

WATERBORNE CONTAMINANTS IN TUMBLING CREEK CAVE, MISSOURI

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Abstract

Tumbling Creek Cave (TCC) is an educational and research cave and a National Natural Landmark in southern Missouri. TCC's recharge area is 2,349 ha, located in a rural area, and part of the Ozark Underground Laboratory. TCC has the highest recorded biodiversity of any American cave west of the Mississippi River. TCC harbors three endangered species: Gray bats (*Myotis grisescens*), Indiana bats (*M. sodalis*) and the Tumbling Creek cavesnail (*Antrobia culveri*). The latter declined severely since the early 1990s, is nearly extinct and is the focus of this study.

The major goals of this study were to analyze SPMD and POCIS samplers in the cave stream for organic contaminants, and to search suitable habitat in the area for cavesnails, including cave streams, springs and groundwater. These goals were accomplished between 2002 and 2007:

Wells, springs and caves in the immediate TCC area were examined for fauna, but no cavesnails were found. Cavers were supported in searches of 25 caves in Taney County. Although a few caves had marginal pool habitat, none had suitable stream habitat like TCC.

Of the nonpolar organic compounds analyzed, one out of 28 PAHs (polyaromatic hydrocarbons) and eight out of 30 OCPs (organochlorine pesticides) were found in the samples. Only minute levels (pg/L or parts per quadrillion) of some nonpolar compounds and no polar organic compounds were detected in the TCC stream, lessening concerns about long-term contamination of the system. It is possible that some chemical(s) could have been transported through the system over a short time, and would not have left a trace, but there were no known spills or large industrial waste sites in the area. Siltation, septic system wastes, oxygen depletion and farm dump sites in sinkholes remain as possible causes of the decline in the

Antrobia cavesnail, but siltation is the most likely cause. However, the Tumbling Creek cavesnail may be sensitive to minute amounts of chemicals. Other ecological factors, such as the decline of Gray bats, *Myotis grisescens*, in the cave also could have had an influence on *Antrobia*. Gray bats have recently increased again at TCC because of conservation work.

An assessment of a chip-seal, highway-resurfacing project by the Missouri Department of Transportation found no effect on water quality in the cave, based on analyses of water samples from the road ditch and in cave stream collected at times when tracer dyes demonstrated that road runoff water was present.

Antrobia still exists in small numbers, and a project started in 2006 is providing additional cavesnail habitat (terra cotta tiles) in the stream as well as a potential cavesnail-propagation laboratory in the cave. Conservation work in the recharge area of the cave is creating improvements in the cave ecosystem and groundwater.

Key Words: Tumbling Creek Cave Missouri, water quality, contaminants, nonpolar and polar organic compounds, SPMD samplers, POCIS samplers, siltation, *Antrobia culveri*, cavesnail, *Myotis grisescens*, Gray bat, endangered species, ecology, land use

Introduction

Tumbling Creek Cave (TCC) is an educational and research cave and a National Natural Landmark located on a 1,032-ha (2,550-acre) tract in Taney County, southern Missouri (Figure 1). The cave is in a rural area, part of the Ozark Under-

ground Laboratory, established in 1966 (Aley and Thomson 1971, Thomson and Aley 1971, Neill et al. 2004, Elliott et al. 2005 and 2006). TCC's

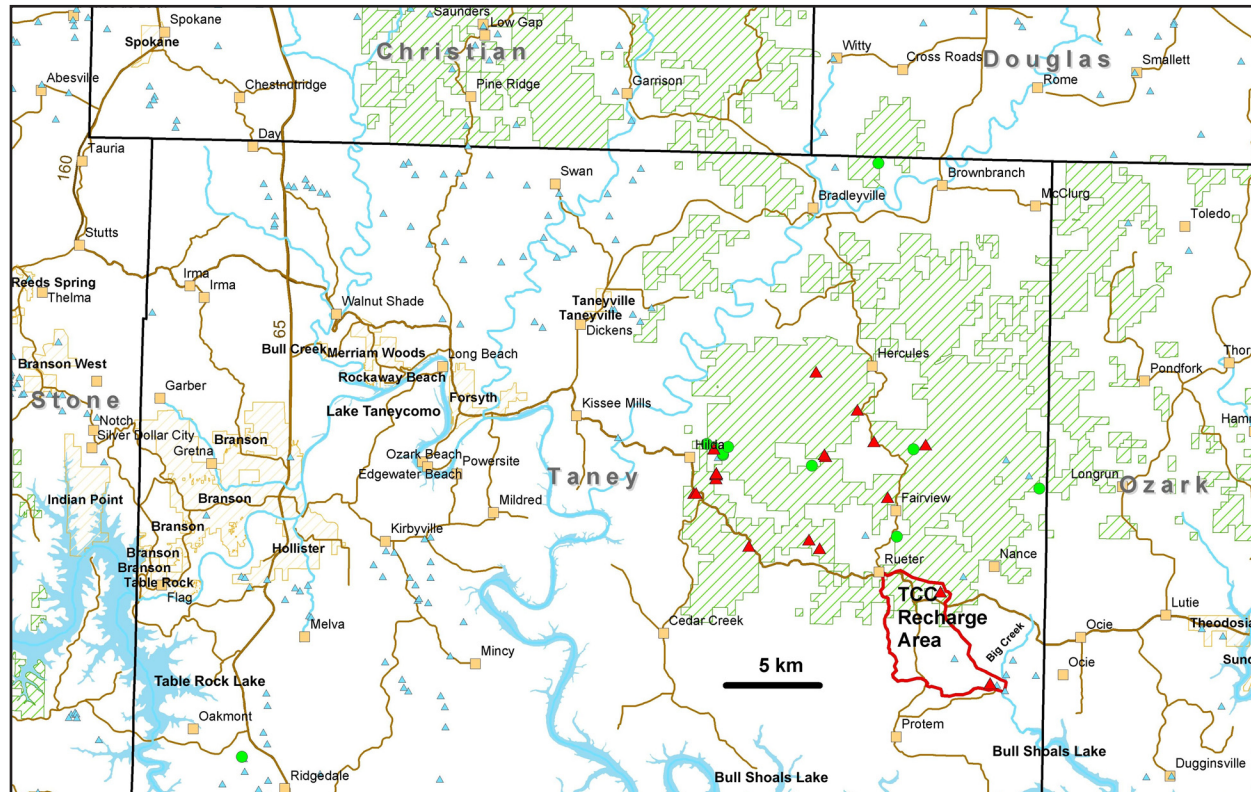


Figure 1 Map of Taney County area showing the location of Tumbling Creek Cave in the southeast, its recharge zone, caves that were targeted for investigation (green dots), caves that were investigated (red triangles) and known springs (blue triangles).

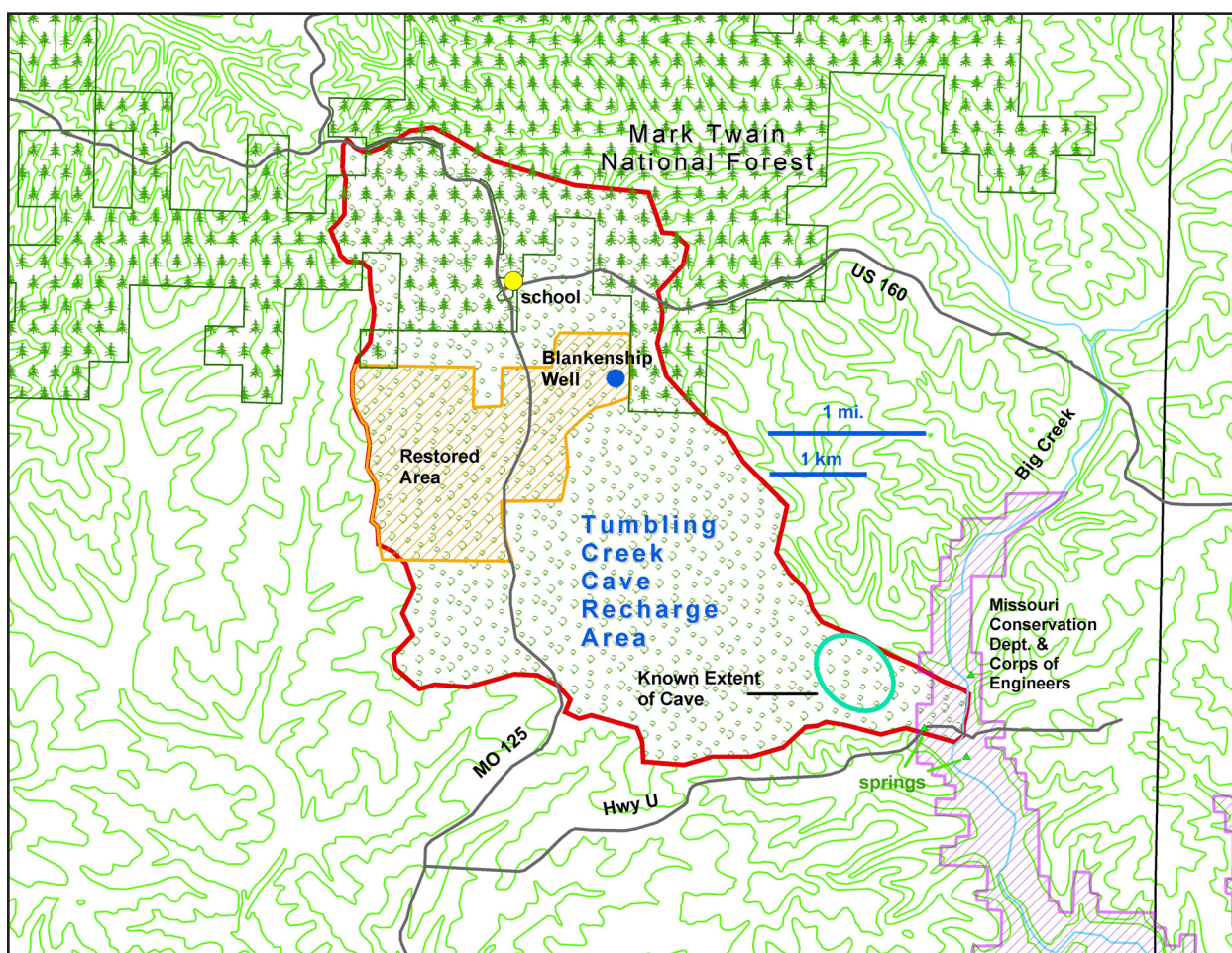


Figure 2 Map of Tumbling Creek Cave recharge area showing the locations of Tumbling Creek Cave, Mark Twain School, Blankenship Well, a restored area, area roads and features.

recharge area is 2,349 ha (5,804 acres, Figure 2). TCC has the highest recorded biodiversity of any American cave west of the Mississippi River, with about 112 species, including 11 or 12 species of obligate cave dwellers, or troglobites (Elliott *in press* 2007). TCC harbors three endangered species: Gray bats, *Myotis grisescens*, Indiana bats, *M. sodalis*, and the Tumbling Creek cavesnail, *Antrobia culveri* (Figure 3). The latter declined severely since the early 1990s, is nearly extinct, and is the focus of this study (U.S. Fish & Wildlife Service 2001, 2003, Ashley 2003).

Three major goals of this study were to

- Analyze SPMD samplers that were deployed in the cave stream in 1995.
- Deploy new SPMD and POCIS samplers in the cave stream for additional analyses.
- Search suitable habitat in the area for cavesnails, including cave streams, springs and groundwater.

SPMD (semi-permeable membrane device) samplers (Figure 4) are useful for investigations of waterborne contaminants because they mimic an organism's fat in their ability to absorb nonpolar organic compounds in a synthetic lipid.

This report discusses other scientific and conservation projects related to the fate of *Antrobia*, with references, tables and figures provided. *Antrobia* still exists in small numbers, and a project started in 2006 is providing additional cavesnail habitat (terra cotta tiles) in the cave stream as well as a potential cavesnail propagation laboratory built in the cave.

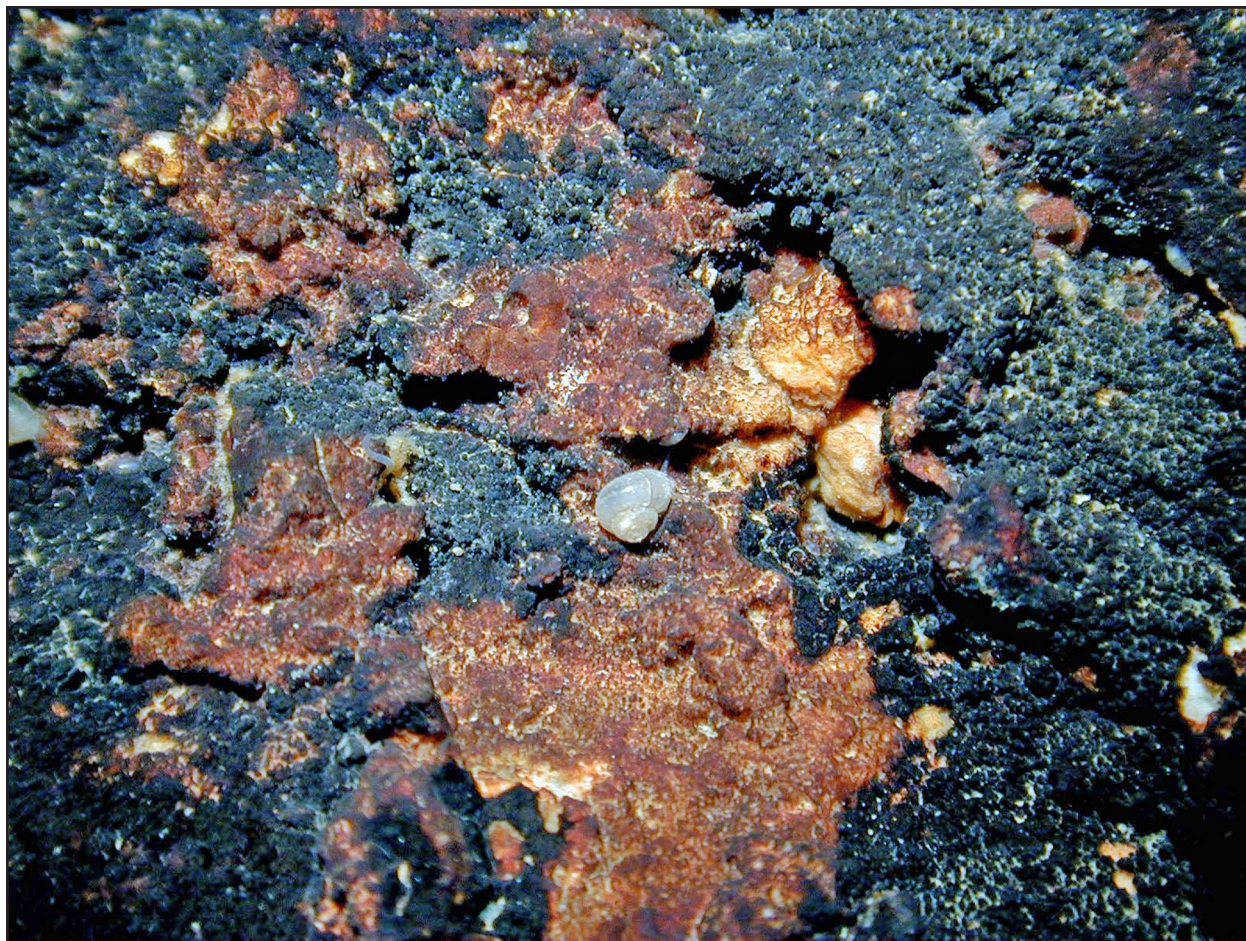


Figure 3 *The Tumbling Creek cavesnail, Antrobia culveri. Photo by David C. Ashley.*



Figure 4 *SPMD deployed by William R. Elliott in Tumbling Creek Cave. The membrane sampler is inserted into a perforated cover and anchored in the stream. Photo by Steve Samoray.*

Materials and Methods

Searches of Caves, Springs, and Wells

In 2002–2003 David C. Ashley, Michael E. Slay, Philip Moss, and William R. Elliott examined groundwater in the TCC area for cavesnails. Samples were taken from the “karst window” near the cave, wellpoint (hand pump) samples along Big Creek, springs on Big Creek and Blankenship Well north of TCC.

A contract between Missouri Department of Conservation (MDC) and Cave Research Foundation (CRF) provided support for eleven volunteer cavers to search for potential cave stream habitat. Field trips were taken in December 2004 and January, February, April, and May 2005. The January trip was largely washed out by extremely heavy rains. The area was limited to Taney County. Most of the sites investigated were in southeastern Taney County, in areas closest to Tumbling Creek Cave (Figures 1 and 2).

Twenty-five caves were searched, besides Tumbling Creek Cave: Stafford Cave, Skull Cave, Tiny “Cave,” Dicus Cave, Spring Cave, Marholtz Cave, Decker Cave, Hercules Lookout Cave, Mores Branch Cave, Hercules Glades Pit, Little Bear Cave, Clayton Cave, California Cave 1, California Cave 2, Gilbert Cave, Twenty-five Sink Cave, Cane Bluff Cave 1, Cane Bluff Cave 2, Cane Bluff Cave 3, Little Cane Bluff Tunnel, Cane Bluff Shelter, Double Cave, Midden Cave, one unnamed cave, and Jack Cave. In addition several other cave locations were found to be in error and duplications of other caves were removed (this included Dicus #2 and Armadillo Cave). Most of the above caves are on land belonging to Mark Twain National Forest. Attempts to investigate several other caves (Fairview Church Cave, Blowing Spring, Willies Pit, China Hole, Coyote Collapse, and Wolf Cave) were stymied by failure to find landowners at home or otherwise get permission to cross lands to access other lands. Absentee landowners are prevalent in the area, and this makes it difficult to easily gain legal access. Additionally several springs in the area of Tumbling Creek Cave (two were resurgences) were investigated for potential “wash-outs” of snails. The entrance zone of the Bear Cave or natural entrance of Tumbling Creek Cave was investigated for snails and checked for wintering bats (there also is an ar-

tificial entrance). Several more trips were taken to areas with potential caves and springs.

Contaminants Study

Background

In 1995 David C. Ashley did a limited study of waterborne contaminants in TCC and Fantastic Caverns (FC, near Springfield, Missouri) using SPMDs from EST Lab in St. Joseph, Missouri. The Fantastic Caverns sample provided a comparison with another large stream cave in Southwest Missouri. Dr Ashley provided his sample extracts for this study, which had been stored in ampules at EST Lab, and they were analyzed in 2006–2007.

SPMD extracts that had been returned from the field were provided by Elliott to the Columbia Environmental Research Center (CERC) for analysis of organic contaminants including polychlorinated biphenyls (PCBs as total PCBs), organochlorine pesticides (OCPs) and polyaromatic hydrocarbons (PAHs). Cresol was evaluated in the full scan PAH analysis and polybrominated diphenyl ethers (PBDEs) also were screened. These chemicals comprise several classes of environmental contaminants.

SPMD preparation and deployment

SPMDs deployed in the field were provided by EST, and they were manufactured as standard size SPMDs (2.5 cm x 152 cm, 85 μ m membrane thickness, 1.64 g triolein) (Huckins et al., 1993, 1996). Post-manufacture SPMDs were sealed in airtight metal cans prior to field deployment. Trip blanks accompanied the samplers to the field and were exposed to air during the deployment and resealed in cans.

The samplers, manufactured by EST Lab, were taken into the field by university and/or state personnel. The 1995, samplers were deployed by David C. Ashley in Tumbling Creek Cave and Fantastic Caverns for 30 days. The 2002–2004 TCC samplers were deployed by William R. Elliott, Steve Samoray, and Philip Moss for 61 days in the cave stream at the foot bridge, inside a perforated stain-

less steel container (Figure 4), then retrieved and returned to EST for dialysis and high performance size exclusion chromatography. The resulting extracts were then solvent-reduced and stored in amber ampules at 3 SPMDs per ampule for the 1995 samples and 1 mL per SPMD for the post-2000 samples.

POCIS deployment

A new type of sampler, the POCIS, for polar organic compounds, was deployed once with a trip blank in Tumbling Creek Cave in 2004. The round sampler was deployed in a solvent-rinsed, air-dried, stainless-steel steamer basket in the cave stream. A representative set of target residues was selected, including various pharmaceuticals, antibiotics, caffeine, and other waterborne polar compounds. A very large array of possible substances could be found in the environment, but not all can be detected in a study with limited duration and funding.

Summary of analytical methods

Sample preparation. Sample extracts were prepared and analyzed for OCPs, total PCBs and PAHs using USGS-CERC standard operating procedures. Total PCBs are reported as a summation of congeners. The following quality control (QC) samples were incorporated into the various analyses:

- Procedural blank—to measure laboratory background and to establish method detection limits,
- Procedure spikes (PCB, OCP, PAH spiked)—to demonstrate recovery through the analytical method,
- SPMD dialysis blank—to demonstrate the background of a freshly prepared SPMD from dialysis step onward,
- SPMD trip blanks—SPMDs that went to the field and were exposed to cave air conditions during deployment and retrieval of the sample SPMDs.

Mixtures of Aroclors 1242, 1248, 1254, and 1260 (in a 1:1:1:1 ratio), OC pesticides (29 compounds), and PAHs (27 compounds) were added to a procedure blank. The following recovery compounds were added to all sample extracts before the cleanup steps described below were performed, including samples used for QC (procedure blank and procedure spike):

- PCB 029 (2,4,5-trichlorobiphenyl),
- PCB 155 (2,2',4,4',6,6'-hexachlorobiphenyl),
- PCB 204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl),
- Deuterated PAH mix (16 priority pollutant PAHs),
- Deuterated p,p'-DDD.

The PCB compounds selected are used for recovery because they are rarely found or are undetectable in Aroclors and they are chromatographically resolvable. The three PCB surrogates are used to correct for analytical recoveries of the PCBs (PCB 029, a trichlorobiphenyl, is representative of more volatile early eluting PCBs (Cl_1 - Cl_3), PCB 155, a hexachlorobiphenyl, is representative of mid-range eluting congeners (Cl_4 - Cl_6), and PCB 204, an octachlorobiphenyl, is less volatile and representative of later eluting PCBs (Cl_7 - Cl_{10})) and several pesticides which are found in the PCB fraction off silica gel fractionation. The deuterated p,p'-DDD is the surrogate for pesticides in the second silica gel fraction. The deuterated PAH mixture compounds are surrogates for the PAHs found. Evaluation of the spikes also gives recovery information. Table 1 lists deuterated PAH surrogates that were added to all samples and QC samples before extraction for PAH analysis. Table 2 lists the 27 native PAH solutions used for standard checks, procedural checks and spiking.

Table 1 Deuterated PAH surrogates that were added to all samples and QC samples before extraction for PAH analysis.

Naphthalene- d_8	Fluoranthene- d_{10}	Benzo[<i>a</i>]pyrene- d_{12}
Acenaphthylene- d_8	Pyrene- d_{10}	Indeno[1,2,3- <i>c,d</i>]pyrene- d_{12}
Acenaphthene- d_{10}	Benz[<i>a</i>]anthracene- d_{12}	Dibenz[<i>a,h</i>]anthracene- d_{14}
Fluorene- d_{10}	Chrysene- d_{12}	Benzo[<i>g,h,i</i>]perylene- d_{12}
Phenanthrene- d_{10}	Benzo[<i>b</i>]fluoranthene- d_{12}	
Anthracene- d_{10}	Benzo[<i>k</i>]fluoranthene- d_{12}	

Table 2 The 27 native PAH solutions used for standard checks, procedural checks and spiking.

Naphthalene	2-Methyl Anthracene	Chrysene
2-Methyl Naphthalene	4,5-Methylene Phenanthrene	Benzo[<i>b</i>]fluoranthene
1-Methyl Naphthalene	1-Methyl Phenanthrene	Benzo[<i>k</i>]fluoranthene
Acenaphthylene	Fluoranthene	Benzo[<i>e</i>]pyrene
Acenaphthene	Pyrene	Benzo[<i>a</i>]pyrene
Fluorene	Retene	Perylene
Dibenzothiophene	1-Methyl Pyrene	Indeno[1,2,3- <i>c,d</i>]pyrene
Phenanthrene	Benzo[<i>b</i>]naphtha[2,1- <i>d</i>]thiophene	Dibenz[<i>a,h</i>]anthracene
Anthracene	Benz[<i>a</i>]anthracene	Benzo[<i>g,h,i</i>]perylene

The SPMDs were dialyzed at EST according to standard procedures. Dialysates were then run through a HPSEC (high performance size exclusion chromatography) cleanup to remove residual lipid and polyethylene waxes from the dialysis. Once they were received for analysis by CERC the extracts were spiked with the appropriate recovery compounds discussed above before proceeding with chemical-class-specific cleanup steps.

In the analytical protocol targeting total PCBs and organochlorine pesticides, a 1 SPMD equivalent amount of the extract was analyzed. The extracts were spiked with recovery compounds and then fractionated on a two-layered octadecyl silica/activated silica gel column into two fractions: one fraction containing PCBs and six of the targeted OCPs (SODS-1), and a second fraction contain-

ing the remainder of the OCPs (SODS-2). The sample extracts were adjusted to a final volume of 1 mL and two instrumental internal standards were added: PCB congeners 030 and 207 (40 ng each).

The resulting fractions were prepared for gas chromatography with electron capture detection (GC/ECD). The sample extracts were adjusted to a final volume of 1 mL. Two instrumental internal standards were added: PCB congeners 030 and 207 (40 ng/mL each).

For PAH analysis a 1-SPMD equivalent portion of the extract was split after dialysis and HPSEC. The 1-SPMD equivalent portions were spiked with recovery compounds and the extracts were purified by potassium silicate preparative column chromatography and a silica gel (3% water deactivated) preparative column chromatography. The resulting extracts were evaporated to ~100 μ L

final volume and prepared for gas chromatography with mass spectrometry by adding an instrumental internal standard *p*-terphenyl-*d*₁₄.

Summary of gas chromatographic method for total PCBs.

PCBs were measured in SODS-1 fractions by gas chromatography with electron capture detection (GC/ECD). Analyses were performed using Hewlett-Packard 5890 Series II GCs with cool, on-column capillary injection systems and Hewlett-Packard model 7673 autosamplers. For all analyses, a 3-m section of 0.53 mm i.d. uncoated and deactivated capillary retention gap (Agilent, Palo Alto, CA) was attached to each analytical column by a Press-Tight[®] (Restek Corp., Bellefonte, PA) union. The analytical columns were 60-m x 0.25-mm x 0.25 μ m DB-5 (5% phenyl-, 95% methylsilicone, Agilent, Palo Alto, CA) and DB-17HT[®] (0.25 μ m 50% phenyl-, 50% methylsilicone, Agilent, Palo Alto, CA). The H₂-carrier gas was pressure regulated at 25 psi. The temperature program for the PCB analysis was as follows: initial temperature 60°C, immediately ramped to 150°C at 15°C/min, then ramped to 250°C at 1°C/min, and finally ramped to 320°C at 10°C/min, and held for 1 min. The ECD temperature was 330°C.

PCBs were matched and identified on one GC capillary column with known PCB peaks from the standards. For this report single column matching and ID were used on the DB5 column as the analysis called for total PCBs only. The total was determined as a sum of congeners from the one column. The capillary GC/ECD data were collected, archived in digital form, and processed using a PerkinElmer chromatography data system, which included the model 970 interface and version 6.1 of Turbochrom Workstation chromatography software (PerkinElmer, Waltham, MA), on a microcomputer. A mix of several Aroclors is used to produce the PCB congener calibration standards. These standards have been quantified based on pure, primary PCB standards (Accustandard, New Haven, CT) and are used as secondary standards. Up to nine levels of calibration for each individual congener are used to quantify approximately 140 congeners in the samples. In terms of total-PCB concentrations, the calibration curve covers a range from 10 to 8000 ng/mL.

The “method detection limits” (MDLs) for individual PCB congeners and for total PCBs were based on procedural blank (PB) results following

the method outlined by Keith *et al.* (1983, 1991). Briefly, a mean (\bar{X}_{PB}) and standard deviation (SD) are determined using PB, trip blank SPMD and dialysis blank SPMD results. This produces a MDL (ng/SPMD) calculated using the following formula:

$$MDL = \bar{X}_{PB} + 3(SD_{PB})$$

The MDL is then expressed in units of concentration, e.g. mass of analyte per SPMD. If a concentration is below its respective MDL it will be censored with a “< MDL” (where MDL is a value).

The “method quantitation limits” (MQLs) for congeners is calculated in the same manner as above using the following formula:

$$MQL = \bar{X}_{PB} + 10(SD_{PB})$$

Data that fall between the MDL and MQL were censored in all the data tables. However, data above the MQL have a greater degree of confidence—i.e. when the analyte signal is 10 or more times greater than the standard deviation of the measurement there is a 99% probability that the true concentration of the analyte is within $\pm 30\%$ of the calculated concentration.

Recoveries of analytes are monitored by the following measures:

- Procedural internal standards spiked into each sample,
- PCB/OCP/PAH-spiked blank.

Three procedural standards are used to account for analytical recoveries of the PCBs: PCB 029, a trichlorobiphenyl, is representative of more volatile early eluting PCBs (Cl₁ - Cl₃), PCB 155, a hexachlorobiphenyl, is representative of mid-range eluting congeners (Cl₄ - Cl₆), and PCB 204, an octachlorobiphenyl, is less volatile and representative of later eluting PCBs (Cl₇ - Cl₁₀).

Summary of gas chromatographic method for pesticides and PBDEs. Pesticides were measured by GC/ECD using Hewlett-Packard 5890 Series II GCs with cool on-column capillary injection systems and Hewlett-Packard model 7673 autosamplers. For all analyses, a 3-m section of 0.53 mm

i.d. uncoated and deactivated capillary retention gap (Agilent, Palo Alto, CA) was attached to each analytical column by a Press-Tight® (Restek Corp., Bellefonte, PA) union. The analytical columns were 60-m x 0.25-mm x 0.25 µm DB-5 and DB-17 phase columns. The H₂-carrier gas was pressure regulated at 25 psi. The temperature program for the PCB analysis was as follows: initial temperature 60°C, immediately ramped to 150°C at 15°C/min, then ramped to 250°C at 1°C/min, and finally ramped to 320°C at 10°C/min, and held for 1 min. The ECD temperature was 330°C.

The dual column method accurately identifies and quantifies pesticide peaks from one or both columns based upon known standards. The GC/ECD data were collected, archived in digital form, and processed using a PerkinElmer chromatography data system, which included the model 970 interface and version 6.1 of Turbochrom Workstation chromatography software, on a Pentium III microcomputer. Six levels of organochlorine pesticide standards (29 components) were used for calibration, with each pesticide at concentrations ranging from 0.1 to 80 ng/mL. Concentrations are expressed as nanograms of analyte per SPMD (ng/SPMD). Detection limits were calculated as discussed above for PCB congeners.

Polybrominated diphenyl ethers (PBDEs), flame retardants, were screened using a nine-congener standard with three calibration levels—1 ng/mL to 200 ng/mL. Recoveries of analytes are monitored by the following measures:

- Procedural internal standards spiked into each sample,
- OCP-spiked control fish analyzed with each set.

Two recovery-method compounds are used to account for analytical recoveries of the pesticides in the two analyzed fractions: PCB 029, a trichlorobiphenyl and *d*₈-p,p'-DDD. Since the PBDE values were just a screen they were not corrected for analytical recovery.

Summary of gas chromatographic mass spectrometric method for polyaromatic hydrocarbon.

The sample extracts were adjusted to a final volume of ~100 µL and the instrumental internal standard p-terphenyl-*d*₁₄ (100 ng) was added.

Sixteen perdeuterated and 27 native PAHs were measured in the PAH fraction from silica gel by GC/MS in the full scan mode. Analyses were performed using a CE Instruments 8000Top GC with cool on-column capillary injection systems and an AS800 autosampler (2 µL injected) interfaced with a Voyager quadrupole mass spectrometer (Thermo-Finnigan Corp., San Jose, CA). For all analyses, a 2.5 m section of 0.53 mm i.d. uncoated and deactivated (Restek Corp., Bellefonte, PA) capillary retention gap was attached to the front of each analytical column by a Press-Tight® (Restek Corp., Bellefonte, PA) union. The analytical column was a 50 m x 0.20 mm Ultra-2 (0.11 µm 5% phenyl-, 95% methyl-silicone, Agilent, Palo Alto, CA). Helium carrier gas was flow-regulated at 1 mL/minute. The temperature program for the PAH analysis was: initial temperature 60°C, hold time 2.5 minutes, ramped to 300°C at 5°C/minute, and held for 15 minutes. The direct transfer line to the mass spectrometer was maintained at 305°C.

The mass spectrometric method acquired full scan data (*m/z* 50-550 0.75s scan time) from 12.5 to 60 minutes. The photomultiplier was set to 350V. The mass spectrometer was tuned using PFTBA (*m/z* 50-614). This method is confirmatory, where background-corrected spectra in samples are compared with standard spectral libraries and with authentic spectra acquired from the calibration standards. The data were collected, archived in digital form, and processed using the Thermo-Finnigan XCalibur GC/MS data system. Depending on the dynamic range required, calibration up to eleven levels of calibration standards, ranging from 0.250-625 pg/µL were analyzed with an analytical set.

Method detection limits were estimated from low-level standards and the blanks determined by both the signal-to-noise ratio of the peak in the quantitation ion channel and the gradual loss of unique characteristics of the background-corrected mass spectrum.

For the positive identification and quantification of each PAH, the following criteria were established and met in this study:

- Peak areas for the selected ion responses must be greater than three times background noise.
- Native ion peaks must occur at relative retention times (to the perdeuterated surrogate) that are equivalent to those for the correspond-

ing calibration standards.

The m/z pattern for the major ion responses in the background-corrected mass spectrum must closely match that of the calibration standard for each specific analyte.

Highway study. In November 2006 Ozark Underground Laboratory (Aley 2007), in cooperation with Missouri Department of Transportation (MODOT), studied the potential runoff of petroleum hydrocarbons from a “chip seal project” on U.S. Highway 160. About 3,725 m (15,500 ft.) of highway crosses the northern part of the recharge zone of TCC, 4.8 km or more from accessible portions of the cave stream (Figure 2). The highway was treated with a emulsified asphalt and rock chips. This is a relatively new road-surfacing method that reduces hydrocarbon runoff. A dye trace was conducted after the application, and water samples were taken from the cave stream and tested at a commercial laboratory for Total Purgeable Hydrocarbons (TPH) at a detection limit of 0.1 mg/L (0.1 ppm).

Results

MDC’s cave conservation program staff took 11 field trips to TCC from October 2003 to July 2004. Trips included field work as well as participating in meetings of the Cavesnail Working Group and the draft and revision of the federal recovery plan.

In October 2003, the first SPMDs were collected and exchanged with new ones. Lab reports from the October collection indicated low levels of some possible combustion products captured on both the sampler and the field blank, but little else. These combustion products could have come from cave dust, residues from fires in the area, or from a carbide lamp used by a person who retrieved samplers. Electric headlamps were used thereafter. In February 2004 an SPMD sampler in the cave stream was exchanged for a POCIS (polar organic chemical integrative sampler) a new type of sampler from EST Lab, similar to the nonpolar SPMD sampler.

In October 2003, Elliott and Steve Samoray visited Stafford Cave on private land in northern Taney County. No cavesnails were found in Stafford, it appeared to be a small, wet-weather

resurgence with only a muddy pool and no appropriate habitat.

Groundwater sampling from Blankenship Well, the Karst Window, and wellpoint samples yielded a few small invertebrates, including a few remains of shells, but none appeared to be *Antrobia culveri*.

In March 2004 a large chute gate was built at the entrance of Tumbling Creek Cave to provide greater security from intruders, while affording the Gray bats an ample flyway. Eighteen people from different organizations worked on the project. Simultaneously, OUL workers removed the internal “barrel gate” far inside the cave. This opened a larger flight path for the bats. Insofar as the Gray bat colony is an important component of the cave community, and may contribute directly or indirectly to the nutrient flow into the cave stream, the cave gate may be a step in the recovery of both the Gray bat and the cavesnail at this site. The total cost was \$25,000 paid from MDC funds. Gray bats began using the gate immediately.

In April, May, June, and July of 2004, Elliott and others recorded seven bat exit flights at the cave gate using a digital 8 camcorder and NIR (near-infrared) illumination, so as to avoid disturbance of the bats (Elliott et al. 2006).

MDC’s cave conservation program staff took 18 field trips to Tumbling Creek Cave from July 2004 through June 2005. Trips included field work as well as participating in meetings of the Cavesnail Working Group. The group met on May 22-23, 2005, at Ozark Underground Laboratory. On May 23 Paul Johnson and Stephanie Clark from the Tennessee Aquarium Research Institute observed 67 cavesnails when they crawled upstream from the usual transect. Some were upstream of the last bat area, some downstream of “Bill’s Bath,” and a few were in the tributary stream. This find gave hope for attempting a captive propagation study in the cave, first using a suitable surrogate species for testing.

In 2004 we estimated a population of about 19,000 Gray bats in May, peaking at 34,000 in July, which was up from the previous visual count in July 1998 of about 12,000 Gray bats. The July counts were about 32,000 in 2005 and 37,000 in 2006. In August and September the numbers vary from night to night, probably because of emigration and immigration to/from other caves. We continue to monitor the population with NIR and TIR (ther-

mal infrared) imagery.

Of the caves checked in the area in 2004-2005, the following had streams with some gravel substrate in them: Spring Cave, Dicus Cave, Hercules Lookout Cave, Clayton Cave, and Gilbert Cave (Figure 2). Decker Cave should be reinvestigated. However, probably none of these stream caves are extensive enough to support cavesnail populations. The best sites were Gilbert Cave and Hercules Lookout Cave; both were extensively studied by Dr Mick Sutton and found to lack aquatic snails.

MDC and the USFWS assisted the owners, who bought a nearby, abused farm with their own funds. With cost-share funds they replanted 70,000 trees to restore the mostly cleared land. They oversaw the planting of native species, such as black oak, northern red oak, white oak, black gum, black walnut, green ash, dogwood, redbud, sycamore, and a few short-leaf pines. Other native species will re-establish naturally from the surrounding areas. Another cost-share project with the National Park Service helped to clear the land of trash, which was dumped or buried in several places. These projects probably have already helped to clear the cave stream of sediments, and may be aiding in the re-appearance of cavesnails. Increased input of Gray bat guano may also be important for the long-term recovery of the cavesnail (Elliott and Aley 2006).

MDC's Private Lands Division and the USFWS worked with the Mark Twain School in the recharge area to correct a leaking sewage lagoon that could have affected the cave ecosystem. A modern peat-filter septic system was implemented in 2005-06 with at least \$90,000 in donations from local, state and federal sources.

Analytical results are given in Tables 3-6, condensed from Tables 1-8 in Elliott and Echols (2007). EST Lab provided analyses of the first round of samples in 2003, but could not continue because of problems with laboratory instruments (Table 3). Total PCBs exceeded those detected in the trip blank in 2002, but not in 1995. Those exceeding the 2002 trip blank residues in 1995 included acenaphthene, fluorene, phenanthrene, anthracene, and fluoranthene. In 2002 acenaph-

thylene and acenaphthene exceeded the trip blank results, as did fluoranthene, pyrene, and total PCBs. These minute amounts were not quantitated to water concentrations.

MDC contracted with CERC to complete the analyses in 2006-2007. CERC found one of 28 PAHs (Table 4) and 8 out of 30 OCPs (Table 5). Two brominated flame retardants (PBDEs) out of nine congeners were detected, but not quantitated to water concentrations (Table 6).

CERC's analysis of SPMDs (Table 4) found the PAH, benzo[g,h,i]perylene, in TCC and FC on several occasions. "Legacy organochlorine pesticides" and PCBs (commonly used in the past and persistent in the environment) were evaluated in the SPMDs from 1995 and 2003, and they were below detection or quantitation limits of the methodology for almost of the compounds evaluated. Those that were detected were typically barely above the MQL. Five OCPs were found in TCC, and six were found in FC. TCC had somewhat higher concentrations of 4 OCPs than did FC. In the 1995 samples, pesticides that we detected were pentachlorobenzene, HCB, PCA, dieldrin, oxychlordane, cis-chlordane, trans-nonachlor and p,p'-DDT. The highest level found was for cis-chlordane at 5.6 ng/SPMD in FC. For the 2003 TCC samples there were fewer hits for pesticides—only HCB, delta-BHC and endosulfan I. Levels of PAHs were likewise typically below the calculated MDLs or MQLs for each compound, except for low levels (1-2 ng/SPMD) of benzo[e]pyrene (but less than the trip blank) and benzo[g,h,i]perylene from several of the samples. These two PAH compounds have low water-solubility and would typically be associated with sediment or particulate organic carbon. Sediment samples from TCC collected in 2004 were below detection limit levels of the same list of OC pesticides, total PCBs and PAHs, including benzo[e]pyrene and benzo[g,h,i]perylene. It is uncertain what the source of these two PAHs was. Cresol was also evaluated in the GC/MS analysis with the PAHs because creosoted timbers had been found buried within the recharge area, none was found in any of the samples.

Table 3 EST Laboratory analysis of TCC cave stream samples in $\mu\text{g}/\text{SPMD}$ (ng/SPMD for total PCBs). Results that exceeded trip blank levels are in bold.

Analyte	Dialysis Blank	EST# 02-379 Trip Blank	30001E 1995	EST# 02-380 Bridge 2002	EST# 02-381 Weir 2002
Naphthalene	0.36	1.3	0.06	0.13	0.03
Acenaphthylene	ND	0.04	0.03	0.09	0.04
Acenaphthene	ND	ND	0.01	0.14	0.1
Fluorene	ND	ND	0.05	ND	ND
Phenanthrene	ND	ND	0.28	ND	0.04
Anthracene	ND	ND	0.02	ND	ND
Fluoranthene	ND	ND	0.08	0.08	ND
Pyrene	ND	ND	ND	0.16	ND
Benzo(a)Anthracene	ND	ND	ND	ND	ND
Chrysene	ND	ND	ND	ND	ND
Benzo(b)Fluoranthene	ND	ND	ND	ND	ND
Benzo(k)Fluoranthene	ND	ND	ND	ND	ND
Benzo(a)Pyrene	ND	0.7	ND	0.49	ND
Indeno(123-cd)Pyrene	ND	ND	ND	ND	ND
Dibenzo(ah)Anthracene	ND	ND	ND	ND	ND
Benzo(ghi)Perylene	ND	ND	ND	ND	ND
Total PCBs ng	6.21	18.47	14.03	21.27	41.33

Table 4 PAHs detected in cave waters in pg/L (parts per quadrillion).

Detected	Fantastic Caverns 10/16/95	TCC Upstream 10/16/95	TCC Downstream 10/16/95	TCC at Bridge 5/20/03	TCC at Bridge 10/29/03	TCC at Bridge 12/10/03
	11			6.8	8.9	7.5

Table 5 OCPs detected in the cave stream in pg/L (parts per quadrillion).

Analyte	Fantastic Caverns 10/16/95	TCC Upstream 10/16/95	TCC Downstream 10/16/95	TCC at Bridge 5/20/03	TCC at Bridge 10/29/03	TCC at Bridge 12/10/03
Hexachloro-benzene (HCB)			35	3.9	6.8	
Pentachloro-anisole (PCA)	29		58			
Dieldrin	4.4	5.5	6.3			
Oxychlordane			18			
cis-Chlordane	67					
cis-Nonachlor	11					
trans-Nonachlor	43					
p,p'-DDT	42		38			

Polybrominated diphenylethers (PBDEs) were evaluated in SPMDs from Tumbling Creek Cave and Fantastic Caverns, sampled in 1995 (Table 6). Low levels of PBDE-47 and 99 were detected in the 1995 SPMD samples, but it is most likely because

of contamination from indoor air or indoor dust. There were no trip blanks with the 1995 SPMDs to verify whether these are due to this contamination, therefore, the PBDE data probably are not valid for those samples.

Table 6 PBDEs (fire retardants) detected in cave waters in ng/SPMD, not quantitated to water concentrations. ND = none detected. The 1995 results probably are not valid because a trip blank was not collected to evaluate airborne contaminants.

Cave	PBDE-47	PBDE-99
Fantastic Caverns 10/16/95	10	20
TCC Upstream 10/16/95	9.5	15
TCC Downstream 10/16/95	7.6	11
TCC at Bridge 5/20/03	ND	ND
TCC at Bridge 10/29/03	ND	ND
TCC at Bridge 12/10/03	ND	ND

Quality control (QC) associated with samples showed that laboratory procedure blanks were either ND (not detected) or less than the MDL for all of the compounds evaluated. Trip blanks showed some low background for some pesticides—lindane, some of the pp-DDTs—and PCBs. There were also background levels of PAHs in the trip blanks. Most of the background levels of PAHs were <1 ng/SPMD, but naphthalene, methyl-naphthalene and phenanthrene all were found at 10-20 ng/SPMD. These are typically problematic as background PAHs in SPMDs. PBDEs in the trip blanks were found at 2-3 ng/SPMD for congeners 47 and 99, which are both typically found in indoor air and/or dust backgrounds.

Recovery spikes for OCPs, PCBs and PAHs added to the extracts that were received at CERC, and they were processed through several cleanup steps, analyses were good, typically within acceptable QC parameters (50-125%). Procedure spike recoveries were also in acceptable QC range for most of the compounds.

Water concentrations can be estimated from SPMD data using an Excel® spreadsheet calculator developed from calibration data. When modeling these low levels of persistent organic pollutants we used the detection limit or quantitation limit values to estimate potential levels in water. For ex-

ample, the MDL for total PCBs was determined to be 22 ng/SPMD and the MQL was determined to be 41 ng/SPMD, the water concentration estimated from these two values would be < 75 and < 140 pg/L, respectively. The water temperature in Tumbling Creek Cave was 13.9° C over a long period, but is not precisely known for Fantastic Caverns. All water concentrations were estimated for the OCPs, PCBs, and PAHs and are presented in Tables 3 and 4 using a calibration point of 18° C. Any value with an MDL/MQL was flagged as MDL/MQL in water concentration tables.

Very few studies showing the levels of these compounds in cave waters or groundwater have been published. In 2002 the Oklahoma Conservation Commission (Twin Cave Water Quality and Pollution Source Assessment, Final Report) found concentrations of technical chlordane in all of the samples analyzed ranging from 0.071 µg/L to 0.22 µg/L. Two samples had low levels of p,p'-DDT and p,p'-DDE (<0.009 µg/L). USGS evaluated surface water, groundwater and sediments in 1992-1995, and they had limited hits for pesticides or other semivolatile chemicals in the Ozark Region (Petersen et al. 1998). The Tumbling Creek Cave samples had estimated chlordane concentrations in water ranging from 0.001 µg/L to 0.016 µg/L, however the concentration values were below MDLs and

MTLs in the SPMDs and the estimated water concentrations are not valid.

The POCIS sampler deployed in 2004 contained no detectable residues of any type, with detection limits similar to the nonpolar compounds.

No TPH and no dye were detected by a commercial laboratory in the cave stream after a rain event that followed the chip-seal application on Highway 160 by about eight days.

Discussion

Only minute levels (pg/L or parts per quadrillion) of some nonpolar organic contaminants were detected in the TCC stream, far below those allowed by drinking water and other standards. Neither polar organic compounds nor petroleum hydrocarbons were detected. These results lessen concerns about long-term contamination of the cave system. However, it is possible that some non-persistent chemical(s) could have been transported through the system over a short time and could have left no trace. However, there were no significant, known spills or large industrial waste sites in the area.

The Oklahoma Conservation Commission (2002) studied contaminants in Twin Cave, Delaware County, Oklahoma, using SPMDs. They found small amounts of 48 organic compounds, including chlordane, 4,4' DDE and 4,4' DDT, legacy organochlorines now banned from use. Using large volume injections, they also found caffeine and o-benzyl-p-chlorophenol, indicators of human waste contamination. Yet Twin Cave is in a rural area, like Tumbling Creek Cave. No contaminants were found at unusual levels, even though volatile chemicals had been found previously, probably caused by episodic dumping of waste materials into a sinkhole. Toxicity testing led to the conclusion that the levels found were not threatening to cave fauna, but they used standard test species, *Ceriodaphnia dubia*, a "daphnia," and *Pimephales promelas*, the fathead minnow. These species may not be representative of troglobitic species, the sensitivities of which are generally unknown.

Neill et al. (2004) characterized agricultural practices around Tumbling Creek Cave and their influence on water quality. Neill et al. discussed sources of chloride and nitrate from a now-restored area near TCC, which had been cleared of

trees by a previous owner between the late 1980s and mid-1990s, and converted to little more than a cattle feed lot. Chlorides could have come from salt blocks for the cattle, and nitrates from cattle waste, but were diluted greatly by the time they entered the cave stream. The restored area, also within the TCC recharge area, apparently provided cleaner waters to the system. By 2004 a leaking sewage lagoon was discovered at the Mark Twain School within the recharge area, but its contribution to nitrate in the cave stream has not been quantified.

Neill et al. discussed possible metal sources in sinkhole dumps within the area. John Besser and Kathy Echols at CERC are examining cave stream sediments for metals. Sediment samples from TCC collected in 2004 had below-detection-limit levels of OC pesticides, total PCBs and PAHs.

Elliott and Aley (2006) discussed studies in the cave, land use practices and corrective measures recently undertaken in the recharge area. In early 2006 the Working Group constructed a cavesnail propagation laboratory in the cave. The laboratory apparatus is being tested at this time, using local well water and cave water piped in through plastic line or garden hose, respectively. A test surrogate species, *Physa haley*, from Perry County, Missouri caves, died off after being tested in well water in the propagation laboratory. Further tests indicated that *Physa* thrives in cave water. At this time we do not know what killed the *Physa*. There will be further work on this problem.

In 2006 32 terra cotta tiles were placed in the cave stream by Paul Johnson and Stephanie Clark at the request of the Working Group (Figure 5). The tiles provide a clean surface above the sediments for the snails. From February 2007 to May 2008 David C. Ashley observed cavesnails crawling on the tiles over four trips, an encouraging development.

The MODOT/OUL study of Highway 160 found no petroleum hydrocarbons in the TCC road ditch or cave stream in water samples collected at appropriate times. This result does not eliminate road spills as a potential threat to the cave, but it is reassuring that the road now can be maintained with a low-impact method.

Our study has eliminated many potential, persistent organic carbon contaminants from serious consideration as causes of the decline in *Antrax*. POCIS samplers could still be used to detect pharmaceuticals, antibiotics, caffeine and other waterborne polar compounds. A very large array of

possible substances could be found in the environment, but not all could be detected in this study because of limited duration and funding.

Siltation was one of several possible factors in the cavesnail's decline outlined in the Fish & Wildlife Service's recovery plan (2003) and Elliott and Aley (2006). Siltation in TCC may be decreasing, as measured by turbidity (Figure 6). Additional data are needed to be sure that this is a real trend. Septic system wastes such as nitrates and ammonia, farm dump sites in sinkholes, and oxygen depletion remain as possible influences. Based on observations by Tom Aley and Cathy Aley, David C. Ashley, and others, we conclude that an unusual influx of sediments was the probable cause of the decline in the Tumbling Creek cavesnail, but other factors may have contributed to the decline. We have little or no information on the sensitivity of aquatic snails to chemicals, as illustrated by the die-off of *Physa* in the new laboratory apparatus.

Other ecological factors, such as the decline of

Gray bats for decades in the cave, also could have had an adverse impact on *Antrobia*. Gray bats have increased again since 2004 at TCC because of conservation work at the cave. We do not yet know to what extent bacterial biofilms on the rocks in the cave stream provide food for the cavesnail, and if that biofilm is especially nourished by Gray bat guano. We do not know if guano caused significant oxygen depletion as well. TCC and many Ozark cave streams also have black coatings of manganese oxide on the cobbles and bedrock, which may be deposited with microbial influence, the details of which are not known to us.

The "Tumbling Creek Cave Ecosystem" is now recognized by MDC and its partners as a "Conservation Opportunity Area" within the new initiative called the "Comprehensive Wildlife Strategy." This is a long-term, statewide conservation planning effort that recognizes certain areas for their high biodiversity, wildlife and natural resources (Elliott 2006).



Figure 5 Drs Stephanie Clark and David C. Ashley, March 30, 2006, with terra cotta tiles placed in Tumbling Creek for the cavesnail, *Antrobia culveri*.

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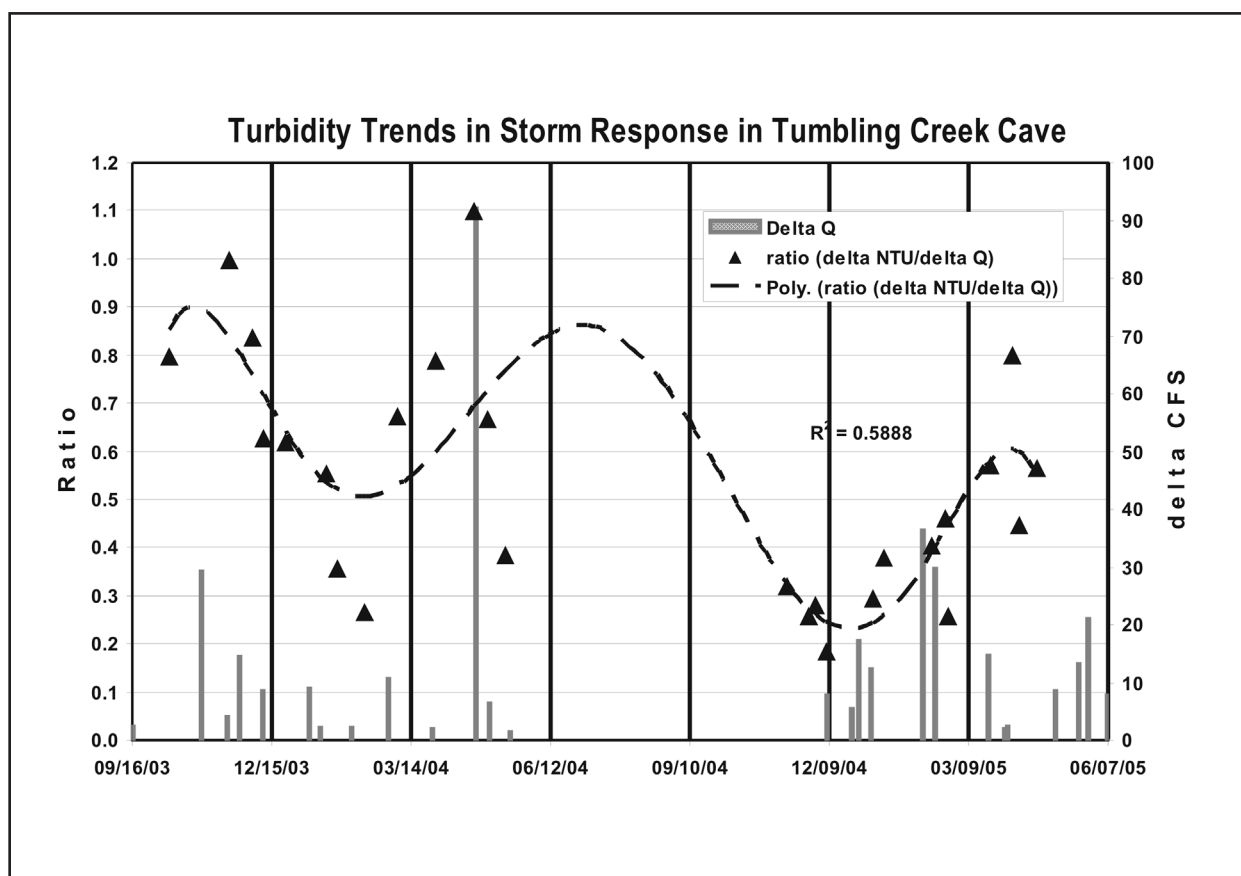


Figure 6 Turbidity trends in storm response in Tumbling Creek, using the delta values for both turbidity and discharge, as measured with in-stream sensors and a data logger. There are two apparent trends: more intense storms and turbidity in the summer and a trend towards lower ratios of turbidity to discharge from 2003 to 2004. Graph by Philip Moss, OUL.

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