Development of Tolerance Values for Kentucky Crayfishes

Kentucky Environmental and Public Protection Cabinet
Department for Environmental Protection
Division of Water
Water Quality Branch
Watershed Management Branch

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Cover photograph description: *Cambarus dubius*, Morgan County, Kentucky

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Development of Tolerance Values for Kentucky Crayfishes

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Executive Summary

Crayfish have effectively populated nearly every type of aquatic ecosystem in Kentucky. Approximately 53 species of crayfish can be found in Kentucky, making this a relatively diverse state. Crayfish species diversity reaches a global climax in the southeastern United States. Many species have small or limited ranges, which has been noted as a major contributing factor behind their potential and realized imperilment. Threats to crayfish conservation include channelization, excessive sedimentation, impoundments, pollution and invasive species. It has been estimated that 19% of the North American (north of Mexico) crayfish taxa are endangered, 13% threatened, 15% of special concern, 2 species are probably extinct and 48% of all crayfish species deserve some type of protective conservation status.

Studies that examine the sensitivity of crayfish to environmental impairments may be key to their conservation. Considering the diversity of crayfish species, it is logical to suspect that many are vulnerable to the same suite of human-induced factors that affect other aquatic organisms. Defining tolerance values (TVs) for crayfish on a species level may aid or at least create a starting point in the management of important resources for crayfish conservation.

The Kentucky Division of Water (KDOW) uses published TVs for macroinvertebrates to calculate the modified Hilsenhoff Biotic Index (mHBI). The KDOW uses the mHBI as an important metric to assess water quality for point and nonpoint source impacts, compliance and ambient monitoring, as well as for mandatory 305(b) and 303(d) reports. Average TV of a water quality sampling site can also be used as a meaningful, stand-alone index, especially when studying long-term ambient water quality and its recovery potential. This is useful when studying stream restoration projects or the effects of best management practices (BMPs) to control nonpoint source pollution.

Sampling by KDOW has shown that there may be some discrepancy in the published TVs of some crayfish and their occurrence in Kentucky streams. We felt that this merited an examination of a wide array of physicochemical, habitat and biological variables to test their effects on crayfish occurrence and abundance in high quality and impaired streams. This study was also initiated to reassign TVs to all cambarids in Kentucky in an attempt to improve the accuracy of water quality surveys.

Crayfish were collected from 38 1st or 2nd order streams (as depicted on 1:24,000 scale topographic maps) with catchment areas ranging from 80 to 780 ha during March–May 2000. Sampling locations were in the upper Cumberland and upper Kentucky River basins within ecoregions 68 (Southwestern Appalachians) and 69 (Central Appalachians). Reference sites (n = 23) were located in high-quality, densely forested, relatively undisturbed watersheds. Test sites (n = 15) were located in watersheds that had been impacted by point and nonpoint sources.

A 100-m study reach was established for each site at the first riffle where prime crayfish habitat was identified. Riffles, pools and runs (n = 3) were quantitatively sampled using a 0.25 m^2-quadrat sampler. We also made a qualitative search at each site. In addition, we also evaluated 18 habitat, 4 physicochemical and 8 biological variables at each site.

A Discriminant Function Analysis (DFA) used percent embeddedness, canopy cover, conductivity and total habitat score to best distinguish reference from test sites. Box and Whisker plots were used to illustrate that there was no difference between reference and impaired sites regarding catchment area, slope, elevation and riffle substrate size.

A total of 8 species of crayfish (n = 244) were collected from the two river basins: *Cambarus bartonii* (n = 49), *C. buntingi* (n = 4), *C. cumberlandensis* (n = 3), *C. distans* (n = 59), *C. parvoculus* (n = 12), *C. robustus* (n = 40), *C. rusticiformis* (n = 9) and *Orconectes cristavarius* (n = 39).

Overall, species of *Cambarus* were more abundant within the study area than *O. cristavarius*. *Cambarus* spp. occurred in 95% of all the streams sampled while *O. cristavarius* was found in 16% of the streams sampled. The cumulative results of a Spearman's correlation analysis illustrated a pattern that depicted the eastern Kentucky *Cambarus* spp. as favoring reference quality stream conditions while *O. cristavarius* was correlated with impaired conditions. Specifically, the genus *Cambarus* was correlated with 20 variables depicting reference conditions ($p < 0.05$), including an increase in canopy cover and total habitat score, and a decrease in conductivity and percent riffle embeddedness. *Orconectes*

cristavarius was correlated with 21 variables depicting impairment within test streams ($p < 0.05$), including decrease in canopy cover and total habitat score, and an increase in conductivity and percent riffle embeddedness. *Cambarus* spp. were found at 100% of the reference sites and 86% of the test sites, while *O. cristavarius* were found at no reference sites and 40% of the test sites.

We used a DFA to determine which variables may have accounted for the presence or absence of these genera at the study sites. The stepwise DFA model chose conductivity, total habitat score, bank vegetation and sediment deposition to discriminate between *Cambarus* spp. and *Orconectes cristavarius* occurrence.

The KDOW ecological database, EDAS, contained 1,331 locations with records of Cambaridae taxa, including 5 genera, 35 species and 4,992 individuals. We recalculated TVs for crayfish in Kentucky at the family level, at the genus level for the 4 most abundant genera found in Kentucky (*Cambarus*, *Fallicambarus*, *Orconectes*, and *Procambarus*) and 21 species. Other Kentucky genera with $n < 10$ records in EDAS were assigned the adjusted TV corresponding to their genus or family.

We believe that it is reasonable to assume that species such as *C. parvoculus* (TV = 2.37) and *C. distans* (TV = 3.98) that were more susceptible to a suite of anthropogenic impairments were once more widespread. Other crayfish, such as *O. cristavarius* (TV = 5.42), that flourished in the eastern Kentucky test sites but were conspicuously absent from reference sites, may have expanded their ranges as humans colonized North America and impacted water quality. If the assumptions about these data are true, they might indicate a disturbing scenario where intolerant crayfish may be out-competed not only by introduced species but also by more tolerant native crayfish that occur naturally within the same basin.

1.0 Introduction

Crayfish species diversity reaches a global climax in the southeastern United States (Taylor et al. 1996, Taylor 2000), and Kentucky is a relatively diverse state with 53 species (Schuster unpublished data 2003). Taylor (2000) reported that 68% of all U.S. species are endemic to an area south and east of (and including) the state of Kentucky. Cambarid crayfish have effectively populated nearly every type of aquatic ecosystem, including streams, lakes, swamps, caves, springs, meadows and forests with hardpan soils, and ephemeral ditches (Taylor 2000). This extensive adaptive radiation has led to a high degree of taxonomic diversity and endemism that has limited the natural range of many species (Schuster 1997, Taylor 2000). Taylor et al. (1996) identified small natural range as a major contributing factor behind the potential or realized impairment of the group. Species with small ranges may be extremely vulnerable to extirpation due to one-time catastrophic events, and they are particularly vulnerable to habitat destruction or degradation (Gilpin and Soule 1986, Rabinowitz et al. 1986, Taylor et al. 1996). More specifically, threats to crayfish conservation include channelization, excessive sedimentation, impoundments and pollution (Hobbs and Hall 1974, Taylor et al. 1996). Lodge et al. (2000) stated that the greatest threat faced by native crayfish might be the introduction of nonindigenous crayfish taxa. Taylor et al. (1996) found that 19% of the North American (north of Mexico) crayfish taxa were endangered, 13% threatened, 15% of special concern, 2 species have probably gone extinct and 48% deserve some type of protective conservation status.

Studies that examine the sensitivity of crayfish to environmental impairments are lacking and may be key to their conservation. Considering the diversity of crayfish species, it is logical to suspect that many are vulnerable to the same suite of human-induced factors that affect other aquatic organisms (Butler 2003). Defining tolerance values (TVs) for crayfish on a species level may aid, or at least create a starting point, in the management of important resources for crayfish conservation.

The Kentucky Division of Water (KDOW) uses published TVs (e.g., Lenat 1993, NCDENR 2001) for macroinvertebrates to calculate the modified Hilsenhoff Biotic Index (mHBI) to complete water quality assessments. The mHBI score is based on the average TVs of macroinvertebrates (genus and species) collected semi-quantitatively at a monitoring location. Tolerance values are based on a 1–10 scale, as is the mHBI (i.e., 1 being the least tolerant, 10 the most tolerant). Hilsenhoff (1977, 1982, 1987, 1988) designed the HBI to measure the amount of organic pollution in a stream. Other studies (e.g., Lenat 1993, McMurray and Schuster 2001) have shown that this biotic index is sensitive to multiple stressors. The mHBI is used as an important index to assess water quality for point and nonpoint source impacts, compliance and ambient monitoring, as well as for mandatory 305(b) and 303(d) reports required by the Clean Water Act. Lillie and Schlesser (1994) reported the usefulness of using the mean TV of the entire sample (riffle, runs and pools) as an index in itself. They found that it displayed little spatial and temporal variability and may be valuable for estimating a stream's long-term ambient water quality and its recovery potential. This may become a particularly useful metric when studying the effects of best management practices (BMPs) used to control nonpoint source pollution and in stream restoration projects.

The published TVs for *Orconectes* spp. (2.6) and *Cambarus* spp. (7.6) (NCDENR 2001) agree with the majority of the literature (Malley 1980, Berril et al. 1985, Hollet et al. 1986, Davies 1989, DiStefano et al. 1991, France and Collins 1993) concerning the sensitivity of these two genera to degrees of acidification and changes in water quality. A TV of 7.6 effectively designates all species of *Cambarus* as being tolerant, and a TV of 2.6 denotes all *Orconectes* spp. as being intolerant. However, sampling in Kentucky has shown that *Orconectes* spp. can be highly abundant and dominant in degraded streams. In contrast, *Cambarus* spp. can be abundant and occur with relatively high species diversity in high quality Kentucky streams as well as in impaired systems. We felt that this merited an examination of a wide array of physicochemical, habitat and biological variables to test their effects on crayfish occurrence and abundance in high quality and impaired streams. This study was also initiated to reassign TVs to all cambarids in Kentucky in an attempt to improve the accuracy of our water quality surveys. Rosenberg et al. (1986) suggested that using the most precise level of identifications when assessing streams is particularly important in surveys which may help guide management decisions, such as requiring a discharger to meet strict effluent regulations, applying BMPs or restricting development (Lenat and Resh 2001).

There has been much debate and interest in the taxonomic resolution of macroinvertebrates collected for the purpose of bioassessments (e.g., Lenat and Resh 2001, Bailey et al. 2001). From our observations, we suspected that species in the family Cambaridae would show considerable variability in their tolerances to pollution, especially within the genus *Cambarus*. It has been well documented that macroinvertebrate families may contain taxa with a wide range of TVs at the genus/species level (Lenat and Resh 2001). For example, the North Carolina TVs for taxa within the family Baetidae range from 9.84 (*Callibaetis* sp.) to 1.62 (*Diphetor hageni*) (NCDENR 2001). Lewis (1974) found that species of *Stenonema* ranged from fairly tolerant to intolerant to pollution, and Lenat (1993) went on to define the ranges of tolerance within the genus from 0.13 (*S. meririvulanum*) to 7.18 (*S. femoratum*). Using genus/species data may also be important in terms of allowing reasonably sensitive detection of biological impairment using biologic metrics. For example, Hawkins and Norris (2000) and Hawkins et al. (2000) indicated that multivariate models based on family levels of macroinvertebrate identifications did not perform as accurately as species-level models in the mountain streams of California where extensive adaptive radiation had occurred and this may be true of similar areas, such as the mountainous streams of Appalachia.

This study examines how physicochemical, habitat and biological variables affect the occurrence and abundance of crayfish in high quality versus degraded streams in the Cumberland Plateau of eastern Kentucky. This region of Kentucky provided a relatively diverse study area. Hobbs (1969) proposed that the crayfish genus *Cambarus*, and perhaps *Orconectes*, had the Cumberland Plateau as the epicenter of their evolutionary radiation.

2.0 Study Area Description

The sites used in this study were originally selected to document regional expectation criteria for benthic communities in the Eastern Coalfield region in Kentucky (Pond and McMurray 2002). Crayfish were collected from 38 1st or 2nd order streams (as depicted on 1:24,000 scale topographic maps) with catchment areas ranging from 80 to 780 ha during March–May 2000. Sampling locations were contained within the upper Cumberland and upper Kentucky River basins in ecoregions 68 (Southwestern Appalachians) and 69 (Central Appalachians) (Figure 1). Reference sites (n = 23) were located in high-quality, densely forested, relatively undisturbed watersheds. Test sites (n = 15) were located in watersheds that had been impacted by various point and nonpoint sources (Appendix A).

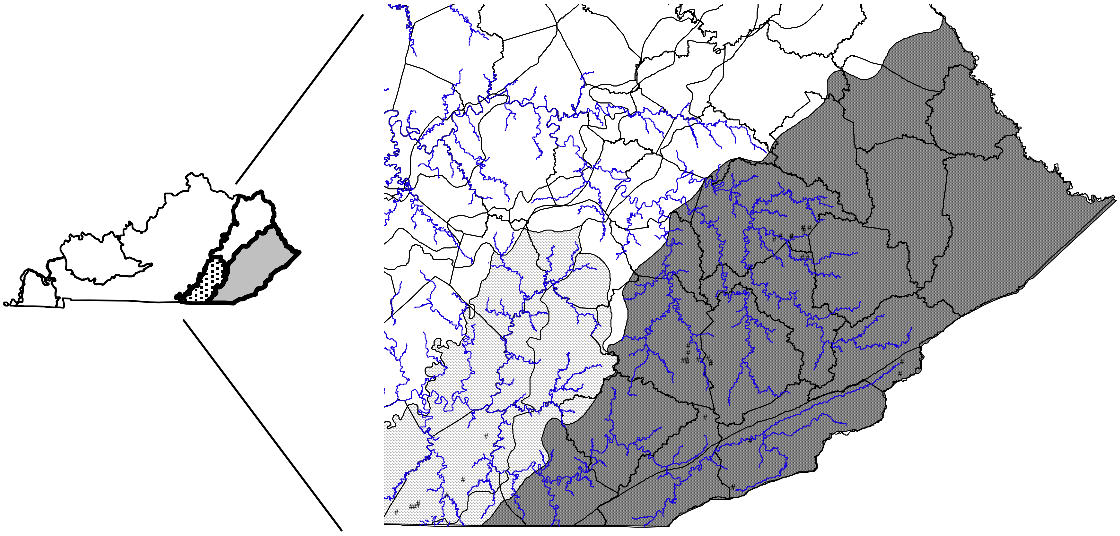

Figure 1. Generalized map of study area showing the Central Appalachian (gray area) and the Southwestern Appalachia (stippled) ecoregions.

The following ecoregion descriptions are taken from Woods et al. (2002). The ecoregions are typified as being unglaciated, mixed mesophytic forest type with a mesic temperature regime, a mean annual precipitation of 101.6–129.5 cm/year and a mean temperature range of -7–31.6 ˚C. The Southwestern Appalachian ecoregion covers an area of 5,294 km^2 and is mostly forested with limited cropland and pastureland. This ecoregion is composed of low hills, ridges, rolling uplands and valleys. Streams have moderate to high gradients with cobble and boulder substrates (as defined by Wentworth 1922). Lower gradient streams are also present with gravel or sandy bottoms. Some of Kentucky's highest quality streams occur in this ecoregion. However, logging, coal mining and livestock impair streams in this ecoregion. The dissected hills, narrow ridges, deep coves and mountains of the Central Appalachians cover an area of 15,840 km^2 and are mostly forested, with extensive coal mines, gas fields, pastureland and some cropland. Streams in this region have moderate to high gradients and cobble or boulder substrates. Water quality impairments include logging, coal mining and oil and gas production. In some cases, degradation from acid mine runoff and sedimentation are extensive (e.g., McMurray and Schuster 2001).

3.0 Methods

3.1 Data Collection for Eastern Kentucky Headwater Streams Study

In a 100-m study reach at each site estimates of canopy cover, forested riparian zone width, stream width and substrate size were made at 5 transects spaced every 20-m throughout the 100-m reach following the methods described in Pond and McMurray (2002) (Table 1). Percent riffle embeddedness and substrate sizes were estimated using 0.25-m^2 quadrats as described in Pond and McMurray (2002). Habitat features (Table 1) were assessed at each site following Barbour et al. (1996). Physicochemical measurements of pH, temperature, conductivity and dissolved oxygen were made with a Hydrolab® Surveyor 4/MiniSonde (Hydrolob-Hach Company, Loveland, CO) (Table 1).

Crayfish were quantitatively collected in the 100-m study reach using a 0.25 m^2-quadrat sampler (Figure 2) modified from DiStefano (2003). The sampler was constructed using 1.9 cm PVC pipes, standing 60.96 cm high and covered on three sides with 5 × 3 mm rectangular mesh. A 60.96 × 60.96 × 60.96 cm bag net (10 mm mesh) with weights on the bottom and floats on the top (Memphis Net and Twine Co., Memphis, TN) was attached to the rear of the sampler. Flaps (15 cm in depth, 5 × 3 mm mesh) were attached to the bottom of the front and sides of the sampler to allow for an uneven substrate to prevent individuals from escaping beneath the sampler.

Using the sampling device, we targeted prime crayfish habitat in riffles, pools and runs (n = 3) at each site. Prime crayfish habitat was defined as cobbles, boulders (as defined by Wentworth [1922]), leaf packs, root mats and any other available habitat that crayfish may have utilized for cover as perceived by the authors. Crayfish were also collected using qualitative methods. These specimens were either hand picked or collected using a D-frame dipnet (800 × 900 μm mesh). Qualitative searches were conducted to ensure that all possible species of crayfish were accounted for in the collection. When an additional species was collected, it was counted as 1 crayfish merely to note its presence. For example, if 4

Table 1. Habitat, biological and physicochemical variables correlated with crayfish collected from eastern Kentucky headwater streams (March–May 2000).

	Variables	
Habitat	Physicochemical	Biological
Bank stability*	Conductivity	mHBI
Bank Vegetation width*	Dissolved Oxygen	Taxa richness
Canopy cover	pH	%Ephemeroptera
Channel alteration*	Temperature	%EPT
Distance to source of stream		%Chironomids + Oligochaetes
Elevation		%Primary Clingers
Embeddedness*		%Scrapers
Epifaunal substrate*		
Frequency of riffles*		
Latitude and Longitude		
Percent riffle embeddedness		
Riparian zone width		
Sediment deposition*		
Slope		
Stream width		
Substrate size		
Total Habitat Score*		
Velocity depth regime*		
Watershed size		

* Variables derived from Environmental Protection Agency/Rapid Bioassessment Protocol Habitat Assessment Sheet

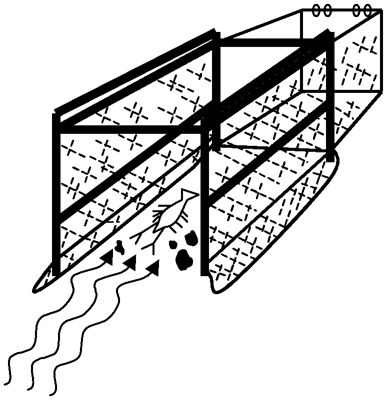

Figure 2. Sampling device used to collect crayfish from eastern Kentucky headwater streams, March–May, 2000

Cambarus bartonii and 3 *C. distans* were collected quantitatively and 4 *C. robustus* were collected qualitatively, the total number of crayfish counted for analysis would be 8.

All crayfish were initially preserved in 95% ethanol and then transferred to 70% ethanol for final preservation. Crayfish were identified to genus and an effort was made to identify all specimens to the species level using Hobbs (1972, 1989) and Schuster (2002 unpublished).

Macroinvertebrates were quantitatively collected at each site using a 0.25-m^2 quadrat and a macroinvertebrate kicknet following the methods described in Pond and McMurray (2002). Crayfish captured using this method were added into the total count since the two quadrats were the same effective size. Elevation, latitude and longitude, distance to source of the stream and watershed size were determined from 7.5-minute USGS topographical maps (1:24,000 scale) using ARCVIEW GIS software (ESRI, Redlands, CA).

3.2 Data Analysis for Eastern Kentucky Headwater Streams Study

For all of the sites used in this study, Pond and McMurray (2002) evaluated 33 metrics for discriminatory efficiency, redundancy, variability and sensitivity to create a subset of metrics that could best distinguish reference from test sites. Physical habitat parameters and water quality variables of test and reference sites were also compared using a Mann-Whitney *U* test, a Principle Components Analysis (PCA), a Discriminant Function Analysis (DFA, *F*) (SYSTAT, version 7.0, SPCC, Inc., Point Richmond, CA) and box and whisker plots to determine if these sites could be successfully discriminated using these variables (Pond and McMurray 2002).

A Spearman’s correlation analysis (*r*) (StatMost, DataMost Corporation, Salt Lake City, UT) was used to determine the relationship of each crayfish genus and species found with 18 habitat, 4 physicochemical, and 6 biological variables (Table 1). We used a DFA to distinguish variables that may have accounted for the presence or absence of crayfish genera at sites.

3.3 Data Analysis for Reassignment of Crayfish Tolerance Values

The KDOW ecological database, Ecological Data Application System (EDAS, Microsoft ACCESS software), was used to calculate new crayfish TVs. The database was populated with collections from 1978–present that were sampled using various methods (KDOW 2002) as part of water quality surveys conducted throughout the Commonwealth. The database was queried to locate the average TV of each site where crayfish taxa occurred ($n \geq 10$). The average TV for each site in EDAS was calculated by averaging the TV of every macroinvertebrate collected from the site. We used the average TV of all the sites where a particular crayfish taxon was found and used this new average to assign new TVs to the crayfish species. For example, *C. striatus* was found at 11 sites, the average TV for these sites was 5.66, and this number was assigned as the TV for *C. striatus*. We assumed this would provide a conservative reflection of the crayfish's water quality preference. If there were fewer than 10 records for any species, the new genus or family level TV was assigned to those taxa until more records can be attained from future collections. We also used box and whisker plots to graphically examine the ranges and median values of TVs of the crayfish taxa contained in our database.

4.0 Results and Discussion

Pond and McMurray (2002) examined our reference and test sites using various statistical methods to test which environmental variables could distinguish reference from test sites (Appendices B, C and D). Their DFA chose percent embeddedness (*F* = 3.47), canopy cover (*F* = 7.65), conductivity (*F* = 1.76) and total habitat score (*F* = 1.76) to classify 43 reference and test sites with 98% accuracy (Figure 3). No difference between reference and impaired sites could be identified for catchment area, slope and riffle substrate size (Figure 4) (Mann-Whitney, p < 0.01) (Pond and McMurray 2002). This was integral to the design of this study for comparison of reference and test sites as crayfish faunas may change as slope, drainage area, and elevation vary. In addition, reference sites differed from test sites in riffle embeddedness, conductivity, riparian width and canopy cover (Figure 4). The results for temperature are also displayed in Figure 4; however, these measurements were not taken at similar times of the day so their usefulness is questionable.

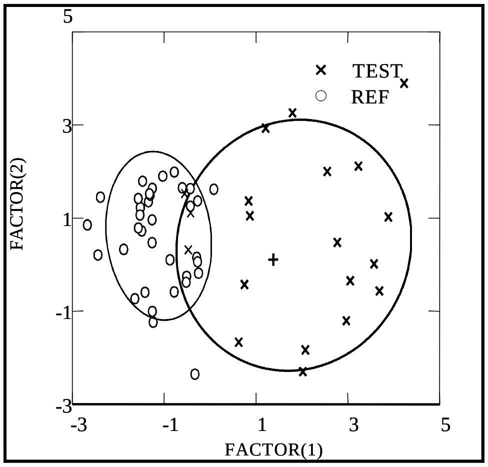

Figure 3. Discriminant root scores using %embeddedness, conductivity, canopy and total habitat score (2000–2001 data, from Pond and McMurray [2002]).

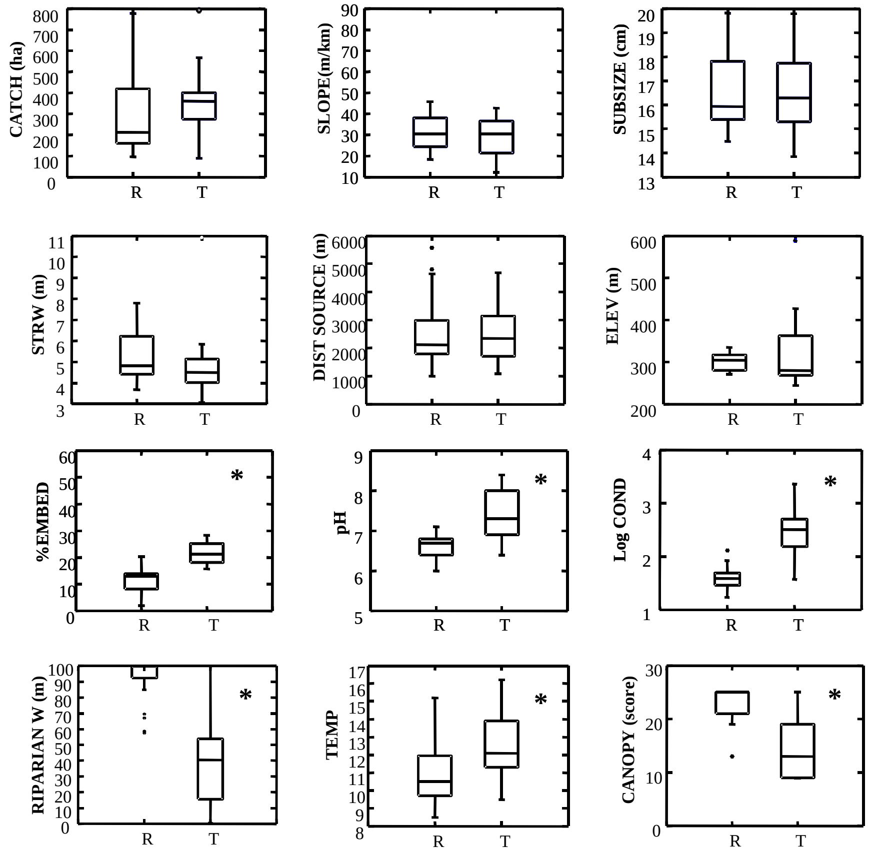

Figure 4. Selected environmental variables from reference and test sites (2000 calibration dataset, from Pond and McMurray [2002]). An * indicates variables that had significant differences between reference and test sites (Mann-Whitney, $p < 0.01$)

A total of 8 species of crayfish (n = 244) were collected from the two river basins: *Cambarus bartonii* (n = 49), *C. buntingi* (n = 4), *C. cumberlandensis* (n = 3), *C. distans* (n = 75), *C. parvoculus* (n = 19), *C. robustus* (n = 40), *C. rusticiformis* (n = 9), and *Orconectes cristavarius* (n = 37) (Appendix E). We also collected 8 *Cambarus* that could not be identified to species. No statistical correlations using Spearman's correlation analysis ($p > 0.05$) existed with any measured variable and the abundance of *C. buntingi* or *C. cumberlandensis*, possibly due to their low sample sizes. *Cambarus rusticiformis* abundance was correlated with an increase in slope ($p < 0.05$, Table 2); however, this variable was not indicative of water quality in this study. Likewise, *C. robustus* was significantly correlated with %scrapers and dissolved oxygen (D.O.) ($p < 0.05$, Table 2), which were not indicative of water quality in this study. *Cambarus bartonii* was correlated with 1 habitat variable and 3 biological variables depicting reference conditions ($p < 0.05$, Table 2). *Cambarus distans* was correlated with 4 habitat variables and 1 physicochemical variable that depicted reference conditions ($p < 0.05$, Table 2). *Cambarus parvoculus* was positively correlated with reference sites ($p < 0.05$) and had a negative relationship to test sites (p <

0.05, Table 2). *Cambarus parvoculus* was also positively correlated with an increase in Total Habitat Score ($p < 0.05$, Table 2).

Table 2. Results of Spearman correlation analysis of *Cambarus* species and variables collected from eastern Kentucky headwater streams (March–May 2000).

Taxa	Habitat/Physicochemical Parameters
Cambarus bartonii (n = 75)	Frequency of Riffles (*r* = 0.38, p = 0.02)
	mHBI (*r* = -0.35, p = 0.03)
	%Chironomids+Oligochaetes (*r* = -0.35, p = 0.03)
	%Scrapers (*r* = 0.50, p = 0.003)
C. distan (n = 59)	Bank Vegetation (*r* = 0.39, p = 0.02)
	Canopy cover (*r* = -0.51, p = 0.002)
	Conductivity (*r* = -0.33, p = 0.04)
	Dissolved Oxygen (*r* = -0.46, p = 0.004)
	Slope (*r* = 0.53, p = 0.001)
	Total Habitat Score (*r* = 0.34, p = 0.03)
	Velocity/Depth Regime (*r* = 0.43, p = 0.008)
C. parvoculus (n = 19)	Reference Sites (*r* = 0.44, p = 0.007)
	Temperature (*r* = -0.47, p = 0.003)
	Test sites (*r* = -0.44, p = 0.007)
	Total Habitat Score (*r* = 0.51, p = 0.002)
C. robustus (n = 40)	Dissolved Oxygen (*r* = 0.4, p = 0.01)
	%Scrapers (*r* = 0.05, p = 0.004)
C. rusticiformis (n = 9)	Slope (*r* = 0.36, p = 0.03)

Admittedly, these correlations are somewhat weak, perhaps due to small sample size. However, evaluating *Cambarus* at a genus level (n = 207) offered more revealing results. Specifically, the genus *Cambarus* was correlated with 20 variables depicting reference conditions ($p < 0.05$, Table 3). In contrast, *O. cristavarius* was correlated with 21 variables depicting impairment within test streams (p <

Table 3. *Cambarus* spp. and *Orconectes cristavarius* correlations with variables measured in eastern Kentucky headwater streams (March–May 2000).

Taxa	Habitat/Physicochemical Parameters
Cambarus spp. (n = 207)	Canopy cover (r = 0.32, p = 0.04)
	Channel Alteration (r = 0.37, p = 0.02)*
	%Clingers (r = 0.31, p = 0.05)
	Conductivity (r = -0.51, p = 0.002)
	Embeddedness (r = -0.45, p = 0.005)*
	Epifaunal Substrate (r = 0.51, p = 0.001)*
	Frequency of Riffles (r = 0.34, p = 0.04)*
	EPT (r = 0.37, p = 0.02)
	mHBI (r = -0.42, p = 0.009)
	%Chiro+Oligo (r = -0.45, p = 0.005)
	%Embeddedness (r = 0.48, p = 0.005)
	%Ephemeroptera (r = 0.45, p = 0.005)
	%Scrapers (r = 0.52, p = 0.001)
	pH (r = -0.47, p = 0.003)
	Sediment Deposition (r = 0.45, p = 0.005)*
	Reference Sites (r = 0.44, p = 0.007)
	Temperature (r = -0.47, p = 0.003)
	Test sites (r = -0.44, p = 0.007)
	Total Habitat Score (r = 0.51, p = 0.002)*
	Velocity Depth Regime (r = 0.005, p < 0.05)*
Orconectes cristavarius (n = 37)	Bank Vegetation (r = -.64, p < 0.0001)*
	Canopy cover (r = -0.51, p = 0.002)*
	Channel Alteration (r = -0.56, p = 0.0003)*
	Clingers (r = -0.58, p = 0.0003)
	Conductivity (r = 0.59, p = 0.003)
	Embeddedness (r = -0.45, p = 0.005)*
	Dissolved Oxygen (r = 0.32, p = 0.045)
	EPT (r = -0.47, p = 0.004)
	Epifaunal Substrate (r = -0.45, p = 0.006)*
	Frequency of Riffles (r = -0.31, p = 0.05)*
	mHBI (r = 0.48, p = 0.003)
	%Chiro+Oligo (r = 0.48, p = 0.003)
	%Embeddedness (r = 0.43, p = 0.008)
	%Ephemeroptera (r = -0.54, p = 0.0008)
	pH (r = -0.55, p = 0.0006)
	Reference Sites (r = -0.61, p = 0.0001)
	Riparian Width (r = -0.054, p = 0.0008)*
	Taxa Richness (r = -0.38, p = 0.02)
	Temperature (r = 0.34, p = 0.032)
	Test sites (r = -0.61, p = 0.0001)
	Total Habitat Score (r = -0.51, p = 0.002)*

*From USEPA Habitat Assessment Sheet; positive correlations denote a relationship with reference conditions while negative correlations denote relationships with degraded conditions.

0.05, Table 3). The stepwise DFA model chose conductivity (*F* = 18.09), total habitat score (*F* = 5.00), bank vegetation (*F* = 8.90) and sediment deposition (*F* = 6.86) as variables that classified the 38 records with 88% accuracy (Figure 5). An internal jackknife test of the data classified the sites with a 12% misclassification rate. Overall, the 4-variable discriminant model was highly significant (Wilk's λ = 0.326, *F* = 18.125, p < 0.00001). The Spearman's correlation analysis (Table 3) likewise correlated *Cambarus* occurrence with an increase in total habitat score and a decrease in conductivity and sediment deposition. The correlations for *O. cristavarius* occurrence included a decrease in total habitat score and increases in conductivity and percent riffle embeddedness (similar to sediment deposition). Other notable correlations included *Cambarus* occurrence with increases in canopy cover and EPT, and decreases in channel alteration and mHBI (p < 0.05, Table 3), while *O. cristavarius* occurrence was correlated with a decrease in bank vegetation and canopy cover and an increase in mHBI score (p < 0.05, Table 3). *Cambarus* spp. were found at 100% of the reference sites and 86% of the test sites, while *O. cristavarius* were found at no reference sites and 40% of the test sites. *Cambarus* were more abundant in the study area and were collected at 95% of all sites, while *O. cristavarius* was found at only 16% of the sites.

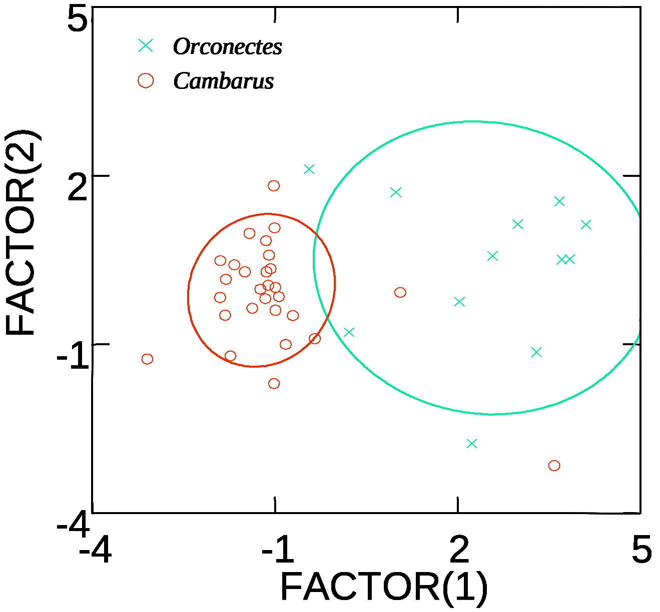

Figure 5. Discriminant root scores using total habitat score, bank vegetation and sediment deposition.

Cambarus spp. were correlated with low pH values and *O. cristavarius* were correlated with higher levels (p < 0.05, Table 3). Reference sites were generally more acidic than test sites, and this observation concurred with previously published research. For example, Malley (1980) reported that Ca^+ uptake by *Orconectes virilis* was inhibited during the postmolt stage when subjected to pH levels below

5.75, and uptake ceased altogether below 4.00 and resulted in mortality. Other researchers suggest *Cambarus* spp. to be more tolerant of acidity than *Orconectes* spp. (Collins et al. 1981, Berrill et al. 1985, Hollet et al. 1986, DiStefano et al. 1991). However, France (1993) reported that pH in central Ontario lakes had a dramatic influence on the presence or absence of crayfish, regardless of genus. Davies (1989) reported a complete population collapse of *Orconectes virilis* in response to a gradual experimental whole-lake acidification project in northwestern Ontario where the population suffered when pH was lowered to 5.64 from 6.49, and complete eradication occurred when the average pH reached 5.09–5.13. Using the results from previous research and this dataset, we concur that many *Cambarus* spp. are more tolerant of low pH levels than *Orconectes cristavarius*. However, when compared to a myriad of habitat, physicochemical and biological metrics, as a group, *Cambarus* spp. were less tolerant of water quality impacts than *Orconectes cristavarius.* Although the genus level correlations are still somewhat weak, the cumulative results illustrate a definitive pattern that depict the eastern Kentucky *Cambarus* spp. as favoring reference quality stream conditions while *Orconectes cristavarius* was correlated with impaired conditions (Table 3).

4.1 Adjusting Crayfish Tolerance Values in Kentucky

The KDOW database (EDAS) contained 1,331 collection locations of Cambaridae taxa, including 4 genera, 35 species and 4,992 individuals. Table 4 lists a recalculated TV for the family level, 35 crayfish species and a genus level TV for the 4 most abundant genera found in Kentucky (*Cambarus*, *Fallicambarus*, *Orconectes*, and *Procambarus*). The database had only two accounts of *Barbicambarus cornutus*, a monotypic genus endemic to the Green River system, that were associated with water quality data. We therefore assigned it the family level TV (5.58). Likewise, *Cambarellus* was assigned the family level TV since no species of *Cambarellus* has yet been collected by KDOW. Additional crayfish taxa that populated EDAS and for which we had fewer than 10 records were assigned their corresponding genus level TV until more records can be obtained (Table 4).

The family Cambaridae contains species that exhibit a wide range of water quality preferences. For example, *C. parvoculus* occurred at sites with a median TV of 3.0 that ranged from 2.5 – 3.5 (excluding outliers) (Figure 6) and had an average TV of 3.07 (Table 4), while *Procambarus clarkii* was found at sites with a median TV approaching 7.8 that ranged from 6.1 – 8.1 (Figure 6) and had an average TV of 7.44 (Table 4). *Cambarus distans* displayed the most variability as it was collected from sites with average TVs ranging from 2.2 – 6.8 (excluding outliers) (Figure 6). We plotted the top occurring cambarid genera from EDAS using box plots to visually examine their median and range of average TVs. The genus *Cambarus* displayed the most variability of average TVs and occurred at sites with the widest

Table 4. Former and recalculated family, genus and species level Kentucky crayfish tolerance values (TVs) (n = number of collection sites). Bolded values represent published TVs (NCDENR 2001).

Taxa	Former TV	Recalculated TV
Cambaridae (n = 1,331)	5.00	5.58
Barbicambarus cornutus (n < 10)	5.00	5.58
Cambarellus spp. (n < 10)	--	5.58
Cambarus spp. (n = 463)	**7.62**	5.14
C. bartonii cavatus (n = 21)	7.50	4.09
C. buntingi (n < 10)	7.50	5.14
C. cumberlandensis (n = 23)	7.50	4.54
C. diogenes (n = 50)	7.50	7.16
C. distans (n = 54)	7.50	4.39
C. dubius (n < 10)	7.50	5.14
C. friaufi (n < 10)	7.50	5.14
C. graysoni (n < 10)	7.50	5.14
C. ortmanni (n = 12)	7.50	5.75
C. parvoculus (n = 14)	7.50	3.07
C. robustus (n = 26)	7.50	4.43
C. rusticiformis (n = 15)	7.50	4.62
C. sciotensis (n = 26)	7.50	5.59
C. sphenoides (n < 10)	7.50	5.14
C. striatus (n = 11)	7.50	5.66
C. tenebrosus (n = 24)	7.50	5.92
Fallicambarus fodiens (n = 10)	5.00	6.53
Orconectes spp. (n = 768)	**2.60**	5.71
O. barrenensis (n < 10)	2.60	5.71
O. bisectus (n < 10)	2.60	5.71
O. burri (n < 10)	2.60	5.71
O. cristavarius (n = 88)	2.60	5.42
O. compressus (n < 10)	2.60	5.71
O. durelli (n < 10)	2.60	5.71
O. immunis (n = 23)	2.60	7.36
O. juvenilis (n = 56)	2.60	5.90
O. kentuckiensis (n < 10)	2.60	5.71
O. placidus (n = 12)	2.60	4.91
O. putnami (n = 82)	2.60	5.62
O. rusticus (n = 177)	2.60	5.98
O. sanborni (n = 10)	2.60	6.18
O. tricuspis (n < 10)	2.60	5.71
Procambarus spp. (n = 52)	**9.50**	6.82
P. acutus acutus (n = 27)	9.50	6.58
P. clarkii (n = 15)	9.50	7.44

range of TVs (Figure 7). *Cambarus* TVs ranged from 2.37 (*C. parvoculus*) to 7.16 (*C. diogenes*) and species within the genus occurred at sites with average TVs ranging from 2.5–9.1. Discounting *Fallicambarus*, which is monotypic in Kentucky (*F. fodiens*), *Orconectes* spp. displayed the least amount of variability in their range of average TVs (3.50–8.00) (Figure 7). The genus *Orconectes* also exhibited low variability in TVs, ranging from 4.48 (*O. placidus*) to 7.55 (*O. immunis*) (Figure 7). Interestingly, *F. fodiens* and *Procambarus* spp. occurred at sites with higher average TVs (5.50–7.00 and 5.00–8.50, respectively) (Figure 7).

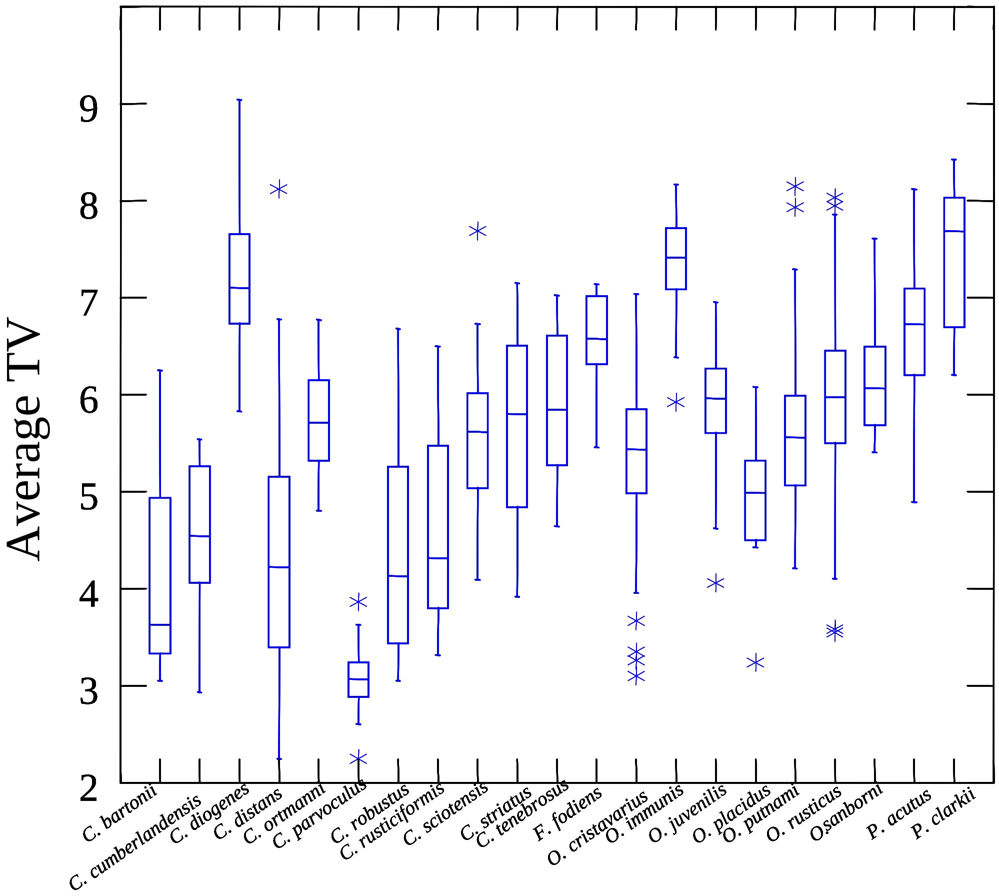

Figure 6. Average tolerance value (TV) variation among selected crayfish species from the KDOW ecological database (EDAS, 2003)

4.2 Conservation Summary

Variables such as elevation, slope and drainage area could have had an effect on the current distribution of crayfish species. Since the reference and test sites from our eastern Kentucky dataset did not significantly differ in regard to these variables, we believe it reasonable to assume that species such as

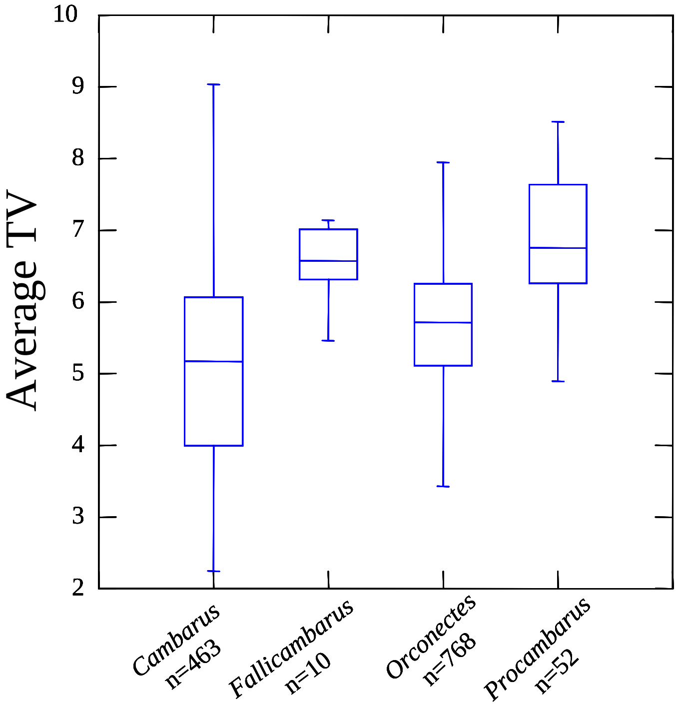

Figure 7. Average tolerance value (TV) variation among selected crayfish genera from the KDOW ecological database (EDAS, 2003)

C. parvoculus (TV = 2.37) and *C. distans* (TV = 3.98) that were less tolerant to a suite of anthropogenic impairments were once more widespread. Other crayfish, such as *O. cristavarius* (TV = 5.42) that flourished at test sites but were conspicuously absent from reference sites, may have expanded their ranges as humans colonized North America and impacted water quality. If our assumptions about these data are true, they might indicate a disturbing scenario where intolerant crayfish may be out-competed not only by introduced species but also by more tolerant native crayfish that occur naturally within the same basin. Without protection of our high quality streams, sensitive species with small ranges could conceivably be driven to extirpation by more tolerant crayfish. Our results suggest that it will be important to determine if currently threatened crayfish species exhibit the same intolerances to disturbance as some of the eastern Kentucky *Cambarus* species in this study.

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Appendix A
Site Locations For Reference and Test Sites

SiteID	Condition	Stream Name	RM	Order	Area (mi^2)	Basin	Ecoregion	County	Latitude	Longitude
02006027	TEST	HATCHELL BR	0.1	1	0.35	U. CUMBERLAND	SW	MCCREARY	36.86816	-84.3669
02006030	REF	JACKIE BR	0.1	2	1.14	U. CUMBERLAND	SW	WHITLEY	36.90527	-84.2791
02006031	REF	CANE CR	0.3	1	0.65	U. CUMBERLAND	SW	WHITLEY	36.76649	-84.30595
02008017	REF	ROCK CR1	0.2	1	0.82	U. CUMBERLAND	SW	MCCREARY	36.64218	-84.70962
02008018	REF	WATTS BR	0.1	2	2.2	U. CUMBERLAND	SW	MCCREARY	36.65685	-84.65647
02008019	REF	PUNCHEONCAMP BR	0.1	2	1.7	U. CUMBERLAND	SW	MCCREARY	36.65766	-84.64091
02008020	REF	ROCK CR2	0.1	2	0.63	U. CUMBERLAND	SW	MCCREARY	36.66325	-84.62916
02008021	REF	ROCK CR3	0.1	1	0.37	U. CUMBERLAND	SW	MCCREARY	36.66859	-84.62849
02008023	TEST	COFFEY BR	0.1	2	1.25	U. CUMBERLAND	SW	MCCREARY	36.69082	-84.51865
02014004	TEST	JENNEYS BR	0.3	1	0.66	U. CUMBERLAND	SW	MCCREARY	36.7366	-84.45815
02041003	REF	BROWNIES CR	14.1	2	2.3	U. CUMBERLAND	CA	HARLAN	36.6981	-83.44046
02041004	TEST	BROWNIES CR2	0.1	1	0.31	U. CUMBERLAND	CA	HARLAN	36.69928	-83.4399
02042002	TEST	EWING CR	0.2	2	3.06	U. CUMBERLAND	CA	HARLAN	36.8389	-83.37168
02046004	REF	PRESLEY HOUSE BR	0.2	2	0.9	U. CUMBERLAND	CA	LETCHER	37.06656	-82.7916
02046005	TEST	FRANKS CR	3.0	2	1.36	U. CUMBERLAND	CA	LETCHER	37.03002	-82.8015
04050007	TEST	FUGATE FORK	0.2	2	2.6	KENTUCKY	CA	BREATHITT	37.46033	-83.2353
04050008	TEST	JENNY FORK	0.1	1	0.45	KENTUCKY	CA	BREATHITT	37.45763	-83.19653
04050010	REF	CLEMONS FORK	3.9	2	0.8	KENTUCKY	CA	BREATHITT	37.48593	-83.13222
04050011	REF	FALLING ROCK BR	0.1	1	0.41	KENTUCKY	CA	BREATHITT	37.47624	-83.1388

04050012	REF	JOHN CARPENTER FORK	0.2	1	0.58	KENTUCKY	CA	BREATHITT	37.48239	-83.12843
04050013	REF	SHELLY ROCK FORK	0.1	1	0.55	KENTUCKY	CA	BREATHITT	37.48165	-83.15128
04050014	REF	MILLSEAT BR	0.7	2	0.58	KENTUCKY	CA	BREATHITT	37.48242	-83.15023
04050015	REF	LITTLE MILLSEAT BR	0.1	2	0.82	KENTUCKY	CA	BREATHITT	37.47224	-83.1466
04050017	TEST	WILLIAMS BR	0.6	2	1.08	KENTUCKY	CA	PERRY	37.39329	-83.15638
04050018	TEST	CANEY CR	0.75	2	2.5	KENTUCKY	CA	BREATHITT	37.44875	-83.2611
04052017	REF	LITTLE DOUBLE CR	0.7	2	1.5	KENTUCKY	CA	CLAY	37.1312	-83.5983
04052018	REF	RF BIG DOUBLE CR2	0.7	2	1.46	KENTUCKY	CA	CLAY	37.08907	-83.6184
04052019	REF	LF BIG DOUBLE CR	0.5	2	0.6	KENTUCKY	CA	CLAY	37.08321	-83.60373
04052020	REF	RF ELISHA CR	2.1	2	2.35	KENTUCKY	CA	LESLIE	37.07628	-83.51512
04052021	REF	BIG MF ELISHA CR	0.2	1	0.82	KENTUCKY	CA	CLAY	37.0815	-83.51472
04052022	REF	LF ELISHA CR	0.6	2	2.47	KENTUCKY	CA	LESLIE	37.09225	-83.52559
04052023	REF	RF BIG DOUBLE CR	0.2	2	1.53	KENTUCKY	CA	CLAY	37.09037	-83.60673
04052024	TEST	RED BIRD CR	86.05	2	1.4	KENTUCKY	CA	BELL	36.91241	-83.54094
04052025	TEST	MUD LICK BR	0.2	1	1.1	KENTUCKY	CA	BELL	36.91261	-83.53675
04052027	TEST	SPRUCE BR	0.1	2	0.95	KENTUCKY	CA	CLAY	36.95668	-83.53017
04052028	TEST	GILBERTS LITTLE CR	0.2	2	1.47	KENTUCKY	CA	CLAY	37.09083	-83.56353
04052029	TEST	ARNETTS FORK	0.9	2	1.42	KENTUCKY	CA	CLAY	37.11115	-83.59735
04052030	REF	SUGAR CR	2.1	2	3.05	KENTUCKY	CA	LESLIE	37.12376	-83.5243

Appendix B
Mean Physical and Chemical Variables From All Sites

StationID	StreamName	Condition	Date	Area (Mi^2)	DO	pH	Spec. Cond.	Temp	%Embed	RipWidth (m)	StrWidth (m)	Canopy Score	SubSize (cm)	Elevation (m)	Slope (m/km)
02006027	HATCHELL BRANCH	TEST	4/19/00	0.4	9.3	6.4	37.2	14	28	>100.0	3.59	20	16.5	280	42.7
02006030	JACKIE BRANCH	REF	4/20/00	1.1	9.7	6.6	22.1	11.3	2	>100.0	7.55	20	18.4	274	30.5
02006031	CANE CREEK	REF	4/25/00	0.7	8.5	6.2	19.5	12	15	>100.0	4.26	20	18.5	293	27.4
02008017	UT ROCK CREEK1	REF	4/17/00	0.8	9.5	6.3	38.6	11.9	13	>100.0	4.53	18	14.5	305	39.6
02008018	WATTS BRANCH	REF	4/17/00	2.2	9.1	6.2	27	12.4	17.3	57.5	4.57	20	15.4	280	30.5
02008019	PUNCHEONCAMP BRANCH	REF	4/18/00	1.7	10.7	6.7	26.2	10.4	13.3	>100.0	7.8	20	15.3	280	39.6
02008020	UT ROCK CREEK3	REF	4/18/00	0.6	10.6	6.7	38.9	10.5	22.8	>100.0	3.74	18	15.3	274	82.3
02008021	UT ROCK CREEK2	REF	4/18/00	0.4	10.1	6.9	30.9	10.5	6.5	>100.0	3.97	16	18.1	271	73.2
02008022	UT BS FK CUMBERLAND	REF	4/18/00	0.9	9.7	7	41.8	11	12.3	>100.0	4.98	20	15.9	232	54.9
02008023	COFFEY BRANCH	TEST	4/19/00	1.3	10.1	6.8	66.9	10.7	18	21.5	5.65	10	16.3	274	36.6
02014004	JENNEYS BRANCH	TEST	4/19/00	0.7	9.7	7.3	189	12.9	37.5	50	4.36	4	14	357	30.5
02041003	BROWNIES CREEK	REF	4/26/00	2.3	10.2	6.8	60.2	10.2	5.5	85	6.66	20	17.6	494	22.3
02041004	BROWNIES CREEK2	TEST	4/26/00	0.3	8.7	6.7	95	13.1	9.3	>100.0	3.56	16	16	503	64.1
02042002	EWING CREEK	TEST	4/26/00	3.1	8	7.4	485	15.5	16.5	59	10.9	4	16.2	354	25.3
02046004	PRESLEY HOUSE BRANCH	REF	4/27/00	0.9	10.2	6.1	17.1	8.6	2.5	>100.0	4.82	20	18	543	85.3
02046005	FRANKS CREEK	TEST	4/27/00	1.4	9.1	7.1	324	11.7	15.8	2.6	5.83	16	15.3	588	73.1
04050007	FUGATE FORK	TEST	4/10/00	2.6	10.9	8.1	610	14.9	22.8	1.6	5.38	8	19.9	244	18.3
04050008	JENNY FORK	TEST	4/10/00	0.5	11.3	7.6	635	13.3	19.5	>100.0	5.13	16	14.6	268	36.6
04050009	BEAR BRANCH	TEST	4/10/00	1.5	11.6	8	431	16.2	20	30.501	3.1	4	18.7	268	27.4
04050010	CLEMONS FORK	REF	4/10/00	0.8	13	6.8	83.4	12.7	7.25	>100.0	6.5	20	15.8	317	18.3
04050011	FALLING ROCK BRANCH	REF	4/11/00	0.4	13.3	6.7	41.4	8.9	13.3	>100.0	4.46	20	19.3	293	45.7
04050012	JOHN CARPENTER FORK	REF	4/11/00	0.6	12.9	6.8	38.8	9.1	8.5	>100.0	4.59	20	15.8	317	24.4

04050013	SHELLY ROCK FORK	REF	4/11/00	0.6	12.2	7.1	39.4	10.1	12.3	>100.0	4.62	20	15.1	305	36.6
04050014	MILLSEAT BRANCH	REF	4/11/00	0.6	12.7	7.4	130	10.9	9.5	>100.0	4.36	20	15.9	305	24.4
04050015	LITTLE MILLSEAT BRANCH	REF	4/12/00	0.8	10.9	7.1	40.2	10.4	11.8	>100.0	4.05	20	14.7	280	24.4
04050017	WILLIAMS BRANCH	TEST	4/12/00	1.1	15.7	8.4	1228	10.5	23.8	30.5	3.05	10	14.6	268	18.3
04050018	CANEY CREEK	TEST	4/12/00	2.5	13.5	8.1	153	11.6	28.3	40.5	4.01	6	16.1	244	12.2
04052017	LITTLE DOUBLE CREEK	REF	3/29/00	1.5	11.3	6.9	60	9.1	13.4	67	5.15	14	15.8	280	30.5
04052018	RIGHT FORK BIG DOUBLE CREEK2	REF	3/29/00	1.5	11	6.4	38.3	9.4	7.8	73	5.96	8	16.5	329	36.6
04052019	LEFT FORK BIG DOUBLE CREEK	REF	3/29/00	0.6	11.2	6.4	48.4	10	18.6	>100.0	3.68	14	16.3	329	30.5
04052020	RIGHT FORK ELISHA CREEK	REF	3/30/00	2.4	11.5	6.8	49.2	11	13.1	>100.0	6.66	16	16.6	317	18.3
04052021	BIG MIDDLE FORK ELISHA CREEK	REF	3/30/00	0.8	10.3	6.5	54.3	13.1	14.1	>100.0	5.71	14	15.1	317	36.6
04052022	LEFT FORK ELISHA CREEK	REF	3/30/00	2.5	9.8	6.4	45	15.2	10.6	58.8	6.1	20	15.7	329	18.3
04052023	RIGHT FORK BIG DOUBLE CREEK	REF	4/ 5/00	1.5	12.4	6.4	35	8.5	20.3	69.5	5.99	18	18.1	317	24.4
04052024	RED BIRD CREEK	TEST	4/ 5/00	1.4	13.7	6.9	505	11.9	25.3	0.4	4.41	6	17.6	421	30.5
04052025	MUD LICK BRANCH	TEST	4/ 5/00	1.1	11.1	6.5	157	12.1	19.4	53.9	4.48	10	16	415	30.5
04052026	LAWSON CREEK	TEST	4/ 5/00	1.5	10.6	7	436	12.9	18.1	15.5	4.73	4	16.9	427	30.5
04052027	SPRUCE BRANCH	TEST	9/ 6/00	1	12.3	7.3	161	9.5	21.3	77	4.71	14	18.3	363	67.1
04052028	GILBERTS LITTLE CREEK	TEST	4/ 6/00	1.5	11.4	7	63	11.3	24.1	52.2	4.49	4	17.7	280	36.6
04052029	ARNETTS FORK	TEST	4/ 6/00	1.4	10.6	6.7	56	13.9	16.3	52.503	4.98	8	18.3	293	21.3
04052030	SUGAR CREEK	REF	4/ 6/00	3.1	10.9	6	26.3	12.5	13.1	>100.0	6.33	16	19.8	317	21.3

Appendix C
RBP Habitat Assessment Scores From All Sites

StationID	StreamName	Condition	Date	Total HabScore	BankStab	Bank VegP	Cha FlowS	Chan Alter	Embed-dedness	EpiFau Sub	FreqOf Riffles	RipVegZ W	SedDep	Vel/Dep Regime
02006027	HATCHELL BRANCH	TEST	4/19/00	154	16	18	15	18	11	16	18	20	7	15
02006030	JACKIE BRANCH	REF	4/20/00	179	18	20	15	20	19	18	17	20	17	15
02006031	CANE CREEK	REF	4/25/00	163	17	19	15	19	14	16	15	20	13	15
02008017	UT ROCK CREEK1	REF	4/17/00	176	16	18	15	20	17	19	18	20	14	19
02008018	WATTS BRANCH	REF	4/17/00	174	18	18	15	17	16	18	18	18	18	18
02008019	PUNCHEONCAMP BR	REF	4/17/00	186	18	18	15	18	19	20	19	20	19	20
02008020	UT ROCK CREEK3	REF	4/18/00	176	18	18	15	19	16	19	19	20	17	15
02008021	UT ROCK CREEK2	REF	4/18/00	170	16	18	15	19	14	18	18	20	17	15
02008023	COFFEY BRANCH	TEST	4/19/00	146	14	10	15	16	13	16	17	12	15	18
02014004	UT JENNEYS BRANCH	TEST	4/19/00	110	15	11	15	11	8	10	11	9	9	11
02041003	BROWNIES CREEK	REF	4/26/00	176	16	16	15	18	18	19	19	18	18	19
02041004	BROWNIES CREEK2	TEST	4/26/00	166	13	16	15	17	18	19	19	20	14	15
02042002	EWING CREEK	TEST	4/26/00	107	4	4	15	14	14	7	15	13	5	16
02046004	PRESLEY HOUSE BR	REF	4/27/00	187	20	20	15	20	19	18	17	20	19	19
02046005	FRANKS CREEK	TEST	4/27/00	140	14	14	15	10	17	18	18	2	14	18
04050007	FUGATE FORK	TEST	4/10/00	136	15	16	15	14	13	16	19	2	10	16
4050008	JENNY FORK	TEST	4/10/00	138	14	15	15	15	12	12	16	18	6	15

4050010	CLEMONS FORK	REF	4/10/00	180	18	18	15	20	19	19	19	20	16	16
4050011	FALLING ROCK BR	REF	4/11/00	160	12	14	15	19	18	18	18	20	11	15
4050012	JOHN CARPENTER FORK	REF	4/11/00	174	16	17	15	18	17	19	19	20	15	18
4050013	SHELLY ROCK FORK	REF	4/11/00	171	18	18	15	19	17	16	17	20	15	16
4050014	MILLSEAT BRANCH	REF	4/11/00	175	16	18	15	19	17	19	19	20	15	17
4050015	LITTLE MILLSEAT BR	REF	4/12/00	169	15	16	15	17	17	18	18	20	15	18
4050017	WILLIAMS BRANCH	TEST	4/12/00	128	14	12	15	15	10	14	18	8	11	11
4050018	CANEY CREEK	TEST	4/12/00	144	16	15	15	14	13	15	16	10	15	15
4052017	LITTLE DOUBLE CREEK	REF	3/29/00	173	16	14	15	20	18	19	20	19	17	15
4052018	RT FK BIG DOUBLE CR 2	REF	3/29/00	172	18	18	15	20	15	18	19	19	15	15
4052019	LT FK BIG DOUBLE CREEK	REF	3/29/00	162	14	14	15	18	14	18	20	20	14	15
4052020	RIGHT FORK ELISHA CR	REF	3/30/00	174	18	18	15	16	15	19	19	20	15	19
4052021	BIG MIDDLE FK ELISHA CR	REF	3/30/00	161	14	16	15	17	16	17	19	20	12	15
4052022	LEFT FORK ELISHA CREEK	REF	3/30/00	171	16	16	15	18	16	19	19	18	16	18
4052023	RT FK BIG DOUBLE CREEK	REF	4/ 5/00	147	5	6	15	15	15	19	20	20	14	18
4052024	RED BIRD CREEK	TEST	4/ 5/00	133	14	14	15	13	13	13	17	1	14	19
4052025	MUD LICK BRANCH	TEST	4/ 5/00	144	18	16	15	11	13	16	16	13	10	16
4052027	SPRUCE BRANCH	TEST	4/ 6/00	150	12	14	15	16	11	17	19	20	7	19
4052028	GILBERTS LITTLE CREEK	TEST	4/ 6/00	132	16	17	15	14	13	11	16	8	11	11
4052029	ARNETTS FORK	TEST	4/ 6/00	154	17	15	15	15	16	17	18	10	15	16
4052030	SUGAR CREEK	REF	4/ 6/00	181	18	18	15	19	17	19	19	20	17	19

Appendix D
Metric Values For All Sites

StationID	Program	Stream Name	CollDate	Area (Mi^2)	MBI	G-TR	G-EPT	mHBI	m%EPT	%Ephem	%Chir+Olig	%Clng
02006027	TEST	HATCHELL BRANCH	4/19/00	0.35	57.48	29	18	3.69	46.25	30.63	0.30	16.52
02006030	REF	JACKIE BRANCH	4/20/00	1.14	82.24	53	25	2.94	62.53	43.40	4.85	69.81
02006031	REF	CANE CREEK	4/24/00	0.65	79.56	52	26	2.66	77.95	32.29	3.56	50.11
02008017	REF	UT ROCK CREEK 1	4/12/00	0.82	85.51	57	30	3.25	62.02	40.867	2.56	75.48
02008018	REF	WATTS BRANCH	4/17/00	2.2	90.23	46	25	3.14	84.97	66.67	1.77	74.32
02008019	REF	PUNCHEONCAMP BR	4/18/00	1.7	92.40	55	30	2.89	82.29	70.19	2.68	64.20
02008020	REF	UT ROCK CREEK 2	4/18/00	0.63	89.21	56	26	2.68	74.92	52.10	1.95	76.88
02008021	REF	UT ROCK CREEK 3	4/18/00	0.37	85.88	39	19	2.49	81.82	70.74	0.85	69.03
02008023	TEST	COFFEY BRANCH	4/19/00	1.25	75.50	41	21	3.24	78.65	48.54	11.24	45.62
02014004	TEST	JENNEYS BRANCH	4/19/00	0.66	48.15	37	13	5.71	27.74	6.19	28.54	53.49
02041003	REF	BROWNIES CREEK	4/26/00	2.3	70.92	52	31	2.93	50.10	18.38	2.22	34.55
02041004	TEST	BROWNIES CREEK2	4/26/00	0.31	61.45	39	24	2.53	36.32	18.25	0.71	23.21
02042002	TEST	EWING CREEK	4/26/00	3.06	42.66	25	11	4.88	32.20	20.34	33.90	18.64
02046004	REF	PRESLEY HOUSE BR	4/27/00	0.9	73.71	46	24	2.64	72.14	26.01	2.79	42.41
02046005	TEST	FRANKS CREEK	4/27/00	1.36	81.07	42	25	3.41	80.24	56.99	5.32	50.91
04050007	TEST	FUGATE FORK	4/10/00	2.6	55.71	43	13	3.87	45.65	1.85	16.89	49.08
04050008	TEST	JENNY FORK	4/10/00	0.45	65.79	42	19	3.05	84.05	2.37	9.70	42.46
04050010	REF	CLEMONS FORK	4/10/00	0.8	90.33	59	30	2.55	74.12	51.97	2.69	68.74
04050011	REF	FALLING ROCK BR	4/11/00	0.41	88.85	57	32	2.79	71.69	46.86	2.37	68.76

04050012	REF	JOHN CARPENTER FK	4/12/00	0.58	76.71	40	22	2.98	59.94	42.98	0.88	63.16
04050013	REF	SHELLY ROCK FORK	4/11/00	0.55	85.58	38	20	2.41	78.84	62.09	0.70	73.26
04050014	REF	MILLSEAT BRANCH	4/11/00	0.58	82.02	53	31	2.45	75.42	24.92	7.41	61.95
04050015	REF	LITTLE MILLSEAT BR	4/12/00	0.82	86.84	44	28	2.61	79.69	57.59	0.45	60.71
04050017	TEST	WILLIAMS BRANCH	4/12/00	1.08	21.65	25	5	5.82	1.74	0.00	75.96	12.89
04050018	TEST	CANEY CREEK	4/12/00	2.5	37.02	36	10	5.42	9.62	5.13	44.23	28.85
04052017	REF	LITTLE DOUBLE CR	3/29/00	1.5	80.42	27	19	2.16	94.26	64.09	0.00	49.93
04052018	REF	RF BIG DOUBLE CR 2	3/29/00	1.46	81.07	46	22	2.39	68.77	46.53	3.00	63.25
04052019	REF	LF BIG DOUBLE CR	3/29/00	0.6	87.42	52	25	2.55	74.42	54.09	1.53	69.69
04052020	REF	RF ELISHA CREEK	3/30/00	2.35	83.11	48	31	2.63	72.03	47.97	4.50	50.00
04052021	REF	BM FORK ELISHA CR	3/30/00	0.82	83.18	57	28	2.82	74.35	55.90	5.54	38.01
04052022	REF	LF ELISHA CREEK	3/30/00	2.47	85.67	42	25	2.52	81.80	69.32	0.52	50.95
04052023	REF	RF BIG DOUBLE CR	4/ 5/00	1.53	84.59	40	22	2.45	82.27	59.31	4.71	64.67
04052024	TEST	RED BIRD CREEK	4/ 5/00	1.4	49.67	28	13	4.66	42.14	13.21	10.06	27.67
04052025	TEST	MUD LICK BRANCH	4/ 5/00	1.1	85.22	42	24	2.49	75.52	60.00	0.90	63.58
04052027	TEST	SPRUCE BRANCH	4/ 6/00	0.95	91.75	43	26	2.39	88.17	76.10	1.16	74.26
04052028	TEST	GILBERTS LITTLE CR	4/ 6/00	1.47	33.64	32	11	5.33	5.94	2.74	28.31	6.39
04052029	TEST	ARNETTS FORK	4/ 6/00	1.42	74.55	27	20	2.09	97.06	51.47	0.00	30.14
04052030	REF	SUGAR CREEK	4/ 6/00	3.05	88.51	54	29	2.79	73.04	52.07	2.30	70.28

Appendix E
Station Condition and Taxa

StationID	Condition	StreamName	FinalID	Individuals	CollDate
04050011	REF	FALLING ROCK BRANCH	Cambarus bartonii cavatus	2	04/11/00
04052023	REF	RIGHT FORK BIG DOUBLE CREEK	Cambarus bartonii cavatus	3	04/05/00
04052018	REF	RIGHT FORK BIG DOUBLE CREEK2	Cambarus bartonii cavatus	5	03/29/00
04052017	REF	LITTLE DOUBLE CREEK	Cambarus bartonii cavatus	16	03/29/00
04052019	REF	LEFT FORK BIG DOUBLE CREEK	Cambarus bartonii cavatus	7	03/29/00
04052022	REF	LEFT FORK ELISHA CREEK	Cambarus bartonii cavatus	2	03/30/00
04050018	TEST	CANEY CREEK	Cambarus bartonii cavatus	1	04/12/00
04052025	TEST	MUD LICK BRANCH	Cambarus bartonii cavatus	4	04/05/00
04050012	REF	JOHN CARPENTER FORK	Cambarus bartonii cavatus	5	04/12/00
04052021	REF	BIG MIDDLE FORK ELISHA CREEK	Cambarus bartonii cavatus	1	03/30/00
04052029	TEST	ARNETTS FORK	Cambarus bartonii cavatus	3	04/06/00
02042002	TEST	EWING CREEK	Cambarus buntingi	2	04/26/00
02041003	REF	BROWNIES CREEK1	Cambarus buntingi	2	04/26/00
02006030	REF	JACKIE BRANCH	Cambarus cumberlandensis	2	04/20/00
02006027	TEST	HATCHELL BRANCH	Cambarus cumberlandensis	1	04/19/00
02046005	TEST	FRANKS CREEK	Cambarus distans	7	04/27/00
02006027	TEST	HATCHELL BRANCH	Cambarus distans	1	04/19/00
04050013	REF	SHELLY ROCK FORK	Cambarus distans	2	04/11/00
04050014	REF	MILLSEAT BRANCH	Cambarus distans	1	04/11/00
02046004	REF	PRESLEY HOUSE BRANCH	Cambarus distans	3	04/27/00
02008021	REF	ROCK CREEK2	Cambarus distans	1	04/18/00
02006030	REF	JACKIE BRANCH	Cambarus distans	1	04/20/00
02006031	REF	CANE CREEK	Cambarus distans	2	04/24/00
02008017	REF	ROCK CREEK1	Cambarus distans	9	04/12/00
02008018	REF	WATTS BRANCH	Cambarus distans	6	04/17/00
02008019	REF	PUNCHEONCAMP BRANCH	Cambarus distans	10	04/18/00
02041004	TEST	BROWNIES CREEK	Cambarus distans	18	04/26/00
02008020	REF	ROCK CREEK3	Cambarus distans	2	04/18/00
02008023	TEST	COFFEY BRANCH	Cambarus distans	3	04/19/00
02041003	REF	BROWNIES CREEK1	Cambarus distans	4	04/26/00
04050013	REF	SHELLY ROCK FORK	Cambarus distans	1	04/11/00
02042002	TEST	EWING CREEK	Cambarus distans	1	04/26/00
04052027	TEST	SPRUCE BRANCH	Cambarus distans	1	04/06/00
04052024	TEST	RED BIRD CREEK	Cambarus distans	1	04/05/00
04052028	TEST	GILBERTS LITTLE CREEK	Cambarus distans	1	04/06/00
02041004	TEST	BROWNIES CREEK2	Cambarus parvoculus	5	04/26/00
02041003	REF	BROWNIES CREEK1	Cambarus parvoculus	2	04/26/00
04052023	REF	RIGHT FORK BIG DOUBLE CREEK	Cambarus parvoculus	1	04/05/00
04052021	REF	BIG MIDDLE FORK ELISHA CREEK	Cambarus parvoculus	2	03/30/00
02041004	TEST	BROWNIES CREEK	Cambarus parvoculus	5	04/26/00
04052027	TEST	SPRUCE BRANCH	Cambarus parvoculus	1	04/06/00
04050014	REF	MILLSEAT BRANCH	Cambarus parvoculus	1	04/11/00
04052028	TEST	GILBERTS LITTLE CREEK	Cambarus parvoculus	2	04/06/00

04052023	REF	RIGHT FORK BIG DOUBLE CREEK	Cambarus robustus	1	04/05/00
04052019	REF	LEFT FORK BIG DOUBLE CREEK	Cambarus robustus	1	03/29/00
04052018	REF	RIGHT FORK BIG DOUBLE CREEK2	Cambarus robustus	1	03/29/00
04052029	TEST	ARNETTS FORK	Cambarus robustus	4	04/06/00
04052021	REF	BIG MIDDLE FORK ELISHA CREEK	Cambarus robustus	2	03/30/00
04050018	TEST	CANEY CREEK	Cambarus robustus	2	04/12/00
04050008	TEST	JENNY FORK	Cambarus robustus	1	04/10/00
04050015	REF	LITTLE MILLSEAT BRANCH	Cambarus robustus	10	04/12/00
04052030	REF	SUGAR CREEK	Cambarus robustus	7	04/06/00
04052025	TEST	MUD LICK BRANCH	Cambarus robustus	1	04/05/00
04052028	TEST	GILBERTS LITTLE CREEK	Cambarus robustus	1	04/06/00
04050010	REF	CLEMONS FORK3	Cambarus robustus	1	04/10/00
04050012	REF	JOHN CARPENTER FORK	Cambarus robustus	6	04/12/00
04050011	REF	FALLING ROCK BRANCH	Cambarus robustus	1	04/11/00
04050017	TEST	WILLIAMS BRANCH	Cambarus robustus	1	04/12/00
02008023	TEST	COFFEY BRANCH	Cambarus rusticiformis	6	04/19/00
02008021	REF	ROCK CREEK2	Cambarus rusticiformis	1	04/18/00
02008020	REF	ROCK CREEK3	Cambarus rusticiformis	2	04/18/00
02008020	REF	ROCK CREEK3	Cambarus sp	3	04/18/00
04052020	REF	RIGHT FORK ELISHA CREEK	Cambarus sp	3	03/30/00
04050018	TEST	CANEY CREEK	Cambarus sp	2	04/12/00
04050007	TEST	FUGATE FORK	Orconectes cristavarius	1	04/10/00
04052028	TEST	GILBERTS LITTLE CREEK	Orconectes cristavarius	6	04/06/00
04050018	TEST	CANEY CREEK	Orconectes cristavarius	18	04/12/00
04052029	TEST	ARNETTS FORK	Orconectes cristavarius	1	04/06/00
04052024	TEST	RED BIRD CREEK	Orconectes cristavarius	5	04/05/00
02014004	TEST	JENNEYS BRANCH	Orconectes cristavarius	6	04/19/00