

Modified Index of Biological Integrity
Protocol for Subterranean Streams
in and around Mammoth Cave National Park

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Section 1.0 Introduction

Section 1.1 Mammoth Cave National Park

Mammoth Cave National Park is an international treasure, as recognized by its designation as an International Biosphere Reserve in 1990. The cave is the longest in the world, has outstanding geological, anthropological and hydrological components, and provides niches for a unique community of troglobitic organisms. Some of these organisms are known only from this cave system or a few adjacent systems, and one is federally-listed as an endangered species. The preparation for a biomonitoring program for the subterranean streams in and around Mammoth Cave National Park began in the early 1990s. Dr. Thomas L. Poulson developed a survey methodology which is extremely useful for both spatial and temporal comparisons of community level characteristics. His work provided the framework for developing indices as monitoring tools in subterranean waters (Poulson 1990, 1992a, 1992b).

Section 1.2 Biological Monitoring

The idea of biological indicators of pollution resulted in the use of the saprobitic index (the degree of pollution) in Europe at the turn of the century (Rosenberg and Resh, 1993). This technique uses the presence (and, in later forms, the proportion) of insect species with known ecological tolerances. This required a great deal of taxonomy and toxicology to be done at the species level. North American monitoring focused on the known ecological limits for a few representative species from different taxa (vertebrate and invertebrate). The precision and ease of water chemistry measurements increased until they became dominant in water quality monitoring

programs. By the 1970s, equilibrium theory and new views on biological diversity generated new interest in the use of biomonitors (Parrish and Wagner, 1983 and Hilsenhoff, 1988). Species diversity indices were incorporated into policy making programs (Alabama Department of Environmental Management, 1990). Diversity is a community level characteristic and is therefore not responsive to population and individual variations. Tolerances of individuals, structures of populations, and community characteristics were incorporated into a new set of benthic invertebrate indices (Nuzzo, 1986; Lenat, 1988; Plafkin et al, 1989). In 1981, Karr used data from fish communities to develop the index of biological integrity. This index included information from individuals, populations, and the community (fish only) into a single value.

Section 1.3 Index of Biological Integrity

The first index of biological integrity was published by James Karr in 1981. This biomonitoring protocol used data gained by electro-fishing to rank streams according to the degree of human impact. The variables (metrics) were stratified to incorporate data on individuals, populations, and the fish community. Particular variables were chosen for their correlation to estimated levels of impact. Hundreds of electro-fishing sites were included in the original database (Karr, 1993). The variance on individual metrics were standardized to a score of one, three, or five. The highest possible overall score was recorded for sites with a five for each metric score. IBI total scores were then ranked from least-impacted (higher scores) to most impacted (lower scores). The IBI was fast, easily calculated, easily interpreted, and powerful (Hughes and Noss, 1992). Modifications to this protocol include regional adjustments (Miller *et al*, 1988; Steedman, 1988; Oberdorff, T and R. M. Huges, 1992; Simon, 1992; Minns *et al*, 1994 Lyons *et al*, 1995; Paller *et al*, 1996) to monitor other fish communities and utilizing other

aquatic communities (Ohio EPA, 1988; Kerans and Karr, 1994). A statistical validation for the IBI was reported in 1994 (Fore, L. S. *et al*, 1994). Thomas Poulson began to develop the idea of creating a modified IBI for the subterranean streams of Mammoth Cave in the early 1990s (Poulson, 1990; Poulson, 1992a; and Poulson 1992b). He used his community data from the 1960s and 1970s to demonstrate the usefulness of biomonitoring. The development of the modified IBI began in 1993 (Pearson, W. D. and T. G. Jones, 1994 & 1995; Jones *et al*, 1995). This protocol is a direct result of that work.

Section 2.0 Methods and Materials

Section 2.1 Measurements Taken

The measurements taken to complete a stream survey can be stratified according to the frequency of data collection. General habitat data is collected just once for each stream segment. Water quality samples should be collected once during each survey. Specific measurements on individual cavefish and cave crayfish are collected intermittently along the survey route. Rock count data (small invertebrate densities and size estimates) are also collected intermittently throughout the survey. Data on large-organism species composition and size estimates are made continuously along the entire survey segment.

Stream geomorphology and substrate characteristics should be collected during a pre-survey route-finding trip. This type of data can be collected on the initial survey of a stream segment, but this lengthens the trip time considerably, and could result in fatigue-induced errors

and the compromising of safety. A survey starting point (i.e. a known tributary, a unique formation, etc.) is chosen and should be tied to an existing map or a set of cave-survey records. Characterizations of stream width, depth, and substrate composition should be noted over short distances (100 meters or less). The total length of the survey segment should be estimated by pacing, use of existing maps, or direct measurement. Significant features (tributaries, large pools, waterfalls, and formations) should be noted along the survey route. This location data is not currently included in the IBI calculations, but it can be very important in interpreting the results. Low-flow estimations should be made along the stream segment for comparison. Surveys made during high-flow conditions are subject to extreme variation and should be avoided. As a practical consideration, if the observers cannot see the bottom of the pooled sections of the stream (unless the depth exceeds 1-1.5 m) a useful survey cannot be made.

The amount of coarse particulate matter (CPOM, a general measure of potential food resources) in the substrate of a stream segment is estimated by taking grab samples of substrates containing visible organic deposits at depositional areas along the stream. At least three samples should be collected. A sample is collected by filling a small (1 liter) plastic bag full of sediment. The collections should be evenly distributed along the stream segment. Some high-gradient streams have limited depositional areas, in which case samples should be taken from pool bottoms. The samples are stored in a cooler (short-term) until frozen (long-term storage). Analysis of the samples will be covered in section 4.1. Subsequent CPOM samples only need to be collected if the stream segment geomorphology is significantly altered. Natural phenomena (e.g. major flooding events) and/or human disturbances (e.g. increased siltation due to surface construction) can alter stream geomorphology. New CPOM data may be needed only every few

years in stable, highly resource limited (little visible CPOM) stream segments, while more variable stream segments (e.g. transition habitats such as Mystic River) may require annual reassessments of CPOM.

At the beginning of every survey, observations of flow and water clarity should be made to determine if the low-flow, clear conditions necessary for gathering useful, comparable data are present. Surveys made during high or rising water levels are not comparable to those made during stable low-flows. Flow is measured using a fishing bobber on one meter of monofilament fishing line. The end of the line is held just above the water, and the float is moved upstream until the line tightens. It is released and timed until it stops after traveling two meters. The cross-sectional area of the stream is measured at the point of flow measurement. Water quality samples (at least three) are collected along the stream segment above areas disturbed by the investigators. The collection locations should be near prominent, identifiable features such as tributaries. If such features do not exist, then the samples are taken at equal intervals along the stream segment. Samples are collected in sterile, plastic bottles. The sample should be at least 500 ml. The collected water samples are stored in a cooler outside of the cave. Simple wet chemistry (alkalinity, turbidity, sulfates, and total hardness) should be completed within 24 hours by EPA approved techniques. Total phosphorus, total nitrogen, and chlorides can be measured from samples that have been stored frozen. Temperature, conductivity, and pH can be measured with "pocket instruments" during the survey (see Appendix 1 for sources).

Specific biological data is collected on troglobitic fish and crayfish. Every third or fourth

cavefish is captured using a 10 cm x 20 cm (mesh= 3mm) aquarium net. No more than thirty cavefish should be sampled. Total length (the distance from the most anterior point of the jaws to the most posterior point of the compressed caudal fin) and standard length (the distance from the most anterior point of the jaws to the end of the vertebral column) are both measured using a 15 cm ruler. The ruler is mounted on an aluminum measuring board. The cavefish should be handled gently in the soft, fine-meshed net to minimize the disruption of the mucus coat. A small glass or nalgene volumetric cylinder is filled with an initial amount of stream water. This amount should be large enough to completely cover the fish when submerged. The initial and ending volumes are recorded. While still in the cylinder, the fish is examined with the halogen dive light for damage, the presence of food in the gut, fat body conditions for *Typhlichthys subterraneus*, and signs of reproduction. These signs can be the presence of maturing eggs in the body cavity, eggs held in the gill chamber, or fry in the gill chamber. The fish is then released at its capture location.

Troglobitic crayfish are also captured with the aquarium net. At least twenty crayfish should be sampled, including individuals from all size classes. Typically, at the densities encountered, this will mean that every 4th or 5th individual encountered will be captured. Crayfish are removed from the net by gripping the crayfish at the middle of its carapace with the thumb and forefinger. Carapace length (the distance from the tip of the rostrum to the posterior edge of the carapace) is measured using the 15 cm ruler. The captured crayfish is examined for damage, food in the gut, and the presence of eggs in the body cavity, or glued to the swimmerets (biramous appendages on the venter of the tail segments). The sex and reproductive form of male crayfish are observed and recorded. Male crayfish have the first and second (counting from the most anterior pair) pairs of swimmerets (on the venter of their tail) modified for sperm transfer. Both

pairs are elongated and lay in an anteriorly-directed groove between the bases of the walking legs. Female crayfish have unmodified swimmerets and an oval-shaped plate (annulus ventralis) between the bases of the fourth and fifth pairs of walking legs. Males are divided into "first form" (reproductively-active) and "second form" (not reproductively active) life stages. The modified swimmerets (hemi-penes) of a first form male are highly sclerotized and angular. The hemi-penes of second form males are soft and non-angular (lobes instead of angles). The ischiatic hooks (located on the ventral base of the second pair of walking legs) are also enlarged and angular on first form males. Any other notable characteristics of the captured troglobitic fish and crayfish should be noted (e.g. unusual coloration, body conformation, tumors, parasites, diseases, etc.).

The last data set gathered during a survey consists of species composition and size estimates. The observer walks/crawls/swims along the stream segment and identifies each macro-organism seen and estimates its size. The size estimates are of total body length for all organisms except crayfish (carapace length). The actual measurements made on large organisms improve the accuracy of the observers estimates of size for organisms not captured. During the initial survey of an area or when the observers are fatigued, the number of actual measurements on captured organisms should be increased to maintain accuracy.

Population data for smaller invertebrates is obtained by making counts on selected rocks taken from the stream bed. Rock counts are made both during and after the walking survey. An aluminum ring which encloses 0.033 m² is laid on the substrate, and any small rocks inside the circle are slowly lifted and searched for invertebrates. The length, width, and depth of each rock is estimated in centimeters and recorded. Six such quadrats are searched at each survey segment. Family, generic, and specific characters are impossible to distinguish in the field, so small

invertebrates are only identified to order. However, the limited distribution of many species and the low diversity within most orders may allow us to infer family, genus, or even species at some locations. Each organism is counted and total body lengths are estimated. Estimations are checked by periodic actual measurements made with a 15-cm scale. After all small rocks in the quadrat have been examined, that part of any large rocks inside the circle are examined. The substrate itself is then scanned for invertebrates. The quadrat can be used to obtain density data in isolated pools where invertebrates are concentrated. The six quadrat samples are supplemented with counts of invertebrates on individual rocks. This practice allows large rocks to be sampled and allows for a more uniform coverage of substrate types. At least twenty individual rocks should be sampled during every survey.

Section 2.2 Walking Surveys

A walking survey team has two to five members. The recorder enters survey information in a water-proof notebook as it is called out by the observer or observers. The recorder also makes a running notation of time and location as each survey proceeds. One observer is utilized if the stream is less than 1.5 meters wide. A second observer is added if the stream segment is wider. The rest of the survey team serve as guides, safety standby personnel, gear haulers, apprenticing surveyors, and bucket carriers. Buckets are used when discrete sections (i.e. pools) must be surveyed to their ends before stopping to make species specific (for troglobitic fish and crayfish only) measurements. Any organism captured should be returned to their approximate original location.

The observers primary responsibility is to gather data on the community composition for the given stream segment. All observers must be able to identify all of the common taxa present in the subterrean, aquatic environments of Mammoth Cave National Park. Each observer must have a halogen dive light, measuring board, and at a set of volumetric cylinders (see Appendix 1 for gear check sheets).

As the survey begins, the observers, working upstream (when possible) scan the stream with dive lights. The size of each organism seen is estimated and this value is called back to the recorder. Organisms are identified and recorded by the first letter of their genus and species to speed the recording process. For example, "CT 36" is a *Cambarus tenebrosus* 36 mm in carapace length. For detailed information on the various length measurements see section 2.1.

The recorder trails the observers 5 to 15 meters. The distance increases in deeper, still waters, particularly those confined by vertical walls. The reason for this is that waves created by the recorder will obscure the vision of the observers and can contribute to the disturbance of bottom sediments, making a survey less accurate. The observers should always regulate the distance between themselves and the rest of the party to maintain optimum viewing conditions.

When two observers are used, they each survey half of the stream as they walk side by side. Organisms near the middle of the stream should be signaled as counted by one of the two observers. For example, one observer will call out while pointing to the specimen, "I have this Ts." This will prevent missing or duplicating an organism. Size estimates should be checked periodically by capturing organisms and measuring them. As the observers begin to tire, they should check their size estimates more often. During very long surveys, the observers should stop to eat, drink, and rest in a dry area.

All data is recorded with pencil in a waterproof notebook. The data call-out rate is regulated by the recorder to prevent inaccuracies or loss of information. This is most important when using two observers. Notes on the flow rates, water clarity, and water level are important in the interpretation of IBI values. The location of the organisms along a survey route is noted by the running time log and significant landmarks (i.e. 3:45pm, now passing the first right-hand tributary). Any unusual conditions are noted (ie. oily sheen on water at base of waterfall). When the data call out rate is slow (as when the observer is moving slowly through a deep pool), the recorder or other party members can carry out rock counts to supplement the quadrat surveys made by the observers. The recorder is the head of the survey team, and is responsible for its quality, preventing duplications, assessing accuracy, completeness, and maintaining the running time and location logs.

The rest of the survey team is made up of people helping and/or learning. The team should not exceed five individuals and in most locations four is the best number. The cave, the streams, and the organisms can all be impacted by human traffic, and the ambient noise level increase and wave generation will make the surveying more difficult. All monitoring teams must have at least one observer who has monitored the stream segment prior to the actual survey. The quality of data generated will drop significantly if the observer is not trained and has not practiced the survey technique. The accuracy and consistency of size estimation should be practiced. We have experimented with this by using lengths of white, insulated wire to simulate isopods of 1-12 mm in length and dowels of 20-120 mm for fish models.

A variation of the walking survey is employed when the average water depth exceeds one meter. Wetsuits must be used to prevent hypothermia and provide the personal comfort necessary

for reliable data collection. Each member of the team is responsible for every other member. Everyone must periodically speak to and observe the other members of the team. Each member must be instructed to volunteer honest assessments of injuries, fatigue, and chilling. Exhaustion and hypothermia can occur slowly and are not always noticed by the victim. Glazed eyes, inability to concentrate, verbal concerns about being cold, shivering, reduced coordination, and slack faces are warning signs of danger. Energy and temperature can be increased by resting in a dry area, eating, and drinking. If unsafe injuries, chilling, or exhaustion appear imminent, the survey should cease and all team members should leave the cave together. Naturally, all other standard caving safety rules regarding equipment, surface watches, vertical caving techniques, etc., must be observed.

Section 2.3 Snorkel Surveys

Snorkel surveys are preformed in base-level streams in Mammoth Cave. Two observers are always used since these stream segments are wide as well as deep. The observers are in full wetsuits (6 mm thick) with gloves, booties, and hoods. A full gear list can be found in Appendix 1. The observers swim along even with each other. Each observer surveys half of the stream width. The walls of the stream should be within each surveyor's vision. Many organisms concentrate along irregularities on the wall so more attention is paid to the sides of the passage than the open center water. Organisms along the boundary are pointed to as they are counted. A short distance is observed, then the surveyor rights himself or herself in the water and calls out the data to the recorder. The recorder rides in a small inflatable boat in order to keep the notebook

dry. A short paddle is used to propel the boat. Any other team members should also be in boats to prevent heat loss due to inactivity. The boat(s) also provide an emergency transport system if the snorkeling members of the party should be incapacitated by illness, injury, or hypothermia. The data collected is the same, but the capture of troglobitic fish and crayfish is much more difficult and therefore the numbers of measured specimens are reduced. Two major differences in the data gathered during the snorkel surveys are the common occurrence of both epigean fishes and the Mammoth Cave shrimp (*Palaemonias ganteri*). Epigean fish are identified and their total lengths are estimated. Centrarchids and minnows are only identified to family (unless captured). Shrimp are extremely difficult to see, and all observers should have the experience of seeing them under natural conditions before surveys are attempted. The observers must move slowly and carefully if they hope to count shrimp with any accuracy. This species is federally endangered and should not be disturbed or collected. Total body length is estimated. Rock counts and CPOM samples are taken in shallow water sections along the base-level streams.

Snorkel surveys cannot be as long as walking surveys due to the increased exposure to cold water. Hypothermia is extremely dangerous during some snorkel surveys since dry land may not be available along the survey route (e.g. Roaring River). The recorder is responsible for speaking to and watching the observers closely. The observers must inform other team members if they begin to get cold. The importance of this type of cooperation and mutual observing cannot be over-emphasized.

Section 3.0 Analysis of Data

Section 3.1 Geomorphology Data and CPOM Samples

The area of the stream segment surveyed is calculated by multiplying the average width of the section by the length of that section. All sections are added together to arrive at the total surveyed area. This value is then used to calculate large organism densities. The substrates of the sections are then combined to describe substrate composition (e.g. Substrate = 10% fines, 40% sand, 30% small rock, and 20% bedrock). Geomorphology data is used to help interpret IBI scores.

CPOM samples can be stored frozen until the researcher has gathered enough samples for batch processing. Each sample is thawed in a refrigerator to minimize bacterial metabolism. The sample is homogenized by shaking and kneading, and three subsamples are placed in foil drying dishes. The dishes are identified by codes embossed on the bottom of each foil pan. A typical subsample will weigh 30 to 50 grams. The subsamples are dried at 110 degrees Celsius for 48 hours. The subsamples are weighed to the nearest 0.01 gram on an electronic balance and then ignited at 550 degrees Celsius for 24 hours. The subsamples are removed from the muffle furnace and allowed to cool for 15 minutes, after which they are again weighed to the nearest 0.01 gram. The initial weight minus the ending weight is the weight loss by ignition. Most carbon compounds will be oxidized at this temperature. The mean sample weight loss and the standard deviation is calculated using the subsamples. A grand mean and standard deviation of weight loss are calculated for each stream using the replicate samples. Mean weight loss by ignition represents the relative amount of useable carbon available for use by the primary heterotrophs. This information can be used to rank the streams from most-resource limited to least-resource

limited.

Section 3.2 Water Quality Data

A mercury-in-glass thermometer is used to record water temperature at the beginning of each survey. Conductivity and pH are measured at the survey site using “pocket instruments” (see Appendix 2 for suppliers). These instruments should be calibrated regularly using standard solutions and a certified thermometer. Flow is calculated as the current velocity (as measured by the fishing bobber) times the cross-sectional area of the stream. Alkalinity, total hardness, sulfates, total nitrogen, total phosphorus, and chloride content are calculated using EPA approved titration and/or colorimetric techniques. The results are recorded in standardized EPA units.

Section 3.3 Community Composition Data

A spread sheet data base is created for the community data. Large organism density is calculated as the number of each species seen over the area surveyed. All large organism densities were standardized by converting to the number of organisms/5000 m². The 5000 m² area was selected to conform to the data presentation units used by Tom Poulson in Mammoth Cave National Park over the past 20 years. Size estimate data was grouped by taxa for each survey.

Section 3.4 IBI Metrics

Our metrics can be grouped into those requiring simple, proportional, and relatively

complex calculations. Table one lists the metrics used and their scoring. The simple metrics are calculated using either species lists or density data. The next group of metrics are based on either the proportion of individuals who are placed in the troglobitic/troglophilic groups or the proportion of individuals who represent recent recruitment. The final metric, condition coefficient, has the most complex calculation. This formula has been used for many years by fisheries biologists to monitor the general health of fish populations. Eleven metrics were chosen for the modified index of biotic integrity.

Number of Troglobitic/Troglophilic Fish Species (#1)

The number of troglobitic/troglophilic fish species metric is calculated by totaling the number of cave-adapted fish species observed for a site. Three species are possible: *Amblyopsis spelaea*, *Typhlichthys subterraneus*, and *Chologaster agassizi*. For the present database, the number of cave-adapted fish species decreases with increasing impact.

Total Number of Small Invertebrate Taxa (#2)

Many of the small invertebrates can not be identified to the specific level without dissection and/or microscopic examination. This limits the accuracy of the taxonomic data. The scoring for this metric is based on the concept of an optimum species number. In cave environments, a characteristic, low number of taxa/species is expected in an unimpacted stream. A reduced number of taxa may indicate impact, whereas an increased number of taxa/species beyond the optimum may also indicate impacts resulting in the presence of epigeal forms. The optimum number of field-identifiable, small invertebrate taxa appears to be two or three (Table 1).

Total Number of Taxa (#3)

This metric is calculated by summing the numbers of taxa observed during rock counts

and large organism surveys at a site. Three to five taxa appear to be the optimum number of total taxa. A total taxa number lower or higher is considered to be indicative of human impact.

Total Density of Large Organisms (#4)

The fourth metric, total density of large organisms, is based on the concept of cave habitats being resource limited. If the densities of fish, crayfish, shrimp, and salamander larva increase above 400 organisms per 5000 m², impact (in the form of enrichment) is indicated. This metric is calculated by adding the densities of all large organisms together. All densities are extrapolated to the number of organisms per 5000m².

Percentage of Troglobitic Invertebrate Taxa (#5)

This metric is calculated by dividing the number of troglobitic individuals by the total number of individuals. The highest proportion of troglobitic individuals are found in unimpacted streams.

Percentage of Troglobitic/Troglophilic Fish Individuals (#6)

This metric is calculated by dividing the number of troglobitic fish by the total number of fish. The numbers of epigean fishes increase with increasing human impact.

Percentage of Troglophilic Crayfish Individuals (#7)

This metric is calculated by dividing the number of *Cambarus tenebrosus* individuals by the total number of crayfish individuals at each site. *Cambarus Tenebrosus* is found in many unimpacted cave streams in very low numbers (one to three individuals). In highly enriched habitats, it can dominate the crayfish population.

Isopod Recruitment (#8)

Isopods are recorded in the surveys at total lengths of 3 to 13 millimeters. The lower level

taxonomy of isopods requires sacrificing specimens, but our survey protocol does not allow for the collection of individuals. This metric is the percentage of isopod individuals observed at a site that represent recent recruitment. Several detailed isopod studies have been completed at Mammoth Cave by Dr. Julian Lewis. In upper level streams, isopods began reproducing at four millimeters total length (Lewis, 1988). Therefore, isopods four millimeters or less were considered juveniles. A negative relationship between reproduction and the degree of human impact is assumed.

Troglobitic Fish Recruitment (#9)

This metric is the percentage of troglobitic fish individuals observed at a site that represent recent recruitment. Unpublished data from our mark and recapture studies suggest very slow growth rates for both *A. spelaea* and *T. subterraneus*. Slow growth allows for biomagnification of toxins. The total lengths considered to represent recent recruitment (40 mm for *A. spelaea* and 30 mm for *T. subterraneus*) were based on unpublished growth rates that we have obtained elsewhere, allowing for what may be normal gaps in annual reproduction. Impacted sites show reduced recruitment. Recovering sites may initially show unusually high levels of recruitment.

Orconectes pellucidus Recruitment (#10)

This metric is the percentage of troglobitic crayfish individuals observed at a site that represent recent recruitment. Individuals with a carapace length of ≤ 20 mm are considered juveniles (Hobbs, 1973). Outliers in this metric are often the result of isolated, mass reproduction that is common with this mobile and fecund species. Optimum recruitment levels appear to be 10 to 40% juveniles in the population.

Condition Coefficients of Troglobitic Fishes (#11)

This metric is calculated as: $\text{volume (ml)} \times 10^5 / \text{total length(mm)}^3 = \text{condition coefficient}$. Mean individual condition coefficients for each site are thus calculated. The scoring of this metric is complicated because the coefficients are species specific, and both species are not found at all study sites. We avoided this problem by determining separate IBI scores for each species and then averaging the two scores when both species were present. This results in some metric values that are not 1, 3 or 5.

Section 3.5 IBI Calculation

Calculation of the index value for a site is simple and straight forward. Each metric scores a one, three, or five (see condition coefficient for troglobitic fishes for an exception) based on the criteria in Table 1. The scores for each metric are then added for a total score. The higher the score, the less-impacted, or more pristine the stream. Interpretation will rely upon access to previous index scores at the site and/or similar sites. Water quality data, habitat descriptions, and the input of a biologist familiar with the system are also essential for management decisions based on the modified IBI. Appendix 3 presents a sample IBI calculation to aid in the application of the index.

Table 1. The scoring criteria for each of the eleven modified cave IBI metrics.

Metrics	Score		
	1	3	5
1. Number of Troglobitic / Troglophilic Fish Species	0 species	1 species	2 or 3 species
2. Total Number of Invertebrate Taxa	0 invertebrate taxa or more than 5 taxa	1 taxon or 4 or 5 taxa	2 or 3 taxa
3. Total Number of Taxa	1 to 2 taxa or 9 or more taxa	6 to 8 taxa	3 to 5 taxa
4. Total Density of Large Organisms	more than 800 organisms/5000m ²	400 to 800 organisms/5000m ²	less than 400 organisms/5000m ²
5. Percentage of Troglobitic Invertebrate Taxa	less than 70%	70% to 85%	more than 85%
6. Percentage of Troglobitic / Troglophilic Fish Individuals	less than 45%	45% to 99%	100 %
7. Percentage of Troglophilic Crayfish Individuals	more than 20%	20% to 5%	less than 5%
8. Isopod Recruitment	less than 10%	10% to 25%	more than 25%
9. Troglobitic Fish Recruitment	more than 50%	none or 21% to 49%	less than 20%
10. <i>Orconectes pellucidus</i> Recruitment	more than 60%	less than 10% or 41% to 60%	10% to 40%
11a. Condition Coefficients for <i>Amblyopsis spelaea</i>	less than 0.70	0.70 to 0.90	greater than 0.90
11b. Condition Coefficients for <i>Typhlichthys subterraneus</i>	less than 0.90	0.90 to 1.10	greater than 1.10

Section 4.0 References and Appendices

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Section 4.2 Appendix 1: Equipment Lists

Table 1. A checklist of general cave gear.

- Two pairs of thick, non-cotton socks
- pair of boots
- thermal underwear (both top and bottom)
- coveralls
- knee pads (B&C Wunder-wear preferred)
- gloves
- helmet
- main light source
- gate keys
- cave pack
 - 2 independent light sources
 - drink
 - cave food (light weight, high calorie)
 - first aid kit
 - 20 feet of tubular webbing
 - knife (survival tool better)
 - plastic bag and candles (emergency heat source)
 - matches
 - spare batteries for main light source

Table 2. A checklist of gear needed for biological walking surveys.

one halogen dive light for each observer
spare light bulbs for each halogen dive light
one set of spare batteries for each dive light
one measuring board for each observer (15 cm ruler glued on a piece of angled aluminum)
spare measuring board
15cm x 20cm aquarium net for each observer + spare
one set of volumetric cylinders
several sandwich-sized ziplock bags (for CPOM samples)
permanent marker (to code CPOM samples)
fishing bobber on one meter long piece of monofilament line (current measurement)
aluminum ring with area of 0.033 m^2
waterproof notebook (4" x 6" - Rite in Rain)
several spare pencils
thermometer
conductivity probe
pH probe
three sterile water sample bottles
maps of survey segments

Table 3. A checklist of additional gear needed for a snorkeling biological survey.

Full wetsuit
 hood
 gloves
 farmer john
 shorty step-in
 booties
15 cm x 20 cm aquarium net on a string
small inflatable boot
paddle for boat
extra halogen dive light
chemical warming pacs

Table 4. A checklist of extra gear needed for a vertical caving trip.

Static rope of appropriate length
Sit-harness for each person
rappel device for each person (figure 8, etc.)
ascending gear for each person
spare ascender on pigtail for each person
three 20 foot pieces of tubular webbing
one pair of etriers or aiders
one rescue pulley
four spare carabiniers
rope pad

Section 4.3 Appendix 2: Suppliers

Cave Gear

B&C Wunderwear
306 Eakin, East
Blacksburg, VA 24060
Phone (703) 552-5305

Bob & Bob
P.O. Box 441-N
Lewisburg, WV 24901
phone (304) 772-5049
fax (304) 772-3076

Campmor
P.O. Box 700-R
Saddle River, NJ 07458-0700

Inner Mountain Outfitters
102 Travis Circle
Seaford, VA 23696
e-mail: alexsproul@aol.com

Speleoshoppe
Box 297
Fairdale, KY 40118
phone (502) 367-6292
fax (502) 367-6990

Snorkeling Gear

Kentucky Diving Headquarters
3928 Shelbyville Rd
Louisville, KY 40207
phone (502) 897-6481

Louisville Dive Center
Bardstown Rd
Louisville, KY
phone (502) 852-6844

Underwater Kinetics (dive lights)
1020 Linda Vista Dr.

San Marcos, CA 92069
phone (619) 744-7560

Biological Equipment

Forestry Suppliers Inc.
205 W. Rankin St.
P.O. Box 8397
Jackson, MS 39284-8397

Hach Company
Dept. 98
Denver, CO 80281-0198
phone 1-800-227-4224

Section 4.4 Appendix 3: IBI Example

Imagined River

Stream surface area 3500m² (conversion factor to 5000m² for densities=1.43)

Large organisms observed during survey

Troglobtic fish	Number seen	#/5000m ²
<i>Amblyopsis spelaea</i>	15	21
<i>Typhlichthys subterraneus</i>	5	7

Epigeal fish		
<i>Cottus carolinae</i>	2	3

Large invertebrates		
<i>Palaemonias ganteri</i>	20	29
<i>Orconectes pellucidus</i>	53	76

Rock Count	Number seen	#/1000cm ²
Isopods	28	16
Amphipods	4	2
Snails	1	1

Number of rocks surveyed 37

Total area of the rocks 1700 cm² (conversion factor to #/1000cm² density = .588)

1. Number of Troglobitic/Troglophilic Fish Species

2 species were seen.

Table one scores a 5

2. Total Number of Invertebrate Taxa

5 taxa were seen.

Table one scores a 3

3. Total Number of Taxa

8 taxa were seen in total.

Table one scores a 3

4. Total Density of Large Organisms

21+7+3+29+76=136

Table one scores a 5

5. Percentage of Troglobitic Invertebrate Taxa

A total of $20+53+28+4+1=106$ troglobitic invertebrate individuals seen. No epigean forms seen. 100% of the organisms observed are troglobitic.

Table one scores a 5

6. Percentage of Troglobitic/Troglophilic Fish Individuals

$15+5=20$ troglobitic fish seen. 2 epigean fish seen for a total of 22. 20 divided by 22 equals 90.9% of the observed population troglobitic.

Table one scores a 3

7. Percentage of Troglophilic Crayfish Individuals

No troglophilic crayfish were seen. 0% of the observed population is troglophilic.

Table one scores a 5

8. Isopod Recruitment

A spread sheet is used to determine what percentage of the population observed is equal to or less than 4mm long. $N=28$. 10 individuals are 4mm or less. 10 divided by 28 equals 35.7.

Table one scores a 5

9. Troglobitic Fish Recruitment

A spread sheet is used to determine what percentage of the population observed is equal to or less than 30mm long for *T. subterraneus* and 40mm or less for *A. spelaea*. 1 out of 5 T.s. are equal to or less than 30 mm long. 1 divided by 5 equals 20% for T.s.. 0 out of 15 A.s. are 40 mm long or less. 0 divided by 15 equals 0%.

Table one scores a five for both species. The total metric score is then $5+5=10$, $10/2=5$

10. *Orconectes pellucidus* Recruitment

A spread sheet is used to determine what percentage of the population observed is equal to or less than 20mm long. 16 out of 53 crayfish were 20mm long or less. 16 divided by 53 equals 30.2%.

Table one scores a 5

11. Condition Coefficients of Troglobitic Fishes

A spreadsheet is used to calculate the formula: $\text{volume (ml)} \times 10^5 / \text{total length (mm)}^3 =$ condition coefficient for an individual. The individual coefficients are added together and their total divided by the number of fish measured. T.s. $N=2$, mean condition coefficient = 1.23. A.s. $N=7$, mean condition coefficient = 0.84.

Table one scores these values as T.s. = 5 and A.s. = 3, the combined score is $5+3=8$; $8/2=4$

Total Index Score

metric 1 = 5

metric 2 = 3

metric 3 = 3

metric 4 = 5

metric 5 = 5

metric 6 = 3

metric 7 = 5

metric 8 = 5

metric 9 = 5

metric 10 = 5

metric 11 = 4**Overall index score = 48 out of a possible 55**

A general interpretation would be that this is a very high score (87% of the total possible). A more in-depth interpretation would require considerations of the water quality data, CPOM, number of specimens measured for particular metrics, and habitat descriptions. The greatest value of this overall score would be in comparison to the scores recorded at the site in the past, as a benchmark against which future scores could be compared, or in comparison to scores at other sites.

Appendix 2

Appendix 2 COL-1. Length frequencies of *Amblyopsis spelaea* observed at Colossal River in 1994.

Size Class (total length in mm)	Date
	08/9&10/94
61-65	1
81-85	1
86-90	<u>2</u>
Total	4

Appendix 2 COL-2. Length frequencies of *Typhlichthys subterraneus* observed at Colossal River in 1994.

Size Class (total length in mm)	Date
	08/9&10/94
46-50	2
51-55	3
61-65	2
Total	7

Appendix 2 COL-3. Length frequencies of *Palaemonias ganteri* observed at Colossal River in 1994 .

Size Class (total length in mm)	Date
	08/9&10/94
26-30	1
Total	1

Appendix 2 COL-4. Length frequencies of *Orconectes pellucidus* observed at Colossal River in 1994.

Size Class (carapace length in mm)	Date
	08/9&10/94
0-5	7
6-10	5
11-15	2
16-20	6
21-25	9
26-30	8
31-35	4
36-40	<u>2</u>
Total	43

Appendix 2 COL-5. Length frequencies of isopods
observed at Colossal River in 1994.

Size Class (total length in mm)	Date
	08/9&10/94
3-4	3
5-6	11
7-8	1
Total	15

Appendix 2 MYS-1. Length frequencies of *Amblyopsis spelaea* observed at Mystic River in 1993-95.

Size Class (Total length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
11-15	5	0	0
16-20	0	2	0
31-35	0	0	2
41-45	0	2	0
46-50	2	1	0
51-55	0	1	1
56-60	0	1	2
61-65	0	2	0
66-70	2	1	2
71-75	1	0	1
76-80	<u>1</u>	<u>1</u>	<u>0</u>
Totals	11	11	8

Appendix 2 MYS-2. Length frequencies of *Typhlichthys subterraneus* observed at Mystic River in 1993-95.

Size Class (Total length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
26-30	1	0	0
31-35	1	0	0
36-40	3	0	1
41-45	2	0	0
46-50	0	1	0
51-55	<u>0</u>	<u>0</u>	<u>1</u>
Totals	7	1	2

Appendix 2 MYS-3. Length frequencies of *Palaemonias ganteri* observed at Mystic River in 1993-95.

Size Class (total length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
6-10	0	8	37
11-15	5	16	65
16-20	2	9	89
21-25	2	0	44
Totals	9	33	235

Appendix 2 MYS-4. Length frequencies of *Orconectes pellucidus* observed at Mystic River in 1993-95.

Size Class (carapace length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
0-5	0	2	0
6-10	0	1	2
11-15	8	17	11
16-20	14	18	10
21-25	16	18	40
26-30	12	9	12
31-35	11	8	5
36-40	5	2	3
41-45	<u>1</u>	<u>0</u>	<u>0</u>
Totals	67	75	83

Appendix 2 MYS-5. Length frequencies of *Cambarus tenebrosus* observed at Mystic River in 1993-95.

Size Class (carapace length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
	3		
40-45	<u>1</u>	<u>0</u>	<u>0</u>
Totals	1	0	0

Appendix 2 MYS-6. Length frequencies of isopods observed at Mystic River in 1993-95.

Size Class (total length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
3-4	9	6	4
5-6	15	4	2
7-8	<u>15</u>	<u>5</u>	<u>4</u>
Totals	39	15	10

Appendix 2 MYS-7. Length frequencies of amphipods observed at Mystic River in 1993-95.

Size Class (total length in mm)	Date		
	8/7&21/93	9/3/94	10/26/95
1-2	4	0	0
3-4	3	1	0
5-6	<u>1</u>	<u>1</u>	<u>0</u>
Totals	8	2	0

Appendix 2 EFT-1. Length frequencies of *Amblyopsis spelaea* observed at Eyeless Fish Trail, 1993-95.

Size Class (total length in mm)	Date		
	7/12/93	7/15/94	7/24/95
11-15	0	0	1
31-35	0	1	0
86-90	0	0	1
91-95	0	0	1
Totals	0	1	3

Appendix 2 EFT-2. Length frequencies of *Typhlichthys subterraneus* observed at Eyeless Fish Trail, 1993-95.

Size Class (total length in mm)	Date		
	7/12/93	7/15/94	7/24/95
26-30	1	0	0
31-35	0	1	2
36-40	3	2	1
41-45	3	2	1
46-50	3	2	2
51-55	2	2	0
56-60	0	3	3
61-65	<u>0</u>	<u>2</u>	<u>1</u>
Totals	12	14	10

Appendix 2 EFT-3. Length frequencies of *Orconectes pellucidus* observed at Eyeless Fish Trail, 1993-95.

Size Class (carapace length in mm)	Date		
	7/12/93	7/15/94	7/24/95
0-5	0	1	0
6-10	3	0	0
11-15	2	1	1
16-20	2	5	3
21-25	5	7	6
26-30	9	7	7
31-35	1	5	2
36-40	<u>0</u>	<u>1</u>	1
Totals	*22	27	20

* carapace lengths estimated from total lengths

Appendix 2 EFT-4. Length frequencies of *Cambarus tenebrosus* observed at Eyeless Fish Trail, 1993-95.

Size Class (carapace length in mm)	Date		
	7/12/93	7/15/94	7/24/95
56-60	1	0	0
61-65	<u>0</u>	<u>1</u>	<u>0</u>
Totals	*1	1	0

* carapace length estimated from total length

Appendix 2 EFT-5. Length frequencies of isopods observed at Eyeless Fish Trail, 1993-95.

Size Class (total length in mm)	Date		
	7/12/93	7/15/94	7/24/95
0-2	0	0	0
3-4	8	8	4
5-6	0	1	3
7-8	<u>0</u>	<u>1</u>	<u>8</u>
Totals	8	10	15

Appendix 2 EFT-6. Length frequencies of *Antroselates spiralis* observed at Eyeless Fish Trail, 1993-95.

Size Class (total length in mm)	Date		
	7/12/93	7/15/94	7/24/95
0-2	4	3	0
3-4	<u>0</u>	<u>6</u>	2
Totals	4	9	2

Appendix 2 GTA-1. Length frequencies of
Amblyopsis spelaea observed at Golden Triangle in
1993-95.

Size Class (Total length in mm)	Date	
	7/15/94	7/24/95
16-20	1	0
21-25	0	1
26-30	1	0
51-55	0	1
56-60	0	1
61-65	1	0
66-70	1	0
71-75	0	1
76-80	2	0
81-85	0	1
86-90	2	1
91-95	0	1
111-115	0	1
Totals	8	8

Appendix 2 GTA-2. Length frequencies of
Typhlichthys subterraneus observed at Golden
Triangle in 1993-95.

Size Class (Total length in mm)	Date	
	7/15/94	7/24/95
21-25	0	1
Totals	0	1

Appendix 2 GTA-3. Length frequencies of
Palaemonias ganteri observed at Golden Triangle in
1993-95.

Size Class (total length in mm)	Date	
	7/15/94	7/24/95
6-10	0	5
11-15	1	11
16-20	6	11
21-25	<u>5</u>	18
Totals	12	45

Appendix 2 GTA-4. Length frequencies of
Orconectes pellucidus observed at Golden Triangle
in 1993-95.

Size Class (carapace length in mm)	Date	
	7/15/94	7/24/95
21-25	2	0
26-30	0	3
36-40	<u>0</u>	<u>1</u>
Totals	2	4

Appendix 2 GTA-5. Length frequencies of *Cambarus tenebrosus* observed at Golden Triangle in 1993-95.

Size Class (carapace length in mm)	Date	
	7/15/94	7/24/95
61-65	<u>1</u>	<u>0</u>
Totals	1	0

Appendix 2 L&N-1. Length frequencies of *Typhlichthys subterraneus* observed at L&N Railroad Cave in 1993-95.

Size Class (Total length in mm)	Date		
	7/25/93	8/3/94	8/25/95
25-30	1	1	2
31-35	3	1	0
36-40	4	3	0
41-45	1	3	2
46-50	5	4	1
51-55	3	0	4
56-60	2	0	1
61-65	0	0	1
Totals	19	12	11

Appendix 2 L&N-2. Length frequencies of *Orconectes pellucidus* observed at L&N Railroad Cave in 1993-95.

Size Class (carapace length in mm)	Date		
	7/25/93	8/3/94	8/25/95
1-5	0	5	0
6-10	7	18	9
11-15	14	15	13
16-20	16	11	17
21-25	12	10	8
26-30	6	3	3
31-35	<u>0</u>	<u>1</u>	<u>0</u>
Totals	55	63	50

Appendix 2 L&N-3. Length frequencies of *Cambarus tenebrosus* observed at L&N Railroad Cave in 1993-95.

Size Class (carapace length in mm)	Date		
	7/25/93	8/3/94	8/25/95
41-45	1	1	0
46-50	<u>0</u>	<u>2</u>	<u>1</u>
Totals	1	3	1

Appendix 2 L&N-4. Length frequencies of isopods observed at L&N Railroad Cave in 1993-95.

Size Class (total length in mm)	Date		
	7/25/93	8/3/94	8/25/95
1-2	13	0	0
3-4	61	15	1
5-6	32	6	13
7-8	<u>0</u>	<u>1</u>	<u>18</u>
Totals	106	22	32

Appendix 2 L&N-5. Length frequencies of amphipods observed at L&N Railroad Cave in 1993-95.

Size Class (total length in mm)	Date		
	7/25/93	8/3/94	8/25/95
1-2	1	1	0
3-4	8	0	1
5-6	<u>6</u>	<u>0</u>	<u>0</u>
Totals	15	1	1

Appendix 2 UHR-1. Length frequencies of *Typhlichthys subterraneus* observed at Upper Hawkins River in 1993-95.

Size Class (Total length in mm)	Date		
	11/5/93	11/13/94	8/14/95
11-15	0	0	1
16-20	6	0	2
21-25	12	2	2
26-30	11	10	4
31-35	14	12	14
36-40	15	12	14
41-45	18	9	20
46-50	8	12	23
51-55	5	7	10
56-60	3	4	5
61-65	2	2	0
66-70	<u>1</u>	<u>0</u>	<u>0</u>
Totals	95	70	95

Appendix 2 UHR-2. Length frequencies of *Orconectes pellucidus* observed at Upper Hawkins River in 1993-95.

Size Class (carapace length in mm)	Date		
	11/5/93	11/13/94	8/14/95
1-5	6	0	0
6-10	104	25	88
11-15	65	54	78
16-20	49	53	47
21-25	55	72	49
26-30	16	20	12
31-35	4	10	9
36-40	<u>2</u>	<u>2</u>	<u>1</u>
Totals	301	236	284

Appendix 2 UHR-3. Length frequencies of *Cambarus tenebrosus* observed at Upper Hawkins River in 1993-95.

Size Class (carapace length in mm)	Date		
	11/5/93	11/13/94	8/14/95
51-55	<u>1</u>	<u>0</u>	<u>0</u>
Totals	1	0	0

Appendix 2 UHR-4. Length frequencies of isopods observed at Upper Hawkins River in 1993-95.

Size Class (total length in mm)	Date		
	11/5/93	11/13/94	8/14/95
3-4	56	16	1
5-6	41	12	20
7-8	28	14	40
9-10	<u>4</u>	<u>4</u>	0
Totals	129	46	61

Appendix 2 LHR-1. Length frequencies of *Amblyopsis spelaea* observed at Lower Hawkins River in 1993-95.

Size Class (Total length in mm)	Date		
	11/6/93	11/12/94	8/14/95
76-80	0	0	1
86-90	0	0	1
111-115	0	0	1
116-120	0	0	1
Totals	0	0	4

Appendix 2 LHR-2. Length frequencies of *Typhlichthys subterraneus* observed at Lower Hawkins River in 1993-95.

Size Class (Total length in mm)	Date		
	11/6/93	11/12/94	8/15/95
16-20	3	1	0
21-25	1	2	0
26-30	0	2	0
31-35	4	2	2
36-40	0	1	4
41-45	0	0	2
46-50	<u>1</u>	<u>0</u>	<u>0</u>
Totals	9	8	8

Appendix 2 LHR-3. Length frequencies of *Orconectes pellucidus* observed at Lower Hawkins River in 1993-95.

Size Class (carapace length in mm)	Date		
	11/6/93	11/12/94	8/15/95
6-10	9	11	9
11-15	11	9	12
16-20	12	15	12
21-25	36	21	56
26-30	20	17	30
31-35	<u>2</u>	<u>4</u>	<u>1</u>
Totals	90	77	120

Appendix 2 LHR-4. Length frequencies of *Cambarus tenebrosus* observed at Lower Hawkins River in 1993-95.

Size Class (carapace length in mm)	Date		
	11/6/93	11/12/94	8/15/95
41-45	1	0	0
46-50	1	1	1
51-55	0	0	1
56-60	<u>1</u>	<u>0</u>	<u>0</u>
Totals	3	1	2

Appendix 2 LHR-5. Length frequencies of isopods
observed at Lower Hawkins River in 1993-95.

Size Class (total length in mm)	Date		
	11/6/93	11/12/94	8/16/95
1-2	9	0	0
3-4	30	11	0
5-6	20	2	3
7-8	13	4	2
9-10	<u>7</u>	<u>1</u>	<u>0</u>
Totals	79	18	5

Appendix 2 ROR-1. Length frequencies of *Amblyopsis spelaea* observed at Roaring River in 1993-95.

Size Class (Total length in mm)	Date	
	10/1/94	12/9/95
6-10	0	1
11-15	1	0
16-20	0	2
26-30	1	2
31-35	4	0
36-40	0	3
41-45	1	5
46-50	2	2
51-55	5	3
56-60	12	5
61-65	8	7
66-70	14	2
71-75	11	2
76-80	7	2
81-85	2	1
86-90	4	1
91-95	2	0
96-100	7	0
116-120	0	2
121-125	2	1
145-150	<u>1</u>	<u>0</u>
Totals	84	41

Appendix 2 ROR-2. Length frequencies of
Typhlichthys subterraneus observed at Roaring
 River in 1993-95.

Size Class (Total length in mm)	Date	
	10/1/94	12/9/95
21-25	1	0
31-35	2	1
36-40	0	0
41-45	3	0
46-50	2	1
51-55	4	3
56-60	3	1
61-65	0	0
71-75	0	<u>1</u>
Totals	15	7

Appendix 2 ROR-3. Length frequencies of *Chologaster agassizi* observed at Roaring River in 1993-95.

Size Class (Total length in mm)	Date	
	10/1/94	12/9/95
46-50	1	0
51-55	1	0
56-60	<u>1</u>	<u>0</u>
Totals	3	0

Appendix 2 ROR-4. Length frequencies of *Palaemonias ganteri* observed at Roaring River in 1993-95.

Size Class (total length in mm)	Date	
	10/1/94	12/9/95
6-10	1	0
11-15	1	3
16-20	7	5
21-25	2	11
26-30	1	3
Totals	12	22

Appendix 2 ROR-5. Length frequencies of *Orconectes pellucidus* observed at Roaring River in 1993-95.

Size Class (carapace length in mm)	Date	
	10/1/94	12/9/95
0-5	0	0
6-10	8	7
11-15	8	0
16-20	8	12
21-25	25	16
26-30	41	8
31-35	9	5
36-40	<u>4</u>	<u>2</u>
Totals	103	50

Appendix 2 ROR-6. Length frequencies of *Cambarus tenebrosus* observed at Roaring River in 1993-95.

Size Class (carapace length in mm)	Date	
	10/1/94	12/9/95
41-45	1	0
51-55	<u>1</u>	<u>0</u>
Totals	2	0

Appendix 2 ROR-7. Length frequencies of isopods observed at Roaring River in 1993-95.

Size Class (total length in mm)	Date	
	10/1/94	12/9/95
3-4	27	3
5-6	13	9
7-8	7	9
9-10	<u>1</u>	<u>0</u>
Totals	48	21

Appendix 2 ROR-8. Length frequencies of *Antroselates spiralis* observed at Roaring River in 1993-95.

Size Class (total length in mm)	Date	
	10/1/94	12/9/95
0-2	1	1
3-4	1	6
5-6	<u>0</u>	<u>2</u>
Totals	2	9

Appendix 2 TSP-1. Length frequencies of *Amblyopsis spelaea* observed at the Shrimp Pools in 1993-95.

Size Class (Total length in mm)	Date		
	7/26/93	8/19/94	12/9/95
26-30	0	0	1
31-35	0	1	0
36-40	0	0	1
46-50	0	1	0
51-55	2	0	0
56-60	2	0	0
61-65	2	0	0
71-75	0	1	0
76-80	1	0	0
81-85	0	0	1
Totals	7	3	3

Appendix 2 TSP-2. Length frequencies of *Typhlichthys subterraneus* observed at the Shrimp Pools in 1993-95.

Size Class (Total length in mm)	Date		
	7/26/93	8/19/94	12/9/95
31-35	0	1	1
41-45	1	0	0
46-50	1	1	1
61-65	1	0	0
66-70	<u>0</u>	<u>0</u>	<u>1</u>
Totals	3	2	3

Appendix 2 TSP-3. Length frequencies of *Palaemonias ganteri* observed at the Shrimp Pools in 1993-95.

Size Class (total length in mm)	Date		
	7/26/93	8/19/94	12/9/95
11-15	0	0	2
16-20	<u>0</u>	<u>0</u>	<u>2</u>
Totals	0	0	4

Appendix 2 TSP-4. Length frequencies of *Orconectes pellucidus* observed at the Shrimp Pools in 1993-95.

Size Class (carapace length in mm)	Date		
	7/26/93	8/19/94	12/9/95
5-10	4	7	5
11-15	11	5	0
16-20	3	1	7
21-25	8	8	8
26-30	5	4	0
31-35	1	2	3
36-40	<u>0</u>	<u>0</u>	<u>1</u>
Totals	32	27	24

Appendix 2 TSP-5. Length frequencies of *Cambarus tenebrosus* observed at the Shrimp Pools in 1993-95.

Size Class (carapace length in mm)	Date		
	7/26/93	8/19/94	12/9/95
55-60	<u>1</u>	<u>0</u>	<u>0</u>
Totals	1	0	0

Appendix 2 TSP-6. Length frequencies of isopods observed at the Shrimp Pools in 1993-95.

Size Class (total length in mm)	Date		
	7/26/93	10/1/94	12/9/95
1-2	7	0	0
3-4	28	27	4
5-6	7	13	10
7-8	5	7	9
9-10	<u>0</u>	<u>1</u>	<u>0</u>
Totals	47	48	23

Appendix 2 TSP-7. Length frequencies of *Antroselates spiralis* observed at the Shrimp Pools in 1993-95.

Size Class (total length in mm)	Date		
	7/26/93	10/1/94	12/9/95
0-2	0	1	1
3-4	0	1	7
5-6	0	0	2
Totals	0	2	10

Appendix 2 ESR-1. Length frequencies of *Amblyopsis spelaea* observed at Echo/Styx River System in 1993-95.

Size Class (Total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
16-20	0	1	1
21-25	0	0	1
31-35	0	1	0
36-40	0	3	0
41-45	1	2	0
46-50	1	0	0
51-55	0	0	1
56-60	0	0	1
61-65	0	0	3
66-70	1	2	0
71-75	0	1	0
76-80	0	3	0
81-85	0	2	1
86-90	0	1	1
91-95	0	0	3
96-100	0	1	1
101-105	0	3	0
106-110	<u>0</u>	<u>1</u>	<u>1</u>
Totals	3	21	14

Appendix 2 ESR-2. Length frequencies of *Typhlichthys subterraneus* observed at Echo/Styx River System in 1993-95.

Size Class (Total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
31-35	0	1	0
36-40	0	1	0
41-45	0	0	0
46-50	0	0	2
51-55	0	0	0
56-60	0	0	1
61-65	0	0	1
66-70	0	0	<u>1</u>
Totals	0	2	5

Appendix 2 ESR-3. Length frequencies of *Chologaster agassizi* observed at Echo/Styx River System in 1993-95.

Size Class (Total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
16-20	1	0	0
21-25	2	1	0
26-30	0	1	0
31-35	0	1	0
36-40	0	0	2
41-45	0	1	2
46-50	1	1	3
51-55	0	0	3
56-60	0	2	1
66-70	<u>0</u>	<u>0</u>	<u>1</u>
Totals	4	7	12

Appendix 2 ESR-4. Length frequencies of *Palaemonias ganteri* observed at Echo/Styx River System in 1993-95.

Size Class (total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
11-15	0	1	0
16-20	0	1	0
21-25	<u>0</u>	<u>4</u>	<u>2</u>
Totals	0	6	2

Appendix 2 ESR-5. Length frequencies of *Orconectes pellucidus* observed at Echo/Styx River System in 1993-95.

Size Class (carapace length in mm)	Date		
	10/07/93	10/02/94	12/10/95
6-10	1	0	0
11-15	3	0	1
16-20	9	0	3
21-25	5	7	5
26-30	3	13	12
31-35	2	1	4
36-40	0	1	0
41-45	<u>0</u>	<u>1</u>	0
Totals	23	23	25

Appendix 2 ESR-6. Length frequencies of *Cambarus tenebrosus* observed at Echo/Styx River System in 1993-95.

Size Class (carapace length in mm)	Date		
	11/07/93	10/02/94	12/10/95
36-40	<u>0</u>	<u>1</u>	<u>0</u>
Totals	0	1	0

Appendix 2 ESR-7. Length frequencies of isopods observed at Echo/Styx River System in 1993-95.

Size Class (total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
0-2	0	1	0
3-4	38	11	1
5-6	11	13	3
7-8	9	5	2
11-12	<u>0</u>	<u>0</u>	1
Totals	58	30	7

Appendix 2 ESR-8. Length frequencies of *Antroselates spiralis* observed at Echo/Styx River System in 1993-95.

Size Class (total length in mm)	Date		
	11/07/93	10/02/94	12/10/95
0-2	0	0	1
3-4	2	6	1
5-6	<u>2</u>	<u>1</u>	<u>2</u>
Totals	4	7	4

Appendix 2 HID-1. Length frequencies of *Typhlichthys subterraneus* observed at Hidden River in 1993-95.

Size Class (Total length in mm)	Date		
	10/29/93	8/3/94	10/3/95
15-20	11	0	1
21-25	10	0	2
26-30	0	4	0
31-35	1	9	1
36-40	0	5	2
41-45	2	1	5
46-50	0	0	5
51-55	3	1	2
56-60	<u>2</u>	<u>2</u>	<u>1</u>
Totals	29	22	19

Appendix 2 HID-2. Length frequencies of *Orconectes pellucidus* observed at Hidden River in 1993-95.

Size Class (carapace length in mm)	Date		
	10/29/93	8/3/94	10/3/95
1-5	0	6	2
6-10	4	21	27
11-15	27	13	23
16-20	15	22	21
21-25	16	31	41
26-30	6	13	7
31-35	<u>2</u>	<u>0</u>	<u>1</u>
Totals	70	106	122

Appendix 2 HID-3. Length frequencies of *Cambarus tenebrosus* observed at Hidden River in 1993-95.

Size Class (carapace length in mm)	Date		
	10/29/93	8/3/94	10/3/95
5-10	0	1	0
11-15	3	1	0
16-20	5	1	0
21-25	6	12	2
26-30	4	6	0
31-35	4	5	1
36-40	2	6	0
41-45	1	4	0
46-50	4	2	0
51-55	<u>3</u>	<u>1</u>	<u>0</u>
Totals	32	39	3

Appendix 2 HID-4. Length frequencies of isopods observed at Hidden River in 1993-95.

Size Class (total length in mm)	Date		
	10/29/93	8/3/94	10/3/95
1-2	0	1	0
3-4	1	16	37
5-6	7	33	63
7-8	10	15	59
9-10	4	0	3
11-12	0	0	2
Totals	22	65	164

Appendix 2 HID-5. Length frequencies of amphipods observed at Hidden River in 1993-95.

Size Class (total length in mm)	Date		
	10/29/93	8/3/94	10/3/95
1-2	0	0	1
3-4	0	2	1
5-6	5	0	10
7-8	<u>0</u>	<u>1</u>	<u>0</u>
Totals	5	3	12

Appendix 3

Appendix 3a. The IBI metric scores at each of the eight sites; 1993.

<u>Metric</u>	<u>Location</u>						
	EFT	L&N	UHR	LHR	MYS	ESR	HID
# Trog. Fish Species	3	3	3	3	5	5	3
Total # Invert. Taxa	3	3	3	5	1	5	3
Total # Taxa	5	5	3	5	1	1	3
Total Density Macro-organisms	5	1	3	5	5	5	3
% Trog. Taxa Invert.	3	3	1	1	3	5	1
% Ind. Trog Fish	5	5	5	5	5	1	5
% Troglomorphic Crayfish	5	5	5	5	5	5	1
Isopod Recruitment	5	5	5	5	3	5	1
Trog. Fish Recruit.	5	5	5	3	4	3	1
Trog. Crayfish Recruitment	5	1	1	5	5	3	1
Trog. Fish Condition	*2.5	3	3	*2.5	4	*3.5	3
Total Score	46.5	39	37	44.5	41	41.5	25

* No condition measurements taken, so value estimated from mean of 1994 and 1995 values.

Appendix 3b. The IBI metric scores at each of the ten sites; 1994.

<u>Metric</u>	<u>Location</u>									
	E F T	L & N	C O L	U H R	L H R	M Y S	E S R	R O R	G T A	H I D
# Trog. Fish Species	5	3	5	3	3	5	5	5	3	3
Total # Invert. Taxa	3	3	3	5	5	3	1	1	5	3
Total # Taxa	3	5	1	5	5	1	1	1	5	3
Total Density Macro-organisms	5	1	5	3	5	3	5	5	1	1
% Trog Taxa. Invert.	3	3	3	5	1	3	1	3	1	3
% Ind. Trog Fish	5	5	3	5	5	3	1	1	1	5
% Troglomorphic Crayfish	5	5	5	5	5	5	5	5	5	1
Isopod Recruitment	5	5	3	5	5	5	5	5	1	3
Trog. Fish Recruit.	2	5	3	3	3	5	3	5	3	5
Trog. Crayfish Recruitment	5	1	5	3	3	3	3	5	3	3
Trog. Fish Condition	2	5	3	3	1	5	5	3	1	5
Total Score	43	41	39	45	41	41	35	39	29	35

Appendix 3c. The IBI metric scores at each of the nine sites; 1995.

<u>Metric</u>	<u>Location</u>								
	EFT	L&N	UHR	LHR	MYS	ECO	ROR	GTA	HID
# Trog. Fish Species	5	3	3	5	5	5	5	5	3
Total # Invert. Taxa	3	3	5	5	5	3	3	5	3
Total # Taxa	3	5	5	3	5	1	3	3	3
Total Density Macro-organisms	5	3	3	5	3	5	5	1	3
% Trog. Taxa Invert.	5	3	5	1	5	3	5	5	3
% Ind. Trog Fish	5	5	5	3	5	1	5	3	3
% Troglomorphic Crayfish	5	5	5	5	5	5	5	5	5
Isopod Recruitment	3	1	1	1	5	3	3	1	3
Trog. Fish Recruit.	3	5	3	3	3	4	4	5	5
Trog. Crayfish Recruitment	5	1	1	5	5	5	5	3	3
Trog. Fish Condition	3	5	5	4	3	2	3	3	3
Total Score	45	39	41	40	49	37	46	39	37