

Cumberland Piedmont Network



National Park Service
Inventory and Monitoring Division

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

Standard Operating Procedures

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Contents

	Page
Standard Operating Procedure 1	
Training Personnel—Version 1.0	SOP 1-1
Standard Operating Procedure 2	
Pre-Field Sampling and Safety—Version 1.0	SOP 2-1
Standard Operating Procedure 3	
Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0.....	SOP 3-1
Standard Operating Procedure 4	
Post-Field Sampling—Version 1.0.....	SOP 4-1
Standard Operating Procedure 5	
Data Management—Version 1.0	SOP 5-1
Standard Operating Procedure 6	
Data Analysis and Reporting Version 1.0	SOP 6-1
Standard Operating Procedure 7	
Revising the Protocol—Version 1.0.....	SOP 7-1

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Training Personnel—Version 1.0

This standard operating procedure (SOP) discusses the training of personnel involved in all aspects of protocol implementation. Implementation of this protocol requires an overall project leader plus three to four other persons. The project leader, a permanent employee intimately familiar with field sites and with years of experience employing sampling techniques, performs monitoring activities and is responsible for training sessions. Field sampling crews will typically be comprised of the project leader (or designated field crew leader) and three field crew members.

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Training

Training sessions are required for all project crew members particularly crew members new to the project. New crew members must undergo the entire gamut of training sessions to be proficient in all protocol activities and able to assist with any task. Training sessions will include: an orientation session summarizing this protocol, data processing, data entry, sampling dry runs, pre-sampling event briefings, safety procedures, and white-nose syndrome decontamination requirements and procedures. On-the-job training is of enormous value toward honing new crew members' skills at various protocol tasks. Experienced crew members absent from the project for six months or more will attend a sampling dry run and re-train at office tasks (e.g., data entry) with current crew members. Once the project attains a critical mass of reliable crew members, training sessions will be conducted as needed. Table SOP1-1 below is a detailed list of protocol tasks crew members need to master.

An important quality assurance step and an essential component for the collection of credible, high-quality data on cave aquatic biota and their associated covariates is a well-trained and experienced field crew leader. Crew leaders must be thoroughly familiar with SOP 2 *Pre-Field Sampling and Safety—Version 1.0*, SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*, SOP 4 *Post-Field Sampling—Version 1.0*, along with the Cumberland Piedmont Network's Water Quality Monitoring Protocol SOP 4 *Field Measures—Version 1.2* (Meiman 2018). Crew leaders will also be given hands-on training by the project leader, or an experienced crew leader designated by the project leader, for each of the tasks detailed therein. A few crew leaders from among network and park staff should be trained (and should participate in sampling on a regular basis) in case scheduled crew leaders are unavailable for particular sampling events. Proper adherence to SOP 2 should serve to refresh and “recalibrate” the crew leaders' ability to prepare for sampling, locate sampling sites, and properly record the information to ensure the integrity of the data collected.

All personnel involved with the handling and processing of equipment or data (i.e., hardcopy and electronic formats) following field sampling must review SOP 4 prior to the first field sampling event each year. Transient personnel (e.g., university interns, Student Conservation Association interns, cooperators, short-term or temporary NPS employees) can serve as field crew members and assist with data entry. Review of SOPs 2, 3, 4, and SOP 5 *Data Management—Version 1.0* is necessary for transient personnel.

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 must be emphasized. Trainers must stress the importance of exact adherence to SOP instructions to prevent bias in measurements among years. Lack of strict adherence to protocols will inhibit our ability to detect long-term trends. The crew must never deviate from established protocols or alter data sheets or the database without first consulting with the project leader. Any changes in procedures must be in accordance with SOP 7 *Revising the Protocol—Version 1.0* and clearly documented in the SOP revision history and the project database by the project leader in conjunction with the data manager.

Training must emphasize the critical importance of careful behavior in the cave and consistent adherence to the latest national decontamination guidance to minimize the risk of spreading the fungus which causes white-nose syndrome in bats. At the end of training sessions observer accuracy for taxon identification, size estimation, condition, and habitat characteristics will be documented and evaluated. Finally, training will also include an overview of safety issues that may be encountered by field crews. Prior to participating in their first field sampling event each sampling year, all field personnel involved must review SOP 2, and **read and sign** the “Field Work in Caves” Job Hazard Analysis (JHA), in which they agree to follow all safety guidelines.

Table SOP1-1. Detailed list of protocol tasks at which project crew members must be proficient.

Training Location		Protocol Task
Field	Office	Use of various checklists and data sheets (SOP 2: Pre-Field Sampling, CUPN Water Quality Monitoring Protocol SOP 4—Version 1.2, SOP 3: Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics)
Field	Office	Calibration and use of water quality grab sampling sensors (CUPN Water Quality Monitoring Protocol SOP 4—Version 1.2)
Field	Office/ Lab	Lab analysis of water samples (CUPN Water Quality Monitoring Protocol SOP 4—Version 1.2)
Field	Office	Installing and servicing a HOBO U20L water level and temperature data logger (SOP 3—Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics)
Field	Office	Downloading continuously sampled surface meteorological data from specific park SCAN Station website (SOP 4—Post-Field Sampling)
Field	–	Taxon identification for detection/nondetection sampling (SOP 3—Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics)
Field	Office	Primary: identification and size estimation of cave aquatic biota. Secondary: recognition of eggs, larvae (in shrimp and crayfish), fat bodies and DELTS (in cavefish) and food in gut. Review of available photos and preserved specimens collections are a part of this training.
Field	–	Evaluation of habitat characteristics for detection/nondetection sampling (SOP 3—Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics)
Field	Office	Use of field data sheets (CUPN Water Quality Monitoring Protocol SOP 4—Version 1.2, SOP 3—Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics)
–	Office	Data Entry (CUPN Water Quality Monitoring Protocol SOP 4—Version 1.2, SOP 3—Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics, SOP 4—Post-Field Sampling, SOP 5—Data Management)
Field	Office	Proper safety procedures (SOP 2—Pre-Field Sampling and Safety)

Helpful Hints for a Successful CAB Sampling Event

- Hot soup/tea/chocolate in thermal flask should be included as a safety item.
- Exposure suit is the safest, most comfortable equipment for snorkeling observers.
- Recorders need thicker wetsuits (e. g., 3–5mm) due to less activity in kayak.
- Back rest (i.e., stadium seat) needed in kayak for recorder's comfort.
- Backpacks with hip support have better weight distribution for hauling heavy equipment to and from cave stream reaches.
- A dry bag or sealed container helps keep loose items in the kayak organized.

Literature Cited

Meiman, J. 2018. Cumberland Piedmont Network water quality monitoring: Standard operating procedures. Cumberland Piedmont Network. Mammoth Cave, Kentucky. Available at: <https://irma.nps.gov/DataStore/Reference/Profile/2253371> (last accessed September 2018).

Standard Operating Procedure 2

Overview

Revision Log

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Contents

	Page
Sample Frame	SOP 2-3
General Sampling Approach.....	SOP 2-5
Schedule	SOP 2-7
Staffing.....	SOP 2-7
Communication and Coordination with Park.....	SOP 2-8
Logistics	SOP 2-9
Safety	SOP 2-9
Field Information Sheets and Cave Stream Reach Maps.....	SOP 2-23
GPS Setup	SOP 2-23
Communication and Crew Oversight.....	SOP 2-26
Summary of Yearly Staff Roles	SOP 2-28
Supplies and Equipment	SOP 2-31
Literature Cited	SOP 2-37

Sample Frame

The major objectives of this protocol are to monitor changes in the abundance and occupancy of selected cave aquatic biota at selected CUPN cave parks over time. However, randomly selecting CAB sampling sites from among 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 is index sites (reaches) selected based on the professional judgement of network and park staff. The limited number of subjectively selected underground stream or river reaches are scattered among several karst watersheds and subbasins in three CUPN cave parks.

We have adopted a common sampling design to allocate permanent 40-meter (131.2-foot [ft]) transects to random locations in each selected cave stream reach. We used the Generalized Random Tessellation Stratified (GRTS) algorithm (Stevens and Olsen 2004) in an unstructured, equal-probability, spatially-balanced design. Unlike simple random sampling, GRTS produces approximate spatial balance, reducing the variance in separation distances between adjacent transects. However, we expect some drawn transect locations will be rejected as unsampleable in the field because of factors such as safety or resource damage considerations, and will need to be replaced by oversample transect locations. In GRTS, the replacement sample for a rejected transect location can occur anywhere in the frame, so the relative inclusion probabilities are equal and independent of the distribution and extent of unsampleable locations. The GRTS design has been successfully applied in many National Park Service inventory and monitoring protocols and is a flexible, efficient, and statistically robust approach that accommodates many of the difficulties commonly encountered in field sampling (e.g., sampling frame errors, inaccessibility) and allows inclusion of new or replacement sample transects as necessary (Stevens and Olsen 2004). Line segment locations obtained from GRTS sample draws are used as transect locations. The sampling in each park employs a two-year serially alternating panel design with approximately half the total sample allocated to each panel (Table SOP2-1). One panel of plots is surveyed each year.

Table SOP2-1. Summary of panel design and sampling effort to monitor cave aquatic biota, habitat characteristics, water quality, and water quantity in cave stream reaches at three parks in the Cumberland Piedmont Network.

Panel	Park	Number of sampled cave stream reaches per park/Months sampled/Panel subtotal	Number of sampling events ¹ per park/Panel subtotal	Number of surveys ² per park/Panel subtotal	Estimated Field Effort per sampling event (person hours) ³	Estimated Field Effort per panel (person hours) ⁴
Panel 1	MACA	4 /July-Oct	8	16	–	–
Subtotal	1 park	4 cave stream reaches	8 sampling events	16 surveys	44	352
Panel 2	MACA	1 / July-Aug	2	4	–	–
	CUGA	1 / Aug-Sept	2	4	–	–
	RUCA	1 / Sept-Oct	2	4	–	–
Subtotal	3 parks	3 cave stream reaches	6 sampling events	12 surveys	44	264
Total	3 parks	7 cave stream reaches	14 sampling events	28 surveys	–	616

¹A complete sampling of the physical, chemical, and biological data of all transects in one cave stream reach completed by two independent teams in a single 11-hour day (i.e., two sampling events/cave stream reach × N cave stream reaches/park).

²A survey is the complete sampling of the physical, chemical, and biological data of all transects in one cave stream reach by one team in a single 11-hour day (i.e., 2 teams/sampling event × 2 sampling events/cave stream reach × 1 sample/team × N cave stream reaches/park). The number of transects per sample will vary among stream reaches.

³Minimum of four personnel (two teams/sampling event × two person/team × 11 hours/person).

⁴44 person hours/sampling event × N sampling events/panel.

General Sampling Approach

Sampling Schedule

Monitoring of cave aquatic biota among the three CUPN parks is divided into two panels with the full complement of all selected cave stream reaches sampled every two years (Table SOP2-1 and Table SOP2-2). Cave aquatic biota sampling events at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM are scheduled during July through October when precipitation is minimal and risks to safety and visibility are minimized due to baseflow conditions in cave stream reaches. 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 inches [in]) of rain would not be cause for concern regarding safety or 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. For example, if the Green River level is above 4.27 meters (14.0 ft) on the USGS Mammoth Cave Ferry Gage conditions are unsuitable for monitoring base level streams.

A sampling event is a complete sampling of the physical, chemical, and biological data of all permanent transects in one cave stream reach completed by two independent teams as described in SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, Habitat Characteristics, and Water Quality/Quantity—Version 1.0*. Ideally, all sampling events at the four scheduled caves in Panel 1 will take a minimum of 30 days total to complete each year and will be scheduled between July 1 and October 31 (Table SOP2-1). Ideally, all sampling events at the three scheduled caves in Panel 2 will take a minimum of 21 days total to complete each year and will be scheduled between July 1 and September 30 (Table SOP2-1). Every attempt will be made to schedule specific sampling dates for each cave stream reach as close as possible to the same dates each time it is sampled to minimize inter-annual variation and minimize adding unaccounted-for uncertainty to the occupancy and abundance estimates. To minimize intra-annual variation and immigration/emigration, the two non-consecutive sampling events are performed within as short an interval as possible between samples, normally within a week—but a **minimum** of two days apart—to allow site conditions to resettle. In most cases, all sampling tasks within a cave, including travel times, are expected to be completed within approximately 11 hours. Round trip travel times between office or lodging site and the cave entrance should range from 10 minutes to 3.5 hours.

Table SOP2-2. Serially-alternating panel design used for monitoring cave aquatic biota at selected cave stream reaches among three Cumberland Piedmont Network parks. All cave stream reaches in each panel are monitored biennially. Although not shown, every stream reach is surveyed twice in the scheduled sampling year. Resampling occurs within as short an interval as possible between samples, within 3–4 days—but a minimum of 2 days apart—to allow site conditions to resetttle.

Panel	2018	2019	2020	2021	2022	2023	2024	2025	2026
1	XX	–	XX	–	XX	–	XX	–	XX
2	–	XX	–	XX	–	XX	–	XX	–

Water Quality and Quantity Sampling

Monitoring of water quality and water quantity in cave stream reaches will employ two sampling methods: opportunistic grab sampling and continuous sampling.

Opportunistic Grab Sampling of Water Quality

All procedures involved in the preparation for opportunistic grab sampling for water quality data are described in SOP 5 *Sample Collection, Preservation, and Transportation—Version 1.2* of the CUPN Water Quality Monitoring Protocol (CUPN 2018). Opportunistic grab samples will be collected at each cave stream reach as described in SOP 4 *Field Measures—Version 1.2* of the CUPN Water Quality Monitoring Protocol (CUPN 2018). Data on most water-quality parameters will be gathered at the cave stream reach, from the same location as the HOBO data loggers, with pre-calibrated handheld sensors. Measured parameters include specific conductance, water temperature, dissolved oxygen, and pH. Water samples will be collected at the reach and analyzed in the lab for turbidity, nitrates, and *E. coli* by trained NPS personnel as described in the CUPN Water Quality Monitoring Protocol (CUPN 2018). Replicate quality assurance and quality control (QA/QC) samples for parameters measured with the handheld sensor and in the lab will be collected at two randomly-selected sampling events each year.

Continuous Sampling of Water Quality and Water Quantity

HOBO U20L data loggers in stilling wells will continuously sample water level, the height of the water column above the data logger, and water temperature at a programmed interval in all monitored cave stream. Water level will be used as an indirect measure of the physical forces acting on cave aquatic ecosystems through periodic flooding. Procedures associated with the use of HOBO U20L data loggers will generally follow those detailed in Curdts (2017). One-time field trips focused on data logger installation activities will be performed by a team of two to three persons, depending on cave-specific factors (e.g., human safety and distance from the entrance to the cave stream reach). It will take approximately five days to complete these initial field trips focused on data logger installation activities at the seven cave stream reaches. Annual cave water level and temperature field sampling activities broadly include data logger deployment, service, replacement, and removal (see SOP 3), all of which are performed by the project leader or designated field crew leader. Field crew members may assist by serving as data recorder, data logger spotter, measurer, or photographer (of data loggers and landmarks). This process is only performed at the beginning of the second sampling

event of the field season. After the second CAB surveys of the season, the water-level data from each sampled cave stream reach are retrieved from the data logger and uploaded to Aquarius.

Abundance, Detection/Nondetection Data, and Habitat Characteristics

Visual surveys of cave aquatic biota and their habitat are the standard sampling approach for this protocol. This typically 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 data recorder during independent timed counts. Visual survey is also used to evaluate hypothesized important habitat characteristics (e.g., percent substrate composition of transects or pools). Visual survey is the primary method used by CAB researchers throughout the world and is considered a standardized technique (Pearson and Boston 1995; Pearson and Jones 1998; Trajano 2001; McCandless 2005; Graening et al. 2010).

Schedule

Variable weather and personnel workloads preclude the scheduling of sampling events to specific annual dates (Table SOP2-3). Exact sampling dates and field personnel will be scheduled and logistics (e.g., lodging, vehicles) organized at least two months in advance of sampling trips. Backup dates will also be scheduled in advance. Flexibility is encouraged when scheduling because the project leader may want to take advantage of periods of dry or stable weather. To minimize inter-annual variation, sampling dates for each cave will be scheduled as close as possible to the same dates each scheduled year. At Mammoth Cave NP, some sampling dates will be scheduled around Cave Research Foundation expedition dates to take advantage of volunteers with caving expertise. The project leader is responsible for contacting potential field personnel to schedule specific sampling (and backup) dates.

Staffing

The CAB monitoring protocol will be implemented by a minimum of four persons (typically one field crew leader and three crew members). Field crews will consist of CUPN staff, volunteers, park staff, university interns, Student Conservation Association interns, American Conservation Experience interns, or other cooperators. The involvement of park staff and volunteers is essential to the implementation of this protocol, and it is the project lead's responsibility to recruit their assistance each year. It is the network program manager's responsibility to obtain intern assistance each year through various cooperative agreements available to the Cumberland Piedmont Network. The field crew leader and at least one crew member must possess good identification skills for local cave aquatic biota and be thoroughly familiar with the sampling methods. The project leader typically serves as the field crew leader, but there should always be a trained backup as a contingency. All field crew members should be well-trained so the field crew is a cohesive, efficient unit. New crew members or experienced crew members who miss a field season must review and be trained in all relevant SOPs (Table SOP1-1) and undergo re-training, as needed. Field personnel must also be adept at traveling through caves, including crawling, descending or ascending, and climbing where necessary. Caving skills are important to ensure that field personnel can safely access caves

and maneuver through passages without causing resource damage or sustaining injury. Implementation of the protocol also requires the use of cameras, GPS units, radios or satellite communicators, and other field equipment (see equipment checklists in Table SOP2-6 through Table SOP2-9).

Table SOP2-3. List of cave aquatic biota caves by Cumberland Piedmont Network (CUPN) park, species of management concern status, sampling panel number, and co-located CUPN cave vital signs. Both panels are sampled every other year (see Table SOP2-2). Note that although two entrances are listed for Russell Cave NM, they are part of the same cave stream reach.

Park Name	Cave Name	Species of Mgmt Concern? (Y/N)	Sampling Panel #	Sampling Date Range	Co-located Cave Vital Sign
CUGA (1 cave)	Gap Cave	Y	2	Aug-Sept	Cave Bats
MACA (5 caves)	Bridge Avenue	Y	2	July-Aug	–
	Eyeless Fish Trail/Golden Triangle	Y	1	July	Cave Crickets
	Mystic River	Y	1	August	–
	Shrimp Pools/Roaring River	Y	1	September	–
	Styx/Echo River	Y	1	October	Cave Bats/ Cave Crickets
RUCA (1 cave)	Main Entrance	N	2	Sept-Oct	Cave Bats
	Picnic Entrance	N	2	Sept-Oct	Cave Bats

Communication and Coordination with Park

A current National Park Service (NPS) research permit must be obtained to perform CAB monitoring. Mammoth Cave NP allows multi-year permits to cover long-term monitoring projects whereas permits for Cumberland Gap NHP and Russell Cave NM must be renewed annually. The status of CAB monitoring research permits is verified and applications for new permits submitted at the Research Permit and Reporting System “Quick Link” on the [NPS Integrated Resource Management Application website](#). The project leader ensures the parks’ designated resources management contact has the current approved protocol for monitoring cave aquatic biota and a list of sampling locations so any compliance issues can be addressed. The project leader is responsible for year-end reporting requirements for each permit.

In addition to permits, the project leader emails the key park contact at each park at least two weeks prior to each sampling event to provide the planned or proposed dates CAB monitoring field work is

scheduled. When possible or when requested by park staff, the sampling crew should stop by the park resources management office for brief introductions and to meet any logistical needs such as picking up keys and vehicle hang tags. If required by a park, the project leader will fill out a cave entrance request form for each cave to be sampled during that trip and obtain a proper approval signature. Brief follow-up trip reports will be completed within two weeks after each sampling trip. However, because sampling at Mammoth Cave NP occurs throughout the summer, a single trip report for that park will be completed within two weeks after the end of the sampling season.

Logistics

Once the specific dates (including backup dates) for each sampling trip have been selected, the project leader will reserve a CUPN or rental vehicle for transport to and within parks. Sampling at Mammoth Cave NP may require a four-wheel drive vehicle. The project leader will attempt to reserve in-park lodging, where available, as soon as specific field sampling dates are selected each year. If no in-park lodging is available, the project leader will secure nearby commercial lodging within government rates.

Organizing Supplies and Equipment

The checklists provided in Tables SOP2-6 through SOP2-9 include a pre- and post-event logistical checklist, a checklist of supplies and equipment needed for samplers, data recorders, and water-quality samplers, and a personal supply checklist for crew members. Print out and read checklists well in advance of each sampling event. This allows time to prepare documents and organize equipment, make needed repairs, check batteries and order equipment, if necessary.

Safety

The Cumberland Piedmont Network considers the occupational health and safety of its employees, cooperators, and volunteers to be of primary importance, and is committed to ensuring that all field crews receive adequate training on NPS safety procedures, incident reporting, and emergency response prior to field work. Working in a cave environment can present some particular safety hazards, and certain activities (e.g., vertical caving) necessitate specialized training and experience. All field crew members should read and sign the Job Hazard Analysis form (Table SOP2-4).

Job Safety

An important tool used to promote safe conduct is the Job Hazard Analysis (JHA; formerly called a Job Safety Analysis). This approach is consistent with the NPS Occupational Safety and Health Program (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 participating field personnel must **read and sign** the related JHA (Table SOP2-4) and agree to follow all safety guidelines during sampling.

Responding to an Incident

Life-Threatening Medical Emergency

- **Call 9-1-1 or park emergency number. When available, use park radios to contact dispatch.** Administer first aid to the best of your knowledge, ability and training. If appropriate, transport to emergency room. Directions to the nearest hospital from each park and emergency numbers will be updated and printed every January, prior to field sampling, by the project leader or designated field crew leader. Copies of the hospital directions and emergency numbers will be included on the trip plan (Figure SOP2-3) associated with each sampling event.
- As soon as it is practical to do so, inform your supervisor and the park's emergency contact.
- For injured NPS staff and volunteers, complete Worker's Compensation paperwork. For contractors and cooperators, follow your organization's procedures for documenting accidents.

Non-Emergency Incidents

- For a non-emergency incident that may require medical attention, contact supervisor immediately after incident. If the supervisor cannot be reached, inform the park's emergency contact.
- Seek medical attention, if needed.

For injured NPS staff and volunteers, complete Worker's Compensation paperwork (must be done within 48 hours of incident). For contractors and cooperators, follow your organization's procedures for documenting accidents.

Cumberland Piedmont Network JOB HAZARD ANALYSIS

Job Description:	Field Work in Caves
Date of last update:	2 August, 2013
NPS Division with primary responsibility for this JHA:	Inventory and Monitoring Division for the Southeast Region
Last updated by:	Rick Toomey
Reviewed by:	Teresa Leibfreid, Rickard Olson, Steven Thomas, Rickard Toomey, Kurt Helf
Approved by:	Teresa Leibfreid
Required standards and general notes:	Cave entry permits must be obtained and approved prior to any caving activity. Surface watch and call-in time must also be established prior to the trip; surface watch should be informed of party exiting cave ASAP (e.g., via cell phone or radio). Appropriate staff must be informed of trip plan when conducting surveys in remote sections of cave. Trip leaders must be appropriately trained and prepared for the type of trip they are leading. Each team member must be prepared for the type of trip and familiar with first aid kit locations along the nearest travel route (if any). All personal protection equipment (PPE) will be inspected and tested prior to using, and all equipment will be adequate for the type of trip. Always cave with an appropriate party size for activity and cave area, caving alone is usually not appropriate.
Personal protective equipment:	UIAA approved helmet with four-point suspension chin strap, three reliable independent light sources, knee-pads (as needed), elbow-pads (as needed), gloves, treaded boots with good soles and ankle support, sufficiently warm clothing, chemical heat pack, compact first aid kit.
Typical tools, equipment & supplies:	Side-mounted pack, adequate drinking water, adequate quick-energy food supply, extra batteries, watch, cave maps, compass, hand-line.

Table SOP2-4. Job Hazard Analysis (JHA) for performing monitoring work in Cumberland Piedmont Network cave parks.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Planning Cave Trip and Communication	Not being prepared and not following plan/itinerary. Lack of leadership and communication. Failing to establish a reliable surface watch and reasonable call-in time. Planning a caving trip that will exceed the abilities of any team member. These abilities include physical condition, technical skills and psychological aspects.	One person for each trip will be designated as the trip leader. This person is responsible for providing leadership and clear communication concerning safety, minimizing impact to the cave resource and achieving the trip goals. Ensure that trip plans are within the range of all team members. Discuss trip plans with team members and make sure each member understands the trip plans, is prepared to meet the challenges of the trip in terms of physical condition, technical skills and psychological aspects. Gather appropriate maps, GPS data, and route information so the team can efficiently find caves they are going to and their objectives within the caves. Establish a reliable surface contact person (surface watch) and reasonable call-in time. Ensure designated surface watch and Law Enforcement (LE) are informed when conducting surveys in remote sections of cave. Check expected weather and stream conditions for day and sites.
Emergency Preparedness	Not knowing emergency procedures.	Know who to contact and how to reach them in the event of a life-threatening or non-life-threatening emergency. Have current CPR and first aid certification, and know the certification status of co-workers.
Preparing Equipment	Not bringing proper equipment to achieve the planned objectives. Equipment worn, broken or inoperable due to lack of proper maintenance. Team member not knowing how to properly use caving equipment.	Trip members will make sure they have the proper personal equipment for the trip. Trip leader will supply protocol-specific checklist of necessary equipment. Each trip member is responsible for regularly checking, cleaning and ensuring their caving equipment is in proper working order. All team members will have the training and knowledge as to proper usage of each piece of equipment used for their specific trip.
Entering Cave	Entrance Zone animals Rock fall	While in the entrance area of a cave all team members should be alert and aware that skunks, venomous snakes, spiders, and other potentially hazardous animals may be found. Avoid treading on accumulated guano or middens. Due to high fluctuation in temperature and moisture near cave entrances, some entrance areas can be particularly prone to loose rocks. Move carefully and thoughtfully so as not to dislodge rocks.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Horizontal Caving (general)	Getting lost	Caves can be confusing, and route finding in the cave can be tricky. As you travel to the area(s) where you will be working, pay attention to the route you take. Each person on the party should be able to find his/her way out of the cave. It helps to look back at junctions as you travel through them. Doing this gives you a look at what the junction will look like when you come back. If needed, removable flagging can be used to mark junctions. Permanent markers, like arrows, should not be placed in the cave without specific permission.
	Exposed climbs	Always use three points of body contact on cave surfaces to minimize risk of falling. Where feasible, use a hand-line or belay.
	Slippery surfaces / Falling	Everyone will wear footwear with good traction and a caving helmet with a chinstrap. Everyone should move in a careful, controlled manner to avoid falling. When climbing, test all holds to ensure that they can withstand the force being placed upon them.
	Low / small areas	Trip leaders should ensure all members are able to negotiate low/small areas on caving route. Remaining calm and thinking through what one must do to get through low/small areas is key. Traveling head-first through low/small areas that slope steeply downward can present challenges and should be undertaken with extreme care.
	Exertion / Exhaustion	Each team member should have adequate knowledge of the length and duration of trip prior to heading into the cave, and should have cave-specific physical conditioning. People in good physical condition need less water and are less prone to injury. Push your endurance limit in gradual increments. Avoid overloading your pack; be creative to reduce weight and bulk. Prior to the trip, the trip leader should inquire about people with known physical conditions and treatment needs. Groups should avoid overexertion, and should stop at least every hour to eat and drink. Group speed should be tailored to the slowest person on the team. Should the trip become too much for one trip member, the whole trip plan will be modified to achieve a safe trip.
	Temperature-related issues	Ensure team members are appropriately dressed for continued movement – a lightweight long-sleeved shirt and lightweight, durable pants are usually sufficient and prevent overheating.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Horizontal Caving (general)	Temperature-related issues (cont)	Ensure team members have adequate cold-weather clothing in their packs such as a balaclava and long-sleeved polypropylene shirt. Explain to team members about the colder temps while not moving and the necessity of wearing these items to prevent hypothermia. Keeping clothing dry is important.
	Overdue party	Trip leaders must always establish a reliable surface watch prior to embarking on a trip. This person must be briefed on what time to expect the team to return (or call-in) and whom to contact in the event the team does not exit on time. Location of the team, number of participants and travel route maps will be made available to the surface watch. Trip leaders will allow a reasonable amount of time for the team to exit the cave. If the team becomes lost, they will remain where they are and wait for the surface watch to notify search and rescue.
	Dehydration	All team members will be properly hydrated before entering the cave and drink sufficient water or electrolyte replacement drinks during the trip to maintain a proper hydration and avoid cramps.
	Minor injuries	Self-rescue using compact first aid kit in pack or in caches.
	Major injuries	When possible, at least one team member stays with injured party while other team member(s) goes for help. In many areas of Mammoth Cave near toured sections, telephones may allow you to notify help without exiting the cave.
	Rock fall	Cavers should locate themselves in places where they will not be exposed to rockfall from team members above them. Cavers will move carefully and thoughtfully so as not to dislodge rocks. Should a team member accidentally dislodge a rock or drop equipment they will clearly yell "Rock!" to inform team members below of the impending danger. Team members below should be alert and step away (not look up). All team members will wear a caving helmet with chinstrap. Helmets should not be removed when in area with potential for rockfall.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Wading or snorkeling Additional required equipment: Thermal protection in water (such as wetsuit or drysuit, neoprene shorts, neoprene shirt, neoprene socks, exposure suit as appropriate for specific condition), mask and snorkel, fins (if snorkeling), inflatable kayak/boat, paddle (if needed for specific work).	Total submersion Hypothermia	When possible, monitoring in cave streams and rivers should be performed during the dry season so that water levels and flooding potential are low (and visibility high). Always check weather reports before performing monitoring in cave streams or rivers. Be aware of antecedent conditions and consider current soil saturation. If there is a high chance of heavy rain in the surrounding area the trip should be postponed. Always work in areas with adequate air space between the water surface and the cave ceiling. Know alternative exit routes. Personal flotation devices are unnecessary when snorkeling because wetsuits or exposure suits (which are needed due to temperature) make the individual positively buoyant and so they will float on the surface. When wading use neoprene socks and shorts to keep a warm film of water in contact with skin. For work in deeper water, wetsuits or even drysuits should be used to reduce chance of hypothermia. The thickness of wetsuit (or choice to use a drysuit or exposure suit) will depend on the water temperature, length of potential exposure, and type of exposure (wading chest deep, or swimming). Chemical heaters that last for 8–10 hours are highly recommended to deal with hypothermia. They can be wrapped in a rag and inserted into the exposure suit to supply heat to the snorkeler's core. A heat tab stove can be used to heat up a hot meal (e.g., beef stew) for energy and warmth. Performing physical exercise is also a good way to warm someone up.
Vertical Caving Additional required equipment: Rope of adequate length, harness outfitted with standard (or preferred) ascending and descending gear, and Quick Attachment Safety	Miscommunication resulting in someone entering the rockfall zone while another team member is in a position to dislodge rocks or while another team member is still on rope. Ropes and or rigging materials worn or damaged. Unsafe rigging. Not conducting a thorough check prior to rappelling.	Clear signals will be used to avoid miscommunications. "On Rope!" will be clearly shouted when entering the rockfall zone with the intent to rappel or ascend a rope. "Off Rope!" will be clearly shouted after getting off rope and exiting the rockfall zone. A clearly shouted "OK!" from the other team members should acknowledge either of these commands. All ropes and rigging materials will be inspected for wear or damage before use. If necessary damaged or worn materials will be retired. All rigging will be inspected before use to ensure that it is safe. If determined not to be safe, the rigging will be modified if possible or the trip halted until the rigging can be made safe. During rigging, a figure eight knot will be tied at the end of the rope prior to the first rappel to prevent accidental "short-ropeing." All vertical caving participants will go through a pre-rappel checklist; often called "Checking the Chain."

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
	Rope damage encountered while on rope.	A butterfly knot will be used to eliminate the damaged section of rope from the life supporting rope. A note will be left both at the top and bottom of the rope informing cavers of the situation.
	Incident occurs where caver is forced to change over to ascent while descending or to descent while ascending.	Everyone participating in vertical caving will be required to have the equipment, training and knowledge to perform changeovers from ascent to descent and descent to ascent.
	Object becomes jammed in rappel device.	Everyone participating in vertical caving will be required to have the equipment, training and knowledge to safely lock off their rappel device and remove the jammed object without using a knife.
	Difficulty in passing a rebelay, traverse or other complex rigging situation.	All persons traveling to sections of cave with complex riggings will be required to have the equipment, training and knowledge to safely negotiate these complex riggings.
	Caver becomes exhausted, unconscious or injured resulting in immobilization on rope.	When someone becomes immobilized on rope, it is critical to remove that person from rope as soon as possible, usually by talking them through the problem, or lowering them to the ground. Ideally every trip should have at least one person knowledgeable of small-party vertical rescue techniques. Single-rope pickoffs are a last resort.
	Unsafe use of cable ladders	Cable ladders are often convenient for short pitches (less than 30 feet [9.1 meters {m}]); however, they can be tricky to use. People using cable ladders should have knowledge and experience in their use. A belay should be used when either descending or ascending on cable ladders.
	Unsafe use of ladders	Although they are not used very frequently, hard-sided ladders (such as extension ladders, step ladders, collapsible ladders, or sectional ladders) may be used for some types of vertical caving. Be sure that ladders are appropriately secured. It may be appropriate to belay climbers on ladders, depending on mounting and exposure. With collapsible ladders, be sure that ladder is locked, to prevent collapse while climbing. For sectional ladders, be sure sections are locked together and that ladder is used with an appropriate rise and run ratio. Be aware of (and clean off) mud on rungs to prevent slipping on the ladder.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
General Foot Travel (on surface)	Falling or tripping due to stream crossings, wading, wet areas, poor footing, uneven terrain, loose/rolling rocks and heavy pack.	Use caution at all times. Walk carefully, watching footing. Wear appropriate boots for conditions, especially stream wading. Stay aware of your feet. Address blisters and hot spots promptly. Avoid carrying excessive weight or unbalanced loads. When walking on a steep slope, lean upslope. Ensure that stems and vines are alive and can support your weight before relying on them. Use extreme caution traversing wet rocks, streams, steep slopes or blowdown areas. Proceed cautiously, test footing carefully, and use a sturdy pole or walking stick for balance.
Working Outdoors During Storms	Being struck by falling trees or branches. Being struck by lightning.	Listen to the weather forecast each morning (park radio and/or internet). Plan or adjust field work to avoid being out in thunderstorms. Postpone work if safety will be compromised by storm conditions. If you see or hear a thunderstorm coming, retreat from high ground and exposed areas. Go inside a sturdy building, vehicle, or cave, if possible. If you can't get inside and if you feel your hair stand on end, lightning is about to strike. Make yourself the smallest target possible and minimize contact with the ground. Crouch down on your pack on the balls of your feet and keep your feet close together. Place your hands on your knees and lower your head. During a thunderstorm members of the crew should stay separated by at least 10 feet (3 meters [m]).
Poisonous Plants (especially poison ivy)	Contamination/toxicity from contact with poisonous plants.	Learn to identify poison ivy in its many growth forms. Wear long sleeves and pants. Be aware of poison ivy and avoid coming in direct contact with it. Thoroughly wash hands, equipment, and clothes with Tecnu or similar specialized soap after working in areas with poison ivy.
Bee, Wasp, or Yellow-jacket Stings	Multiple stings from disturbing or stepping into nest areas.	Be alert to hives in brush, ground holes, or hollow logs. Watch for insects traveling in and out of one location. If you or anyone you are working with is known to have allergic reactions to bee stings, tell the rest of the crew and the field crew leader. Make sure you carry emergency medication with you at all times, and that your co-workers know where you keep it. Wear long sleeve shirts and pants, tuck in shirt. Bright colors and metal objects may attract bees or wasps. If you are stung, a cold compress may bring relief. If stinger is left behind, scrape it off of skin. Do not use tweezers as this squeezes the venom sack, worsening the injury. If the victim develops hives, asthmatic breathing, tissue swelling or a drop in blood pressure, seek medical help immediately.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Bites from Mosquitoes, No-see-ums, and Chiggers	Itchy reactions to multiple bites.	Avoid sitting on the ground or on logs, especially in dry sunny grassy areas. Use insect repellants. Do not apply Permethrin, Permanone, or greater than 30% DEET directly to skin, only to clothing. Wear long sleeves and pants. Carry after-bite medication to reduce skin irritation.
Venomous snakes	Being bitten by a venomous snake.	Wear snake gaiters when in known snake habitat. Be alert for snakes in thick vegetation and rocky habitats. Look before putting hands or feet in places out of immediate view. Treat all bites as if envenomation has occurred. Seek medical attention immediately and/or call 911 for help. Keep the person calm and move as little as possible to reduce spread of the venom. Immobilize the bitten area and keep it at or below the level of the heart. Remove rings, watches, shoes, etc. before swelling begins in earnest. Monitor the person's vital signs—temperature, pulse, rate of breathing, and blood pressure—if possible. If there are signs of shock (such as paleness), lay the person flat, raise the feet about a foot, and cover the person with a blanket. Do not use tourniquets or suction devices. Do not cut the area around the bite. Do not apply ice or flush the wound with water. Do not give the person stimulants or pain medications unless a doctor tells you to do so. Do not give the person anything by mouth. <i>(Compiled from NIH, JAMA, and American College of Emergency Physicians)</i>
Ticks	Contracting diseases transmitted from ticks.	Use tick avoidance precautions, such as pre-treating clothing with permethrin, tucking pants into socks and shirt into pants. Avoid sitting directly on the ground, especially in leaf litter and check packs for ticks before putting them on. Wear clothes (including pants and long-sleeved shirts) that are light colored and check for ticks on clothing periodically throughout the day. Conduct a thorough tick check every evening after completing field work. Know how to identify tick life forms, and the symptoms of tick-borne diseases.
Walking through Thick Vegetation	Cut, scratched, or bruised by vegetation; eye or ear injuries.	Shield your eyes and face with your hands, glasses, or hat when moving through tall thick brush. Keep your head and eyes pointed somewhat downward so your head hits obstacles before your eyes. Wear pants and long-sleeved shirts to protect bare skin. Look before you grab vegetation to avoid grasping thorny stems. Do not follow closely behind other people to avoid having branches snap back at you.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Working in Heat, Humidity, or Cold	Heat exhaustion, sunburn, dehydration, hypothermia.	Evaluate the weather forecast each morning and plan field work accordingly. Carry and drink plenty of water. Take extra breaks during extreme weather events. Adjust the work routine to minimize exposure to extreme heat and humidity. Take adequate garments for all possible weather conditions. Choose clothing that will keep you warm even if it gets wet.
Hazard Trees	Being struck by falling trees or branches.	Look up. Be alert for “widow-makers”, storm-damaged trees with large broken limbs, and unstable standing dead trees. Do not spend extended time in an area with hazard trees.
High Water Stream Crossings	Injuries from falling and/or drowning.	Thoroughly investigate area to find safest crossings. Wear appropriate foot gear for stream crossings. It is safer to wade through high water, rather than rock hop across a stream trying to keep your boots dry. Unbuckle your pack and be prepared to jettison gear should you lose your balance or fall in. Use a sturdy pole or walking stick for balance.
Carrying a Pack and Other Equipment	Injuries from improper packing, adjustment, and lifting of backpacks. Injuries from improper carrying of gear.	Learn how to properly pack, adjust, lift, and carry a pack. When hand-carrying gear, keep one hand free. If carrying long equipment, be aware of other people and never swing around quickly. Avoid allowing a long piece of equipment to project up and behind you, where you cannot see it.
Vehicle Operation	Injuries from vehicle accident; damage to vehicle.	Perform pre-operational check of vehicle (oil, tire pressure, tire condition, fluids, wipers, brakes, lights, gas, etc.). Report all needed repairs to the crew leader or supervisor promptly. Do not use the vehicle if it is unsafe. Wear seat belts with shoulder harnesses whenever vehicle is in motion. (E.O. 13043 4-16-97) Do not use cell phones or text while driving. Only NPS employees, volunteers, or authorized cooperators and contractors are allowed to operate or ride in a government vehicle. Ensure full visibility from all windows and mirrors. Clean windshield regularly. Always ride inside the vehicle. Properly store and secure all tools, equipment, and cargo so that they will not shift during sudden starts or stops. Plan your travel before you start. Know your route. Practice defensive driving; be alert to potential hazards. Obey all traffic laws and speed limits. Adjust speed to changing weather or traffic conditions. Allow adequate following/stopping distance. Avoid distractions such as eating or adjusting navigation/GPS units while driving. Be alert for pedestrians or bicyclists using roadways. Be watchful for wildlife crossing roads, especially at early morning, dusk, and after dark. Do not drive if fatigued. Stay alert! Do not exceed the 12 hour limit for driving/work hours.

Activity	Potential Hazards	Recommended (unless otherwise stated) Safe Action or Procedure
Driving on Gravel, Dirt, or Un-maintained Roads	Injuries from vehicle accident; damage to vehicle.	Maintain a slow and safe speed for changing road conditions, such as loose gravel, large potholes, washed out road, fallen trees or rocks. Be alert on narrow roads for oncoming vehicles and log trucks. Be prepared to slow down, pull over, or stop with little notice. Many roads require four-wheel drive and/or high-clearance vehicles for safe passage. Use the appropriate vehicle for the terrain. Do not exceed the capacity of your vehicle or driving ability. When in doubt, turn around or back out. Use spotters to assist in navigating obstacles and assessing water depth at stream crossings.

Job Hazard Analysis (JHA) for performing monitoring work in CUPN cave parks.

Please sign your name below to certify that you have read the Job Hazard Analysis and fully understand the Safety Standard Operating Procedures for the Cumberland Piedmont Network protocol for long-term monitoring of cave aquatic biota.

PRINT NAME

SIGNATURE

DATE

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Field Information Sheets and Cave Stream Reach Maps

Field Information Sheets describing conditions at each CAB stream reach help orient new crew members, increase efficiency, and greatly improve the consistency of data gathering through time. The Field Information Sheet in Figure SOP2-1 provides a template for the types of information to be included. A Field Information Sheet will initially 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 by the CUPN GIS specialist in conjunction with the project leader. Cave Stream Reach Maps are used for plotting and labeling the locations of permanent transect markers, and the locations of HOBO data loggers and their permanent landmarks, to assist the field crew with their relocation during sampling events. Cave Stream Reach Maps will consist of an overview map of all transects in the entire cave stream reach. The overview map (Figure SOP2-2, top) is also divided up into a number of subsections that provide greater detail on the locations of transects, data loggers, and their permanent landmarks (Figure SOP2-2, bottom). For transects containing a series of pools a separate Pool Map will be created so that each pool can be separately surveyed for cave aquatic biota (see SOP 3). After each sampling event, as necessary, the project leader will update the cave stream reach maps by plotting newly established data logger or landmark locations. Pool maps may also need to be periodically updated if increased flood intensity or increased drought length grow or shrink pools, respectively.

All field information sheets, cave stream reach maps, and pool maps will be printed on waterproof marine paper/Rite-in-the-Rain™ paper (8.5 × 11 inch size). All field data sheets are printed on waterproof, looseleaf Rite-in-the-Rain™ paper (4 5/8 × 7 inch size) and retained in a zippered field book (Figure SOP2-2, top). Field data sheets and maps filled out in pencil or permanent ink can be disinfected after surveying by immersion in an appropriate solution following current national decontamination protocols. Other non-permanent ink may smear during disinfection, so be sure to use permanent ink. While some print cartridges use permanent ink, others do not, so we will experiment with locally printed forms prior to field sampling. Because they contain sensitive information (e.g., cave location coordinates), extra copies of field information sheets and cave section maps will be maintained by the project leader with the secure project files.

GPS Setup

Using DNRGarmin (free) or similar software, the CUPN GIS specialist will upload cave location and parking location coordinates into the CUPN GPS unit(s) or handheld device. Immediately after uploading the plot coordinates, it is critical to check the GPS unit's map page to ensure that the cave locations are shown correctly and are in the correct projection.

UPDATED: 27 April, 2017

PARK: MACA

CAVE NAME: Apocryphal Cave



**GPS: N-XX-XX-XX
W-XX-XX-XX**

*****CAPPED -#XXX***
ROAD GATE KEY: Y
CAVE GATE KEY - XXXX**

DIRECTIONS: Park on *Imaginary Road* X miles north of the ferry. Walk X feet down Apocryphal Hollow on an abandoned road. The entrance is on the east side about X feet east of the creek bed.

ENTRANCE: Small entrance possibly dug open originally, in bottom of collapsed sink at base of hill, locked gate.

DESCRIPTION: "Beyond entrance, drops down a small crack into a sandy hands-and-knees crawl to a belly crawl, that leads to river level, requires ropes after this and drops to a muddy, slippery slog along a ledge above a river"- SRM

FIELD CREW SIZE: Best/Minimum=3; Maximum=4.

SPECIAL EQUIPMENT NEEDED: All transects require walking/wading.

CULTURAL NOTE: historic signatures

BIOLOGY: Base level CAB (e.g., cave crayfish, cavefish, cave shrimp).

HOBO DATALOGGER INFORMATION:

Landmark is 15 meters upstream of beginning of transect 1; 1.5m stalactite on left side of passage just before it turns sharply to left; large pool at turn.
HOBO U20L-02#10663142 **installed** 10/12/2017; **serviced** 8/15/2018. Stilling well is anchored in large pool on left side of passage just before sharp turn.

Figure SOP2-1. Field Information Sheet template for CAB monitoring in CUPN parks.

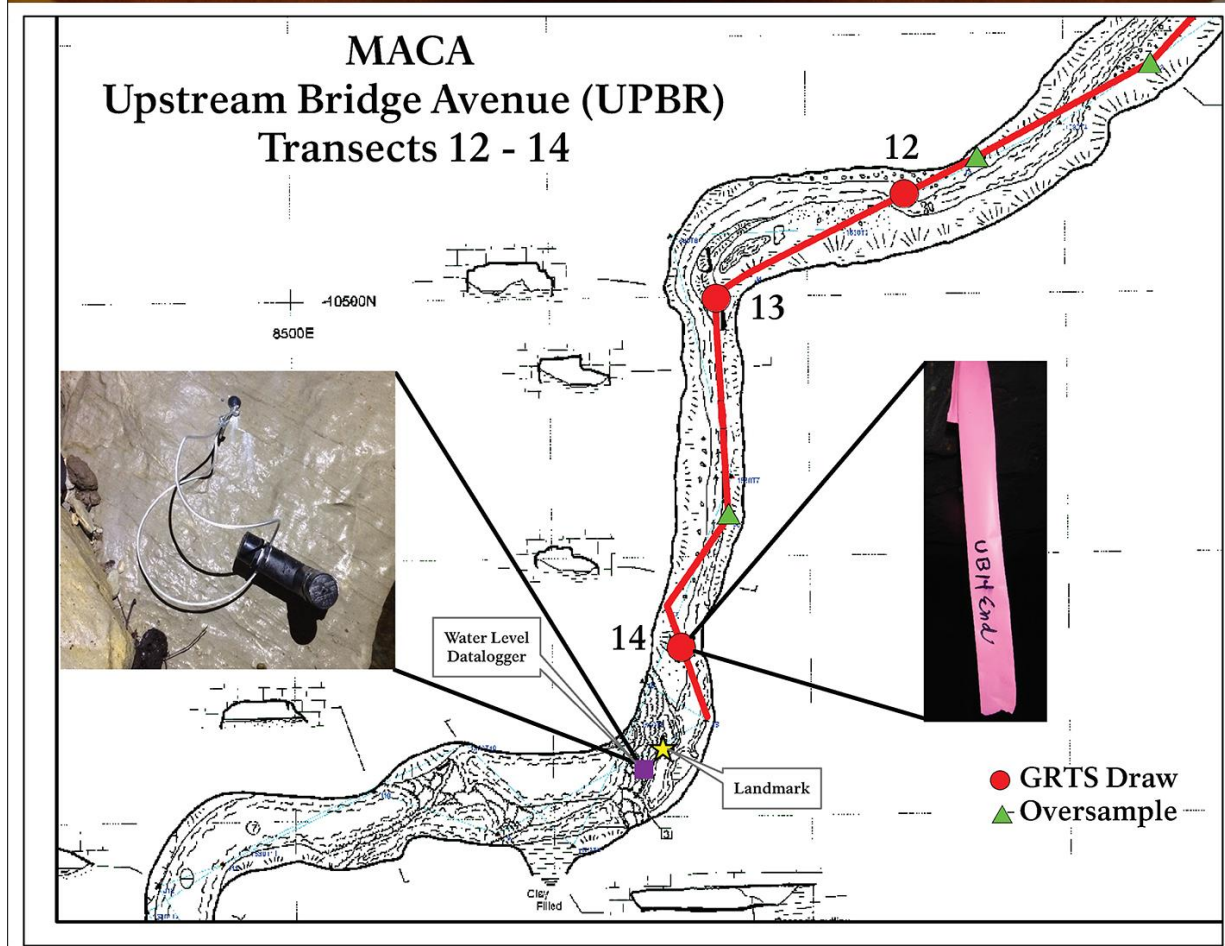
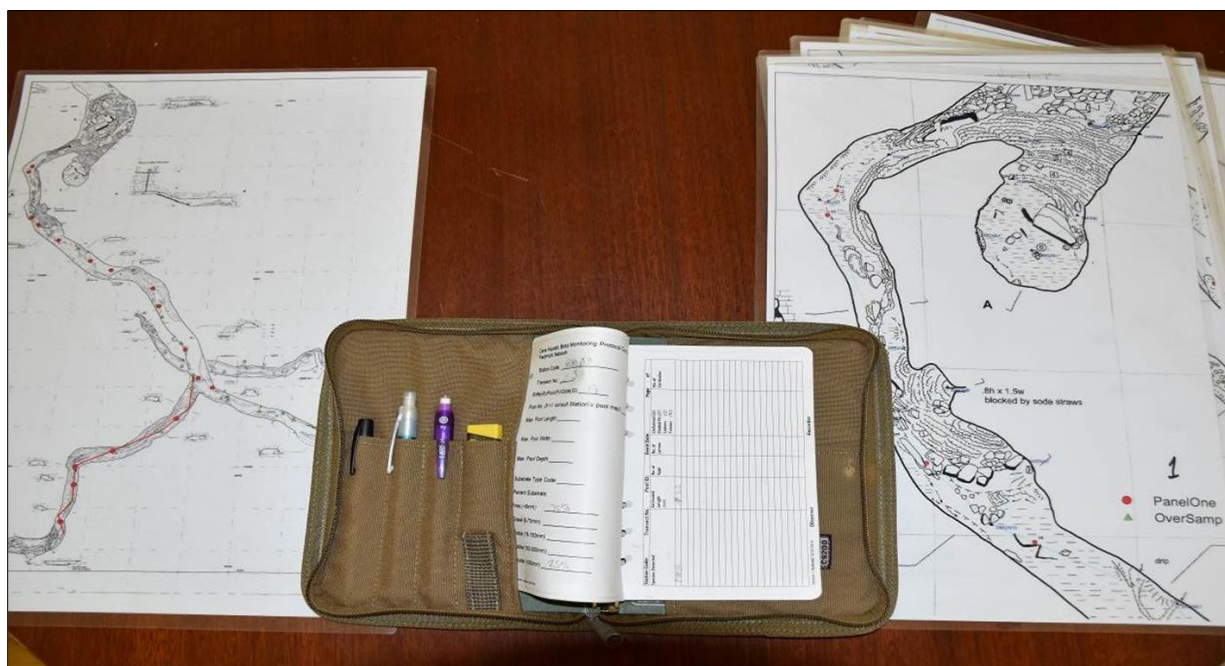


Figure SOP2-2. Overview cave stream reach map with all subdivided sections and zippered field book (top). Mockup of Cave Stream Reach map section for CAB monitoring in CUPN parks (bottom).

Communication and Crew Oversight

Prior to each trip (i.e., multiple days sampling at a park, or periodically at Mammoth Cave NP), a trip plan will be filed with a CUPN contact (i.e., someone in the office during the trip) and the park (Figure SOP2-3). A trip plan will include items such as trip purpose, sampling dates and times, daily check-in/check-out procedure, field crew members, contact information, and sampling sites.

When available, the field crew should carry a cell phone plus an NPS radio or satellite communicator when traveling in remote areas at Cumberland Gap NHP and Mammoth Cave NP. The cave selected for monitoring at Russell Cave NM is a front-country cave (i.e., not in remote areas but within shouting distance from the visitor center and housing area), therefore Russell Cave was omitted from the list. In all situations, crew members will comply with park policies regarding the use of radios while conducting field work. Remember, radios, cell phones, and satellite communicators do not work inside caves. Radios must be programmed to the appropriate frequencies for each park. Crew members should know which frequencies to use, how to contact park radio dispatch, and be familiar with proper radio etiquette. If available, crew members should be familiar with proper use of a satellite communicator. In areas with cell phone coverage, crew members are encouraged to carry a cell phone in the field for safety and to call the project leader concerning protocol questions. It is essential for the crew to maintain regular contact with the project leader throughout the field season. Establish a regular weekly telephone call time between the field crew leader (or crew member, as appropriate) and project leader to review the week's work. The update should cover questions or problems related to caves, protocol, logistics, and scheduling.

CUMBERLAND PIEDMONT NETWORK (CUPN) TRIP PLAN Cave Aquatic Biota Monitoring		
PARK: Cumberland Gap National Historical Park (CUGA)		
DATES: Week of August 28, 2017		
ESTIMATED TIME: The field crew anticipates arriving at the park on Monday, August 28 at approximately 1:00 pm (EST). They will check-in with a representative of Resource Management Division. Field activities will likely occur between the hours of 9:00 am and 7:00 pm and should be completed by Saturday, September 4. The crew will check-out with staff at the Visitor Center.		
PURPOSE: To conduct cave aquatic biota monitoring and service/deploy water level dataloggers inside selected caves.		
DAILY PROCEDURE: A surface contact person ("surface watch") will be established before <u>each</u> trip to a cave. Clearly established with the contact person will be: 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 contact person will be given a copy of this trip plan, as well as, travel route maps (surface and subsurface) for each cave to be visited.		
VEHICLE: Ford Explorer (white), license plate: I515510		
FIELD CREW:		
NAME	MOBILE PHONE	
Kurt Helf (CUPN)	270-784-XXXX	
Stuart Daw (CRF)	606-246-XXXX	
Joe Settles (CRF)	606-246-XXXX	
Terri Brown (UT Knoxville)	865-850-XXXX	
CONTACT INFO:		
NAME	OFFICE PHONE	MOBILE PHONE
Jenny Beeler (CUGA)	606-246-XXXX	865-850-XXXX
Amy Settles (CUGA)	606-246-XXXX	606-670-XXXX
Cumberland GAP Tunnel	606-248-XXXX	N/A
Teresa Leibfreid (CUPN)	270-758-XXXX	270-784-XXXX
SITES:		
Gap Cave		
Nearest Hospital Contact Info: Middlesboro ARH Hospital Phone number: (606) 242-1100		
From the Visitor Center take the ramp onto US-25E N		
Use the left turn lanes to turn left on Cumberland Avenue (next stoplight).		
The hospital is 2.1 mi down Cumberland Avenue on the right.		
Address: 3600 W Cumberland Ave, Middlesboro, KY 40965		

Figure SOP2-3. Example of sampling event trip plan to be filed with a Cumberland Piedmont Network contact and a park prior to monitoring cave aquatic biota in CUPN parks.

Summary of Yearly Staff Roles

Timing and staff roles for primary tasks are presented in Table SOP2-5.

Table SOP2-5. List of major protocol tasks, responsible staff, and schedule for monitoring cave aquatic biota in selected Cumberland Piedmont Network parks.

Task	Staff	Timing
Acquire/renew the appropriate permits (i.e., NPS, USFWS) as necessary.	Project Leader	February
Propose panel-specific sampling event dates and backup dates for field crews.	Project Leader	Late April
Upload cave entrance location and parking location coordinates into CUPN GPS unit(s) as needed. Print maps, if necessary.	GIS Specialist	Late April
Finalize panel-specific Sampling Event Trip Plan.	Project Leader	Late April
Email panel-specific (i.e., MACA and CUGA/RUCA) resources management contacts at least two weeks in advance to confirm sampling event dates, send Trip Plan, and to arrange logistical support (e.g., keys, lodging, reserve vehicle).	Project Leader	Two weeks prior to sampling event (i.e., July–October)
Prepare Field Books with Field Information Sheets, Cave Stream Reach Maps, Pool Maps (as needed), Cave Stream Reach Field Data Sheets (i.e., sampling event metadata, habitat characteristic metadata, and CAB), CAB Field Guide, and equipment for sampling.	Project Leader/Field Crew Leader	Early June
Arrange and conduct field crew training for sampling (i.e., new crew members or refresher for experienced crew members absent from field sampling for more than six months).	Project Leader/Field Crew Leader	Late June/early July
Prepare sample bottles and calibrate instruments, per CUPN Water Quality Monitoring Protocol SOP 4 <i>Field Measures—Version 1.2</i> , the day before the sampling event(s).	Field Crew Member	Day prior to sampling event
Participate in CAB field sampling. Project Leader oversees field crew during field season and provides logistic and administrative support.	Project Leader/Field Crew Leader/Field Crew Member(s)	July–October
Deploy, service, remove, or replace water level and temperature and HOBO data loggers in cave stream reach.	Project Leader/Physical Scientist/Field Crew Leader/Field Crew Member	July–September (second sampling event)
Coordinate directly with panel-specific (i.e., MACA and CUGA/RUCA) resources management contacts when conducting sampling events.	Project Leader	Ongoing
Ensure field work meets quality standards.	Project Leader/Field Crew Leader	Ongoing

Task	Staff	Timing
Ensure effective communication between park staff, field crew, and other CUPN staff.	Project Leader/Field Crew Leader	Ongoing
After sampling event: download, sort, and rename any camera image files to folder on CUPN server; store or decontaminate all field books/field data sheets, file decontaminated field data sheets, and updated Field Information Sheets; upload data from HOBO data logger to folder on CUPN server. Hold post field sampling "tailgate session".	Field Crew Leader/Field Crew Members	Ongoing
Submit brief sampling trip report to park-specific resources management contact within two weeks after sampling event.	Project Leader	See panel-specific months
Enter all CAB sampling data into master database as soon as practicable after data are collected. Field data sheets are scanned and stored on CUPN server. Hardcopy forms are stored in project leader's file cabinet.	Crew Member(s)/Data Intern	Early November
Enter all WQ grab sampling data into NPSTORET as soon as practicable after data are collected. WQ grab sampling data sheets are scanned and stored on CUPN server. Hardcopy forms are stored in project leader's file cabinet.	Crew Member(s)/Data Intern	Early November
Upload all HOBO data logger data into AQUARIUS as soon as practicable after data are collected. HOBO U20L Water Level Field Data Sheets are scanned and stored on CUPN server. Hardcopy forms are stored in project leader's file cabinet.	Physical Scientist	Early November
Conduct data verification on field data sheets once data from hardcopy sheets are entered into database.	Data Manager/Field Crew Member/Data Intern	Mid–Late November
Conduct data validation after records are entered into database.	Project Leader/Field Crew Member/Data Intern	Mid–Late November
Inventory the condition of all equipment and prepare a list of required repairs or replacements.	Field Crew Member/Data Intern	Mid–Late November
Update Field Information Sheets in database, as necessary.	Project Leader	Mid–Late November
Data from CAB database, NPStoret, and Aquarius are imported into statistics/graphing packages (R and Sigmaplot) for analysis. Data uploaded to IRMA.	Project Leader/Field Crew Leader	Late November
Data analysis and (status/trends) report writing.	Project Leader	January–February
Ensure final electronic data sets are archived and backed-up following NPS guidelines.	Project Lead/Data Manager/Physical Scientist/Field Crew Member	January–February
Ensure archives are processed according to NPS guidelines.	Curatorial Specialist	January–February

Task	Staff	Timing
Review and revise monitoring protocol and standard operating procedures on a yearly basis.	Project Leader	January–February
Prepare, repair, or purchase supplies and equipment for upcoming sampling year.	Project Leader/Field Crew Leader	January–February
Establish and refine annual and seasonal budgets.	Project Leader/Monitoring Coordinator/Network Manager	January–February

Supplies and Equipment

Required equipment and supplies for monitoring:

1. pre-sampling event logistical checklist (see below),
2. observer and recorder field equipment checklists (Tables SOP2-6 and SOP2-7),
3. water quality sampler checklist (Table SOP2-8), and
4. personal equipment checklist (Table SOP2-9) are listed below.

Equipment must be organized and maintained in good working condition. Print out the checklists provided in this section and use to assemble caving gear and sampling supplies and equipment, and calibrate all necessary equipment prior to departure for each sampling event. The project leader or field crew leader must sign and date a copy of the pre-sampling event logistical checklist (see below) used after each sampling event.

Pre-Sampling Event Logistical Checklist

1. Ensure research permit(s) for scheduled park(s) are up to date.
2. Finalize panel-specific sampling event dates and backup dates for field crews.
3. Email panel-specific (i.e., MACA and CUGA/RUCA) resources management contacts at least two weeks in advance to confirm sampling event dates, and send and file Trip Plan.
4. Arrange logistical support at least two weeks in advance:
 - a. Reserve road gate and cave entrance keys, as needed.
 - b. Reserve lodging, as needed.
 - c. Reserve appropriate vehicle in advance.
5. Review supplies/equipment checklist and check for working condition and battery condition—replace as needed. Complete/sign and date checklist.
6. Prepare Field Books with:
 - a. Field Information Sheets
 - b. Cave Stream Reach Maps,
 - c. Pool Maps (as needed)
 - d. Cave Stream Reach sampling event metadata field sheet
 - e. Cave Stream Reach habitat characteristic metadata field data sheet
 - f. Cave Stream Reach CAB field data sheet
 - g. CAB Field Guide
 - h. Cave stream reach specific HOBO U20L Data logger Site Setup and Maintenance Field Data Sheet
7. Project leader or designated crew leader conducts pre-sampling training and briefing for crew member(s).
8. Review Job Hazard Analysis (JHA) form. Get signatures from field crew member(s) in advance of field sampling.
9. Establish “surface watch”.*

*(Unnecessary if sampling event is part of CRF Expedition)

Checklist the morning of the sampling event

1. Give a copy of the trip plan and Cave Entrance Authorization form (Mammoth Cave NP only) to the surface watch. Sign out field crew with destination, vehicle(s), surface contact person, and approximate return time on park/office message board (if required by park).
2. Project leader or designated field crew leader conducts pre-field sampling briefing for crew member(s), as necessary.

Checklist after the sampling event

1. Project leader or designated field crew leader informs the surface watch of the field crew exiting the cave ASAP (e.g., via phone or radio).
2. Project leader or designated field crew leader conducts post-field sampling “tailgate session” for crew member(s).

Crew Leader’s Signature/Date: _____

Table SOP2-6. Observer field equipment checklist.

Number of Items Required	Description
☐ 1	Transparent SciOptic grain size chart
☐ 1	Mask, fins, and snorkel (as needed)
☐ 1	Wetsuit/exposure suit (as needed)
☐ 1	Mask/wrist-mounted dive light and held-held backup
☐ 1	Mask mounted camera (as needed)
☐ 1	Additional dive lights and batteries (as needed)

Table SOP2-7. Data Recorder field equipment checklist.

Number of Items Required	Description
☐ 1	Field Book with set of panel-specific, pre-printed field data sheets in Rite-in-the-Rain looseleaf binder. This includes: ☐ Field Information Sheet ☐ Cave Stream Reach Maps ☐ Cave Stream Reach Sampling Event Metadata Sheets ☐ Cave Stream Reach Habitat Characteristic Metadata Sheets ☐ Cave Stream Reach Field Data Sheets ☐ CAB Abbreviations
☐ 1	Park-specific CAB Field Guide
☐ 1	Cave stream reach specific HOBO U20L Data logger Site Setup and Maintenance Field Data Sheet
☐ 1	Pool Maps (as needed)
☐ 1	Fiberglass tape (50 meters)
☐ 2	Mechanical pencils
☐ 1	Digital watch with stopwatch function
☐ 1	Laser Disto
☐ 1	Wetsuit (as needed)

Table SOP2-8. Water-quality sampler field equipment checklist.

Number of Items Required	Description
☐ 1	Tool Box (with pH, DO probes, thermometer)
☐ 2	Box with extras (probes and devices)
☐ 1	Water Quality Monitoring Program Rite-in-the-Rain® fieldbook
☐ 1	Suunto® watch (for reading barometric pressure to calculate DO)
☐ 1	pH and SPC Calibration solutions
☐ 1	DI H ₂ O
☐ 20	Nitrate-Nitrogen sample bottles
☐ 1	1, 10, 100 mg/L Mixed Nitrate Standards
☐ 1	Turbidity device box
☐ 1	Camera
☐ 1	Cooler
☐ 1	Ice
☐ 1	Extensible water collecting pole
☐ 1	Black binder with manuals for troubleshooting

Table SOP2-9. Personal field equipment checklist.

Number of Items Required	Description
☐ 1	Coveralls or caving suit
☐ 1	Pair of boots in good condition
☐ 1	Caving helmet with light
☐ 2	Independent back up light sources (headlamp + flashlight)
☐ 1	Backpack
☐ 1	Work gloves
☐ 1	Pair knee pads
☐ 1	Wet or dry suit with appropriate neoprene hood, neoprene gloves and neoprene socks (as necessary)
☐ 1	Extra batteries for all personal battery operated equipment
☐ 1	Set of extra clothing to change into after wet suit trips (as needed)
☐ 2	Large plastic garbage bags to store contaminated or wet clothing/gear (as needed)
☐ 1	Water bottles and lunch (warm soup/tea in tough insulated container, as needed)
☐ 1	Liquid waste bottle
☐ 1	Mylar emergency blanket*
☐ 1	First aid kit*
☐ 1	Two-way radio/cell phone*
☐ 1	Emergency signal device*
☐ 1	Set of gate and cave keys*
☐ 1	Waterproof shuttle and coupler for HOBO data logger*
☐ 1	Set of vertical gear when required. To include: static rope of appropriate length, sit harness, rappel device, ascending gear, spare carabiners and ascender. Rescue pack to include a selection of slings and webbing, etriers, carabiners, and pulleys.

*Crew leader

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National Park Service
Inventory and Monitoring Division



Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0

This standard operating procedure (SOP) gives step-by-step instructions for conducting a sampling event in underground stream reaches and completing field data sheets to collect long-term monitoring data on cave aquatic biota (CAB), habitat characteristics, and data on water quality and quantity.

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Contents

	Page
Glossary	SOP 3-4
Overview.....	SOP 3-5
Cave Stream Reach Sampling	SOP 3-5
Timing of Sampling.....	SOP 3-6
Water Quality and Water Quantity	SOP 3-6
Opportunistic Grab Sampling of Water Quality.....	SOP 3-6
Continuous Sampling of Water Level and Temperature	SOP 3-7
Step by Step Instructions for Data Logger Site Setup and Maintenance.....	SOP 3-7
Detection/Nondetection Sampling	SOP 3-11
Direct Visual Counts of Cave Aquatic Biota	SOP 3-11
General Step By Step Instructions for CAB Surveys.....	SOP 3-14
Walking/Wading Surveys Step By Step Instructions	SOP 3-17
Snorkeling Surveys	SOP 3-24
Additional Equipment Required.....	SOP 3-24
Snorkeling Surveys Step-By-Step Instructions	SOP 3-25
Literature Cited	SOP 3-30
Appendix A: HOBO Water Level/Temp Data Logger Field Data Sheet and Instructions for Filling out the Sheet.	SOP 3-32
Appendix B: Mock-up Example of a Cave Stream Reach Specific Field Information Sheet for Monitoring CAB in Three Parks in the Cumberland Piedmont Network	SOP 3-35
Appendix C: Mock-up Example of a Cave Stream Reach Map for Monitoring CAB in Three Parks in the Cumberland Piedmont Network	SOP 3-36
Appendix D: Data Dictionary for Cave Stream Reach Metadata, Habitat Characteristics, and CAB Field Data Sheets and Instructions for Filling out the Sheet.....	SOP 3-37
Appendix E: Field Guide to Common Cave Aquatic Biota at Mammoth Cave National Park	SOP 3-45
Common Aquatic Invertebrates.....	SOP 3-45

Amphipods	SOP 3-47
Isopods.....	SOP 3-48
Common Aquatic Vertebrates	SOP 3-51
Cave fishes.....	SOP 3-53
Surface fishes.....	SOP 3-55

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.

Geomorphic channel units—Descriptors of channel shape and velocity in a transect. Riffles, glides, and pools are the three descriptors used in this protocol.

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—Organisms 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 of it goes to a common outlet. Used interchangeably with basin.

Overview

Prior to a sampling event the project leader, or designated field crew leader, assembles all required group caving gear and sampling supplies or equipment. All field personnel review this SOP and associated equipment checklists (see Tables SOP2-6 through SOP2-9) by early to mid-June of each year. In late fall or early winter the project leader revises any sections based on new information (e.g., field information sheets) and lessons learned from field sampling the previous year. Prior to conducting a sampling event the project leader trains all field crew on their previously assigned tasks. Finally, each field season, just prior to field work, all field crew members must read and sign the related Job Hazard Analysis (JHA) in SOP 2 *Pre-field Sampling and Safety—Version 1.0* and agree to follow all safety guidelines during sampling. Newly added field crew members must also read and sign the JHA.

Cave Stream Reach Sampling

To facilitate sampling, cave stream reaches were subdivided into a number of permanent 40-meter (131.2-ft) transects via a Generalized Random Tessellation Stratified sampling draw prior to the first sampling event (see SOP 2). Reasons for using 40-meter (131.2-feet [ft]) transects included:

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. 40-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;
3. striking a balance between the competing interests of transect length and sample size; particularly true for short cave stream reaches (Table SOP3-1); and
4. finally, the number of transects in any given cave stream reach (Table SOP3-1) was subject to the constraints of fitting round trip travel time to the field site, preparation time in the field, reach length, and survey method into an 11-hr field day.

To address this protocol's monitoring objectives, we will collect two kinds of data during a sampling event in the following order: (1) water-quality and water-quantity data (i.e., CUPN protocol monitoring objective 3) including cave stream habitat characteristics; and (2) CAB detection/nondetection data (i.e., CUPN protocol monitoring objectives 1 and 2).

Table SOP3-1. Cave aquatic biota monitoring sites among Cumberland Gap National Historical Park (CUGA), Mammoth Cave National Park (MACA), and Russell Cave National Monument (RUCA).

Park	Watershed	Cave Stream Reach	Approx. Length (m)	Sampling Technique	Estimated Number of Transects
CUGA	Clinch River Subbasin	Gap Cave	4,000	Walk/Wade	27
MACA	River Styx Spring/ Echo River Spring	Styx/Echo River	1,150	Walk/Snorkel	17
MACA	Turnhole Spring	Bridge Avenue	450	Walk/Wade	9
MACA	Echo River Spring	Mystic River	1,800	Walk	18
MACA	Echo River Spring	Shrimp Pools/Roaring River	4,400	Walk/Snorkel	10
MACA	Pike Spring	Eyeless Fish Trail/Golden Triangle	1,100	Walk	15
RUCA	Doran Cove	Picnic/Main Entrance stream	2,800	Walk/Wade/Snorkel	28

Timing of Sampling

Sampling events are scheduled during the dry season (i.e., July–October) since base or low flow conditions provide the best opportunity for detecting cave aquatic biota. Sampling events occur as soon as conditions and personnel resources permit. Due to limited resources, it is impractical to sample all sites in all three CUPN parks in one year so we divided them into two separate panels: Panel 1=four Mammoth Cave National Park (MACA) sites, and Panel 2=one Mammoth Cave NP site, one Cumberland Gap National Historical Park (CUGA) site and one Russell Cave National Monument (RUCA) site. Sampling events (including one replicate per site) will be conducted at one panel per dry season such that, conditions permitting, 14 events will be completed every two years. During a given field season all cave stream reaches (=sites) in the panel are sampled independently by two teams consisting of two trained field personnel each. A replicate visit of each cave stream reach normally occurs within three to four days—a **minimum** of two days—to allow site conditions to resettle.

Water Quality and Water Quantity

Opportunistic Grab Sampling of Water Quality

Unless otherwise noted, water-quality data are collected at each scheduled cave stream reach using methods detailed in *SOP 4 Field Measures—Version 1.2* of the CUPN Water Quality Monitoring Protocol (Meiman 2018). The order in which sampling teams conduct their (staggered) surveys is

decided before the surveys begin: teams are designated “Team 1” and “Team 2.” Team 2 is responsible for the collection of all water-quality and water-quantity data. Team 1 readies their equipment and begins their CAB survey while team 2 collects opportunistic grab samples with pre-calibrated handheld sensors from the same location as the data logger (see below) and the data are recorded on custom Rite-in-the-Rain™ field data sheets as detailed in SOP 4 *Field Measures—Version 1.2* of the CUPN Water Quality Monitoring Protocol (Meiman 2018). The parameters measured with handheld sensors in cave stream reaches include specific conductance, water temperature, dissolved oxygen, and pH. Once the handheld sensors stabilize, measurements are made by taking three successive readings and recording the median value (SOP 5 *Sample Collection, Preservation, and Transportation—Version 1.2*; Meiman 2018). Team 2 then collects water samples which are subsequently analyzed by trained NPS personnel in the lab for turbidity, nitrates and *E. coli*. Quality control and quality assurance (QA/QC) replicates are collected during two randomly selected sampling events per year. During these events the handheld sensor measurements are repeated and separate water samples are collected for lab analyses.

Continuous Sampling of Water Level and Temperature

Water level, the height of the water column above the data logger, and water temperature are sampled continuously in all monitored cave stream reaches by HOBO U20L data loggers in stilling wells. Water level is used as an indirect measure of the physical forces acting on cave aquatic ecosystems through periodic flooding. 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 generally follow those detailed in Curdts (2017). Data logger sampling intervals are set to collect water level and temperature data at one-hour intervals. Cave water level and temperature field sampling activities broadly include data logger deployment, service, replacement, and removal (see Appendix A in this SOP), all of which are performed by Team 2. Field crew members may assist by serving as data recorder, data logger spotter, measurer, or photographer (of data loggers and landmarks). This process is only performed at each cave stream reach at the beginning of the second sampling event of the field season.

Step-by-Step Instructions for Data Logger Site Setup and Maintenance

- Given the significant flooding that may occur in most monitored cave streams the HOBO U20L data loggers are housed in a solid 2-inch PVC well pipe known as a stilling well (Figure SOP3-1). Stilling wells dampen the effects of flooding spates on the water level inside the pipe and protect the data logger. Vented sections, created by drilling quarter-inch holes in top and bottom sections of the pipe, allow water and air into the pipe. Stilling wells are installed in a location upstream of each cave stream reach in which monitoring transects are located. Stilling well locations are determined by the project leader and the hydrologist based on field experience and previous water-quality and water-quantity monitoring efforts. The stilling wells are securely mounted to a stable structure such as a staff gauge, post, piling, cave stream passage walls, or a large, immovable limestone rock (Figure SOP3-1). The latter two mounting options require drilling two small holes with a hammer drill. One hole is to secure the stilling well, with a concrete anchor, to the wall or rock (Figure SOP3-2, left). The second hole tethers the stilling well to a second anchor point, via a cable that passes

through the stilling well and the data logger, to prevent the loss of the stilling well should it be dislodged at the first anchor point (Figure SOP3-2, right). Note the cable also secures the data logger inside the stilling well so as to maintain a constant depth (Figure SOP3-2, left).

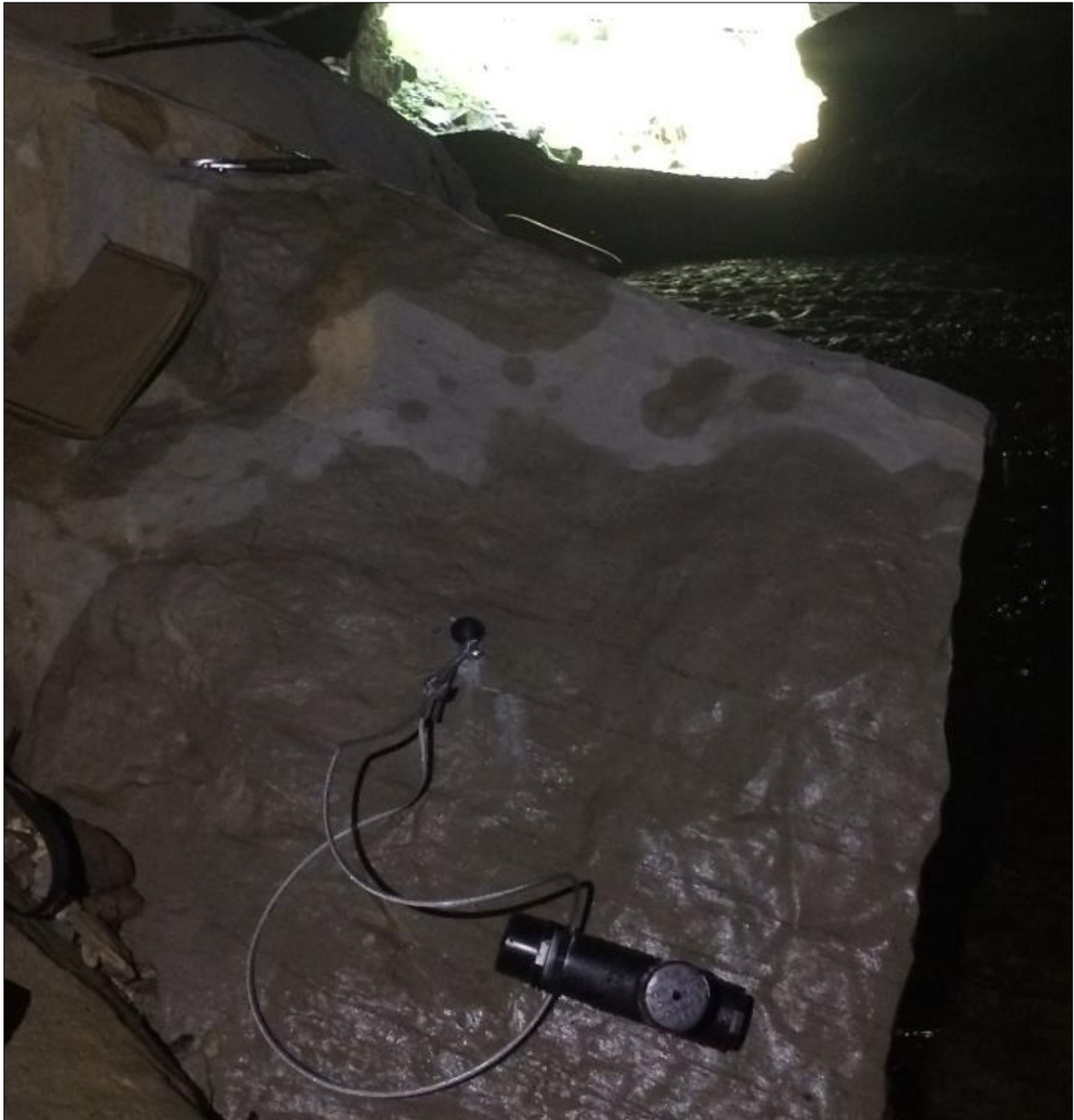


Figure SOP3-1. Stilling well containing HOBO data logger installed on large limestone boulder just above the cave stream's low water level inside RUCA's Main Entrance.



Figure SOP3-2. Concrete anchors are used to secure the stilling well at two points. One anchor point secures the stilling well and data logger (left) and the other anchor point secures a tether in case the stilling well is dislodged (right). Note these images were not taken during an installation inside a cave entrance.

- After the stilling well is installed a permanent landmark must be selected to use as a reference point for its relocation. Detailed written descriptions of the stilling well and specific landmark locations, including azimuth, inclination, distance from an obvious permanent landmark (e.g., a permanent benchmark), and the distance from the top of the stilling well to the water surface are recorded on the HOBO Water Level/Temp Data logger field data sheet in the “Initial Install or Resurvey Only” section (Appendix A, this SOP). The stilling well is also photographed in situ. Finally, the approximate location of each stilling well and corresponding landmark must be plotted and labeled on the field copy of the Cave Stream Reach Map. Refer to the specific cave stream reach’s Field Information Sheet (see Appendix

B, this SOP) and Cave Stream Reach Map (see Appendix C, this SOP) to navigate through the cave to the stilling well.

- The data recorder for Team 2 completes the fields “Park Code,” “Cave Name,” “Crew Member,” “Date,” “Time,” “Cave Stream Reach,” and “Data Logger Location Name” in the “General Information” section of the HOBO U20L Data Logger – Site Setup and Maintenance Field Data Sheet (see Appendix A, this SOP).
- As necessary, deploy initial pre-launched (in the office) HOBO U20L Data Logger at locations in caves in which data loggers were not previously installed. **Because the data logger’s battery life only extends five years it is important to check “Battery Status” during the launching process and record it on the HOBO Water Level/Temp Data logger field data sheet.**
 - Use the Install activity if a pre-launched data logger is scheduled to be reinstalled in a permanent location at which a data logger had previously collected data in the past. In this case, siting information and a data logger location description are left blank (previously collected).
- **On the second sampling event of the field season** Team 2 services each previously installed HOBO U20L data logger located in the cave stream reach. This includes:
 - data uploading to the appropriate model of data shuttle (as per checklists in Tables SOP2-8 and SOP2-9); the data shuttle must be connected to a computer in the office beforehand, in order to update its date and time, making sure to note park-specific time zone (MACA and RUCA are in Central and CUGA is in Eastern Time Zone),
 - relaunching the data logger (i.e., restarting automatic data collection),
 - examining,
 - cleaning, and
 - redeploying the HOBO. Record required metadata on the HOBO Water Level/Temp Data logger field data sheet (see Appendix A, this SOP).
- Location information for previously installed data loggers should have been included on the cave’s Field Information Sheet and should have been plotted on the cave’s Cave Stream Reach Map to assist with relocating the data logger.
- Replace a previously installed HOBO U20L data logger with a pre-launched data logger **every third panel rotation** (i.e., when its battery is nearing exhaustion) or when there is some doubt about the reliability of the previously installed data logger. Examples of this include the data transfer was not successful, the data logger did not relaunch, the LED is not blinking after replacing battery, or there are signs of external damage to the data logger. **Prior to replacement it is important to record the “Battery Status” of the new data**

logger, checked during the launching process, on the HOB0 Field Data Sheet. Record required metadata on the HOB0 Water Level/Temp Data logger field data sheet (see Appendix A, this SOP), **including** metadata listed under “Servicing existing (above) data logger”—even if data from the HOB0 will be uploaded in the office.

- Remove (and **do not** replace) a previously installed HOB0 U20L data logger from a location when:
 - there is some doubt about the reliability of the previously installed data logger and no pre-launched replacement data logger is available;
 - it is no longer safe or prudent for a data logger to be placed in that location (e.g., repeated theft/vandalism of data loggers, change in stability of cave ceiling/walls/floor);
 - or previously instructed to do so by the Project Lead.

Record required metadata on the HOB0 Water Level/Temp Data logger field data sheet (see Appendix A, this SOP) **including** applicable metadata listed under “Servicing existing (above) data logger”—even if data from the HOB0 will be uploaded in the office.

Detection/Nondetection Sampling

Direct Visual Counts of Cave Aquatic Biota

Visual survey is the primary method used by CAB researchers throughout the world and is considered a standardized technique (Pearson and Boston 1995; Pearson and Jones 1998; Trajano 2001; McCandless 2005; Graening et al. 2010). As such, visual surveys of cave aquatic biota are the standard sampling approach for this protocol. Visual surveys involve observers 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 a data recorder.

Prior to beginning a survey, field personnel split into two separate teams each consisting of an observer and a recorder. It is important for the project leader or field crew leader to establish roles in advance and maintain good communication in the cave to ensure accurate counting. The teams conduct staggered, independent 15-minute counts of cave aquatic biota by walking, wading or snorkeling in cave stream transects. The use of staggered 15-minute timed searches is a trade-off among the competing interests of standardizing search time between two different techniques (i.e., walking, wading versus snorkeling) maximizing the number of transects surveyed per cave stream reach, and minimizing cost. Observers scan the transect for cave aquatic biota, call out the species code, level of confidence in their identification (if in doubt), and the estimated size of all vertebrates, crayfish, and cave shrimp detected in each transect, and recorders write the data on the CAB field data sheet (see Figure SOP3-3 and Appendix D in this SOP).

Sampling techniques differ depending on whether the cave stream reach being sampled is wadeable or nonwadeable. Wadeable stream reaches or pools are generally shallow and transects can be

surveyed while walking along the bank. In the **rare** circumstance where a wadeable stream reach or pool is wide or extends across the entire passageway, the observers must perform a single survey by roughly dividing the transect in half while wading upstream side by side, keeping their surveys as independent as possible. Transects in cave stream reaches with a majority of nonwadeable habitat must be surveyed by snorkeling observers while recorders paddle inflatable kayaks behind.

While there may be concern regarding the effect of the first survey team's passage through the transect on the second survey team's data, it is unclear whether CAB behavior is affected by disturbance to the same degree as surface aquatic biota. Means and Johnson (1995) collected and tagged *Troglichthys (Amblyopsis) rosae* to track their movements in Logan Cave and indicated "...the cavefish would move away from the collector, but slowly enough that it could easily be netted; sometimes the cavefish moved directly toward the netter." The latter observation matches other worker's experiences in the field in that cavefish may be indifferent or even attracted to surveyors (personal observation). While visual surveys are biased toward the detection of conspicuous individuals, this works in the CAB monitoring protocol's favor, particularly in deeper passages, since many target species are pigmentless or depigmented (e.g., cavefish or cave crayfish) and highly visible. Visual surveys for smaller, less conspicuous cave aquatic biota (e.g., cave shrimp) by trained observers have been successfully implemented in the past (Pearson 2009). Finally, we will collect limited quantitative and qualitative data on CAB habitat covariates (Figure SOP3-3) because physical characteristics of streams and pools are important determinants of habitat quality for benthic macroinvertebrates and fish (Fitzpatrick et al. 1998; Brasher et al. 2011; TCEQ 2014). Field personnel sample site habitat covariates such as qualitative assessment of habitat type (e.g., Riffle/Pool/Glide), quantitative measurement of substrate particle size, and qualitative assessment of substrate type (e.g., % Granules, and % Pebbles).

Cave Aquatic Biota Monitoring Protocol—Cumberland Piedmont Network Sampling Event Metadata Field Data Sheet	Cave Aquatic Biota Monitoring Protocol—Cumberland Piedmont Network Habitat Characteristic Metadata Field Data Sheet
Park ID.: _____	Cave Stream Reach: _____
Cave Stream Reach: _____	Event Date: ____/____/____
Crew (circle leader): _____	Transect No.: _____ Team No.: _____
Event date: ____/____/____	Observer: _____ Recorder: _____
Start: _____ End: _____	<u>Stream</u>
Water quality/quantity	Circle only one Riffle(R) Glide(G) Pool(P)
Collect two grab samples: Field _____ Lab _____	R = shallow, swift flow, turbulent, partially exposed substrate
Download data from datalogger	G = slow flow, nonturbulent
(second sampling event only): _____	P = disjunct from stream, no flow
Hydrologic observations / notes	☐ Cover Searched
_____	Estimated Percent Searched _____
_____	Pool # (If >1 consult transect's pool map): _____
_____	Max. Pool Length: _____
_____	Stream/Pool Width: ____/____/____
_____	Stream/Pool Depth: ____/____/____
_____	(Percent) Substrate:
_____	Fines (<1 mm) Sand _____ Silt _____
_____	Granules (> 1–4 mm) _____
_____	Pebbles (> 4–64 mm) _____
_____	Cobbles (> 64–256 mm) _____
_____	Boulders (> 256 mm) _____
_____	Bedrock _____
Reviewed by (rev'r/date) _____	SOP version 1.0 updated 08/06/2018
Data entered (enterer/date) _____	
Data verified (verifier/date) _____	
SOP version 1.0 updated 12/18/2017	

Figure SOP3-3. Separate field data sheets used by sampling teams to record metadata during implementation of the Cave Aquatic Biota monitoring protocol. The field data sheet on the left is where cave stream reach-specific metadata, in addition to data entry and verification dates, are recorded by Team 2, one per cave stream reach. The field data sheet on the right is where data on transect-specific habitat characteristics are recorded generally once per transect. However, if there are multiple pools in a transect, then a separate habitat characteristic metadata field data sheet is used for each pool. Searchable cover in/under which cave aquatic biota may be detected, as well the proportion in the transect searched, are also noted.

“Collect 2 grab samples,” “Field”, “Lab” and “Download data from data logger” are filled in (the latter only on the second sampling event of the field season) at the beginning of the sampling event. “Start” and “End” times are filled in at the beginning and the end, respectively, of the sampling event. “Hydrologic Observations/Notes” can be filled in as needed.

- The cave stream reach habitat characteristic metadata field data sheet (Figure SOP3-3, right; Appendix D, Figure D-2) is used by each team for each surveyed transect during each sampling event.
- The cave stream reach CAB field data sheet (Figure SOP3-4) is used by each team for each surveyed transect during each sampling event (but see “Walking/Wading Surveys Step By Step Instructions” below for an exception).
- As CAB data are received from observers, the data recorder repeats back what the observer said and ensures that data are entered legibly into the correct fields on the CAB field data sheet (Figure SOP3-4). Observers and recorders must be intimately familiar with the abbreviation codes for the commonly encountered stygobionts/stygophiles in the region (Figure SOP3-5). Infrequently observed organisms (e.g., frogs) are entered in the “Notes” column. The data recorders retain all field data sheets, abbreviation code sheets, and field guides in the zippered field book at all times while in the cave so as not to damage or lose them.
 - Repeated monitoring visits increase the possibility of damage to cave resources so best management practices should be observed. They include the following:
 - To reduce pressure on cave resources, the three CUPN parks with accessible cave aquatic habitat are split into two panels. Panel 1 includes the four Mammoth Cave NP cave stream reaches and Panel 2 is one Cumberland Gap NHP Park cave stream reach, one Mammoth Cave NP cave stream reach, and one Russell Cave NM cave stream reach. Each is surveyed every other year.
 - A small field crew size is used to minimize the impact on cave resources caused by monitoring yet still collect rigorous data and maintain safety.
 - In general, but particularly through fragile cave sections, travel slowly and deliberately to minimize the chances of impact. Between transects proceed in a single-file line to confine impact to a narrow path.

Codes for common MACA stygobionts

MACROINVERTEBRATES

Crustaceans
Copepoda (COPE)

● Amphipods
Crangonyx barri (CRBA)
C. forbesi (CRFO)
Stygobromus vitreus (STVI)
● *S. exilis* (STEX)
Amphipoda (UNAM)

Decapods
● *Cambarus tenebrosus* (CATE)
Orconectes pellucidus (ORPE)
Palaemonias ganteri (PAGA)

Isopods
Caecidotea stygia (CAST)
C. bicrenata whitei (CBWH)
Isopoda (UNIS)

Flatworms
Sphalloplana percoeca (SPPE)
S. buchanani (SPBU)

Mollusc
● *Antroselates spiralis* (ANSP)

● **FISH/NEKTON**

Stygobiotic Fish
Amblyopsis spelaea (AMSP)
Typhlichthys subterraneus (TYSU)

● *Forbesichthys papilliferus* (FOPA)
Cottus carolinea (COCA)
Osteichthyes (UNFI)

AMPHIBIANS

● Salamanders
Eurycea lucifuga (EULU)
Plethodon glutinosus (PLGU)
P. dorsalis (PLDO)
Pseudotriton ruber (PSRU)
Caudata (UNSA)

Figure SOP3-5. Example of field book page with codes, customized for Mammoth Cave NP, for commonly encountered stygobionts/stygophiles in Cumberland Piedmont Network cave streams. Note codes are printed on pre-perforated Rite-in-the-Rain™ paper template for use in Rite-in-the-Rain™ field binder No. 9200.

- Members of the field crew consult the cave stream reach map (see Appendix C, this SOP) to locate the transects in the cave stream reach scheduled for sampling. Transects labeled on the maps match those on markers in the field. Three transect markers are used in the field to indicate the start, middle, and end of each sampling transect (Figure SOP3-6). All transect markers are deployed in the field ahead of sampling surveys and require occasional replacement. Transect markers used in wadeable reaches are typically either pin flags or flagging tape tied to convenient formations (Figure SOP3-6). Transect markers in nonwadeable reaches are typically poker chips, anchored to the cave passage above the water line, with dabs of highly visible fluorescent nail polish. Two different poker chips and nail

polish colors (i.e., green and red) are used to mark the start and end, respectively, of each transect. If one or both transect markers cannot be located the teams may move on to the next transect in the sequence. **All attempts should be made to locate the transect markers before moving on.**



Figure SOP3-6. Transect markers indicating starting and ending points of a transect.

Walking/Wading Surveys Step-By-Step Instructions

- During a walking and wading survey the two observers, equipped with hand-held dive lights, perform timed (i.e., 15 minute) independent surveys in each permanent 40-meter (131.2-ft) transect. A data recorder was previously assigned to each observer to record data on all cave aquatic biota detected by observers on the field data sheets. Transects within a cave stream reach may contain lotic (e.g., characterized by moving water, including streams) or lentic (e.g., characterized by still water, including pools) sites.
 - If the transect is easily surveyed from its bank, with a narrow perennial stream or shallow pools, observers begin their survey at opposite ends of the transect or pool, on opposite sides of the transect, and work their way toward the other end (Figure SOP3-7).



Figure SOP3-7. Observer surveying pool for organisms while the recorder writes data.

- In transects containing multiple pools, a characteristic of intermittent streams, teams are issued a separate map of all surveyed pools in the transect. Only pools with a surface area roughly greater than 1 square meter (10.8 square feet [ft²]) should be surveyed. Recorders consult their pool map and record the number of the pool at which the team begins their survey and at each subsequent pool in the transect. For example, if a transect has three pools according to the pool map, then three separate cave stream reach habitat characteristic metadata field data sheets (Appendix D, Figure D-2) and at least one CAB field data sheet (Figure SOP3-4) would be filled in. Over time, pool configurations may change significantly, which will necessitate redrawing the maps.
- If the stream or pool contained in a transect is greater than 2 meters (6.6 ft) wide or extends across the entire passageway, the observers each survey one side of the transect while wading together up the middle. They go against the current if they are wading in a stream, so their view of the organisms is not reduced by muddied water and the mud and silt is carried downstream (Figure SOP3-8). Turbulent flow in riffles or destructive interference waves generated by the two observers wading in a pool can reduce the visibility of cave aquatic biota from above the water's surface. To mitigate reduced visibility the observers submerge their flashlights and scan their half of the transect (Figure SOP3-8). Observers help each other keep track of cave aquatic biota so that no double counts are recorded. Since independent observations are impossible in these situations, only one set of data are recorded on one recorder's field data sheet to avoid confusion (i.e., each observers' half transect is combined). If this technique is used it must be used throughout the entire transect. The recorder

from Team 2 is noted on the CAB field data sheet as the Recorder, both observer's initials are recorded as the Observer(s), and the "Combined Survey" box is checked (Figure SOP3-4).

- The data recorder on Team 2 acts as the designated timekeeper. Because sampling intensity is doubled with two observers the countdown is 7:30 (i.e., 7 minutes, 30 seconds) **in these specific situations only**. The recorder starts the 7:30 countdown on the stopwatch while simultaneously saying "Begin." The observers begin their survey of the transect.
- The designated timekeeper twice verbally alerts the observer as to the time they have remaining to survey the transect. This helps observers apportion their time evenly throughout the transect:



Figure SOP3-8. Observers wading through the transect while one of the two recorders writes data. Note observer shining submersible flashlight beneath surface to enhance detection of organisms and mitigate destructive interference waves caused by wading.

- Once at the approximate halfway point (i.e., 3 minutes 45 seconds).
- Once when there is one minute remaining.

- Surveys in silt- or mud-bottomed transects that must be waded may not require the entire timed interval because turbidity created by the observers' passage effectively eliminates the possibility of resurvey during the allotted time. If possible, in these cases any portion of the stream or pool visible from the bank should be carefully surveyed before wading.
- The observers carefully survey each 40-meter (131.2-ft) transect for all cave aquatic biota for 15 minutes. If cover (e.g., large cobble, woody snags, leaf packs, trash) is present the observer must scale their time spent searching through/under cover to its proportion in the transect. Cave stream reach maps will contain estimates of the amount of cover in transects, based on previous estimates, to assist the observers with managing their survey time. When an organism is detected, the observer tries not to reveal its location to the other team's observer (e.g., no pointing or exclamations) but calls out the species code, identification confidence (as necessary), size estimate, and the type of cover (where appropriate) in/under which the cave aquatic biota was detected, to their designated data recorder. When possible, additional pertinent information such as deformities, injuries, eggs, or larvae should also be reported.
- As CAB data are received from the observer, the data recorder repeats what the observer said and records the data in the appropriate column on the field data sheet (Figure SOP3-4). The recorder must also record the geomorphic channel unit (i.e., riffle, glide, pool) wherein the observer detected each organism. Deformities, injuries, eggs, and larvae are recorded in the "Notes" row at the bottom. The recorder also ensures data are written legibly into correct fields on the field data sheet. Observers and recorders must be intimately familiar with the abbreviation codes for the commonly encountered stygobionts/stygophiles in the region (Figure SOP3-5).
 - While observers are chosen for their ability to identify cave aquatic biota in the field, all data recorders are equipped with a field guide to common cave aquatic biota, which is specific to the region in which the park is located, to assist in identification (see Appendix E in this SOP for Mammoth Cave NP example). The field guides contain information necessary to identify cave aquatic biota in the field including images, field characteristics (e.g., size range), and species codes. If necessary, observers can consult with recorders to identify biota in question. "Unknown" codes are provided for all difficult to identify taxa and if consulting the field guide holds up the process (Figure SOP3-5). Recorders pause their stopwatch because time taken to identify biota in question does not count against the 15-minute survey time.
 - Observers estimate length of vertebrates, crayfish, and cave shrimp. For salamanders, estimate the animal's length from its snout to the base of its tail. For fishes, total length is estimated as the distance from the closed mouth to the extreme tip of the tail fin. For crayfishes and cave shrimp, carapace length

is measured as the distance from the rostrum (i.e., the pointed end of the ‘nose’) to the edge of the carapace. Estimated length is recorded to the nearest 5 millimeters (0.2 inches [in]).

- If the observer does not detect any organisms in the transect, the recorder writes “ND” prominently on the field data sheet. **Non-detections are data!**
- The data recorder twice verbally alerts the observer as to the remaining time they have to survey the transect. This helps the observers to apportion their time evenly throughout the transect:
 - Once at the approximate halfway point (i.e., 7 minutes 30 seconds).
 - Once when there is one minute remaining.
 - At the halfway point in the time allotted for the first team’s survey (i.e., 7:30) the data recorder on Team 2 resets their stopwatch to 15 minutes and, if the first team has not doubled back, they begin their survey of the same transect.
 - At the end of the 15-minute countdown the designated timekeeper says “Stop.” The designated timekeeper resets the stopwatch for the next transect.
- After each transect has been surveyed for cave aquatic biota each team ensures the completeness of their CAB field data sheets (Figure SOP3-4).
- After a stream or mapped pool is surveyed for CAB each team records a set of independent habitat characteristic measurements on the habitat characteristic metadata field sheet (Figure SOP3-3, right):
 - Determination of the transect’s dominant geomorphic channel unit (Fitzpatrick et al. 1998), i.e., riffle, glide, or pool. To be considered a discrete habitat type, the geomorphic channel unit must be roughly greater than the average stream width. Transects may contain more than one geomorphic channel unit, but only the dominant unit is recorded here. Riffles are relatively shallow areas of the channel where water flows swiftly over completely or partially submerged obstructions to produce surface turbulence. Glides are areas with moderate depth and little or no surface turbulence. Velocities can be high or low, but the key feature is little apparent surface turbulence. Pools are disjunct from the stream, with no velocity or surface turbulence, and may be deeper than riffles or glides. Only pools with a surface area greater than 1 square meter (10.8 ft²) are surveyed.
 - The stream’s (perennial streams taking up the entire 40-meter (131.2 ft) transect will not need their length measured) or pool’s maximum length, its width and its depth.
 - Length and width are measured, to the nearest centimeter, with a laser disto. Team members stand at opposite ends of the pool, one team member holds

the laser disto with its bottom at the edge of the pool, aims the laser disto at a target (e.g., field notebook) held by the team member at the opposite edge of the pool, and measures the distance to the target. One measurement is taken at the length of each pool. The pool's length is roughly divided into four sections and its width and depth are measured at three points of intersection along its length. Thus, if the pool is a 10-meter (32.8 ft) long transect its width and depth are measured at 2.5, 5.0, and 7.5 meters (8.2, 16.4, and 24.6 ft). Depth is measured, to the nearest centimeter, at the point of intersection of its length and width. Pools under 90 millimeters (3.5 in) deep can be measured with the SciOptic grain size chart (Figure SOP3-9). Pools over 90 millimeters (3.5 in) deep are measured with the gauging tape.

- Substrate characteristics are also sampled by each team at the three intersections where width and depth are measured. A minimum of 10 particles per team must be measured.
 - At each sample point the observer leans down and samples the first particle they touch. If the particles are less than 1 millimeter (0.04 in), the sample should be rubbed between the forefinger and thumb. Sand feels gritty and silt feels smooth; the sample must be recorded as sand or silt.
 - As each sample is retrieved the diameter of the particle's b-axis, the axis perpendicular to the longest axis (Brasher et al. 2011), is measured with the SciOptic grain size chart (Figure SOP3-9).
 - The recorder writes the measurements as hash marks within the range of the particle size categories under the "Substrate" section of the Habitat Characteristic Metadata field data sheet (Appendix D, Figure D-2 in this SOP).
 - Occasionally, a quick overview of a transect or, more likely, a pool suggests it has a uniform substrate. Observers may also scatter some substrate onto the transparent SciOptic grain size chart (Figure SOP3-9) to confirm their observations of substrate grain size and composition. These quick samples must be made at the three sections where width and depth are measured. If the overview is confirmed the recorder writes the measurement as "100%" within the range of the particle size categories under the "Substrate" section of the Habitat Characteristic Metadata field data sheet (Appendix D, Figure D-2 in this SOP).

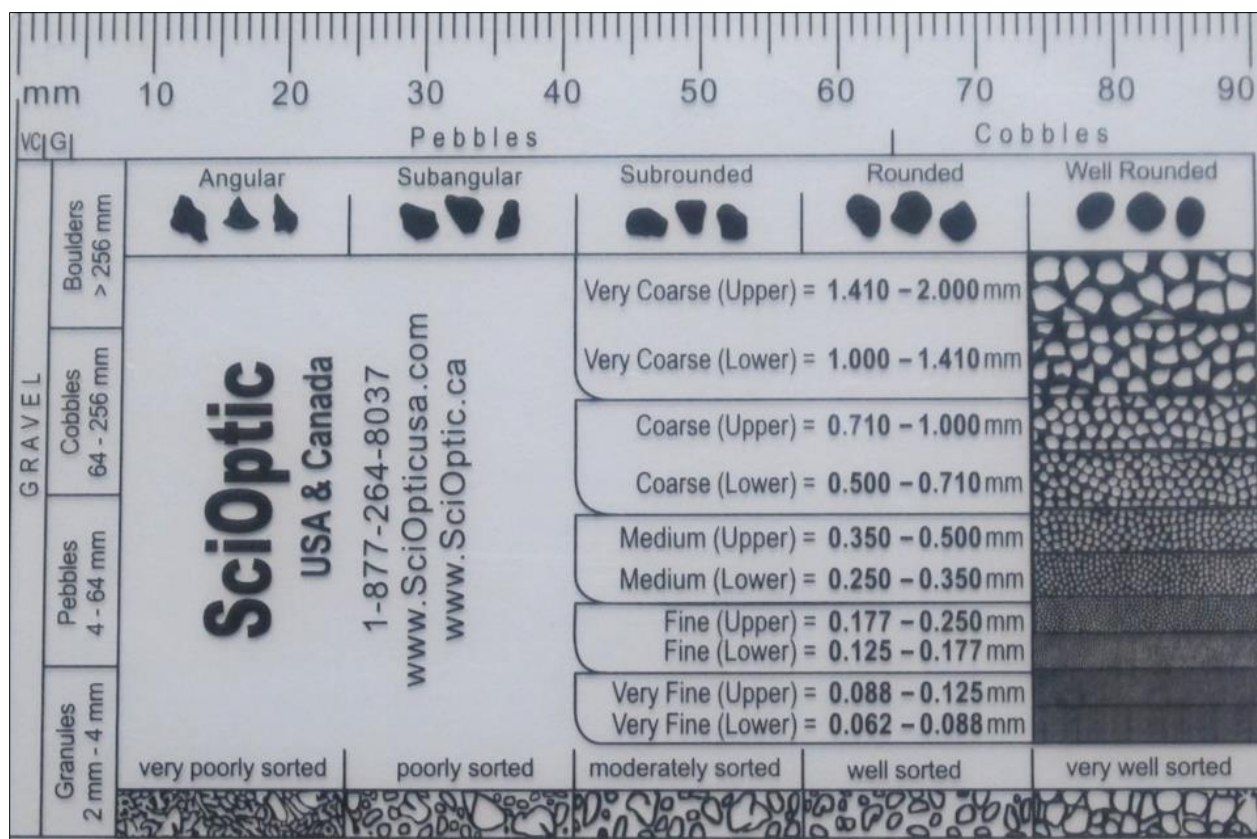


Figure SOP3-9. Transparent SciOptic grain size chart. Used, as necessary, as an adjunct to the overview of the transect's substrate composition observers make during their survey of the transect.

- After each transect has been surveyed for habitat characteristics each team ensures the completeness of all data sheets before moving to the next transect.
- All previous steps in the general and specific CAB survey section are repeated for all transects as necessary.
- When work is complete, the field crew exits the cave in a safe manner. The data recorder from Team 2 enters the time the teams exit the cave on the cave stream reach-specific metadata field data sheet.
- The field crew leader secures all cave gates and locks as required.
- The field crew leader informs the surface watch of the field crew exiting the cave as soon as possible (e.g., via phone or radio).
- The field crew follows all applicable gear decontamination procedures as described in the White-nose Syndrome section of SOP 4 *Post-Field Sampling—Version 1.0* under “Decontamination of Gear.”

Snorkeling Surveys

Additional Equipment Required

- Inflatable kayak with paddle (2)
- Double action hand pump(s)
- Wetsuit/ exposure suit
- Mask, fins, and snorkel
- Additional dive lights and batteries

In sampling reaches where there is no shoreline, or water depths regularly exceed 1.25 meters (4.1 ft; e.g., MACA's Roaring River) snorkeling must be used to survey the reach (Figure SOP3-10). During snorkeling surveys, the observer snorkels the transect while the data recorder follows from shore, wades, or paddles behind the observers in a one-person inflatable kayak (Figure SOP3-11). The observers are in full wetsuits (6 millimeters [0.2 in] thick) with gloves, booties, hoods, masks (prescription ground if needed), snorkels, and fins. Suitable exposure suits (Figure SOP3-10) may be used instead of wetsuits. The recorder must also wear a wetsuit (not an exposure suit) to prevent hypothermia, but fins and mask are superfluous. The recorder's primary responsibilities are to maintain field notes, making sure to record the observer's observations, obtain water-quality data, and preserve the integrity of the kayak. The kayak also serves as an emergency transport system if a member of the party should be incapacitated by illness, injury, or hypothermia (Figure SOP3-11). The possibility of hypothermia affecting the observers is a concern at these sites (see Table SOP2-7) because there may not be access for the observers to get out of the water. The recorders are responsible for watching the observers closely, speaking to them often, and asking them about their physical condition. Every member of the group must monitor the reactions and conditions of other members of the team. The importance of this type of cooperation and mutual observation cannot be overemphasized. A separate half-day trip prior to and after the surveys will be required to transport and stage heavy equipment (e.g., inflatable kayaks and exposure suits) to nonwadeable sampling sites that require greater than 15 minutes travel time, as indicated on the cave's field information sheet (see Appendix B in this SOP). Separate trips will reduce the sampling crew's fatigue associated with carrying additional heavy equipment to the site and inflating kayaks.



Figure SOP3-10. Observers snorkeling nonwadeable transect.



Figure SOP3-11. Data recorder following observer in one-person, inflatable kayak.

Snorkeling Surveys Step-By-Step Instructions

- All personnel put on their wetsuit or exposure suit at the site and assist each other as needed.
- The field crew enters the water and ensures their equipment is functioning properly:

- Observers ensure their exposure suits have no leaks and the rubber seals at their neck and wrists are watertight.
- Data recorders ensure their kayaks are properly inflated and they are seated comfortably with data sheets at the ready (Figure SOP3-11).
- Once both teams have arrived at the first transect and are ready, both data recorders start the 15-minute countdown on their stopwatches simultaneously.
- The first team begins its timed, independent survey: the observer begins snorkeling the transect (Figure SOP3-10) while the data recorder paddles a few meters behind (Figure SOP3-11).
 - The observer slowly swims along a zigzag path upstream through the 40-meter (131.2-ft) transect, carefully searching in open water and under ledges (Figure SOP3-12) using a hand-held dive light(s) if necessary. Some organisms concentrate along irregularities on the wall so pay attention to the sides of the passage. Note: the only safe technique to search under ledges without an air space is to shine a dive light under the ledge. **Under no circumstances should observers attempt to swim under ledges without an air space.**

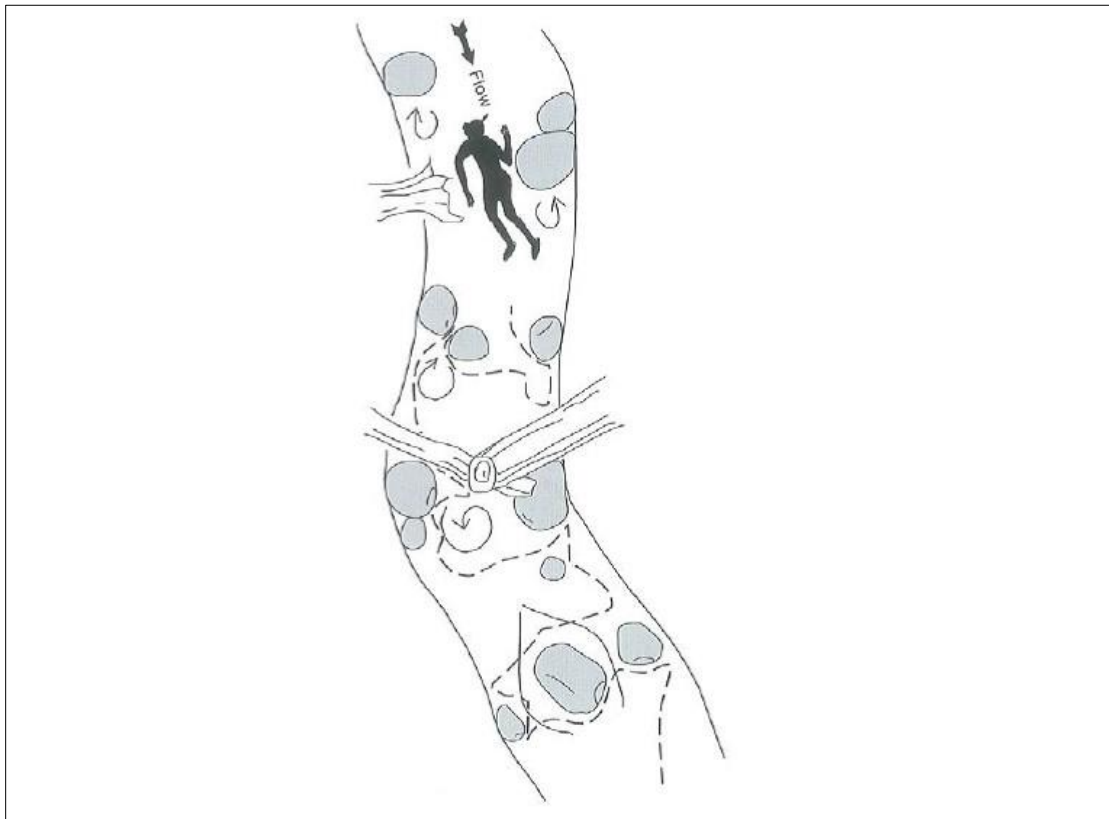


Figure SOP3-12. A snorkeler surveying in a single pass zigzags through the site while moving upstream. The dashed line approximates the surveyor's path (Thurow et al. 2006; Apperson et al. 2015). The recorder follows some distance behind in an inflatable kayak.

- The observers carefully survey the 40-meter (131.2-ft) transect for all cave aquatic biota. If cover (e.g., large cobble, woody snags, leaf packs, trash) is present, and within reach, the observer must scale their time spent searching through and under cover to its proportion in the transect. Cave stream reach maps will contain estimates of the amount of cover in transects, based on previous estimates, to assist the observers with managing their survey time. Data recorders also assist observers manage their survey time with verbal alerts regarding elapsed survey time (see below). When an organism is detected the observer tries not to reveal its location to the other observer (e.g., no pointing or exclamations) but calls out the species code, their identification confidence (as necessary), size estimate, and the type of cover (where appropriate) in/under which the cave aquatic biota was detected, to their designated data recorder. Additional pertinent information such as deformities, injuries, eggs, or larvae should also be reported.
- As CAB data are received from the observer, the data recorder repeats what the observer said and records the data in the appropriate column on the field data sheet (Figure SOP3-4). It is the recorder's job to note and record the geomorphic channel unit (i.e., riffle, glide, pool) wherein the observer detected each organism on the CAB field data sheet. Riffles are relatively shallow areas of the channel where water flows swiftly over completely or partially submerged obstructions to produce surface turbulence. Glides are areas with moderate depth and little or no surface turbulence. Velocities can be high or low, but the key feature is little apparent surface turbulence. Pools are disjunct from the stream, with no velocity or surface turbulence, and may be deeper than riffles or glides. Deformities, injuries, eggs, or larvae are recorded in the "Notes" column. The recorder ensures data are written legibly into correct fields on the field data sheet. Observers and recorders must be intimately familiar with the abbreviation codes for the commonly encountered stygobionts/stygophiles in the region (Figure SOP3-5).
 - While observers are chosen for their ability to identify cave aquatic biota in the field, data recorders are equipped with a field guide (see Appendix E) to common cave aquatic biota that are specific to the region in which the park is located to assist in identification. The field guides contain information necessary to identify cave aquatic biota including images, field characteristics (e.g., size range), and species codes (see Appendix E in this SOP for Mammoth Cave NP example). If necessary, observers consult with recorders to identify biota in question. "Unknown" codes are provided for all difficult to identify taxa and if consulting the field guide holds up the process (Figure SOP3-5). Recorders pause their stopwatch because time taken to identify biota in question does not count against the 15-minute survey time.
 - Observers estimate length of vertebrates, crayfish, and cave shrimp. For salamanders, estimate the animal's length from its snout to the base of its tail.

For fishes, total length is estimated as the distance from the closed mouth to the extreme tip of the tail fin. For crayfishes and cave shrimp, carapace length is measured as the distance from the rostrum (i.e., the pointed end of the ‘nose’) to the edge of the carapace. Estimated length is recorded to the nearest 5 millimeters (0.2 in).

- If the observer does not detect any organisms in the transect, the recorder writes “ND” prominently on the field data sheet. **Non-detections are data!**
- The data recorder twice verbally alerts the observer as to the remaining time they have to survey the transect. This helps the observers to apportion their time evenly throughout the transect:
 - Once at the approximate halfway point (i.e., 7 minutes 30 seconds).
 - Once when there is one minute remaining.
 - At the halfway point in the time allotted for the first team’s survey (i.e., 7:30 [7 minutes, 30 seconds]) the second team’s data recorder resets their stopwatch to 15 minutes and they begin their survey of the same transect.
 - At the end of the 15-minute countdown the data recorder says “Stop” and resets the stopwatch for the next transect.
- After each transect has been surveyed for cave aquatic biota each team ensures the completeness of their CAB field data sheets (Figure SOP3–4).
- Where practical (i.e., the reach is not too deep) each team records the following set of independent habitat characteristic measurements on the habitat characteristic metadata field sheet (Figure SOP3-3, right):
 - Measure depth to the nearest centimeter, at the point of intersection of its length and width. Pools under 90 millimeters (3.5 in) deep can be measured with the SciOptic grain size chart (Figure SOP3–9).
 - Reaches over 90 millimeters (3.5 in) deep are measured with the gauging tape.
 - Substrate characteristics are also sampled by each team at the three intersections where depth is measured. A minimum of 10 particles per team must be measured.
 - At each sample point the observer leans down and samples the first particle they touch. If the particles are less than 1 millimeter (0.04 in), the sample should be rubbed between the forefinger and thumb. Sand feels gritty and silt feels smooth; the sample must be recorded as sand or silt.

- As each sample is retrieved the diameter of the particle's b-axis, the axis perpendicular to the longest axis (Brasher et al. 2011), is measured with the SciOptic grain size chart (Figure SOP3-9).
 - The recorder writes the measurements as hash marks within the range of the particle size categories under the "Substrate" section of the Habitat Characteristic Metadata field data sheet (Appendix D, Figure D-2 in this SOP).
 - Occasionally, a quick overview of a transect suggests it has a uniform substrate. Observers may also scatter some substrate onto the transparent SciOptic grain size chart (Figure SOP3-9) to confirm their observations of substrate grain size and composition. These quick samples must be made at the three sections where width and depth are measured. If the overview is confirmed the recorder writes the measurement as "100%" within the range of the particle size categories under the "Substrate" section of the Habitat Characteristic Metadata field data sheet (Appendix D, Figure D-2 in this SOP).
- If the snorkeled reach is too deep or sampling the substrate could affect visibility, it may be impractical for observers to use the SciOptic grain size chart (Figure SOP3-9) to assist with substrate size quantification. In this case, each observer and recorder team makes an overview while moving through the transect and determines a consensus estimate for its percent substrate composition. The recorder writes the percentages, to the nearest 5%, within the range of the particle size categories under the "Substrate" section of the Habitat Characteristic Metadata field data sheet (Appendix D, Figure D-2 in this SOP).
- After each transect has been surveyed, data recorders ensure the completeness of all data sheets before moving to the next transect.
- Both teams move on to the next transect to be surveyed (as appropriate).
- When all transects have been surveyed, both teams return to the starting point where they then exit the water.
 - The field crew removes wet gear and changes into dry caving clothes for the return to the surface.
 - Deflate and secure inflatable kayaks left behind for replicate surveys so they do not float away.
- When work is complete, the field crew exits the cave and moves in a safe manner. The data recorders enter the time the teams exit the cave on the cave stream reach-specific metadata field data sheet.

- The field crew leader secures all cave gates and locks as required.
- The field crew leader informs the surface watch of the field crew exiting the cave as soon as possible (e.g., via phone or radio).
- The field crew follows all applicable gear decontamination procedures as described in the White-nose Syndrome section of SOP 4 under “Decontamination of Gear.”

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Appendix A: HOBO Water Level/Temp Data Logger Field Data Sheet and Instructions for Filling out the Sheet.

CUPN_CAB_HOBO_Data_Form_20170620

HOBO U20L Data Logger – Site Setup and Maintenance Field Data Sheet

General Information

Park Code: _____ Cave Name _____

Crew Member _____ SOP Version #: _____

Date _____ Time (hhmm, 24-hr format) _____ (LST)

mm dd yyyy

Cave Stream Reach _____

Datalogger Location Name _____

HOBO Serial Number _____ Model _____

Battery Status _____

Datalogger Location Description _____

Datalogger Photos _____

Landmark Name _____

Landmark Description _____

Landmark Photos _____

Siting information:

Azimuth _____ degrees

Inclination _____ degrees (+ or -)

Distance _____ meters

Datalogger installation status and accompanying notes (check all that apply):

_____ Installed (above) datalogger today.

_____ Servicing existing (above) datalogger.

Datalogger undisturbed? Y N

Data transfer successful? Y N

Datalogger relaunched? Y N

_____ This (above) datalogger is being replaced today by:

HOBO Serial Number _____

Model _____

Battery Status _____

_____ This (above) datalogger is being removed, but **NOT** replaced today.

Notes: _____

Data recorder initials _____ Reviewed by: _____

Office Use Only:

Raw data file uploaded to server by _____

on ____/____/____

Renamed raw data file filename: _____

Raw data file processed & validated by _____

on ____/____/____

Final processed and validated file filename: _____

Final data file completeness:

Temp: _____% RH: _____%

Notes: _____

Entered into DB by _____ on ____/____/____

Scan made by _____ on ____/____/____

Figure A-1. Example of a HOBO U20L Data Logger—Site Setup and Maintenance Field Data Sheet.

Table A-1. Field name and descriptions for HOBO U20L Data Logger – Site Setup and Maintenance Field Data Sheet.

Field Name	Field Description
Park Code	Unique 4-letter park acronym (i.e., CUGA, MACA, RUCA).
Cave Entrance Name	The unique name of the cave entrance containing the cave stream reach being sampled.
Crew Member	First initial and last name of the primary crew member who carries out the data logger installation or maintenance.
SOP Version #:	SOP version number under which the field data sheet was created. Example: “1.0”
Date	The date that data are being collected in mm/dd/yyyy format (e.g., 05/17/2011 for May 17, 2011).
Time	The time, to the nearest minute, when new data logger was installed, or the existing data logger was relaunched or removed. Use 24-hours format. All four digits should be filled in. Examples: 0630 (6:30 am) and 1350 (1:50 pm). Use local standard time (LST)—make sure to use correct time zone.
Cave Stream Reach	Letter code of the stream/river reach surveyed. The cave stream reach code should be five characters or less. To ensure metadata sheets are matched with the correct biota field data sheets this is also recorded at the top of this data sheet. Codes are listed on the cave stream reach map (Appendix C). Example: “UPBR” = Upstream Bridge Avenue. Where possible, cave stream reach codes should match station name codes used for NPSTORET in the CUPN Water Quality Monitoring protocol (Meiman 2018).
Data logger Location Name	Unique name assigned (in the field) to a permanent location where a data logger was initially installed. Within a cave, each specific HOBO data logger location will be assigned a name beginning with “H1”, followed by “H2”, and so forth.
HOBO Serial Number	Record the serial number printed on the side of the existing data logger, or if no data logger is present, record the # of the new data logger being installed.
Model	Record the HOBO data logger model type (e.g., U20-L).
Battery Status	Checked and noted during the launching process (e.g., “Good”)
Data logger Location Description	A detailed description of the permanent location where the newly installed HOBO data logger is placed.
Data logger Photos	Record frame (i.e., image) number(s) of photos taken of newly installed HOBO data logger in situ.
Landmark Name	Unique name assigned (in the field) to a permanent landmark that will be used as a reference point in the cave for relocating the specific location where a data logger was initially installed. Within a cave, each landmark will be assigned a name beginning with “L1”, followed by “L2”, and so forth.
Landmark Description	A detailed description of the permanent landmark and its location.
Landmark Photos	Record frame (i.e., image) number(s) of photo(s) taken of the landmark.

Field Name	Field Description
Siting information: Azimuth	Record the compass bearing in degrees (0 - 359) from the permanent landmark to the data logger using a compass or disto.
Siting information: Inclination	Record the inclination obtained by a clinometer or disto from the permanent landmark to the data logger. Record as a positive or negative whole integer.
Siting information: Distance	Record the distance in meters obtained by a measuring tape, laser range finder, or disto from the permanent landmark to the data logger.
Data logger installation status and accompanying notes (check all that apply):	<header only>
Installed data logger today	Place an "X" or "√" on this line if there was no data logger at this location upon arrival today.
Servicing existing data logger	Place an "X" or "√" on this line if an existing data logger is being serviced today (e.g., data offloaded).
Data logger undisturbed?	Circle Y if the data logger was undisturbed when the crew arrived today. Circle N if the data logger was disturbed when the crew arrived today.
Data transfer successful?	Circle Y if the data transfer was successful today. Circle N if the data transfer was not successful today.
Data logger relaunched?	Circle Y if the data logger successfully relaunched today. Circle N if the data logger did not successfully relaunch today.
This data logger is being replaced today by:	Place an "X" or "√" on this line if an existing data logger is being replaced today. Record: 1) the serial number of the data logger being installed today, 2) the HOBO data logger model type of the data logger being installed today, and 3) the battery status of the HOBO data logger being installed today.
This data logger is being removed, but NOT replaced today	Place an "X" or "√" on this line if an existing data logger is being removed from the permanent data logger location and not being replaced by another data logger today (it may be replaced in the future).
Notes:	Record notes related to the data logger installation and maintenance event. May include: damage to data logger, shuttle problems, missing or moved data logger, disturbance at cave entrance, etc.
Data recorder initials	Initials of individual filling-out the data form in the field. Occasionally, one (or more) persons will call out data, while another records the data.
Reviewed by	Initials of individual who reviewed the completed data form for HOBO Temp/RH data logger installation and maintenance elements. Ideally this should be someone other than the individual who filled-out the data form and is someone intimately familiar with the data form.

Appendix B: Mock-up Example of a Cave Stream Reach Specific Field Information Sheet for Monitoring CAB in Three Parks in the Cumberland Piedmont Network

UPDATED: 27 April, 2017

PARK: MACA

CAVE NAME: Apocryphal Cave



GPS: N-XX-XX-XX
W-XX-XX-XX

CAPPED -#XXX
ROAD GATE KEY: Y
CAVE GATE KEY: XXXX

DIRECTIONS: Park on *Imaginary Road* X miles north of the ferry. Walk X feet down Apocryphal Hollow on an abandoned road. The entrance is on the east side about X feet east of the creek bed.

ENTRANCE: Small entrance possibly dug open originally, in bottom of collapsed sink at base of hill, locked gate.

DESCRIPTION: "Beyond entrance, drops down a small crack into a sandy hands-and-knees crawl to a belly crawl, that leads to river level, requires ropes after this and drops to a muddy, slippery slog along a ledge above a river"- SRM

FIELD CREW SIZE: Best/Minimum=3; Maximum=4.

SPECIAL EQUIPMENT NEEDED: All transects require walking/wading.

CULTURAL NOTE: historic signatures

BIOLOGY: Base level CAB (e.g., cave crayfish, cavefish, cave shrimp).

HOBO DATALOGGER INFORMATION:

Landmark is 15 meters upstream of beginning of transect 1; 1.5m stalactite on left side of passage just before it turns sharply to left; large pool at turn.

HOBO U20L-02#10663142 **installed** 10/12/2017; **serviced** 8/15/2018. Stilling well is anchored in large pool on left side of passage just before sharp turn.

Figure B-1. Field information sheet template for CAB monitoring in CUPN parks.

Appendix C: Mock-up Example of a Cave Stream Reach Map for Monitoring CAB in Three Parks in the Cumberland Piedmont Network

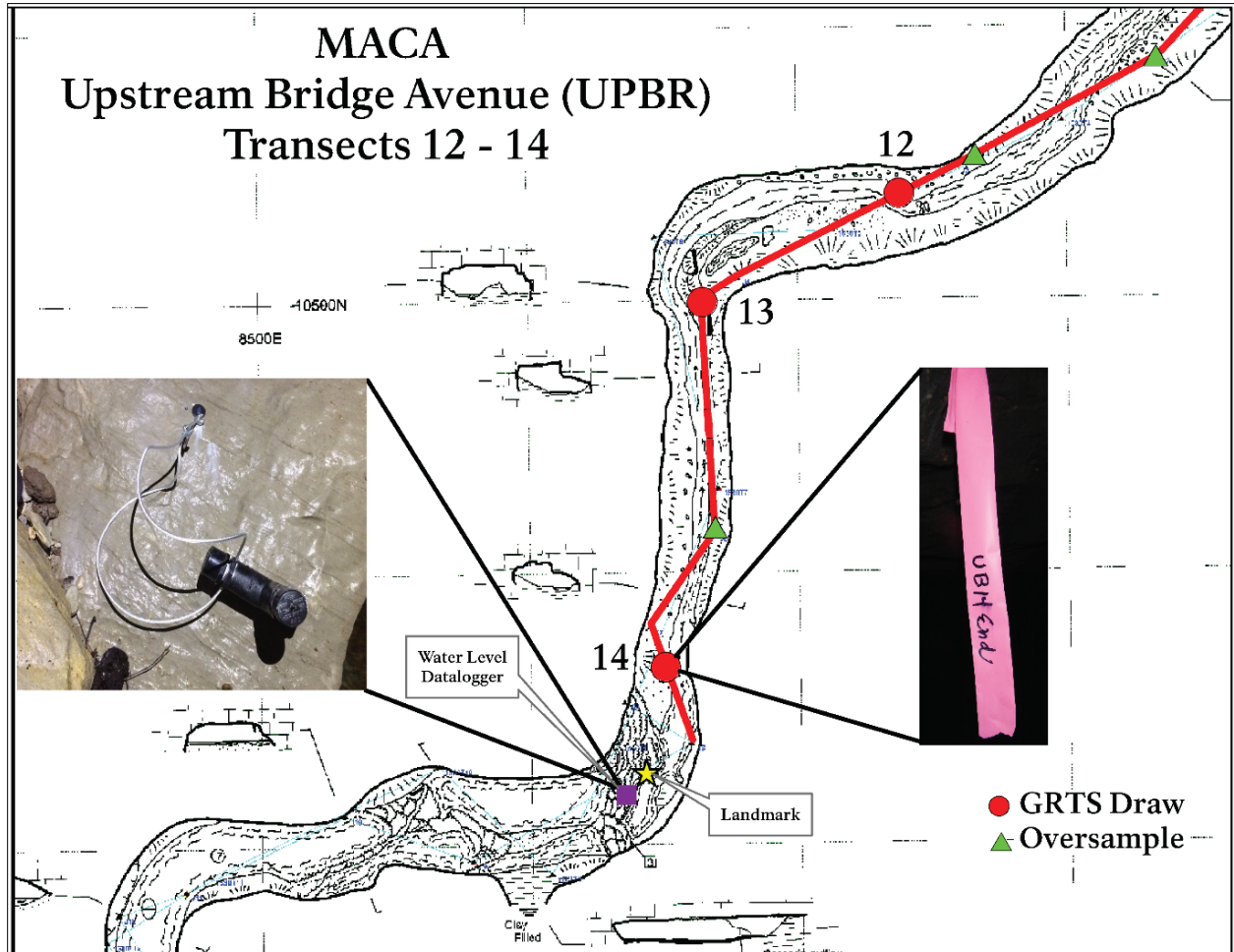


Figure C-1. Mockup of cave stream reach map section for CAB monitoring in CUPN parks.

Appendix D: Data Dictionary for Cave Stream Reach Metadata, Habitat Characteristics, and CAB Field Data Sheets and Instructions for Filling out the Sheet

Cave Aquatic Biota Monitoring Protocol—Cumberland Piedmont Network Sampling Event Metadata Field Data Sheet Park ID.: _____ Cave Stream Reach: _____ Crew (circle leader): _____ _____ Event date: ____ / ____ / ____ Start: _____ End: _____ Water quality/quantity Collect two grab samples: Field _____ Lab _____ Download data from datalogger (second sampling event only): _____ Hydrologic observations / notes _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ Reviewed by (rev'r/date) _____ Data entered (enterer/date) _____ Data verified (verifier/date) _____ SOP version 1.0 updated 12/18/2017
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Figure D-1. Example of the sampling event metadata field data sheet used by each sampling team during implementation of the Cave Aquatic Biota monitoring protocol. The above sheet is where cave stream reach-specific metadata, in addition to data entry dates, are recorded, once per cave stream reach.

Table D-1. Instructions for filling out sampling event metadata sheet used by sampling team 2 during implementation of the Cave Aquatic Biota monitoring protocol. The above sheet is where cave stream reach-specific metadata, in addition to data entry dates, are recorded, one per cave stream reach.

Cave Stream Reach Sampling Event Metadata	Field Description
Park ID	The four-letter code for the park unit being sampled. Example: "MACA".
Cave Stream Reach	Letter code of the stream/river reach surveyed; where applicable this is the same code as that used in the CUPN Water Quality Monitoring Protocol. Codes are listed on the cave stream reach map (Appendix C). Example: "UPBR".
Crew	Names of all field crew participating in a given survey; first initial and spelled out last name. The crew leader's name is circled. Example: "KHelf, RToomey, ROlson, SThomas".
Event Date	Starting date for the event in mm/dd/yyyy format. To ensure metadata sheets are matched with the correct biota field data sheets this is also recorded at the top of each Cave Aquatic Biota Detection/Nondetection field data sheet. Example "08/03/2016".
Start	Starting time for the survey (i.e., arrival at the cave entrance); recorded in 24-hour clock in hhmm format. Example: "1000".
End	Ending time for the survey (i.e., exit from the cave entrance); recorded in 24-hour clock in hhmm format. Example: "1900".
Collect 2 grab samples: Field/Lab	Check to ensure these data are collected during each sampling season. The blank must be filled in with an "X" to indicate the samples were collected. Example: "X"
Download data from data logger	Check to ensure these data are downloaded on the second sampling event of the sampling season. The blank must be filled in with an "X" to indicate the samples were collected. Example: "X"
Hydrologic Observations/ Notes	Note any phenomena pertinent to the water quality, biological activity, unusual odors, missing parameters (i.e., when a scheduled parameter is not collected).
Reviewed by	Initials of field crew leader who reviewed field data sheet for completeness. Example: "KHelf"
Data Enterer/Date	Identity of enterer and date on which data from field data sheet were entered in the cave aquatic biota monitoring database. Example: "CB/10/12/2016".
Data Verifier/Date	Identity of verifier and date on which data from field data sheet were verified in the cave aquatic biota monitoring database. Example: "KLH/10/15/2016".

**Cave Aquatic Biota Monitoring Protocol—Cumberland Piedmont Network
Habitat Characteristic Metadata Field Data Sheet**

Cave Stream Reach: _____

Event Date: ____/____/____

Transect No.: _____ Team No.: _____

Observer: _____ Recorder: _____

Stream

Circle only **one** Riffle(R) Glide(G) Pool(P)

R = shallow, swift flow, turbulent, partially exposed substrate

G = slow flow, nonturbulent

P = disjunct from stream, no flow

☐ Cover Searched

Estimated Percent Searched _____

Pool # (If >1 consult transect's pool map): _____

Max. Pool Length: _____

Stream/Pool Width: ____/____/____

Stream/Pool Depth: ____/____/____

(Percent) Substrate:

Fines (<1mm) Sand _____ Silt _____

Granules (> 1–4 mm) _____

Pebbles (> 4–64 mm) _____

Cobbles (> 64–256 mm) _____

Boulders (> 256 mm) _____

Bedrock _____

SOP version 1.0 updated 08/06/2018

Figure D-2. A field data sheet where data on transect-specific habitat characteristics are recorded by both teams once per sampling season. However, if a transect contains multiple pools one transect-specific field data sheet is filled in for each pool.

Table D-2. Instructions for filling out habitat characteristic metadata sheet used by each sampling team during the implementation of the cave aquatic biota protocol. The sheet is where data on transect-specific habitat characteristics are recorded generally once per transect. However, if a transect contains multiple pools one transect-specific field data sheet is filled in for each pool.

Habitat Characteristics Metadata	Field Description
Cave Stream Reach code	Letter code of the stream/river reach surveyed. The cave stream reach code should be five characters or less. To ensure metadata sheets are matched with the correct biota field data sheets this is also recorded at the top of the biota data sheet (Figure SOP3-2). Example: "UPBR" = Upstream Bridge Avenue. Codes are listed on the cave stream reach map (Appendix C).
Event Date	Starting date for the event in mm/dd/yyyy format. To ensure biota field data sheets are matched with the correct metadata sheets this is also recorded at the top of each Cave Aquatic Biota Detection/Nondetection field data sheet. Example "08/03/2016".
Transect Number	Transect number as indicated on the reach map and written on the transect marker. While transects are numbered sequentially, starting upstream, they may not always be surveyed in numerical sequence; especially if the access point on the reach is on its downstream end. For example, the reach in MACA's Bridge Avenue is split into upstream and downstream portions. The first five transects in upstream Bridge Avenue are surveyed in reverse order, to prevent organisms being disturbed and skewing data, so there is the potential for confusion. Thus, it is important to keep careful track of which transect was surveyed. To ensure metadata sheets are matched with the correct biota data sheets this is also recorded at the top of the CAB field data sheet (Figure SOP3-2). Example: "15".
Team No.	The order in which sampling teams conduct their (staggered) surveys is decided before the surveys begin: teams are designated "Team 1" and "Team 2". Team number connects the habitat characteristic metadata field sheet to the CAB field data sheet each team fills in during their staggered surveys of all transects in a cave stream reach. Example: "1".
Observer	The first initial and full last name of the crew member acting as the observer for a specific team. There will be two distinct observers, each assigned to a specific recorder, during each survey. Example: "ROlson"
Recorder	The first initial and full last name of the crew member acting as the data recorder for a specific team. There will be two distinct data recorders, each assigned to a specific observer, during each survey. Example: "RToomey"
Riffle(R)/Pool(P)/Glide(G)	Three types of geomorphic channel units will be used to describe habitat at spatial scales relevant to characterizing what constitutes suitable habitat for CAB. The geomorphic channel unit in the transect is recorded. To be considered a discrete habitat type the geomorphic channel unit must be roughly $\geq$ the average stream width. Riffles are relatively shallow areas of the channel where water flows swiftly over completely or partially submerged obstructions to produce surface turbulence. Pools are disjunct from the stream with no velocity or no surface turbulence, and may be deeper than riffles or glides. Only pools with a surface area $> 1 \text{ m}^2$ are to be surveyed. Glides are areas with moderate depth and little or no surface turbulence. Velocities can be high or low, but the key feature is little apparent surface turbulence. Example: "G".
Cover Searched	Check box is checked if the transect contained searchable cover (e.g., large cobble, woody snags, leaf packs, trash) within reach.

Habitat Characteristics Metadata	Field Description
Estimated Percent Searched	Estimate of the proportion of cover searched in the transect. Example: "50%".
Pool No.	Transects with multiple pools have a "pool map" associated with that transect wherein each pool has been assigned a unique number. One field data sheet would be filled in for each pool. The pool number associated with the pool being surveyed must be noted. Transects with only one pool are labeled with the number 1. Example: "P1".
Max. Pool Length	Measured once per sampling season by both survey teams. Maximum length of <u>each</u> surveyed pool in a transect to the nearest decimeter. Example: "4.8m".
Stream/Pool Width	Measured once per sampling season by both survey teams. Width of <u>each</u> surveyed transect/pool in a transect is measured to the nearest decimeter in three locations. Example: "1.5m".
Stream/Pool Depth	Measured once per sampling season by both survey teams. Depth of <u>each</u> surveyed transect/pool in a transect is measured to the nearest decimeter in the three locations where pool width is measured (at the mid-point of the width, not necessarily the deepest point). Example: "0.1m".
Percent Substrate	Measured once per sampling season by both survey teams. In wadeable reaches ten particles are measured with the SciOptic grain size chart in the three locations where depth is measured. In nonwadeable reaches each time estimates the percent of each substrate in the current transect: (1) Fines (<1mm) subdivided into sand or silt; (2) Granules (>1-4mm); (3) Pebbles (>4-64mm); (4) Cobbles (>64-256mm); (5) Boulders (>256mm), and (5) Bedrock. Example: "Cobbles 50%". For transects/pools with obviously uniform substrate, the transparent SciOptic grain size chart may be used to quickly determine grain size.

Cave Stream Reach:		Transect No.		Team No.		Event Date:		Page of		☐ Combined Survey
Species Detected	ID Confidence Certain, Probable Uncertain	Est. Length (mm)	Cover Open, Lg Cobble Woody Snag, Trash	Geomorphic Channel Unit Riffle, Glide, Pool#	Species Detected	ID Confidence Certain, Probable Uncertain	Est. Length (mm)	Cover Open, Lg Cobble Woody Snag, Trash	Geomorphic Channel Unit Riffle, Glide, Pool#	

Notes:

CAB Field Data Sheet SOP Version 1.0 updated 08/28/2018 **Observer(s):** _____ **Recorder**_____

Table D-3. Instructions for filling out Cave Aquatic Biota Detection/Nondetection field data sheet for monitoring cave stream reaches in three parks in the Cumberland Piedmont Network.

CAB Detection/ Nondetection Field Data Sheet	Field Description
Cave stream reach	Letter code of the stream/river reach surveyed. The cave stream reach code should be five characters or less. Codes that match the station codes used in the CUPN Water Quality Monitoring protocol (Meiman 2018) should be used wherever possible. To ensure metadata sheets are matched with the correct biota field data sheets this is also recorded at the top of this data sheet. Example: "UPBR" = Upstream Bridge Avenue.
Transect No.	Transect number as indicated on the cave stream reach map and written on the transect marker. While reaches are numbered sequentially, starting upstream, transects may not always be surveyed in numerical sequence; especially if the access point on the reach is on its downstream end. For example, the cave stream reach in MACA's Bridge Avenue is split into upstream and downstream portions. The first five transects in upstream Bridge Avenue are surveyed in reverse order, to prevent organisms being disturbed and skewing data, so there is the potential for confusion. Thus, it is important to keep careful track of which transect was surveyed. To ensure metadata sheets are matched with the correct biota data sheets this is also recorded at the top of the biota data sheet (Figure SOP4-3). Example: "15".
Team No.	There are two distinct teams, each with an observer and a recorder, during each survey. The order in which sampling teams conduct their (staggered) surveys is decided before the surveys begin: teams are designated "Team 1" and "Team 2". In addition to "Cave stream reach", and "Transect No.", team number connects the habitat characteristic metadata field sheet to the CAB field data sheet each team fills in during their staggered surveys of all transects in a cave stream reach. Example: "1".
Event Date	Starting date for the event in mm/dd/yyyy format. To ensure metadata sheets are matched with the correct biota field data sheets this is also recorded at the top of the Sampling Event Metadata field data sheet. Example "08/03/2016".
Page_of_	Filled in by the recorder for reaches requiring multiple pages of Cave Aquatic Biota Detection/Nondetection field data sheets. Example: "Page 6 of 8".
Combined Survey	If the stream or pool contained in a transect is > 2 m wide or extends across the entire passageway, the observers each survey half the transect while wading together up the middle, against the current if they are wading in a stream, so that neither's view of the organisms is reduced by muddied water and the mud and silt is carried downstream. Only one set of data are recorded on one recorder's field data sheet to avoid confusion (i.e., each observers' half transect is combined). The other recorder notes this situation on their field data sheet (e.g., "One set of data only; see Team 1's field data sheet"). If this technique is used it must be used throughout the entire transect. The recorder from Team 2 is noted on the CAB field data sheet as the Recorder, both observer's initials are recorded as the Observer(s), and the "Combined Survey" box is checked (Figure SOP3-4).
Species Detected	The four letter code indicating the species detected by the observer. <u>All</u> species, whether stygobiotic or epigean, are identified to the lowest taxonomic level possible. See "Abbreviations for common stygobitic/stygophilic invertebrates and vertebrates" (Figure SOP3-3). See SOP3 (Appendix E and following) for the appropriate park-specific field guide to common cave aquatic biota and their appropriate abbreviations. Example: "UNFI".
ID Confidence	In cases where species identification is not certain record confidence level as either C=certain, P=probable, or U=uncertain. Example: "C".

CAB Detection/ Nondetection Field Data Sheet	Field Description
Estimated Length (mm)	The estimated length of all organisms encountered during the survey. For fish, total length is estimated as the distance from the closed mouth to the extreme tip of the tail fin. For salamanders, estimate the animal's length from its snout to the base of its tail. For crayfish and shrimp, carapace length is measured as the distance from the rostrum (i.e., the pointy end of the 'nose') to the edge of the carapace. Estimated length should be recorded to the nearest millimeter. Example: AMSP "105".
Cover	Used to distinguish whether CAB are detected in/under cover or in the open (O). Cover to be searched consists of large cobble ca. 100-250 mm diameter (LC); woody snags are submerged branches or logs ≥ 1 cm in diameter and other organic debris (WS); leaf packs are accumulated wads or clumps of leaves and other organic debris (LP), and submerged/emergent trash (T). Example: "LP".
Geomorphic Channel Unit	Geomorphic Channel Unit. Three types of geomorphic channel units will be used to describe habitat at spatial scales relevant to characterizing what constitutes suitable habitat for CAB. The geomorphic channel unit in the transect is recorded in the cell. To be considered a discrete habitat type the geomorphic channel unit must be roughly $\geq$ the average stream width. Riffles (R) are relatively shallow areas of the channel where water flows swiftly over completely or partially submerged obstructions to produce surface turbulence. Pools (P#) are disjunct from the stream with no velocity or no surface turbulence, and may be deeper than riffles or glides. There may be multiple pools in a transect wherein each pool was assigned a unique number on the pool map (P#). Transects with multiple pools will have one habitat characteristic metadata sheet for each pool. Only pools with a surface area > 1 m ² are to be surveyed. Glides (G) are areas with moderate depth and little or no surface turbulence. Velocities can be high or low, but the key feature is little apparent surface turbulence. Example: "P1".
Notes	Deformities, injuries, eggs, or larvae are recorded in the "Notes" column. Multiple individuals in a transect are recorded with hash marks.
Observer(s)	The first initial and full last name of the crew member acting as the observer. Specific transects will require a combined survey, where two observers divide the transect in half, in which case there will be two names. Example: "ROlson"
Recorder	The first initial and full last name of the crew member acting as the data recorder. There will be two distinct data recorders, each assigned to a specific observer, during each survey. Example: "RToomey"

Appendix E: Field Guide to Common Cave Aquatic Biota at Mammoth Cave National Park

Photographs by Rick Olson unless otherwise noted. All photographs used with permission.

Common Aquatic Invertebrates



Cave crayfish (*Orconectes pellucidus*) 10-75mm

Species Code: ORPE

Eyeless, white to yellowish, slender with long chelipeds (claws).

Found in all subsurface aquatic habitats, even out of water on occasion, but mostly above base level.
Can be abundant.

Note eggs carried beneath tail.



Stygophilic crayfish (*Cambarus tenebrosus*) 15-150mm

Species Code: CATE

Small-eyed, dark-greenish brown, robustly built surface crayfish often found in caves.

Found in all subsurface aquatic habitats but typically above base level and near entrances.



Kentucky Cave Shrimp (*Palaemonias ganteri*) 4-25mm

Species Code: PAGA

Federally listed endangered species. Unlike other cave crustaceans found in Mammoth Cave *P. ganteri* is nearly transparent but lightly pigmented near the head.

Also, unlike other cave crustaceans, it can be found swimming in the water column, on the underside of the water surface, and occasionally grazing on substrate.

Found in base level habitat, especially with sandy or silty water; can be found stranded in pools high within base level flooding range. *Note food in gut.*



Amphipods

Four cave-dwelling amphipods are found in the Mammoth Cave region. Amphipods are *laterally compressed*, with their tail curled, giving them a c- shape. Note four large eggs in ovaries.

Stygobromus exilis, *S. vitreus* 2-4mm

Species Code: STEX, STVI

S. exilis and *S. vitreus* are both white and cannot be distinguished in the field, except by habitat:

S. vitreus occurs only in high level flood pools and intermittent streams whereas *S. exilis* is found in shallow permanent streams on down to base level. Both are stygobites and small.

Crangonyx barri, *C. forbesi* 2-7mm

Species Code: CRBA, CRFO

The stygophilic *C. barri* occurs in streams and drip pools near the surface where food availability is highest.

Larger individuals exhibit an opalescent sheen and two tiny white eyespots on their head; when found in the twilight zone they can also be pigmented.

Crangonyx barri are larger than *Stygobromus* spp.



Isopods

Cannot be distinguished in appearance but can be told apart by their habitat. Isopods are *dorsoventrally compressed*. The long antennae are at the head end (top right) and there is a short pair of cerci at the posterior end (bottom left).

Found in cave streams and pools.

Caecidotea stygia, *C. bicrenata* 2-15mm long

Species Code: CAST, CABI

Caecidotea stygia occurs only in upper level areas lacking fish or crayfish.

In the presence of fish or crayfish the species is C. bicrenata; their ranges may overlap.

Isopods can be found beneath flat rocks in flowing water or grazing on the sides of rimstone or flood pools.



Snail (*Antroselates spiralis*) 1-2mm

Species Code: ANSP

This is the only aquatic snail occurring within the dark zone.

The snail can be found on the undersides of large flat rocks in base-level pools and streams.

It has not been reported from north of the Green River within Mammoth Cave National Park but should be looked for there.



Flatworms (*Sphalloplana percoeca*, *S. buchanani*) 3-6 mm long when extended

Species Code: SPPE, SPBU

Both species are white.

Their shape changes constantly as they slowly move across the substrate; they can also be found moving along the underside of the surface of quiet pools.

Sphalloplana buchanani is more slender, has a transparent rim around the perimeter of its body and has more prominent auricles at its head end (see pictures).

Stygobiotic flatworms are found in flood or drip pools sometimes in large aggregations when feeding on corpses or feces. They are also found beneath rocks in gravelly streams.

Sphalloplana percoeca can occur in flood pools that periodically dry up.

Common Aquatic Vertebrates

Plethodontidae sp.

Typically found out of the water.

Common Salamanders



Red Salamander (*Pseudotriton ruber*) 100-150mm

Species Code: PSRU

Typically found in pools and stream runs (larvae pictured). Iris of eye yellow.



Cave Salamander (*Eurycea lucifuga*) 100-150mm

Species Code: EULU

Typically terrestrial but may be found in pools



Slimy Salamander (*Plethodon glutinosus*) 120-170mm
Species Code: PLGU



Zig Zag Salamander (*P. dorsalis*) 60-90mm
Species Code: PLDO

Cavefishes

Three members of the cave fish family Amblyopsidae-two eyeless and depigmented stygobionts and one stygophile- ranges overlap in the Mammoth Cave region.



Southern Cavefish (*Typhlichthys subterraneus*) Up to 60mm

Species Code: TYSU

Relatively slender build. Smaller, more rounded head compared to the northern cave fish.

Off-white color.

Note the distinct, white circular fat bodies approximately where the eyes would be located and near base of tail.

Usually found above base level in streams with rock and gravel substrate. Overlaps into the upper range of base-level water and may occur with the northern cave fish.



Northern Cavefish (*Amblyopsis spelaea*) Up to 150mm

Species Code: AMSP

Relatively stout build. Larger, more angular head than the southern cave fish.

Pinkish white color.

Unlike the southern cave fish *there are no fat bodies visible on the head of the northern cave fish*.
Usually found in base-level streams.

Can overlap into known range of the southern cave fish. Photograph by Dante Fenolio.

Surface fishes

Surface fishes may also be found well within the dark zone. The most common surface species found in cave waters are the banded sculpin, several species of *Notropis* minnows, several species of sunfish, and madtom and bullhead catfish.



Spring fish (*Forbesichthys papilliferus*) Up to 120mm

Species Code: FOAG

Resembles the stygobiotic fishes in build but note the *presence of eyes and brownish-green color*.

Mainly in spring outlets but may occur within the dark zone of base-level streams.



Banded Sculpin (*Cottus carolinae*) Up to 160mm

Species Code: COCA

Usually, *distinct dark bands across body.*

Can be found in twilight zone but also well into the dark zone.

Typically found resting on the bottom of streams at or near base level.

**National Park Service
Inventory and Monitoring Division**



Post-Field Sampling—Version 1.0

This standard operating procedure describes procedures (SOP) to follow after completion of a sampling event in cave stream reaches in the Cumberland Piedmont Network (CUPN). This covers the point when field personnel leave the cave after a sampling event (i.e., complete sampling of the physical, chemical, and biological data of all transects in one cave stream reach by two independent teams in a single day), to the start of data entry (covered in SOP 5 *Data Management—Version 1.0*). This SOP also covers procedures to follow at the end of a sampling season. All field personnel should review this SOP by early to mid-June of each year as well associated equipment checklists (see Tables SOP2-6 through SOP2-9). In late fall or early winter the project leader revises any sections based on new information (e.g., Field Information Sheets) and lessons learned from field sampling the previous year.

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Contents

	Page
Logistics	SOP 4-4
Transit from Field to Office	SOP 4-4
Arrival at Office/Post Field Season	SOP 4-4
Disposition of Hardcopy Field Data Sheets, Digital HOBO Data, and Photo/Video Processing	SOP 4-5
Handling Field Data Sheets	SOP 4-5
Sampling Event CAB Field Data Sheets (Figures SOP3-3 and SOP3-4 and SOP3-Appendix D)	SOP 4-5
Sampling Event HOBO U20L data logger cave stream reach setup and maintenance field data sheet (SOP3-Appendix A).....	SOP 4-6
Scanned Field Data Sheet Renaming Examples.....	SOP 4-6
Cave Stream Reach Map, CAB Abbreviations Sheet, and CAB Field Guides (Figure SOP2-2 and SOP3-Appendix C, Figure SOP3-5 and SOP3-Appendix E, respectively)	SOP 4-7
Water Quality Sampling Field Data Sheets (Figure SOP4-2; CUPN 2017 and CUPN Water Quality Monitoring Protocol SOP 3.2; Meiman 2018).....	SOP 4-8
Scanning Field data sheets.....	SOP 4-8
Step-by-Step Instructions for Processing Water Quality Field Data Sheets	SOP 4-9
Processing HOBO U20L Data Files.....	SOP 4-11
Downloading and Processing Surface Meteorological Data Files	SOP 4-12
Step by Step Instructions (SCAN station)	SOP 4-12
Photo Processing	SOP 4-14
Photos	SOP 4-14
Upload the image files from the camera to the appropriate image folder on the CUPN drive.	SOP 4-14
Decontamination	SOP 4-14
Criteria for Decontamination When Caving In Mammoth Cave National Park	SOP 4-15
Decontamination Procedures.....	SOP 4-15

Contents (Continued)

Page Approaches to Reducing Decontamination Needs	SOP 4-16
Transport and Storage.....	SOP 4-16
Equipment Organizing/Cleaning	SOP 4-16
Literature Cited	SOP 4-17

Logistics

The project leader is responsible for post-field season communication and coordination with park staff, as well as logistics. Before leaving the park, the sampling crew must stop by the park resources management office to dispense with remaining logistical items, such as returning keys and vehicle hang tags. Complete brief follow-up Trip Reports will be completed within two weeks after each sampling trip. Because sampling at Mammoth Cave NP occurs throughout the summer, a single Trip Report for that park will be completed within two weeks after the end of the sampling season.

Transit from Field to Office

- Upon exiting the cave contact the designated surface contact person via cell phone, radio, or satellite communicator.
- If necessary, use the GPS unit to navigate to the vehicle.
- Secure all road gates used.
- Prior to entering the vehicle, office, or place of lodging if no vehicle was used, follow the most recent national guidance regarding the proper storage of all gear that was either used in the cave or that entered the cave entrance area. This is to reduce the possibility of transmitting *Pseudogymnoascus destructans* fungal spores. (Available at <https://www.whitenosesyndrome.org/resource/national-white-nose-syndrome-decontamination-protocol-april-2016> [last accessed July 2018] and stored on the CUPN server).
- Project leader or designated field crew leader conducts post-field sampling “tailgate session” for crew member(s).

Arrival at Office/Post Field Season

1. Upon returning from a cave entrance with a long transit between the cave entrance and the office or place of lodging, immediately contact the designated surface contact person via cell phone, radio, or satellite communicator.
2. Sign in field team on park or office message board (if required by park).
3. Remove gear and refuse from field vehicles.
4. Carefully clean and decontaminate gear using the latest national White Noise Syndrome decontamination guidance (see link above or obtain from the CUPN server). Field data sheets, notebooks, and maps filled out in pencil or permanent ink and printed on Rite-in-the-Rain[™] paper that were exposed to a cave must be disinfected following current national decontamination protocols. For gear, this step is performed as needed (e.g., between caves) otherwise field data sheets and gear may be double-bagged and disinfected post field season.

5. After the end of the field season check for damage to equipment and caving gear requiring repairs or replacement, and return equipment and gear to the appropriate office or person for proper storage.
6. For NPS staff or volunteers injured in a non-emergency incident during the field trip, complete Worker's Compensation paperwork (must be done within 48 hours of incident).
7. Field crew leaders or members should hold a brief “tailgate session” to discuss any issues that arose with the monitoring procedures with the project leader.
8. An after-action review will be held a day or two after each sampling event (i.e., multiple days sampling at a park, or periodically at Mammoth Cave NP) to find out how it went, whether any safety concerns are present, and if there are suggestions for improving operations.

Disposition of Hardcopy Field Data Sheets, Digital HOBOT Data, and Photo Processing

Handling Field Data Sheets

Upon return to CUPN headquarters at Mammoth Cave NP, and after disinfected field data sheets, notebooks, or maps have thoroughly dried, they will be returned to the project leader to be retained in a physical park-specific file folder labeled with the unique cave stream reach code, and the year of the sampling event, “<Cave Stream Reach> <Sampling Event Year>.” After confirmation from the project leader that all field data sheets are present, post-processing of data files, revising the field data sheets, and database entry and QA/QC procedures are completed (Figure SOP4-1). As soon as possible the field data sheets will be scanned and stored to specific folders on the network server in the following directory: Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working Data\.

Sampling Event CAB Field Data Sheets (Figures SOP3-3 and SOP3-4 and SOP3-Appendix D)

Upon completion of fieldwork, the cave stream reach's Sampling Event Metadata field sheet, as well as the Habitat Characteristic Metadata and CAB field data sheets associated with all its transects (plus pools, as necessary), must be scanned as a single PDF. These files will be saved using the naming convention <cave stream reach letter code>_CAB_<transect#>_<date>.pdf where cave stream reach letter code is the four letter code assigned to a particular cave stream reach on the field data sheet, transect# is the sequential number of the transect on the field data sheet, and date is the date on the field data sheet as YYYYMMDD (see example below). In cases where transects contain pool(s), files will be saved utilizing the naming convention <cave stream reach letter code>_CAB_<transect #>_<pool#>_<date>.pdf where pool number is the pool number on the field data sheet (taken from the pool map). These sheets are stored to park specific folders on the network server in the following directory:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\Scanned_Field_Data_Sheets\Park Code\CAB_Field_Data_Sheets

Sampling Event HOBO U20L data logger cave stream reach setup and maintenance field data sheet (SOP3-Appendix A)

Field data sheets will be saved utilizing a naming convention <cave stream reach letter code>_<HOBO location name>_<serial number>_<date>.pdf where cave stream reach letter code is the four letter code assigned to a particular cave stream reach on the field data sheet. The HOBO location name is the unique name assigned to the permanent location where a data logger was initially installed, the serial number is the number printed on the side of the data logger, and date is the date on the field data sheet as YYYYMMDD (see example below). Data logger data files and cave stream reach setup and maintenance field data sheets are stored in the following directory:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\
Data\Working_Data\Scanned_Field_Data_Sheets\Park Code\HOBO_Field_Data_Sheets

Scanned Field Data Sheet Renaming Examples

A cave stream reach with the letter code “UPBR” is sampled at Mammoth Cave NP, on February 27, 2015. Upon completion of field work all Sampling Event Metadata, Habitat Characteristic, and CAB field data sheets associated with a specific transect (plus pools, as necessary) will be scanned as a single .pdf with the following naming convention:

- UPBR_CAB_T#_(Pool#)_20150227.pdf

During the same sampling event a HOBO data logger with serial number 675503 was serviced at permanent data logger location named “H1.” Upon completion of the fieldwork, post-processing of data files, revising the field data sheet, and database entry and QA/QC procedures, the project leader determines that the field data sheet is complete. The file name that will be used for the scan is:

- UPBR_H1_675503_20150227.pdf

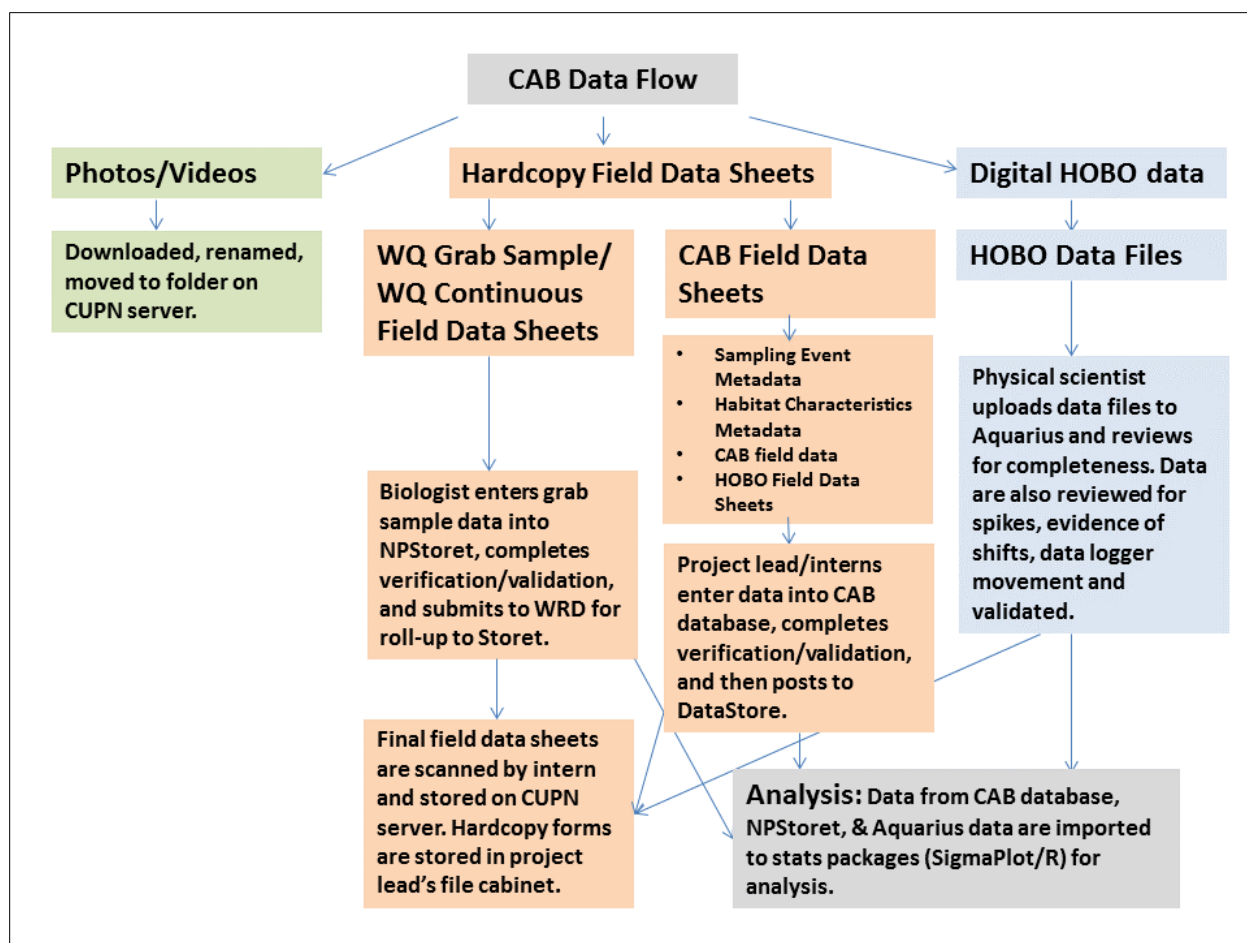


Figure SOP4-1. Data flow chart for processing of CAB images, field data sheets, and digital data files.

Cave Stream Reach Map, CAB Abbreviations Sheet, and CAB Field Guides (Figure SOP2-2 and SOP3-Appendix C, Figure SOP3-5 and SOP3-Appendix E, respectively)

Cave stream reach maps (and Pool Maps), CAB abbreviations sheets, and CAB field guides that are not used as field data sheets are less likely to be changed over time and so are laminated for reuse. After Cave Stream Reach Maps are created by the CUPN GIS Specialist they are stored in the following directory:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Docs\Field_Data_Sheets\Cave Stream Reach Maps\Park Code

Files will be saved, in cave stream reach specific folders (Mammoth Cave NP only), utilizing a naming convention <cave stream reach letter code>_Map_<Section#>_<date>.pdf. Cave stream reach letter code is the four letter code assigned to a particular cave stream reach on the field data sheet, section# is the section number of the map (see Figure SOP2-2, top), and date is the date of the map's creation as YYYYMMDD.

For example: UPBR_Map_Section1_20130125.pdf

After they are created by the project lead CAB Abbreviations Sheet and Field Guides are stored, respectively, in the following directory:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Docs\Field_Data_Sheets\CAB Abbreviations\Park Code Abbreviations

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Docs\Field_Data_Sheets\CAB Abbreviations\Park Code Field Guide

Water Quality Sampling Field Data Sheets (Figure SOP4-2 and CUPN Water Quality Monitoring Protocol SOP 3 Collection of Field Data—Version 1.2; Meiman 2018)

File naming convention <cave stream reach letter code>_WQ_<date> where cave stream reach letter code is the four letter code assigned to a particular cave stream reach on the field data sheet, and date is the date on the field data sheet as YYYYMMDD (see example below)

For example, ECHO_WQ_20160315

These sheets are stored to park-specific folders on the network server in the following directory:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working
Data\Scanned_Field_Data_Sheets\Park Code\PDFs_WQ_Hardcopy_Data

Scanning Field Data Sheets

Before scanning field data sheets, review them for obvious errors (e.g., no cave name at the top of the sheet) and make sure that all pages are present and in the correct order (see Figure SOP4-1). Scan settings must be set to “black” and “text only.” After scanning all field data sheets from an event on the CUPN’s Konica printer/scanner and saving to a user box (use Project Leader’s):

- From an NPS computer with Adobe Acrobat Version 9.0 or later, open web browser and go to <http://10.147.161.137/>
- Select “Box” on the ribbon at the top of the screen, hit “Next,” enter the “User Box No.” that corresponds to Project Leader’s user box, and hit “OK.” No password is necessary.
- Download and open the file on your computer.
- In Adobe Acrobat choose File > Save As > Reduced Size PDF.
- Choose Make compatible with version 9.0 or later.
- Save files in designated folders on the network server according to CUPN’s naming convention given above:

Examples: UPBR_CAB_T1_20150227.pdf

UPBR_H1_675503_20150227.pdf

Note: File size should be in the neighborhood of 1.0 MB or less. Also, it is important that you thumb through each page of the scan to make sure all pages scanned correctly and are legible.

- Files will be saved/copied to their park specific folder on the network file server at Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\.
- Once the scanned file has been reduced, renamed, reviewed and copied to the network file server, go back to the User Scanner box and delete the original scanned file.
- The original hard copy field data sheets will be returned to the project leader for storage.

Step-by-Step Instructions for Processing Water Quality Field Data Sheets

The following is a step by step process for preparing the field data sheets for entry into NPSTORET.

1. Figure SOP4-2 is a mock up of the CUPN water-quality field data sheet. Once sampling is complete, some fields will need to be processed back at the office or in the lab and results will need to be filled in to complete the data sheet. Fields that will typically need to be filled in post-sampling include: “Precipitation past week,” “*E. coli*,” and “NO₃.” Precipitation past week will be calculated by accessing precipitation data on the SCAN station website (<https://www.wcc.nrcs.usda.gov/scan/>) (last accessed August 2018); see “Processing Surface Meteorological Data Files” below. *E. coli* and NO₃ will be obtained from tests conducted at the lab (see Water Quality Monitoring Standard Operating Procedure 4 *Field Measures*—Version 1.2; Meiman 2018).

QAQC ☐

Park MACA

Station Code UPBR

Project CAB

General Information

Date	<u>4.15.2016</u>	
Collection Time and Time Zone	<u>1321</u>	<u>CDT</u>
Sampler(s)	<u>K. HELF</u> <u>R. TOOMEY</u>	
Precipitation past week (cm)	<u>1.18</u>	
Weather Code	<u>1</u>	
Flow Condition	<u>NORMAL</u>	

Photos Taken ☒

Samples Taken ☒

Hydrologic Observations

<u>Water clear.</u>
<u>No visible flow.</u>

Discharge

Estimated Total Discharge	☐ l/sec ☐ CFS
<u>N/A</u>	

Parameters

Air Temp. (°C)	<u>11</u>
Water Temp. (°C)	<u>9.7</u>
SPC (µS)	<u>424</u>
pH (SU)	<u>7.8</u>
DO conc (mg/l)	<u>5.42</u>
DO sat (%)	<u>68.3</u>
Turbidity (NTU)	<u>3.7</u>
E.Coli (MPN/100ml)	<u>148.3</u>
NO ₃ (mg/l NO ₃)	<u>2.6</u>
ANC (mg/l CaCO ₃)	

Figure SOP4-2. Example of water-quality field data sheet. Note: “Station Code: UPBR” identifies the cave stream reach in which the data were taken.

- During lab testing, an *E. coli* data sheet and NO₃ data sheet are used to record results (see Water Quality Monitoring SOP 4 *Field Measures—Version 1.2*; Meiman 2018). Once lab tests/lab data sheets are complete, those results are hand recorded in the field data sheet above.
- Once the water-quality field data sheet is completely filled in, it is then copied and scanned along with the *E. coli* and NO₃ data sheets.
- The scanned copy is emailed to the CUPN Hydrogeologist for pre-validation (see Water Quality Monitoring SOP 8 *Data Analysis and Reporting—Version 1.2*; Meiman 2018).
- During pre-validation, the CUPN Hydrogeologist will make any changes or edits if necessary to the digital copy and email it back to the data manager.
- The CUPN biologist will then use this (potentially) revised copy for data entry into NPSTORET (see SOP 5 *Data Management—Version 1.0*) and permanently store it at:
Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\Scanned_Field_Data_Sheets\Park Code\PDFs_WQ_Hardcopy_Data.

File naming convention <cave stream reach>_WQ_YYYYMMDD (e.g., ECHO_WQ_20160315).

7. The hardcopies/originals will also be given to the project lead for filing/future archiving.

Processing HOBO U20L Data Files

Instructions for downloading data to the data shuttles (storage modules) for use with HOBO data loggers used in this protocol will generally follow methods in SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*, but more specifically in Curdts (2017). After the data are downloaded to the data shuttle in the field, the data are uploaded to Aquarius in the office where post-processing occurs (e.g., evaluation for outliers). The files are renamed using the standard naming convention (see below). Data files from the data shuttles are saved to the park or cave stream reach specific (Mammoth Cave NP only) folders located at the following path:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\HOBO_Offload\Park Code

Field crews will have maintained a record associated with each handling of a HOBO data logger at a cave stream reach on the field data sheet (Appendix A in SOP3—*Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics*) including the data logger serial number, the date and time the data logger was either installed or serviced or replaced, the unique data logger location name, and whether the data transfer in the field was successful.

Therefore, **do not continue with processing of HOBO data files, beyond copying them to the network server as described above, unless you have the HOBO Data logger field data sheet(s) at hand.**

All HOBO data files in Z:\...\HOBO_Offload are downloaded to the park code/cave stream reach (MACA only) level in the “working data” directory before renaming. For example, HOBO data files from Upstream Bridge Avenue in the HOBO_Offload folder should be **downloaded** into:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\HOBO_Offload\Park Code\

Data files should be renamed as <cave stream reach>_<serial number>_<date>.hobo where the HOBO cave stream reach is the unique name assigned to the permanent location where a data logger was initially installed. The serial number is the number printed on the side of the data logger, and the date is the date recorded on the field data form when the data logger was downloaded inside- or removed from- the cave in YYYYMMDD format. These unique names will distinguish them from other meteorological data files from other protocols (e.g., cave bat monitoring).

Example: UPBR_H1_675503_20150227.hobo

Downloading and Processing Surface Meteorological Data Files

Within two weeks after the second sampling event of the season, prior to performing data analysis, correlative data on precipitation, gathered continuously by Soil Climate Analysis Network (SCAN) or National Atmospheric Deposition Program (NADP) weather stations will be downloaded to the appropriate park subfolder here:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\Surface_Met_Data\Park Code\

Surface meteorological data downloaded from either a [SCAN](#) station site page (last accessed August 2018) or [NADP](#) station (last accessed August 2018) are preferred. As necessary, (e.g., if a nearby site is not available or is decommissioned) data may also be accessed through the [National Oceanic and Atmospheric Administration's Climate Reference Network](#) (accessed August 2018). Data files downloaded from SCAN stations are processed in the following way. The physical scientist, project leader or designated field crew member downloads the data file from the appropriate SCAN station website (Figure SOP4-3), renames the files using the standard naming convention (see below), and checks the files for completeness.

Step-by-Step Instructions (SCAN station)

1. Surface meteorological data are accessed from the interactive map on the SCAN station website. The user must select the station from a map of stations and click on “site map.”
2. The first column, “Select Report Content”, is where the surface meteorological parameters to be downloaded are selected; these are accessed by scrolling down past the “Individual elements” divider and clicking on “Atmospheric 2003-08-28” (Figure SOP4-3).
3. The second column, “Select Time Series,” is where the desired time interval is selected; in this case it is “Hourly.”
4. The third column, “Select Format,” gives three choices of data format which, in this case, is “csv.”
5. The fourth column “View Current” can be ignored.
6. The fifth column, “View Historic,” is where the desired year, month, and days corresponding to a week prior to the date of the sampling events associated with specific sampled cave stream reach are selected. Thus, for a sampling event on July 15, 2017 the data for July 8, 2017 to July 14, 2017 are downloaded. Once the time period is selected the data are downloaded by clicking the “View Historic” button.

United States Department of Agriculture
NRCS Natural Resources Conservation Service
 National Water and Climate Center

SCAN Site: Mammoth Cave
 State: Kentucky
 Site Number: 2079
 County: Hart
 Latitude: 37 deg; 11 min N
 Longitude: 86 deg; 2 min W
 Elevation: 800 feet
 Reporting since: 2003-08-28

Questions about this site.

View Soils Pedon Report
 View Daily Sensor Descriptions
 View Hourly Sensor Descriptions
 View Sensor History

Site notes:

- Data is provisional and subject to revision.
- More site notes.
- Photograph is of the Mammoth Cave SCAN site.
- 2017-June-29 National Water and Climate Center

☐ Check to view temperatures in degrees Fahrenheit. Default is degrees Celsius.

Select Report Content	Select Time Series	Select Format	View Current	View Historic
Standard SCAN (no chart) (2003-08-28)	Daily	table	Last 30 days	2003
All Sensors (no chart)	Hourly	csv	Last 7 days	2004
Soil Moisture & Temperature (2003-08-28)	12 AM	chart	Last 24 hours	2005
Soil Moisture (2003-08-28)	3 AM		Water Year	2006
Soil & Air Temperature (2003-08-28)	6 AM		Calendar Year	2007
Precipitation 2003-08-28	9 AM			2008
Atmospheric 2003-08-28	12 PM			2009
Wind (2003-08-28)	3 PM			2010
Solar (2003-08-28)	6 PM			2011
===Individual elements===	9 PM			2012

☐ Check here to view reports in new window.

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Figure SOP4-3. Soil Climate Analysis Network (SCAN) website from which validated surface meteorological data (i.e., surface temperature, RH, and precipitation), collected by MACA's SCAN station, are downloaded.

- A pop up window will appear and the data are copied to the folder associated with the month of the sampling event: Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Data\Working_Data\Surface_Met_Data\Park Code\
- After the file is downloaded as a “.csv” file it must be checked for completeness. This file includes more parameters than needed and so unneeded parameters, other than temperature, relative humidity, and precipitation, can be deleted.
- The merged data file is renamed as “SCANXXXX_<date>.csv” “SCANXXXX” is the unique site number assigned to Mammoth Cave NP’s SCAN station location, and “date” is the date of the specific sampling event on which the cave stream reach was sampled as recorded on the field data sheet. The date format used is YYYYMMDD. Example: “SCAN2079_20150227.csv.”

Photo Processing

Photos

Crew members will download all photos taken during a sampling event from image cards (e.g., CAB photos, data logger photos) in digital cameras and rename photo files using a standard naming convention (e.g., <cave stream reach letter code>_<transect #>_(<pool #>)<CAB letter code>_<date>).

For example, UPBR_ T1_ORPE_20150227

Upload the image files from the camera to the appropriate image folder on the CUPN drive.

Image transfer can take a substantial amount of time and is best performed as soon possible (i.e., within a few days of the sampling event). Remove the SD card from the digital camera and plug into the SD card slot on the project leader's computer. The "Computer" window opens and the "SD Disk" icon is double clicked to access the images. In a separate window, the "Raw" folder with the appropriate park code and cave stream reach name into which the images will be dragged and dropped, is accessed via the following folder structure:

Z:\Vital_Signs\Vital_Signs_Prj\Cave_Aquatic_Biota\Images\Park Code\Raw_Images

A window with the title "Copying..." opens automatically, indicating the image files are being transferred from the SD card to the folder. A bar graph and time remaining clock indicates the progress of the image transfer. Once all the images are transferred, the "Copying..." window will automatically close. When the process is finished the number of images in the cave entrance folder window is compared to that of the "SD Disk" folder to ensure the integrity of the transfer. In addition, several of the transferred photos in the cave entrance folder window are randomly examined to ensure images were not corrupted during transfer. The images are stored as ".jpg" files with annotation for date, time, and exposure parameters automatically provided by the camera. Once all raw images have been transferred the images on the SD card are to be deleted. **Even if the memory card has a large amount of space, it is best to delete the images each day to avoid later confusion or having to pause during field work to delete old images in order to free space on the memory card.**

To save space on the CUPN server once the image files are renamed they will be moved from the "Raw_Images" subfolder to a dedicated external hard drive and the raw image files deleted from the "Raw_Images" subfolder. The dedicated external hard drive will have the following file structure:

Cave Aquatic Biota\Images\Park Code\.

Decontamination

Since the initial discovery of a northern long-eared bat (*Myotis septentrionalis*) with white-nose syndrome (WNS) in Mammoth Cave NP in January 2013, multiple additional species have been found with the disease and multiple hibernacula are known to be contaminated with the fungus that causes the disease. Decontamination procedures adopted by the park will continue in order to

minimize the risk of inadvertent human-assisted transmission among caves inside and outside of the park. These procedures include decontamination of cave gear, including everything worn or taken into a cave. The criteria below only covers Mammoth Cave NP because it is the only park in the monitoring protocol wherein multiple cave entrances are visited.

Criteria for Decontamination When Caving In Mammoth Cave National Park

Gear will require decontamination before conducting another sampling event in three cases:

- between cave systems or entrances of known infected status and cave systems or entrances of unknown infected status,
- between two cave systems or entrances of unknown infected status, or
- before using equipment at another park.

Decontamination Procedures

The minimal acceptable decontamination standards for cave work at Mammoth Cave NP follow the current national decontamination protocol available from the U.S. Fish and Wildlife Service (USFWS). Standards for cave work are available at the USFWS's main WNS website at:

<https://www.whitenosesyndrome.org/resource/national-white-nose-syndrome-decontamination-protocol-april-2016> (last accessed August 2017).

The procedures are summarized as follows:

1. Scrub contaminated submersible gear to remove mud and other dirt.
2. Scrubbed, contaminated submersible gear should either be:
 - a. If the item is relatively small (e.g., hand held waterproof flashlight) it may be wiped down with Isopropyl Alcohol. The recommended minimum contact time is one minute.
 - b. Large items (e.g., equipment packs) should be immersed in hot water [i.e., greater than or equal to 55°C (131°F)] for a minimum of 20 minutes, thoroughly rinsed, and air dried.
3. Decontamination procedures for non-submersible gear, such as electronic equipment and field notebooks (as well as hard, non-porous surfaces), can involve the use of a number of different commercially available cleaning products. Consult the Applications/Products chart on page one of the linked pdf file above. Alternatively, some electronics can be placed in sealed plastic bags (or wrapped) and the plastic bags can be discarded between caves. Follow manufacturer's recommendations pertaining to personal protective equipment for use with all decontamination chemicals. Material Safety Data Sheets for each product must be available upon request from designated park personnel.

Approaches to Reducing Decontamination Needs

There are several approaches that will reduce the amount of gear that needs to be decontaminated and reduce the risk of transmission by error. They include:

1. Reduce the amount of gear being taken into the cave or field that is needed for safe and effective work.
2. Gear (such as extra lights) which may not be used during a cave trip can be bagged in sealed plastic bags. If the bags are not opened in the cave, the gear inside will not require decontamination. However, the outside of the bags themselves will be considered contaminated and will require disposal or decontamination.
3. Clothing coverings, such as disposable rubber booties and disposable coveralls (made of Tyvek[®] or similar fabrics), may be used to reduce the risk of contamination of clothing and shoes. However, it is important to be sure that coverings do not tear or otherwise allow clothing to be exposed to the cave sediments. Also, boot coverings can affect footing and should only be used when it is clear they will not compromise safety.
4. Some gear (especially less expensive or difficult to decontaminate gear) could be dedicated for use in only one cave system entrance or cave (such as Mammoth Cave Ridge system entrances, or Little Beauty Cave). Dedicated gear should be stored in labeled containers to reduce confusion and cross-contamination among gear.
5. The use of submersible rubber boots, such as wellies or muckboots, may simplify decontamination of footwear.
6. Remove outer clothing and footwear, place in a sealed plastic bag, and change into clean clothing and footwear prior to entering a vehicle.

Transport and Storage

Careful double bagging of potentially contaminated gear after exiting the cave but before placing it in vehicles is essential to reduce the potential for contaminating vehicles and other gear. Vehicles that have been used to haul dirty gear not properly stored should also be decontaminated. After gear is decontaminated, personnel do not let it come in contact with gear that has not been decontaminated or placed in storage containers or car trunks that have been used to haul dirty cave gear.

Equipment Organizing/Cleaning

Clean the interior of all vehicles used in the field. Clean and repair all equipment, per the above standards, prior to returning them to their designated storage space. All reference manuals must be re-shelved on their appropriate bookshelf. Other reference materials and extra data sheets need to be filed in their appropriate filing cabinet. These documents are currently stored in the project leader's office at the CUPN headquarters, in Mammoth Cave NP.

Literature Cited

- Curdts LT. 2017. Continuous water level data collection and management using Onset HOBO® data loggers: A Northeast Coastal and Barrier Network methods document. Natural Resource Report. NPS/NCBN/NRR—2017/1370. National Park Service. Fort Collins, Colorado. Available at: <https://irma.nps.gov/DataStore/Reference/Profile/2237862> (last accessed September 2018).
- Meiman, J. 2018. Cumberland Piedmont Network water quality monitoring: Standard operating procedures. Cumberland Piedmont Network. Mammoth Cave, Kentucky. Available at: <https://irma.nps.gov/DataStore/Reference/Profile/2253371> (last accessed September 2018).

Standard Operating Procedure 5

Overview

Revision Log

[illegible]

Contents

	Page
Acknowledgements.....	SOP 5-3
Database Overview	SOP 5-3
Data Quality	SOP 5-10
Database Administration.....	SOP 5-13
Directory Structure.....	SOP 5-16
Metadata.....	SOP 5-17
Protected Data.....	SOP 5-17
Data Delivery	SOP 5-17
Natural History Collections	SOP 5-18
Literature Cited	SOP 5-21

Acknowledgements

This SOP benefitted greatly from the content in Bingham et al. (2016).

Database Overview

The Cumberland Piedmont Network utilizes three separate databases for storage, processing and retrieval of data collected as part of this monitoring effort. This includes two customized Microsoft Access applications and one commercial off-the-shelf application.

Cave Aquatic Biota—Core Data

The CAB database will store the core CAB data collected per guidance found in SOP 3 *Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*. This includes data on counts of cave aquatic biota, detections/nondetections of cave aquatic biota, habitat characteristics, and associated metadata. These data are recorded on paper field data sheets. Copies of these field data sheets and a data dictionary for each can be found in Appendix D in SOP 3.

Development of the Cumberland Piedmont Network's CAB database will be initiated after this protocol's review process is complete and the protocol has received final approval. The Inventory and Monitoring Division (IMD) central office has agreed to provide database development support for this and other 2017 protocols. A technical assistance request for this database development assistance has been submitted by the network, requesting a Microsoft Access application that meets CUPN needs and IMD database standards (Frakes et al. 2015). Microsoft Access is an accepted platform for databases within IMD (Frakes et al. 2015).

NPSTORET—Water Quality

NPSTORET (NPSTORET.mdb) is a Microsoft Access desktop application developed and regularly updated by NPS's Water Resources Division (WRD). NPSTORET.mdb, and the associated back-end databases contain all the forms, reports, and modules that a network needs to enter and complete QA/QC steps locally.

The Cumberland Piedmont Network will submit a copy of the back-end file that contains current year's finalized data to WRD. The Water Resources Division ultimately transmits all records flagged as final to the Environmental Protection Agency's (EPA) STORET database. EPA's STORET is a national data warehouse facilitating STORage and RETRieval of water quality data to the national and international community of water quality professionals.

Additional background information about STORET and NPSTORET can be found at the [Vital Signs Water Quality Data Management and Archiving web page](#) (last accessed September 2018).

Step-by-Step Instructions for NPSTORET Data Entry

The following is the step-by-step process for entering collected data from the water-quality grab sample field data sheet (Figure SOP4-2 in SOP 4) into NPSTORET.

1. Figure SOP5-1 is the first window to appear once the NPSTORET database is opened in Microsoft Access. In order for the five components (Projects, Stations, Metadata, Results, Reports and Stats; and Exit) to be active, first click on the organization (#1) responsible for the data, and the name of the person entering the data (#2). Once the five components are active, click on the “Results” button (#3) to go to the next window.

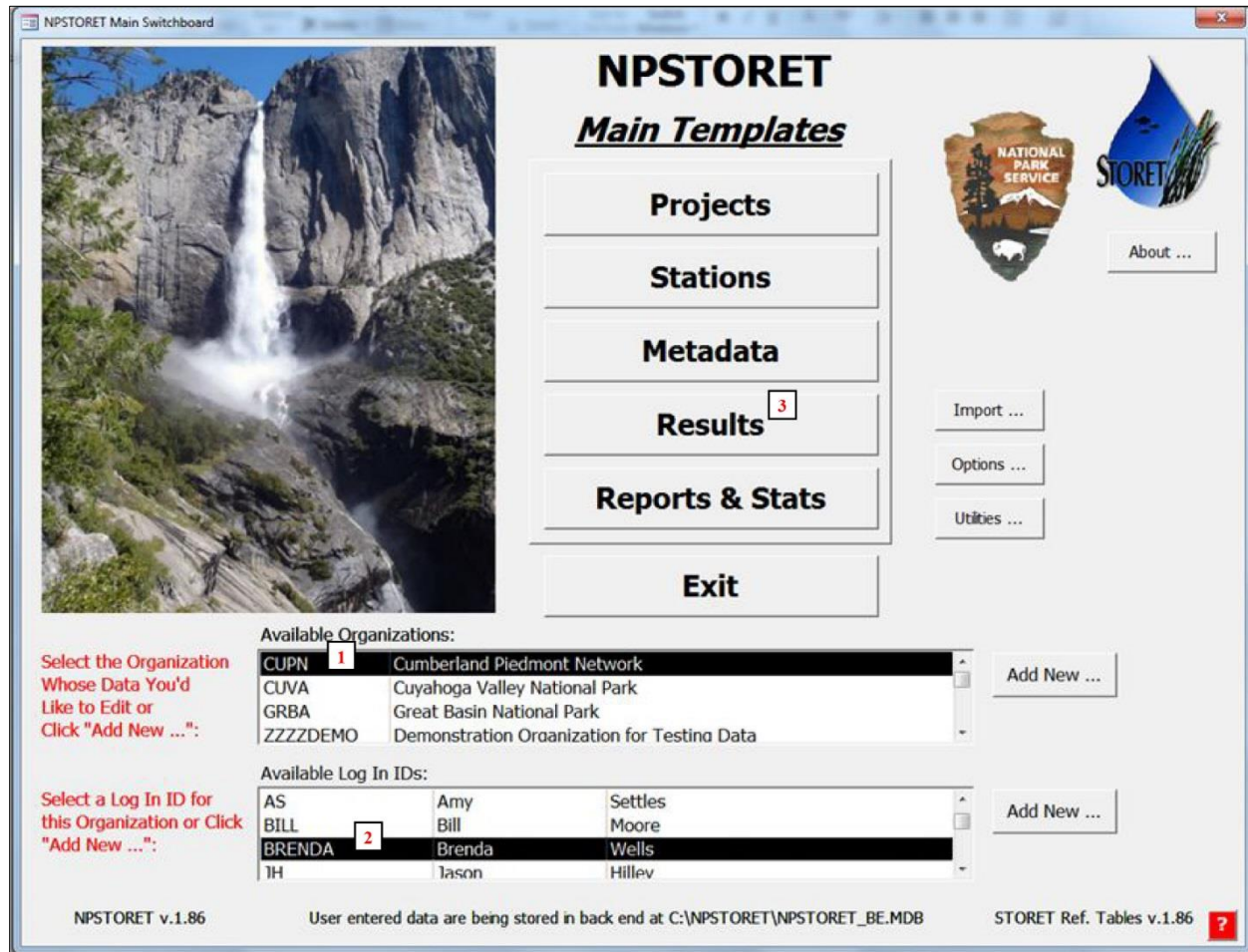


Figure SOP5-1. Switchboard for NPSTORET.mdb, a Microsoft Access desktop application developed by NPS Water Resources Division.

1. Figure SOP5-2 opens after clicking the “Results” button. Under “Available Projects” click on the project (#4) for which you are entering data, so that it is highlighted. Next, under “Available Station Input Groups:” click on “Stations Assigned to the Selected Project” (#5). This ensures only stations connected with your project will show up in the next window.
2. Under “Available Characteristic Input Groups” click on “Characteristics Assigned to the Selected Project” (#6). This ensures the characteristics connected with your project will show up in the next window. Click “OK” (#7) to go to the next window.

Select a Project and Station and Characteristic Input Groups for Results Entry

Available Projects:
Ordered by ProjectID (Ascending)

ProjectID	Project Name
LIRI_WQ	CUPN WQ Monitoring, LIRI
MACA_PP	Mammoth Cave National Park Phytoplankton Survey
MACA_UP	Mammoth Cave National Park Upland Pond Survey
MACA_WQ	CUPN WQ Monitoring, MACA
NISI_WQ	CUPN WQ Monitoring, NISI
RUCA_WQ	CUPN WQ Monitoring, RUCA

Available Station Input Groups:

Stations Assigned to the Selected Project

All the Organization's Stations

Available Characteristic Input Groups:

Characteristics Assigned to the Selected Project

All the Organization's Characteristics

AUTO: UNATTENDED SAMPLING SUPPORT

OK Cancel

Figure SOP5-2. NPSTORET form where users select the appropriate project, sampling sites and characteristics before data entry. Note that the appropriate projects for this protocol will be CUGA_CAB, MACA_CAB, and RUCA_CAB.

1. Figure SOP5-3 opens after clicking “OK.” Collected handwritten data from the water-quality field data sheet (Figure SOP4-2 in SOP 4) are entered in the fields below. This one page encompasses all the data for one particular cave stream reach. To fill out data for the next cave stream reach, click the “Add New Visit” (Visit = Sampling Event) button (#6). The

fields used in cave aquatic biota, along with a corresponding step-by-step process, are numbered below:

NPSTORET Result Entry Template

Quick Filter for Station: Quick Filter for Visit Start Date:

Choose Input Group: All Project Characteristics Jump to Activity:

Station ID: LIRI_EFLR 1 Station Name: East Fork Little River

Start Date: 3/18/2015 Start Time: 14:07 Zone: CST Vis# 00001

End Date: End Time: Zone:

Visit Comment:

Activity ID: EFLR03182015 Activity Type: NPS Regular/Routine QC: ☐

Depth: Units: Custody ID: Relative Depth: Person(s): Mary Shew

Activity Comment: Clear. Kral's water plantain still hanging in there.

Add New Visit Delete Visit Add New Activity Repeat Activity Delete Activity

Visit: 449 of 449 Pictures: 0 Activity: 1 of 1

Double-click a record to pop up an alternate data editing form Change Status to all: Clean Activity Auto Fill

	Local Name	Detection Condition	Result Value	Units	Value Status	Value Type	Statistic Type	Remark Code	Measure Qui	Comment
13	Ar Temp	Detected and Quantified	24	°C	Final	Actual				
14	Water Temp	Detected and Quantified	12.7	°C	Final	Actual				
15	SPC	Detected and Quantified	32.5	µS/cm	Final	Actual				
16	pH	Detected and Quantified	5.75	None	Final	Actual				
17	DO sat	Detected and Quantified	102.4	%	Final	Actual				
18	DO conc	Detected and Quantified	10.82	mg/l	Final	Actual				
19	ANC Lab	Detected and Quantified	1.9	mg/l CaCO3	Final	Actual				
20	Flow	Detected and Quantified	NORMAL		Final	Actual				
21	Discharge	Detected and Quantified	1699	l/sec	Final	Estimated				
22	Weather	Detected and Quantified	1		Final	Actual				
23	Precip Past Week	Detected and Quantified	0.28	cm	Final	Estimated				
24	Turbidity	Detected and Quantified	1.5	NTU	Final	Actual				
25	e. Coli	*Present <QL	*Present <QL	MPN/100ml	Final	Actual	MPN			
26	Nitrate as NO3 CUPN	Detected and Quantified	3.51	mg/l	Final	Calculated				

Record: 14 Filter Print Import Options ... Close Results

Figure SOP5-3. The Result Entry Template for WQ data in NPSTORET wherein CAB results are entered from the water quality field data sheet.

1. Enter the Station ID, which is the four-letter code assigned to the park being sampled, plus the CAB cave stream reach code.
2. Enter the Start Date, which is the date the cave stream reach was sampled.
3. Enter the Start Time, which is the time the sampler arrived at the specific cave stream reach.
4. Enter the time zone, which is a drop down list of all time zones (please choose the correct time zone at the time of the sampling).
5. This is an option field which can be used for anything noteworthy of the sampling event as a whole (e.g. "Logsdon River at 0.8' on old gauge"). Also used for additional sampling personnel that are not regular samplers ("Additional Sampler: John Doe, CRF Volunteer"). Cave stream reach-specific comments are entered in Activity Comment (#11).
6. The Add New Visit button is for creating a blank results entry template page for the next cave stream reach. Click when finished entering all data for that cave stream reach.

7. If photos were taken at this cave stream reach, click the “Pictures” button to attach photo image files to this record.
8. Enter the Activity ID, which is the specific name (created by the user or computer generated) for the record. This is an example of how CAB users will be generating their own Activity ID using the CAB cave stream reach code plus the Start Date (MMDDYYYY).
9. Enter the Activity Type, which is a drop down list of many different activity types. For the most part, CUPN uses either the “NPS Regular/Routine” or “NPS Quality Control Regular/Routine Replicate” for recording quality assurance and quality control (QA/QC) samples.
10. Choose the Person(s) sampling the site. One or many observers can be listed. The leader for that day is listed first and any additional regular observers can be listed next.
11. This is an optional field which can be used for comments regarding conditions/observations at the station being sampled.
12. When entering the QA/QC results, simply click the “Repeat Activity” button. This will give you a replicate page for the same site that will pull in the same result information from the top half of the “Result Entry Template” page and leave the bottom half of the result fields blank to input your QA/QC results.
13. Enter the result value (°C) of the Air Temperature from the field data sheet “Air Temp” into NPSTORET. This value is retrieved from setting a thermometer out while sampling and recording data. The thermometer is checked after grab sampling and the result is recorded in the water-quality field data sheet.
14. Enter the result value (°C) of the Water Temperature from the field data sheet “Water Temp” into NPSTORET. This value is retrieved from the Oakton Dissolved Oxygen meter and recorded in the water-quality field data sheet.
15. Enter the result value (µS/cm) of the Specific Conductivity from the field data sheet “SPC” into NPSTORET. This value is retrieved from the Oakton 300 pH/SPC meter and recorded in the water-quality field binder.
16. Enter the result value (percent saturation) of the Dissolved Oxygen Saturation from the field data sheet “DO sat.” into NPSTORET. This value is retrieved from the Oakton Dissolved Oxygen meter and recorded in the water-quality field data sheet.
17. Enter the result value (mg/l) of the Dissolved Oxygen Concentration from the field binder “DO conc.” into NPSTORET. This value is retrieved from the Oakton Dissolved Oxygen meter and recorded in the water-quality field data sheet.
18. Flow (Flow Severity) within NPStoret is based on a standardized EPA characteristic domain list with the choices being: Dry, Low, Normal, Above Normal, Flood, No Flow or Interstitial. For this protocol sampling events will only be scheduled during base flow conditions, where “Low” is defined as, “Base flow for a stream or flow within roughly 10% to 20% of base flow condition.” Thus in the early stages of this protocol, in the absence of any historical record or photographs of flow conditions, it is expected that field crews will only choose

“low” on the field form (binder). If another option is selected by the field crew in the field binder, this record should be flagged for review by the protocol lead and/or CUPN hydrologist.

19. This is a drop down list. Pick the number from the drop down list that best represents the weather at the time of the site sampling: 0=Cloudless, 1=Cloudy or Partly, 2=Overcast, 3=Drifting Snow, 4=Fog or Dust, 5=Drizzle, 6=Rain, 7=Snow, Sleet, Hail, 8=Rain Showers, 9=Thunderstorms to correspond to value in field binder. These numbers are also listed on the front of the water quality field binder for the sampler to refer to, pick one, and record in the “Weather Code” field of the water quality field binder.
20. The “Precipitation the Past Week” is calculated after the sampling day, back at the office. Log on to [SCAN station website](#). After selecting the location on the interactive map, click on the “History” tab and enter the date one day prior to the sampling date. Starting with that day, calculate the sum total of precipitation up through seven days prior and record that value (cm) “Precipitation past week” into NPSTORET.
21. Enter the result value (NTU) of Turbidity from the field binder “Turbidity” into NPSTORET. This value is analyzed in the lab by collecting a sample of water and putting the sample into a spectrometer or turbidity reading meter. The meter will give the value which is then recorded in the water quality field data sheet.
22. Enter the result value (MPN) for *E. coli* from the field data sheet “*E. coli*” into NPSTORET. Water samples for *E. coli* values are collected and preserved on ice until processed in the lab. Bacterial samples must be processed and incubated within 24 hours of taking them from the source water (see CUPN Water Quality Monitoring Protocol SOP 4 *Field Measures—Version 1.2* [Meiman 2018] for detail on testing/calculating results for *E. coli*).
23. Enter the result value (mg/l) for nitrates from the field data sheet “NO₃” into NPSTORET. Water samples for nitrate values are collected and preserved in ambient temperature. The samples are taken back to the lab for testing (see CUPN Water Quality Monitoring Protocol SOP 4 *Field Measures—Version 1.2* for detail on testing/calculating results for nitrates) (Meiman 2018). Results are achieved through a calibration curve against nitrate standards.

AQUARIUS – Water Level Data

AQUARIUS is commercial off-the-shelf software developed and marketed by Aquatic Informatics. It is a storage and retrieval system for continuous time-series data.

As detailed in SOP 3 *Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*, water level and temperature are continuously monitored with HOBO U20L data loggers. Data will be stored, processed and available for retrieval in AQUARIUS. NPS users can access AQUARIUS on the Natural Resource Stewardship and Science (NRSS) Fort Collins server including the Springboard interface (Figure SOP5-4). The Springboard allows users to import or append data, manage folders, locations and field visits; as well as data correction, exporting and reporting.

The National Park Service has a limited number of service-wide licenses for its use. In order to utilize one of these licenses and subsequently make a remote desktop connection to the NRSS server, users must first request access from Dean Tucker at the Water Resources Division (WRD). Once access has been established, WRD will email a Remote Desktop Protocol file (RDP) that will be used to make the remote desktop connection. Users should save the RDP to their local hard drive. A desktop shortcut can optionally be created for quicker access.

Cumberland Piedmont Network staff are in the initial stages of learning and incorporating AQUARIUS into their workflows. However, we intend to draw heavily from a continuous water-level data management guidance document produced by the NPS Northeast Coastal and Barrier Network (Curdts 2017). Curdts (2017) is a well-written and extremely thorough document which includes step-by-step details on using AQUARIUS to process and archive water level data (Section 4.4). Once this protocol is approved and HOBO U20L data loggers are deployed, specific workflows to meet network needs and responsibilities will be established and this SOP will be updated to reflect those details.



Figure SOP5-4. Opening screen shot to launch AQUARIUS Springboard, which is the interface used by NPS for data management activities.

Other Data Formats

In addition to paper forms, data will be collected in various media. Their mention within this SOP is not to provide step-by-step instruction (refer to the overview section of this SOP), but rather to make readers seeking an overview of data management aware of other data. Many of the steps for processing and cataloging these other forms of data can be found in SOP 4.

Digital photos

CAB photographs to document presence of disease, CAB photographs to aid in species identification, photographs as image markers of field data forms, photographs to document miscellaneous incidental observations (e.g., unique cave formation or a rare species), or photographs to document field work are all examples of images that will need to be processed. Another category of photographs will be taken to aid in documenting the location of HOBO data loggers deployed in-cave. This will entail a photograph of the data logger in situ, as well as photographs of landmarks within caves.

Field paper

In addition to the field data sheets found within SOP 3, other paper field data sheets include Cave Stream Reach Maps and Cave Field Information Sheets. In addition to maintaining copies accessible for field crews, these items will also need occasional updating. Examples include adding new data logger locations on cave maps or updating the equipment list needed within a cave. These updated products will need to be properly versioned and made available to field crews.

GPS data

Coordinates for cave locations or access points (e.g., parking locations).

Data Quality

For protocols submitted in 2017, the NPS IMD Leadership Team has stipulated that a data quality standards document must be developed prior to final approval. The stated purpose of this document is to document the standards used by the network for activities related to the collection, processing, storage, analysis, and publication of monitoring data. This means data quality standards must be established and QA/QC procedures identified that will result in meeting or exceeding the established quality standards.

The IMD has defined quality assurance and quality control (QA/QC) as (DeVivo 2016):

- **Quality Assurance (QA).** The set of activities designed to ensure that data, products, or services meet defined standards of quality with a stated level of confidence. Quality assurance focuses on ensuring that data are collected to and managed to the highest practicable quality, at or above stated data quality standards.
- **Quality Control (QC).** Operational techniques and activities that are used to ensure that data or products meet designated requirements for quality. Quality control procedures focus on testing data for quality and can include both an evaluation of whether data quality meet data quality objectives and/or procedures to correct data.

Quality assurance and quality control procedures are required parts of all protocols, and SOPs must provide the details about how the QA procedures are to be carried out (DeVivo 2016). Data that do not meet quality standards must be annotated or flagged in the system of record (i.e., database). Annotations or flags must also be used to indicate where data are in the certification process (i.e., data processing level). Per IMD guidance (DeVivo 2016) the certification levels available to this protocol in the absence of a full quality assurance plan (QAP) include:

- **Raw Data.** Data in their state of original collection, either in the form of paper or electronic field forms, electronic sensor files, or digital media (photo, video, or audio). These data generally have been collected following some QA procedures, but have not undergone any QC procedures.
- **Provisional Data.** Provisional data are those data that have undergone incomplete QA/QC procedures (as defined in the QAP) and are not suitable for general use. Provisional data may be used in some cases, however, depending on the amount of QA/QC completed, and the intended use.
- **Accepted Data.** Data that have undergone all QA and QC procedures as defined in a published protocol and associated SOPs. Used in cases where a QAP has not been developed and approved.

The goal is to articulate quality assurance and quality control procedures within this SOP and other CAB protocol documents that when followed will result in “Accepted Data” and achieve the stated data quality standards established in the standards document. It should be noted that QA/QC is an iterative process designed to not only identify and correct deficiencies but also to improve procedures to reduce the likelihood of repeated errors. Additions and clarifications to procedures are anticipated as workflows are tested and quality standards are refined.

This protocol incorporates a number of QA activities to ensure data are collected and managed to meet specified requirements of quality. These activities include:

- Proper training of field crews (SOP 3 *Training Personnel—Version 1.2*, CUPN Water Quality Monitoring Protocol [Meiman 2018], SOP 1 *Training Field Personnel—Version 1.0*)
- Clearly articulated field methods including calibration of equipment (SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*, CUPN Water Quality Monitoring Protocol SOP 4 *Field Measures—Version 1.2* [Meiman 2018])
- Use of customized field forms that are reviewed for completeness and accuracy prior to concluding the field sampling session (SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics*, CUPN Water Quality Monitoring Protocol SOP 3 *Collection of Field Data—Version 1.2* [Meiman 2018])
- Use of expected species lists, field guides, and other identification aids (SOP 3 *Field Methods: Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics—Version 1.0*)

- Establishing a chain of custody and repository for all data products (SOP 4 *Post-Field Sampling—Version 1.0*)

Prior to commencement of database use, the individual tasked with data entry (e.g., field crew leader, crew member, or data intern) will have reviewed this SOP and received hands-on training from the project leader or data manager in the use of the database(s) and demonstrate a level of competency and understanding of their assigned tasks. Ideally, the user will also have experience collecting the data to be entered. Data entry will be completed as soon as practical after field data collection.

Data entry forms in the CUPN Cave Aquatic Biota database will be patterned as much as possible to mimic the paper field forms. All the database applications utilized for this protocol contain, or in the case of the CAB database will contain, quality components such as pick lists and validation rules to further limit the possibility of incorrect or incomplete data entries.

Accurate data entry is an integral step in ensuring the overall success of any long-term monitoring effort. Data recorded on field data forms will have been written legibly. However, when in doubt as to what is written on the form due to poor hand writing, **do not guess**. Data forms are initialed and reviewed in the field. Check with the individual who initialed the forms to determine what is printed on the form. If confusion remains, flag the record in the database and make a note for the project leader to review during later data processing steps. After each field data form is entered, the person entering the data will print their initials and date in the reserved space on the field data forms (Appendix D in SOP 3).

Database entries will be reviewed against the original data forms to ensure data have been copied from the paper field data sheets accurately (i.e., verification). In addition to manual checks, basic queries summarizing species counts, date ranges, and maximum and minimum values will be utilized to aid the data entry technician in verifying data are accurate and complete. Graphical outputs are also available in both AQUARIUS and NPSTORET to aid in identifying potential outliers in the data.

At present a 100% verification check is planned for water quality and CAB detection data. If a pattern of data entry errors is found, steps will be reviewed by the data manager and project leader to correct deficiencies and avoid a repeat of future errors. Once all routines are complete the user will flag records indicating verification is complete. Once verification is complete, field data forms will be returned to the location designated by the project leader. The project leader will also be informed that this step has been completed.

After data verification is finished, the data will be reviewed by the project leader, who will again check the data for completeness, as well as logical inconsistencies. The primary questions throughout the validation process should be, “Do these data make sense and were established quality standards achieved?” While range limits can and will be established in the database applications to assist in answering these questions, additional checks are needed. For water quality, a duplicate (i.e., QA/QC) sample will be collected for each water quality sampling event and resultant values compared between the two samples. Generally the standards are to achieve a relative percent difference (RPD)

of no more than 10% between a sample and its duplicate. For readings taken with field sensors, QA/QC sample results must overlap the resolution of the stabilization criteria of the primary sample. Specific standards for each parameter and additional information on collection can be found in the CUPN Water Quality Monitoring Protocol (SOP 4 *Field Measures—Version 1.2*; Meiman 2018). Additional tools for data validation are available in NPStoret (CUPN Water Quality Monitoring Protocol SOP 6 *Data Management—Version 1.2*; Meiman 2018).

For CAB detection data and habitat data, the project leader will be looking for values outside the norm and whether there is agreement between sampling efforts. Checks will include looking for species out of range. For example, a cave shrimp detection may have been recorded on a datasheet from a perched cave stream and entered “correctly” in the database. However, the value is very likely in error because cave shrimp are not found above base level streams (Pearson and Jones 1998). To aid in this comparison reach-specific species lists could easily be added to the database. Species lists will also be examined as a function of site and observer, both within and between sampling events to determine if misidentification, particularly for easily confused species, is a problem. Differences in habitat variables such as pool measurements and habitat type will also be explored.

During the entry, verification, and validation phases, the project leader is responsible for the integrity and security of the data. The project leader must ensure consistency between field data forms and the database by noting how and why any changes were made to the data on the original field forms.

Changes made to the field forms should not be made via erasure, but rather through marginal notes (preferred) or attached explanations.

Once data processing steps are complete and observations are flagged to indicate this—including flags and annotations of observations that do not meet quality standards—the project leader will submit original field forms to the curatorial specialist for archival. The electronic version of the data will be turned over to the data manager for archiving and storage. This electronic version of the data is used for all subsequent data activities including analyses, reporting and dissemination.

Database Administration

Data Updates

Data sets are rarely static. They often change through additions, corrections, and improvements made following the archival of a data set. There are three main caveats to this process:

- Only make changes that improve or update the data while maintaining data integrity.
- Be prepared to recover from mistakes made during editing.
- Document changes.

Any editing of archived data is accomplished jointly by the project leader and data manager. Every change must be documented and accompanied by an explanation that includes pre- and post-edit data descriptions.

Naming Convention and Version Control

Version control allows project staff to manage changes made to data sets or documents, and provides an auditable trail of individual versions. To safeguard against loss or damage to data, changes or edits should not begin without having in place the ability to back up and start over. Version control is saving a copy of a file before beginning to work on it, and using an established naming convention to do so.

This project will utilize a front-end/back-end Microsoft Access application to manage core data. The front-end will contain the forms, queries, and reports for interacting with the data which are stored in the back-end application or file.

The recommended naming convention for both files is to begin with the network acronym, followed by an underscore (“_”) followed by the protocol name (i.e., CUPN_CaveAquatic). For the front-end application (i.e., the user interface) this is followed by another underscore and the version number. The back-end file (i.e., the one containing data) should include the date of the latest changes made to the data using a YYYYMMDD format. Thus the recommended naming convention for this protocol would look like the following:

CUPN_CaveAquatic_v1.30

CUPN_CaveAquatic_20170715

Based on the names of these files the user can discern that these are indeed the Cumberland Piedmont Network’s Cave Aquatic Biota protocol applications. The front-end is version 1.30 and changes were last made to the backend on July 15, 2017.

Any data editing or entry session should begin by making a backup of the current back-end data file. This is a simple task that is accomplished by doing a copy and paste routine and adding “_backup” to the end of the file name. For example, entering data to the above file on July 16, 2017 the following data files should be in the working data folder:

CUPN_CaveAquatic_20170716

CUPN_CaveAquatic_20170715_backup

It is also good practice to maintain an edit log throughout the data processing for a sampling season, to keep track of what changes were made at each step of the way (i.e., backup file). This makes it easier to know which backup file to select, if for some reason you have to “start over” at some point. For suggestions of what information should go into this edit log consult with the data manager.

Data entry or editing should **never** be done in a file labeled backup (or in a file with “Archive” in its name). File naming and proper version control practices are extremely important to the long-term integrity of the data. It can also save both the project leader and the data manager a significant amount of time and frustration. If any questions arise regarding this process ask the data manager.

It is also recommended that reports and presentations be appended with date at the end of the file name. This will show which file is the last report or the most recent presentation given.

Data Backups

Tape backups of all project data stored on CUPN's server are conducted regularly. A full backup is conducted at the end of each work week (i.e., every Friday night), and incremental backups each night, Saturday through Thursday. With this approach, data are backed up every night and the tape is changed on Friday, giving a full week's backup on each tape. Utilizing 12 tapes provides 12 weeks' worth of daily backups before tapes are recycled (i.e., put back in the rotation). In addition to these short-term backups, a historical backup is made and retained by the data manager on an annual basis. All tape backups with the exception of the historical backup are conducted by Mammoth Cave National Park (MACA) information technology (IT) staff.

Directory Structure

The various databases, images, reports, and GIS coverages used and generated by the monitoring program create a large number of files and folders to manage. Poor file organization leads to confusion and potential data corruption. Figure SOP5-5 depicts the file organization structure for a typical monitoring project.

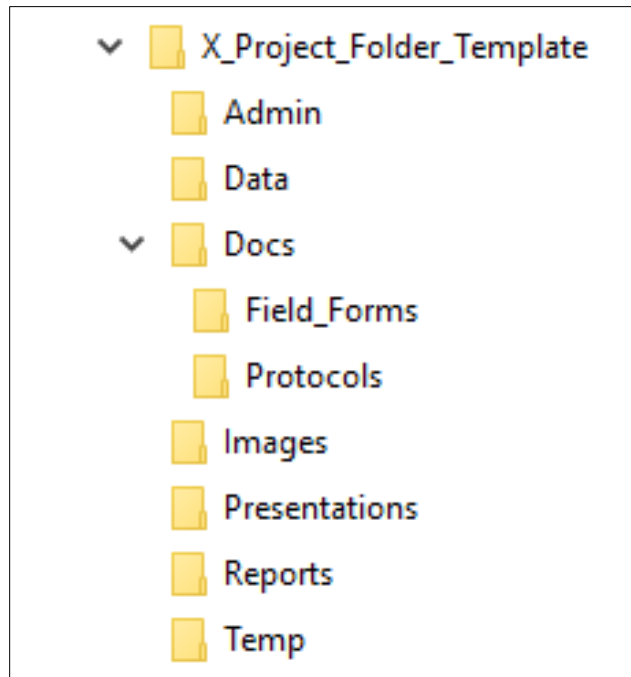


Figure SOP5-5. Project folder structure for the Cumberland Piedmont Network.

It is recommended that folder naming conventions adhere to the following:

- No spaces or special characters in the folder name.
- Use the underbar (“_”) character to separate words in folder names.
- Try to limit folder names to 20 characters or fewer.
- Dates should be formatted as YYYYMMDD (this leads to better sorting than other date naming conventions).

The Cumberland Piedmont Network has established this template directory structure for each vital sign monitoring project to serve as a basic starting point and provide consistency across projects. A similar directory structure contains archived files (CUPN_Archive\). Both the working and archive directories are accessible via the MACA Local Area Network (LAN), with access permissions maintained by the data manager and MACA IT staff.

Without due diligence, file folders will become bloated with draft documents and multiple working copies of the same files. An annual review of archive and temporary files and backups will be done

by the project leader and data manager. A good time for this review to take place is at the end of each season once annual reporting requirements have been completed and products disseminated.

Metadata

Metadata has been defined as structured information about the content, quality, and condition of data. It can provide information about the “core data” such as when and where they were collected, or provide end users some level of confidence in the quality of the data, if for example they were collected via documented field methodologies. All CUPN projects are required to conform to Federal Geographic Data Committee (FGDC) guidelines. NPS has its own guidance within the confines of FGDC guidelines. The goal of metadata is to make data available and inform users of its usability. With this in mind, CUPN staff will make every reasonable effort to fully document and make data available.

Protected Data

The National Park Service does not define “sensitive” data per se, but does distinguish “protected” (from the Freedom of Information Act) data. Generally, data can be protected when releasing the information will “result in harm, theft, or destruction of the resource” (Draft RM 66B, 2010). Such information must not be shared outside NPS, unless a signed confidentiality agreement is in place.

This project will involve the occasional collection and management of cave entrance locations and observations of the Kentucky cave shrimp (*Palaemonias ganteri*), a federally endangered species (16 U.S.C. 1533 et seq.). Per Executive Order and/or resource confidentiality laws these data could potentially be classified as protected. Final determination of what data are considered protected is made by the park, never by NPS IMD staff. While the location information for some of the cave entrances to be sampled is already in the public realm, the Cumberland Piedmont Network will consider all locational data associated with this protocol as protected. This includes GPS coordinates and written descriptions that might facilitate access to and subsequent harm, theft or destruction of resources.

Prior to posting a data set in a publicly-accessible location, the data manager will delete locational information of cave entrances and replace with park centroids. Reports and other publicly disseminated releases will also be reviewed to avoid inadvertent releases. CUPN staff will continue to work with park resource contacts to ensure protected data are identified, labeled, and protected from inadvertent release.

Data Delivery

Inventory and Monitoring Division data should generally be published to the Data Store (<https://irma.nps.gov/DataStore/>; last accessed August 2018) within one year of data collection (National Park Service 2016). A data set reference will be created and linked to a master project reference. References for reports, resource briefs and other products produced from this project will

also be created and linked to the master project reference. Instructions on reference creation and upload of holdings can be found on the Data Store website under the help section.

Natural History Collections

Natural history collections consist of specimens taken from the living and non-living components of the natural world. Often these items are collected as part of a scientific research project in order to: (1) serve as vouchers documenting research; (2) document an area's natural features; (3) provide a better understanding of a natural process; or (4) document changes in the environment over time.

The NPS maintains natural history collections primarily to voucher, or document, the presence of plants, animals, fossils, rocks, minerals, and other resources in a park at a particular place and time. Researchers and resource managers use this information for science and resource management decisions. Parks use research results and specimens in exhibits and as the basis for education and interpretive programs. Natural history collections and their associated records, which are managed as archival collections, document the park's natural environment, geological history, current conditions and changes over time.

Field data, objects, specimens and features obtained for preservation during inventory, monitoring, research and study projects, together with associated records and reports, will be managed over the long term within the museum collection and catalogued into the Interior Collection Management System (ICMS).

The Interior Collection Management System is a collection management system for museum collections throughout the U.S. Department of the Interior. It is used to catalog objects, specimens, and archival materials. This is the NPS museum standard and will be followed according to guidelines established by that program. Specimens, digital photographs, and archival material will be handled according to procedures established in the NPS museum handbook (Polk 2011). Final storage location will be in designated repositories decided by the collection park using loan agreements where needed.

Archives

Administrative histories, collections documentation, exhibits, publications, interpretation, cultural and natural resource management studies, and scholarly research are all dependent upon having well organized and accessible archives and manuscript collections. Without these documents, park interpretation, administrative histories, structural and vegetative preservation, scientific research, and resource management would be greatly diminished, and staff, visitors and scholars could only speculate upon the history of the parks as well as their management.

The preservation of archival and manuscript collections is dependent upon providing appropriate resources, including trained staff, a proper environment, and appropriate storage equipment and supplies. Once a document is damaged, it can never be fully repaired, even with the most costly restorative procedures. Under appropriate conditions, most agents of deterioration that affect collections can be greatly reduced. The design and long-term maintenance of archival conditions is,

therefore, a crucial part of a park's collections management program. Further, it is essential to develop basic procedures on access, usage, duplication, and conservation, as well as to complete the archival processing work of arrangement and description.

Any material (preferably originals, but it can be copies) associated with the project (photos, maps, field notes, and recordings) will be archived and entered into ICMS. After these materials are archived and entered into ICMS they will be returned to the park or a repository requested by the park along with their corresponding ICMS records. All final electronic data sets and material associated with this protocol will be archived and curated according to NPS policy and procedures.

Curatorial Procedures

The project leader is responsible for turning over any and all material related to the project to the CUPN curatorial specialist. A list of steps in the CUPN curatorial process is provided in Figure SOP5-6. Projects have two main components: Archives and Specimens.

CUPN Curatorial Procedures

- Step 1** Project Leader turns over all project materials and specimens to the CUPN curatorial specialist.
- Step 2a** All archival materials are transferred to archival housing by the CUPN curatorial specialist or a designated student intern.
- Step 2b** Specimens are counted by the CUPN curatorial specialist.
- Step 3** The park curator is contacted by the CUPN curatorial specialist to request an accession number and catalog number(s) for that project.
- Step 4** For specimens only: the CUPN curatorial specialist either curates specimens or arranges for professional curation.
- Step 5** The CUPN curatorial specialist enters all information connected with each specimen and/or archival material(s) into ICMS.
- Step 6** For specimens only: NPS labels are printed and placed with specimen(s).
- Step 7** If applicable, photos are taken of specimen(s) and attached to respective ICMS record.
- Step 8a** If park is final repository, archives and/or specimens will be sent to park with associated ICMS records and DI-105 (receipt for property form) by CUPN curatorial specialist.
- Step 8b** If park is unable to store items, the park will choose a repository that abides by NPS standards. The CUPN curatorial specialist will send ICMS records to the park and then create a loan agreement between park and final repository. CUPN will deliver final archives and/or specimen(s) to final repository.

Figure SOP5-6. Stepwise procedures for handling specimens, digital photographs, and archival materials associated with Network vital signs monitoring activities.

Archives

Every project has materials to be archived. Once the project leader has turned over the materials related to the project, not including actual specimens, they are placed in archival housing (including stable acid-free papers, folders, and boxes) for conservation by the CUPN curatorial specialist or an appointed student intern. The CUPN curatorial specialist will then notify the park curator to request one accession number and catalog number(s) for that project. The information is entered into ICMS by the CUPN curatorial specialist. If the park will be the final repository, the archives are sent to the park with the associated ICMS records and a DI-105 (receipt for property form). If the park is unable to store the archives, the park will choose a repository that abides by NPS standards. The CUPN

curatorial specialist is responsible for creating a loan agreement between the park and the repository, as well as the final delivery of the archives.

Specimens

Although there are no plans to collect specimens as part of this project there may be occasion for collection to occur (e.g., fresh dead specimen). If there are specimens, they will need to be curated (prepared for long-term storage). For inventory projects, the amount of specimens will be tallied to obtain the same number of catalog numbers and one accession number from the park. For ongoing monitoring projects, the new specimens are tallied every time they are turned in to the project leader to receive the same number of catalog numbers, keeping the same accession number for the duration of the project. The CUPN curatorial specialist is responsible for either curating the specimens or finding a professional to do so. Once the specimens have been curated, all information connected with each specimen is entered into ICMS by the curatorial specialist. A standard NPS label is created from this information through ICMS and attached to the specimen. If applicable, photos are taken of the labeled specimen and digitally attached to the respective ICMS record. If the park is the final repository, the specimens are sent to the park with the associated ICMS records and a DI-105 (receipt for property form). If the park is unable to store the specimens, the park will choose a repository that abides by NPS standards. The CUPN curatorial specialist is responsible for creating a loan agreement between the park and the repository as well as the final delivery of the specimens.

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Standard Operating Procedure 6

Data Analysis and Reporting Version 1.0

This standard operating procedure describes the process for conducting analyses of cave aquatic biota (CAB) monitoring data and covariate data collected at selected cave stream reaches in the Cumberland Piedmont Network (CUPN) and producing reports based on these analyses. The tasks described in this SOP cover the period of time from when all digital data have been uploaded to the designated folders on the network’s server; all manual data from detection/nondetection data sheets have been entered into the database; and all data quality processing steps, as detailed in *Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Data Quality Standards* (Helf et al. 2018a) have been completed. The steps for data handling and processing prior to data analysis are detailed in SOP 4 *Post-field Sampling—Version 1.0* and SOP 5 *Data Management—Version 1.0*.

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Contents

	Page
Overview.....	SOP 6-3
Routine Data Summaries and Analysis	SOP 6-3
Hierarchical Models for Cave Aquatic Biota Data Analysis.....	SOP 6-4
N-mixture Model Framework.....	SOP 6-5
Occupancy Model Framework	SOP 6-6
Analyses of Estimates for Influence of Covariates	SOP 6-7
Detection Parameters.....	SOP 6-8
Abundance and Occupancy Parameters	SOP 6-8
Downloading and Processing Data Files for Analysis	SOP 6-9
Downloading and Processing Surface Meteorological Data Files	SOP 6-11
Data Analysis	SOP 6-11
Formatting Data For Analysis Using ‘unmarked’	SOP 6-11
General Step By Step Analysis Instructions.....	SOP 6-12
Single-sampling Season Estimates of Abundance and Occupancy for Selected Species.....	SOP 6-16
Multi-sampling Season Estimates of Abundance and Occupancy for Selected Species.....	SOP 6-17
Analyses of Estimates for Comparison and Trends.....	SOP 6-17
Uncertainty of Estimates and Detectable Levels of Change	SOP 6-17
Planned Reporting Schedule and Format	SOP 6-19
Literature Cited	SOP 6-25

Overview

This SOP describes the process for conducting analyses of cave aquatic biota (CAB) monitoring data and covariate data collected at selected cave stream reaches in the Cumberland Piedmont Network (CUPN). The tasks described in this SOP cover the period of time from when all digital data have been uploaded to the designated folders on the network's server; all manual data from detection/nondetection data sheets have been entered into the database; and all data quality processing steps, as detailed in *Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Data Quality Standards* (Helf et al. 2018a) have been completed. The steps for data handling and processing prior to data analysis are detailed in SOP 4 and SOP 5.

This SOP also provides information on the reports that will be produced from implementing the CAB monitoring protocol in Cumberland Gap National Historical Park (CUGA), Mammoth Cave National Park (MACA), and Russell Cave National Monument (RUCA). Reports generated by this monitoring project will consist of three major types: (1) Trip Reports, (2) Annual Summary Reports, and (3) Trend Analysis Reports. Trip Reports will be written to briefly summarize sampling trips for park staff. The Annual Summary Reports and Trend Analysis Reports will be generated to provide park management and other interested parties technical and interpretive information about the status and trends being detected in cave aquatic biota and hypothesized relationships with selected covariates. The schedule and format for each report type, as well as reporting responsibilities, is discussed later in this SOP.

Routine Data Summaries and Analysis

The data collected under this protocol's sampling design support the estimation of abundance, occupancy, and detectability of cave aquatic biota in selected cave stream reaches. The sample frame is a number of selected cave stream reaches subdivided into a number of permanent 40-meter (131.2-foot [ft]) transects with an unstructured, equal-probability, spatially balanced approach using a Generalized Random Tessellation Stratified sampling (GRTS) draw. Cave aquatic biota sampling events are conducted at cave stream reaches during the driest part of the year, to reduce within sampling season variation in CAB detectability, during which only cave stream reaches in that year's panel are surveyed on two dates separated by a minimum of two days. During a CAB sampling event, two independent teams, consisting of an observer and a recorder, survey all transects in a cave stream reach and collect data on selected water quality and quantity parameters, CAB detection and nondetection, and habitat characteristics. Abundance and occupancy estimates are based on counts of CAB found only within each 40-meter (131.2-ft) transect.

Data from the CUPN Cave Aquatic Biota Monitoring Project will result in the following products:

- Annual status summary of CAB monitoring data
 - Size range of species detected (e.g., Figure SOP6-1),
 - Species richness and lists,
 - Abundance and occupancy estimates (when possible) (e.g., Table SOP6-4),

- Habitat use and summarized habitat characteristics, and
- Water quality/quantity summary statistics (e.g., Table SOP 6-5),
- Trend analysis of abundance (common species) and occupancy (uncommon species) over time, produced at least 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 (i.e., abundance and occupancy).

A list of the sampling methods and associated primary measures to be collected, derived, and used during data analysis is provided in Table SOP6-1.

Hierarchical Models for Cave Aquatic Biota Data Analysis

All monitoring objectives (below), and subsequent analyses of the supporting data, address the primary goal of this protocol: to identify status and trends in, and test hypothesized explanatory variables relevant to, CAB abundance and occupancy over time and space within monitored cave stream reaches for resource managers at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM. The protocol was designed to address the following monitoring objectives:

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

Monitoring Objective 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.

Monitoring Objective 3: Sample selected cave stream habitat characteristics and water-quality and water-quantity parameters that we hypothesize are relevant to understanding cave aquatic biota abundance, occupancy, or detectability within monitored cave stream reaches in three parks in the Cumberland Piedmont Network.

Table SOP6-1. Major sampling methods, and primary measures and variables that will be collected or calculated and used for analysis during implementation of the protocol for monitoring cave aquatic biota.

Sampling Category	Sampling Method	Measures	Variables
CAB Sampling (see SOP 3)	Direct Visual Observation (CAB Detection/Nondetection)	Total # CAB counted per taxon per transect	abundance and occupancy of cave stream reaches estimated length CAB detected (mm) CAB size category (small, med., and large)
	Direct Visual Observation & Assessment (Habitat Characteristics)	Habitat type and measurements Substrate type	pool, riffle, glide max pool length, mean transect/pool width mean transect/pool depth percent makeup of substrate categories
Cave Stream Water Quality/Quantity Sampling (see Curdts 2017, Meiman 2018)	Opportunistic Grab Sampling (handheld sensors)	Temp (°C) Specific Conductance (SC) pH Dissolved Oxygen (DO) Turbidity (samples analyzed in lab) Nutrients (samples analyzed in lab)	water temp (°C) SC pH DO mean, max/min Turbidity levels of NO ₃ and <i>E. coli</i>
	Continuous Sampling (HOBO data loggers)	Water level Temp (°C)	mean, max/min water level prior week mean, max/min water temp (°C) prior week

Monitoring Objectives 1 and 2 will be addressed with hierarchical models (HM), specifically N-mixture and occupancy models, respectively. Hierarchical models are a flexible framework in which a complex system may be modeled through the use of multiple submodels. This framework is particularly suited to inference about abundance, occupancy, and species richness 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 nature of hierarchical models will be useful for the reliable estimation of occupancy and abundance of imperfectly detected cave aquatic biota. We will also use selected covariates, as stated in monitoring Objective 3, in an attempt to account for variation in abundance, occupancy, and detectability among cave stream 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 the probability of detecting individual organisms (p) and unobserved abundance (N) (Kéry and Royle 2015a):

1. State process: $N_i \sim \text{Poisson}(\lambda)$
2. 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 *N*-mixture framework is 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 (i.e., no local extinction or colonization), no false positives (i.e., double-counting or species misidentification), individuals are detected independent of one another (i.e., no schools or flocks), detection probability is homogenous for all individuals at cave stream reach i during sampling event j , and that 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 in *Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Protocol Narrative*; Helf et al. 2018b). 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).

1. State process: $z_i \sim \text{Bernoulli}(\psi)$
2. 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/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/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/nondetection state does not change within a sampling 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.

One of the limitations of hierarchical models is the assumption that the population being sampled is closed to births, deaths, emigration, and immigration during the survey period. Given the high mobility of some stygobionts or periodic flooding events this assumption is almost certainly violated if the interval between surveys is long. However, 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. In a 16-month study Hobbs III (1978) found *O. i. inermis* movement was largely restricted to a median area of approximately 21 m (68.8 ft) with some modest movement occurring both up- and downstream. Large movements tended to be downstream and were mostly correlated with flooding due to rainfall or melting snow and ice (Hobbs III 1978). A seven-month study of *T. rosae* movement by Means and Johnson (1995) showed significant differences in mean daily movement between the largest (8.1 meters per day [26.6 ft per day]) and smallest (0.8 meters per day [2.6 ft per day]) recaptured cavefish but that they tended to stay in the reach where they were first captured. While the capture of untagged adult cavefish during all 11 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) it is also consistent with a small percentage of the cavefish population going undetected during a given survey. Finally, some lack of closure simply changes the interpretation of λ to include all individuals associated with a cave stream reach during the survey period (Kéry and Royle 2015a). Regardless, we will conduct replicate surveys as close together as possible (i.e., normally within a week) to avoid violating the assumption of closure.

We will use hierarchical models to produce temporal estimates of change in species abundance and occupancy from our spatially and temporally replicated count data of specified cave stream reaches. We will use functions ‘pcount’ and ‘occu,’ in the R package (R Core Team 2017) ‘*unmarked*’ (Fiske et al. 2015) to test predictions regarding the effect of selected covariates on the abundance, occupancy, and detectability of cave aquatic biota among cave stream reaches (Table SOP6-2).

Analyses of Estimates for Influence of Covariates

Explanatory variables (covariates, predictors) may serve at least two purposes in the analyses. First, covariates may explain “nuisance variation” over time or space in detection probabilities, thereby increasing the precision of estimates of the parameters of primary interest (i.e., abundance and occupancy). Second, covariates may be of interest in their own right. The coupling of hydrogeology and biology (Bonacci et al. 2009) enables us to formulate *a priori* hypotheses regarding the factors that influence the distribution and abundance of cave aquatic biota among cave streams at Cumberland Gap NHP, Mammoth Cave NP, and Russell Cave NM (Table SOP6-2). For instance, water quality or other measurements may be predictive of local variation in cave stream reach abundance or occupancy, and thus useful for management. Potential covariates specific to cave stream reaches include habitat characteristics and, based on whether the watershed in which they are located is recharged by lands outside or inside park boundaries, water-quality parameters such as dissolved oxygen, nitrates, and *E. coli*. Possible covariates specific to sampling events include turbidity, water level, and Julian Date (Table SOP6-2). Covariates will be used together with the generalized linear models intrinsic to the ‘unmarked’ package to estimate their effects on parameters of interest. Akaike Information Criterion (AIC) will be used to rank candidate models and calculate Akaike weights (Burnham and Anderson 2002).

Detection Parameters

We selected water level, turbidity, specific conductance, observer, Julian date, and survey method as covariates that may affect CAB detectability (Table SOP6-2). We include water level because increased water levels may reduce the ability of observers to see through deeper water. Turbidity was also included as a covariate because it may reduce the ability of observers to detect cave aquatic biota; we predict an interaction effect between these two covariates. Decreased specific conductance is correlated with increased stage and flow rate in glides and riffles (Anthony et al. 2003; Raeisi et al. 2007), indicative of a flood pulse potentially hazardous to more delicate cave aquatic biota such as cave shrimp. Observer could be correlated with detectability due to variation in experience or visual acuity among field personnel. Julian date is used to explain variation not attributed to other parameters (e.g., seasonal variation). Given the difference between the sampling methods used to survey wadeable versus nonwadeable cave stream reaches (i.e., walking or wading double survey, walking or wading combined survey, and snorkeling) we will also include survey method as a covariate for detection probability.

Abundance and Occupancy Parameters

We selected water level, dissolved oxygen, habitat type, nitrates, and *E. coli* as covariates that may affect CAB abundance (Table SOP6-2). Reproduction in stygobionts can be highly seasonal and may occur during the flooding season when food availability and water level are high (Niemiller and Poulson 2010). Over time, increased water level may increase habitat availability when juvenile cave aquatic biota, having been recruited into the population and dispersed throughout the cave stream reach, are left behind in more pools during the dry season. Thus, increased water level is expected to decrease CAB abundance but increase their distribution particularly with respect to occupancy of pools. Watersheds recharged by lands outside park boundaries can contribute to the degradation of cave stream reaches through chemical and thermal pollution via backflooding, flow reversals, or

runoff (Trimboli et al. 2016; Meiman 2005). For example, following runoff-producing rainfall events, Mammoth Cave NP's cave streams and springs recharged by watersheds with heavy agricultural land use show a sudden rise in nutrients, bacteria, and sediment (Meiman 2005). Moderate levels of nitrate-nitrogen and fecal coliform bacteria 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). Thus, we predict increased CAB abundance and occupancy at moderate levels of pollution over those at low levels. High levels of nutrients and bacteria in cave streams or river reaches, however, are suspected of causing local extinctions in stygobiotic amphipods, due to reduced dissolved oxygen as a result of biological oxygen demand, in Kentucky (Lewis 1995), Arkansas (Graening and Brown 2003), and Illinois (Taylor et al. 2003; Panno et al. 2006). Thus, we predict decreased CAB abundance and occupancy at low levels of dissolved oxygen. Temperature and pH, though potentially relevant to CAB biology, are not expected to affect their detectability, abundance, or occupancy because of their similarity among watersheds and cave stream reaches.

Downloading and Processing Data Files for Analysis

Data collected in the field (see SOP 3—*Collection of Abundance, Detection/Nondetection Data, and Habitat Characteristics*) are stored in three separate databases, two customized Microsoft Access applications and one commercial application (see SOP 5) data handling functions (Table SOP6-3). These formats will be imported into or extracted with statistical analysis and graphing software (e.g., SigmaPlot, 'unmarked' R package). Correlative surface meteorological data will be gathered from web-based sources (see SOP 4).

Table SOP6-2. Example of parameters included in the abundance and occupancy models for *Orconectes pellucidus*, their predicted effect on detectability, abundance, and occupancy, and the models in which the parameters will be included. An “x” between the two covariates indicates a hypothesized interaction between parameters whereas a “+” indicates an additive effect. WL=water level, SC=specific conductivity, JD=julian date, TB=turbidity, DO=dissolved oxygen, HT=habitat type, NO3=nitrate, EC=*Escherichia coli*. Predicted relationships are based on the literature and our own hypotheses. NA=no hypothesized relationship.

Parameter	D: Effect on detection A: Effect on abundance O: Effect on occupancy	Models for detection	Models for abundance	Models for occupancy
Water level (WL)	D: Decreased detection with increased WL A: Decreased abundance with increased WL O: Increased occupancy with increased WL	WL WL x TB WL + HT	WL WL + HT	WL WL + HT
Specific conductance (SC)	D: Decreased detection with increased SC A: NA O: NA	SC SC x HT	NA	NA
Julian date (JD)	D: Homogeneity between sampling events A: NA O: NA	JD	NA	NA
Turbidity (TB)	D: Decreased detection with increased TB A: NA O: NA	TB WL x TB TB x HT	NA	NA
Dissolved Oxygen (DO)	D: NA A: Decreased abundance with decreased DO O: Decreased occupancy with decreased DO	NA	DO DO + HT DO x HT DO + NO ₃ DO + EC DO + HT + EC DO + NO ₃ + EC DO + NO ₃ + EC + HT	DO DO + HT DO x HT DO + NO ₃ DO + EC DO + HT + EC DO + NO ₃ + EC DO + NO ₃ + EC + HT

Parameter	D: Effect on detection A: Effect on abundance O: Effect on occupancy	Models for detection	Models for abundance	Models for occupancy
Habitat type (HT)	D: Decreased detection in Riffles A: Decreased abundance in Riffles O: Decreased occupancy in Riffles	HT WL + HT SC + HT TB x HT	HT WL + HT DO + HT DO x HT DO + HT + EC	HT WL + HT DO + HT DO x HT DO + HT + EC
Nitrate (NO ₃)	D: NA A: Increased abundance with increased NO ₃ O: Increased occupancy with increased NO ₃	NA	NO ₃ NO ₃ + EC DO + NO ₃ DO + NO ₃ + EC	NO ₃ NO ₃ + EC DO + NO ₃ DO + NO ₃ + EC
<i>E. coli</i> (EC)	D: NA A: Increased abundance with increased EC O: Increased occupancy with increased EC	NA	EC NO ₃ + EC DO + EC DO + HT + EC DO + NO ₃ + EC	EC NO ₃ + EC DO + EC DO + HT + EC DO + NO ₃ + EC

Downloading and Processing Surface Meteorological Data Files

Previously downloaded correlative data on precipitation can be accessed at the appropriate park subfolder here:

Z:\Vital_Signs\Vital_Signs_Prj\CaveAquatic_Biota\Data\Working_Data\Surface_Met_Data\Park Code\

The data file is named “SCANXXXX_<date>.csv”. “SCANXXXX” is the unique site number assigned to Mammoth Cave NP’s SCAN station location, and “date” is the date of the specific sampling event on which the cave stream reach was sampled as recorded on the field data sheet. The date format used is YYYYMMDD. Example: “SCAN2079_20150227.csv.”

Data Analysis

Formatting Data For Analysis Using ‘unmarked’

The essential data structure is a matrix with row dimension equal to the number of transects and column dimension equal to the number of independent sampling events (Table SOP6-3). Entries indicate a single observer’s detection (1) or non-detection (0) of the target species for each sampling event. Dataframes for N-mixture models, for CAB whose abundance can be reliably estimated, is very similar to occupancy models except that observers record counts. Data structure for CAB

covariates is the same as that for CAB abundance. Analyses to estimate abundance and occupancy in unmarked, aside from their differences in modeling distributions, follow a similar, general three-step process (R commands in ‘unmarked’ are given in `Courier New` font):

General Step-By-Step Analysis Instructions

1. Import data and set up for analysis
 - a. Load `unmarked` library
 - b. Check data structure
 - i. `str(data)` displays compact list of contents
 - ii. Plot data
 - iii. Clean up data as necessary

Table SOP 6-3. Mockup of proper format for a CAB occupancy dataframe for a single CAB species from one sampling season at one cave stream reach. Survey data are CAB detections (1) and nondetections (0) by two independent observers along with habitat classifications (i.e., 1=Riffle, 2=Glide, 3=Pool) and substrate composition. Abundance dataframes have a similar appearance except the detection/nondetection data are replaced with count data.

Transect	Survey 1	Survey 2	Survey 3	Survey 4	Habitat	Fines	Granules	Pebbles	Cobble	Boulders
1	1	1	1	1	1	0.0	0.2	0.8	0.0	0.0
2	0	0	0	0	1	0.0	0.4	0.6	0.0	0.0
3	1	1	0	0	1	0.0	0.5	0.5	0.0	0.0
4	0	0	0	0	1	0.0	0.6	0.4	0.0	0.0
5	1	0	1	0	1	0.0	0.6	0.4	0.0	0.0
6	0	0	0	0	3	0.0	0.8	0.2	0.0	0.0
7	1	1	1	1	3	0.7	0.3	0.0	0.0	0.0
8	0	0	0	0	3	0.8	0.2	0.0	0.0	0.0
9	0	0	0	0	2	1.0	0.0	0.0	0.0	0.0
10	0	0	0	0	2	0.9	0.1	0.0	0.0	0.0
11	1	0	1	0	2	0.9	0.1	0.0	0.0	0.0
12	1	1	0	0	2	0.7	0.2	0.0	0.0	0.0
13	0	0	0	0	2	0.5	0.5	0.0	0.0	0.0
14	1	1	0	0	1	0.0	0.4	0.6	0.0	0.0

- c. Format data for unmarked dataframe
 - i. Strip out detection/nondetection data or count data (y)
 - ii. Strip out covariates
 - 1. siteCovs
 - 2. obsCovs
 - iii. Standardize covariates
 - 1. Compute means and standard deviations of covariates
 - 2. Original covariate value – mean/standard deviation
- d. Create the `unmarkedFrame(Occu/PCount)` object (umf)
 - i. Provide count data (y)
 - 1. `umf <- unmarkedFramePCount (y = C,`
 - 2. Where C is an integer matrix with:
 - a. M rows (= number of transects)
 - b. J columns (= number of surveys)
 - ii. Provide site covariates (siteCovs)
 - 1. `umf <- unmarkedFramePCount (y = C,`
`siteCovs = data.frame (e.g., DO),`
 - 2. Where siteCovs is a dataframe with:
 - a. M rows (= number of transects)
 - b. K columns (= number of covariates)
 - iii. Provide observation covariates (obsCovs)
 - 1. `umf <- unmarkedFramePCount (y = C,`
`siteCovs = data.frame (e.g., DO),`
`obsCovs = list (e.g., Date))`
 - 2. Where obsCovs is a named list of dataframes with
 - a. M rows (= number of transects)

- b. J columns (= number of surveys)
 - e. Summarize data and examine
 - i. `summary(umf)`
 - ii. Command provides summary information on imported data:
 - 1. Total #sites
 - 2. Observations at site:
 - a. Max. #
 - b. Mean #
 - c. Sites with at least one detection
 - d. Tabulates # of detections/nondetections
 - 3. Summary of all Site- and Observation-level covariates
 - a. Minimum, Median, Mean, Maximum
 - b. First and third quartile
2. Fit some models to estimate detectability and abundance or occupancy
 - a. Start with the global model (including all covariates and interactions) for detectability and abundance or occupancy.
 - i. Fit the different models for detectability, using the global model for abundance or occupancy. Select one or a few detectability models based on:
 - 1. p-values
 - 2. AIC scores
 - ii. Function `pcount` fits the *N*-mixture model
 - iii. Function `occu` fits the Occupancy model
 - iv. Proceed to abundance or occupancy model selection, this time using the most global detectability model that is within 5 AIC units of the best detectability model.
 - v. Select one or a few abundance or occupancy models within 5 AIC units of the best model.

- vi. Fit any additional models needed (e.g., the set of occupancy models with other selected detectability models).
- b. To assess models with multi-model inference
 - i. Put fitted models into “`fitList`”
 - ii. Rank models by AIC
 - iii. Best has the lowest AIC value
 - iv. AIC weight indicates % chance model is the best one
 - v. Null model should be near the bottom ranked
 - vi. Can use `predict()` function to produce predictions from best fitted models
- 3. Analyze the results with a parametric goodness-of-fit (GoF) test in `parboot` or package `AICmodavg`
 - a. Uses parameter estimates from fit models to simulate new data, fits model to simulated data, and computes fit statistics for each model fit.
 - i. Error sums-of-squares
 - ii. Standard Chi-square
 - iii. Freeman-Tukey statistic
 - b. Residual diagnostics with `residuals(fm)` and plot against predicted values or covariates.
 - i. Where does model fit?
 - ii. Where does model not fit?

Cave stream reaches in each park were not probabilistically sampled from the set of all cave stream reaches and so are not a representative sample from the set of all cave stream reaches in their respective watersheds or parks. Therefore, no quantitative inferences can be made to the set of all cave stream reaches. Hypothesis or significance tests comparing cave aquatic species or communities among cave stream reaches or among watersheds will be used judiciously. Tests of estimated abundance and occupancy between cave stream reaches in pristine versus compromised watersheds are limited to the particular monitored cave stream reaches or watersheds and not all pristine or compromised cave stream reaches in their respective parks. Rather, the logic is that if a pattern is observed in this set of monitored cave stream reaches it may be worth managing resources as if that pattern is pervasive across many or all cave stream reaches or watersheds.

Occupancy and abundance estimates will be summarized by species and (where applicable) sampling season and included in summary tables (e.g., Table SOP6-4). These tables will contain standard errors, as well as estimates of all possible relevant parameters (e.g., local extinction and colonization). Confidence intervals will be provided for all parameter estimates, as well as an indication of the method of computation (e.g., selection of top Akaike Information Criteria (AIC) model, model-averaged estimates).

Table SOP6-4. Population abundance (λ) and occupancy (ψ) estimates, and associated detection probabilities (p), for sampled area of a cave stream reach in Mammoth Cave National Park obtained during a pilot test in 2015. All parameters are reported with associated standard errors. Dashes denote models that did not converge. Note the low p and high ψ estimates for species COPE suggest this model is suspect and should be flagged. Models were fit using the R package ‘unmarked’.

Species	Abundance (<i>N</i> -mixture)				Occupancy			
	λ	se(λ)	p	se(p)	ψ	se(ψ)	p	se(p)
CAST	4.85	0.56	0.73	0.05	0.46	0.113	0.88	0.09
CATE	–	–	–	–	–	–	–	–
COPE	–	–	–	–	1.00	0.28	0.05	0.04
ORPE	18.99	3.67	0.39	0.09	0.90	0.07	0.97	0.03
STVI	0.98	0.49	0.41	0.20	0.63	0.29	0.40	0.20
TYSU	5.28	1.78	0.36	0.12	0.94	0.11	0.69	0.10

Single-sampling Season Estimates of Abundance and Occupancy for Selected Species

Where data are sufficient, our analyses will focus on obtaining estimates and confidence intervals of CAB abundance and occupancy within and among cave stream reaches. To evaluate our hypotheses regarding CAB abundance and occupancy of cave stream reaches, we will start with a global model to estimate detectability and abundance or occupancy. We will fit different models for detectability, using the global model for abundance or occupancy. We will select one or a few models based on their p -values and Akaike Information Criteria (AIC) scores. Next, we will test *a priori* models of abundance and occupancy incorporating the most global model for detectability within five AIC units of the best model.

We will include explanatory covariates to investigate the relationships between CAB abundance, occupancy and detectability, habitat characteristics, and water quality/quantity. Abiotic variables used as covariates (e.g., water temperature) will be tested for multicollinearity and those with r greater than 0.5 will be excluded from the same model (Graham 2003). The candidate models will be evaluated by Akaike Information Criteria, and AIC weights used to obtain model-averaged estimates of occupancy (Burnham and Anderson 2002; Kéry and Royle 2015b). Analysis will be conducted using the ‘occu’ function in the ‘unmarked’ package and will be focused on the estimation of

local occupancy, as well as total occupancy across cave stream reaches. Where feasible, analyses based on count models will be used to provide estimates of abundance corrected for imperfect detection (Royle 2004). The data structure for these models is similar to that for occupancy analysis, except that the entries for the data matrix will involve counts of all cave aquatic biota detected (Table SOP6-3). Analysis will be conducted using the ‘pcount’ function in the ‘unmarked’ package and will be focused on the estimation of local abundance, as well as total abundance across cave stream reaches (Table SOP6-4). We will assess the selected models’ goodness-of-fit with the parametric bootstrap approach which uses parameter estimates from fit models to simulate new data, fits model to simulated data, and computes fit statistics for each model fit (Kéry and Royle 2015a). When necessary, we will perform residual checks to investigate patterns where selected models show lack of fit and whether additional covariates should be added (Kéry and Royle 2015a). Finally, when possible we will use selected models to generate predictions regarding the effects of covariates on CAB abundance. When possible, single season estimates of occupancy and abundance will be included in Annual Summary Reports.

Multi-sampling Season Estimates of Abundance and Occupancy for Selected Species

The single-season analyses of occupancy described above assume that the occupancy state of sites remains fixed over the duration of the field season. By contrast, a longer-term (multi-season) analysis of site occupancy may reveal turnover in site occupancy, with some previously occupied cave stream reaches becoming unoccupied and vice-versa, and increases or decreases in abundance. In this protocol we will attempt to use multi-season analyses, which explicitly estimate abundance and occupancy at each season (year), along with parameters describing local extinction (ϵ) and colonization (γ). We will also obtain estimates and confidence intervals of the probability of initial site occupancy (ψ), local extinction ϵ and colonization γ for cave aquatic biota where data are sufficient. Candidate models will be constructed that assume homogeneous or time- and site-varying detectability, constancy or time variation in ϵ and γ , and explanatory covariates. Candidate models will be evaluated by AIC, and AIC weights used to obtain model-averaged estimates of ψ , ϵ , and γ . These analyses will be included in Trend Analysis Reports.

Analyses of Estimates for Comparison and Trends

Although occupancy and abundance can simply be estimated and reported statistically, comparisons between cave stream reaches, species groups, and trends over time are of interest. Comparisons of interest (e.g., cave stream reaches in “pristine” vs. contaminated watersheds) will be integrated into ‘unmarked’ abundance and occupancy models via group (factor) effects, with effects of factor levels estimated by means of generalized linear models. Dynamic models for trend will be constructed either via direct estimation using N -mixture or occupancy analysis, or via fitting of generalized linear models (e.g., binomial or Poisson regression models) to a time series of estimates from separate, single-season analyses.

Uncertainty of Estimates and Detectable Levels of Change

Estimates of status and temporal trends in abundance or occupancy have uncertainty about them, usually expressed as standard errors or confidence intervals. To be informative to resource management, the resulting estimates must have low enough uncertainty to help differentiate among

alternatives. For this protocol, the alternatives are more likely to be presence of species in the seven different cave stream reaches, differences in abundances either among species or among cave stream reaches (perhaps correlated to among-reach covariates), or trends over time.

The simulations testing the feasibility of N -mixture models (see Figures 6–8 in the protocol narrative) 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 in the CUPN’s cave aquatic biota monitoring protocol narrative 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 detection probability of 0.4 (Table SOP6-4) abundances less than one individual cave aquatic biota per 10 meters (32.8 ft) risk substantial overestimation of the abundances. Abundances above one per meter (3.2 ft), 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 (Niemiller et al. 2018) to increase detection probabilities used to estimate CAB abundance and occupancy.

The simulations for temporal trends in abundance (see CUPN’s cave aquatic biota monitoring protocol narrative, 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 total effort and 20-meter (65.5-ft) transects are equivalent to a design with 4,800 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 CAB with low abundances. For the slower decline of 5% per year (13-plus 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 m 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) indicate this design will have greater than 80% power to detect the 5% per year decline in proportion of sites occupied by large CAB before a 50% total decline (Conroy 2016, unpublished simulation data).

Further analyses showed that trend estimates were unbiased, but that normal-theory confidence intervals for those estimates included the true value well less than the nominal 90% or 95% of the time (see Figure 8 in *Monitoring Cave Aquatic Biota at Selected Parks in the Cumberland Piedmont Network: Protocol Narrative*; Helf et al. 2018b). 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 either profile confidence intervals or confidence intervals based on parametric bootstrapping will provide better coverage. However, the problematic normal theory confidence intervals indicate that 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.

The target power for monitoring objectives 1 and 2 is as follows:

- Detect a 50% decline (at 5% per year) 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).
- Detect a 50% decline (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).

Planned Reporting Schedule and Format

Efficient, timely reporting on monitoring results is the responsibility of the project leader and is critical in assisting park resource managers in management decisions. Brief Trip Reports will be written to summarize sampling trips for park staff and will include important observations such as signs of direct and indirect human disturbance in/at caves. Trip Reports will be completed within two weeks after each sampling trip, and will be sent directly to the park resources management primary contact. Annual Summary Reports, providing park superintendents and resource managers with a preliminary data summary of the current status and short-term shifts of any parameter(s) relevant to the resource (e.g., Tables SOP6-4 and 5 and Figure SOP6-1), will be produced separately for each park in January after the preceding year’s monitoring efforts and subsequent data summaries are completed.

The Trend Analysis Report will be generated at least every sixth year, beginning six years after the collection of data using methods established in this monitoring protocol. Six years will allow three full cycles of monitoring in both panels. The Trend Analysis Report will consist of updated analyses using all data in both graphic and tabular statistical test summary formats (e.g., a table similar to Table SOP6-4 and Figures SOP6-2 and SOP6-3), evaluations and predictions of relationships between key ecosystem drivers/attributes/stressors and key population measures, a Natural Resource Condition Summary Table (see section below, Table SOP6-7), and interpretive discussion. This comprehensive report will place the observed results in both a regional and historical context by relating them to 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 target audience will be park natural resource managers, CUPN staff, and other Inventory and Monitoring networks. Outside of the National Park Service, the target audience can include the state departments of natural resources, the U. S. Fish and Wildlife Service, as well as the broader scientific community.

Table SOP6-5. 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. These data will be included as part of the Annual Summary Report submitted to parks.

Park	Watershed	Cave Stream Reach	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

Trip Reports will be informal (unpublished) communications, but the Annual Summary Reports and Trend Analysis Reports will be submitted for publication in the NPS's Natural Resource Publications Management Report series. The Annual Summary Report will use the Natural Resource Data Series (NRDS) format. The NRDS format is basically an annual status report which is intended for a quick release of accurate, raw data with any statistical analyses and interpretation limited to guidelines described in the monitoring protocol. The NRDS format will present cumulative data in a graphic format reflecting observed trends in the data. The purpose of this format is to provide park managers and other viewers with a visual portrayal of resource status over time. Where inspection of trends graphics will support easy detection of large and rapid changes in resource condition. The Annual Summary Report will include a summary water-quality table (Table SOP 6-5), species lists by stream reach, size range and abundance by species and stream reach (Figure SOP 6-1), a Natural Resource Condition Summary table (Table SOP6-7), and when possible, single season estimates of occupancy and abundance similar to that in Table SOP6-4. The Trend Analysis Report will use the Natural Resource Report (NRR) format. The NRR format is a series intended for comprehensive information and analysis about natural resources and related topics concerning lands managed by the NPS. The NRR format will include quantitative data that are accompanied by analysis and interpretation.

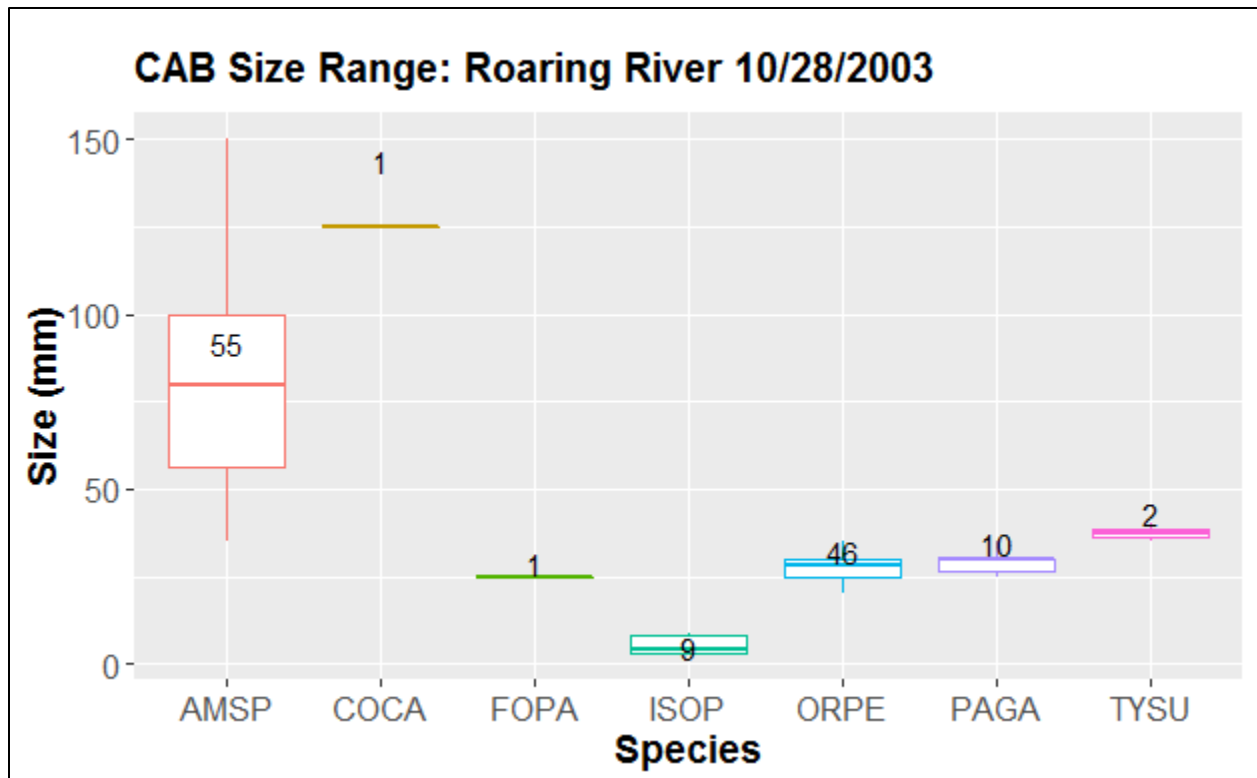


Figure SOP6-1. Boxplot showing size ranges for various CAB species detected during one sampling season. The bottom, middle (line), and top of the box correspond to the 25%, 50% (median), and 75% quantiles. The lower and upper 'whiskers' correspond to smallest and largest observation ± 1.5 times the interquartile range. Numbers are abundances of detected CAB. Data are from Mammoth Cave NP IBI sampling in 2003. These data will be included as part of the annual status report submitted to parks.

Regular reporting will be the responsibility of the project leader. Preparation of the quantitative elements for all reports will be the responsibility of the project leader working in collaboration with appropriate staff. Peer review of Annual Summary and Trend Analysis reports will follow the guidelines in *Peer Review, Publication, and Administrative Recordkeeping Requirements for Scholarly and Science Information Disseminated by the NPS Inventory and Monitoring Division* implemented in 2015.

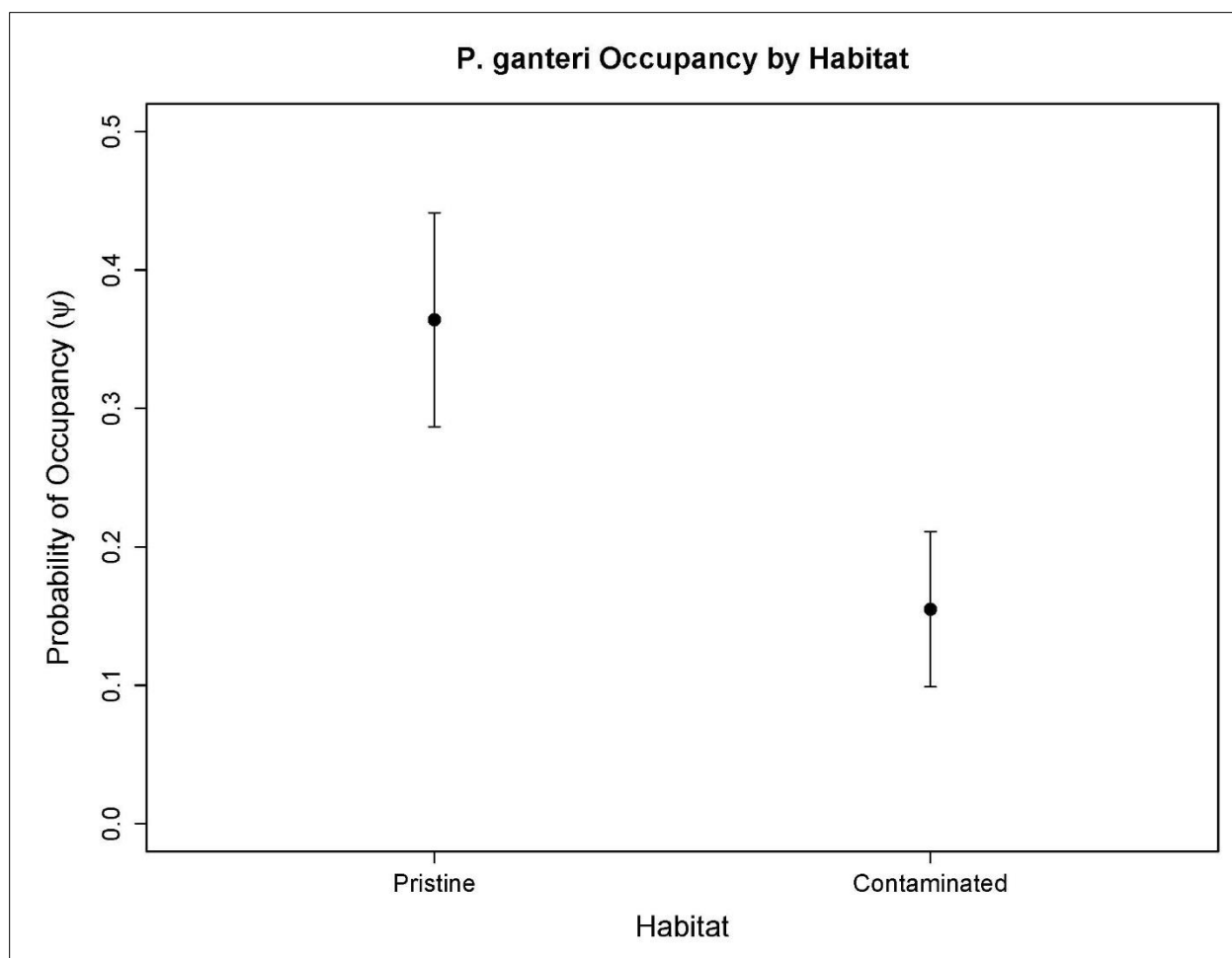


Figure SOP6-2. Mockup of graph depicting *P. ganteri* occupancy as a function of overall water quality. Data are simulated based on R code in Fiske et al. (2015). Error bars are ± 1 SE. These data will be included as part of the Trend Analysis Report submitted to parks.

Natural Resource Condition Summary Table

As previously mentioned, the Annual Summary Report will contain a Natural Resource Condition Summary Table intended to provide a snapshot assessment of the status and trend in the condition of the park's CAB resources being monitored. Reference conditions that represent "healthy" ecosystem parameters, and regulatory standards (such as those related to air or water quality) provide the benchmark to describe current resource status. In the absence of regulatory standards for cave aquatic biota, we will use a combination of collected data, models, and expert opinion from literature and personnel to interpret key measures and choose reference conditions for each measure that assign values of "Good," "Caution," and "Significant Concern" for cave aquatic biota at selected park caves. "Good" represents acceptable or desired conditions; "Caution" indicates a problem may exist; "Significant concern" indicates undesired conditions in need of management correction.

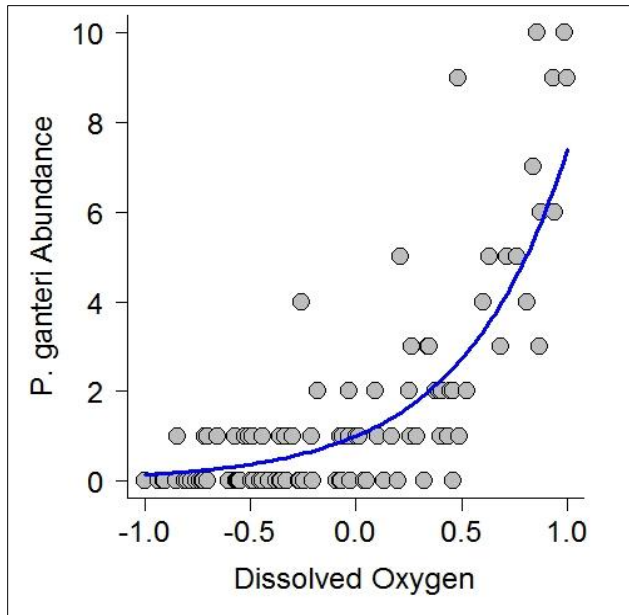


Figure SOP6-3. Abundance of *P. ganteri* as a function of dissolved oxygen content (mg/L), an indicator of water quality, among MACA cave stream reaches (blue line: expected abundance; gray circles: realized abundance). Data are simulated based on R code in Kéry and Royle (2015a). These data will be included as part of the Trend Analysis Report submitted to parks.

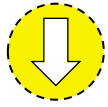
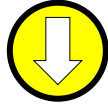
Park managers and cooperators will also receive annual summaries via oral presentations, and results (excluding any sensitive location information) will be made available publicly through the Cumberland Piedmont Network website, newsletters, and professional forums, as appropriate.

The Status and Trend symbols used in the summary table below (Table SOP6-6) are summarized in the following key. The background color represents the current condition status, the direction of the arrow summarizes the trend in condition, and the thickness of the outside line represents the degree of confidence in the assessment. Table SOP6-7 contains an example (at Mammoth Cave National Park) of what the Natural Resource Condition Summary table might look like in an Annual Summary Report for this monitoring protocol. The number of rows could be expanded to add more species for which data are available.

Table SOP6-6. Symbols used in the NPS's Natural Resource Condition Summary Table.

Condition Status		Trend in Condition		Confidence in Assessment	
	Warrants Significant Concern		Condition is Improving		High
	Warrants Moderate Concern		Condition is Unchanging		Medium
	Resource is in Good Condition		Condition is Deteriorating		Low

Table SOP6-7. Mockup of a Natural Resource Condition Summary Table to include in an Annual Summary Report for monitoring CAB at MACA.

Priority Resource or Value	Indicator of Condition	Specific Measure	Condition Status/Trend	Rationale and Data Sources for Resource Condition	Reference Condition and Data Source	Notes
Wildlife Communities	Cave Aquatic Biota	Species richness		6 out of 6 expected common CAB species were documented at MACA. (2004 Survey).	Maintain current species richness	–
Wildlife Communities	Cave Aquatic Biota	Estimated abundance- (PAGA)		Estimated mean abundance was 250 Kentucky cave shrimp (<i>Palaemonias ganteri</i>) during the 2020 surveys of four cave stream reaches where <i>P. ganteri</i> (PAGA) is known to occur.	318 shrimp estimated mean abundance among four PAGA cave stream reaches in 1993 (Pearson 1998)	–
Wildlife Communities	Cave Aquatic Biota	Estimated occupancy (PAGA)		While estimated mean occupancy was 0.8 among cave stream reaches in which PAGA is known to occur, it was only encountered in two of four cave stream reaches during the 2020 surveys.	The estimated mean occupancy among PAGA cave stream reaches was 0.6; PAGA known to occur in at least four MACA cave stream reaches (Pearson 1998).	–

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National Park Service
Inventory and Monitoring Division



Revising the Protocol—Version 1.0

This standard operating procedure explains how to make changes to the Cave Aquatic Biota (CAB) monitoring protocol narrative for the Cumberland Piedmont Network (CUPN) and accompanying SOPs, and how to track these changes. Any changes in the CAB monitoring protocol will adhere to the guidelines contained in this SOP. Personnel tasked with editing the protocol narrative or any one of the SOPs need to follow this procedure in order to eliminate confusion regarding how data are collected and analyzed. All observers must be familiar with this SOP in order to identify and use the most current methodologies, and should see the revision history log attached to the protocol narrative and each SOP.

Revision Log

[illegible]

Revision Process

All edits require review for clarity and technical soundness. Oversight of the revision process for all protocols is the responsibility of the I&M Regional Program Manager (RPM). When a potential modification to a protocol is identified, the project leader consults with the RPM and the CUPN Network Program Manager (NPM), who decide whether the change should be considered “small” or “significant.” Small changes or additions to existing methods will be reviewed in-house by CUPN staff designated by the NPM. However, if a substantial change is sought, such as a significant change in data collection methods, then the RPM coordinates the outside review required. Reviewers will be selected based on the type of change (i.e., changes in sampling sites versus changes in sampling design or sampling analysis). Regional and national staff of the National Park Service, as well as the U.S. Geological Survey, with familiarity in ecological research and data analysis could be utilized as reviewers. Experts in CAB monitoring, research, and statistical methodologies outside of the National Park Service will be utilized in the review process as needed.

Proposed changes, depending upon their magnitude and nature, could also have significant implications for data management. The database may have to be edited by the data manager to accompany changes in the protocol narrative and SOPs. The data manager must be consulted prior to implementing changes to this protocol. Immediately after the changes to the protocol narrative or SOP(s) have been made, inform the data manager, so the new version number can be incorporated in the metadata of the project database.

Major and Minor Revisions

Edits and protocol versioning must be documented in the Revision History Log that accompanies the protocol narrative and each SOP. Log changes in the protocol narrative or SOP being edited only.

- Version numbers will be incremented by decimals (e.g., Version 1.6 to Version 1.7) when there are minor modifications that do not significantly alter the underlying methods or procedures used to generate data values for the protocol’s existing data set.
- Version numbers will be incremented by a whole number (e.g., Version 1.3 to Version 2.0) when significant change(s) are made to the protocol. Significant changes include:
 - the addition of monitoring objectives;
 - changes to the sampling design;
 - addition of new parameters;
 - addition of a new sampling method; and
 - changes to reporting requirements.

Communicating Changes

Once edits have been incorporated the new versions must be posted on the Integrated Resource Management Application (IRMA) data store and in the “Narratives” or “SOP” subfolder for the project. Updates will also be needed within the protocol tracker for this protocol on IRMA. The project leader is responsible for forwarding copies to all known individuals with a previous version of the affected protocol narrative or SOP. The project leader must provide a copy of each previous version to the CUPN curatorial specialist for archiving.