



Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network

Protocol Narrative—Version 1.0

Natural Resource Report NPS/CUPN/NRR—2018/1705



ON THE COVER

Clockwise from top left: Unidentified cave amphipod (Photo by Kurt Lewis Helf), *Caecidotea* sp. (Photo by Rick Olson), *Palaemonias ganteri* (Photo by Michael Durham), *Gyrinophilus palleucus* (Photo by Kurt Lewis Helf), *Orconectes pellucidus* (Photo by Rick Olson), *Amblyopsis spelaea* (Photo by Rick Olson).

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Revision Log

Version numbers will be incremented by a whole number (e.g., Version 1.3 to Version 2.0) when a change is made that significantly affect requirements or procedures. Version numbers will be incremented by decimals (e.g., Version 1.6 to Version 1.7) when there are minor modifications that do not affect requirements or procedures for publication in the series.

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Contents

	Page
Revision Log.....	iii
Figures.....	ix
Tables.....	xi
Executive Summary	xiii
Acknowledgments.....	xvii
Glossary	xix
Introduction.....	1
Background and Objectives	5
Overview of Network Parks with Cave Aquatic Biota	5
Conceptual Model for the Cave Aquatic Ecosystem.....	5
Rationale for Monitoring Cave Aquatic Biota and Issues Being Addressed	8
Historical Development of Cave Aquatic Biota Monitoring at Mammoth Cave NP	10
Measurable Objectives	13
Sampling Design.....	15
Site Selection.....	15
Adding Sites	16
Uncertainty of Estimates and Detectable Levels of Change	16
General Sampling Approach	22
Cave Stream Reach Sampling	22
Timing of Sampling.....	23
Field Methods	25
Pre-field Season Preparation	25
Communication and Coordination with Park Staff and Logistics	25
Permitting and Compliance	25
Job Safety Review	26

Contents (continued)

	Page
Review of Field Information Sheets, Cave Stream Reach Maps, and Field Sampling SOPs.....	26
Supplies and Equipment	27
Training of field crews on data collection	27
Cave Stream Reach Sampling	27
Opportunistic Grab Sampling of Water Quality.....	27
Continuous Sampling of Water Quantity	28
Direct Visual Counts of Cave Aquatic Biota	29
Variables and Measurements.....	30
Post-Field Sampling	31
Data Handling, Analysis, and Reporting	33
Data Collection.....	33
Overview of Database Approach.....	34
Data Entry.....	34
Quality Review	34
Data Archiving and Curation.....	35
Electronic Data Sets	35
Archives.....	35
Metadata Procedures	35
Protected Data	35
Planned Routine Data Summaries and Analysis	35
N-mixture Model Framework.....	36
Occupancy Model Framework	36
Data Analyses	37
Planned Reporting Schedule, Format, and Delivery	39
Procedure for Revising the Protocol.....	42

Contents (continued)

	Page
Personnel Requirements and Training	43
Staff Roles, Responsibilities, and Qualifications	43
Network Program Manager	43
Project Leader.....	43
Field Crew Leader	44
Field Crew Members	45
Physical Scientist.....	46
Data Manager	46
GIS Specialist	47
Curatorial Specialist	47
Data Intern.....	47
Training Procedures.....	47
Operational Requirements	49
Annual Workload and Field Schedule.....	49
Facility and Equipment Needs.....	49
Startup Costs and Budget Considerations	50
Literature Cited	53

Figures

	Page
Figure 1. Location of the 14 parks within the Cumberland Piedmont Network including the parks at which cave aquatic biota will be monitored (red dots).....	2
Figure 2. Broad conceptual model of the cave aquatic ecosystem developed for the <i>Cumberland Piedmont Network Vital Signs Monitoring Plan</i> (Leibfreid et al. 2005)	6
Figure 3. Cave stream conceptual model demonstrating the hypothesized links between threat source drivers on the surface, such as off-park land development, the resultant measurable perturbations to the subsurface habitat, such as water quality (e.g., decreased dissolved oxygen), and general hypothesized correlations (i.e., up and down arrows) with measurable subsurface attributes such as population metrics of cave aquatic biota.	7
Figure 4. Water on its way to the Green River (light blue continuous line) moves (brown continuous lines) over and through local drainage basins outside and inside Mammoth Cave National Park and carries organic matter and contaminants through cave streams (dark blue lines)	9
Figure 5. Constant detectability is unlikely for most species.....	11
Figure 6. Ratio of estimated CAB abundance to expected abundance per transect as a function of individual detection probability p , for combinations of total effort (4800, 2400, & 1200 m), mean abundances (noted as mean per site or transect), 60 sites, 40 m transect lengths, and 2 visits per year	17
Figure 7. Power curves for $\alpha=0.05$ and 0.10 as a function of monitoring duration and CAB detectability; the total effort is 2,400 meters (7,874 ft), 60 sites, 20-meter (65.6-ft) transect length, and 2 visits	19
Figure 8. Coverage of the 95% confidence intervals for the estimated temporal trends and CAB detectability; the total effort is 2400 meters (7,874 ft), 20-meter (65.6-ft) transect length, 120 sites, and 2–4 visits.....	21
Figure 9. A snorkeler surveying in a single pass zigzags through the cave stream reach while moving upstream.....	30
Figure 10. Mockup of graph depicting significant differences in <i>P. ganteri</i> occupancy as a function of overall water quality	38
Figure 11. Boxplot showing size ranges for various CAB species detected at a cave stream reach during one sampling season.....	40

Tables

	Page
Table 1. Legal Status/Type Localities of cave aquatic biota species commonly observed at monitored parks.....	1
Table 2. Estimated abundance (λ) and occupancy (ψ) of cave aquatic biota, and associated CAB detection probabilities (p), for Bridge Avenue stream reach sampled during the January 2015 pilot efforts	12
Table 3. Target cave aquatic biota (CAB) groups and species for cave stream monitoring along with their planned derived data and park of origin	13
Table 4. Cave aquatic biota monitoring sites at Cumberland Gap NHP (CUGA), Mammoth Cave NP (MACA), and Russell Cave NMt (RUCa).....	15
Table 5. Major sampling methods, and primary measures and variables that will be collected or calculated during implementation of the protocol for monitoring cave aquatic biota.....	31
Table 6. Mock-up example of summary water quality data of cave stream temperature, specific conductance (SC), pH, dissolved oxygen (DO), turbidity, NO ₃ , and <i>E. coli</i> from five MACA cave stream reaches during two separate sampling events by opportunistic grab sampling	41
Table 7. Estimated Fiscal Year 2018 budget for implementing the protocol for cave aquatic biota monitoring at four cave streams at Mammoth Cave National Park (MACA; Panel 1)	51

Executive Summary

The mission of the National Park Service is to conserve unimpaired the natural and cultural resources and values of the National Park System for the enjoyment of this and future generations. To uphold this goal, the Director of the National Park Service approved the Natural Resource Challenge to encourage national parks to focus on the preservation of the nation's natural heritage through science, natural resource inventories, and expanded resource monitoring (National Park Service 1999). Through the Challenge, 270 parks in the National Park System were organized into 32 inventory and monitoring networks.

All Inventory and Monitoring (I&M) networks within the National Park Service have identified high priority park vital signs, indicators of ecosystem health, which represent a broad suite of ecological phenomena operating across multiple temporal and spatial scales. The intent is to develop a balanced and integrated suite of vital signs that meets the needs of park management and will accommodate unanticipated environmental conditions and management questions in the future. Three parks in the Cumberland Piedmont Network (CUPN) have selected cave aquatic biota (CAB) in subsurface waters as a high priority vital sign for long-term monitoring. These parks include Cumberland Gap National Historical Park (CUGA), Mammoth Cave National Park (MACA), and Russell Cave National Monument (RUCA). Cave aquatic biota were selected for their status as federally-listed endangered species, federal species of management concern, and state species of concern. Given that many cave aquatic biota are unique to the location where they are found, it is likely that CUPN cave aquatic ecosystems harbor greater biodiversity than is currently known. In fact, Edmonson and Lee counties, home to Mammoth Cave NP and Cumberland Gap NHP, rank second and fourth, respectively, among the six counties containing 25% of the known CAB diversity in the contiguous United States.

Threats to these cave stream communities include chemical and thermal contamination from surface water flow due to land use inside and outside park boundaries. Runoff from large-scale agriculture increases the nutrient load and *Escherichia coli* in water flowing into subsurface streams and may alter the structure of stygobiont communities adapted to nutrient-poor conditions. Weather and climate change are expected to significantly alter hydrologic regimes across the Southeast. Increasingly intense or frequent flood pulses moving through cave streams may significantly modify the cave aquatic habitat and structure of CAB communities. Collecting systematic, long-term data to explain the abundance and distribution of CAB metapopulations will provide resource managers with the necessary data to make informed decisions regarding which habitats they should prioritize for protection.

Monitoring objectives addressed by this protocol include:

1. For common cave aquatic biota, estimate status and long-term trends in abundance within monitored cave stream reaches in three parks in the Cumberland Piedmont Network.

2. For uncommon target cave aquatic biota, determine status and long-term trends in the probability of occupancy and proportion of reaches occupied within monitored cave stream reaches in three parks in the Cumberland Piedmont Network.
3. Sample selected cave stream habitat characteristics and water-quality and water-quantity parameters that we hypothesize are relevant to gaining an understanding of cave aquatic biota abundance, occupancy, or detectability within monitored cave stream reaches in three parks in the Cumberland Piedmont Network.

This monitoring protocol is focused on monitoring a suite of cave aquatic biota that regularly inhabits selected caves within the three CUPN cave parks. We identified seven cave streams among the three cave parks as conducive to visual survey for cave aquatic biota while walking, wading, or snorkeling. Due to logistical constraints, all three cave parks cannot be visited during a single sampling season so they are divided into two separate panels. The sampling season occurs during the dry season (July–October) when the probability of detecting cave aquatic biota during sampling events is highest due to low water levels and low turbidity.

Sampling events, consisting of two independent surveys of each cave stream on two nonconsecutive dates, will be conducted at one panel per sampling season such that, conditions permitting, all cave streams in both panels will be surveyed four times every two years. A single sampling event consists of two teams, one observer and one recorder each, collecting data on water quality and quantity, conducting staggered, independent visual surveys for cave aquatic biota in a predetermined number of noncontiguous, 40-meter (131.2-feet [ft]) transects while also collecting data on their habitat characteristics. Water-quality and water-quantity data are also collected (Objective 3), upstream of the transects to be surveyed. Samples are collected using opportunistic grab sampling and water level measurement, and additional data are obtained from water level/temperature data loggers. Visual surveys for cave aquatic biota (Objectives 1 and 2) typically involve the observer moving slowly and quietly upstream and, using a powerful headlamp or dive light, conducting a timed count in each transect. Each detected cave aquatic biota, along with a size estimate and any noticeable characteristics (e.g., damage), are called out to the data recorder. Finally, field personnel collect limited qualitative data on habitat characteristics (Objective 3) such as habitat type (e.g., riffle, pool, or glide) and percent substrate composition (e.g., fines, granules, or pebbles). Sampling techniques differ depending on whether the cave stream being sampled is wadeable or nonwadeable. Wadeable cave streams or pools are generally shallow, making transects easier to survey while walking along the bank. Cave streams with a majority of nonwadeable habitat must be surveyed by snorkeling observers, with recorders paddling inflatable kayaks behind.

The primary goals of this protocol are to identify changes in CAB abundance and occupancy for resource managers at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM. In order to meet these goals, we must account for the variation in detectability over time and space to provide reliable data for effective resource management. Hierarchical models are a flexible framework in which a complex system may be modeled using multiple submodels. This framework is particularly suited to inference regarding abundance, occupancy, species richness, and demographic

characteristics because it can accommodate multiple data sets, sources of variability, scales of measurement, and varying data quality inherent in the kinds of data we will collect.

Data handling procedures will be in place to ensure data collected under this protocol are of an acceptable quality and are available for current and future data users. Data recorded in the field onto field data sheets or collected by data loggers will be entered and stored in several databases. Water-quality and water-quantity data will be stored in NPSTORET and AQUARIUS databases, respectively, and CAB detection/nondetection data will be stored in a Microsoft® Access database to be developed by the Cumberland Piedmont Network in cooperation with National Park Service contractors. Data verification and validation will be conducted before electronic data sets are archived. Queries and reports in the database will be used to retrieve data for analysis and to generate Annual Summary Reports and six-year Trend Analysis Reports for park management.

This protocol details the why, where, how, and when of the network's CAB monitoring project. As recommended in Oakley et al. (2003), it consists of a protocol narrative and a set of standard operating procedures detailing the steps required to collect, manage, and disseminate the data representing the status and trends of cave aquatic biota and associated habitat parameters in the three network parks. The protocol is a living document continually updated as new information acquired through monitoring and evaluation leads to the refinement of program objectives and methodologies. Changes to the protocol are carefully documented in a revision history log. The use of the protocol ensures that a scientifically credible story about the ecological condition of cave aquatic biota and their responses to park management actions, land use changes, and other stressors can be told to park visitors and managers alike. These long-term data can contribute to the development of informative models of relationships between cave aquatic community dynamics and key environmental factors and management actions specific to each park.

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Glossary

Generalized Random Tessellation Stratified (GRTS) design—a spatially balanced survey design that results in a representative sample of the target population. The design includes an additional oversample only used if initially selected samples are discarded.

Glide—a relatively shallow part of a stream with low velocity and little or no surface turbulence

Intermittent stream—a stream in which, at low flow, dry reaches alternate with flowing ones along the stream length.

Nonwadeable—sections of a stream where an investigator must float from one end of the reach to the other, even though the reach may contain some pools that can be waded.

Perennial stream—a stream that carries some flow at all times.

Pool—a small part of the reach with little velocity, commonly with water deeper than surrounding areas.

Reach—a subunit of a stream chosen to represent a uniform set of physical, chemical, and biological conditions. Reaches are the primary sampling unit for this protocol.

Riffle—a shallow part of the stream where water flows swiftly over completely or partially submerged obstructions to produce surface agitation.

Sampling Event—a complete sampling of the physical, chemical, and biological data of all transects in one cave stream reach; completed by two teams in a single day.

Stygobiont/Stygobiotic—organism(s) restricted to living in the cave aquatic habitat.

Stygophile/Stygophilic—organisms that may be found living in either the cave or surface aquatic habitat but are not restricted to either.

Survey—generally refers to sampling biological data in a transect in a cave stream reach but may also include the collection of physical and chemical data.

Transect—a 40-meter (131.2-ft) permanent subunit of a sampled cave stream reach parallel with its flow. Transects are the secondary sampling unit within which physical, chemical, and biological data are collected.

Wadeable—sections of a stream where an investigator can wade from one end of the reach to the other, even though the reach may contain some pools that cannot be waded.

Watershed—the area of land where all of the water that falls in it and drains off it goes to a common outlet. Used interchangeably with basin.

Introduction

This protocol is focused on monitoring cave aquatic biota within three parks in the Cumberland Piedmont Network (CUPN; Table 1 and Figure 1). The underground streams in these CUPN parks support obligate cave aquatic organisms (i.e., stygobionts) adapted to living in nutrient-poor conditions. Some large or easily observed stygobionts are state species of concern (e.g., the Northern Cavefish, *Amblyopsis spelaea*), federal species of management concern (e.g., the Mammoth Cave Crayfish, *Orconectes pellucidus*), or federally listed endangered species (i.e., the Kentucky Cave Shrimp, *Palaemonias ganteri*). Several species were found at Cumberland Gap NHP and Mammoth Cave NP (Table 1). Given that many stygobionts are unique to the site(s) where they are found (i.e., endemic) among all taxonomic groups and at all scales of measurement (Gibert et al. 2009) it is likely CUPN cave aquatic ecosystems harbor greater biodiversity than is currently known. It is possible the Mammoth Cave area has more than one species of cave shrimp because *P. ganteri* is found in cave streams on both sides of the Green River, a potential geographic barrier to genetic exchange between populations. Many cave-dwelling taxa look alike but are genetically distinct species (Trontelj et al. 2009; Niemiller et al. 2012; Ethridge et al. 2013).

Table 1. Legal Status/Type Localities of cave aquatic biota species commonly observed at monitored parks. Status abbreviations: S1=Extremely rare, S2=Very rare, S3=Rare to uncommon; SOMC=Species of management concern, LE=Listed endangered, SOC=Species of concern; -=No status. Park abbreviations: CUGA=Cumberland Gap National Historical Park, MACA=Mammoth Cave National Park, and RUCA=Russell Cave National Monument.

Cave Aquatic Biota (CAB) Species	State/Federal Legal Status	Park	Type Locality
<i>Amblyopsis spelaea</i>	S3/SOMC	MACA	Y
<i>Orconectes pellucidus</i>	S3/SOMC	MACA	Y
<i>Stygobromus vitreus</i>	S1/-	MACA	Y
<i>Palaemonias ganteri</i>	S1/LE	MACA	Y
<i>Typhlichthys subterraneus</i>	S2S3/SOMC	MACA/RUCA	N/N
<i>Caecidotea stygia</i>	-/-	MACA	Y
<i>Bactrurus angulus</i>	S1/SOC	CUGA	N
<i>Stygobromus cumberlandus</i>	S1S2/SOC	CUGA	Y
<i>Caecidotea cumberlandensis</i>	S1/-	CUGA	Y
<i>Gyrinophilus palleucus palleucus</i>	High Mgmt. Concern/-	RUCA	N

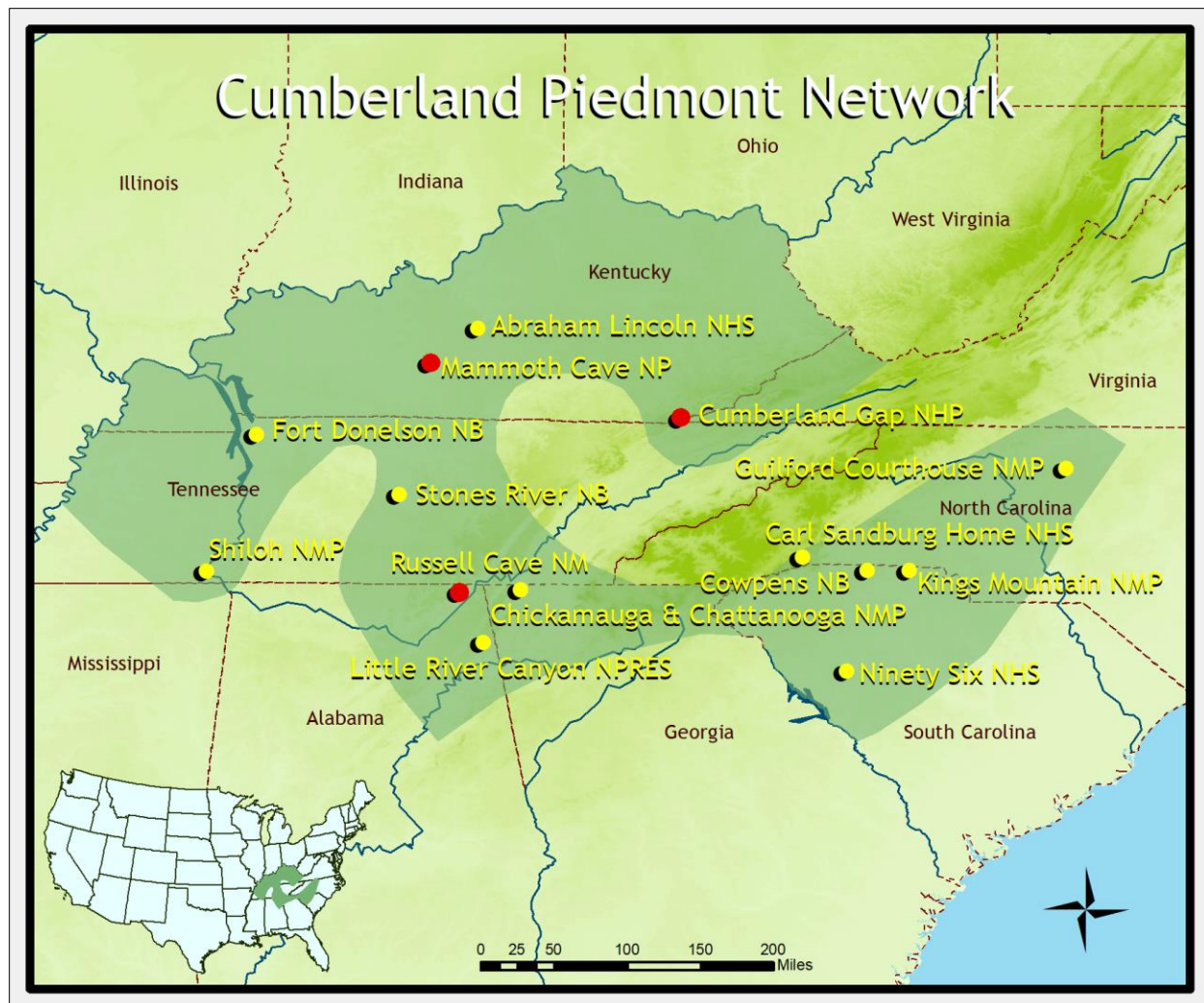


Figure 1. Location of the 14 parks within the Cumberland Piedmont Network including the parks at which cave aquatic biota will be monitored (red dots).

Cave aquatic biota are supported by a continuous supply of water that transports organic matter (as well as contaminants) from the surface as it moves over and through local and regional karst watersheds. The community of aquatic biota found in upper level cave streams and pools, in the unsaturated zone (partially water-filled channels above the water table), is often a mixture of surface and stygobiotic organisms. Water moving through the unsaturated zone ultimately reaches the water table at base level, or the saturated zone, and flows out of springs to the surface along its associated master stream. Occasionally, the CAB community in base level cave streams and pools associated with spring outlets is also a mixture of surface and stygobiotic organisms. More commonly, the community at this level is composed of stygobionts that have adapted to extremely food-limited conditions, such as the unpigmented, eyeless Kentucky Cave Shrimp *P. ganteri*. This simplified description of karst ecohydrology in the CUPN cave streams, selected for this monitoring protocol, is important for understanding the significant interplay between their nonliving and living components (Bonacci et al. 2009).

This narrative contains the background justification for monitoring, the objectives, and the sampling design. It is also an overview of how the Cumberland Piedmont Network will implement the CAB monitoring protocol with respect to field data collection using field data forms, data management, data analysis, and reporting. Network staff will review the methods at the end of each field season and update the associated standard operating procedures (SOPs) as necessary prior to initiation of the next field season. In addition, all staff and volunteers regularly participating in any component of the monitoring work must read all applicable SOPs prior to their involvement.

Background and Objectives

Overview of Network Parks with Cave Aquatic Biota

Jackson County, Alabama; Lee County, Virginia; and Edmonson County, Kentucky (home to Russell Cave NM [RUCA], Cumberland Gap NHP [CUGA], and Mammoth Cave NP [MACA], respectively) rank first, fifth, and sixth, respectively, among the 10 counties accounting for greater than 25% of endemic cave obligate fauna in the contiguous United States (Culver et al. 2003). Edmonson and Lee counties rank second and fourth, respectively, among the six counties containing 25% of the known CAB diversity in the contiguous United States (Culver et al. 2003). Threats to these cave stream communities include contamination due to land use both inside and outside park boundaries, and altered hydrologic regimes due to global climate change (Meiman 2005; Kunkel et al. 2013; Monahan and Fisichelli 2014).

Conceptual Model for the Cave Aquatic Ecosystem

The development of conceptual ecological models to identify key system components, linkages, and processes is a critical step in the design of a long-term monitoring program. The need for conceptual ecological models has been well established (Elzinga et al. 2001; Noon and Dale 2002). Conceptual models improve the planning process for monitoring by explicitly stating key elements of our understanding of system dynamics, which facilitates discussion, evaluation, and refinement of the monitoring program (Maddox et al. 1999). Given the complexity of natural systems and the variety of factors that influence ecological processes, there is an obvious need for conceptual modeling as a tool to help organize information and refine understanding of system components and interactions.

The CUPN Vital Signs Monitoring Plan (Leibfreid et al. 2005) describes the major drivers, stressors, and primary ecosystem attributes associated with cave ecosystems in the network. We relied on that document, in addition to the literature and personal experience, to determine key ecosystem drivers, attributes, and stressors of the cave aquatic ecosystem that should be considered during the development of the monitoring protocol (Figure 2). Ecosystem drivers are major natural forces, such as climate and hydrological cycles, and natural disturbances, such as droughts and floods, with large-scale influences on the attributes of monitored systems. Stressors are defined as perturbations to a system that are either (a) foreign to that system, or (b) natural to the system but applied at an excessive or insufficient level (Barrett et al. 1976). Stressors can include natural disturbances such as flooding but more frequently represent anthropogenic alterations of natural processes or structures (e.g., water pollution, pesticides, cave development; Noon and Dale 2002). Ecosystem attributes are any inherent measurable biotic or abiotic factor or environmental process.

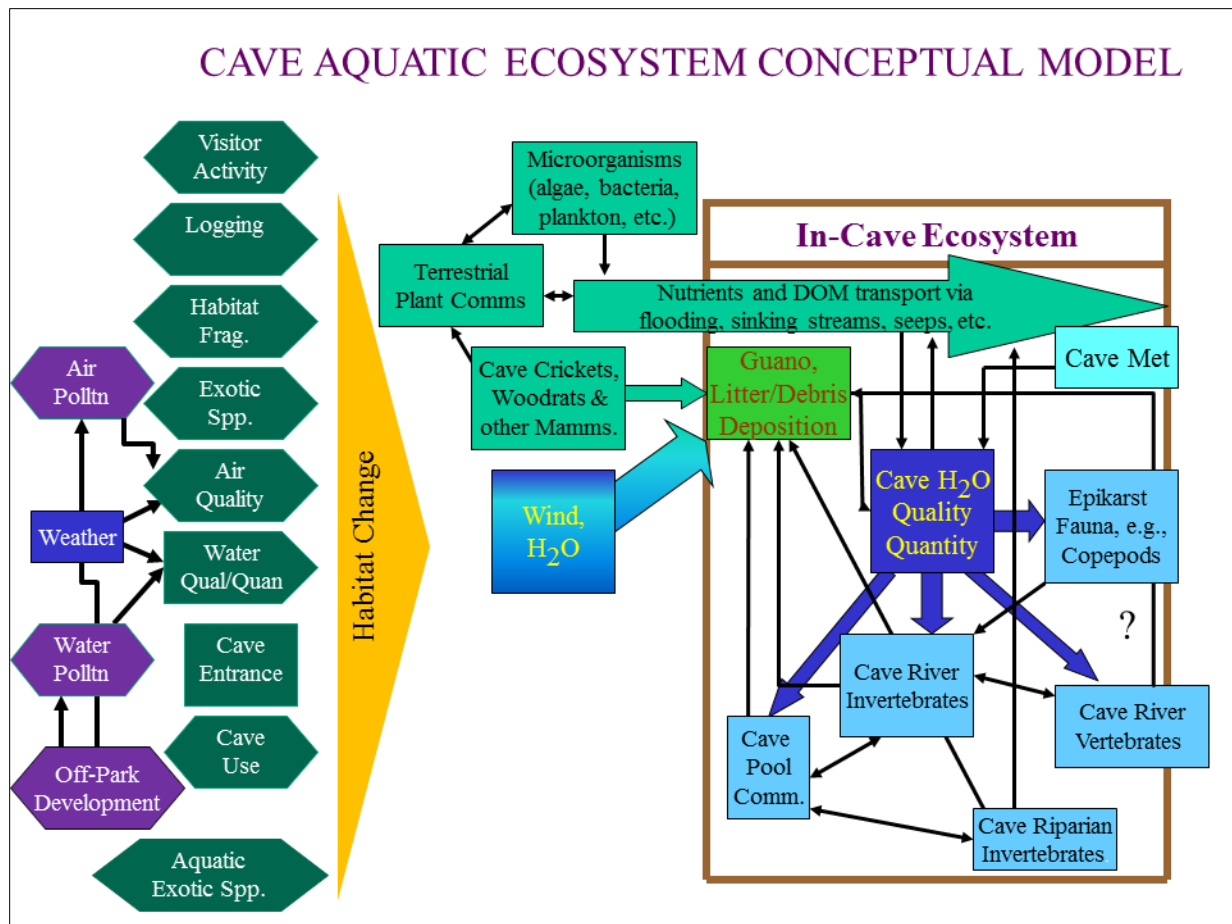


Figure 2. Broad conceptual model of the cave aquatic ecosystem developed for the *Cumberland Piedmont Network Vital Signs Monitoring Plan* (Leibfreid et al. 2005). Model depicts putative interactions among surface drivers, stressors and attributes, and subsurface drivers, stressors and attributes. Note: “Cave Met” is short for Cave Meteorology and “DOM” stands for Dissolved Organic Matter.

We used the broad cave aquatic ecosystem conceptual model (Figure 2) to clarify and focus on hypothesized relationships among a subset of measurable attributes and CAB population abundance, distribution, and community composition (Figure 3). For example, weather and climate change is a major ecosystem driver in our cave conceptual model because it is expected to significantly alter hydrologic regimes across the Southeast (Monahan and Fisichelli 2014). Increasingly intense or frequent flood pulses moving through cave streams, as measured by water level, may significantly modify the cave aquatic habitat and structure of stygobiont communities (Holsinger and Culver 1988). Continuing regional and local development are non-point and point sources, respectively, (i.e., drivers) in that human activities on the surface can affect the living and non-living components of the cave aquatic ecosystem. Runoff from large-scale agriculture increases the nutrient load (e.g., NO₃) and *Escherichia coli* in water flowing into subsurface streams (Meiman 2005) and may alter the structure of stygobiont communities (Simon and Buikema 1997; Graening and Brown 2003; Lewis et al. 2015).

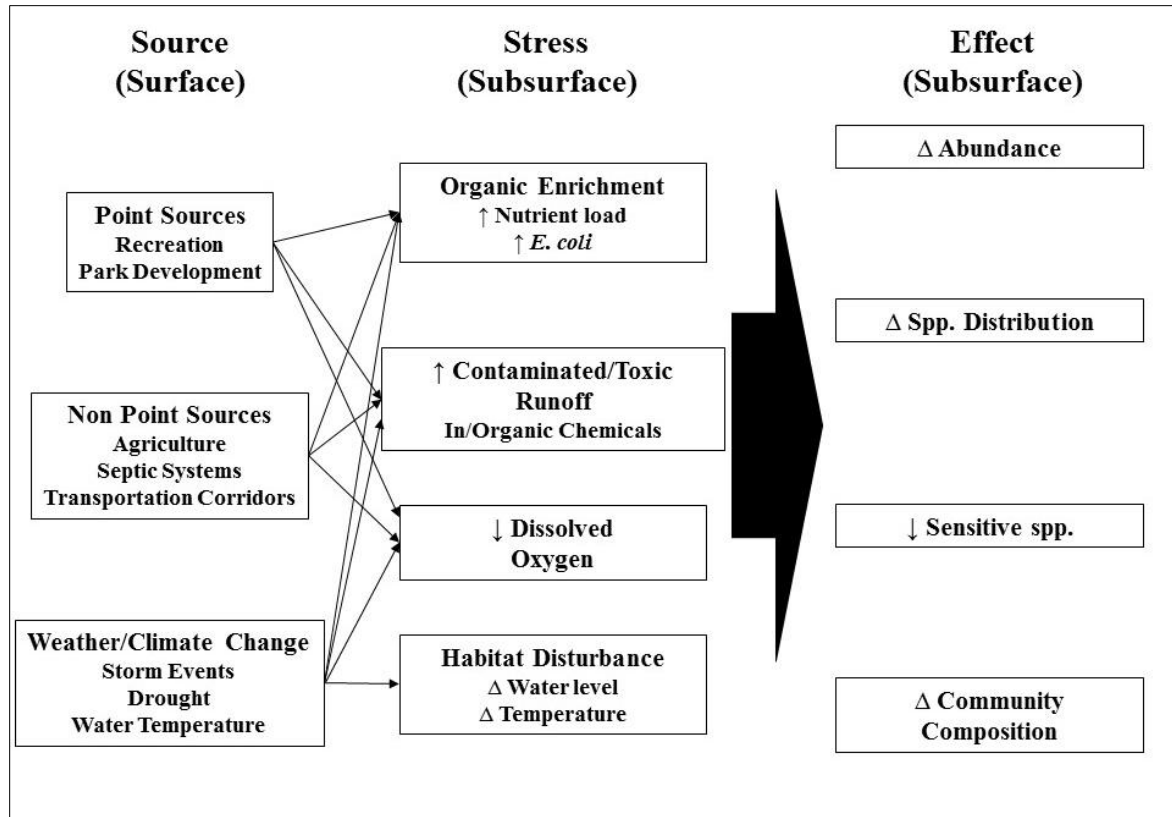


Figure 3. Cave stream conceptual model demonstrating the hypothesized links between threat source drivers on the surface, such as off-park land development, the resultant measurable perturbations to the subsurface habitat, such as water quality (e.g., decreased dissolved oxygen), and general hypothesized correlations (i.e., up and down arrows) with measurable subsurface attributes such as population metrics of cave aquatic biota.

Selected drivers, attributes and stressors will be monitored at CUPN parks either under this monitoring protocol (e.g., water quality), or other monitoring programs (i.e., vegetation community, water quality and quantity, land use change, and weather). When possible, with careful attention to matching temporal and spatial scales, we will examine how trends in these other data sets might correlate with estimates of CAB abundance, CAB occupancy, and observed changes in their habitat use. Stygobionts co-occur in caves with other currently monitored biota (i.e., cave crickets and bats) as well as those planned for monitoring (i.e., woodrats) in CUPN parks. Stygobionts are strongly linked to water-quality and water-quantity parameters, such as nutrient levels and dissolved oxygen (Panno et al. 2006; Lewis et al. 2015), but weakly linked with the aforementioned cave biota. This reflects the multi-faceted approach for monitoring park resources adopted by the Cumberland Piedmont Network wherein ecological indicators were chosen from broad categories such as ecosystem drivers, stressors, focal resources of parks, and key properties of ecosystem integrity (Leibfreid et al. 2005). Ecological integration among system drivers, components, structures, and functions was considered when CUPN vital signs were selected. Thus, the results obtained from monitoring the selected suite of vital signs should provide a comprehensive understanding of the ecosystem condition of selected park caves, as well as the status of resources of management interest.

Rationale for Monitoring Cave Aquatic Biota and Issues Being Addressed

Cave ecosystems were selected as one of several focal resources during workshops at four CUPN parks with major cave systems in 2002 (i.e., Mammoth Cave NP) and late 2011 (i.e., Chickamauga & Chattanooga National Military Park, Cumberland Gap NHP, and Russell Cave NM). The cave river ranked first among MACA's ecosystem "sub-models" at the end of the MACA Prototype Long-term Ecological Monitoring program's ecosystem attribute ranking and prioritization process in 2003 (Leibfreid et al. 2005). Among the attributes in the cave river ecosystem sub-model, cave aquatic biota was ranked second at Mammoth Cave NP. Resources management personnel at Russell Cave NM and Cumberland Gap NHP ranked cave aquatic biota as first and fifth, respectively, at their cave vital signs prioritization and selection workshops held in 2011. These rankings established the significance of monitoring CAB abundance and distribution at Cumberland Gap, Mammoth Cave, and Russell Cave.

Cave aquatic biota populations are vulnerable to chemical and thermal contamination from surface water flow because energy and contaminants are distributed within and between cave streams as a function of the watershed's hydrogeology (Figure 4). For example, following runoff-producing rainfall events, MACA's cave streams and springs recharged by basins with heavy agricultural land use show a sudden rise in nutrients, bacteria, and sediment (Meiman 2018). Moderate levels of nitrate-nitrogen and fecal coliform can result in an increase in the abundance of stygobiotic primary consumers such as cave isopods and amphipods (Simon and Buikema 1997; Graening and Brown 2003). High levels of nutrients and bacteria in cave stream and river reaches, however, are suspected of causing local extinctions in stygobiotic amphipods, due to reduced dissolved oxygen, in Kentucky (Lewis 1995), Arkansas (Graening and Brown 2003), and Illinois (Taylor et al. 2003; Panno et al. 2006). Finally, backflooding or reverse flowing springs can act as conduits for surface waters that carry food resources, contaminants, and water (with temperatures significantly outside cave normal range) into cave streams (Trimboli et al. 2016).

Some authors recommend regular, long-term monitoring efforts of rare, endemic, or listed endangered species, while others recommend including common species such as *O. pellucidus*, *A. spelaea*, and *Typhlichthys subterraneus* (Hobbs 1994; Pearson and Boston 1995; Pearson and Jones 1998; McCandless 2005; Pearson 2009). As top predators in the cave aquatic ecosystem *O. pellucidus*, *A. spelaea*, and *T. subterraneus* likely play a significant role in the food web (Helf and Olson 2017). McCandless (2005) recommends regularly monitoring MACA's Mystic River for *A. spelaea* since it has been abundant there in the past. Other MACA cave streams with reported abundant cave aquatic biota include Roaring River, River Styx, Echo River, and Logsdon River (Pearson and Jones 1998). The recent discovery of a genetically distinct cavefish species (i.e., *A. hoosieri*) also argues for long-term monitoring of common species, since it strongly suggests cave streams may contain more species than previously thought (Chakrabarty et al. 2014). Niemiller et al. (2012) suggest *Typhlichthys* spp. diversity is vastly underestimated with the implication that park metapopulations, as defined by Kéry and Royle (2015a), likely contain at least one species genetically distinct from other look-alike species in the region. McCandless (2005) found *A. spelaea* population clusters coincided with surface watersheds and so watersheds should be suitable for separating populations.

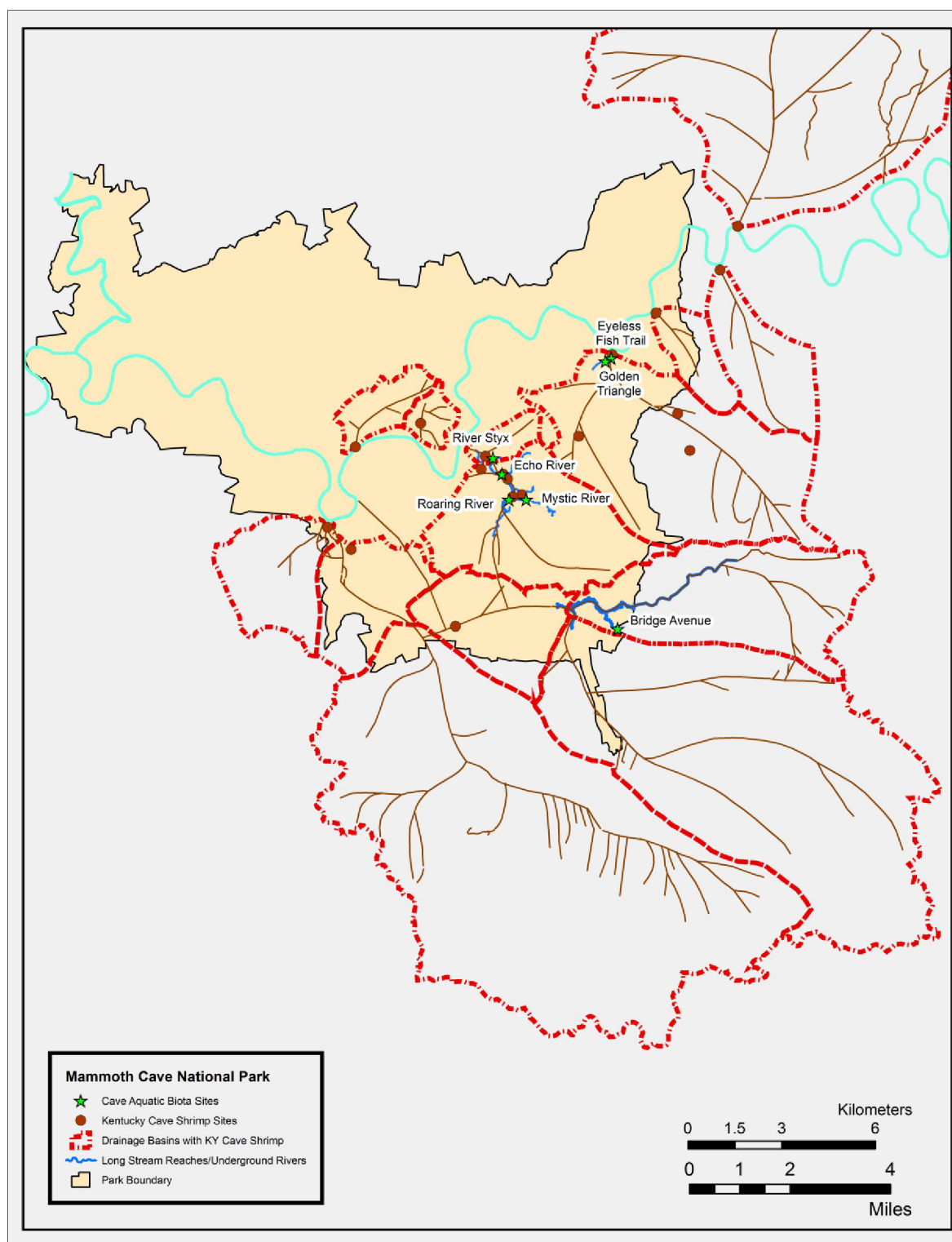


Figure 4. Water on its way to the Green River (light blue continuous line) moves (brown continuous lines) over and through local drainage basins outside and inside Mammoth Cave National Park and carries organic matter and contaminants through cave streams (dark blue lines). See Table 4 for a list of stream reaches planned for initial cave aquatic biota monitoring at all three Cumberland Piedmont Network parks.

Historical Development of Cave Aquatic Biota Monitoring at Mammoth Cave NP

James Karr developed the Index of Biotic Integrity (IBI) as a broadly based, multi-metric index that integrated information from individual, population, and community levels in order to assess the degree of human impact on fish communities (Karr 1981). Individual metrics are standardized to a score of one, three, or five, and then summed to produce a total score. Total IBI scores are ranked from least-impacted (high scores) to most impacted (low scores). Thomas Poulson developed the idea of using a modified Index of Biotic Integrity for MACA's cave aquatic ecosystem. It was developed and tested in the late 1980s and early 1990s by William Pearson and Tom Jones (Poulson 1990, 1991, 1992; Pearson and Jones 1993, 1996, 1998). The finalized cave aquatic ecosystem Index of Biotic Integrity introduced some effective field methods and tools for collecting data (Fitzpatrick et al. 1998).

A review of the modified Index of Biotic Integrity by CUPN ecologists, to determine whether it could be adapted to meet the Inventory and Monitoring Division's protocol needs, revealed several potential problems with the author's choices of IBI metrics and their interpretation. Three of the IBI's scoring criteria (e.g., percent of stygobiotic invertebrate taxa) clearly penalize ecotonal sites despite their expectation of high diversity due to the presence of both surface and subsurface organisms (Pearson and Jones 1996). The assumptions underlying their Isopod Recruitment metric, a negative correlation between isopod reproduction and human impact on their habitat, is contradicted by the literature which suggests moderate pollution may enhance isopod reproduction (Simon and Buikema 1997, Graening and Brown 2003). Indeed, the stygobiotic isopod *Caecidotea bicrenata* was among the first cave aquatic biota found to have recolonized Hidden River Cave after sewage influx was diverted (Lewis et al. 2015). Most importantly, the use of both isopod recruitment and stygobiotic fish recruitment as IBI metrics reveals a critical faulty assumption implicit to all the metrics developed for the modified Index of Biotic Integrity. It assumes all cave aquatic biota are always detected when they are present at a location, or that they all have the same probability of detection (Figure 5).

Three detectability states are possible: (1) present and detected; (2) present but undetected; and (3) absent. Without measuring detectability, the last two states are not distinguishable from one another. The several orders of magnitude size range among cave aquatic biota (i.e., 2–150 millimeters [mm] or 0.07–5.9 inches [in]) certainly influences their detectability and thus some metrics (e.g., total density of large organisms) have a disproportionate effect on IBI scores. Another implicit assumption of the IBI approach to account for is all stygobionts are identified with 100% accuracy; this is unlikely with smaller taxa. Difficulties inherent to sampling in cave environments are also likely a significant contributor to variation in detecting target cave aquatic taxa.

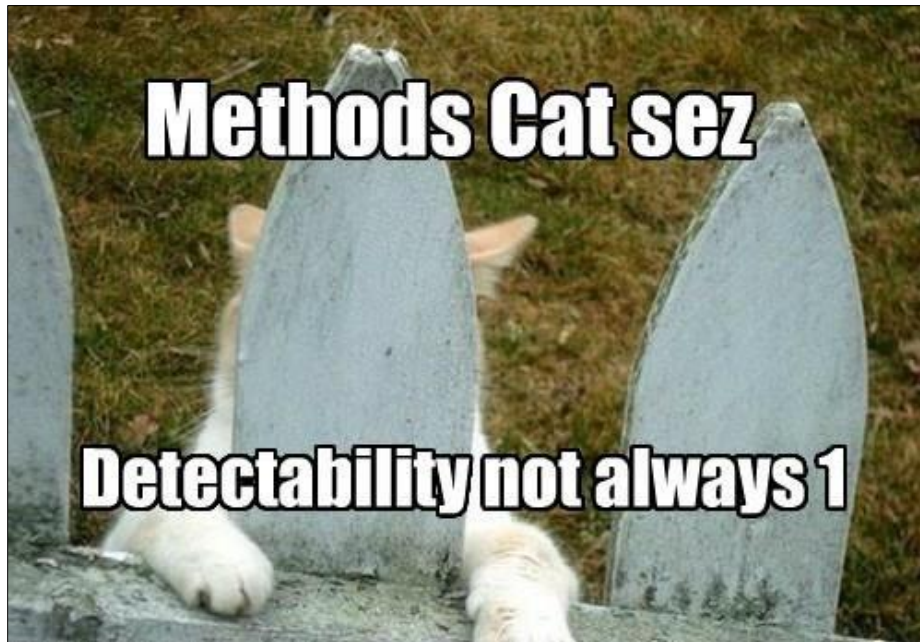


Figure 5. Constant detectability is unlikely for most species.

Abiotic factors that undoubtedly affect detectability, such as turbidity, can also be a “serious constraint on data gathering” (Pearson 2009). Furthermore, reach length at Mammoth Cave NP was not controlled for in the IBI analysis so comparing scores among reaches that differed by orders of magnitude in length or watershed size ignores the well-established relationship between area and species diversity (Rosenzweig et al. 2011). It is clear that accounting for the effects of abiotic and biotic factors on detectability for abundance and occupancy estimation should be incorporated as part of an efficacious, scientifically rigorous, and cost-effective method for monitoring cave aquatic biota that meets the needs of park management.

In January 2015, the protocol lead and park staff conducted two pilot sampling events at a MACA stream reach (Bridge Avenue) where we obtained count data on commonly observed cave aquatic biota. During each event, two separate teams consisting of an observer and recorder made timed, independent counts of cave aquatic biota in 20 non-contiguous 20-meter (65.6-ft) transects. To estimate the occupancy and abundance of cave aquatic biota we detected during the pilot, Dr. Chris Sutherland (University of Massachusetts-Amherst) used the R package ‘unmarked’ to fit *N*-mixture and occupancy models to these data (Table 2). We detected six CAB species in the field but only four frequently enough for analysis: two small (i.e., 2–15 mm [0.07–0.5 in]) crustaceans, a large (i.e., 1–8 centimeters [cm] or 0.3–3.1 in) fish species, and a large (i.e., 1–8 cm [0.3–3.1 in]) crustacean (*Stygobromus vitreus*, *Caecidotea stygia*, *T. subterraneus* and *Orconectes pellucidus*, respectively). Probability of detection at the reach level may be correlated with size of the organism. However, during the pilot (lack of) color may have played a role: *S. vitreus* is small and transparent whereas the other cave aquatic biota are much larger and white or pink. The estimates of CAB detectability for abundance (detectability per individual) were lower but with more abundant cave aquatic biota there are multiple chances to see at least one individual among multiple transects which makes for more

robust occupancy modeling. Additional cave aquatic biota we plan to monitor but did not detect during the 2015 pilot (e.g., *P. ganteri* and *A. spelaea*), are at least as abundant and detectable as the cave aquatic biota we did detect (Table 3). Results from this pilot effort were informative in the development of what we believe to be realistic, long-term measurable objectives for monitoring cave aquatic biota.

Table 2. Estimated abundance (λ) and occupancy (ψ) of cave aquatic biota, and associated CAB detection probabilities (p), for Bridge Avenue stream reach sampled during the January 2015 pilot efforts. All parameters are reported with associated standard errors. Dashes denote models that did not converge. Note that the low p and high ψ estimates for Copepod suggest this model is suspect and should be flagged. Models were fit using the R package ‘unmarked.’

Species	Abundance (N-mixture)				Occupancy			
	λ	se(λ)	p	se(p)	ψ	se(ψ)	p	se(p)
<i>C. stygia</i>	4.85	0.56	0.73	0.05	0.46	0.11	0.88	0.09
<i>C. tenebrosus</i>	–	–	–	–	–	–	–	–
<i>Copepod</i>	–	–	–	–	1.00	0.28	0.05	0.04
<i>O. pellucidus</i>	18.99	3.67	0.39	0.09	0.90	0.07	0.97	0.03
<i>S. vitreus</i>	0.98	0.49	0.41	0.20	0.63	0.29	0.40	0.20
<i>T. subterraneus</i>	5.28	1.78	0.36	0.12	0.94	0.11	0.69	0.10

Table 3. Target cave aquatic biota (CAB) groups and species for cave stream monitoring along with their planned derived data and park of origin. Most species in more numerous CAB groups will be collected at the level of order only as they cannot be distinguished in the field. Some of these target CAB taxa may be distinct enough to be identified to the species level (e.g., *Gammarus minus*) or common enough to derive abundance estimates (e.g., *C. stygia*). Most, however, are likely rare enough to only derive occupancy estimates (e.g., *Orconectes australis australis*). Note: At any given park the list of potential CAB detected is much longer than shown and, while they are not targeted for monitoring, will be part of species richness data.

Target CAB Group	Target CAB Species	Derived Data	Park
Crustaceans—Crayfish	<i>Orconectes pellucidus</i>	Abundance	MACA
	<i>Cambarus hamulatus</i>	Occupancy	RUCA
	<i>C. tenebrosus</i>	Occupancy	All
Crustaceans—Shrimp	<i>Palaemonias ganteri</i>	Occupancy	MACA
Crustaceans—Amphipods	<i>Stygobromus vitreus</i>	Occupancy	MACA
	<i>S. exilis</i>	Occupancy	MACA
	<i>S. cumberlandus</i>	Occupancy	CUGA
	<i>Crangonyx barri</i>	Occupancy	MACA
	<i>C. forbesi</i>	Occupancy	MACA
	<i>Bactrurus angulus</i>	Occupancy	CUGA
	<i>Gammarus minus</i>	Occupancy	CUGA/RUCA
Crustaceans—Isopods	<i>Caecidotea stygia</i>	Abundance	All
	<i>C. bicrenata whitei</i>	Occupancy	MACA
	<i>C. b. bicrenata</i>	Occupancy	RUCA
	<i>C. cumberlandensis</i>	Occupancy	CUGA
Fish	<i>Amblyopsis spelaea</i>	Occupancy	MACA
	<i>Typhlichthys subterraneus</i>	Abundance	MACA/RUCA
	<i>Forbesichthys papilliferus</i>	Occupancy	MACA
	<i>Cottus carolinea</i>	Occupancy	All
Amphibians	<i>Gyrinophilus palleucus palleucus</i>	Occupancy	RUCA

Measurable Objectives

At a minimum, the Cumberland Piedmont Network will provide resource managers with data on the occupancy (i.e., continued presence in the park) of all target cave aquatic biota (Table 3), species richness, and selected habitat characteristics, water-quality and water-quantity parameters in

monitored cave streams at three parks. Abundance estimates derived for a subset of common target CAB species will enable the network to determine long-term changes in their abundance, population demographics, and distribution. Occupancy estimates for less common target cave aquatic biota, derived from a summary of count data, will provide park resource managers with information regarding long-term changes in their distribution. Selected water-quality and water-quantity parameters and cave stream habitat characteristics will be used as covariates to examine their effects on CAB distribution, abundance, and detectability.

The Cumberland Piedmont Network's primary monitoring objectives are:

1. For common cave aquatic biota, estimate status and long-term trends in abundance within monitored cave stream reaches in three parks in the network.
2. For uncommon target cave aquatic biota, estimate status and long-term trends in the probability of occupancy and proportion of reaches occupied within monitored cave stream reaches in three parks in the network.
3. Sample selected cave stream habitat characteristics (e.g., riffle, pool, or glide and substrate composition) and water-quality (e.g., specific conductance, dissolved oxygen, pH, and nitrates) and water-quantity (e.g., continuous water level) parameters that we hypothesize are relevant to gaining an understanding of cave aquatic biota abundance, occupancy, or detectability within monitored cave stream reaches in three parks in the network.

Sampling Design

Site Selection

The major objectives of this protocol are to monitor changes in the abundance and distribution of selected cave aquatic biota over time. However, randomly or systematically selecting CAB sampling sites from all known cave streams is not practical due to accessibility, spatial heterogeneity in the abundance and distribution of target species, budgetary constraints, and resource and safety concerns. Therefore, our sampling frame consists of a limited number of sites, subjectively selected using the professional judgement of CUPN ecologists, the CUPN hydrologist, and MACA resource management specialists. These sites are located in underground streams and rivers scattered among several karst watersheds and subbasins in three CUPN cave parks.

Cave streams in all three CUPN cave parks are relatively well known because of their history of monitoring, inventory, or mapping. Field assessments by CUPN and park personnel were used to further narrow down the list of possible stream reaches. At Cumberland Gap NHP and Russell Cave NM, our choices of easily accessible cave streams to monitor were narrowed to one per park (Table 4). Both Cumberland Gap NHP and Russell Cave NM's cave streams were inventoried for many years by Holsinger and Culver (1988) and for many months by Hobbs (1994). Network and park personnel selected the more extensive group of potential monitoring cave streams at Mammoth Cave NP through careful review of previous inventory and monitoring efforts, cave mapping expeditions, and field assessments, as well as consideration of available operational requirements (Table 4). Most of MACA's cave stream reaches identified for monitoring in this protocol are legacy sites previously monitored by Pearson and Jones (1998) using the modified IBI technique. Seven stream reaches were subjectively chosen for their potential range of degradation (i.e., watersheds receive contaminated runoff from agricultural land use outside the park) as well as being conducive to biological survey while walking, wading, or snorkeling (Table 4). Thus, the area of inference for this protocol is limited to the underground stream reaches selected for monitoring.

Table 4. Cave aquatic biota monitoring sites at Cumberland Gap NHP (CUGA), Mammoth Cave NP (MACA), and Russell Cave NMt (RUCA). Note: Figure 4 shows the locations of MACA cave streams.

Panel	Park	Watershed	Cave Stream	Approx. Length (m)	Sampling Technique	Estimated Number of Transects
2	CUGA	Clinch River Subbasin	Gap Cave	4,000	Walk/Wade	27
1	MACA	River Styx Spring/ Echo River Spring	Styx/Echo River	1,150	Walk/Snorkel	17
2	MACA	Turnhole Spring	Bridge Avenue	450	Walk/Wade	9
1	MACA	Echo River Spring	Mystic River	1,800	Walk	18
1	MACA	Echo River Spring	Shrimp Pools/Roaring River	4,400	Walk/Snorkel	10

Table 4 (continued). Cave aquatic biota monitoring sites at Cumberland Gap NHP (CUGA), Mammoth Cave NP (MACA), and Russell Cave NMt (RUCA). Note: Figure 4 shows the locations of MACA cave streams.

Panel	Park	Watershed	Cave Stream	Approx. Length (m)	Sampling Technique	Estimated Number of Transects
1	MACA	Pike Spring	Eyeless Fish Trail/Golden Triangle	1,100	Walk	15
2	RUCA	Doran Cove	Picnic/Main Entrance stream	2,800	Walk/Wade/Snorkel	28

The following criteria were used to select cave stream reaches at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM:

- Cave stream reaches with optimal conditions for long-term monitoring including ease of accessibility (i.e., few reaches requiring technical caving skills), riffle(s), pool(s), or glide habitat structure(s), sufficient abundances of target species, sufficient room for sampling teams, and minimal disturbance to sensitive resources;
- Areas previously studied that could provide historical data;
- Unimpacted versus impacted cave stream reaches for comparison (Mammoth Cave only);
- Presence of federally listed endangered or state listed species of concern; and
- Available operational resources.

Adding Sites

Cave stream reaches that meet site selection criteria but are not monitored under this protocol are located in each park, including Mammoth Cave NP. If sufficient resources are available to add new cave stream reaches to a park's list they will be considered for inclusion in this protocol. Beyond the scope of this protocol, occasionally specific research questions or inventory needs may arise related to monitoring of cave aquatic biota at CUPN parks; participation by the project personnel will be considered on a case-by-case basis in consultation with the CUPN program manager, and any supervisors as appropriate.

Uncertainty of Estimates and Detectable Levels of Change

Estimates of status and temporal trends in abundance or occupancy are uncertain and usually expressed along with standard errors or confidence intervals. For monitoring results to inform resource management, the estimates must have an uncertainty low enough to help differentiate among alternatives. For this protocol, the alternatives might be explicit management alternatives, but are more likely to be presence of species in the seven different reaches, differences in abundances

either among species or among reaches (perhaps correlated to among-reach covariates), or trends over time.

The simulations testing the feasibility of N -mixture models (*Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Supplemental Materials*; Philippi 2018) provide estimates of the uncertainty for many combinations of parameter values, not just the final design. It is important to note that the simulations for temporal trend assumed annual sampling. Figure 6 gives the ratio of the estimated abundance to the simulated value for a range of detection probabilities (X axis) and abundances and total monitoring effort (Y axis). At an individual CAB detection probability of 0.4, abundances less than four individuals per 40 meters (131.2 ft) risk substantial overestimation of CAB abundances. Abundances above 40 individuals per 40-meters (131.2-ft) transect, or greater detection probabilities, generate estimates nearly all within a factor of two of the true value. We intend to investigate or develop the use of environmental DNA (e.g., Niemiller et al. 2018) to increase detection probabilities used to estimate CAB abundance and occupancy.

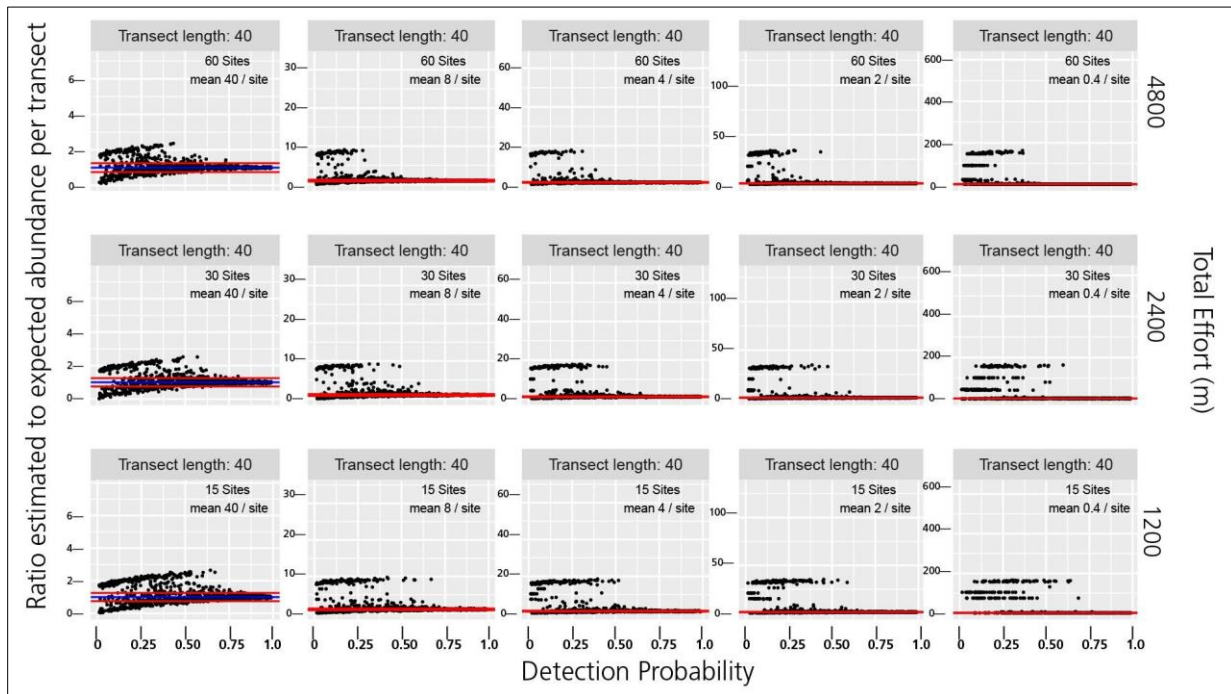


Figure 6. Ratio of estimated CAB abundance to expected abundance per transect as a function of individual detection probability p , for combinations of total effort (4800, 2400, & 1200 m), mean abundances (noted as mean per site or transect), 60 sites, 40 m transect lengths, and 2 visits per year. The X axis is detection probability; the Y axis is the ratio of the estimated abundance per transect to the expected abundance per transect; so 1 is an accurate estimate. The horizontal blue line is the expected value of 1; the red horizontal lines indicate estimates 25% above or below the true value. Columns of figures have the same mean abundances; rows of figures have the same number of total effort.

The simulations for temporal trends in abundance (Figure 7) only included transect lengths of 10 or 20 meters (32.8 or 65.6 ft), not the 40 meters (131.2 ft) in this final design. In theory however, the simulations with 2,400 meters (7,874 ft) total effort and 20-meter (65.5-ft) transects are equivalent to a design with 4,800 m total effort, 40-meter (131.2-ft) transects, and halved abundances. Figure 7 shows this sampling design's ability to detect a 50% decline (vertical red line) for park-wide inference. At 10% decline per year (6-plus years to 50 % decline; two left columns with "decline: 0.9"), even with the moderate year to year fluctuations built into the simulation, there was substantial power to detect population declines except for cave aquatic biota with low abundances. For the slower decline of 5% per year (13+ years to 50% decline; two right columns with "decline: 0.95") power is slightly higher possibly because the longer data set compensates for the masking effect of annual variation. This design will have greater than 80% power to detect the 5% per year decline before a 50% total decline for mean abundances of 0.25 per 10 meters (32.8 ft) and above (0.5 per 10 meters [32.8 ft] in the figure). Similarly, power analyses for temporal trends in occupancy only included transect lengths of 20, 25, and 30 meters (65.6, 82.0, and 98.4 ft, respectively) which indicates this design will have greater than 80% power to detect the 5% per year decline in proportion of sites occupied by large cave aquatic biota before a 50% total decline (M. Conroy 2016, unpublished simulation data).

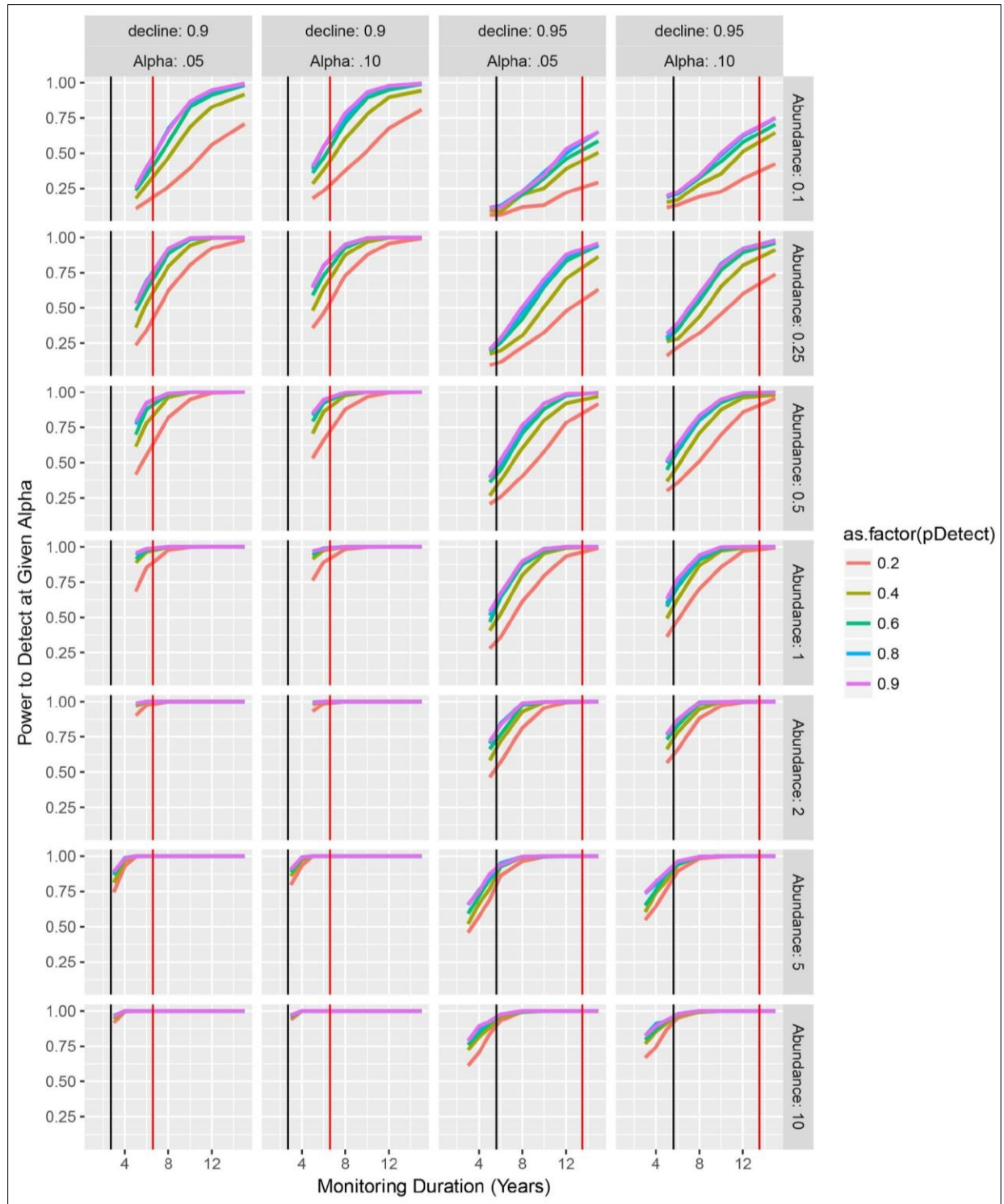


Figure 7. Power curves for $\alpha=0.05$ and 0.10 as a function of monitoring duration and CAB detectability; the total effort is 2,400 meters (7,874 ft), 60 sites, 20-meter (65.6-ft) transect length, and 2 visits. Vertical reference lines are the time of 25% (black) and 50% (red) cumulative decrease from the initial CAB abundances. Note that CAB abundance values in the row faceting are per m; the range from the top to bottom row is from 0.1 to 10 per 10 meters (0.33 to 32.8 ft).

Further analyses showed that trend estimates for abundance were unbiased, but that normal-theory confidence intervals for those estimates included the true value less than the nominal 90% or 95% of the time (Figure 8). The magnitude of this under-coverage increases with true abundance and detection probabilities; those species with the most power to detect trends will also have the least reliable normal theory confidence intervals. It is likely that confidence intervals based on parametric bootstrapping will provide better coverage. However, the problematic normal theory confidence intervals indicate the naïve estimator may be detecting “significant” trends even in the absence of trends. After a few cycles of data collection at all three CUPN parks we will re-assess.

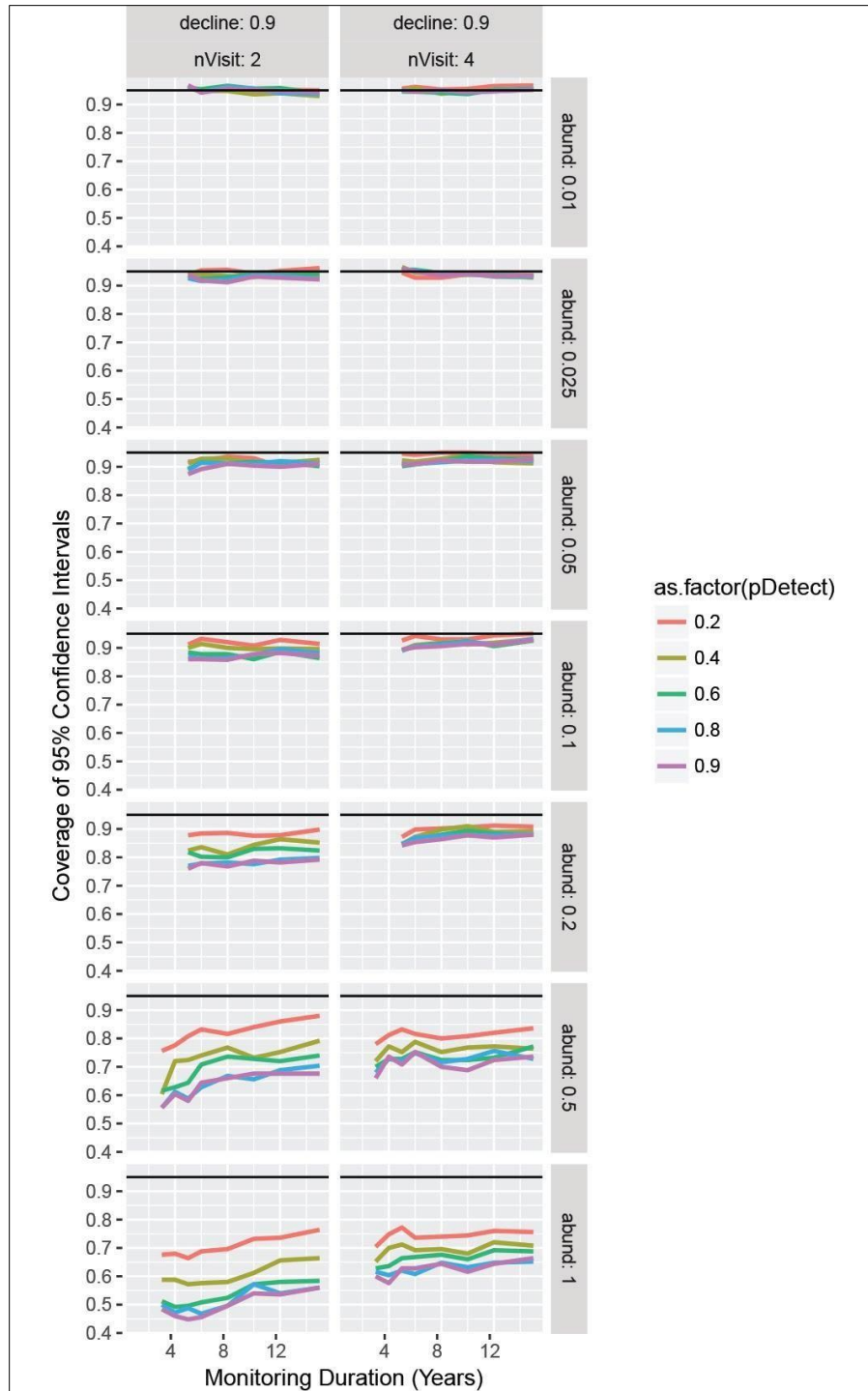


Figure 8. Coverage of the 95% confidence intervals for the estimated temporal trends and CAB detectability; the total effort is 2400 meters (7,874 ft), 20-meter (65.6-ft) transect length, 120 sites, and 2–4 visits. The Y axis is the fraction of the simulations with that set of parameter values that generated 95% confidence intervals for the temporal trend that included the true (simulated) value. Values near 0.95 are good. Values above 0.95 reflect overly conservative confidence intervals. Values below 0.95 reflect overly aggressive (overconfident) confidence intervals. Note that CAB abundance values in the row faceting are per m; the range from the top to bottom row is from 0.1 to 10 per 10 meters (0.33 to 32.8 ft).

The target power for monitoring Objectives 1 and 2 for each park are:

- Detect a 50% decline in abundance (at 5% per year or 10% per panel cycle) with a power of greater than or equal to 0.8 in abundance of common (i.e., greater than 0.25 per 10 meters [32.8 ft]) target cave aquatic biota (monitoring Objective 1) across all sites.
- Detect a 50% decline in the proportion of occupied sites (at 5% per year) with a power of greater than or equal to 0.8 in occupancy of uncommon (i.e., less than 0.5 per 10 meters [32.8 ft]) target cave aquatic biota (monitoring Objective 2).

General Sampling Approach

A sampling event consists of two teams, one observer and one recorder each, collecting data on selected water-quality and water-quantity parameters, CAB detection and nondetection, and habitat characteristics in a predetermined number of transects at selected cave stream reaches. Water-quality and water-quantity data are collected using opportunistic grab sampling and water level and temperature data loggers, respectively. Opportunistic grab samples are collected with pre-calibrated handheld sensors in a location upstream of transects to be surveyed; this is done at the same location as the data logger in its stilling well. Data logger locations will be determined by the project leader and the hydrologist based on field experience and previous water-quality and water-quantity monitoring efforts. Data loggers will be set to collect hourly data on water level and temperature. Visual surveys are the standard sampling approach for gathering CAB detection data and nondetection data. This involves the observer moving slowly and quietly upstream or around a pool using a powerful headlamp or dive light to search for and call out all cave aquatic biota detected to the recorder during independent timed counts. Visual survey is the primary method used by CAB researchers throughout the world and is considered to be a standardized technique (Pearson and Boston 1995; Pearson and Jones 1998; Trajano 2001; McCandless 2005; Graening et al. 2010).

Cave Stream Reach Sampling

Prior to the first survey, cave stream reaches were subdivided into a number of permanent 40-meter (131.2-ft) transects with an unstructured, equal-probability, spatially balanced approach using a Generalized Random Tessellation Stratified (GRTS) draw with the R package '*spsurvey*' (Kincaid and Olsen 2016). The GRTS design has been successfully applied in many NPS inventory and monitoring protocols and is a flexible, efficient, and statistically robust approach that accommodates the difficulties we may encounter during field sampling (e.g., lack of surveyable habitat) and allows us to replace transects as necessary while still maintaining spatial balance. Generalized Random Tessellation Stratified draws provide clear and valid inference relative to other sampling designs. Unlike random or systematic sampling, GRTS draws also provide maximum flexibility for replacing transects in a dynamic habitat (e.g., shifting substrates cover formerly flowing stream). The oversample allows us to replace transects or increase sample size while maintaining spatial balance and valid inference (unlike stratified sampling or judgement).

Our reasons for using 40-meter (131.2-ft) transects include:

1. Reduction of likelihood of CAB movement outside the transect for even the most wide-ranging taxa (Hobbs III 1978; Means and Johnson 1995);
2. Forty-meter (131.2-ft) long transects, particularly those in snorkeled reaches, are amenable to piloting the use of the double-observer method which allow the use of more statistically powerful analyses in future; and
3. Striking a balance between the competing interests of transect length and sample size; this is particularly true for short cave stream reaches. The number of transects in any given reach (Table 4) was subject to the constraints of fitting round trip travel time to the cave stream reach, preparation time in the field, reach length, and survey method into an 11-hour field day. Each sampling event includes two independent surveys of all transects in the cave stream reach conducted by two teams of two trained field personnel. Two sampling events will occur during each sampling season totaling four surveys per cave stream per sampling season.

Timing of Sampling

Sampling events are scheduled during the dry season (i.e., July–October) when precipitation is minimal and baseflow conditions provide safe conditions and the highest probability of detecting cave aquatic biota. Generally, antecedent conditions dictate how precipitation events affect safety and sampling conditions in any given cave stream reach. For example, during drought conditions 12.7 millimeters (0.5 in) of rain would not be cause for concern regarding either decreased safety or increased turbidity because the ground would absorb the runoff. However, if the ground is saturated the same amount of rain would lead to increased turbidity and decreased detectability of stygobionts in cave stream reaches. River levels, obtained by direct observation or checking stream gage sites on the web, are also a good indicator of sampling conditions. Sampling events will occur as soon as conditions and personnel resources permit.

Due to limited resources, it is impractical to sample all stream reaches in all three CUPN parks in one year so they are divided into two separate panels: Panel 1 = 4 MACA reaches; Panel 2 = 1 MACA reach, 1 CUGA reach, and 1 RUCA reach (Table 4). Sampling events (consisting of two independent surveys of each transect) will be conducted at one panel per dry season such that, conditions permitting, 14 events will be completed every two years. The two-year panel design is a tradeoff between the number of sites surveyed and the interval between return visits. A two-year panel enables us to collect data with a short interval between visits at a minimum number of park-prioritized sites. This panel design should enable us to detect trends in CAB abundance and occupancy relatively quickly and minimize the possibility of missing perturbations to local CAB communities between sampling years. During a given sampling year all scheduled cave stream reaches will be visited and then revisited as temporally close together as possible (i.e., at least two days and normally three to four days apart). Replicate sampling visits will be separated by a minimum number of days to allow conditions to resettle. The minimum number of days between replicate sampling visits will be based on the dominant habitat in a given reach. For example, reaches

consisting mostly of perennial stream can be revisited within two days because water clarity recovers more quickly in disturbed flowing water than it does in disturbed isolated pools.

Field Methods

Pre-field Season Preparation

Recurring pre-field season activities include:

- communication and coordination with park staff;
- logistics;
- permitting and compliance;
- job safety review;
- review of field information sheets, cave stream reach maps, and field sampling SOPs;
- gathering and maintenance of required supplies and equipment; and
- training of field crews on data collection and safety.

Communication and Coordination with Park Staff and Logistics

The project leader is responsible for pre-field season communication and coordination with park staff and logistics (Refer to Table SOP 2-1 in SOP 2 *Pre-Field Sampling and Safety—Version 1.0*; Helf et al. 2018a). The project leader will email the key park contacts **at least** two weeks prior to each sampling event to provide the proposed dates (and backup dates) and locations where CAB monitoring fieldwork is scheduled to take place. Once specific dates for each sampling event are selected, the project leader secures a vehicle and makes other necessary arrangements (e.g., access to cave entrances at Mammoth Cave NP may require a four-wheel-drive vehicle or a boat). Specific recommendations for accessing each cave entrance and detailed directions to each cave stream reach are provided in the form of a field information sheet for each selected cave (Figure SOP 2-1 in SOP 2; Helf et al. 2018a). The project leader will attempt to reserve in-park lodging as soon as field sampling dates are selected each year. If no in-park lodging is available, nearby commercial lodging at government rates will be secured by the project leader.

Permitting and Compliance

The project leader will obtain a current National Park Service (NPS) research permit for each park scheduled for CAB monitoring. The permits are completed in November of each year (if possible, permits are requested for two or more years to reduce the amount of paperwork). Some parks allow multi-year permits to cover long-term monitoring projects, while others require annual renewal. The project leader will ensure each park has the current protocol for monitoring cave aquatic biota and a list of sampling locations so compliance issues can be addressed. The project leader will obtain the appropriate state and federal permits (e.g., NPS Institutional Animal Care and Use Committee, and U.S. Fish and Wildlife Service [USFWS]).

Job Safety Review

The Cumberland Piedmont Network considers the occupational health and safety of its employees, cooperators, and volunteers to be of utmost importance. The network is committed to ensuring all field crews receive adequate training on NPS safety procedures, incident reporting, and emergency response prior to fieldwork. Working in a cave environment can present safety hazards, and certain activities (e.g., vertical caving) necessitate specialized training and experience. Between field seasons, field crews involved with specialized cave work must maintain their physical condition and skills appropriately as time and opportunities allow. An important tool used to promote safe conduct is the Job Hazard Analysis (JHA). This approach is consistent with NPS Directors Order 50 and Reference Manual 50B for Occupational Health and Safety (National Park Service 2008). The JHA process is to (1) identify hazards associated with field and laboratory settings, as appropriate, and (2) develop approaches to mitigate those hazards. Prior to conducting the first CAB sampling event each year, all field personnel involved must **read and sign** the related JHA in SOP 2 (Table SOP 2-7; Helf et al. 2018a) and agree to follow all safety guidelines during sampling. Prior to each sampling event all participating field personnel must **review** the JHA and agree to follow all safety guidelines during sampling.

Prior to the first CAB sampling event each year a trip plan is filed with a CUPN contact (i.e., someone in the office during the trip) and the park (see the example trip plan, Figure SOP 2-3 in SOP 2; Helf et al. 2018a). A trip plan will include items such as trip purpose, sampling dates and times, daily check-in and check-out procedure, field crew members, contact information, and cave stream reaches. A surface contact person (“surface watch”) will be established before each trip to a cave. Clearly establish with the contact person: (1) the time to expect the crew to return (or call-in after exiting the cave); and (2) whom and when to notify (i.e., the response lead) in the event the crew does not contact on time and after attempting to contact the crew. The surface contact person will be given a copy of the trip plan, as well as travel route maps (surface and subsurface) for each cave to be visited. After sampling work is completed, a post-field review or “tailgate session” will be held to discuss any safety or other procedural concerns.

Review of Field Information Sheets, Cave Stream Reach Maps, and Field Sampling SOPs

Field information sheets describing conditions at each CAB stream reach will help orient new crew members, increase efficiency, and greatly improve the consistency of data gathering through time. The Field Information Sheet template (Figure SOP 2-1 in SOP 2; Helf et al. 2018a) provides an outline of the types of information to be included. A field information sheet will be developed for each cave prior to the first sampling. Before every field-sampling event, each appropriate information sheet will be reviewed. For each cave stream reach to be surveyed, Cave Stream Reach Maps with delineated transects will be created and used as a means of including a spatial component to the counts in order to assess distribution of cave aquatic biota within a cave stream reach. The Cave Stream Reach Maps will also be used for plotting and labeling the locations of HOBO data loggers and permanent landmarks. SOP 2 (Figure SOP 2-2; Helf et al. 2018a) provides an example of a Cave Stream Reach Map. Each appropriate Cave Stream Reach Map will be reviewed prior to every field-sampling event.

Supplies and Equipment

The project leader, or designated field crew leader, is responsible for organizing, preparing, repairing, or purchasing supplies and equipment for sampling. Prior to each field-sampling event the project leader, or designated field crew leader, will assemble all required group caving gear and sampling supplies or equipment (see Tables SOP2-3, SOP2-4, SOP2-5, and SOP2-6 in SOP 2; Helf et al. 2018a). This includes instrument calibration (e.g., water-quality and water-quantity sensors). Field crew members, including observers, recorders, and water samplers will maintain their individually assigned caving gear to make sure it is ready for safe use (Table SOP 2-6 in SOP 2; Helf et al. 2018a). Equipment and caving gear will be checked for damage requiring repairs or replacement and returned to the appropriate office or person for proper storage. At the end of each sampling season the project leader or designated field crew leader will inventory the condition of all equipment, prepare a list of required repairs or replacements, and complete and sign and date a copy of the appropriate checklist (see SOP 2; Helf et al. 2018a).

Training of field crews on data collection

Training sessions are necessary for all project crew members, especially crew members new to the project. Training sessions include: an orientation session summarizing this protocol, pre-sampling event briefings, sampling dry runs, data entry, data processing, safety procedures, and white-nose syndrome decontamination requirements and procedures. Training includes demonstration and practice of all measurement procedures. Once the project attains a critical mass of reliable crew members, training sessions for some tasks will be conducted as needed. However, sampling dry runs will be held prior to **each** sampling season so all field crew members can “re-calibrate” their ability to identify cave aquatic biota and estimate their size. A more detailed list of protocol tasks that all crew members need to master can be found in Table SOP1-1 in SOP 1 *Training Personnel—Version 2.0* (Helf et al. 2018a). The project leader, a permanent employee intimately familiar with cave stream reaches and with years of experience employing this protocol’s sampling techniques, will conduct training sessions. It is important for the project leader or field crew leader to establish roles in advance and maintain good communication to ensure efficient and accurate work.

Cave Stream Reach Sampling

Opportunistic Grab Sampling of Water Quality

To address CUPN’s monitoring Objective 3, we will collect most water-quality data at all cave stream reaches at each sampling event, using methods detailed in the *Cumberland Piedmont Network Water-Quality Monitoring: Standard Operating Procedures* (Meiman 2018). Data on water-quality parameters are gathered at the reach, from the same location as the data logger (see below), by opportunistic grab sampling with pre-calibrated handheld sensors. Measured parameters include specific conductance, water temperature, dissolved oxygen, and pH. During each sampling event water samples will also be collected from the same location as the data logger, to be analyzed in the lab for turbidity, nitrates and *E. coli* by trained NPS personnel. A quality assurance/quality control (QA/QC) replicate sample for the handheld sensor and lab parameters will be collected and analyzed during two randomly selected events per year.

Continuous Sampling of Water Quantity

The HOBO U20L data loggers in stilling wells will sample water level, the height of the water column above the data logger, and water temperature continuously in all monitored cave stream reaches. Water level will be used to generate the frequency and magnitude of flooding events as a way of measuring the physical forces acting on cave aquatic ecosystems during periods of high and low flow. Water temperature will be used as a covariate to investigate its effects on detectability and occupancy. Procedures associated with the use of HOBO U20L data loggers will generally follow those detailed in Curdts (2017). Cave water-quantity field sampling activities broadly include data logger deployment, service, replacement, and removal. Field personnel should refer to the cave-specific Field Information Sheet and Cave Stream Reach Map to navigate through the cave to the data logger(s). After the second CAB surveys of the season, the water-level data from each sampled cave stream reach are downloaded from the data logger to a waterproof shuttle and uploaded to Aquarius. Correlative data on precipitation, gathered continuously by Soil Climate Analysis Network weather stations, will be downloaded from USDA National Water and Climate Center websites prior to performing data analysis.

Initial pre-launched automated HOBO U20-L pressure transducers (hereafter, data loggers) will be deployed in cave stream reaches as practicable. Procedures associated with installation of stilling wells for HOBO U20L data loggers will generally follow those detailed in Curdts (2016). Data logger sampling intervals will be set to collect water level and temperature data at one-hour intervals. Because the data logger's battery life only extends five years, its "Battery Status" is recorded on the HOBO Water Level and Temp Data field data sheet during the launching process. Required metadata related to data logger deployment will be recorded on the HOBO Water Level and Temp Data logger field data form (Appendix A in SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*; Helf et. al 2018a). After the data logger's initial deployment, a permanent landmark (e.g., a distinct cave rock formation, cave survey station) is selected to use as a reference point for its relocation. Detailed written descriptions of the data logger and landmark locations are recorded on the field data form, both the landmark and the data logger are photographed in situ, and the approximate location of each data logger and corresponding landmark is plotted and labeled on the field copy of the Cave Stream Reach Map.

Each previously deployed data logger located in cave stream reaches will be serviced. This process includes data offloading, redeployment (i.e., restarting automatic data collection), examining, cleaning, and reinstallation. Required metadata related to data logger servicing will be recorded on the field data form (see Appendix A in SOP 3; Helf et al. 2018a). Location information for previously deployed data loggers is included on the cave's Field Information Sheet. When the previously deployed data logger is nearing the end of its two-year rotation or there is some doubt about its reliability (e.g., data transfer was not successful) it will be replaced with another pre-launched data logger. Required metadata related to data logger replacement will be recorded on the field data form (see Appendix A in SOP 3; Helf et al. 2018a).

A previously deployed data logger will be removed from a location when:

- it is nearing the end of its battery life;

- there is some doubt about the reliability of the previously deployed data logger and no pre-launched replacement is available;
- it is no longer safe or prudent for a data logger to be placed in that location (e.g., loss or damage due to flood intensity); or
- it was previously instructed by the Project Lead. Required metadata related to data logger removal will be recorded on the Field Data Form (see Appendix SOP 3-A in SOP 3; Helf et al. 2018a).

Direct Visual Counts of Cave Aquatic Biota

To address the CUPN's monitoring Objectives 1 and 2, the field personnel split into two teams who conduct staggered, independent 15-minute counts of cave aquatic biota by walking, wading or snorkeling in cave stream transects. Observers carefully search the water column, bottom, and any cover (e.g., rocks, woody debris), call out any species detected, its estimated size, and where they were found (e.g., open or under cover) and recorders write the data on the CAB field data sheets. Sampling techniques differ depending on whether the cave stream reach being sampled is wadeable or nonwadeable. Wadeable stream reaches are generally shallow so their transects can be surveyed while walking along the bank. In the rare circumstance where a wadeable stream reach is wide or extends across the entire passageway, observers must perform one survey by roughly dividing the transect in half. In this situation, observers work together to avoid double-counting cave aquatic biota while wading upstream side by side. Transects in cave stream reaches with a majority of nonwadeable habitat must be surveyed by snorkeling observers, with recorders paddling inflatable kayaks behind (Figure 9). While visual surveys are biased toward the detection of conspicuous individuals this works in the protocol's favor, particularly in deeper streams, since many CAB target species are pigmentless or depigmented (e.g., cavefish or cave crayfish) and highly visible. Visual survey for smaller, less conspicuous cave aquatic biota (e.g., cave shrimp) by trained observers has nevertheless been very effective in the past (Pearson 2009). Cave aquatic biota are later categorized into qualitative size classes. Finally, we will collect limited qualitative data on CAB habitat covariates using USGS National Water-Quality Assessment (NAWQA) methods for biological surveys (Fitzpatrick et al. 1998). Field personnel will score cave stream reach habitat covariates such as habitat type (e.g., riffle, pool, or glide). Observers also determine a consensus estimate of the percent substrate composition for the transect or pool; a transparent SciOptic grain size chart may be used to standardize their observations of substrate grain size and composition (e.g., gravel and pebbles).

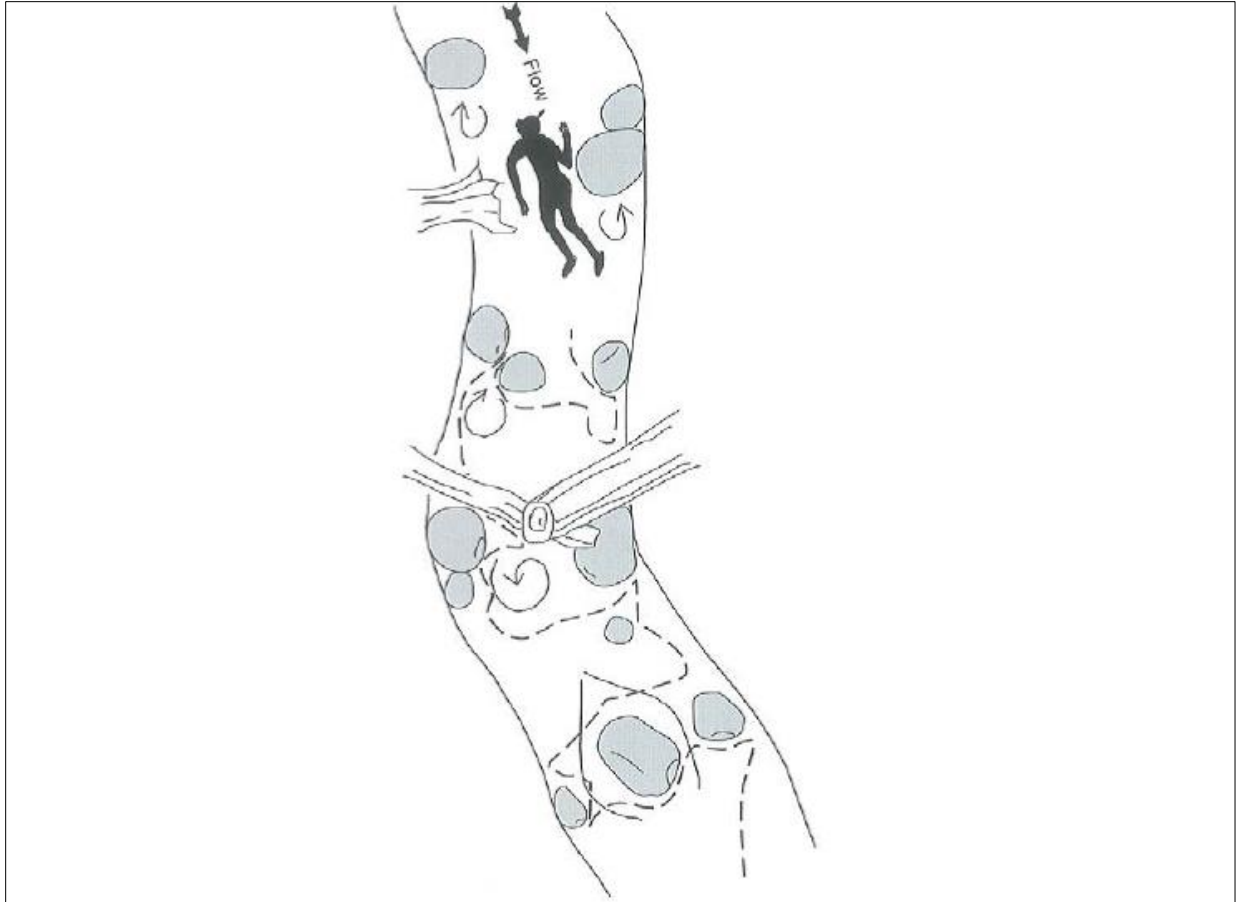


Figure 9. A snorkeler surveying in a single pass zigzags through the cave stream reach while moving upstream. The dashed line approximates the surveyor's path (Thurrow et al. 2006, Apperson et al. 2015). The recorder follows some distance behind in an inflatable kayak.

All parameters were determined to be appropriate for use as covariates in our analyses through an extensive review of the relevant literature and field visits (Pearson and Jones 1996, Simon and Buikema 1997, Fitzpatrick et al. 1998, Meiman 2018). A sequential list of major tasks the field crew will follow during each sampling event can be found in the “Step by Step Instructions” section of SOP 3.

Variables and Measurements

Table 5 provides a summary of the primary measurements and variables that will be collected during field sampling and post-field sampling analyses. Detailed descriptions of each, collection methods, and secondary measurements (e.g., start time of sampling event, distance from landmark to the data logger) are included in SOP 3 (Helf et al. 2018a), SOP 4 *Post-Field Sampling—Version 1.0* (Helf et al. 2018a), and SOP 6 *Data Analysis and Reporting—Version 1.2* (Helf et al. 2018a). The standards used by the Cumberland Piedmont Network for activities related to the collection, processing, storage, analysis, and publication of monitoring data as described in this narrative and its associated SOPs can be found in *Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Data Quality Standards* (Helf et al. 2018b).

Table 5. Major sampling methods, and primary measures and variables that will be collected or calculated during implementation of the protocol for monitoring cave aquatic biota.

Sampling Category	Sampling Method	Measures	Variables
CAB Sampling (see SOP 3; Helf et al. 2018a)	Direct Visual Observation (CAB Detection/Nondetection)	Total # CAB counted per taxon per transect	abundance occupancy of cave stream reaches mean, max/min CAB length (mm) location detected (under rock, in woody snag, open; riffle or non-riffle) CAB size category (small, medium, large)
	Direct Visual Observation & Assessment (Habitat Characteristics)	Habitat type and measurements Substrate type	pool, riffle, glide percent cover searched max pool length, mean transect/pool width mean transect/pool depth percent makeup of substrate categories
Cave Stream Water Quality and Water Quantity Sampling (see Curdts 2017, Meiman 2018)	Opportunistic Grab Sampling (handheld sensors)	Temp (°C) Specific Conductance (SC) pH Dissolved Oxygen (DO) Turbidity Nutrients (samples analyzed in lab) Bacteria	water temp (°C) SC pH DO mean, max/min Turbidity levels of NO3 and E. coli
	Continuous Sampling (HOBO data loggers)	Water level Temp (°C)	mean, max/min water level mean, max/min water temp (°C)

Post-Field Sampling

Procedures to follow after completion of a field sampling event can be found in SOP 4. Recurring post-field sampling activities include the following categories:

- logistics (e.g., return keys or hang tags, write trip report);
- transit from the field to the office;
- arrival at the office;
- clean and decontaminate gear following the latest national WNS guidance;
- handling field data forms;
- processing HOBO data logger data files; and

- equipment inventory, safety check, and storage.

The tasks detailed in SOP 4 (Helf et al. 2018a) cover the approximate period of time from when field personnel have left the cave after a sampling event (or HOBO data logger deployment, collection, replacement, or removal) has been completed to the point of starting data analysis (covered in SOP 6; Helf et al. 2018a). This SOP also covers procedures to follow at the end of a sampling season. The project leader is responsible for post-field season communication and coordination with park staff, as well as logistics. No voucher specimens are planned for collection under this monitoring protocol. However, specimens may be collected at the request of the park's resource managers or researchers with current permits.

The designated surface contact person will be promptly notified by cell phone, radio, or satellite communicator when the field team has left the cave. Any NPS staff or volunteers injured in a non-emergency incident during field sampling will complete required Worker's Compensation paperwork (must be done within 48 hours of incident). After a sampling event is complete a post-field "tailgate session" will be held so all field crew members can discuss any safety or other issues that arose with the monitoring procedures with the project leader. At the end of each sampling season the project leader or designated field crew leader will inventory the condition of all equipment and prepare a list of required repairs or replacements.

Data Handling, Analysis, and Reporting

This section provides an overview of the procedures for data handling, analysis and reporting. Additional detail and context can be found in the CUPN Data Management Plan (Moore et al. 2005), as well as task-specific SOPs for this protocol, including SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0* (Helf et al. 2018a), SOP 4 *Post-Field Sampling—Version 1.0* (Helf et al. 2018a), SOP 5 *Data Management—Version 0.2* (Helf et al. 2018a), SOP 6 *Data Analysis and Reporting—Version 1.2* (Helf et al. 2018a), and SOP 7 *Revising the Protocol—Version 1.0* (Helf et al. 2018a).

Data and information are the basic products of scientific research (Michener and Brunt 2000). With this in mind, the Cumberland Piedmont Network’s success will be judged by its ability to provide relevant high-quality data to current and future end users. The goal is to put data handling procedures in place to ensure high quality data are available, with appropriate context, so that informed resource management decisions can be made. It is important that data handling procedures not only be documented, but also **implemented in a consistent manner**. To accomplish this, staff must be made aware of their assigned roles and responsibilities and receive the tools and training necessary to accomplish their tasks throughout the project life cycle. Data quality is improved by ensuring field crews are properly trained and equipped to minimize the occurrence of ‘alternative’ modes of data collection and to reduce the frequency of incorrect or incomplete field data forms or notebooks. Refer to SOP 2 *Pre-Field Sampling and Safety—Version 1.0* (Helf et al. 2018a) for additional details.

Data Collection

Monitoring data are recorded in the field into a set of pre-printed, customized paper field forms (CUPN Water Quality Monitoring Protocol Figure SOP 3-1 and Appendix D in SOP 3; Helf et al. 2018a):

- Water Quality Grab Sample Field Data Sheets;
- Cave Aquatic Biota Monitoring Field Data Sheets,
 - Sampling Event Metadata,
 - Habitat Characteristics Metadata,
 - CAB Field Data; and
- Water Level/Temp Data logger – Cave Stream Reach Setup/Maintenance Field Data Sheet.

A copy of each of these sheets and instructions on completing the field data sheets (data field description and formatting requirements) is included in SOP 3 (Helf et al. 2018a), along with SOP 3 *Collection of Field Data—Version 1.2* and SOP 4 *Field Measures—Version 1.2* of the CUPN water-quality monitoring protocol’s standard operating procedures (Meiman 2018).

The project leader or field crew leader will designate data recorders for the field-sampling event. These individuals are responsible for accurately and legibly recording all data elements. Other crew members, acting as observers, will call out data. The data recorders will repeat the values before and while recording. This approach creates a real-time double check in the field and is an important step in the QA/QC process. Before leaving the transect, each sampling team will be responsible for reviewing data forms for completeness and legibility and documenting the review by initialing the forms. Upon returning to CUPN headquarters at Mammoth Cave NP, field data sheets (or notebooks) will be decontaminated, dried, and returned to the project leader for storage until post-processing is completed, and they are ready for database entry.

Digital files associated with automated HOBO data loggers will be uploaded to the CUPN file server, and post-processed and validated in Aquarius. Refer to SOP 4 (Helf et al. 2018a) for additional details. The GPS units will be returned to the GIS specialist, who is responsible for stewardship of geospatial data. The field crew leader will alert the GIS specialist if any new waypoints were marked on the GPS units during field sampling.

Overview of Database Approach

The Cumberland Piedmont Network's CAB database will be developed in Microsoft Access. Basic structure of this database will conform to the current National Inventory and Monitoring Program (NPS I&M) standards (Frakes et al. 2015). This application will contain "core" CAB data (i.e., biota detections, habitat characteristics, and associated metadata). In addition, water-quality data will be entered and managed locally in NPStoret, a Microsoft Access application developed by NPS's Water Resources Division. Water level and temperature data will be imported and managed in Aquarius, a commercial off-the-shelf software package recommended and used by multiple Inventory and Monitoring Division networks for continuous data. Additional details on each of these applications can be found in SOP 5 (Helf et al. 2018a).

Data Entry

Data entry is conducted by someone who has been trained in the use of the specific database application and has reviewed the database user manual. This individual should also have familiarity with the data collection procedures and preferably be someone who has participated in field data collection. Data entry should take place as soon as possible following data collection. Data entry forms in the CUPN cave aquatic biota database will be patterned as much as possible to mimic the paper field data forms and the database will have quality components such as pick lists and validation rules to further limit the possibility of incorrect or incomplete data entries. Care should be taken to avoid any potential entry errors. Refer to SOP 5 (Helf et al. 2018a) for additional details.

Quality Review

As part of data entry, database entries are reviewed against the original source data to ensure accuracy (i.e., verification). Refer to SOP 5 (Helf et al. 2018a) for additional details on entry and QA/QC steps. In addition, some basic queries including species count summaries, date ranges, and max and min values, will also be utilized to help the data entry technician verify that data are accurate and complete. Data verification is followed by a review by the project leader, who will check the data for completeness, as well as logical inconsistencies (i.e., data validation). Tools to aid

in validation are incorporated in each of the database applications. Refer to SOP 5 for additional details.

Data Archiving and Curation

All final electronic data sets and material associated with CAB monitoring in the Cumberland Piedmont Network will be archived and curated according to NPS policy and procedures.

Electronic Data Sets

Final verified and validated electronic data sets for CAB monitoring will be archived by the data manager with all supporting documentation, including documentation of data files, and data management procedures. Version control and file naming conventions are used to manage changes made to data sets or documents while safeguarding against loss or damage to data. Data sets destined for archiving will be stored locally within an object-oriented file structure established on the CUPN file server. Tape backups of all project data on the server are regularly conducted. Backup copies of the data are maintained in a secure alternate location. Working GIS files are maintained by the CUPN GIS specialist. Final GIS files will also be archived on a shared network drive.

Archives

Any material (preferably originals but can be copies) associated with the project (e.g., photos, maps, field information sheets, field data forms or notebooks, and video recordings) will be archived and entered into the Interior Collection Management System (ICMS). After these materials are archived and entered into the Interior Collection Management System, they will be returned to the park or a repository requested by the park along with their corresponding ICMS records.

Metadata Procedures

Metadata has been defined as structured information about the content, quality, and condition of data. All geospatial data will be documented with appropriate Federal Geographic Data Committee (FGDC) and NPS metadata standards. For tabular data sets, all database objects (e.g., tables, fields) will be defined and documented in a data dictionary or in Section 5 of a FGDC compliant metadata file, as required.

Protected Data

Generally, data are protected when releasing the information will “result in harm, theft, or destruction of the resource” (National Park Service 2010). Such information must not be shared outside the National Park Service unless a signed confidentiality agreement is in place. Refer to SOP 5 (Helf et al. 2018a) for additional details.

Planned Routine Data Summaries and Analysis

The primary goal of this protocol is to identify changes in CAB abundance and occupancy for resource managers at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM. To meet this goal, we must account for the variation in detectability over time and space, especially in the perpetual darkness of cave environments, to provide reliable data for effective resource management. Accordingly, the unknown, true abundance of an organism (N) may be estimated as the ratio of a

count of organisms (C) over the probability (p), obtained over multiple surveys, that in an occupied habitat an organism will be detected and included in the count (Williams et al. 2002):

$$N = C/p$$

Hierarchical models (HMs) are a flexible framework in which a complex system may be modeled using multiple submodels. This framework is particularly suited to inference about abundance, occupancy, species richness, and demographic characteristics because it can accommodate multiple data sets, sources of variability, scales of measurement, and varying data quality (Yamaura 2013; Kéry and Royle 2015a; Brown et al. 2017). We believe the flexibility inherent in the modular nature of HMs will be useful for the reliable estimation of occupancy and abundance of imperfectly detected cave aquatic biota. Further, with our selected covariates we will attempt to account for variation in abundance among reaches. There is precedent for using these modeling techniques in the estimation of abundance and occupancy of both surface and subsurface aquatic organisms (Krecja and Weckerly 2007; Collier and Fenolio 2009; Schneider et al. 2009; Peterman et al. 2013; Coleman et al. 2016; Mollenhauer and Brewer 2017).

N-mixture Model Framework

The utility of a class of Hierarchical models called N -mixture models lies in their use of two linked Generalized Linear Models (GLMs): a state submodel for the organism's true but unobserved spatial variation in abundance (N), a result of ecological processes; and an observation submodel to account for the measurement error inherent in field counts (n) which is a function of p (i.e., the probability of detecting individual organisms) and unobserved abundance (N) (Kéry and Royle 2015a):

- State process: $N_i \sim \text{Poisson}(\lambda)$
- Observation process: $n_{ij}|N_i \sim \text{Binomial}(N_i, p)$

Where N_i is the abundance at site i ($i = 1, \dots, M$); the *Poisson* distribution is used to model spatial variation in N ; λ is the mean abundance over all sites; n_{ij} is the count at site i during survey j ($j = 1, \dots, J$); the *Binomial* distribution describes the measurement error associated with the observation process; and p is the per individual detection probability.

In the N -mixture model framework, all that is needed to estimate detection probability and abundance is simple counts of unmarked cave aquatic biota replicated in time and space at all sites. However, the HM framework is also robust to less balanced data sets in which replication is not achieved at all sites and times, since information about detectability at replicated sites is “shared” with unreplicated sites. Key assumptions of the N -mixture model are: population closure within a sampling season, no false positives (i.e., double-counting or species misidentification), individuals are detected independent of one another (i.e., no schools or flocks), detection is homogeneous among individuals, and various parametric modeling distribution-specific assumptions hold.

Occupancy Model Framework

Detection/nondetection data, or occupancy data, are useful for making inferences regarding the multiple, linked processes underlying our observations of cave aquatic biota whose abundance cannot

be reliably estimated (Kéry and Royle 2015b). In fact, most of our CAB data will fall into this category for which the so-called site occupancy model (hereafter, occupancy model) is the appropriate model (see Table 3). Occupancy models also use two linked Generalized Linear Models: a submodel for the occupancy state at a site (z) and the proportion of occupied sites (ψ) and an observation submodel accounting for our measurement of a species occurrence (y) conditional on z and detection probability p (Kéry and Royle 2015b).

- State process: $z_i \sim \text{Bernoulli}(\psi)$
- Observation process: $y_{ij}|z_i \sim \text{Bernoulli}(z_i, p)$

Where z_i is the true state of occupancy at site i ($i=1, \dots, M$); the *Bernoulli* parameter ψ is the expected value of z or the probability of occupancy. The observation variable y_{ij} is our detection or nondetection of a species at site i during survey j ($j=1, \dots, J$) and is conditional on z_i ; and p is the detection probability of the species at site i during survey j . In the occupancy model framework, detection probability is at the species level rather than at the individual level as in N -mixture models.

Using repeated measures of detection or nondetection among sites, occupancy models enable us to model structure in occupancy by site and detectability by site and time and express them as functions of covariates, using some link function, or model correlations with random effects. Similar to N -mixture models the occupancy model framework is robust to less balanced data sets in which there are unequal numbers of repeated measures among all sites nor is replication required among all sites (Kéry and Royle 2015b). Replication among as many sites as possible is preferred. Key assumptions of occupancy models are similar to those of N -mixture models: detection or nondetection state does not change within a field season (i.e., closure), no false positive detections (i.e., species misidentification), occupancy probability at one site should be independent of that at another site, species are detected independent of one another (i.e., no schools or flocks), detection is homogeneous among individuals, and Bernoulli modeling distribution assumptions hold.

Data Analyses

Initially, we will use the functions ‘pcount’ and ‘occu’ in the R package ‘*unmarked*’ (Fiske and Chandler 2011) to fit the N -mixture and occupancy models to our replicated counts (Kéry and Royle 2015a). Our objective is to produce temporal estimates of change in CAB abundance and map occupancy to provide park resource managers with understandable results regarding relationships between CAB abundance and occupancy, and cave stream water quality and habitat characteristics (Figure 10).

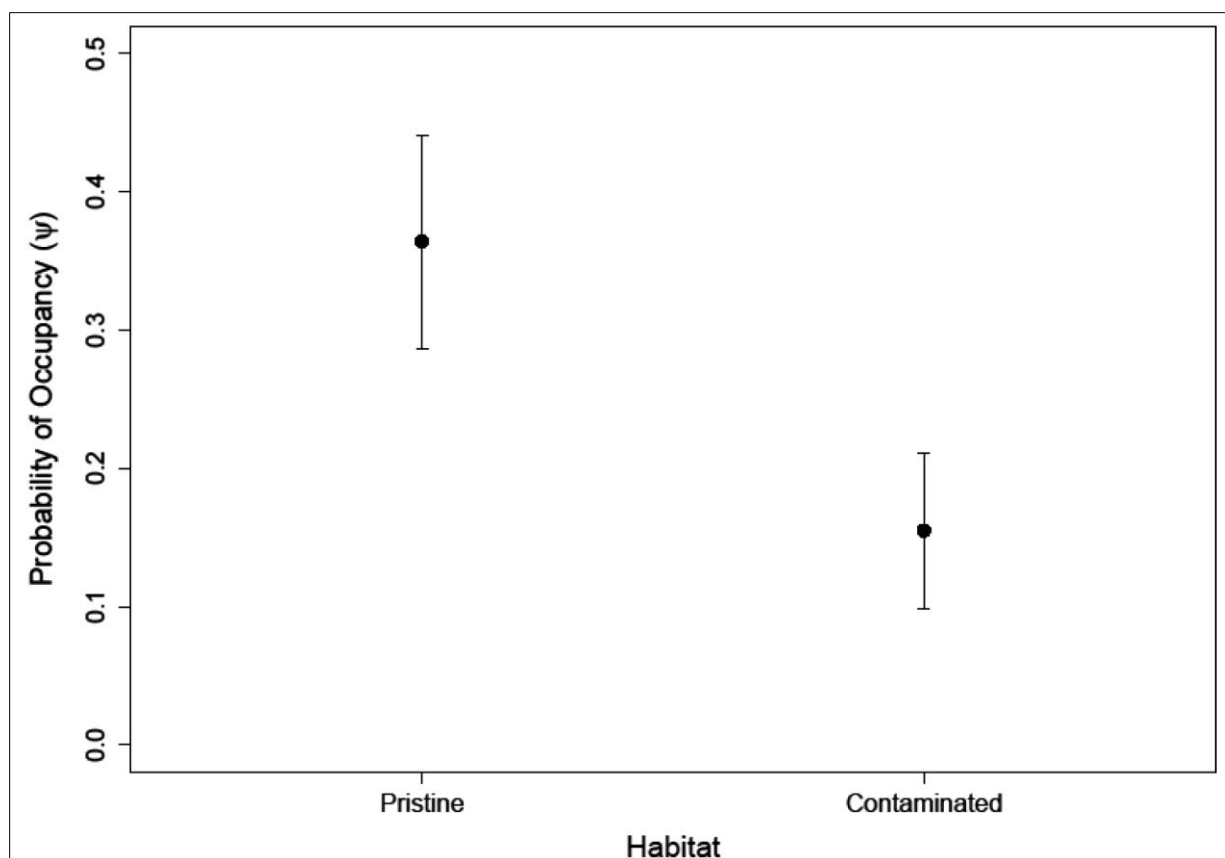


Figure 10. Mockup of graph depicting significant differences in *P. ganteri* occupancy as a function of overall water quality. Data are simulated based on R code in Fiske et al. (2015). Error bars are ± 1 SE.

Data from the CUPN Cave Aquatic Biota Monitoring Project will be analyzed in multiple ways including:

- Summaries of data collected at cave stream reaches during the previous sampling season:
 - Size range of species detected,
 - Species richness and lists,
 - Water quality/quantity summary statistics (e.g., Table 6),
 - Habitat use and summarized habitat characteristics,
 - Abundance and occupancy estimates (when possible) (e.g., Table SOP6-4 in SOP; Helf et al. 2018a).
- Trend analyses of abundance (common species) and occupancy (uncommon species) over time, typically produced every six years (i.e., three complete sampling seasons of both panels) will contain:

- Evaluations and predictions of relationships between key ecosystem drivers, attributes, stressors, and key population measures (Figure 10).

One of the limitations of Hierarchical models is the major assumption that the population being sampled is closed to births, deaths, emigration, and immigration during the survey period. This assumption is almost certainly violated if the interval between surveys is long. Data from two studies suggest minimizing the interval between replicate surveys could reduce the possibility of violating the assumption of closure even for populations of highly mobile stygobionts. Mark-recapture studies of cave crayfish (i.e., *Orconectes inermis inermis*) and cavefish (i.e., *Troglichthys rosae*) found their range of daily activity was generally well within our 40-meter (131.2 ft) transect boundaries. Large movements by *O. i. inermis* tended to be downstream and were mostly correlated with flooding due to rainfall or melting snow and ice (Hobbs III 1978). The capture of untagged adult *T. rosae* during surveys is “consistent with continued immigration of fish into the cave” at a rate of about one cavefish every five days (Means and Johnson 1995). Regardless, we will conduct replicate surveys as close together as possible (i.e., normally within a week) to avoid violating the assumption of closure.

Planned Reporting Schedule, Format, and Delivery

Reports generated by this monitoring project will consist of three major types:

1. Brief follow-up trip reports, a synopsis of monitoring activities, will be completed within two weeks after each sampling trip and sent directly to the park resources management primary contact(s).
2. Annual summary reports provide park superintendents and resource managers with a data summary of the current status of cave aquatic biota and water-quality and water-quantity parameters. The annual summary report will include a summary water-quality table (Table 6), species lists by stream reach, size range and abundance by species and stream reach (Figure 11), when possible single season estimates of occupancy and abundance similar to that in Table SOP6-4 in SOP 6 (Helf et al. 2018a), a Natural Resource Condition Summary table (Figure SOP6-5 in SOP 6; Helf et al. 2018a), and very limited interpretation. Annual summary reports will be produced separately for each park in January after the preceding year’s monitoring efforts and subsequent data analyses are completed. The annual summary report will follow the Natural Resource Data Series (NRDS) format and will be submitted for publication in the NPS’s Natural Resource Publications Management Report series (<http://www.nps.gov/im/report-templates.htm>, last accessed August 2018). The NRDS is basically an annual status report which is intended for a quick release of accurate, raw data. Any statistical analyses and interpretation of the data will be limited by the following guidelines to:
 - a. Summary statistics of covariate parameters and CAB size ranges (e.g., mean, median, maximum, minimum, standard deviation).

- b. Estimates of CAB population abundance and occupancy with associated detection probabilities, including associated standard errors, confidence intervals, and method used to compute estimates.
- c. Reference conditions for specific measures used in the Natural Resource Condition Summary Table are obtained from the most reliable, appropriate and recent data sources (e.g., literature, local/regional subject matter experts) as determined by the project leader.
- d. Interpretation of the data will be limited to general statements or items of interest to park resource managers.

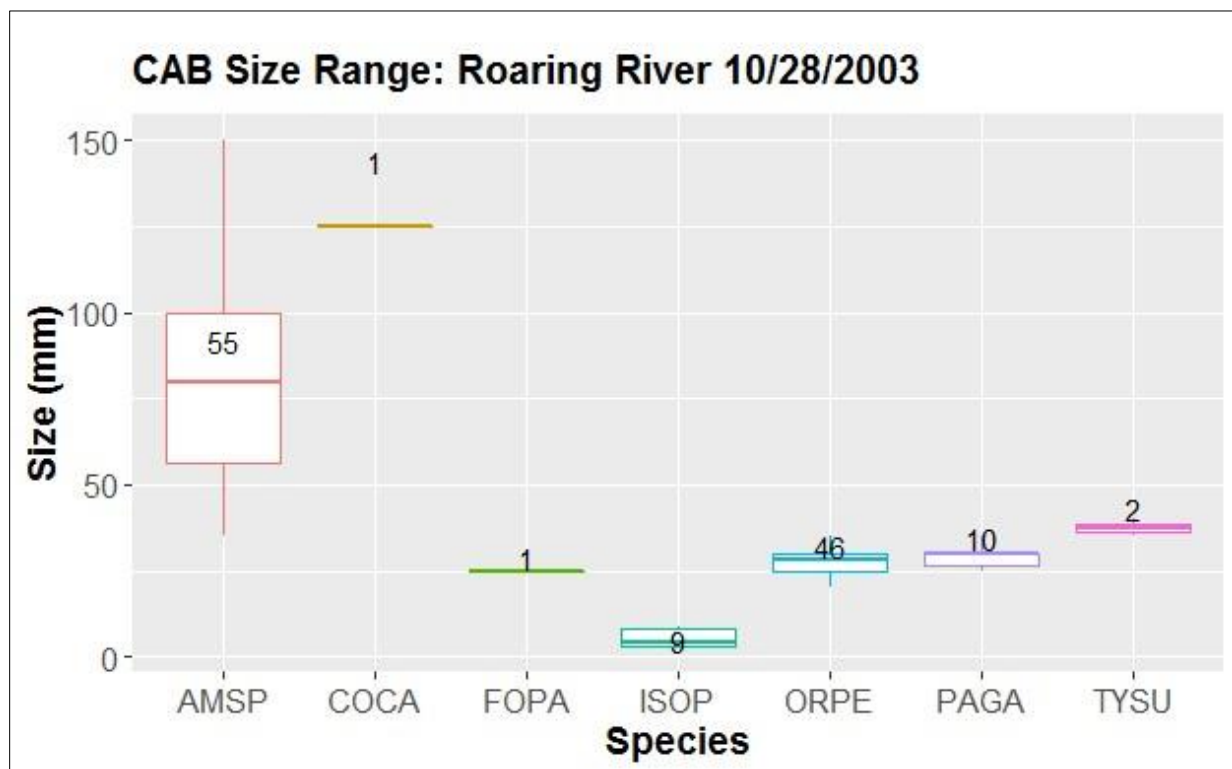


Figure 11. Boxplot showing size ranges for various CAB species detected at a cave stream reach during one sampling season. The bottom, middle (line), and top of the box correspond the 25%, 50% (median), and 75% quantiles. The lower and upper 'whiskers' correspond to smallest and largest observations ± 1.5 times the interquartile range. Numbers are abundances of detected CAB. Data are from MACA IBI sampling in 2003. AMSP=*A. spelaea*, COCA=*C. carolinae*, FOPA=*F. papilliferus*, ISOP=cave isopod, ORPE=*O. pellucidus*, PAGA=*P. ganteri*, TYSU=*T. subterraneus*.

3. A trend analysis report is generated at least every sixth year, beginning six years after the collection of data using methods established in this monitoring protocol. Subsequent trend analyses reports will consist of updated analyses using all data, in both graphic and tabular statistical test summary formats (e.g., Table 2 and Figure 10), evaluations and predictions of relationships between key ecosystem drivers/attributes/stressors and key population

measures, a Natural Resource Condition Summary Table (see Table SOP6-10 in SOP 6; Helf et al. 2018a), and interpretive discussion. This comprehensive report places the observed results in both a regional and historical context by relating them to previous analyses and published literature, discussing the significance of the results relative to changing environmental factors, and, in some instances, providing management recommendations based on these findings. The trend analysis report will use the Natural Resource Reports (NRR) format and will be submitted for publication in the NPS's Natural Resource Publications Management Natural Resource Report series (<http://www.nps.gov/im/report-templates.htm>; last accessed August 2018).

Table 6. Mock-up example of summary water quality data of cave stream temperature, specific conductance (SC), pH, dissolved oxygen (DO), turbidity, NO₃, and *E. coli* from five MACA cave stream reaches during two separate sampling events by opportunistic grab sampling.

Park	Watershed	Cave Stream	Mean cave stream parameters from grab sampling						
			Temp. (°C)	SC (µS)	pH (SU)	DO sat. (%)	Turbidity (NTU)	NO ₃ (mg/L)	<i>E. coli</i> (MPN/100ml)
MACA	River Styx Spring/Echo River Spring	Styx/Echo River	14.0	273	7.70	83.5	2.5	10.35	99
MACA	Echo River Spring	Mystic River	13.7	272	7.19	84.9	11.3	9.14	94
MACA	Pike Spring	Eyeless Fish Trail/Golden Triangle	14.1	241	7.24	80.4	13.2	5.25	172
MACA	Turnhole Spring	Logsdon River	14.5	344	7.22	83.9	22.6	12.32	143
MACA	Echo River Spring	Shrimp Pools/Roaring River	13.2	357	7.50	85.1	20.0	11.45	123

In addition to routine and consistent reporting to parks noted above, products will also be posted to the NPS's Integrated Resource Management Applications portal (IRMA; <https://irma.nps.gov>; last accessed August 2018) hosted by the NPS I&M Division. Formally maintained as separate applications, the IRMA portal incorporates:

- The master database for natural resource bibliographic references, reports, and data sets (Data Store).
- The master database for biodiversity information including species status on parks (i.e., present in park, probably present, etc.), along with observation and voucher information (NPSpecies).

Procedure for Revising the Protocol

Over time, revisions to both the protocol narrative and the SOPs are expected, and those revisions must be carefully documented. The steps for changing the protocol (either the protocol narrative or the SOPs) are outlined in SOP 7 (Helf et al. 2018a). The protocol narrative and each SOP contain a Revision History Log that will be filled out each time the protocol narrative or an SOP is revised to briefly document when and why the change was made, and to assign a new version number to the revised SOP. The new version of the SOP or protocol narrative will be posted on the IRMA data store and archived in the appropriate CUPN Vital Signs Protocol folder on the CUPN network file server.

Personnel Requirements and Training

Staff Roles, Responsibilities, and Qualifications

Major protocol tasks and the staff responsible for each are summarized in Table SOP 2-6 in SOP 2 *Pre-Field Sampling and Safety—Version 1.0* (Helf et al. 2018a). Additional information on personnel requirements is located in the “Staffing” section of SOP 2. The following personnel will be involved in the CAB monitoring project.

Network Program Manager

The CUPN program manager will be responsible for overseeing and coordinating the development and implementation of the CAB monitoring project, as well as the other vital sign protocols. The program manager will ensure the CAB monitoring project is aligned with and contributing toward overall CUPN goals. They also provide support in budget, personnel, and logistical matters.

Project Leader

A CUPN ecologist functions as the CAB monitoring project leader and is responsible for coordinating all aspects of the project, including communication with parks, logistics, training, safety, field work, data collection and management, as well as analysis and reporting. The project leader must be knowledgeable in stygobiont ecology (with emphasis on stygobionts of the southeastern United States) and have experience with the monitoring techniques contained within this protocol. Specific responsibilities and tasks include:

- Ensuring effective communication between park staff, field crew, and CUPN staff.
- Developing, reviewing, revising (as needed) and implementing the monitoring protocol, standard procedures and data forms for field data collection and data handling.
- Developing an annual field schedule and finalizing sampling dates.
- Arranging lodging for field crew.
- Participating in hiring of seasonal field crew or working with the CUPN Volunteer Coordinator to obtain volunteer field crew members.
- Preparing Field Information Sheets for field sampling.
- Inventorying the condition of all equipment, and as needed repairing equipment or purchasing replacements.
- Ensuring field crews receive pertinent training and briefings.
- Supervising field crew during the field season and participating in field sampling.
- Ensuring field crew work meets the desired standards of quality.
- Ensuring sampling work is done in a way that addresses safety hazards.

- Ensuring completed field data forms, photographs, videos and other data are returned from the field and processed in a timely manner.
- Writing and submitting brief sampling trip reports to park staff.
- Obtaining and maintaining the required NPS, USFWS and state permits, including writing and submitting Investigators Annual Reports to the permit reporting system, and other permit reports as required.
- Participating with data manager in QA/QC of data entered into database and archiving of data (both hard copies of data forms and electronic formats).
- Acting as the main point of contact concerning data content.
- Once all data are entered, completing final validation, certification, data analysis, and annual summary and six-year trend reports for each park.

Field Crew Leader

The Cumberland Piedmont Network's CAB monitoring field crew leader is responsible for managing the field crew members as designated by the project leader. The crew leader must be skilled in wildlife biology or ecology, with a strong knowledge of the cave aquatic biota of the southeastern United States. The field crew leader will assist the project leader with training the field crew on data collection; will assemble, assess, safety check, and repair equipment; will oversee and review field data collection; and will oversee gear decontamination and storage. The field crew leader must have experience training others, experience leading groups, and particular experience with all field methods used in this protocol, and conducting fieldwork in caves. They must also be detail-oriented, organized and meticulous about the collection, analysis, and safeguarding of data.

In addition to the responsibilities of a crew member, the field crew leader will perform the following tasks:

- Coordinating directly with appropriate park resource managers or other personnel when conducting fieldwork in a given park.
- Ensuring that all field crew members are trained in proper data collection and gear decontamination and storage procedures.
- Checking for updates to emergency numbers list for each park and printing copies along with directions to hospitals.
- Preparing field data forms or notebooks and Cave Stream Reach Maps for field sampling.
- Assembling, assessing, and repairing equipment.
- Conducting safety briefings prior to sampling events and post-event/season after-action safety/implementation reviews.

- Supervising field crew member(s) assigned to team and ensuring they are collecting data efficiently and correctly.
- Ensuring completed field data forms, photographs, videos and other data are returned from the field and processed in a timely manner.
- Writing and submitting brief sampling trip reports to park staff (if delegated by the project leader).
- In cooperation with the network data manager, ensuring that all data undergo the proper QA/QC procedures (including data verification after data are entered).

Field Crew Members

The field crew members are responsible for assisting with accessing caves, carrying gear, collecting data in the field, handling data, and entering data into the project database. Crew members may be college graduates or students who have strong interest in or experience in ecology, biology, or related natural resource fields. Alternatively, crew members may be volunteers who are experienced cavers (e.g., Cave Research Foundation members), or experienced with field work in general. Crew members involved with data collection or recording must be detail-oriented and must write legibly. All crew members must be able to work in extreme conditions of heat, humidity, cold, rain, darkness, biting and stinging insects, and venomous snakes, and be capable of making long, off-trail bushwhacking trips into remote caves in rugged terrain. Specific tasks (if assigned) for which the crew members will be responsible include:

- Completing mandatory training and reading pertinent protocols, JHAs, and SOPs.
- Ensuring that all necessary gear and equipment are assembled, clean, and functional prior to each trip.
- Safeguarding equipment during field operations to prevent damage or loss.
- Conducting fieldwork in a way that addresses safety hazards.
- Collecting field data accurately, and as described in the SOPs.
- Correctly and legibly recording all data values on the field data forms.
- Completing timesheets and expense reimbursement forms accurately and in a timely manner. Accurately keeping track of volunteer hours, if appropriate.
- Processing completed field data forms, photographs, videos and other data as described in the SOPs, and in a timely manner.
- Entering data accurately into the project database from paper data forms or notebooks and conducting data verification steps.

- When not in the field, assisting data manager and project leader in accomplishing other tasks related to the project, as appropriate.

With few exceptions, the project leader or designated field crew leader will be the lead field team member, supervising the work of field crew members. Field crews will consist of CUPN staff, interns, other cooperators, park staff, or volunteers.

Physical Scientist

The CUPN physical scientist is responsible for the storage and analysis of continuous water-quality data and equipment related to its monitoring. Tasks include archiving field data forms; launching and uploading data loggers; checking, naming, storing and post-processing data files; analyzing continuous water-quality data; and reporting results. Specific tasks for which the physical scientist will be responsible include:

- Ensuring that all field crew leaders are trained in proper collection of continuous water-quality data, including proper calibration, use, servicing, and storage of related equipment.
- Launching, servicing, and replacing water level and temperature automated HOBO data loggers and data shuttles (some of these subtasks may be delegated to the project leader or a trained field crew leader).
- Naming and storing data files offloaded from automated HOBO data loggers.
- Performing QA/QC and post-processing of collected continuous water quality data using Aquarius and archiving of data (both hard copies of data forms and electronic formats).
- Analyzing data and assisting project leader with writing monitoring reports.

Data Manager

The CUPN data manager is responsible for maintaining the Microsoft Access database associated with the CAB monitoring project and will oversee database management. Data entry is conducted at the main CUPN office after field data forms and electronic files have been processed. Along with the project leader, the data manager ensures that all individuals involved in the project are aware of their data management responsibilities. Specific tasks for which the data manager will be responsible include:

- Serving as consultant to all project staff on data management activities.
- Maintaining and updating the database application.
- Providing oversight and training on the use of the database application.
- Coordinating electronic data archival and backup procedures.
- Providing assistance to network program manager and project leader with data summaries and analyses.

- In consultation with the project leader, posting of products to IRMA.

GIS Specialist

The CUPN's GIS specialist is responsible for coordinating GPS data and equipment and maintaining all geospatial data and cave maps (including cave stream reach maps) related to the CAB monitoring project. Tasks include archiving of sampling cave locations and parking locations, and creation of maps for field crews and reports. Geospatial data will be uploaded to IRMA, as appropriate, and will be documented with appropriate Federal Geographic Data Committee (FGDC) and NPS metadata standards. Specific tasks for which the GIS specialist will be responsible include:

- Maintaining a full list and coordinates of all cave stream reaches selected for monitoring.
- Maintaining a geodatabase with information about all cave and parking locations, updated yearly.
- Maintaining all working GIS files associated with the protocol, including archiving the files on a shared network drive.
- Creating and maintaining metadata for all geospatial data using appropriate Federal Geographic Data Committee and NPS metadata standards.
- Maintaining and updating the Cave Stream Reach Maps for each monitored cave.
- Uploading cave location and parking location coordinates into the network GPS unit(s) or handheld device.
- Obtaining GPS units from field team leader or project leader, at end of each field season, and downloading GPS coordinates and metadata associated with any newly marked waypoints.

Curatorial Specialist

Final hard-copy data sets resulting from CAB monitoring will be processed by the CUPN curatorial specialist according to NPS guidelines. They will be responsible for archival housing, contacting park curators to request accession and catalog numbers for the CAB monitoring project, entering information into NPS Interior Collection Management System (ICMS) database, and sending archives to the park (if final repository) or generating loan agreements (if needed) and sending archives to an alternate repository. The curatorial specialist will work with parks to ensure records are uploaded into their master NPS-ICMS database and that archives are delivered to park-designated repositories.

Data Intern

This intern's primary task is to enter data into the master database. When available, an intern can serve to assist the data manager, GIS specialist, and curatorial specialist in their duties.

Training Procedures

An essential component for the collection of credible, high-quality cave aquatic biota and associated water quality and habitat data is well-trained and experienced field crew members. Prior to each field

season, field crews require field training to collect data effectively and efficiently. Training will be conducted by the project leader, or an experienced crew leader designated by the project leader. Training sessions are necessary for **all** project crew members but particularly crew members new to the project. New crew members must undergo training for each of the field sampling methods and office tasks in which they will participate.

Training will include:

- Orientation—which will summarize this protocol
- In-office demonstrations of the less intuitive field equipment (e.g., HOBO data logger servicing)
- Species identification
- Data recording
- Data handling and processing
- Data entry
- Sampling dry runs
- Pre-field sampling event briefings
- Safety procedures

For CAB counting methods, double sampling with trainer and trainee will initially be used as an evaluation tool. A more detailed list of protocol tasks that selected field crew leaders and crew members need to master is provided in Table SOP 1-1 in SOP 1 *Training Personnel—Version 0.2* (Helf et al. 2018a).

Training includes demonstration and practice of all measurement procedures. The critical importance of careful, accurate, and steady data collection to this long-term monitoring project will be emphasized. Trainers will stress the importance of exact adherence to SOP instructions to prevent bias in measurements among years. Training will also include an overview of safety issues that may be encountered by field crews. Prior to conducting the first field sampling event each year, all field personnel involved must **read and sign** the Job Hazard Analysis (JHA), see Table SOP 2-5 in SOP 2 (Helf et al. 2018a). Prior to each multi-event sampling trip (e.g., four consecutive days of sampling at Russell Cave NM), all participating field personnel must **review** the related JHA (Table SOP 2-5 in SOP 2; Helf et al. 2018a) and agree to follow all safety guidelines during sampling.

Operational Requirements

Annual Workload and Field Schedule

The field sampling window begins in July and runs through October. Weather and personnel workloads preclude the scheduling of sampling events to specific annual dates. Replicate surveys will occur as close as possible to the date of the original surveys (i.e., a minimum of days between replicates). A summary of the number of cave streams selected for monitoring in each park, by sampling method and estimated number of transects per site, is given in Table 4. Seven cave streams are scheduled to be surveyed by four-person teams every two years under this project. This will require approximately 14 sampling events (i.e., two sampling events per cave stream) with an estimated 616 person-hours spent in the field (Refer to Table SOP 2-1 in SOP 2 *Pre-Field Sampling and Safety—Version 1.0*; Helf et al. 2018a) every two years. It is important to note each CAB monitoring event **will be completed within an 11-hour field day, allowing for a one-hour cushion within the maximum 12-hour field day**. The number of transects surveyed at each cave stream reach, and the time spent gathering data, is highly dependent on the reach's length, amount of sampleable habitat, survey method used, and "door-to-door" travel times to cave stream reaches. The two snorkeled cave stream reaches (Table 4) will require a separate half-day trip to transport heavy equipment (e.g., kayaks and dry suits) to and from the reach.

All cave stream reaches require a **one-time** special in-cave trip for data logger installation. It will take team(s) of two to three persons about 104 person-hours total to complete the seven special field trips focused on data logger deployment activities. Additional details on estimated workload and schedule for each sampling activity in this monitoring protocol can be found in the "General Sampling Approach" section of SOP 2 (Helf et al. 2018a).

The early spring, summer and winter months will be used for equipment inventory, equipment repair, equipment replacement, permit applications, data entry and QA/QC, data analysis, report writing, review and revision (if necessary) of the protocol based on previous field seasons. Preparations for the upcoming field seasons occur in late spring and early summer. Table SOP 2-6 in SOP 2 (Helf et al. 2018a) gives the annual timing of major protocol tasks.

Facility and Equipment Needs

The logistical and equipment needs of the CAB monitoring crew are detailed in the appropriate checklists in SOP 2 (see Tables SOP 2-2 through SOP 2-6; Helf et al. 2018a). The nature of CAB monitoring work does not require special facilities beyond the normal office space and equipment storage needs currently available at the CUPN headquarters at Mammoth Cave NP. Park housing, with adequate bathing and equipment decontamination facilities, was used for pilot fieldwork at all three CUPN parks with accessible cave streams. Field crews will stay in park housing when available in an effort to trim costs. The project leader will coordinate lodging, when park housing is being used, and transportation needs with park staff. The project leader will reserve a network or rental vehicle for transport to and within parks. Sampling at Mammoth Cave NP sometimes requires a four-wheel drive vehicle or a motorboat. A two-wheel drive vehicle is adequate for accessing the caves selected for CAB monitoring at Cumberland Gap NHP and Russell Cave NM.

Startup Costs and Budget Considerations

Legacy CAB monitoring efforts, a CAB pilot study, assistance with *P. ganteri* collection at Mammoth Cave NP, and mapping or site visits at Cumberland Gap NHP and Russell Cave NM resulted in the development of key partnerships with various agencies, organizations, and individuals. Currently, four CUPN ecologists and biologists have experience with CAB monitoring and the project leader is applying for a USFWS permit for monitoring *P. ganteri* in Kentucky.

In 2016, a five-year cooperative agreement was signed between the Cumberland Piedmont Network and Western Kentucky University (Cooperative Ecosystems Study Unit) for approximately \$29,000 per year to provide student interns in support of vital signs monitoring. Interns will be used as field crew members and will participate in data processing and data entry. Interns acquired through a cooperative agreement between the network and various organizations that provide youth interns (e.g., SCA, ACE) may also assist with field or office tasks in support of this monitoring project.

Startup costs for CAB monitoring project include the following items **already acquired** by the CUPN:

- 10 HOBO U20-L Water level and temperature data loggers (\$2,881).
- Inflatable kayaks and snorkeling platforms (\$1,535).
- GoPro Hero 3+ Cameras (\$1,020).
- Neoprene wetsuits, socks, shirts, shorts (\$1,435).
- Mask (including custom lenses), fins, snorkels (\$1,000).
- Dive lights, rechargeable batteries, hand straps (\$500).
- Miscellaneous equipment and supplies (e.g., Rite-in-the-Rain paper, zippered Rite-in-the-Rain field notebooks, waste bags, hand warmers, fishing line and floats, freezer bags, hammer drill, wall anchors; \$634).
- Preliminary maps, planning, cave stream reach visits (\$1,000 per reach).

Total estimated startup cost for the project (**not** including database or protocol development, vehicles, and individually assigned caving equipment) is approximately \$24,000.

Fiscal Year 2018 cost estimates associated with the CAB monitoring protocol are detailed in Table 7. These costs include salary, travel, and supplies and equipment. Every six years the additional time required for the project leader to perform data analysis and write a comprehensive trend analysis report will add an extra cost to the protocol's budget. We will consider different options to offset the additional expenses associated with producing a trend analysis report, estimated at five pay periods (i.e., roughly two and a half months), including a "pause" or reduction in field sampling and associated activities.

Table 7. Estimated Fiscal Year 2018 budget for implementing the protocol for cave aquatic biota monitoring at four cave streams at Mammoth Cave National Park (MACA; Panel 1). Costs and hours in parentheses are estimates for Panel 2 in which one cave stream each at MACA, Cumberland Gap National Historical Park, and Russell Cave National Monument are sampled. Note estimated costs and hours of office tasks do not vary regardless of panel.

Expense Type	Task Category	Estimated CUPN Costs	Value of Park/ Volunteer Contribution	Estimate Basis
Salary	Field Sampling and Travel Time	\$6,600 (Panel 1) \$4,950 (Panel 2)	\$4,249 (Panel 1) \$3,186 (Panel 2)	Assumes about 176 (132) hours from volunteers or park staff. CUPN Staff Breakdown: \$4,400 (\$3,300) GS-11 ecologist, 88 hr (66 hr) \$2,200 (\$1,650) CUPN Intern, 88 hr (66 hr)
Salary	Office: GIS/Pre-sampling Training, and WQ sample processing	\$5,224	\$772	Assumes about 32 hours from volunteers or park staff. CUPN Staff Breakdown: \$1,800 GS-11 ecologist, 36 hr \$1,680 WQ Processing GS-9 biologist, 20 hr \$1,400 CUPN Intern, 56 hr \$344 GIS Specialist, 8 hr
Salary	Office: Data logger Processing/Data Entry, Data QA/QC, Processing/Data Entry/Data Mgmt/Analysis & Reporting	\$6,408	\$482	Assumes about 20 hours from volunteers or park staff. CUPN Staff Breakdown: \$472 GS-12 ecologist, 8 hr \$4,000 GS-11 ecologist, 80 hr \$752 GS-11 AQ Specialist, 16 hr \$384 GS-9 biologist, 12 hr \$800 CUPN intern, 32 hr
Travel	–	\$1,322	–	Assumes CUGA and RUCA provide reduced rate housing. Breakdown: \$200 lodging \$1,018 per diem \$104 gas

Table 7 (continued). Estimated Fiscal Year 2018 budget for implementing the protocol for cave aquatic biota monitoring at four cave streams at Mammoth Cave National Park (MACA; Panel 1). Costs and hours in parentheses are estimates for Panel 2 in which one cave stream each at MACA, Cumberland Gap National Historical Park, and Russell Cave National Monument are sampled. Note estimated costs and hours of office tasks do not vary regardless of panel.

Expense Type	Task Category	Estimated CUPN Costs	Value of Park/ Volunteer Contribution	Estimate Basis
Supplies and Equipment	–	\$1,000	–	Placeholder for replacement costs (\$600) & annual supplies (\$400).
Totals	–	\$19,232 (Panel 1) \$18,904 (Panel 2)	\$5,503 (Panel 1) \$4,440 (Panel 2)	–

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