield	Grayson	37 35 40	-86 13 04	Wadeable	31.6
MC1	03316885	Meeting Creek at KY 920 near Big Clifty	Grayson	37 34 34	-86 12 59	Wadeable	69.2
NC	03310340	Nosey Creek at Mt. Zion Road near Millerstown	Grayson	37 27 48	-86 04 34	Wadeable	23.8
NFRR	03317400	North Fork Rough River at KY 1073 near Hardinsburg	Breckinridge	37 42 28	-86 20 49	Wadeable	28.2
TWR1	03382550	Tradewater River at Sparkman Road	Christian	36 58 40	-87 30 44	Wadeable	57
UTMF	03320720	UT McFarland Creek 0.1 miles below Flowers Road	Christian	37 03 58	-87 26 45	Wadeable	17.3
WF	03437450	Warrens Fork above Vaughns Grove Road	Christian	36 51 48	-87 19 31	Wadeable	14.2
YBC	03302500	Yellowbank Creek at Cart-Manning Crossing Road Wildlife Management Area	Breckinridge	37 59 44	-86 28 56	Wadeable	40.9

[1] Macroinvertebrate community data not collected

[2] Site type based on Kentucky criteria for the size of drainage area.

The Crawford-Mammoth Cave Uplands ecoregion is composed of hilly uplands containing cliffs and wide karst valleys (Woods and others, 2002). Sinkholes, caverns, springs, and subterranean drainage are common; however, surface drainage can be significant. The mean monthly temperature ranges from -6.11°C to 7.78°C in winter (December to March) and 18.33°C to 32.78°C in summer (June to September); the mean annual precipitation in this area ranges from 107 to 130 centimeters (Woods and others, 2002). Soils in the study area are nutrient-rich and fertile. A mixture of forests, pasture land, and crop land occur; farming is widespread throughout the bioregion.

Study Design and Methods

Nutrient, turbidity, and macroinvertebrate-community data were collected at sampling sites on first- to fourth-order streams in the Crawford-Mammoth Cave Uplands Ecoregion, Kentucky (USEPA Level IV Ecoregion, 71a). The sampling sites (fig.1 and table 1) were selected by the Kentucky Division of Water. Nutrient samples were collected at 22 sites from September 2007 to May 2008. Macroinvertebrate-community samples were collected at 19 sites (table 1). These samples were collected in the fall of 2007 and spring of 2008.

Site Selection

Twenty-two sites were selected in the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion to determine how the biotic integrity of the streams responds to changes in nutrient concentrations and turbidity values. The sampling sites were specifically selected by the Kentucky Division of Water as part of their Reference Reach Program (Kentucky Energy and Environment Cabinet, 2003). This approach is based on the range of natural conditions found in streams with similar physical characteristics and minimal human impact. A typical reference reach watershed contains a high proportion of natural vegetation and has minimal point-source discharges, agricultural land, mining, and urban development.

Sample Collection and Processing Methods

Nutrient and turbidity data were collected by USGS personnel from September 2007 to May 2008 at all 22 sampling sites. Nutrient samples were collected following Kentucky Division of Water Water-Quality Monitoring Standard Operating Procedures (Kentucky Energy and Environment Cabinet, 2005). Turbidity was measured using a multiparameter sonde at the time of nutrient sampling. The macroinvertebrate-community data used in this report was collected by the Kentucky Division of Water. Macroinvertebrate-community data were collected in the spring of 2008 at 19 sampling sites.

Nutrients and Turbidity

Nutrient samples (ammonia as nitrogen (NH_3-N), total Kjeldhal nitrogen as nitrogen (TKN), nitrite plus nitrate as nitrogen (NO_2+NO_3-N), and TP were collected 9–11 times per sampling site from September 2007 to May 2008 by USGS personnel following approved Kentucky Division of Water water-quality monitoring methods (Kentucky Energy and Environment Cabinet, 2005). The sample-collection method used in this study was the nonisokinetic dip-sampling method. All nutrient samples were preserved with sulfuric acid, placed on ice, and transported to the Kentucky Division of Environmental Services Centralized Laboratory Facility for analysis. Nutrient samples were analyzed by colorimetric methods (U.S. Environmental Protection Agency, 1993). Concentrations of nutrients discussed in this report represent their concentrations expressed as either nitrogen or phosphorus. For example, a concentration of nitrate expressed at 10 milligrams per liter (mg/L) refers to a concentration of nitrate as 10 mg/L as nitrogen. In addition, turbidity was measured during each site visit at the time of nutrient sampling by use of a YSI Inc., Model 6920 multi-parameter sonde outfitted with a YSI Inc. 6136 turbidity sensor. The multiparameter sondes were calibrated daily. A statistical summary of the concentration of nutrients and turbidity at each site is found in appendix 1.

Macroinvertebrate Communities

The macroinvertebrate communities were assessed by the Kentucky Division of Water in May 2008 at 19 of the 22 sites (Kentucky Energy and Environment Cabinet, 2008b). Community attributes and metric scores were calculated for the macroinvertebrate-community data, and metric scores were determined by Kentucky Division of Water personnel upon completion of the assessments (Kentucky Energy and Environment Cabinet, 2008b).

Data Analysis

Stressor variables (TP, TN, and turbidity) were analyzed to determine if a gradient (range) was observed, and then the observed data gradients were compared to published trophic-level boundaries (Dodds and others, 1998), in the case of nutrients, and published biological impacts (Klein and others, 2008), in the case of turbidity. The stressor variables were then correlated to the response variable (macroinvertebrate attributes) to identify the statistically and ecologically significant relations among the stressor and response variables. By use of the statistically significant correlations observed as a guide, scatterplots were created between the relations of interest to determine if the relations of interest were ecologically significant. Finally, breakpoints were computed for the ecologically significant relations to determine the concentration of the nutrient variables where the greatest change (breakpoint) was observed in the response variable.

Boxplots of the median stressor variables (TP, TN, and turbidity) were created (TIBCO Spotfire S+® 8.1 for Windows® User's Guide, 2008) for the macroinvertebrate sites. The published trophic-level boundaries (Dodds and others, 1998), in the case of nutrients, and published biological-impact value (Klein and others, 2008), in the case of turbidity, were overlaid on the boxplots. Next, the stressor variables were correlated to the response variable using Kendall tau (τ) rank correlation. Environmental data are not normally distributed, so a nonparametric procedure, such as Kendall tau (τ) is the preferred method for determining relations between variables (Helsel and Hirsch, 2002).

In this report, for a correlation to be considered statistically significant, the Kendall tau rank correlation was required to have, at most, a 10-percent significance level ($\alpha = 0.10$). Although a $| \tau |$ with a significance level of 0.10 is considered significant, there is a possibility of introducing a Type I error in which the relation is declared present when the relation is not present (Helsel and Hirsch, 2002). Several procedures—such as the Bonferroni correction—are available for adjusting the significance level when performing a large number (or "family") of tests simultaneously (Van Sickle, 2003). This adjustment reduces the chances of a Type I error at a specific alpha level. Although useful for reducing Type I error, this technique increases the chance of producing a Type II error, in which no relation is declared when a relation is present. Because ecological significance was weighted higher than statistical significance and the data set was small, no Bonferroni corrections were applied to the data analysis for this report.

Bootstrap regression-tree analysis was used in TIBCO Spotfire S+ (TIBCO Spotfire S+® 8.1 for Windows® user's guide, 2008) to determine the concentration where the greatest change was observed for each ecologically significant stressor-response relation. For the nutrient stressor variables (TP and TN), the mean breakpoints for the ecologically significant attributes from the macroinvertebrate dataset were determined. Confidence intervals for the bootstrap regression-tree analysis were used to determine the confidence intervals at the 90th percentile of the median breakpoints identified in the regression-tree analysis. Bootstrapping simulates the results of repeated experiments based on the observed data by randomly selecting subsets of the observed data. The approach used in the bootstrap regression-tree preserved sampling along a gradient and allowed for the variables of the distribution to change along that gradient. This was done by defining groups of data along the environmental gradient and forcing the bootstrap to resample within those groups, thus preserving the gradient and also the variability of the distribution.

Distribution of the Nutrient and Turbidity Variables

Mueller and Spahr (2006) found that streams within the Midwest, including Kentucky, have some of the highest nutrient loadings in the United States. Although streams for this study mostly were sampled during periods of stable lower flow conditions, which typically have lower nutrient levels (Lowe and others, 2008), many streams within the study were nutrient enriched, based on the trophic classification by Dodds and others (1998). The median values for each of the three stressor variables were calculated from the 9–11 samples collected at each site from September 2007 to May 2008. Stressor variables, specifically TP, and TN (NO_2+NO_3-N plus TKN), were censored to one-half the reporting limit for each variable. The detection limit for TP was 0.01 mg/L with about 6 percent of the samples having values less than the detection limit. For TKN, 88 of 230 of the values recorded (about 37 percent of the samples) had values less than a reporting limit of 0.20 mg/L. For NO_2+NO_3-N, only one percent of the samples had values less than a reporting limit of 0.01 mg/L.

The summary statistics of the nutrient stressor variables used in the macroinvertebrate data-breakpoint analysis are shown in table 2. Within the macroinvertebrate dataset, median concentrations of stressor variables ranged from 0.016 to 0.182 mg/L for TP, and from 0.39 to 4.9 mg/L for TN (table 2). Median turbidity values ranged from 2.4 FNU to 14 FNU (table 2).

Table 2. Summary statistics for nutrient and turbidity variables used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study in Kentucky, 2007–08.

[TP, total phosphorus; mg/L; milligrams per liter; TN, total nitrogen; FNU, Formazin nephelometric units; n, number of samples]

Statistic	Median TP (mg/L)	Median TN (mg/L)	Median turbidity (FNU)
	(n=19)		
Minimum	0.016	0.39	2.4
25th percentile	.023	.79	4.0
Median	.032	1.2	5.4
Mean	.050	1.6	6.4
Maximum	.182	4.9	14

Relations between the Nutrient and Turbidity Stressor Variables

The strongest and most frequently significant correlations (| τ |) between the stressor variables were between concentrations of TP and TN (table 3). This strong correlation suggests the relatively even distribution of nutrient concentrations measured in the streams in the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion. An additional factor that may help understand the trophic status and functioning of streams is turbidity. Turbidity is an optical property defined as the measurement of light commonly scattered at 90 degrees to the incident light by suspended particles in an aqueous medium (Uhrich and Bragg, 2003). Turbidity can be used as a surrogate for suspended sediment (Rasmussen and others, 2009), and it potentially can be used as an indicator of increasing algal biomass caused by nutrient enrichment. Median and maximum turbidity measurements were compared to 25 Formazin nephelometric units (FNU), a turbidity biological impact classification (Klein and others, 2008). Part of the reason for the low median turbidity values in this study was because samples generally were collected during stable, lower-flow conditions. Of the stressor variables studied, turbidity could be the most misleading when assessing the trophic level of streams, because headwater and wadable streams generally have lower turbidity. This is especially true for study designs that sample only during stable, low-flow conditions, but the alternative design of collecting samples only during elevated flow would overestimate turbidity.

Table 3. Absolute Kendall tau correlations among the median stressor variables used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007–08.

[TP, total phosphorus; mg/L, milligrams per liter; TN, total nitrogen; FNU, Formazen nephelometric units; n, number of samples; Bold text, p < 0.10]

Stressor	Median TP (mg/L)	Median TN (mg/L)	Median turbidity (FNU)
		(n=19)	
TP	--	**0.579**	0.223
TN	**0.579**	--	0.129
Turbidity	0.223	0.129	--

Macroinvertebrate Community Attributes, Relations Between Stressor and Response Variables, and Nutrient Breakpoints using Macroinvertebrate Attributes

The macroinvertebrate-community data were evaluated in three ways to aid in the understanding of the distribution of the nutrient concentrations and relations between nutrient concentrations and the macroinvertebrate response variables. First, the stressor variables were (1) examined for distribution, and (2) examined for relations among the stressor variables. Second, the stressor variables were examined for relations between the stressor and macroinvertebrate response variables, and the ecological significant relations were further examined to determine the breakpoints. Third, the computed breakpoints were compared to Dodds' trophic classification levels for nutrients and Klein and others (2008) biological impact classification for turbidity.

Summary of Macroinvertebrate-Community Attributes

Eighteen macroinvertebrate attributes were used to characterize the macroinvertebrate data (Appendix 2). These attributes included taxa richness (TR, 1 attribute), total number of individuals (TNI, 1), relative abundance or total number of insect orders (6), insect families (3), and insect genera (2), feeding classifications (1), and pollution tolerance (3) (table 4). The Hilsenhoff Biotic Index (HBI, Hilsenhoff, 1988), the Hilsenhoff Family Level Biotic Index (Family HBI), and the average pollution tolerance value (Average TV) represent the stress response of the macroinvertebrate assemblage to organic pollution. Both the HBI and Family HBI are based on a 1 to 10 scale, with 1 being the least tolerant and 10 being the most tolerant. Average tolerance values (Average TV) are based on the same scale as the HBI and Family HBI. The macroinvertebrate attributes used are summarized in table 4.

Table 4. Abbreviations, definitions, and summary statistics for macroinvertebrate attributes and metrics for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis in Kentucky, 2007–08.

Attribute	Abbreviation	Definition	Sample number	Minimum	Median	Maxi-mum	Standard deviation
Total Taxa Richness	TR	Total number of distinct taxa present in a sample	19	48.0	75.0	102	14.5
Total Number of Individuals	TNI	Total number of individuals present in a sample	19	1,410	3,202	9,494	2,388
Ephemeroptera, Plecoptera, and Trichoptera Richness	EPT	Total number of distinct taxa in the orders Ephemeroptera, Plecoptera, and Trichoptera	19	4.00	18.0	35.0	8.98
Percentage of Orders Ephemeroptera, Plecoptera, and Trichoptera	EPT	Relative abundance in the orders Ephemeroptera, Plecoptera, and Trichoptera	19	3.65	26.7	58.7	18.9
Hilsenhoff Biotic Index	HBI	Index that summarizes overall pollution tolerance of a benthic arthropod community with a single value	19	4.66	5.59	6.54	0.666
Percentage of Dominant Family	% 5 Dom	Relative abundance of the numerically dominant family relative to total number of organisms in sample	19	38.0	61.8	89.3	12.6
Percentage of Primary Clingers	% ClngP	Relative abundance of organisms that need hard, silt-free substrates to "cling" to	19	28.2	51.4	85.8	17.7
Percentage of Ephemeroptera	% Ephem	Relative abundance of mayflies	19	0.631	15.7	47.6	14.6
Percentage of Chironomidae	% Chiro	Relative abundance of midges	19	8.58	41.9	69.9	17.5
Number of Ephemeroptera, Plecoptera, and Trichoptera Genus Taxa	EPT GenusTax	Total number of distinct taxa at the genus level in the orders Ephemeroptera, Plecoptera, and Trichoptera	19	4.00	18.0	31.0	7.80
Number of Total Genus Taxa	GenusTax	Total number of distinct taxa at the genus level in a sample	19	40.0	65.0	90.0	13.1
Number of Ephemeroptera, Plecoptera, and Trichoptera at the family level	Family EPT	Total number of distinct taxa at the family level in the orders Ephemeroptera, Plecoptera, and Trichoptera	19	3.00	12.0	17.0	4.00
Total Number of Individuals at the Family Level	Family Richness	Total number of family-level macroinvertebrate communities in a sample. Measures the diversity of the macroinverebrate community.	19	19.0	35.0	45.0	7.07
Average Tolerance Value	Average TV	Average of the tolerance values of all species within the benthic arthropod community	19	5.09	5.71	6.64	.485
Modified Percentage of Ephemeroptera, Plecoptera, and Trichoptera Abundance	m% EPT	Abundance of the generally pollution-sensitive insect orders Ephemeroptera, Plecoptera, and Trichoptera	19	.677	22.9	57.5	17.1
Hilsenhoff Family Level Biotic Index	Family HBI	Average-weighted pollution tolerance value of all arthropod identifications at the family level in a sample.	19	5.35	6.11	7.03	0.58
Percentage of Nutrient Tolerance	% Nutrient Tol	1Percent of the composite of 14 nutrient tolerant taxa	19	27.2	58.7	90.2	18.1
Percentage of Oligochaeta	% Oligo	Relative abundance of earthworms	19	.822	4.99	61.3	15.1

[1]Fourtteen taxa include three EPT genera (*Chemumatophyche, Baetis,* and *Stenacron*), one crustacean genus (*Lirceus*), two snail genera (Physella and *Elimia*), two beetle genera (*Psephenus* and *Stenelmis*), one black fly genus (*Simulium*), four midge genera (*Polypedilum, Pheotanytarsus, Cricotopus* and *Chironomus*), and aquatic worms (*Oligochaeta*).

Distribution of the Macroinvertebrate Communities and Their Relations to Nutrient Stressor Variables

Many of the macroinvertebrate-community attributes (13 of 18 attributes) were significantly and ecologically correlated with at least one of the TP or TN statistical measures (table 5). Three attributes, TNI, Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, and Average TV, were chosen as the best macroinvertebrate measures and used for additional detailed investigation. Correlation analysis and scatterplots indicated that these attributes were the most responsive to differences in nutrient concentrations. The TNI index was more strongly correlated with nutrient concentrations than the other attributes. The TNI index was most significantly correlated with both the median concentrations of TP and TN and showed an increasing trend with increasing nutrient concentrations (fig.2). Two additional attributes, EPT richness and Average TV, were also strongly correlated with median concentrations of TP (table 5). The EPT richness decreased as median concentrations of TP increased (fig.3). The EPT taxa are generally intolerant of nutrients, so the richness of EPT taxa would be expected to decrease with higher concentrations of nutrients. A consequence of decreasing diversity and quality of the macroinvertebrate community is the potential negative impact on the fish community. The Average TV increased as median concentrations of TP increased (fig.4). This relation suggests the presence of more tolerant benthic arthropods and poorer water quality.

Table 5. Macroinvertebrate breakpoint summary (n=19) for the stressor variables used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007–08.

[TP, total phosphorus; TN, total nitrogen; mg/L, milligrams per liter; p-value <0.10]

Biological response variable	Stressor variable to biological attribute Kendal tau	Mean bootstrap breakpoint of stressor variable	Lower bootstrap confidence interval	Upper bootstrap confidence interval
Median TP (mg/L)				
Total Taxa Richness	-0.231	0.032	0.026	0.040
Total Number of Individuals	0.488	0.033	0.026	0.040
Number of Ephemeroptera, Plecoptera, and Trichoptera	-0.300	0.032	0.026	0.040
Percentage of Dominant Family	0.275	0.033	0.026	0.040
Percentage of Ephemeroptera	-0.251	0.034	0.026	0.040
Number of Ephemeroptera, Plecoptera, and Trichoptera Genus Taxa	-0.273	0.033	0.026	0.040
Number of Ephemeroptera, Plecoptera, and Trichoptera at the family level	-0.397	0.032	0.026	0.040
Total Number of Individuals at the Family Level	-0.252	0.034	0.026	0.040
Average Tolerance Value	0.380	0.032	0.026	0.040
Modified Percentage of Ephemeroptera, Plecoptera, and Trichoptera Abundance	-0.287	0.035	0.026	0.040
Percentage of Nutrient Tolerance	0.228	0.034	0.026	0.040
Median TN (mg/L)				
Total Number of Individuals	0.571	1.1	0.850	1.23
Number of Ephemeroptera, Plecoptera, and Trichoptera at the family level	-0.287	1.1	0.850	1.23
Percentage of Nutrient Tolerance	0.228	1.0	0.850	1.23

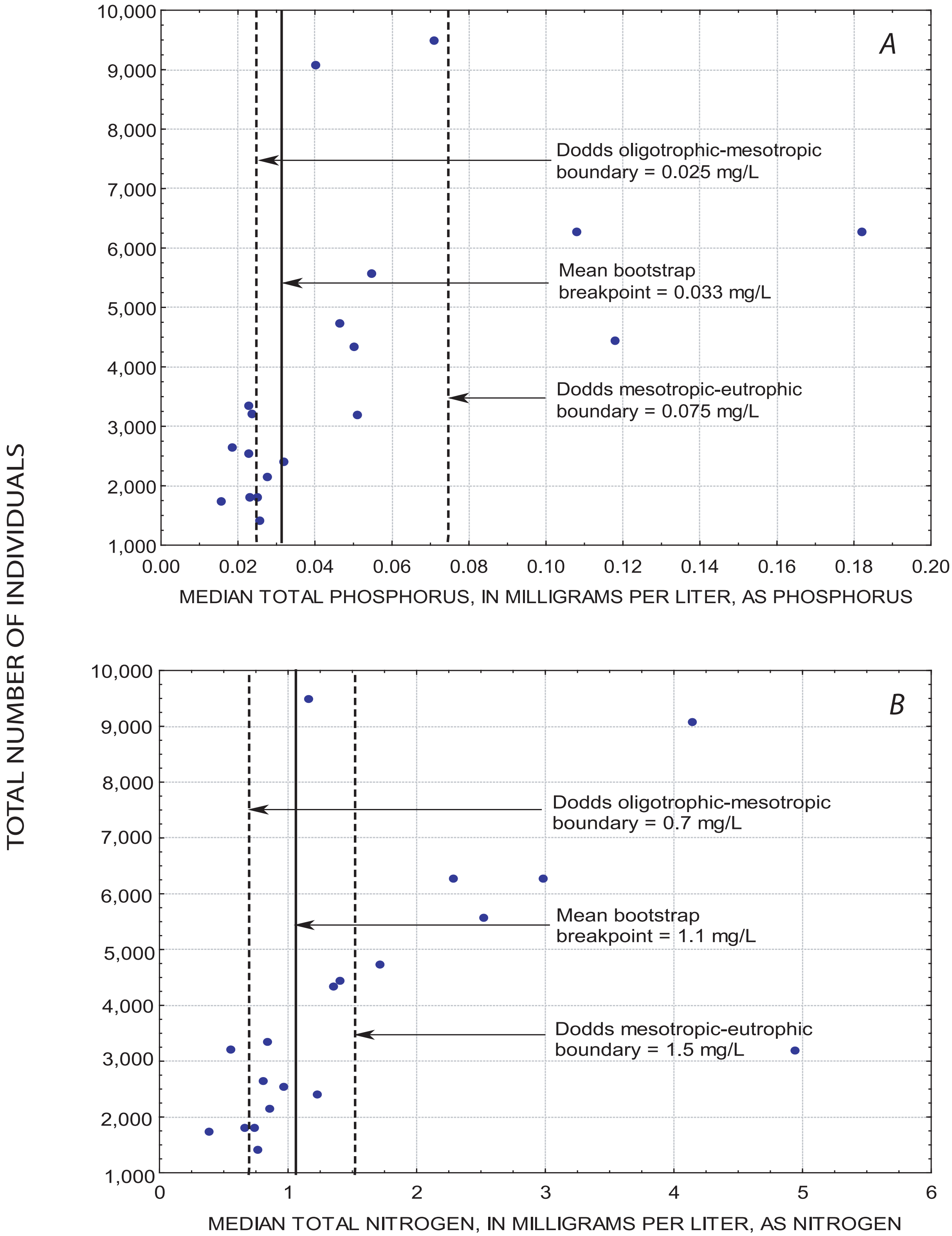


Figure 2. Scatter plot of the A, median total phosphorus concentrations and B, median total nitrogen concentrations to the total number of individuals for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007–08. (Trophic classifications from Dodds and others, 1998.)

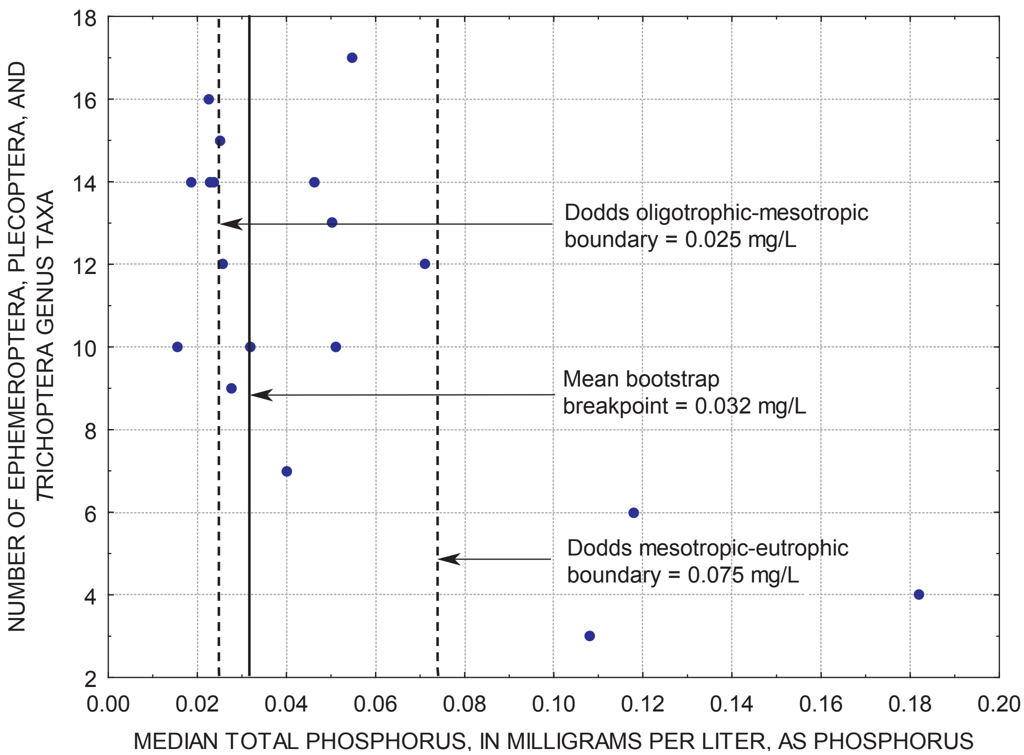


Figure 3. Scatter plot of the median total phosphorus concentrations to the number of Ephemeroptera, Plecoptera, and Trichoptera genus taxa for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007–08. (Trophic classifications from Dodds and others, 1998.)

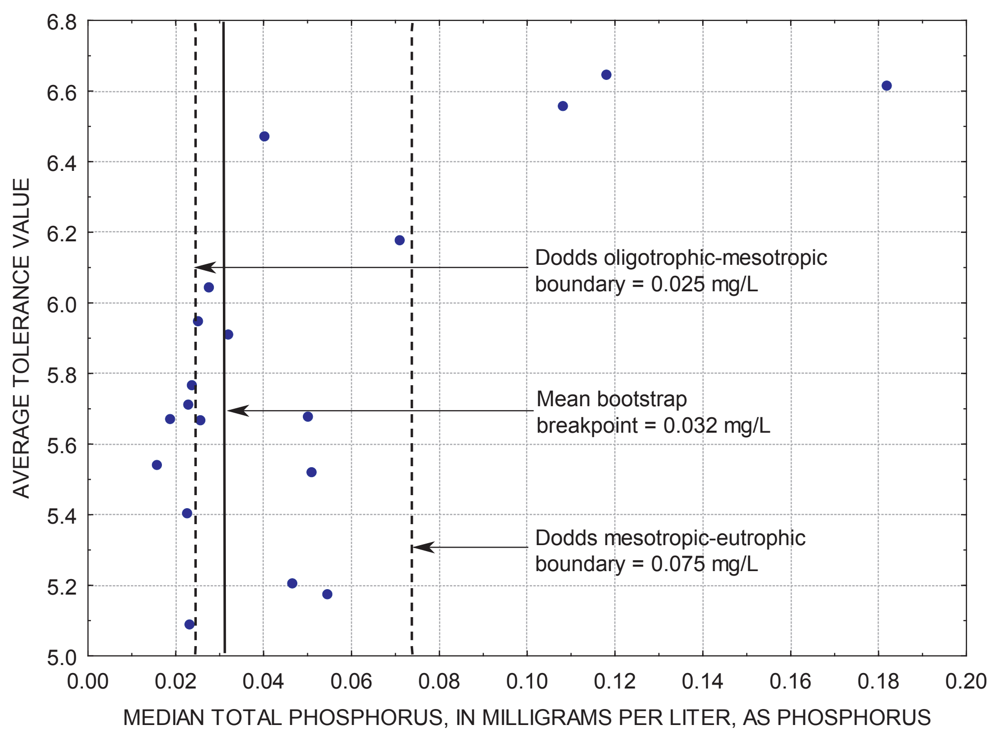


Figure 4. Scatter plot of the median total phosphorus concentrations to the average tolerance value for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08. (Trophic classifications from Dodds and others, 1998.)

Macroinvertebrate-Community Breakpoints of the Nutrient Stressor Variables and Implications for Developing Nutrient Criteria in the Crawford-Mammoth Cave Uplands Ecoregion

Regression-tree analyses were performed to define specific breakpoints (thresholds) in the stressor-response relations. The strongest and most ecologically significant of these relations and their breakpoints are listed in table 5. All of the breakpoint values for TP and TN were statistically significant at p-value < 0.10.

The breakpoint values identified by the regression-tree analysis in the responses to changes in median concentrations of TP were 0.032 mg/L to 0.035 mg/L (table 5). The ranges in the significant breakpoint values in the response to changes in median concentrations of TN were 1.0 mg/L to 1.1 mg/L (table 5). The positive response of the total number of individuals (TNI) index, which is most strongly correlated with nutrients, is shown with respect to median concentrations of TP and TN in figure 2. In general, TNI increased as nutrient concentrations increased. Other strong responses based on correlation analysis and scatterplots were between median concentrations of TP and EPT richness and Average TV attributes. The median concentration of TP increased as EPT richness decreased, with a mean breakpoint of 0.032 mg/L (fig.3). This relation suggests that as the diversity and quality of the macroinvertebrate community decreases, the impact on the fish community potentially becomes more negative. The breakpoint value found for Average TV (0.032 mg/L) matched the EPT richness index. However, the response was positive between Average TV and the median concentration of TP, so as the median concentration of TP increased, the Average TV index increased (fig.4).

The average median concentration of TP (0.033 mg/L) associated with the biological breakpoints in this study (table 5) were lower than the TP breakpoint values of 0.091 mg/L and 0.053 mg/L within Wisconsin (Robertson and others, 2006) and Indiana (Caskey and others, 2010), respectively. In general, the biological breakpoint relations with median concentrations of TP in this study were similar to the USEPA proposed numeric TP criteria (0.037 mg/L) for Aggregated Ecoregion IX (central and western Kentucky) for rivers and streams.

The biological breakpoint values for the median TN concentration (1.1 mg/L) in this study (table 5) are similar with the TN breakpoint value of 1.1 mg/L within Wisconsin (Robertson and others, 2006), and three times lower than the TN breakpoint value of 3.3 mg/L within Indiana (Caskey and others, 2010). The biological breakpoint relations with median concentrations of TN in this study were not similar to the USEPA proposed numeric TN criteria for Aggregated Ecoregion IX (central and western Kentucky) for rivers and streams (0.69 mg/L).

Macroinvertebrate Communities and the Comparison of Trophic Classification for Nutrient Concentrations and Turbidity Values

Trophic state is a classification system designed to “rate” rivers and streams based on the amount of biological productivity occurring in the water. To assess the trophic state of each stream, the breakpoints of the median stressor concentrations for TP and TN were compared to Dodds’ trophic classification. Dodds and others (1998) proposed classifying streams into trophic-state conditions similar to those developed for lakes and reservoirs (U.S. Environmental Protection Agency, 2000). The approach used by Dodds and others (1998) was based upon establishing statistical distributions of TP, TN, and periphyton and seston CHL*a* using existing data. Three trophic-state conditions are classified as oligotrophic, mesotrophic, and eutrophic. Dodds and others (1998) classified the oligotrophic-mesotrophic boundaries of Midwest streams as 0.025 mg/L for TP and 0.70 mg/L for TN, and the mesotrophic-eutrophic boundaries as 0.075 mg/L for TP and 1.5 mg/L for TN.

Based on Dodds’ trophic-state conditions, streams in this study were most often classified as eutrophic, based on the distribution of median TP concentrations (44 percent of values), and mesotrophic-eutrophic, based on the distribution of maximum TP concentrations (94 percent of values) (fig.5). Comparison of the average biological breakpoint for the median concentration of TP (0.033 mg/L) to Dodds’ trophic classification for TP indicates streams in the Pennyroyal Bioregion would be classified as mesotrophic-eutrophic. The average biological breakpoint for the maximum concentration of TP of 0.156 mg/L found in this study was more than two times higher than the trophic levels Dodds and others (1998) describe as hypereutrophic.

The trophic-state stream classifications based on median and maximum TN concentrations were similar to those for the median and maximum TN concentrations, with 56 percent of TN values considered eutrophic and 69 percent of TN values considered mesotrophic-eutrophic (fig.5). The average biological breakpoint for the median concentration of TN (0.93 mg/L) indicates that streams in the Pennyroyal Bioregion would be classified as mesotrophic-eutrophic according to Dodds’ trophic classification for TN. The average biological breakpoint for the maximum concentration of TN (1.8 mg/L) is slightly higher than Dodds’ mesotrophic-eutrophic boundary (1.5 mg/L).

An additional factor that may aid understanding of the trophic status and functioning of streams is turbidity. Turbidity can be used as a surrogate for suspended sediment (Rasmussen and others, 2009), and it could be used as an indicator of increasing algal biomass caused by nutrient enrichment. No biological breakpoints in relation to turbidity measurements were reported in this study, because no significant correlations were shown. However, median turbidity measurements were compared to 25 FNU, a turbidity biological impact

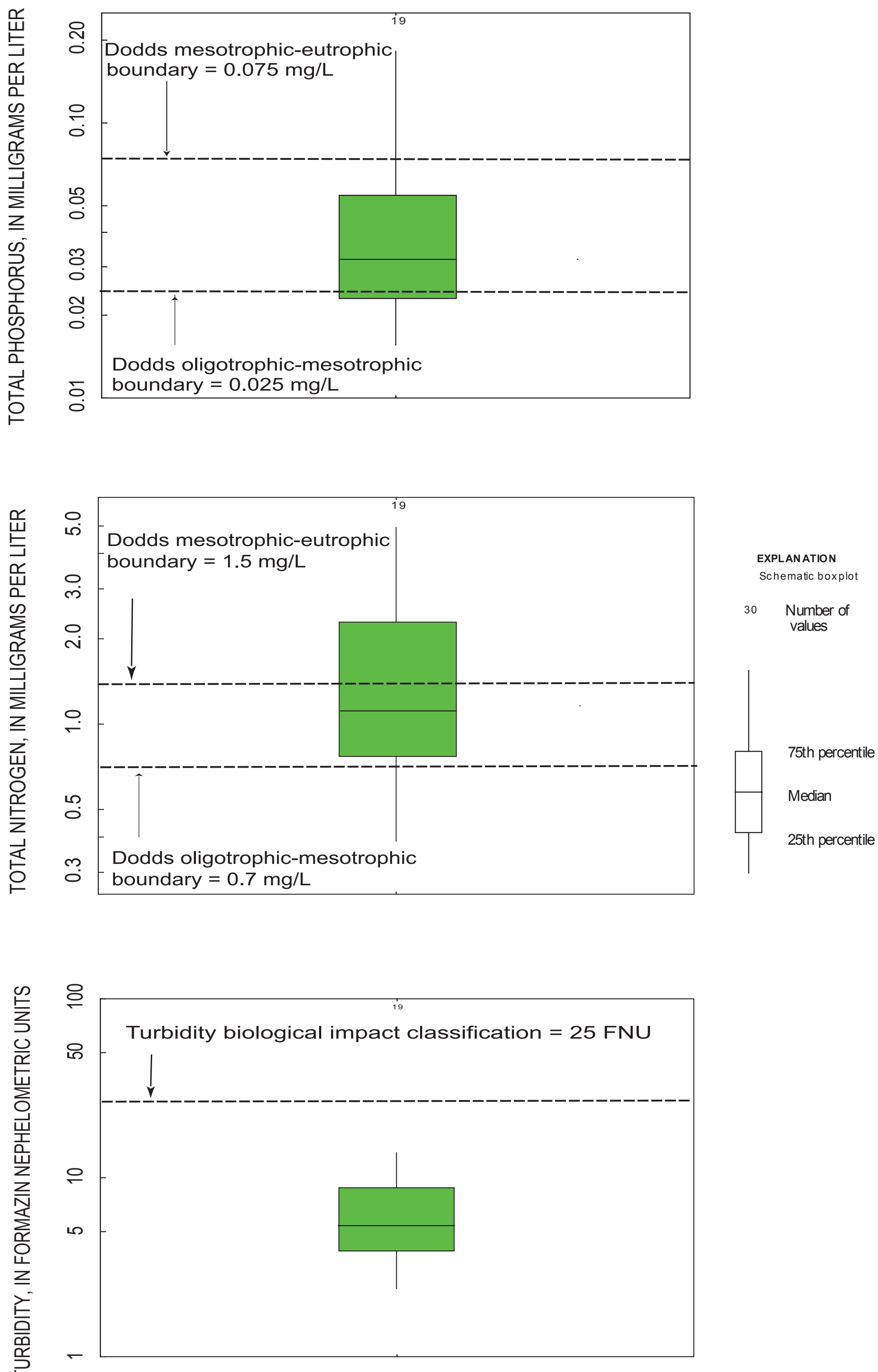


Figure 5. Distribution to the median total phosphorus and total nitrogen concentrations (presented on a log scale) compared to Dodds and others (1998) trophic classifications , and distribution of the median turbidity concentrations compared to Klein and others (2008) turbidity biological impact classification in selected datasets used in the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007–08. (FNU, Formazin nephelometric units.)

classification (Klein and others, 2008) (fig.5). No sites were impacted adversely using median turbidity values for macroinvertebrates, but more than 89 percent of the sites were impacted using maximum turbidity values for macroinvertebrates based on the 25 FNU biological threshold used by Klein and others (2008). Part of the reason for the low median turbidity values in this study was that samples generally were collected during stable, lower flow conditions. Of the stressor variables studied, turbidity could be the most misleading when assessing the trophic level of streams, because headwater and wadable streams generally have lower turbidity. This is especially true for study designs that sample only during stable, low-flow conditions, but the alternative design of collecting samples only during elevated flow would overestimate turbidity.

Summary and Conclusions

Nutrients are essential to the development, health, and diversity of plants and animals in surface waters, yet excessive inputs of nutrients into streams have potential human-health, economic, and ecological consequences. Kentucky is part of the nutrient-rich Midwest, which leads the nation in corn and soybean production; these row-crops require the use of significant amounts of nutrients—primarily from fertilizer and manure for their production. Within the United States, streams sampled within the Midwest have some of the highest nutrient concentrations and loading, which contributes to the hypoxia issue in the Gulf of Mexico. Studies using breakpoint analysis in Wisconsin and Indiana found that indicators, such as biological-community attributes and metrics, could be helpful for developing nutrient criteria for streams in such nutrient-rich regions.

The USGS and the KDOW collected water-quality and biological data in 22 wadable streams throughout the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion from September 2007 to May 2008. These samples were collected to determine if the changes among macroinvertebrate-community attributes and metrics were statistically and ecologically related to changes in stressor (TP, TN, and turbidity) variables. Breakpoint analysis was used for the most statistically significant and ecologically relevant relations to find the concentration of the stressor variables where the greatest change occurred with the biological species and community attributes and metrics.

Median concentrations of stressor variables ranged from 0.016 mg/L to 0.182 mg/L for TP and 0.39 mg/L to 4.9 mg/L for TN within the macroinvertebrate dataset. Median turbidity values ranged from 2.4 FNU to 14 FNU.

Many macroinvertebrate-community (13 of 18) attributes were statistically significant (p-value < 0.10) and ecologically relevant in relation with concentrations of TP and TN, suggesting that nutrients have direct or indirect links with those biological communities in wadable streams in the Crawford-Mammoth Cave Uplands ecoregion within the Pennyroyal Bioregion. Correlation analysis and scatterplots indicated three macroinvertebrate-community attributes (Total Number of Individuals, Ephemeroptera-Plecoptera-Trichoptera richness, and Average Tolerance Value) were the most responsive to differences in nutrient concentrations.

Breakpoints for the macroinvertebrate-community attributes were generally consistent with a mesotrophic-eutrophic status when using median nutrient concentrations. The macroinvertebrate attributes breakpoint values in the responses to changes in median concentrations of TP range from 0.032 mg/L to 0.035 mg/L. The ranges in the significant breakpoint values in response to changes in median concentrations of TN were 1.0 mg/L to 1.1 mg/L.

The goal of the study was not to develop numeric nutrient criteria, but to demonstrate the breakpoint analysis approach between nutrient concentrations and some aspects of macroinvertebrate attributes and metrics. Although the sample size was small, this study found meaningful relations between nutrient concentrations and changes in macroinvertebrate attributes and metrics in the Pennyroyal Bioregion. The average biological breakpoint value for concentrations of TN (1.1 mg/L) in this study was not similar to the USEPA proposed numeric TN criteria for rivers and streams (0.69 mg/L). This finding suggests that setting the TN criteria to that criteria proposed by the USEPA would be conservative and that many rivers and streams in the Pennyroyal Bioregion would be considered impaired, despite the use of biological-community attributes that indicate acceptable water-quality conditions. The average biological breakpoint in relation with median concentrations of TP (0.033 mg/L) was similar to the USEPA proposed numeric TP criteria (0.037 mg/L) for rivers and streams in the study area.

Results from this study demonstrate that macroinvertebrate attributes and metrics can be used to evaluate relations between concentrations of nutrients and macroinvertebrate communities. In addition, breakpoint values in nutrient concentrations can be identified. With additional biological data (such as diatoms, CHL*a* and fish), the biological assessment of macroinvertebrate communities has a greater potential for success in developing and refining numeric nutrient criteria in the Pennyroyal Bioregion.

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Appendixes

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
BC	03320300	DOW03002019	9/24/2007	E0.031	0.72	0.02	0.045	0.74	
			10/22/2007	<0.025	E0.44	E0.01	0.082	0.46	27.0
			11/27/2007	<0.025	<0.2	2.01	0.025	2.21	2.6
			12/18/2007	<0.025	<0.2	1.87	0.024	2.07	1.2
			1/14/2008	<0.025	<0.2	1.29	E0.017	1.49	
			2/14/2008	<0.025	<0.2	1.11	0.027	1.31	4.6
			3/19/2008	E0.049	1.00	0.35	0.164	1.35	125.0
			4/8/2008						1.3
			4/29/2008	<0.025	<0.2	0.02	<0.01	0.22	1.5
			5/13/2008	<0.025	<0.2	0.19	E0.011	0.39	0
			5/19/2008	<0.025	E0.25	0.32	E0.016	0.57	0.6
BDC	03311600	DOW03016002	9/26/2007	<0.025	E0.28	1.08	0.065	1.36	4.7
			10/25/2007	<0.025	0.69	6.11	0.150	6.80	8.4
			11/28/2007	<0.025	E0.28	3.70	0.057	3.98	9.1
			12/18/2007	<0.025	E0.25	3.25	0.055	3.50	8.1
			1/15/2008	<0.025	E0.29	2.83	0.040	3.12	10.1
			2/12/2008	0.118	1.14	0.89	0.325	2.03	105.0
			3/11/2008	<0.025	0.38	2.14	0.045	2.52	4.9
			4/7/2008	E0.038	0.58	2.00	0.063	2.58	3.8
			4/24/2008	<0.025	<0.2	1.87	0.038	2.07	1.7
			5/6/2008	E0.033	E0.42	1.88	0.054	2.30	4.8
			5/27/2008	<0.025	E0.32	2.03	0.051	2.35	6.7
BEAR	03311800	DOW03002019	9/25/2007	E0.034	E0.24	0.09	0.021	0.32	
			10/25/2007	<0.025	0.52	2.05	0.081	2.57	5.6
			11/28/2007	<0.025	E0.25	1.63	0.040	1.88	3.4
			12/19/2007	<0.025	<0.2	1.43	0.033	1.63	2.4
			1/15/2008	<0.025	<0.2	1.11	0.025	1.31	5.3

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.—Continued

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
BEAR	03311800	DOW03002019	2/12/2008	0.110	0.85	0.48	0.155	1.33	40.8
			3/11/2008	<0.025	<0.2	0.64	0.034	0.84	26.0
			4/7/2008	<0.025	E0.27	0.42	0.022	0.69	1.8
			4/24/2008	<0.025	<0.2	0.06	E0.019	0.26	1.4
			5/6/2008	E0.030	E0.24	0.14	E0.013	0.37	0.8
			5/27/2008	E0.044	E0.2	0.25	<0.01	0.45	0.3
BF	03320100	DOW03004020	9/24/2007	<0.025	0.76	0.02	0.064	0.79	
			10/22/2007	0.205	0.92	1.00	0.155	1.92	42.0
			11/28/2007	<0.025	E0.39	3.06	0.038	3.45	3.0
			12/18/2007	<0.025	E0.46	1.90	0.072	2.36	8.8
			1/14/2008	E0.033	E0.47	1.73	0.071	2.20	
			2/14/2008	0.0673	E0.41	1.46	0.071	1.87	15.0
			3/19/2008	E0.049	E0.43	0.64	0.121	1.07	98.0
			4/8/2008	E0.027	E0.22	0.71	0.071	0.93	15.3
			4/29/2008	E0.030	E0.28	0.28	0.028	0.56	2.5
			5/13/2008	0.0612	0.53	0.32	0.041	0.85	4.2
			5/20/2008	E0.045	0.54	0.62	0.042	1.16	4.9
BRB	03317940	DOW03008026	9/25/2007	<0.025	E0.31	0.95	0.044	1.25	
			10/25/2007	<0.025	E0.45	1.66	0.081	2.11	8.6
			11/28/2007	<0.025	E0.22	1.06	0.041	1.28	21.7
			12/18/2007	<0.025	<0.2	1.18	0.031	1.38	4.4
			1/15/2008	<0.025	<0.2	1.13	0.022	1.33	5.2
			2/12/2008	0.089	0.88	0.52	0.168	1.40	122.0
			3/11/2008	<0.025	<0.2	0.80	0.032	1.00	12.0
			4/8/2008	<0.025	E0.24	0.74	0.023	0.98	1.5
			4/24/2008	<0.025	<0.2	0.24	0.027	0.44	2.1
			5/6/2008	<0.025	E0.31	0.30	0.026	0.60	0.5
			5/27/2008	0.0566	<0.2	0.61	0.034	0.81	1.3

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.—Continued

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Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
BRBW	03317395	DOW03008024	11/28/2007	<0.025	<0.2	0.39	0.020	0.59	59.3
			12/18/2007	0.105	<0.2	0.45	0.022	0.65	7.1
			1/15/2008	<0.025	E0.27	0.32	E0.012	0.59	6.5
			2/12/2008	0.055	0.67	0.30	0.091	0.97	4.8
			3/11/2008	<0.025	<0.2	0.29	0.028	0.49	13.0
			4/8/2008	<0.025	E0.24	0.11	E0.016	0.35	1.8
			4/24/2008	<0.025	<0.2	<0.01	<0.01	0.21	2.7
			5/6/2008	<0.025	E0.32	<0.01	<0.01	0.33	0.2
			5/27/2008	0.0575	<0.2	0.12	<0.01	0.32	0.7
CC	03384330	DOW08013004	9/24/2007	E0.028	0.91	0.06	0.121	0.96	
			10/22/2007	E0.040	0.77	0.22	0.168	0.99	3.7
			11/27/2007	0.087	0.71	1.40	0.293	2.11	26.0
			12/18/2007	0.164	E0.46	1.14	0.107	1.60	13.8
			1/14/2008	0.067	E0.32	1.03	0.097	1.35	
			2/13/2008	0.237	0.68	0.72	0.110	1.40	27.8
			3/18/2008	0.592	3.26	0.61	0.461	3.87	319.0
			4/7/2008	0.334	0.65	0.54	0.077	1.19	14.9
			4/28/2008	0.061	E0.303	0.14	0.057	0.44	5.2
			5/12/2008	0.201	0.52	1.00	0.118	1.52	7.3
			5/19/2008	0.538	1.14	0.95	0.163	2.09	8.6
DC	03303203	DOW08035013	9/25/2007	E0.040	E0.32	0.12	E0.016	0.44	
			10/24/2007	<0.025	0.54	1.23	0.104	1.77	6.2
			11/29/2007	<0.025	<0.2	0.96	0.028	1.16	1.9
			12/18/2007	<0.025	<0.2	1.13	0.030	1.33	4.0
			1/15/2008	<0.025	<0.2	0.87	E0.018	1.07	2.5
			2/14/2008	E0.028	<0.2	0.94	0.026	1.14	9.7
			3/11/2008	<0.025	<0.2	0.56	0.033	0.76	17.1

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DC	03303203	DOW08035013	4/8/2008	<0.025	0.54	0.52	0.023	1.06	3.4
			4/24/2008	<0.025	<0.2	0.06	E0.019	0.26	0.7
			5/6/2008	E0.026	<0.2	0.29	E0.011	0.49	3.9
			5/27/2008	<0.025	<0.2	0.21	<0.01	0.41	1.8
ELC	03316110	DOW03012008	9/25/2007	0.080	E0.33	0.18	0.027	0.51	
			10/23/2007	<0.025	0.94	1.30	0.250	2.24	42.4
			11/28/2007	E0.048	E0.41	1.49	0.051	1.90	8.4
			12/19/2007	E0.030	0.56	1.25	0.088	1.81	14.5
			1/15/2008	<0.025	E0.42	1.32	0.050	1.74	
			2/15/2008	<0.025	E0.27	1.44	0.047	1.71	10.7
			3/20/2008	E0.027	E0.44	0.91	0.089	1.35	128.0
			4/8/2008	E0.047	0.60	0.56	0.078	1.16	21.6
			4/29/2008	E0.049	E0.26	0.32	0.020	0.58	3.6
			5/13/2008	E0.049	0.52	0.30	0.047	0.82	5.4
			5/20/2008	0.0586	0.50	0.34	0.033	0.85	6.5
FC	03320200	DOW03004006	9/24/2007	E0.0411	E0.43	0.12	0.032	0.55	
			10/22/2007	<0.025	E0.26	0.05	0.042	0.31	1.5
			11/27/2007	<0.025	E0.24	1.05	0.027	1.29	7.1
			12/18/2007	<0.025	<0.2	1.26	0.024	1.46	3.1
			1/14/2008	<0.025	<0.2	1.01	E0.015	1.21	
			2/14/2008	<0.025	<0.2	0.87	0.025	1.07	6.1
			3/19/2008	0.0546	0.61	0.41	0.093	1.02	117.0
			4/8/2008	E0.035	<0.2	0.39	E0.017	0.59	2.8
			4/29/2008	<0.025	<0.2	0.09	E0.014	0.29	3.2
			5/13/2008	<0.025	<0.2	0.20	<0.01	0.40	1.1
			5/20/2008	<0.025	E0.22	0.23	<0.01	0.45	1.8

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GR	03315180	DOW03018011	9/25/2007	E0.046	E0.39	1.82	0.105	2.21	
			10/23/2007	0.0824	1.51	3.97	0.397	5.48	211.0
			11/28/2007	<0.025	<0.2	9.63	0.059	9.83	5.9
			12/19/2007	<0.025	<0.2	7.80	0.054	8.00	12.4
			1/15/2008	<0.025	<0.2	6.79	0.051	6.99	
			2/15/2008	<0.025	<0.2	6.08	0.049	6.28	9.9
			3/20/2008	E0.027	E0.38	3.51	0.068	3.89	31.7
			4/8/2008	<0.025	<0.2	4.70	0.043	4.90	10.9
			4/29/2008	<0.025	<0.2	4.42	0.036	4.62	3.9
			5/13/2008	<0.025	<0.2	4.33	0.045	4.53	2.4
			5/20/2008	<0.025	<0.2	4.84	0.042	5.04	2.9
HC	03303202	DOW08035004	9/25/2007	0.0613	<0.2	22.10	3.110	22.30	
			10/25/2007	0.318	1.28	1.01	0.493	2.29	10.8
			11/29/2007	<0.025	E0.48	2.19	0.197	2.67	7.3
			12/18/2007	0.552	1.07	1.12	0.234	2.19	4.0
			1/15/2008	0.241	1.01	2.08	0.182	3.09	8.7
			2/14/2008	0.549	0.77	1.06	0.175	1.83	13.4
			3/11/2008	0.302	0.77	0.67	0.169	1.44	1302.0
			4/8/2008	E0.031	0.64	1.21	0.094	1.85	1.8
			4/24/2008	E0.045	E0.41	2.62	0.110	3.03	1.4
			5/6/2008	E0.040	1.03	1.21	0.154	2.24	3.2
			5/27/2008	0.0765	0.58	3.66	0.462	4.24	3.2
LBDC	03311700	DOW03016001	9/26/2007	E0.034	E0.33	0.20	0.050	0.53	
			10/25/2007	<0.025	0.76	4.43	0.154	5.19	6.7
			11/28/2007	<0.025	E0.34	2.72	0.057	3.06	8.0
			12/18/2007	0.0731	E0.25	2.55	0.041	2.80	5.1
			1/15/2008	<0.025	E0.31	2.03	0.047	2.34	12.1
			2/12/2008	0.0921	1.12	0.59	0.243	1.71	105.0

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LBDC	03311700	DOW03016001	3/11/2008	<0.025	<0.2	1.38	0.032	1.58	10.2
			4/7/2008	E0.025	E0.30	1.22	0.041	1.52	4.4
			4/24/2008	<0.025	<0.2	0.90	0.027	1.10	8.7
			5/6/2008	E0.026	E0.23	0.83	0.032	1.06	7.6
			5/27/2008	E0.034	E0.41	1.56	0.046	1.97	5.2
LC	03316870	DOW03008011	9/25/2007	E0.034	E0.31	0.12	<0.01	0.43	
			10/24/2007	<0.025	0.68	0.62	0.084	1.30	57.7
			11/29/2007	<0.025	E0.28	0.63	0.023	0.90	7.8
			12/18/2007	E0.029	<0.2	0.56	0.023	0.76	13.8
			1/15/2008	<0.025	<0.2	0.50	E0.018	0.70	10.0
			2/12/2008	<0.025	0.53	0.41	0.093	0.94	30.3
			3/11/2008	<0.025	<0.2	0.37	0.033	0.57	25.1
			4/8/2008	E0.029	E0.40	0.39	0.037	0.79	24.6
			4/24/2008	<0.025	0.93	0.23	0.041	1.16	3.2
			5/6/2008	<0.025	E0.28	0.22	E0.014	0.50	10.7
			5/27/2008	E0.032	E0.2	0.29	<0.01	0.49	4.6
LMC	03316900	DOW03008025	9/25/2007	E0.037	E0.34	0.05	<0.01	0.39	
			10/24/2007	<0.025	0.57	0.96	0.107	1.52	19.0
			11/29/2007	<0.025	<0.2	0.94	0.023	1.14	0.0
			12/18/2007	<0.025	<0.2	1.05	0.031	1.25	5.6
			1/15/2008	<0.025	E0.23	0.95	E0.017	1.18	5.2
			2/14/2008	E0.028	<0.2	0.91	0.021	1.11	38.8
			3/11/2008	<0.025	<0.2	0.66	0.032	0.86	13.1
			4/8/2008	E0.031	<0.2	0.74	0.027	0.94	6.8
			4/24/2008	E0.034	<0.2	0.16	0.023	0.36	1.7
			5/6/2008	E0.029	E0.28	0.39	E0.014	0.67	3.1
			5/27/2008	0.141	<0.2	0.55	<0.01	0.75	2.1

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.—Continued

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
MC	03316885	DOW03004018	9/25/2007	E0.027	E0.41	0.12	0.026	0.53	
			10/24/2007	<0.025	0.72	1.11	0.168	1.83	43.4
			11/29/2007	<0.025	E0.27	0.98	0.043	1.24	5.1
			12/18/2007	<0.025	<0.2	1.17	0.037	1.37	4.6
			1/15/2008	E0.036	E0.27	1.06	0.025	1.33	6.1
			2/12/2008	0.189	1.65	0.57	0.236	2.22	310.0
			3/11/2008	<0.025	E0.28	0.72	0.051	1.00	28.0
			4/8/2008	E0.027	E0.32	0.77	0.031	1.10	10.2
			4/24/2008	<0.025	<0.2	0.28	0.026	0.48	1.9
			5/6/2008	0.0723	E0.29	0.46	0.023	0.75	7.51
			5/27/2008	E0.043	<0.2	0.58	0.029	0.78	
NC	03310340	DOW03026003	9/25/2007	E0.034	E0.28	0.43	<0.01	0.71	
			10/24/2007	E0.030	E0.36	1.08	0.045	1.44	11.0
			11/28/2007	<0.025	<0.2	0.75	E0.018	0.95	8.9
			12/19/2007	<0.025	<0.2	0.81	0.022	1.01	4.3
			1/15/2008	E0.032	<0.2	0.71	E0.014	0.91	5.3
			2/12/2008	0.065	0.73	0.45	0.147	1.17	189.0
			3/11/2008	<0.025	<0.2	0.55	0.023	0.75	7.6
			4/7/2008	E0.026	E0.20	0.51	E0.019	0.71	4.5
			4/24/2008	<0.025	<0.2	0.36	E0.015	0.56	0.6
			5/6/2008	<0.025	E0.22	0.36	E0.123	0.59	4.9
			5/27/2008	E0.041	<0.2	0.72	E0.016	0.92	1.8
NFRR	03317400	DOW03008014	9/25/2007	0.062	E0.27	0.10	0.027	0.37	
			10/24/2007	<0.025	E0.45	1.00	0.066	1.45	14.3
			11/29/2007	<0.025	<0.2	0.99	0.028	1.19	
			12/18/2007	<0.025	<0.2	0.99	0.028	1.19	4.8
			1/15/2008	<0.025	<0.2	0.82	E0.017	1.02	10.3
			2/14/2008	<0.025	<0.2	0.77	E0.019	0.97	13.9

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.—Continued

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
NFRR	03317400	DOW03008014	3/11/2008	<0.025	<0.2	0.57	0.026	0.77	15.6
			4/8/2008	<0.025	<0.2	0.58	E0.018	0.78	1.7
			4/24/2008	E0.042	E0.42	0.35	0.055	0.77	6.1
			5/6/2008	E0.035	E0.26	0.46	E0.013	0.72	2.6
			5/27/2008	<0.025	<0.2	0.58	0.025	0.78	3.3
TWR	03382550		9/24/2007	0.066	0.90	0.06	0.097	0.96	
			10/22/2007	<0.025	E0.41	0.34	0.044	0.75	9.0
			11/27/2007	<0.025	E0.25	1.08	0.034	1.33	5.4
			12/18/2007	<0.025	E0.24	1.15	0.029	1.39	5.3
			1/14/2008	<0.025	<0.2	0.85	E0.019	1.05	
			2/13/2008	<0.025	<0.2	0.76	0.037	0.96	12.4
			3/18/2008	<0.025	<0.2	0.36	0.020	0.56	2.9
			4/7/2008	<0.025	E0.30	0.48	0.026	0.77	2.5
			4/28/2008	<0.025	<0.2	0.13	E0.015	0.33	1.6
			5/12/2008	<0.025	<0.2	0.16	<0.01	0.36	0.6
			5/19/2008	<0.025	E0.21	0.12	E0.016	0.34	1
UTMF	03320720	DOW03004018	9/24/2007	<0.025	0.51	E0.02	0.114	0.53	
			10/22/2007	<0.025	E0.44	0.23	0.107	0.67	2.4
			11/27/2007	0.116	0.75	5.16	0.168	5.91	5.3
			12/18/2007	0.203	E0.39	4.77	0.115	5.16	4.1
			1/14/2008	E0.048	E0.27	3.93	0.116	4.20	
			2/14/2008	0.144	E0.43	2.63	0.096	3.06	7.6
			3/19/2008	0.673	2.22	0.77	0.284	2.99	124.0
			4/8/2008	<0.025	E0.44	2.28	0.076	2.72	1.2
			4/29/2008	E0.028	E0.23	0.62	0.038	0.85	1.2
			5/12/2008	<0.025	E0.23	1.39	0.067	1.62	0
			5/19/2008	<0.025	E0.41	2.62	0.108	3.03	0.7

Appendix 1. Station number, sample-collection date, nutrient, and turbidity sample results for samples collected for the Crawford-Mammoth Cave Uplands Ecoregion breakpoint analysis study, 2007-08.—Continued

[Sampling site name abbreviations: BC, Buck Creek; BDC, Beaverdam Creek; BEAR, Bear Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeeting Creek; MC, Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; TWR, Trade Water River; UTMF, Unnamed tributary to McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Sample-collection date	Ammonia, in milligrams per liter, as nitrogen	Total Kjehldal nitrogen, in milligrams per liter	Nitrite plus nitrate, in milli-grams per liter, as nitrogen	Total Phosphorus, in milligrams per liter	Total nitrogen, in milligrams per liter	Turbidity, in Formazin nephelometric units
WF	03437450	DOW20014005	9/25/2007	0.071	<0.2	8.54	0.058	8.74	
			10/23/2007	<0.025	1.26	2.88	0.695	4.14	94.3
			11/28/2007	<0.025	<0.2	7.57	0.041	7.77	3.5
			12/19/2007	<0.025	<0.2	7.22	0.040	7.42	3.8
			1/15/2008	<0.025	<0.2	5.70	0.021	5.90	
			2/15/2008	<0.025	<0.2	3.66	0.036	3.86	5.0
			3/20/2008	E0.039	<0.2	2.12	0.044	2.32	14.7
			4/8/2008						4.0
			4/29/2008	<0.025	<0.2	3.07	E0.017	3.27	1.9
			5/13/2008	<0.025	<0.2	3.27	0.024	3.47	2.4
YBC	03302500	DOW08037001	10/25/2007	<0.025	0.60	0.92	0.155	1.52	14.6
			11/30/2007	<0.025	E0.21	0.80	0.030	1.00	2.2
			12/18/2007	<0.025	<0.2	1.03	0.043	1.23	311.8
			1/15/2008	<0.025	E0.39	0.79	0.027	1.17	13.2
			2/11/2008	0.028	<0.2	0.84	0.024	1.04	4.4
			3/11/2008	<0.025	E0.23	0.48	0.051	0.71	14.0
			4/8/2008	E0.048	E0.28	0.40	0.026	0.68	1.8
			4/24/2008	<0.025	<0.2	0.02	0.028	0.22	0.6
			5/6/2008	E0.038	E0.23	0.54	0.027	0.77	3.1
			5/27/2008	E0.036	E0.22	0.07	0.022	0.29	3.6

Appendix 2. Macroinvertebrate community attributes and metrics calculated by site for the Crawford-Mammoth Cave Uplands

[Sampling site name abbreviations: BDC, Beaverdam Creek; BC, Buck Creek; BF, Buck Fork Pond River; BRB, Big Run Branch; BRBW, Big Run Branch at Little Beaverdam Creek; LC, Linders Creek; LMC, Little Meeting Creek; NC, Nosey Creek; NFRR, North Fork Rough River; UTMF, Unnamed tributary to

Sampling site name (refer to figure 1 for location)	USGS station number	KDOW site number	Attribute (refer to table 3 for definitions of attributes)							
			Taxa Richness	Total Number of Individuals	Ephemeroptera, Plecoptera, and Trichoptera Richness	Percentage of Orders Ephemeroptera, Plecoptera, and Trichoptera	Hilsenhoff Biotic Index	Percent-age of Dominant Family	Percent-age of Primary Clingers	Percentage of Ephemeroptera
BDC	03311600	DOW03016002	102	5573	35	29.66	4.684	59.19	51.37	17.2
BC	03311800	DOW03002019	62	1811	18	9.663	5.992	59.41	28.21	3.423
BF	03320100	DOW03004020	75	9494	14	3.654	6.242	89.3	28.82	3.096
BRB	03317940	DOW03008026	48	1742	12	7.921	5.778	61.82	34.67	6.027
BRBW	03317395	DOW03008024	62	2404	14	26.74	6.1	50.08	46.5	23.71
CC	03384330	DOW08013004	60	4434	6	4.127	6.476	56.76	57.55	0.631
DC	03303203	DOW08035013	87	2545	21	47.03	5.027	57.05	60.51	19.01
ELC	03316110	DOW03012008	81	4339	26	29.49	5.585	80.36	85.8	7.743
FC	03320200	DOW03004006	86	3202	23	58.71	4.954	75.48	36.22	47.62
GR	03315180	DOW03018011	80	3192	22	57.51	4.909	70.58	57.08	27.13
HC	03303202	DOW08035004	49	6273	4	14.69	6.419	75.83	29.66	0.813
LBDC	03311700	DOW03016001	79	4727	28	47.93	4.66	51.38	54.36	32.3
LC	03316870	DOW03008011	73	1811	27	27.27	4.713	63.55	73.1	18.27
LMC	03316900	DOW03008025	87	3348	31	18.84	5.394	59.37	77.24	7.974
NC	03310340	DOW03026003	86	2645	20	26.01	5.525	38.03	35.99	15.65
NFRR	03317400	DOW03008014	67	1410	17	54.82	5.148	65.74	47.02	37.23
UTMF	03320720	DOW03004018	58	6273	4	4.065	6.539	76.96	72.86	1.163
WF	03437450	DOW20014005	60	9080	9	44.69	6.384	72.01	33.74	40.92
YBC	03302500	DOW08037001	77	2145	15	17.06	6.078	50.16	61.16	7.692

Ecoregion breakpoint analysis study, 2007-08.

Watson School Road; CC, Crooked Creek; DC, Dorridge Creek; ELC, Elk Lick Creek; FC, Forbes Creek; GR, Gasper River; HC, Hardins Creek; LBDC, McFarland Creek; WF, Warrens Fork; YBC, Yellowbank Creek. USGS, U.S. Geological Survey; KDOW, Kentucky Division of Water]

Attribute (refer to table 3 for definitions of attributes)									
Percentage of Chironomidae	Number of Ephemeroptera, Plecoptera, and Trichoptera Genus Taxa	Number of Total Genus Taxa	Number of Ephemeroptera, Plecoptera, and Trichoptera at the Family Level	Total Number of Individuals at the Family Level	Average Tolerance Value	Modified Percentage of Ephemeroptera, Plecoptera, and Trichoptera Abundance	Hilsenhoff Family Level Biotic Index	Percentage of Nutrient Tolerance	Percentage of Oligochaeta
41.91	31	90	17	45	5.17	23.15	5.39	46.53	5.65
39.53	18	57	15	35	5.95	6.35	6.11	60.46	32.69
69.91	14	67	12	32	6.18	3.42	6.50	29.23	0.82
62.74	11	40	10	22	5.54	7.92	6.49	39.21	3.50
47.25	13	54	10	27	5.91	25.08	6.46	66.51	7.24
47.9	6	53	6	25	6.64	0.68	7.03	80.36	19.82
22.71	19	75	14	38	5.71	25.34	5.60	62.55	2.51
49.08	23	70	13	38	5.68	8.71	6.02	82.92	2.14
34.94	23	78	14	41	5.77	57.46	5.75	27.17	1.72
21.86	20	69	10	36	5.52	33.21	5.40	58.74	2.82
13.5	4	40	4	19	6.61	0.97	6.87	90.24	61.33
32.7	27	75	14	37	5.20	38.38	5.37	43.62	1.73
54.16	21	59	14	32	5.09	26.12	5.51	63.11	2.98
61.02	27	75	16	36	5.40	16.43	5.97	68.07	4.99
33.08	19	73	14	39	5.67	22.95	6.24	50.93	25.82
8.581	17	61	12	35	5.67	51.21	5.35	36.95	2.20
53.22	4	51	3	21	6.56	1.16	6.92	74.53	5.52
15.37	9	54	7	32	6.47	40.94	6.84	41.55	12.05
42.65	14	65	9	32	6.04	14.17	6.48	53.47	6.95

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