Development and Application of the Kentucky Index of Biotic Integrity (KIBI)

Kentucky Department for Environmental Protection
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by

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1.0 Introduction

Under the Federal Clean Water Act (CWA), state statute, and federal and state regulations, the Kentucky Division of Water (KDOW) monitors and assesses the waterways of the Commonwealth. Chemical, physical, and biological data are used to gauge the levels of pollution, degradation, and biotic integrity; and to characterize the structure of aquatic ecosystems in Kentucky. These findings are reported to the U.S. Congress under sections 305b and 303d of the CWA. KDOW established a Reference Reach (RR) Program in the Water Quality Branch (WQB) in 1991 to establish a benchmark to which streams can be compared to within the state. The primary goal of the program was to develop biological indices for diatoms, macroinvertebrates, and fish; develop numerical criteria; and monitor trends (KDOW 1997). The purposes of this document are to establish the reference condition, set criteria using fish as the biological indicator, provide an index that will be reliable and precise in assessing streams for aquatic life use support, and identify exceptional waters.

The Index of Biotic Integrity (IBI) as described by Karr (1981) was used to assess fish community structure and biotic integrity of warmwater Midwestern streams and has proven to be very useful for resource managers. The IBI was comprised of 12 equally weighted metrics that were grouped into three general categories: Species Richness and Composition, Trophic Composition, and Fish Abundance and Condition. Each metric was assigned a 5, 3, or 1 value depending upon whether the obtained value strongly approximates the expected value (5), somewhat approximates the expected value (3), or does not approximate the expected value (1). The individual metric scores were summed, and a total IBI score ranging from 12-60 was achieved. Species richness metrics often varied with region and stream size, while less variation was usually found among other metrics (Karr et al. 1986). Five narrative classifications based on total IBI scores were assigned by Karr (1981) to describe the quality of the fish community at each site.

Development of criteria for an IBI must be region and stream-size specific to correspond with the differences within the ecoregion/basin mosaic (Fausch et al. 1984, Angermeier et al. 2000). In recent years various versions of an index have been developed for different regions (Ohio EPA 1987, Barbour et al. 1999, Hughes and Oberdorff 1999, Maret 1999, Smogor and Angermeier 2001), ecosystems (Minns et al. 1994, Emery et al. 2003) and fauna (Lenat 1993, Deshon 1995, Barbour et al. 1996). The IBI was originally modified by KDOW (1997) for Kentucky and followed the framework of Karr (1981) and Karr et al. (1986). However, no metric evaluation process was performed, criteria were not established for all ecoregions, and scoring of individual metrics was a visual interpretation of a point on a graph, which lead to inconsistencies in scoring by users. Therefore, following the approaches detailed by Barbour et al. (1999), Simon (1999), McCormick et al. (2001), and Smogor and Angermeier (2001), a new index for Kentucky was developed, the Kentucky Index of Biotic Integrity (KIBI). The objectives of the new index were to provide reliable and consistent analysis and application among users, and to cover all regions and wadeable streams in a uniform approach.

1.1 Ecoregions

Kentucky is comprised of seven Level III ecoregions (Figure 1): Southwestern Appalachians (68), Central Appalachians (69), Western Allegheny Plateau (70), Interior Plateau (71), Interior River Valleys and Hills (72), Mississippi Alluvial Plain (73), and Mississippi Valley Loess Plain (74) (Omernik 1987; U.S. EPA 2000). Within the Level III ecoregions, 25 subecoregions have been delineated (Woods et al. 2002), which, in part, overlay the physiographic regions of the state (Quarterman and Powell 1978, Andrews 2000). The Mississippi Alluvial Plain was the only ecoregion that did not cover a significant land area or provide high fish diversity in Kentucky. The three eastern Kentucky ecoregions, Southwestern and Central Appalachians and Western Allegheny Plateau, make up what is commonly known as the Eastern Coalfields. This area has the highest density of forest, greatest topographic relief, and sandstone lithology. The presence of the Daniel Boone National Forest is important for the relatively undisturbed streams in the region. The Interior Plateau ecoregion covers central Kentucky and is the largest ecoregion in the state. The region is limestone based with extensive areas of karst topography, which provides for numerous spring-fed streams and an extensive underground stream network (e.g., Mammoth Cave National Park). The relief is mostly rolling hills and landuse is mostly farmland. The largest urban areas are found in Louisville, Lexington, and northern Kentucky (greater Cincinnati area). The Interior River Valleys and Hills, Mississippi Alluvial Plain, and the Mississippi Valley Loess Plain Ecoregions comprise the western and northwestern part of the state. Low-gradient streams flowing through the alluvial soils of the flat bottomlands typify these regions. Farmland is extensive and channelization of streams frequent. The Interior River Valleys and Hills Ecoregion shows evidence of acid mine drainage in several river systems.

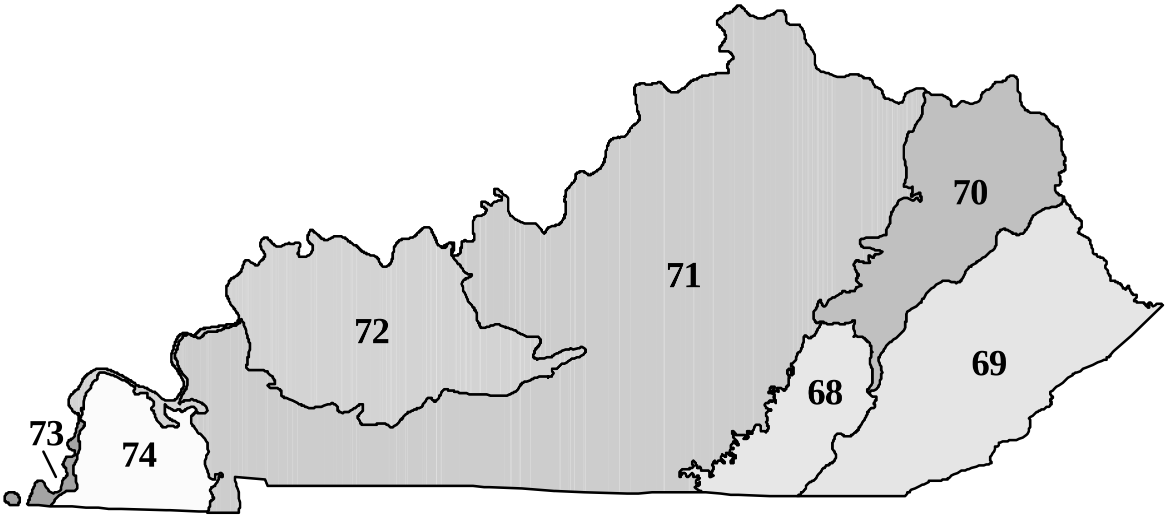

Figure 1. Level III ecoregions. 68=Southwestern Appalachians, 69=Central Appalachians, 70=Western Allegheny Plateau, 71=Interior Plateau, 72=Interior River Valleys and Hills, 73=Mississippi Alluvial Plains, 74=Mississippi Valley Loess Plains.

1.2 River Basins

Burr and Warren (1986) recognized 11 major river basins for Kentucky. KDOW recognizes 12 basins, and differs in classifying the Little Sandy system as a major river basin rather than a minor tributary of the Ohio River, combines the Barren River system within the Green River basin, and subdivides the Cumberland River into upper and lower reaches (Figure 2). The influence of basins on the distribution of aquatic biota provides distinct faunal groups, especially with fishes (e.g., numerous endemics) (Burr and Warren 1986), and mussels (e.g., Cumberlandian fauna) (Cicerello et al. 1991). However, a paradigm can occur when river basins traverse one or more physiographic regions or ecoregions, which commonly occurs in Kentucky. Although a basin, as a whole, can provide distinct faunal groups, review of phenograms in Burr and Warren (1986) indicate a river's fauna can be more affiliated within a region than within its own system as the basin crosses several regions (e.g., Green River Basin). Factors influencing this phenomenon can be primarily attributed to topography, gradient and geology, which dictate features of a stream more locally, such as landuse, in-stream habitat, flow regimes, and temperature. This is supported in the Green and Cumberland river systems, which cover large areas and are the two most diverse river systems in the state and, in addition, with more than 10 endemic species combined. However, Strange (1999) points out habitat quality dictates species persistence in a region and the basin history, in part, provides basin diversity. This was seen in the Kentucky and Green River systems, which both traverse the Interior Plateau ecoregion and encounter similar landuse. Furthermore, the Kentucky River was part of the Teays River system and the Green River was part of the Old Ohio River system (Burr and Warren 1986, Strange 1999), and diversity between these two systems can been seen ecologically and taxonomically. Therefore, the inherent differences between basins and within regions needs to be addressed during index development.

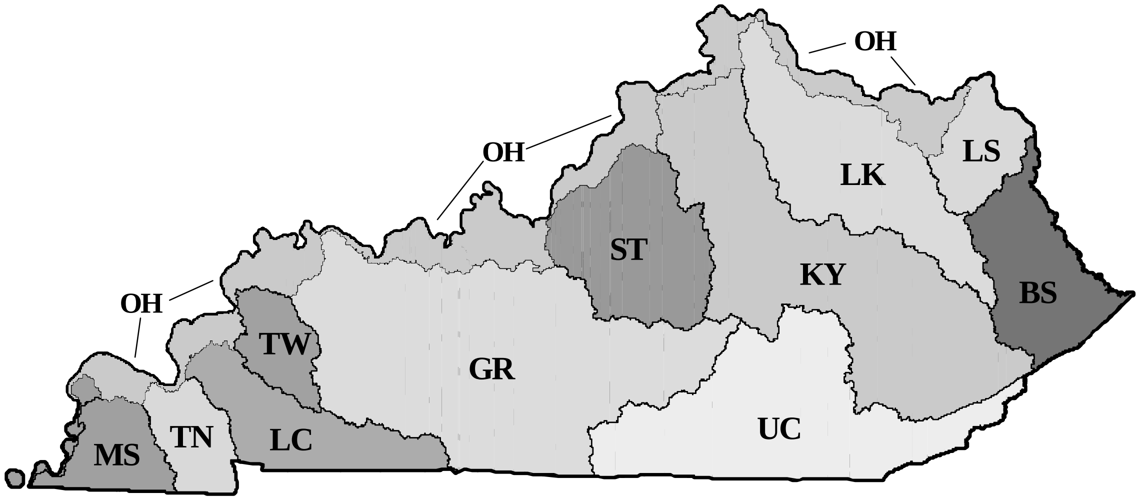

Figure 2. Major river basins. BS= Big Sandy, GR= Green, KY= Kentucky, LC= Lower Cumberland, LK= Licking, LS= Little Sandy, MS= minor tributaries of the Mississippi R., OH= minor tributaries of the Ohio R., ST= Salt, TN= Tennessee, TW= Tradewater, UC= Upper Cumberland.

1.3 Stream Size

As with river basins, stream size can also provide distinct faunal groups. For example, certain species (e.g. *Phoxinus* spp. and *Etheostoma parvipinne*) were typically found in streams less than 10 mi^2, or when encountered in larger streams they represented less than 2 percent of the community (KDOW unpublished data). In contrast, other species (e.g. *Moxostoma* spp. and *Notropis photogenis*) were characteristic of larger bodies of water. KDOW (2002) classified streams into headwater streams (<8 mi^2) and wadeable streams (>12 mi^2). A “gray” area between 8-12 mi^2 existed and best professional judgment was used to classify streams into the respective class. Upon further analysis and observation, this document classifies headwater streams as <6 mi^2, wadeable streams as >10 mi^2, and the “gray” area as 6-10 mi^2. Collection and analysis of large-wadeable and non-wadeable large rivers (>200 mi^2) is ongoing, and separate criteria will be developed.

1.4 Ichthyoregions

Based upon the classification of river basins, and ecoregions and the influence of these regions upon river basins, *a posteriori* regional classification for index criteria was established. The classification scheme was based on review of Burr and Warren (1986) and exploratory multivariate analysis (KDOW unpublished data), which suggested Kentucky might have several distinct fish faunal groups. Review of the taxonomic differences was one aspect of setting regional KIBI classifications, but differences in ecological attributes (e.g. species richness, darter richness, percent tolerants) must be explored in conjunction. For example, the presence of the allopatric sister species, *Etheostoma barrense* and *E. rafinesque* in subecoregion 71g (Wood et al. 2002), provides two distinct species found in one region and in one river system that were considered ecological equivalents and would not influence index results. Another example was the Upper Cumberland River system and in particular the influence of Cumberland Falls. Burr and Warren (1986) showed that fauna above Cumberland Falls was most similar to the Cumberland River below the Falls in the Central and Southwestern Appalachian Ecoregions (see Fig. 17 in Burr and Warren 1986). However, review of physiographic regions shows the Cumberland Mountains physiographic region, which encompasses most of the Cumberland River above Cumberland Falls, to be quite dissimilar to the rest of the Cumberland River system in the Cumberland Plateau region (see Fig. 18 in Burr and Warren 1986). This dissimilarity within a single basin and between two physiographic regions was obviously a result of Cumberland Falls but provides credence that a combination of eco/physiographic regions and river basins is needed for regional criteria classification, particularly since the Cumberland River below the Falls in the Cumberland Plateau has nearly twice the number of species as the Cumberland River above Cumberland Falls. Therefore, the purpose for regional classification for application of the KIBI is not to separate distinct taxonomic groups into regional criteria but to take the inherent nature of the faunal groups and provide an ecologically based regional classification scheme for criteria development.

Six ichthyoregions (Figure 3) were developed to alleviate the influence of basins and regions and typically follow Level III Ecoregion boundaries (Woods et. al 2002). These ichthyoregions were modified into areas to incorporate ecological region and basin similarities or differences. The six ichthyoregions are defined below.

Mountain (MT) This region encompasses all river systems (Big Sandy, Cumberland, Kentucky, Licking, Little Sandy, and minor tributaries of the Ohio River) within the boundaries of the Central (69) and Southwestern Appalachian (68) Ecoregions and the Western Allegheny Plateau (70) Ecoregion, except for the Cumberland River above Cumberland Falls.

Cumberland River above Cumberland Falls (CA) This region encompasses the Cumberland River system above the Cumberland Falls in the Central (69) and Southwestern Appalachian (68) Ecoregions.

Bluegrass (BG) This region includes all river systems (Kentucky, Licking, Salt, and minor tributaries of the Ohio River) that lie within subecoregions (71d, k, and l) of the Interior Plateau (71).

Pennyroyal (PR) This region includes all river systems (Cumberland, Green, Kentucky, Salt, Tradewater, Tennessee, and the minor tributaries of the Ohio River) that lie within subecoregions (71a, b, c, e, f, g, and h) of the Interior Plateau (71), except for the Green River system that lies within subecoregion 71g.

Upper Green River (GR) This region is the Green River system in subecoregion 71g of the Interior Plateau (71).

Mississippi Valley-Interior River (MVIR) This region encompasses all river systems (Lower Cumberland, Green, Tradewater, Tennessee, minor tributaries of the Mississippi River, and minor tributaries of the Ohio River) within the boundaries of the Interior River Valleys and Hills (72), Mississippi Alluvial Plain (73), and Mississippi Valley Loess Plain (74).

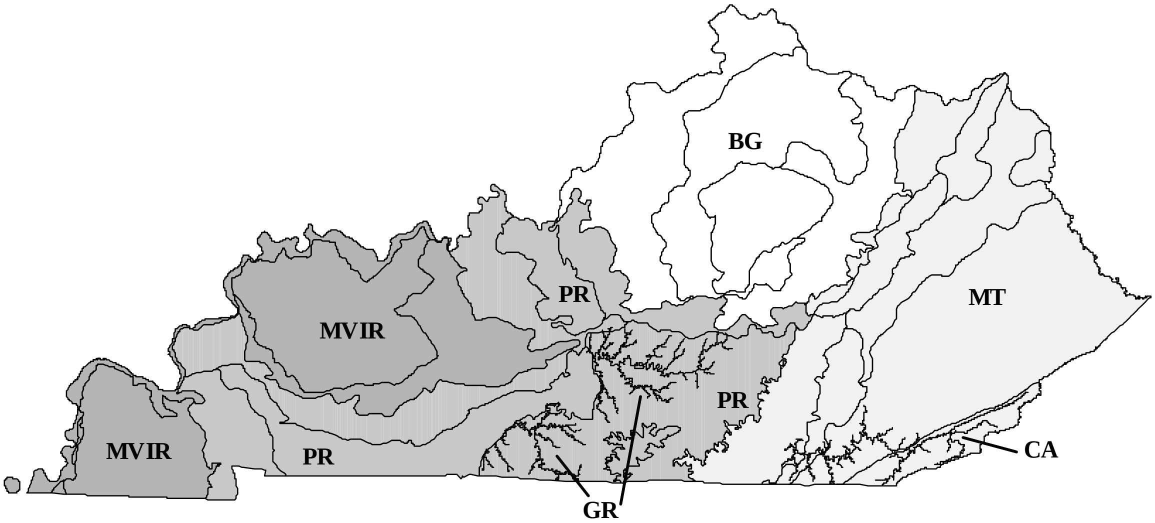

Figure 3. Ichthyoregions. BG= Bluegrass, CA= Cumberland River above Cumberland Falls, GR= Upper Green River, MT= Mountain, MVIR= Mississippi Valley-Interior River, PR= Pennyroyal. Note GR and CA ichthyoregions are river basins within larger ichthyoregions. Solid lines mark Level IV subecoregion boundaries (see Woods et al. 2002).

2.0 Methodology

2.1 Reference Condition

The concept of the reference condition was to establish a network of streams that exhibit the most "natural" conditions for which biotic integrity can be measured against. "Natural" can be defined as the condition of a stream with least or minimal impact to its watershed. The reference streams represent the best conditions available in the state based from data collected within the past 10 years. Following Hughes (1995), a regional reference approach was established to obtain data from streams with similar physical characteristics. This is important since a better understanding of the inherent biological variability and natural potential of the streams in a collective region is necessary (Pond and McMurray 2002). In addition, a regional sampling design was more robust than site-specific control methods and facilitates assessment at various scales (Barbour 1997). Therefore, the objectives of the Reference Reach Program in the KDOW's WQB were to collect and summarize data from least-disturbed streams using a regional framework in order to develop appropriate numerical criteria for bioassessment interpretation. Previous studies on fish (KDOW 1997), algal (KDOW 1998) and macroinvertebrate (KDOW 2000) communities inhabiting Kentucky's reference reach streams helped to develop a framework for establishing reference conditions in select parts of the state.

The reference condition collectively refers to the range of quantifiable ecological elements (i.e., chemistry, habitat, and biology) that are found in natural environments. Determination of reference sites were obtained based on "least" or "minimal" disturbance. Minimally disturbed sites were classified as sites that were most natural for a given region and time. Least disturbed sites were classified as sites that showed some degree of anthropogenic influence but were considered the best sites for a given region and time. Most reference sites fall under least disturbed, because in many regions of Kentucky finding reference streams can be a difficult task because no regions are without areas of human disturbance. Selection of reference quality streams used a combination of narrative and quantitative physical attributes (Table 1). In conjunction, additional agency data were reviewed (e.g., presence/absence of dischargers, confined animal feeding operations, mines, oil and gas development, and land cover) to help select candidate reference reaches. Streams were selected as reference if they met all of the criteria (minimal-disturbance) or exhibited the best (least-disturbance) condition for a region. Stream reaches that failed the criteria were classified as “Test.”

Table 1. Summary of criteria used in the Reference Reach selection process.

Category	Criterion
1) riparian zone condition*	well-developed providing some canopy over the stream; presence of adequate aquatic habitats in the form of root mats, coarse woody debris and other allochthonous material
2) bank stability*	at least moderately stable with only a few areas susceptible to erosion within the sampling station
3) degree of sedimentation*	the substrate is 25 percent or less embedded by fine sediment
4) suspended material	the water is relatively free from suspended solids during base flow conditions
5) evidence of nutrient enrichment	the substrate is relatively free from extensive algal mats that could smother benthic habitats
6) conductivity	conductivity is not highly elevated above what naturally occurs (region-specific)
7) aquatic habitat availability*	there is $\geq$ 70 percent (or >50 percent for low gradient) mix of rubble, gravel, boulders, submerged logs, root mats, aquatic vegetation or other stable habitats available for aquatic organisms
8) presence or absence of trash in the stream	solid waste within the stream and on the streambank is rare or absent
9) evidence of new land-use activities in the watershed	the landuse conditions are unchanged compared to most recent topographic maps or aerial photos
10) accessibility of the site for collection	access to site is obtained within a practical time and manner

*based on RBP habitat scoring procedures (Barbour et al. 1999)

After selection of stream reaches the reference condition was established to compare streams exposed to environmental stressors using defined sampling methodology and assessment criteria. Impairment would be detected if indicator measurements (e.g., biotic indices, habitat rating, nutrient concentrations) fell outside the range of threshold criteria established by the reference condition.

2.2 Fish Data

Fish community data were obtained from the Ecological Data Application System (EDAS) database used by KDOW. A total of 388 collections, 165 reference and 223 test, representing all Level III Ecoregions in Kentucky (Woods et. al 2002) and ichthyoregions ranging in watershed size, 0.9 mi^2 to 198 mi^2, were used from 1993-2003 sampling (Figure 4). The distribution of sites in each ichthyoregion and stream size classification is shown in Table 2. Collections and identifications were conducted by one of three crew leaders from KDOW and/or U.S. Forest Service (Daniel

Boone National Forest) to provide consistency. Given the complexity of stream sampling conditions (e.g., stream size, substrate, and flow regime), sampling techniques ranged from seining (78 sites), backpack electro-fishing (180), to a combination of the two techniques (130). The sites sampled covered all available habitats within a 100-250 meter reach with a total sampling effort of 30-180 minutes. The goal was to thoroughly sample the reach and assure that all of the fish species would likely be collected, except for the most rare species, and their relative abundance accounted. The sampling period ranged from mid-March to mid-October, except for one sample, which was collected the last week of February 2002. Identification and preservation of specimens follows methodology outlined in KDOW (2002).

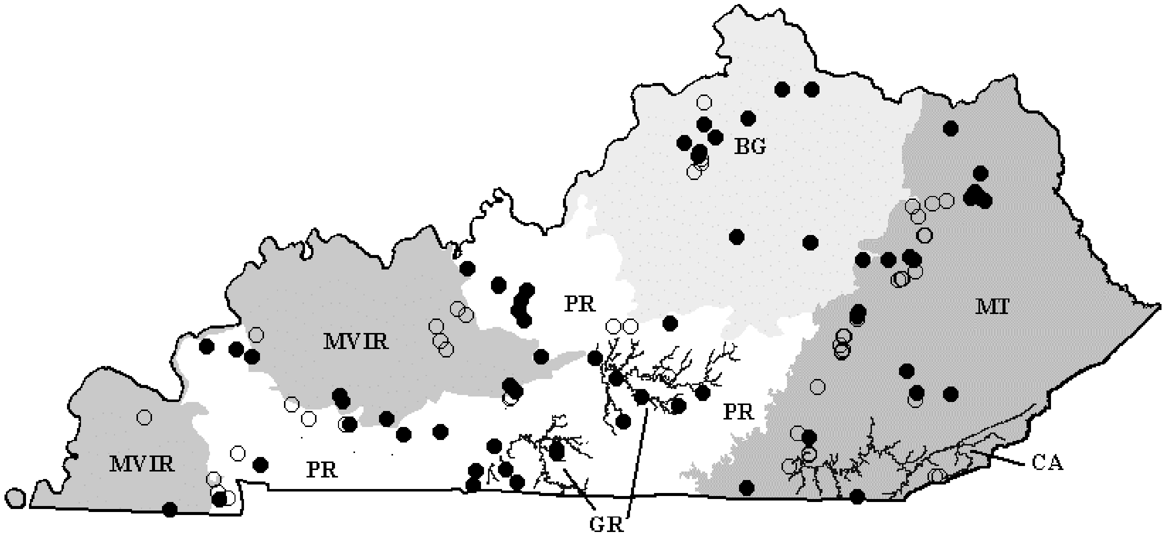

Figure 4. Reference sites within ichthyoregions. Open circles= headwater sites, shaded circles= wadeable sites. BG= Bluegrass, CA= Cumberland River above Cumberland Falls, GR= Upper Green River, MT= Mountain, MVIR= Mississippi Valley-Interior River, PR= Pennyroyal. Note GR and CA ichthyoregions are river basins within larger ichthyoregions.

A fish master taxa list with taxonomic, trophic, tolerance, and ecological classifications is provided in Appendix A. The list was compiled using scientific literature (Ohio EPA 1987, Etnier and Starnes 1993, KDOW 1997, Goldstein and Simon 1999, McCormick et al. 2001), historic data (Burr and Warren 1986, Laudermilk and Cicerello 1998), consultation with ichthyologists and fisheries biologists (L. Page, J. Porterfield, D. Eisenhour, R. Cicerello, pers. com.), and best professional judgment.

Table 2. Number of reference (R) and test (T) sample events for each ichthyoregion.

	Headwater		*Wadeable*			
Ichthyoregions	R	T	R	T	Total R	Total T
CA	12	4	3	11	15	15
BG	6	6	12	32	18	38
GR	1	0	10	4	11	4
MT	23	49	27	54	50	103
MVIR	7	18	16	29	23	47
PR	18	6	30	10	48	16
Totals:	67	83	98	140	165	223

2.3 Chemical and Physical Data

Chemical and physical data collection provided background information for the screening of reference sites and to test metric responsiveness along the parameter gradient. Temperature, dissolved oxygen, pH, and conductivity were collected using a YSI meter, Hydro-Lab meter or similar unit. Grab water samples were collected for ammonia (NH_3) (190 samples), nitrate (N) (193), total phosphorous (TP) (192), and total Kjeldahl nitrogen (TKN) (193). Each nutrient sample was fixed with sulfuric acid and initially preserved on ice. Samples were transferred to a refrigerator for temporary storage before being submitted to the Kentucky Division of Environmental Services laboratory for analysis. TKN and nitrate (N) results were added to represent total nitrogen (TN). A total of 286 Water Quality Branch Habitat Field Sheets (KDOW 2002) were filled out for either high (214 samples) or low gradient (72) stream sites. Field sheets were modified from RBP habitat forms (Barbour et al. 1999). Given the extended period of time during the collection period (10 years) and the initial objective of numerous fish collections, not all sites were represented by each chemical and physical parameter. However, a total of 78 reference and 98 test sites had the complete suite of chemical and physical data in which sample collection occurred on or near the same date as the fish data.

Following a categorical approach used by Ohio EPA (Miltner and Rankin 1998) and Bryce et al. (1999), the chemical and physical parameters were coded to provide a habitat stressor gradient and a nutrient load stressor gradient. Both categorical approaches are outlined below. All of the nutrient data (i.e., statewide reference and non-reference) stored in EDAS were utilized to determine the 25th, 50th, 75th, and 90th percentile distributions for TP (n=594) and TN (n=673) (Table 3). Bioassessment sites were placed into one of six categories (nutrient codes) based upon the percentile rankings for TP and TN at those sites. For example, a code rating of "1" was given to sites having TP and TN concentrations less than the 25th percentile for both parameters. Sites were given a nutrient code rating of "2" if either TP or TN concentrations were less than the 50th percentile for either parameter. A category rating of "3" was given to sites having a TP concentration less than the 75th percentile and a TN concentration less than the 90th percentile. If a site had a TP concentration greater than the 75th percentile irrespective of TN, then the site was placed into category "4." Sites were given a category rating of "5" if both TP and TN concentrations were greater than the 90th percentile. When ammonia concentration (a toxic stressor) was greater than 1.0 mg/l the site was given a category rating of "6."

Table 3. Nutrient code designations. Total Nitrogen (TN) and Total Phosphorus (TP) (in mg/l) derived from dataset corresponding to all biological sample events (after Milton and Rankin 1998).

Code	Nutrient Interaction	Percentile	TP (n=594)	TN (n=673)
1	both $\leq TP_{25}$ TN_{25}	25th	0.014	0.386
2	either $\leq TP_{50}$ TN_{50}	50th	0.045	0.860
3	$\leq TP_{75}$, $<TN_{90}$	75th	0.163	1.763
4	$>TP_{75}$, $<>TN_{90}$	90th	0.710	4.178
5	both $\geq TP_{90}$ TN_{90}			
6	$NH_3 \geq 1.0$ mg L^{-1}			

For the habitat stressor gradient, using the RBP habitat form, the WQB recognized a subset (based on correlation data) of seven of the 13 metrics (both high and low gradient) that appear to be key elements in community performance (epifaunal substrate, embeddedness, sediment deposition, velocity/depth regime, riparian zone width, pool variability, and channel sinuosity). As with the nutrient gradient, a categorical approach was established. Based on the 75th, 50th, 25th, and 10th percentiles of all habitat data stored in EDAS (high gradient = 483; low gradient =112), habitat stress points (0 to 4) were assigned to each of the selected habitat parameters. Stress points were then summed for each sample event and the site was assigned to one of five habitat stress categories (Table 4).

Table 4. Designation of site habitat stress codes using subset of RBP habitat parameters (a.) parameter percentile distributions, (b.) stress point scoring, (c.) stress code assignment.

a.

%ile	Embedded Score	Epifaunal Substrate	Sediment Deposition	Vel/Depth Regime	Riparian Zone	Pool Variability	Channel Sinuosity
75th	18	18	16	18	19	18	17
50th	16	16	13	16	15	16	13
25th	13	11	8	12	10	12	9
10th	8	7	6	9	5	9	6
N=	483	595	595	483	595	112	112

b.

Habitat Parameter %ile	Habitat Stress Points
> 75th	0
50 to 75th	1
25 to 50th	2
10 to 25th	3
<10th	4

c.

Range of Stress Points	Habitat Stress Code
0--4	1
5--9	2
10--14	3
15--19	4
20--24	5

2.4 Metric Screening

The objective of the Kentucky Index of Biotic Integrity (KIBI) was to provide users with a uniform, reliable, and consistent bioassessment tool that would be applicable statewide. To achieve this goal, 42 candidate metrics (Appendix B) were selected from previous studies (Karr 1981, Karr et al. 1986, Ohio EPA 1987, Barbour et al. 1999, and McCormick et al. 2001). For uniform application of each candidate metric retained for the KIBI, the evaluation process was performed on a statewide scale and not for localized regions or river systems. To adjust for regional differences, expectations of the KIBI score for a region would be accounted and numerical criteria for a region would be established separately. This approach allows the index to be consistent in framework development but be cautious of narrative classification regionally. Therefore, candidate metrics represented various attributes of a stream fish community that would potentially show sensitivity to human impacts and predictability to environmental parameters, and would provide uniform application statewide. The metrics represented four categories: taxonomic composition (24 metrics), tolerance (7), trophic (9), and reproductive guilds (2). Individual fish condition metrics were omitted since deformities and anomalies of fish specimens were infrequently observed or reported.

The screening process for each metric included tests for range, variability (within reference dataset), redundancy (within reference dataset), predictability to environmental parameters, and discriminatory power (sensitivity) between reference and test sites. Richness metrics failed if the range was 5 or less or if the range of a relative abundance metric was 65 % or less. Range values were selected to ensure that each ecological parameter in the fish community was typically present and readily derived from collected samples. Variability of a metric (within the reference dataset only) was considered too great (i.e., failed) if the range between the 75th %ile and the 25th %ile was greater than the value of the 25th %ile. To express this numerically, the 25th %ile was subtracted from the 75th %ile and the outcome was divided by the 25th %ile. Values greater than 1.0 were considered to show high variability. Following Barbour et al. (1996) box plots were used to show the sensitivity of a metric between reference and test sites. Metrics failed if the discriminatory power score was 0 (poor) (Figure 5). Metrics that perform relatively similarly between test and reference sites provide little or no information in impairment detection and can confound assessment efforts. To compensate for statewide variability, box plots were used to test the discriminatory power for each metric using the *a posteriori* ichthyoregion classification scheme. Therefore, metrics that performed poorly on the statewide level were still considered since they may exhibit good discrimination within several ichthyoregions. Metrics were tested for predictability with each parameter of the RBP habitat sheet using Spearman's correlation. Natural log transformed values for conductivity (Cond.), ammonia (NH_3), total Kjeldahl nitrogen (TKN), nitrate (N), total nitrogen (TN), total phosphorus (TP), and an interactive parameter (TN*TP) were evaluated for responsiveness using Pearson's correlation analysis. Spearman's correlation analysis was used on categorical data and Pearson's correlation analysis was used on continuous-scale data. Metrics were retained if a good expected response ($r \geq 0.25$, $p < 0.01$) was shown for five or more of the habitat and chemical parameters. Pearson's correlation coefficients of the remaining metrics were used to test for redundancy between paired metrics within the reference dataset. One of the paired metrics was dropped if $r > 0.75$; the paired metric with higher variability and weaker discriminatory power was dropped. Following McCormick et al. (2001), $r > 0.75$ was considered since values higher were believed to provide little new insightful information to the index. Most metrics exhibited a correlation with catchment area that required adjustment.

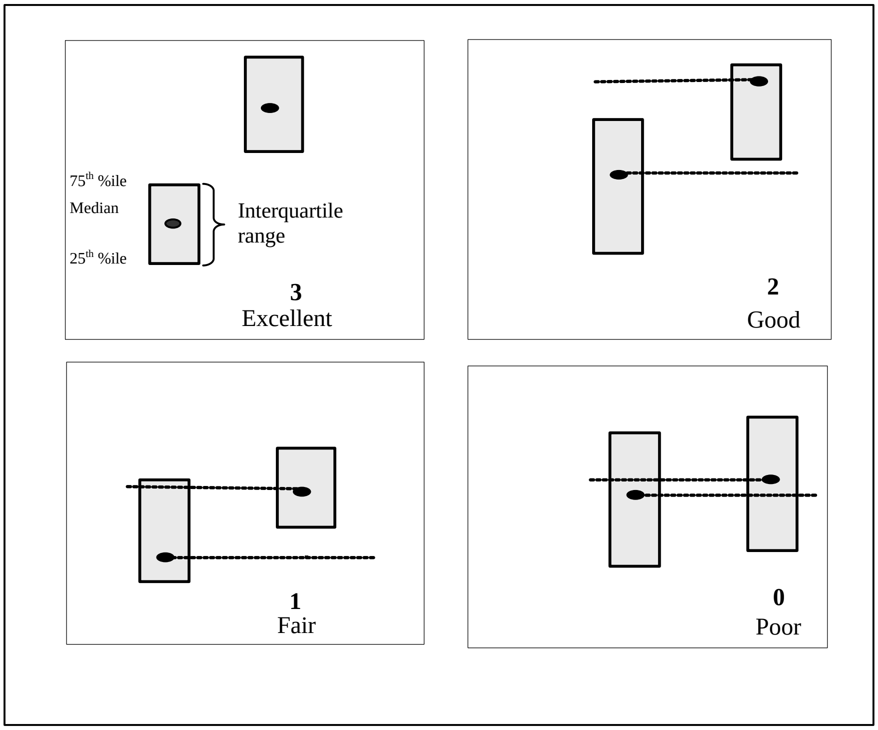

Figure 5. Hypothetical interquartile plots showing sensitivity or discriminatory power rating score criteria (after Barbour et al. 1996).

2.5 Catchment Area Calibration

Regression equations were used to determine the relationship between catchment area and the candidate metrics. Metrics were adjusted for catchment area if $r^2 \geq 0.1$, $p<0.01$. Adjusting for drainage area enhances the metrics' performance to detect disturbances rather than stream-size effects (Smogor and Angermeier 1999). Calibration steps followed Urquhart (1982) and McCormick et al. (2001). The first step was to transform catchment area size (mi^2) to the $\log_{10}$ value for each site (Table 5). Negative response metrics (e.g. %TOL, %FHW, or %OMNI) were inverted to perform as positive response metrics (e.g. DMS, INT). Negative response metrics were metrics that were expected to increase with impairment, and positive response metrics were metrics that were expected to decrease with impairment. Using the reference dataset only, regression equations were established to obtain the expected value for a metric. Linear regression equations were used for richness metrics, and binominal regression equations were used for relative abundance metrics, since they have a defined range (0-100). The use of regression equations was to serve as a substitute for the maximum species richness lines (MSRL) described by Fausch et al. (1984) and Karr et al. (1986). The residuals (difference between the reference expected and actual) were added to a catchment area constant of 38.6 mi^2 (100 km^2) to obtain univariate metric values. This standardized catchment constant value was chosen based on the distribution of the catchment area values and the past study of McCormick et al. (2001). The calibration of the residuals provides a normalized distribution of values from which a rank and percentile can be determined regardless of catchment area. This allows for uniform scoring across all stream sizes.

2.6 Metric and KIBI Scoring

Scores of all metric values were divided by the 95th %ile of the reference dataset for the respective metric, and multiplied by 100 to score the metric on a 0-100 point scale. A continuous scale was used since it was believed to be more responsive with the continuous scale of various environmental parameters than prior categorical scoring (5, 3, 1), as used in Karr's (1981) IBI (Hughes et al. 1998, McCormick et al. 2001). Metrics from sites that performed exceptionally well and scored above 100 were set at 100. Metrics from sites that performed extremely poor and had negative values were set at 0. If collections had 50 or fewer fish individuals, the relative abundance metrics were set at 0. If collections had 51-99 fish individuals, relative abundance metrics were set at 50, unless the metric score was already below 50, then the value was not changed. This automatic scoring of proportional metrics was based on the scoring modification principle used by Ohio EPA (1987) and Simon (1991). The final KIBI score, on a 0-100 point scale, was the average of the remaining Metric Scores. A summary outline of the calculation process is found in Table 5. KIBI application users will be given the Reference Regression Equation (RRE), Catchment Area Constant (CAC), and metric value 95th %ile value for each retained metric. Example metric RRE, CAC, and 95th ile are shown in Table 6. An overall calculation example is found in Appendix C.

Table 5. Scoring outline for metric calculation and KIBI Score.

Metric scoring calculation process:

1) Convert site catchment area (sq. miles) to Log_{10}. This value will represent ‘x’ in the Reference Regression Equation (RRE) (see Table 6).
2) Inverse negative response relative abundance metrics, 100 minus metric’s actual/raw value.
3) Solve for the Expected Value of metric using the Log_{10} of a site’s catchment area as ‘x’ in the RRE (Table 6) of the respected metric.
4) Subtract Actual Value (raw data) from the Expected Value (Step 3) to obtain a Residual Value. This number will be positive or negative based on site quality.
5) To normalize Residual Value data for all catchment areas a Catchment Area Constant (CAC) (Table 6) is used for each metric. CAC is added to the Residual Value (Step 4) to obtain Metric Value.
6) Metric Value is divided by 95th %ile (Table 6) of the reference dataset for the respective metric and multiplied by 100 to equal Metric Score.
7) The average of the retained Metric Scores equals the final KIBI score; KIBI score is a whole integer.

Scoring rules:

1) If Metric Score >100 then score as 100.
2) If Metric Score < 0 then score as 0.
3) If total number of individuals (TNI) $\leq$ 50 then set % metrics at 0.
4) If TNI 51-99, then set % metrics at 50, unless metric value is already under 50 then do not modify.
5) If TNI > 99 then % metric scores are not modified.

Table 6. Example retained KIBI metrics with respective Reference Regression Equations (RRE), Catchment Area Constant (CAC), and 95th %ile of reference metric values provided. Richness metrics use linear regression equations, and relative abundance metrics use binominal regression equations. In RRE ‘x’ = $\log_{10}$ of catchment area and ‘y’ = expected metric value

Example metric variable	**Reference Regression Equations**	**CAC**	**95th %**
A	$y = 10.123x + 4.4279$	20.49	28.2
B	$y = 2.967x + 1.5037$	6.21	9.3
C	$y = -10.326x^2 + 44.989x + 17.575$	58.88	87.8
D	$y = 8.9128x^2 - 59.151x + 98.557$	27.14	61.4

General equation: ((Actual metric value – (RRE)) + CAC)/ 95th %ile * (100) = Metric Score

2.7 KIBI Testing

Box plots were used to determine the discriminatory power rating score (Barbour et al. 1996) for the KIBI on the statewide level. Responsiveness of the KIBI to each chemical parameter was measured using Pearson correlation analysis, and Spearman’s correlation analysis was used for each physical parameter. Box plots were used to show KIBI scores along coded habitat and nutrient stressor gradients. Linear regression was used to test the repeatability and variability of the KIBI within the sample period. Box plots of reference scores for each ecoregion, major river basin, and ichthyoregion were used to determine the best classification scheme and show variability within each. Sites were designated as headwater or wadeable, and box plots were used to test for differences between the stream size and stream condition.

2.8 KIBI Narrative Classification

Narrative classification thresholds for Excellent, Good, Fair, Poor, and Very Poor were established using the reference KIBI scores. Scores greater than the 50th %ile were classified as having "Excellent" biotic integrity; scores between the 5th and 50th %iles were classified as having "Good" biotic integrity. The value of the reference 5th %ile was trisected to have equal intervals representing Fair, Poor and Very Poor biotic condition.

3.0 Results and Discussion

A total of 6 metrics failed the range test, 9 failed the variability test, and 20 failed the discriminatory power test (Table 7). Only 1 metric (DMS) had a discriminatory power rating of 3. The 10 remaining metrics showed responsiveness with the chemical and physical parameters. Only 3 (TR, %OH, and BEN) of the 10 remaining metrics failed redundancy ($r>0.75$). These metrics were dropped because of higher variability than the respective paired metric (NAT, %INSCT, and DMS). The metrics %NutTol and %NutTolCC were dropped because of the similar ecological aspect as %TOL, and prior wide acceptance of %TOL in other fish indices (Karr 1981; Ohio EPA 1987; Barbour et al. 1999). The metrics INT and %FHW failed the variability test but were retained after reexamination within the ichthyoregion and stream size classification schemes indicated specific uses for each metric. Therefore, seven metrics were retained to comprise the aggregate Kentucky Index of Biotic Integrity (KIBI).

Table 7. Metric screening process results.

Metric Abbreviation	Range	Variability	Redundancy	Discriminatory Power
TNI				X
TR			X	
NAT				
DMS				
INT		X		
WC				X
SL				
%INSCT				
%OMNI				X
%TOL				
%DMS		X		
%CrChub				X
%Dar		X		
%InsctCyp				X
%Sucker	X			X
%NutTol				
%NutTolCC				
%Camp		X		X
%TC		X		X
%PIO				X
%Dace	X			X
HW	X			
%HW		X		
SUN	X			X
SUC	X			X
MIN				X
%INT		X		
%SL		X		X
TOL				X
PIO				X
%WC				X
OMNI				X
%OH			X	
%FHW		X		
FHW				X
TC	X			X
%BEN		X		
BEN			X	
%InsctCypTol				X
InsctCypTol				X
%Pelagic				X
Pelagic				X

‘X’ denotes metric failure. Bold and Italic metrics were retained for the KIBI.

3.1 Metric Description

The seven metrics retained for the Kentucky Index of Biotic Integrity (KIBI) were Native Richness (NAT), Darter, Madtom, and Sculpin Richness (DMS), Intolerant Richness (INT), Simple Lithophilic Spawners (SL), Relative Abundance of Insectivorous Individuals, excluding Tolerant Individuals (%INSCT), Relative Abundance of Tolerant Individuals (%TOL), and Relative Abundance of Facultative Headwater Individuals (%FHW). NAT was used only in wadeable streams, and was replaced by %FHW in headwater streams. Environmental parameters that were significantly correlated ($r \geq 0.2$, $p < 0.01$) to metrics were noted.

1. ***Native Species Richness*** (NAT): This is the total number of native species present in a sample. Non-native species were excluded since they were a direct indication of anthropogenic impairment. This is a modification from Karr's (1981) total number of species and was used in several other indices (Robinson and Minshall 1992, Barbour et al. 1999, and Smogor and Angermeier, 1999). NAT was found to have poor sensitivity in headwater streams and will be used only in wadeable streams. A moderate amount of impairment (e.g., increased nutrients or increased temperature) slightly alters the typical habitat, which allows for the presence of species that usually do not inhabit small streams (e.g., *Lepomis* spp.). A replacement metric (%FHW) for headwater streams is described below. NAT was correlated positively with the RBP habitat parameters epifaunal substrate, riparian vegetative zone width, channel alteration, pool variability, pool substrate characterization, and total habitat score. NAT was correlated negatively with conductivity, NH_3, TN, and nitrate (N).

2. ***Darter, Madtom, and Sculpin Richness*** (DMS): This is the total number of the species present in a sample within the tribe Etheostomatini (darters), the genus *Noturus* (madtoms), and the genus *Cottus* (sculpins). These groups, relatively, are intolerant or sensitive to pollution. This metric was a modification of Karr's (1981) Darter Richness metric. DMS was correlated positively with embeddedness, epifaunal substrate, bank vegetative protection, sediment deposition, riparian vegetative zone width, and frequency of riffles, pool variability, pool substrate characterization, channel sinuosity, total habitat score, and channel alteration. DMS was correlated negatively with conductivity, $NH_{3,}$ and TN.

3. ***Intolerant Species Richness*** (INT): This is the total number of intolerant species present in a sample and was originally used by Karr (1981). Members of this metric were believed to represent the first species to disappear after impairment and the last to re-establish after restoration. The metric initially failed the variability evaluation but after examination of the metric regionally, it was found to be less variable and have good discriminatory power. INT was correlated positively with all habitat parameters except for channel flow status and correlated negatively with all chemical parameters except for N.

4. ***Simple Lithophilic Spawning Species Richness*** (SL): This metric is the total number of simple lithophilic spawning species and represents species that require relatively clean gravel and exhibit simple spawning behavior (Ohio EPA 1987; Simon 1991). The metric was considered a habitat metric and was expected to decline with impairment and be particularly sensitive to siltation (Berkman and Rabeni 1987). SL was correlated positively with all habitat parameters except channel flow status, embeddedness, and velocity depth regime, and correlated negatively with all chemical parameters except conductivity, and N.

5. ***Relative Abundance of Insectivorous Individuals*** (%INSCT): This metric is the relative abundance of insectivorous individuals excluding tolerant individuals. The metric is a modification of Karr's (1981) relative abundance of insectivorous cyprinids and Ohio EPA's (1987) relative abundance of insectivorous individuals. %INSCT was correlated positively with embeddedness, epifaunal substrate, sediment deposition, riparian vegetative zone width, channel alteration, velocity depth regime, pool substrate characterization, pool variability, channel sinuosity, and total habitat and correlated negatively with conductivity and NH_3.

6. ***Relative Abundance of Tolerant Individuals*** (%TOL): This metric was originally used by Karr (1981) and represents a proportion of individuals that are pollution tolerant and increase in abundance with impairment (negative response). For scoring, actual %TOL values were inversed to respond like prior positive response metrics. %TOL was correlated positively with embeddedness, epifaunal substrate, sediment deposition, channel alteration, velocity depth regime, pool substrate characterization, pool variability, channel sinuosity, and total habitat score and correlated negatively with conductivity, NH_3, and TKN.

7. ***Relative Abundance of Facultative Headwater Individuals*** (%FHW): The metric was designed to detect the abundance of species that were atypical of headwater streams (e.g., *Lepomis* spp.) or typically exhibit low abundance in small streams (e.g., *Campostoma* spp.), but tend to increase in abundance with impairment (negative response). *Semotilus atromaculatus* was not considered a member since reference and test averages were roughly the same (30%). The metric replaced NAT in headwater streams. For scoring actual %FHW values were inversed to respond like prior positive response metrics. %FHW was correlated positively with embeddedness, epifaunal substrate, bank stability, bank vegetative protection, sediment deposition, riparian vegetative zone width, channel alteration, frequency of riffles, velocity depth regime, and total habitat score, and correlated negatively with conductivity and NH_3.

3.2 Retained Metric Performance

Analysis of the ranges for the seven retained metrics showed each metric passed the statewide screening criteria (Table 8). Metric ranges for each ichthyoregion passed except for DMS in CA (0-4), and INT in the BG (0-2) and CA (0-4). However, the two metrics did meet the criteria in the majority of the remaining ichthyoregions and were strong candidates in the discriminatory power rating and therefore retained.

Table 8. Retained KIBI metric statewide screening results.

Metric Abbreviation	Range	Variability Score	Discriminatory Power
NAT	0-38	0.31	1
DMS	0-12	0.31	3
INT	0-12	1.1	2
SL	0-19	0.51	1
%INSCT	0-90.8	0.43	2
%Tol	0-100	0.31	1
%FHW	0-100	2.0	2

Variability of the metrics statewide was acceptable for all metrics except INT and %FHW (Table 8). However, because of the low range for INT in the BG the variability was elevated. Analysis of INT without BG yielded an acceptable variability score below 1.0 for the remaining combined ichthyoregions. The variability score of %FHW (2.0) was not surprising since the metric was to be indicative of headwater situations, and the initial test was for all stream sizes statewide. Therefore, after analysis of stream size and ichthyoregions, %FHW resulted in a variability score of 1.0 for headwater streams statewide. CA and MT were the least variable ichthyoregions while BG, MVIR, and PR showed the most variability, but BG and PR showed good sensitivity while MVIR was poor.

Responsiveness of each metric to conductivity, NH_3, N, TKN, TN, TP, and TN*TP are shown in Table 9. Each metric was responsive to at least two chemical parameters, which was acceptable for metric selection. All metrics responded to NH_3, and all metrics, except for SL, responded to conductivity. The significance of all metrics correlating to ammonia indicate elevated levels can be critically detrimental to most attributes of the fish community. INT and SL were the most responsive metrics to TN, TP, and TN*TP. None of the relative abundance metrics, %INSCT, %TOL, and %FHW, correlated significantly with TN, TP, TN*TP. NAT was the only metric responsive to nitrate.

Table 9. Pearson's correlation matrix of chemical values vs. fish metric scores and KIBI.

Metrics	Cond.	Ammonia	Nitrate	TKN	TN	TP	TN*TP
NAT	-0.34	-0.25	-0.23	**-0.03**	-0.22	**-0.01**	**-0.11**
DMS	-0.29	-0.31	**-0.09**	**-0.18**	-0.23	**-0.10**	**-0.19**
INT	-0.39	-0.31	**0.00**	-0.30	-0.23	-0.19	-0.24
SL	**-0.13**	-0.42	**-0.18**	-0.28	-0.37	-0.18	-0.31
%INSCT	-0.30	-0.29	**0.04**	**-0.14**	**-0.11**	**-0.11**	**-0.13**
%TOL	-0.23	-0.36	**0.15**	-0.25	**0.13**	**-0.12**	**-0.13**
%FHW	-0.28	-0.24	**0.16**	**-0.14**	**0.02**	**-0.09**	**-0.07**
KIBI	-0.35	-0.37	**-0.03**	-0.20	-0.21	**-0.15**	-0.21

Bolded values are **not** significantly correlated ($p < 0.01$)

Metric responsiveness to habitat data is shown in Table 10. DMS, INT, and SL were the most responsive metrics and were most sensitive to epifaunal substrate, channel alteration, channel sinuosity, riparian zone, and pool variability. None of the metrics were responsive to channel flow status, which could be an effect of the seasonal variability of that parameter. All metrics were responsive to epifaunal substrate, channel alteration, and the total habitat score. All metrics, except %FHW, were correlated with pool variability, pool substrate characterization, and channel sinuosity which most likely can be attributed to the high variability of the %FHW metric in the MVIR ichthyoregion. Overall responsiveness of metrics was greater to the habitat parameters than to the nutrient parameters, suggesting that fish communities were more sensitive to habitat degradation than to water chemistry impairment.

Table 10. Spearman's correlation matrix for all RBP habitat parameter scores and KIBI metric scores.

Metrics	Embeddedness	Epifaunal Sub	Sediment Dep	Bank Stability	Bank Veg Prot	Riparian Zone	Channel Flow	Chan Alteration	Frequency of Riffles	Velocity/Depth Regime	Pool Variability	Pool Sub Char	Chan Sinuosity	Total Habitat
NAT	**0.16**	0.30	**0.11**	**-0.05**	**0.03**	0.19	**-0.07**	0.22	**0.11**	**0.09**	0.44	0.45	0.42	0.22
DMS	0.28	0.39	0.22	**0.14**	0.20	0.32	**0.06**	0.33	0.20	**0.13**	0.44	0.34	0.46	0.40
INT	0.31	0.39	0.25	0.27	0.34	0.22	**0.06**	0.34	0.33	0.24	0.32	0.26	0.51	0.42
SL	**0.07**	0.31	0.18	0.20	0.20	0.21	**0.06**	0.28	0.18	**0.13**	0.36	0.50	0.42	0.34
%INSCT	0.28	0.35	0.23	**0.07**	**0.13**	0.25	**0.13**	0.31	**0.02**	0.25	0.49	0.31	0.42	0.35
%TOL	0.27	0.31	0.29	**0.11**	**0.10**	**0.15**	**0.13**	0.27	**0.03**	0.22	0.57	0.37	0.41	0.32
%FHW	0.34	0.32	0.29	0.31	0.33	0.33	**0.07**	0.44	**0.11**	0.14	**0.22**	**0.18**	**0.16**	0.42
KIBI	0.42	0.49	0.34	0.23	0.29	0.36	**0.13**	0.48	0.18	0.25	0.55	0.38	0.51	0.52

Bolded values were **not** significantly correlated ($p < 0.01$).

The discriminatory power ratings for each metric on a statewide scale (Figure 6) showed DMS, INT, %INSCT, and %FHW were the most sensitive metrics. DMS was the only metric with a rating of 3. NAT had a rating of 1, and the reference %FHW box plot indicated high variability statewide. Analysis of box plots for each metric showed NAT (Figure 7) to be sensitive in wadeable streams and %FHW to be slightly less variable and an excellent discriminator in headwater streams on a statewide level (Figure 8). About half (49%) of the test sites had a %FHW score of 0.0. The poor sensitivity of NAT in headwater streams probably was a result of some test sites having moderately degraded stream conditions, therefore creating an environment for facultative species to invade. Typical reference headwater streams/watersheds in Kentucky were mostly forested and cool with low nutrient levels. With increased degradation in a watershed, temperatures and nutrients increase, providing supportable conditions for more atypical species associated with headwater streams, either in presence or in high abundance (e.g., *Lepomis* spp., *Pimephales* spp., *Campostoma* spp.). The %FHW metric was sensitive to these changes.

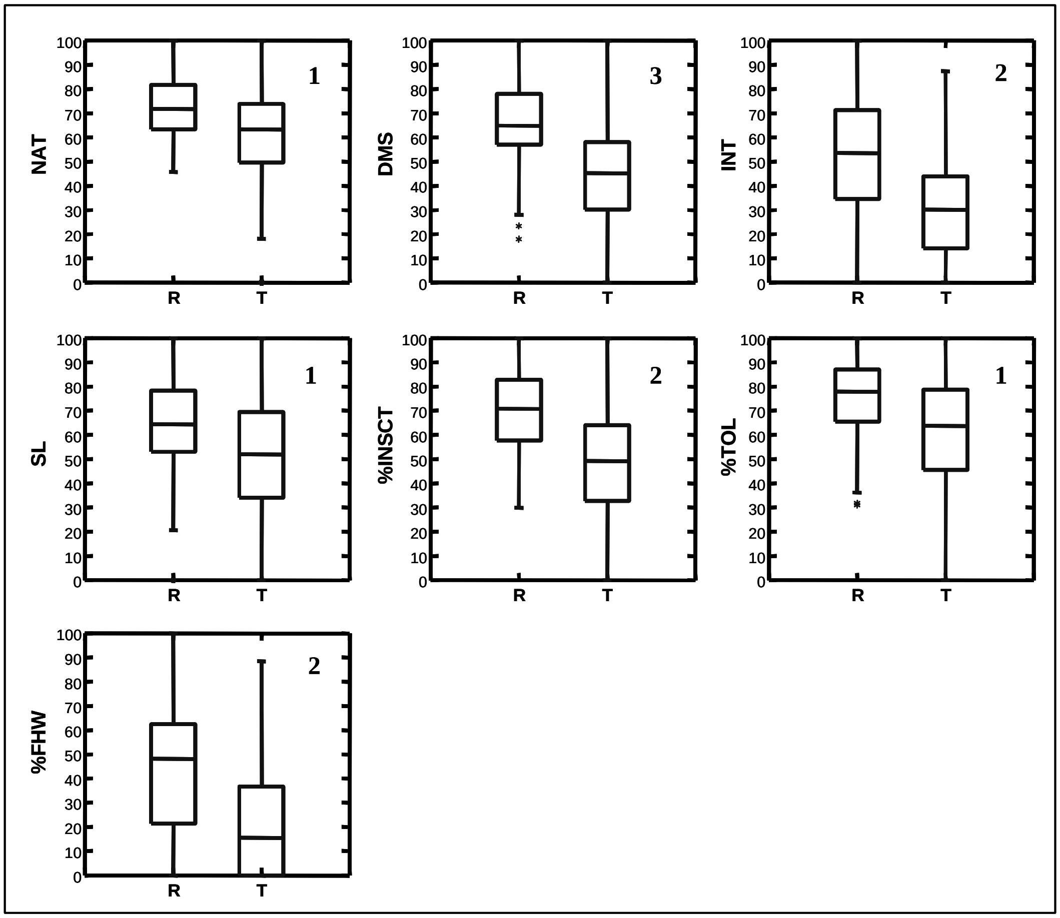

Figure 6. Box plots of scores showing metric discrimination between reference (R) and test (T) sites for all stream sizes on the statewide scale. Discriminatory power rating score is shown in each metric box.

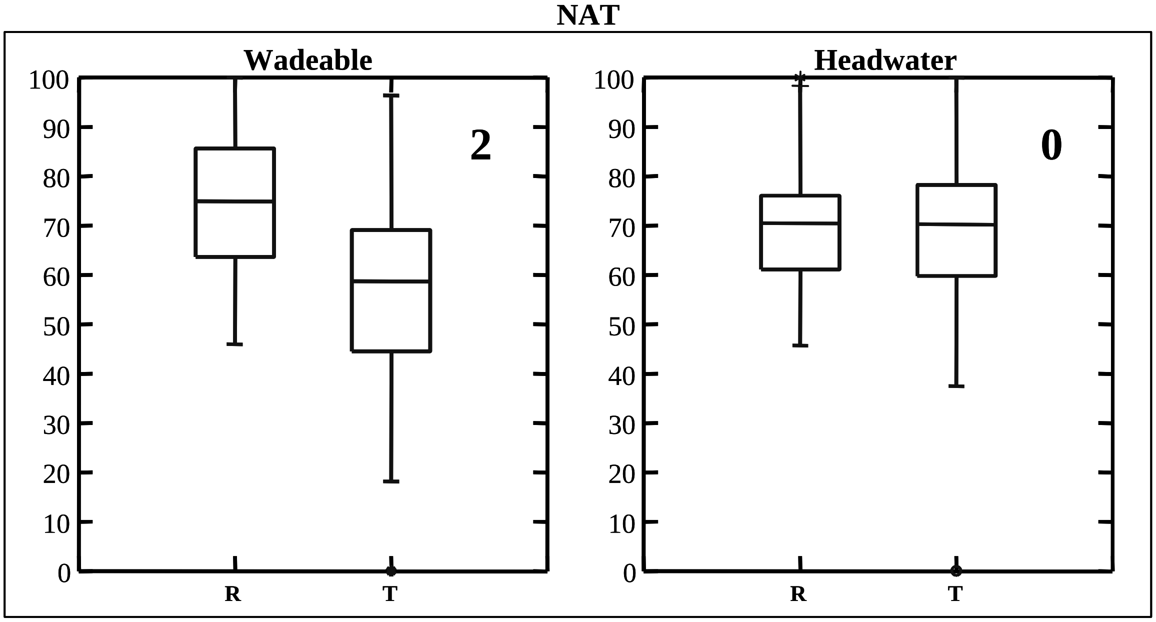

Figure 7. Box plots of scores showing statewide sensitivity of NAT for wadeable and headwater class streams.

%FHW

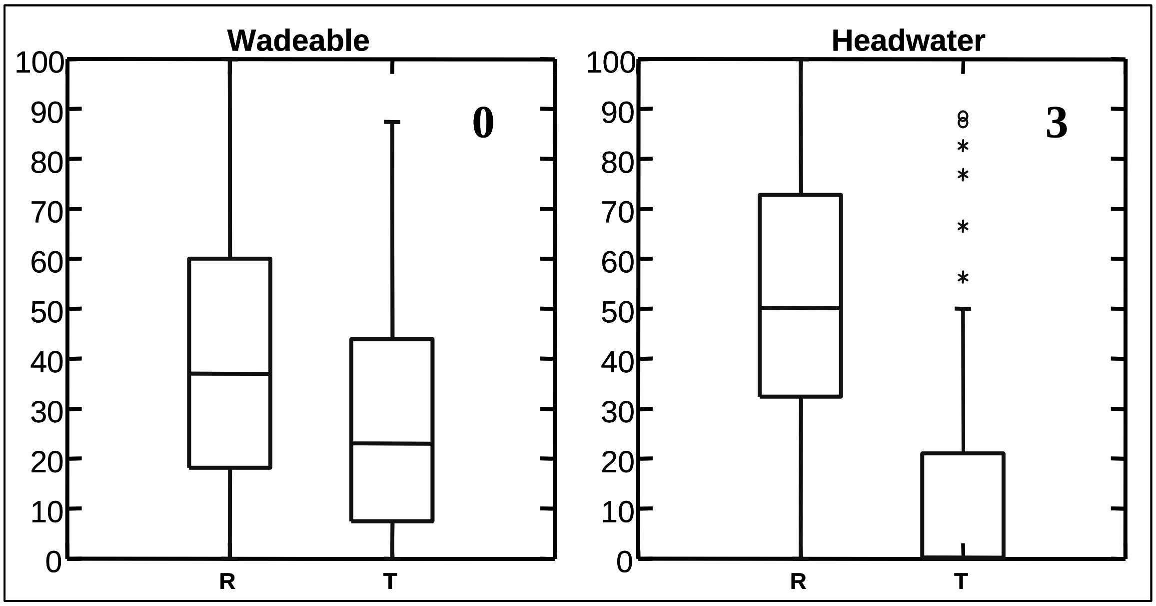

Figure 8. Box plots of scores showing statewide sensitivity of %FHW for wadeable and headwater class streams.

Analysis of discriminatory power for NAT in wadeable streams within each ichthyoregion (Figure 9) showed the metric to be relatively sensitive for all regions except CA and GR. However, even though some test sites scored higher than reference sites, the median values were lower. CA reference sites were less variable, which was probably a result of the small pool of potential species within this region (38). Therefore, reference expectations were probably being defined more consistently than in other regions. The weaker discrimination in the GR was probably a result of the extreme high diversity within this region, which allowed for numerous species to fill niches as sensitive species became fewer. Also, the number of test sites (4) was low and the spectrum of impairment probably was not fully represented. Notably, GR had the highest scoring values among reference sites (87.5) for NAT, which was a result of the high potential richness within the region. This phenomenon played a major role in the exclusion of the GR from PR. DMS discrimination for each region was either excellent or good except for GR (Figure 10). Again, this could be a result of the low number of test sites in the region and less severe degradation as compared to the other regions. INT showed sensitivity in all regions except for BG (Figure 11). The range of INT in BG was 2, which represented more of an indicator of strict presence/absence and not a continuum from which to measure impairment. However, the metric was retained because of the good sensitivity it had in other regions. Therefore, investigators should make note of the low range in the BG and possible exclusion of this metric in regional analysis may be warranted. As with most of the other metrics, SL was relatively sensitive except within the GR (Figure 12). %INSCT showed relatively good discriminatory power for all ichthyoregions except the PR (Figure 13), which may be a result of the variability of the 5 major river basins within this region. Further division of the PR may be needed. %Tol showed the weakest sensitivity of all of the metrics but was retained for reasons already stated (Figure 14). %FHW was used only in headwater streams and showed excellent or good discriminatory power in all regions except for GR and MVIR (Figure 15). A possible reason for weak discrimination in the MVIR would be that headwater streams in this region have been severely altered by agricultural practices (e.g., channelization) and “true” reference conditions do not exist. In the GR, more headwater samples for reference and test sites were 1 and 0 respectively and further sampling needs to be conducted. However, it is suspected %FHW in the GR will perform relatively similarly as it does in the PR and probably with less variability.

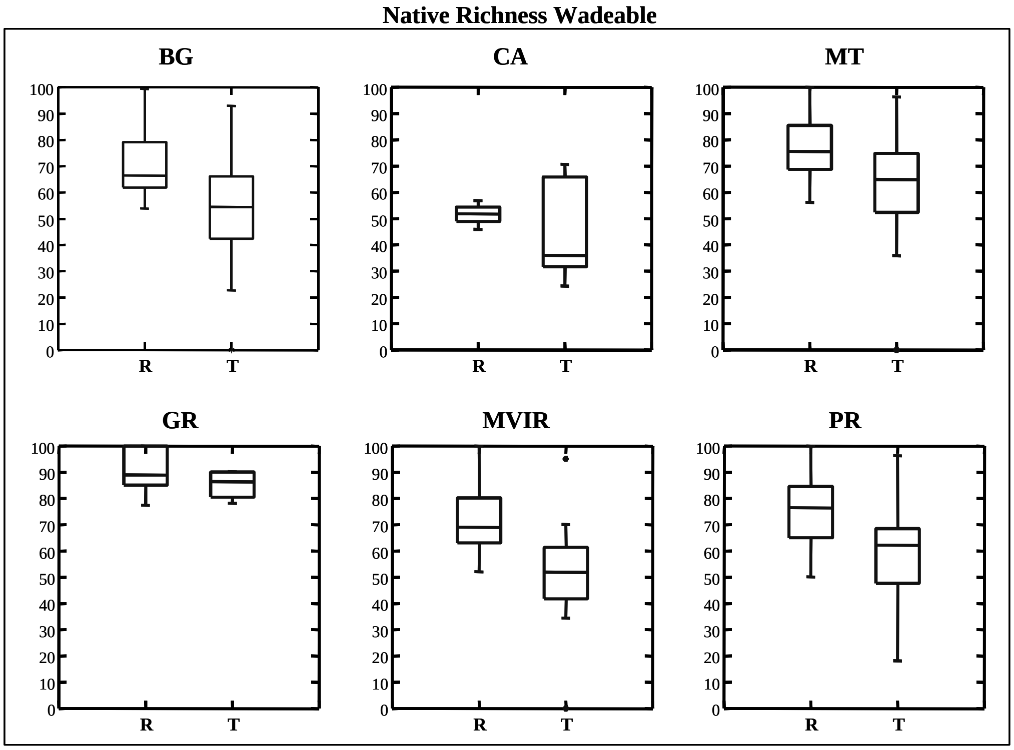

Figure 9. Box plots of scores showing sensitivity of NAT for wadeable streams within each ichthyoregion.

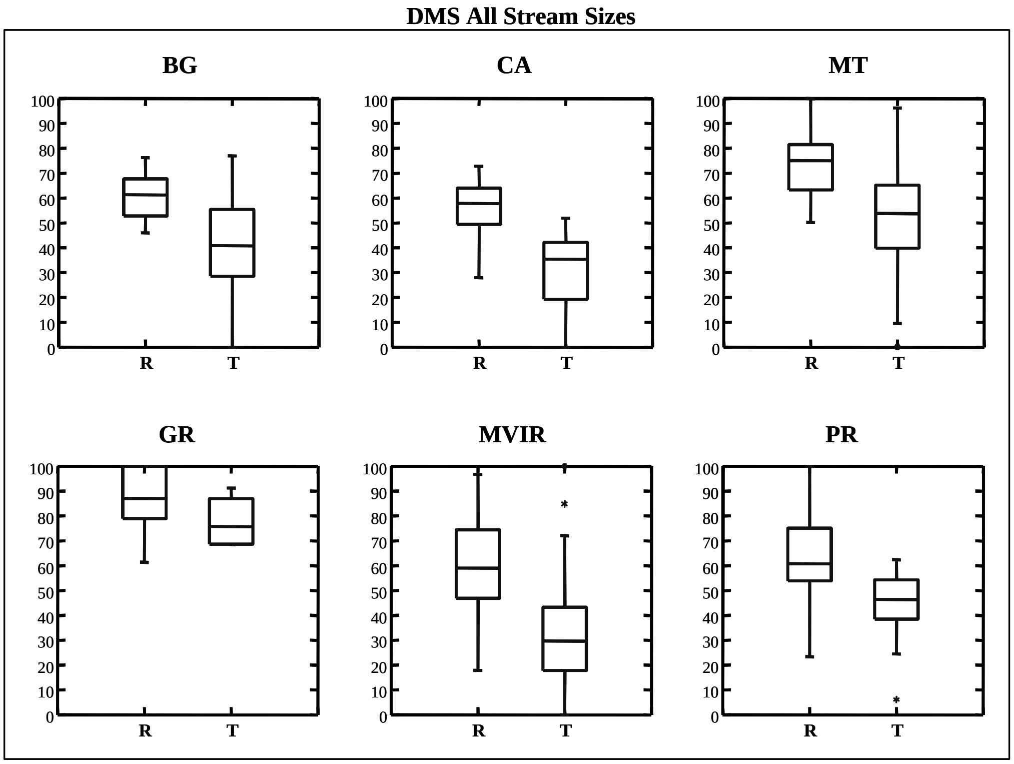

Figure 10. Box plots of scores showing sensitivity of DMS for all stream classes within each ichthyoregion.

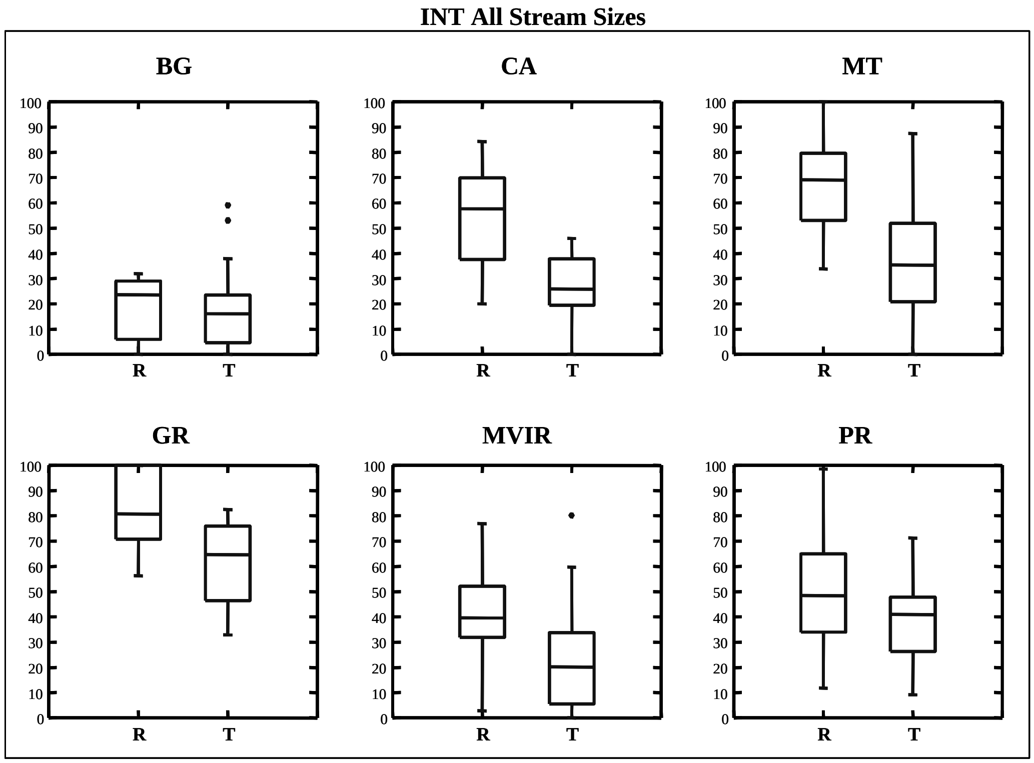

Figure 11. Box plots of scores showing sensitivity of INT for all stream classes within each ichthyoregion.

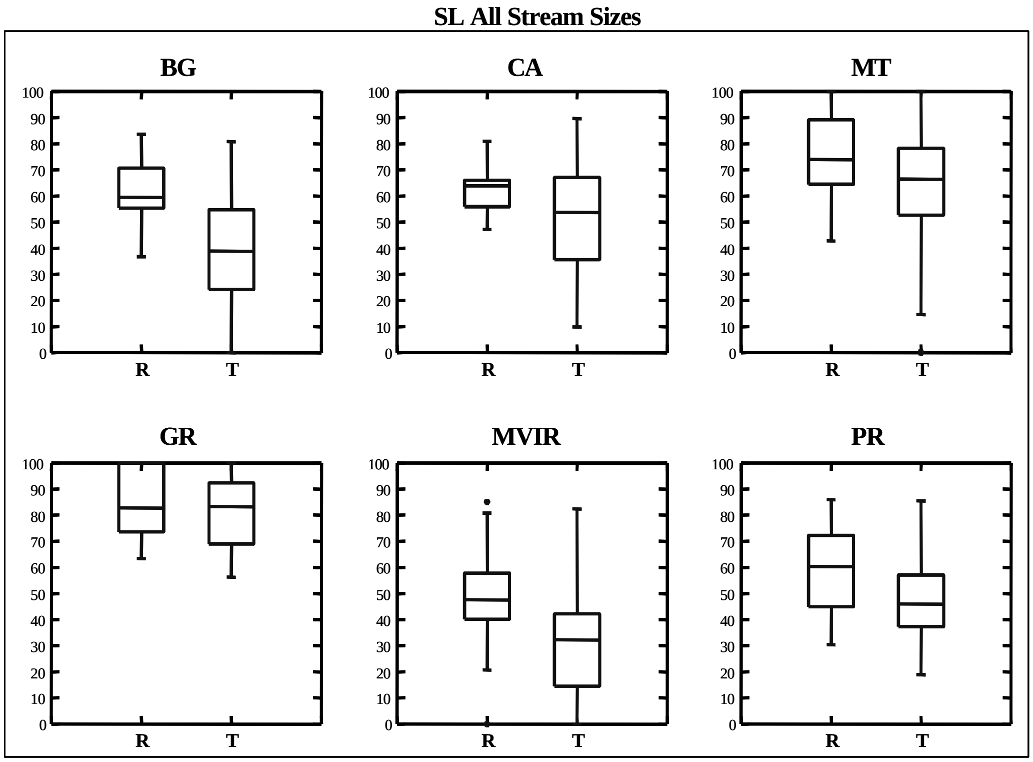

Figure 12. Box plots of scores showing sensitivity of SL for all stream classes within each ichthyoregion.

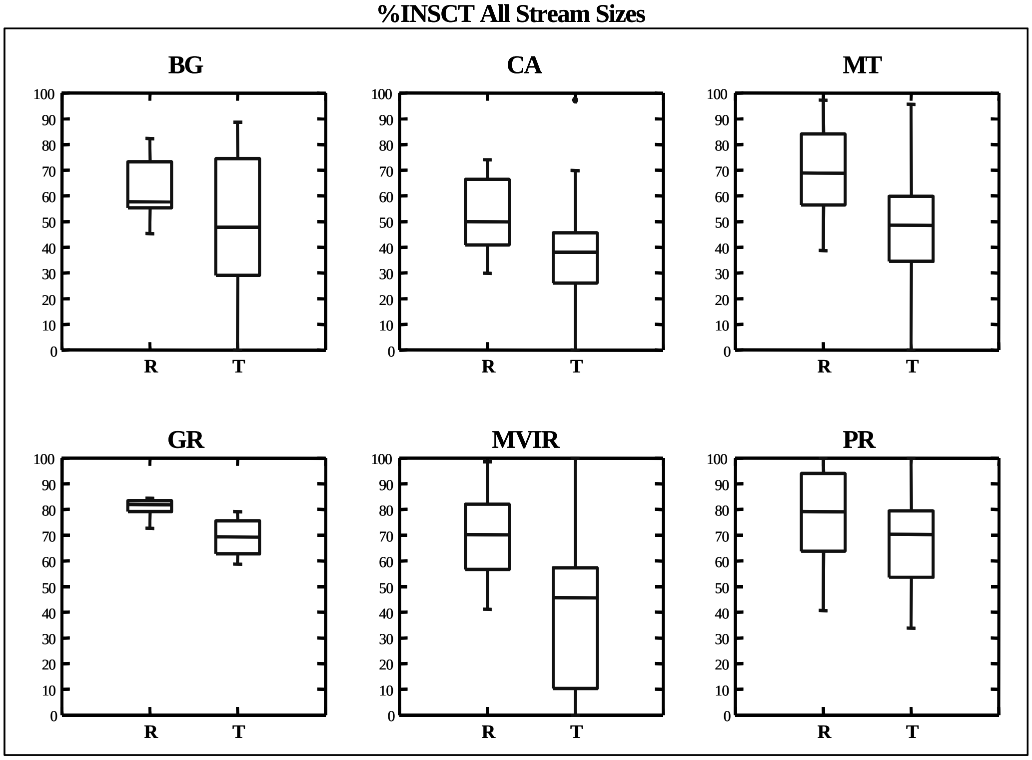

Figure 13. Box plots of scores showing sensitivity of %INSCT for all stream classes within each ichthyoregion.

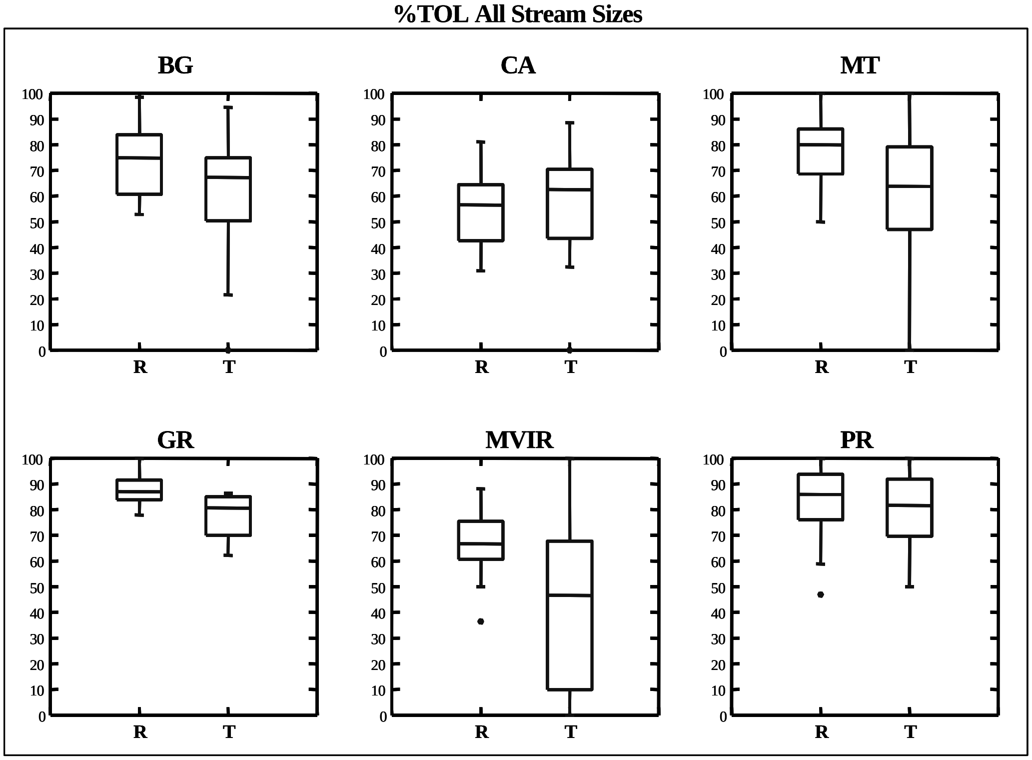

Figure 14. Box plots of scores showing sensitivity of %TOL for all stream classes within each ichthyoregion.

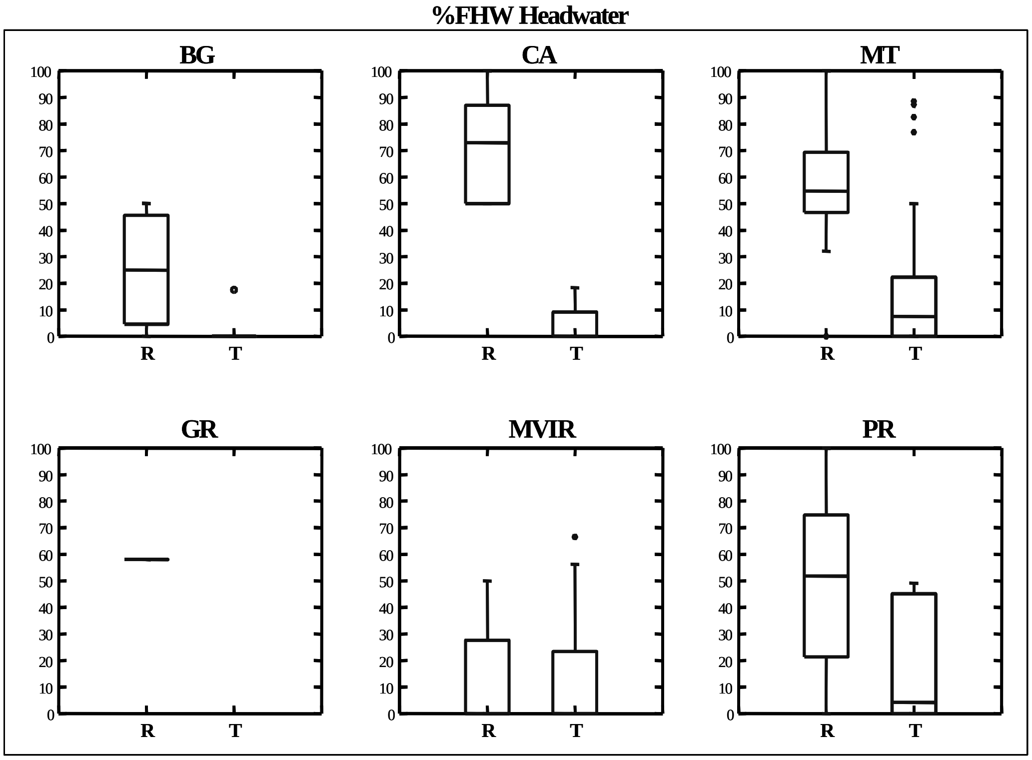

Figure 15. Box plots of scores showing sensitivity of %FHW for headwater streams within each ichthyoregion.

Analysis of redundancy among reference metric scores revealed no metrics were redundant (r>0.75) (Table 11). Therefore, it was considered each metric provided unique community information and the KIBI gave a representative ecological evaluation of the fish community at a site. In addition, metric scores were not significantly correlated (p>0.01) with catchment area, indicating calibration of drainage area for metrics was successful, and a comparison of metrics across wadeable stream sizes was valid.

Table 11. Pearson's correlation coefficient values for metric scores and KIBI

	Log_{10} CA	NAT	DMS	INT	SL	%INSCT	%TOL	% FHW	KIBI
Log_{10} CA	1.00								
NAT	**0.05**	1.00							
DMS	**0.03**	0.59	1.00						
INT	**-0.02**	**0.19**	0.45	1.00					
SL	**0.03**	0.58	0.58	0.45	1.00				
%INSCT	**0.08**	**0.10**	**0.14**	**0.13**	**-0.05**	1.00			
%TOL	**0.07**	**0.15**	**0.15**	**0.19**	**0.08**	0.72	1.00		
% FHW	**-0.06**	-0.46	**0.05**	0.27	**-0.07**	**0.09**	**0.05**	1.00	
KIBI	**0.20**	0.47	0.71	0.69	0.64	0.48	0.54	0.24	1.00

Bolded values were **not** significantly correlated (p< 0.01).

3.3 KIBI Testing

The aggregate KIBI exhibited excellent discrimination between reference and test sites statewide (Figure 16). The level was considered acceptable, and any deficiencies were most likely due to natural variation among communities, regional differences, and possible flaws in reference site selection; thus, index error was considered minimal. The responsiveness of the KIBI to the chemical and physical parameters was significant for most parameters (Tables 9 and 10). The index was significantly correlated with all of the parameters except for nitrate, total phosphorous, and channel flow status. The KIBI was most responsive to ammonia (Figure 17) and conductivity (Figure 18). However, correlation values were fairly low and further analysis of regional correlation is needed upon further data collection. The KIBI showed higher correlation with the physical parameters epifaunal substrate, channel alteration, pool variability, and channel sinuosity (see Table 10). Total habitat score was the most significantly correlated variable with the KIBI (Figure 19).

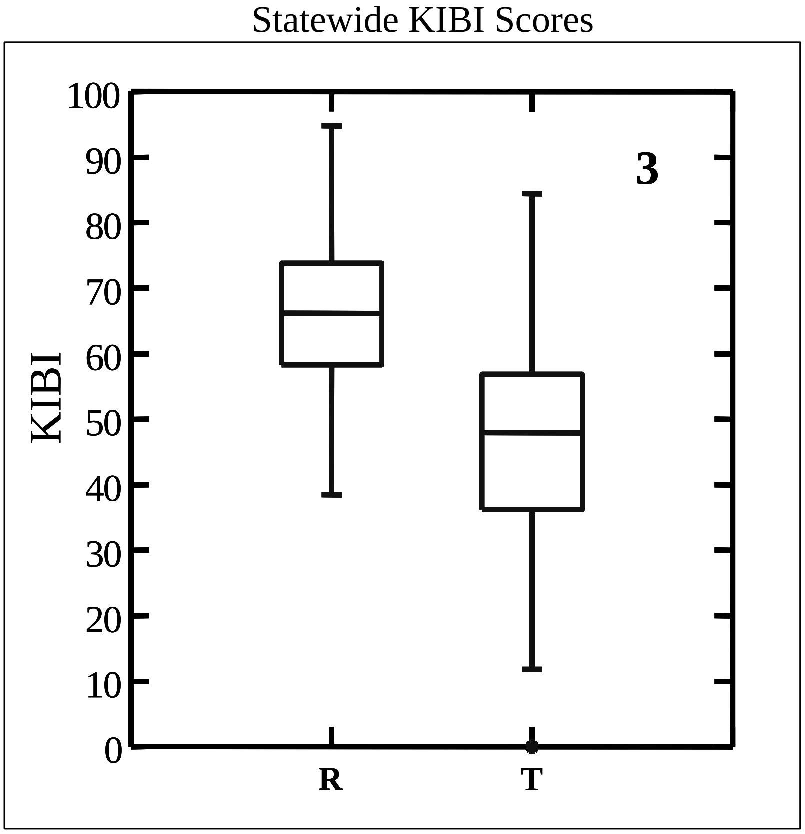

Figure 16. Box plot between reference (R) and test (T) sites statewide, with discriminatory power rating.

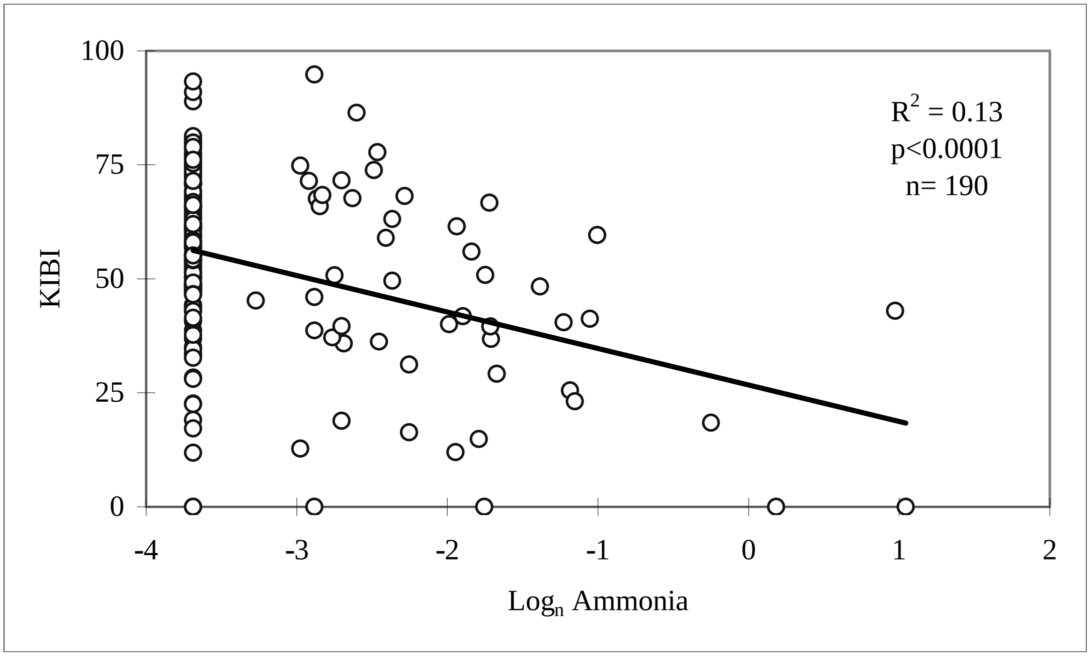

Figure 17. Scatter plot of statewide KIBI score vs. Log$_n$ Ammonia values.

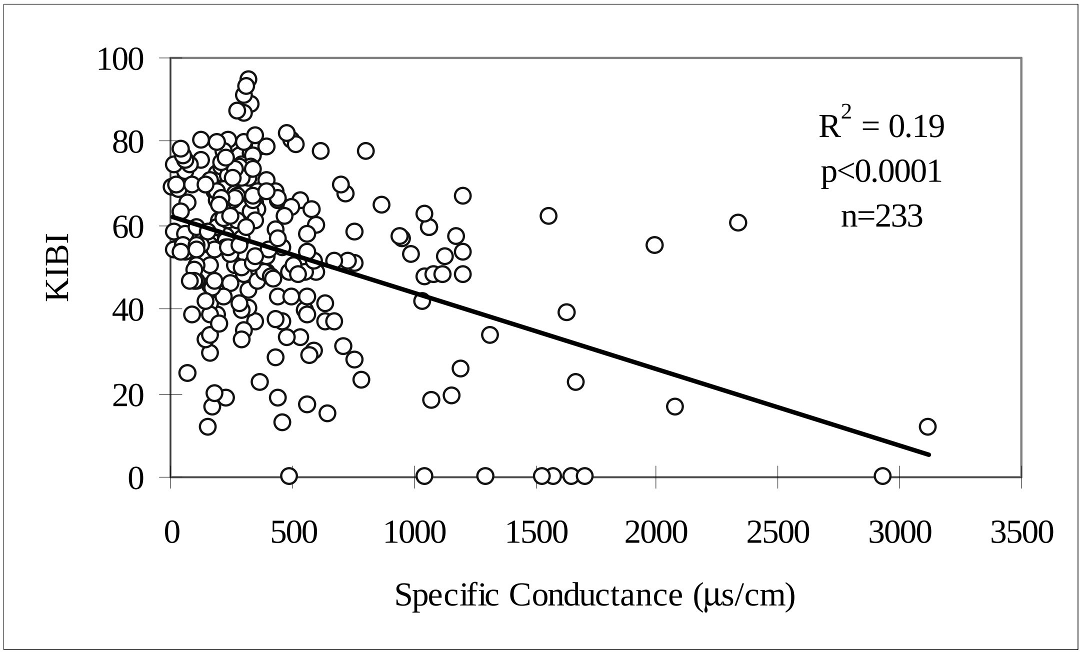

Figure 18. Scatter plot of statewide KIBI scores vs. Specific conductance values.

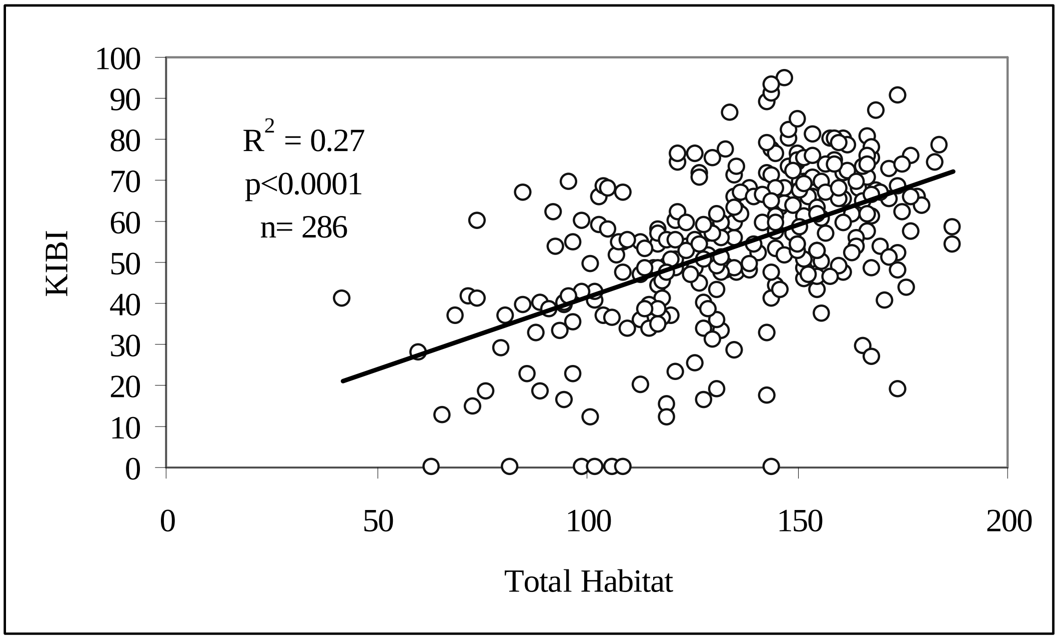

Figure 19. Scatter plot of statewide KIBI scores vs. Total Habitat scores.

The responsiveness of statewide KIBI scores was examined along a habitat and nutrient stressor gradient (Figure 20). Box plots were used to indicate stress responsiveness, categorically, for each variable. Fish communities appeared to be impacted at habitat stress code 3, where the median KIBI score dropped below the lower quartile for codes 1 and 2. As stress codes increased KIBI scores continued to decrease. The range of scores for each habitat code was large except for code 5. This suggests two phenomena: the KIBI was influenced by other parameters outside of habitat (e.g., water chemistry), and once habitat was severely altered the other parameters that may be beneficial for a stream no longer provide enough support for the community.

Nutrient stressors were not as indicative as habitat, and impairment was not conclusive until code 6 (toxic ammonia levels) was reached. Review of statewide KIBI means with sites that had both habitat and nutrient variables, showed sites with nutrient codes 3-5 and habitat codes 1-2 had a mean of value 61, but sites with the same nutrient codes but with a habitat code of 3 had a mean KIBI score of 44. This indicates, as did the correlation values with nutrient and habitat parameters, that the KIBI was more sensitive and responsive to habitat degradation than to nutrient enrichment.

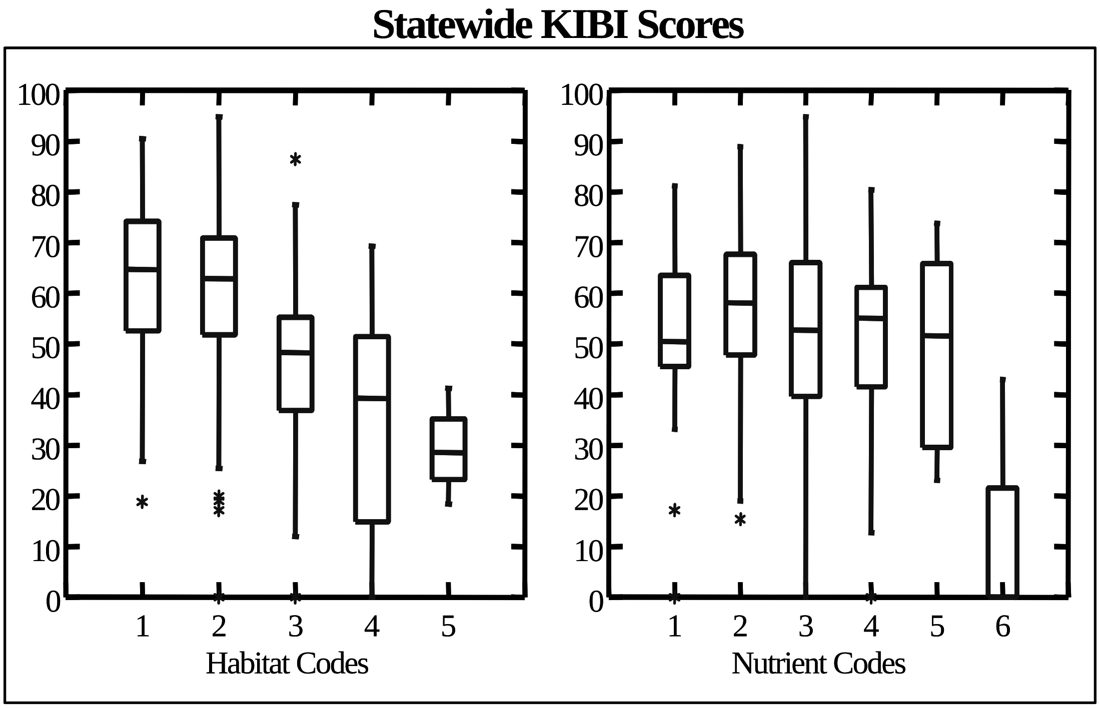

Figure 20. Box plots of statewide KIBI scores with coded habitat and nutrient variables.

Variability of KIBI scores from repeated sample events (n= 17) for a site (combined within-year and between-year samples) was considered stable (Figure 21). The result ($R^2 = 0.75$) was comparable to the findings of McCormick et al. (2001), who reported $R^2 = 0.78$ for within-year sampling and $R^2 = 0.74$ for between-year sampling. Therefore, based on these findings, the collection methodology and KIBI analysis were repeatable. The variation shown was probably a result of several factors, including seasonality, natural community variation, and collector error, and was considered acceptable for stream assessments.

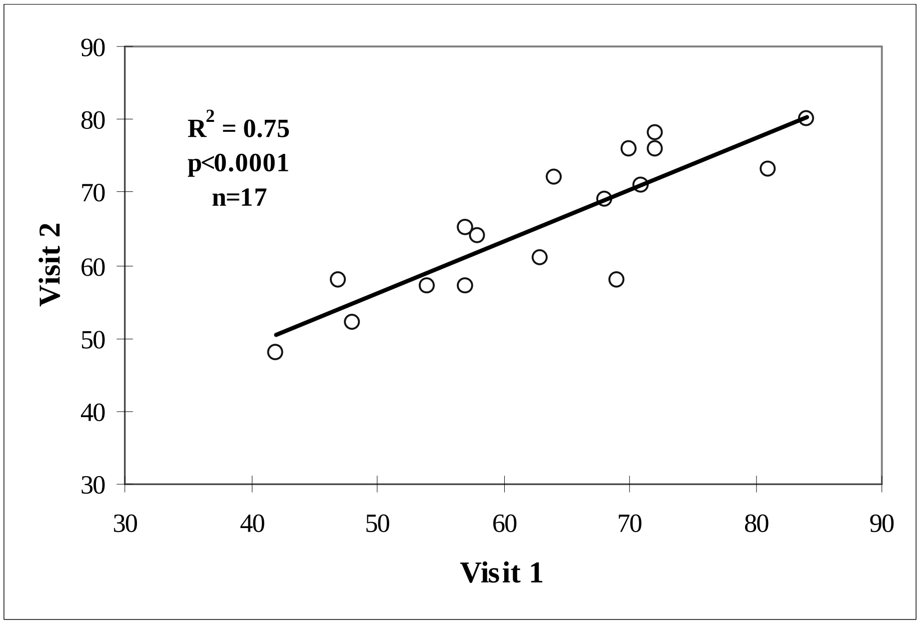

Figure 21. Scatter plot of KIBI scores from repeated site visits.

Box plots were used to show variation among reference sites within stream size classes, river basins, ecoregions, and ichthyoregions. Reference KIBI scores tended to be slightly higher in wadeable streams than in headwater streams, but the differences were minimal (Figure 22). Spearman's correlation coefficient ($r = 0.2$, $p > 0.01$) was non-significant for paired KIBI scores and Log_{10} catchment area (Table 11). McCormick et al. (2001) also found larger streams to score slightly higher. This trend may be a by-product of adjusting metrics that were correlated with catchment area.

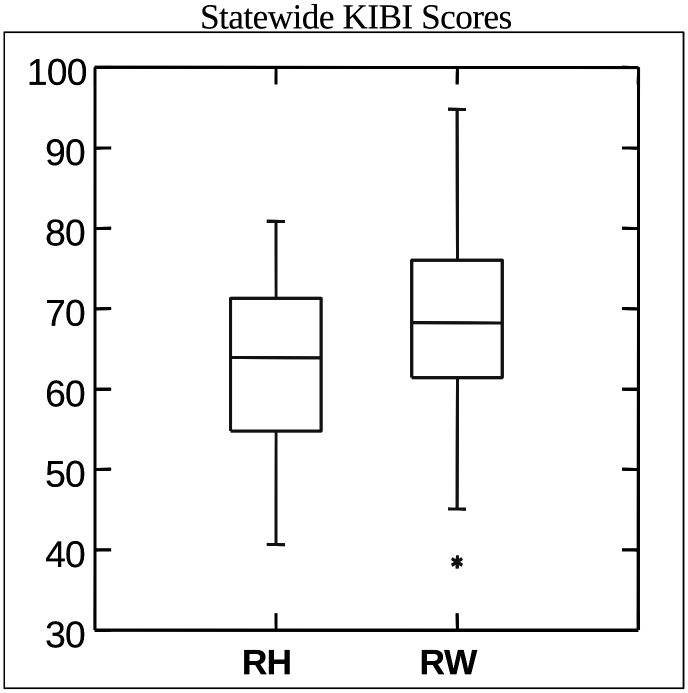

Figure 22. Box plot showing statewide reference headwater (RH) and reference wadeable (RW) KIBI scores.

Box plots of river basins revealed that the most variability among reference sites was within the Green and Kentucky river systems (Figure 23), which was expected since these basins traverse a wide array of geological, topographical, and landuse types. Variability was high within the Interior Plateau (IP) Ecoregion because of the numerous basins (7) within the IP. The greatest difference within the IP was between the Green and Kentucky river systems. Further analysis of the IP was explained by viewing the ichthyoregion box plots. The box plots revealed the median KIBI for the BG, where 14 of the 18 reference sites were from the Kentucky system, was drastically lower than the GR and PR ichthyoregions. In addition, the GR KIBI median was considerably higher than PR. Ichthyoregions, which were a combination of river basins and subecoregions, had the lowest variability in reference KIBI scores among the three classification schemes. Therefore, use of criteria derived from ichthyoregions would provide the least variability and give the greatest confidence in assessment evaluations. However, three ichthyoregions, MT, MVIR, and PR, showed higher variation when compared to the other ichthyoregions. This was probably an influence of river basins within each region since MT, MVIR, and PR had the most number of river basins (4, 6, 7, respectively). Continued modification of regions may be warranted upon further collection and analysis of data.

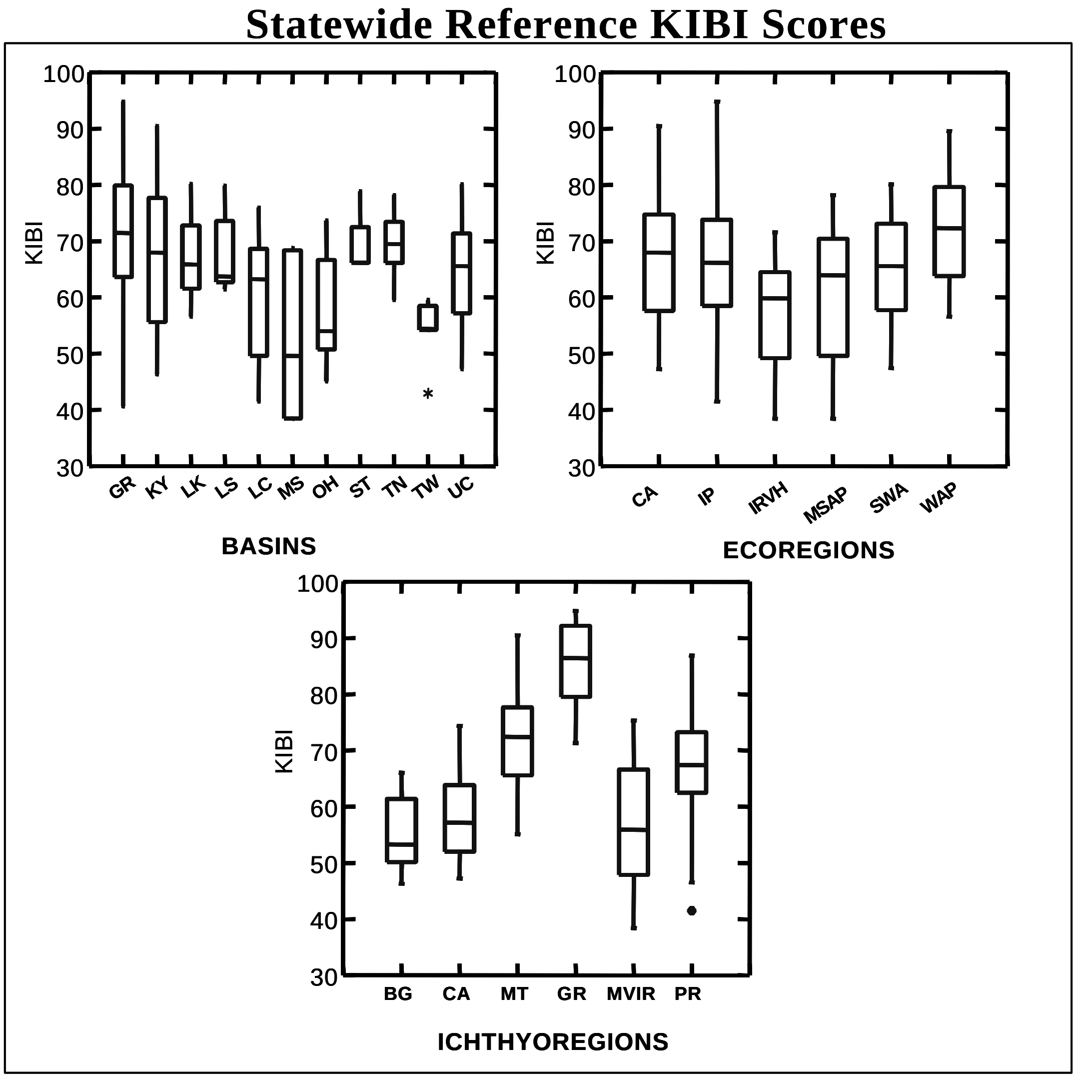

Figure 23. Box plots showing reference KIBI scores for river basins, ecoregions, and ichthyoregions. River Basins: GR=Green, KY=Kentucky, LK= Licking, LS= Little Sandy, MS= minor tributaries of the Mississippi River, OH= minor tributaries of the Ohio River, ST= Salt, TN= Tennessee, TW= Tradewater, and UC= Upper Cumberland, Ecoregions: CA= Central Appalachian, IP= Interior Plateau, IRVH= Interior River Valleys and Hills, MSAP= Mississippi Alluvial Plain, SWA= Southwestern Appalachian, and WAP= Western Allegheny Plateau, and Ichthyoregions: BG= Bluegrass, CA= Cumberland River above the Falls, MT= Mountain, GR= Green River system in subecoregions 71g, MVIR= Mississippi Valley-Interior River, and PR= Pennyroyal.

Box plots were used to show discrimination between reference and test sites for each ichthyoregion (Figure 24). Discrimination power ratings were “Excellent” for each ichthyoregion except for BG. BG had a power rating of 2 (Good). CA and MT demonstrated the best discrimination among regions, primarily because most reference sites were selected from the heavily forested and relatively undisturbed areas of the Daniel Boone National Forest.

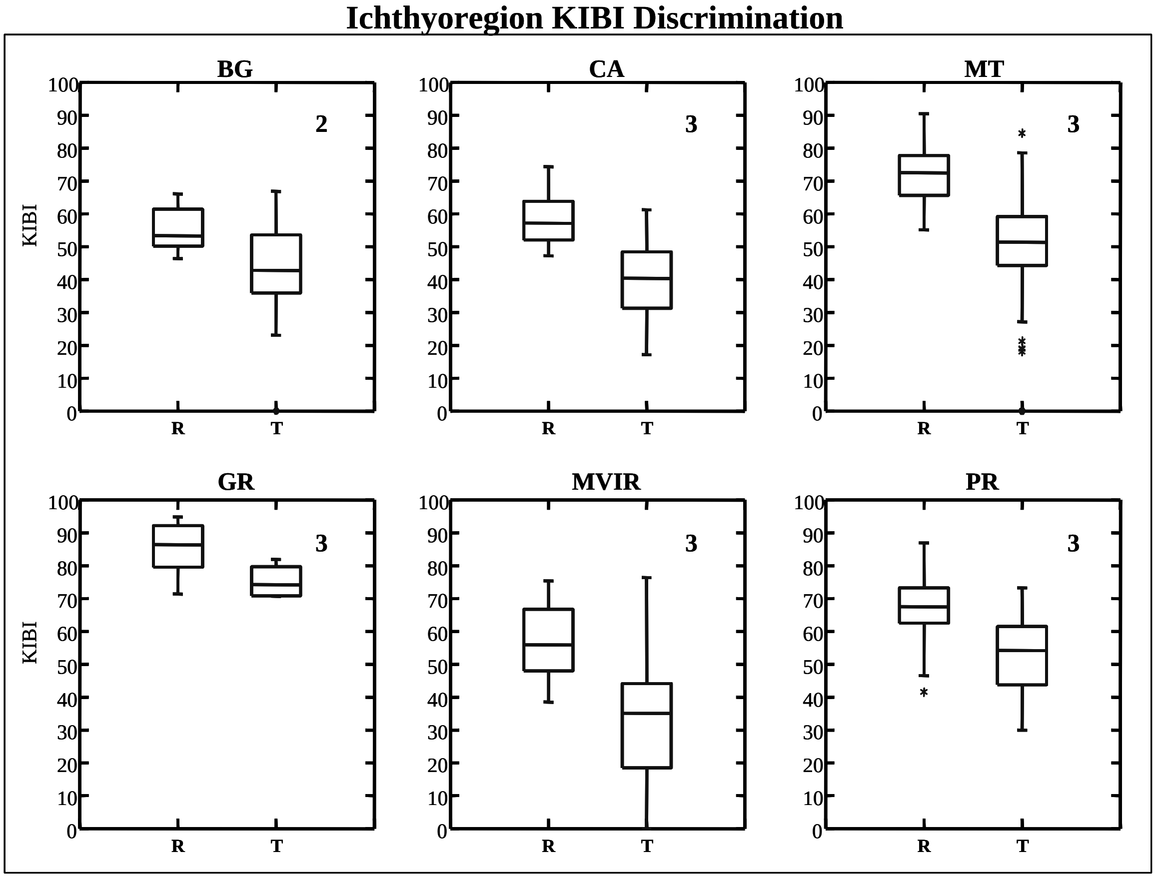

Figure 24. Box plots of scores showing KIBI discrimination for each ichthyoregion, with power ratings.

3.4 KIBI Criteria and Application Notes

For headwater streams, DMS, INT, SL, %INSCT, %TOL, and %FHW metrics comprise the KIBI. NAT, DMS, INT, SL, %INSCT, and %TOL comprise the KIBI for wadeable streams. Calculation of metric scores must follow the methods outlined in Tables 5 and 6 and the example in Appendix C. Table 12 shows each retained KIBI metric with respective Reference Regression Equation (RRE), Catchment Area Constant (CAC), and 95th %ile for each reference metric value. The KIBI score is the average of the six metrics used for wadeable and headwater streams. Criteria thresholds for narrative classifications of “Excellent”, “Good”, “Fair”, “Poor”, and “Very Poor” for each ichthyoregion were established from KIBI percentile distribution scores of the reference dataset (Table 12). The narrative classifications for “Excellent” and “Good” used the 50th and 5th %ile values for all ichthyoregions except for MVIR, respectively. The use of the 75th and 25th %ile thresholds was used for “Excellent” and “Good” in MVIR, respectively. Values below the “Good” threshold value were trisected for “Fair”, “Poor”, and “Very Poor” classifications. The justification for using the 75th and 25th %ile values for the MVIR narrative classifications was based on detrimental legacy landuse and current landuse practices of the region. The majority of the reference streams in this region have been severely modified through agricultural practices (e.g., channelization). Therefore, all reference streams were considered “least-disturbed.” In the CA, BG, GR, MT, and PR numerous reference streams were considered “minimally-disturbed.” Confidence in the reference dataset for these sites was reinforced through higher densities of forested watersheds, greater in-stream habitat scores, and background water chemistry results.

Table 12. KIBI metrics with Reference Regression Equations (RRE), Catchment Area Constant (CAC), and metric value 95th %ile.

KIBI Metrics	Reference Regression Equations	CAC	95th %
NAT*	$y = 10.123x + 4.4279$	20.49	28.2
DMS	$y = 2.967x + 1.5037$	6.21	9.3
INT	$y = 2.6679x - 0.1395$	4.09	7.7
SL	$y = 4.4162x + 0.9526$	7.96	12.5
%INSCT	$y = -10.326x^2 + 44.989x + 17.575$	58.88	87.8
%TOL	$y = -5.4568x^2 + 31.379x + 41.6$	77.65	101.5
%FHW*	$y = 8.9128x^2 - 59.151x + 98.557$	27.14	61.4

* Note: NAT for wadeable streams and %FHW for headwater streams

Table 13. Ichthyoregion scoring criteria. Narrative "Excellent" and "Good" thresholds derived from 50th/5th %ile or 75th/25th %ile and further trisection below "Good" threshold %ile.

Narrative Class	50th/5th %ile	50th/5th %ile	50th/5th %ile	50th/5th %ile	50th/5th %ile	75th/25th %ile
	CA	**BG**	**GR**	**MT**	**PR**	**MVIR**
Excellent	≥56	≥52	≥86	≥71	≥67	≥67
Good	47-55	47-51	76-85	59-70	53-66	48-66
Fair	31-46	31-46	51-75	39-58	35-52	32-47
Poor	16-30	16-30	26-50	19-38	17-34	16-31
Very Poor	0-15	0-15	0-25	0-18	0-16	0-15

The KIBI is a model for evaluating stream health based on fish communities, and as with most bio-assessment models, it was designed to be a tool to gauge stream health. Although 100% accuracy is not expected, the KIBI has been tested, and an acceptable discrimination efficiency of roughly 80% has been obtained. To overcome the inherent flaws of a biological model and achieve an acceptable and reliable level of precision and accuracy, the user must follow the sampling protocol as outlined in KDOW (2002). Also, the user should be familiar with the numerous variables (e.g., stream flow, water clarity, time of day, season) in the project area, including knowledge of the watershed landuse (e.g., forest, residential, agricultural), and other practices upstream of and around the immediate area of the site. Perceived fish community expectations may not be met if simple natural and anthropogenic variables have been overlooked. On the other hand, expectations may be exceeded due to unknown causes; therefore, scrutiny of all possible variables will help in the explanation of a given KIBI score. In addition, when KIBI scores fall close (± 2 points) to the narrative classification thresholds it is recommended the classification contain both categories (e.g., Good/Fair). It is also recommended additional chemical or biological data (diatoms or macroinvertebrates), or an additional fish sample be obtained to help define the condition more clearly.

The KIBI will still be somewhat limited regardless of certain outside influences. The most prominent limitation is assessing sites that approach the extremes of the recommended drainage areas (2-300 mi^2), where the reliability and consistency of the KIBI becomes more uncertain. Therefore, the user needs to be aware of this factor when expectations are not met. The result may be related to catchment area effects instead of an anthropogenic factor. In addition, streams with small drainage areas (<3 mi^2) tend to have fish communities dominated by tolerant species and have naturally low abundances and richness. Therefore, these communities may show little discrimination between high and low quality streams. Streams with very large drainage areas (250-300 mi^2) frequently have complex habitats, often with large deep sections (>2 m) of pool and run, thereby creating difficulties in sampling efficiency. Consequently, reliability and consistency is compromised. Overall, when the sampling protocol is followed, the KIBI is reliable within the recommended drainage areas, as long as the user is aware of all of the possible variables encountered in sampling. To obtain the sampling protocol, fish species classifications, KIBI scoring template, and other fish community information, refer to *Methods for Assessing Biological Integrity of Surface Waters in Kentucky*, (http://www.water.ky.gov/sw/swmonitor/sop/) (KDOW 2002) or contact KDOW for questions, concerns, and/or a copy of the manual on CD-ROM.

4.0 Summary

The Kentucky Index of Biotic Integrity (KIBI), an aggregate of seven metrics (NAT, DMS, INT, SL, %INSCT, %TOL, %FHW), six each for headwater and wadeable streams, has been tested and will serve as an effective model for stream biomonitoring using fish as the biological indicator. The index was designed to reflect environmental changes due to anthropogenic disturbances in a uniform and precise method. Metrics were calibrated for basin size and examined for general fitness across the state by examining their range, responsiveness to disturbances, variability within the reference dataset, discriminatory power between reference and test sites, and redundancy within the reference dataset. An *a posteriori* ichthyoregion classification scheme was established and compared to two other regional classification schemes to determine which format was least variable. Criteria were obtained from the reference distribution of KIBI scores. An outline of the calculation process was provided for users.

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Appendix A. Master fish taxa list with ecological classifications.

Family	Final Identification	Fish Type	NAT	FHW	INSCT	INT	TOL	SL
Acipenseridae	Acipenser fulvescens		X	X				X
Clupeidae	Alosa alabamae		X	X				X
Clupeidae	Alosa chrysochloris		X	X				
Clupeidae	Alosa pseudoharengus			X				
Centrarchidae	Ambloplites rupestris	SUN	X	X				
Amblyopsidae	Amblyopsis spelaea		X					
Ictaluridae	Ameiurus catus			X	X		X	
Ictaluridae	Ameiurus melas		X	X			X	
Ictaluridae	Ameiurus natalis		X	X			X	
Ictaluridae	Ameiurus nebulosus		X	X	X		X	
Ictaluridae	Ameiurus spp.		X	X			X	
Amiidae	Amia calva		X	X				
Percidae	Ammocrypta clara	DAR	X	X	X	X		X
Percidae	Ammocrypta pellucida	DAR	X	X	X	X		X
Percidae	Ammocrypta vivax	DAR	X	X	X	X		X
Anguillidae	Anguilla rostrata		X	X				
Aphredoderidae	Aphredoderus sayanus		X	X	X			
Sciaenidae	Aplodinotus grunniens		X	X				
Lepisosteidae	Atractosteus spatula		X	X				
Cyprinidae	Campostoma anomalum	MIN	X	X				
Cyprinidae	Campostoma oligolepis	MIN	X	X				
Cyprinidae	Carassius auratus	MIN	X	X			X	
Catostomidae	Carpiodes carpio	SUC	X	X				
Catostomidae	Carpiodes cyprinus	SUC	X	X				
Catostomidae	Carpiodes velifer	SUC	X	X				
Catostomidae	Catostomid fry	SUC	X	X			X	X
Catostomidae	Catostomus commersoni	SUC	X	X			X	X
Catostomidae	Catostomus sp.	SUC	X	X			X	X
Centrarchidae	Centrarchus macropterus	SUN	X	X	X			
Cyprinidae	Clinostomus elongates	MIN	X		X	X		X
Cyprinidae	Clinostomus funduloides	MIN	X		X	X		X
Cottidae	Cottus bairdi	COT	X		X	X		
Cottidae	Cottus carolinae	COT	X		X	X		
Percidae	Crystallaria asprella	DAR	X	X	X	X		X
Cyprinidae	Ctenopharyngodon idella	MIN		X				
Gasterosteidae	Culaea inconstans			X	X			
Catostomidae	Cycleptus elongates	SUC	X	X	X	X		X
Cyprinidae	Cyprinella camura	MIN	X	X	X	X		
Cyprinidae	Cyprinella galactura	MIN	X	X	X	X		
Cyprinidae	Cyprinella lutrensis	MIN	X	X				
Cyprinidae	Cyprinella spiloptera	MIN	X	X	X			
Cyprinidae	Cyprinella venusta	MIN	X	X	X			
Cyprinidae	Cyprinella whipplei	MIN	X	X	X			
Cyprinidae	Cyprinid sp.	MIN	X	X				
Cyprinidae	Cyprinus carpio	MIN		X			X	
Clupeidae	Dorosoma cepedianum		X	X				
Clupeidae	Dorosoma petenense		X	X				
Elassomatidae	Elassoma zonatum		X		X			
Cyprinidae	Ericymba buccata	MIN	X	X				

Family	Final Identification	Fish Type	Native	FHW	INSCT	INT	TOL	SL
Cyprinidae	Erimystax dissimilis	MIN	X	X	X	X		X
Cyprinidae	Erimystax insignis	MIN	X	X	X			X
Cyprinidae	Erimystax x-punctatus	MIN	X		X	X		X
Catostomidae	Erimyzon oblongus	SUC	X	X	X			
Catostomidae	Erimyzon sucetta	SUC	X	X				
Esocidae	Esox americanus vermiculatus		X	X				
Esocidae	Esox lucius			X				
Esocidae	Esox masquinongy		X	X				
Esocidae	Esox niger		X	X				
Percidae	Etheostoma asprigene	DAR	X		X			
Percidae	Etheostoma baileyi	DAR	X		X	X		X
Percidae	Etheostoma barbouri	DAR	X		X	X		
Percidae	Etheostoma barrenense	DAR	X		X	X		
Percidae	Etheostoma bellum	DAR	X	X	X	X		
Percidae	Etheostoma bison	DAR	X		X			X
Percidae	Etheostoma blennioides	DAR	X	X	X			X
Percidae	Etheostoma caeruleum	DAR	X		X			X
Percidae	Etheostoma camurum	DAR	X	X	X	X		X
Percidae	Etheostoma chienense	DAR	X		X			
Percidae	Etheostoma chlorosomum	DAR	X		X			
Percidae	Etheostoma cinereum	DAR	X	X	X	X		
Percidae	Etheostoma crossopterum	DAR	X		X			
Percidae	Etheostoma flabellare	DAR	X		X			
Percidae	Etheostoma flavum	DAR	X		X	X		X
Percidae	Etheostoma fusiforme	DAR	X		X	X		
Percidae	Etheostoma gracile	DAR	X		X			
Percidae	Etheostoma histrio	DAR	X	X	X	X		X
Percidae	Etheostoma kantuckeense	DAR	X		X			X
Percidae	Etheostoma kennicotti	DAR	X		X			X
Percidae	Etheostoma lawrencei	DAR	X		X			
Percidae	Etheostoma lynceum	DAR	X	X	X			X
Percidae	Etheostoma maculatum	DAR	X	X	X	X		X
Percidae	Etheostoma microlepidum	DAR	X	X	X	X		
Percidae	Etheostoma microperca	DAR	X		X	X		
Percidae	Etheostoma nigrum	DAR	X	X	X			
Percidae	Etheostoma obeyense	DAR	X		X	X		
Percidae	Etheostoma oophylax	DAR	X		X			
Percidae	Etheostoma parvipinne	DAR	X		X	X		
Percidae	Etheostoma percnurum	DAR	X		X	X		
Percidae	Etheostoma proeliare	DAR	X		X	X		
Percidae	Etheostoma pyrrhogaster	DAR	X		X	X		
Percidae	Etheostoma rafinesquei	DAR	X		X	X		
Percidae	Etheostoma rufilineatum	DAR	X	X	X	X		
Percidae	Etheostoma sagitta	DAR	X		X	X		
Percidae	Etheostoma sanguifluum	DAR	X	X	X	X		
Percidae	Etheostoma simoterum	DAR	X		X	X		X
Percidae	Etheostoma smithi	DAR	X		X	X		X
Percidae	Etheostoma sp.	DAR	X		X	X		X
Percidae	Etheostoma spectabile	DAR	X		X			X
Percidae	Etheostoma squamiceps	DAR	X		X			

Family	Final Identification	Fish Type	Native	FHW	INSCT	INT	TOL	SL
Percidae	Etheostoma stigmaeum	DAR	X	X	X	X		X
Percidae	Etheostoma swaini	DAR	X		X	X		X
Percidae	Etheostoma tecumsehi	DAR	X		X			X
Percidae	Etheostoma tippecanoe	DAR	X	X	X	X		X
Percidae	Etheostoma variatum	DAR	X	X	X	X		X
Percidae	Etheostoma virgatum	DAR	X		X	X		
Percidae	Etheostoma zonale	DAR	X	X	X			X
Percidae	Etheostoma zonistium	DAR	X		X	X		X
Amblyopsidae	Forbesichthys agassizi		X					
Fundulidae	Fundulus catenatus		X	X	X			X
Fundulidae	Fundulus chrysotus		X	X	X			
Fundulidae	Fundulus dispar		X	X	X			
Fundulidae	Fundulus notatus		X	X	X			
Fundulidae	Fundulus olivaceus		X	X	X			
Poeciliidae	Gambusia affinis		X	X	X		X	
Cyprinidae	Hemitremia flammea	MIN	X	X	X			
Hiodontidae	Hiodon alosoides		X	X	X			
Hiodontidae	Hiodon tergisus		X	X	X			
Cyprinidae	Hybognathus hayi	MIN	X	X				
Cyprinidae	Hybognathus nuchalis	MIN	X	X				
Cyprinidae	Hybognathus placitus	MIN		X				
Cyprinidae	Hybopsis amblops	MIN	X	X	X	X		X
Cyprinidae	Hybopsis amnis	MIN	X	X	X			
	Hybrid sp.							
Catostomidae	Hypentelium nigricans	SUC	X		X			X
Cyprinidae	Hypophthalmichthys molitrix	MIN		X				
Petromyzontidae	Ichthyomyzon bdellium		X					
Petromyzontidae	Ichthyomyzon castaneus		X					
Petromyzontidae	Ichthyomyzon fossor		X					
Petromyzontidae	Ichthyomyzon gagei		X					
Petromyzontidae	Ichthyomyzon greeleyi		X					
Petromyzontidae	Ichthyomyzon unicuspis		X					
Ictaluridae	Ictalurus furcatus		X	X				
Ictaluridae	Ictalurus punctatus		X	X				
Catostomidae	Ictiobus bubalus	SUC	X	X				
Catostomidae	Ictiobus cyprinellus	SUC	X	X				
Catostomidae	Ictiobus niger	SUC	X	X				
Atherinidae	Labidesthes sicculus		X	X	X			
Catostomidae	Lagochila lacera	SUC	X	X				X
Petromyzontidae	Lampetra aepyptera		X					
Petromyzontidae	Lampetra appendix		X					
Petromyzontidae	Lamprey ammocoete		X					
Petromyzontidae	Lamprey sp.		X					
Lepisosteidae	Lepisosteus oculatus		X	X				
Lepisosteidae	Lepisosteus osseus		X	X				
Lepisosteidae	Lepisosteus platostomus		X	X				
Lepisosteidae	Lepisosteus spp.		X	X				
Centrarchidae	Lepomis auritus	SUN		X	X		X	
Centrarchidae	Lepomis cyanellus	SUN	X	X	X		X	
Centrarchidae	Lepomis cyanellus X L. macrochirus	SUN		X			X	

Family	Final Identification	Fish Type	Native	FHW	INSCT	INT	TOL	SL
Centrarchidae	Lepomis cyanellus X L. megalotis	SUN		X			X	
Centrarchidae	Lepomis gibbosus	SUN	X	X	X			
Centrarchidae	Lepomis gulosus	SUN	X	X	X			
Centrarchidae	Lepomis humilis	SUN	X	X	X			
Centrarchidae	Lepomis macrochirus	SUN	X	X	X		X	
Centrarchidae	Lepomis macrochirus X L. cyanellus			X			X	
Centrarchidae	Lepomis macrochirus X L. megalotis	SUN		X				
Centrarchidae	Lepomis marginatus	SUN	X	X	X			
Centrarchidae	Lepomis megalotis	SUN	X	X	X			
Centrarchidae	Lepomis microlophus	SUN	X	X	X			
Centrarchidae	Lepomis miniatus	SUN	X	X	X	X		
Centrarchidae	Lepomis sp.	SUN	X	X	X			
Centrarchidae	Lepomis symmetricus	SUN	X	X	X			
Gadidae	Lota lota		X	X				X
Cyprinidae	Luxilus chrysocephalus	MIN	X	X	X		X	X
Cyprinidae	Lythrurus fasciolaris	MIN	X	X	X			
Cyprinidae	Lythrurus fumeus	MIN	X	X	X		X	
Cyprinidae	Lythrurus umbratilis	MIN	X	X	X			X
Cyprinidae	Macrhybopsis aestivalis	MIN	X	X	X	X		
Cyprinidae	Macrhybopsis gelida	MIN	X	X	X	X		
Cyprinidae	Macrhybopsis meeki	MIN	X	X	X	X		
Cyprinidae	Macrhybopsis storeriana	MIN	X	X	X			
Atherinidae	Menidia beryllina		X	X	X			
Centrarchidae	Micropterus coosae			X				
Centrarchidae	Micropterus dolomieu		X	X				
Centrarchidae	Micropterus punctulatus		X	X				
Centrarchidae	Micropterus salmoides		X	X				
Centrarchidae	Micropterus spp.		X	X				
Catostomidae	Minytrema melanops	SUC	X	X	X			X
Moronidae	Morone chrysops		X	X				
Moronidae	Morone chrysops x M. saxatilis			X				
Moronidae	Morone mississippiensis		X	X				
Moronidae	Morone saxatilis			X				
Catostomidae	Moxostoma anisurum	SUC	X	X	X	X		X
Catostomidae	Moxostoma carinatum	SUC	X	X	X	X		X
Catostomidae	Moxostoma duquesnei	SUC	X	X	X	X		X
Catostomidae	Moxostoma erythrurum	SUC	X	X	X			X
Catostomidae	Moxostoma macrolepidotum breviceps	SUC	X	X	X			X
Catostomidae	Moxostoma poecilurum	SUC	X	X	X			X
Catostomidae	Moxostoma sp.	SUC	X	X	X			X
Catostomidae	Moxostoma valenciennesi	SUC	X	X	X			X
	NO FISH							
Cyprinidae	Nocomis biguttatus	MIN	X	X		X		X
Cyprinidae	Nocomis effusus	MIN	X	X	X	X		X
Cyprinidae	Nocomis micropogon	MIN	X	X	X	X		X
Cyprinidae	Notemigonus crysoleucas	MIN	X	X			X	
Cyprinidae	Notropis albizonatus	MIN	X	X	X	X		X
Cyprinidae	Notropis ariommus	MIN	X	X	X	X		X
Cyprinidae	Notropis atherinoides	MIN	X	X				X
Cyprinidae	Notropis blennius	MIN	X	X	X			X

Family	Final Identification	Fish Type	Native	FHW	INSCT	INT	TOL	SL
Cyprinidae	Notropis boops	MIN	X	X	X			X
Cyprinidae	Notropis buchanani	MIN	X	X	X			
Cyprinidae	Notropis hudsonius	MIN	X	X	X			
Cyprinidae	Notropis leuciodus	MIN	X	X	X	X		
Cyprinidae	Notropis ludibundus	MIN	X	X				
Cyprinidae	Notropis maculatus	MIN	X	X				
Cyprinidae	Notropis nubilus	MIN	X	X	X			
Cyprinidae	Notropis photogenis	MIN	X	X	X	X		X
Cyprinidae	Notropis rubellus	MIN	X	X	X	X		X
Cyprinidae	Notropis shumardi	MIN	X	X				
Cyprinidae	Notropis sp.	MIN	X	X				
Cyprinidae	Notropis spp. (sawfin shiner)	MIN	X	X	X	X		X
Cyprinidae	Notropis telescopus	MIN	X	X	X	X		
Cyprinidae	Notropis volucellus	MIN	X	X				
Ictaluridae	Noturus elegans	MAD	X		X	X		
Ictaluridae	Noturus eleutherus	MAD	X		X	X		
Ictaluridae	Noturus exilis	MAD	X		X	X		
Ictaluridae	Noturus flavus	MAD	X		X	X		
Ictaluridae	Noturus gyrinus	MAD	X		X	X		
Ictaluridae	Noturus hildebrandi	MAD	X		X	X		
Ictaluridae	Noturus miurus	MAD	X		X	X		
Ictaluridae	Noturus nocturnus	MAD	X		X	X		
Ictaluridae	Noturus phaeus	MAD	X		X	X		
Ictaluridae	Noturus stigmosus	MAD	X		X	X		
Salmonidae	Oncorhynchus kisutch							
Salmonidae	Oncorhynchus mykiss				X	X		
Cyprinidae	Opsopoeodus emiliae	MIN	X	X		X		
Osmeridae	Osmerus mordax							
Percidae	Perca flavescens		X	X				
Percidae	Percina burtoni	DAR	X	X	X	X		X
Percidae	Percina caprodes	DAR	X	X	X			X
Percidae	Percina copelandi	DAR	X	X	X	X		X
Percidae	Percina evides	DAR	X	X	X	X		X
Percidae	Percina macrocephala	DAR	X	X	X	X		X
Percidae	Percina maculata	DAR	X	X	X			X
Percidae	Percina oxyrhynchus	DAR	X	X	X	X		X
Percidae	Percina phoxocephala	DAR	X	X	X	X		X
Percidae	Percina sciera	DAR	X	X	X	X		X
Percidae	Percina shumardi	DAR	X	X	X			X
Percidae	Percina squamata	DAR	X	X	X	X		X
Percidae	Percina stictogaster	DAR	X		X	X		X
Percidae	Percina vigil	DAR	X	X	X	X		X
Percopsidae	Percopsis omiscomaycus		X		X	X		
Cyprinidae	Phenacobius mirabilis	MIN	X	X	X			X
Cyprinidae	Phenacobius uranops	MIN	X	X	X	X		X
Cyprinidae	Phoxinus cumberlandensis	MIN	X			X		X
Cyprinidae	Phoxinus erythrogaster	MIN	X			X		X
Cyprinidae	Pimephales notatus	MIN	X	X			X	
Cyprinidae	Pimephales promelas	MIN	X	X			X	
Cyprinidae	Pimephales spp.	MIN	X	X			X	

Family	Final Identification	Fish Type	Native	FHW	INSCT	INT	TOL	SL
Cyprinidae	Pimephales vigilax	MIN	X	X			X	
Cyprinidae	Platygobio gracilis	MIN	X	X	X		X	
Polyodontidae	Polyodon spathula		X	X		X		X
Centrarchidae	Pomoxis annularis		X	X				
Centrarchidae	Pomoxis nigromaculatus		X	X				
Ictaluridae	Pylodictus olivaris		X	X				
Cyprinidae	Rhinichthys atratulus	MIN	X	X	X		X	X
Cyprinidae	Rhinichthys cataractae	MIN	X	X	X			X
Salmonidae	Salmo trutta				X			
Salmonidae	Salvelinus fontinalis				X			
Salmonidae	Salvelinus namaycush							
Acipenseridae	Scaphirhynchus albus		X	X				X
Acipenseridae	Scaphirhynchus platorynchus		X	X				X
Cyprinidae	Semotilus atromaculatus	MIN	X				X	
Percidae	Stizostedion canadense		X	X				
Percidae	Stizostedion vitreum		X	X				
Catostomidae	Thoburnia atripinne	SUC	X		X	X		X
Amblyopsidae	Typhlichthys subterraneus		X					
Umbridae	Umbra limi		X		X		X	

FHW= Facultative Headwater, INSCT=Insectivore, INT= Intolerant, NAT= Native, SL= Simple Lithophilic, TOL= Tolerant, COT= Sculpin, DAR= Darter, MAD= Madtom, MIN= minnow, SUC= sucker, SUN= sunfish

Appendix B. Candidate Metrics.

Candidate Metrics	Abbreviation	Response
Rel. Abun. of Benthic Ind.	%BEN	Decrease
Rel. Abun. of Campostoma spp. Ind.	%Camp	Increase
Rel. Abun. of Catostomidae Ind.	%Sucker	Decrease
Rel. Abun. of Clinostomus, Phoxinus, and Rhinichthys Spp. Ind.	%Dace	Decrease
Rel. Abun. of Creek Chub Ind.	%CrChub	Increase
Rel. Abun. of Darter Ind.	%Dar	Decrease
Rel. Abun. of Darter, Madtom, and Sculpin Ind.	%DMS	Decrease
Rel. Abun. of Facultative Headwater Ind.	**%FHW**	Increase
Rel. Abun. of Headwater Ind.	%HW	Decrease
Rel. Abun. of Insectivorous Cyprinid Ind.	%InsctCyp	Decrease
Rel. Abun. of Insectivorous Cyprinids, excluding Tolerant Ind.	%InsctCypTol	Decrease
Rel. Abun. of Insectivorous Ind., excluding Tolerant Ind.	**%INSCT**	Decrease
Rel. Abun. of Intolerant Ind.	%INT	Decrease
Rel. Abun. of Nutrient Tolerant Ind.	%NutTol	Increase
Rel. Abun. of Nutrient Tolerant Ind., excluding Creek Chub Ind.	%NutTolCC	Increase
Rel. Abun. of Omnivorous and Herbivore Ind.	%OH	Increase
Rel. Abun. of Omnivorous Ind.	%OMNI	Increase
Rel. Abun. of Pelagic Ind.	%Pelagic	Decrease
Rel. Abun. of Pioneering Ind.	%PIO	Increase
Rel. Abun. of Simple Lithophilic Spawning Ind.	%SL	Decrease
Rel. Abun. of Tolerant Ind.	**%TOL**	Increase
Rel. Abun. of Top Carnivorous Ind.	%TC	Decrease
Rel. Abun. of Water Column Ind.	%WC	Decrease
Total Fish Ind.	TNI	Decrease
Total Number of Benthic Species	BEN	Decrease
Total Number of Cyprinid Species	MIN	Decrease
Total Number of Darters, Madtoms, and Sculpins Species	**DMS**	Decrease
Total Number of Facultative Headwater Species	FHW	Increase
Total Number of Headwater Species	HW	Decrease
Total Number of Insectivorous Cyprinids, excluding Tolerant Spp.	InsctCypTol	Decrease
Total Number of Intolerant Species	**INT**	Decrease
Total Number of Native Species	**NAT**	Decrease
Total Number of Omnivorous Species	OMNI	Increase
Total Number of Pelagic Species	Pelagic	Decrease
Total Number of Pioneering Species	PIO	Increase
Total Number of Simple Lithophilic Spawning Species	**SL**	Decrease
Total Number of Species	TR	Decrease
Total Number of Sucker Species	SUC	Decrease
Total Number of Sunfish Species	SUN	Decrease
Total Number of Tolerant Species	TOL	Increase
Total Number of Top Carnivorous Species	TC	Decrease
Total Number of Water Column Species	WC	Increase

Metrics in **bold** were selected for the KIBI

Appendix C. Metric and KIBI calculation example.

Actual/Raw Data	**Convert/Invert**
Step: Collect field data and calculate metric parameters	**Step**: Convert CA to Log10 value, inverse negative metric
Stream: Rough River	
Ichthyoregion: PR	
Catchment Area (CA): 54.3	CA= 54.3; Log_{10}_CA= 1.735
TNI: 437	
NAT: 18	
DMS: 7	
INT: 4	
SL: 9	
%INSCT: 81.82	
%TOL: 5.92	100-5.92= 94.08

Reference Regression Equations (RRE)		**Expected**	**Residuals**
Provided: used to calculate expected Value (y), based on reference data		**Step**: use RRE, solve for 'y'; 'x' =log_{10}_CA	**Step**: subtract expected value from actual value
			Actual-Expected= Residual
NAT:	$y = 10.123x + 4.4279$	21.989	18 - 21.989 = -3.989
DMS:	$y = 2.967x + 1.5037$	6.651	7 - 6.651 = 0.349
INT:	$y = 2.6679x - 0.1395$	4.489	4 - 4.489 = -0.489
SL:	$y = 4.4162x + 0.9526$	8.614	9 - 8.614 = 0.386
%INSCT:	$y = -10.326x^2 + 44.989x + 17.575$	64.545	81.82 – 64.545 = 17.275
%TOL:	$y = -5.4568x^2 + 31.379x + 41.6$	79.614	94.08 – 79.614 = 14.466

Catchment Area Constant (CAC)		**Metric Value**	**95th %ile**	**Metric Score**
Provided: used to normalize residual values		**Step**: add residual to CAC	**Provided**: based on the univariate reference dataset for Metric Values	**Step**: divide metric value by 95th %ile value, multiple by 100
		Residual + CAC		
NAT:	20.49	16.501	28.2	58.513
DMS:	6.21	6.559	9.3	70.528
INT:	4.09	3.601	7.7	46.769
SL:	7.96	8.346	12.5	66.769
%INSCT:	58.88	76.155	87.8	86.736
%TOL:	77.65	92.116	101.5	90.755
			KIBI= average Metric Scores	**KIBI**= 70

RULES

1) If TNI <50 then Rel. Abund. metric score = 0

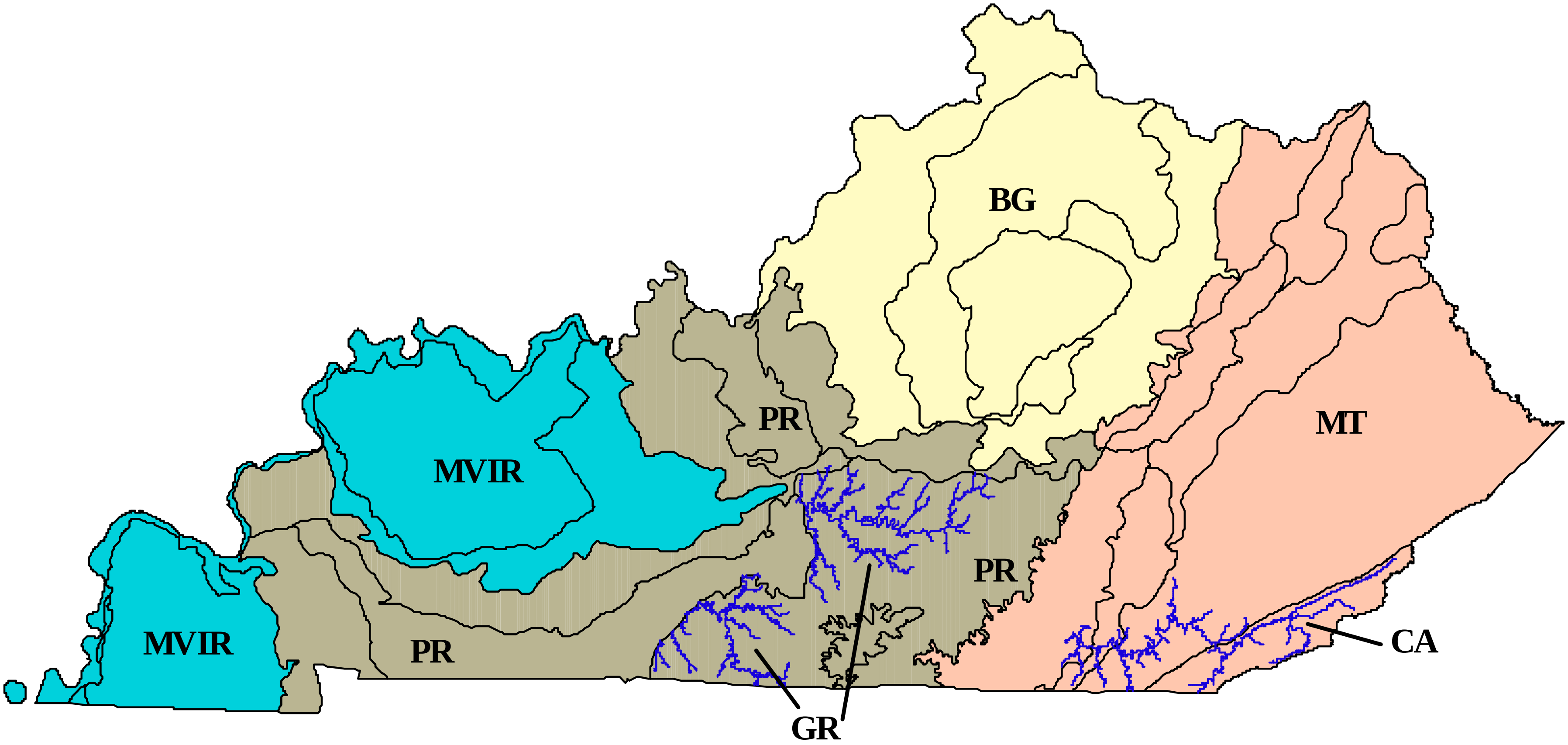
2) If TNI 51-99 then Rel. Abund. metric score = 50, unless metric score is already <50

3) If TNI $\geq$ 100 then Rel. Abund. metric score is not modified

4) If metric score >100 then score = 100

5) If metric score < 0 then score = 0

6) KIBI score is whole integer



Appendix D. Ichthyoregion Map. BG= Bluegrass, CA= Cumberland above the Falls, GR= Green River, MT= Mountain, MVIR= Mississippi Valley-Interior River, PR= Pennyroyal. Note GR and CA ichthyoregions are river basins within larger ichthyoregions. Solid lines mark Level IV subecoregion boundaries (see Woods et al. 2002).