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POTENTIAL EFFECTS OF RECURRENT LOW OXYGEN CONDITIONS ON THE ILLINOIS CAVE AMPHIPOD

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The caves of Illinois' sinkhole plain are the sole habitat of the Illinois Cave amphipod (Gammarus acherondytes)*, a federally endangered species. The sinkhole plain is a hydrologically-connected sequence of karstified limestone that constitutes an extensive karst aquifer which serves as an important source of potable water for area residents. During this investigation, we examined the ground-water quality in caves within two ground-water basins: 1) Illinois Caverns, where the amphipod is now present after previously reported to have been extirpated from the lower reaches, and 2) Stemler Cave, where the amphipod is reported to have been extirpated. The chemical composition of cave streams in Illinois Caverns and Stemler Cave were compared to determine which parameters, if any, could have contributed to the loss of* G. acherondytes *from Stemler Cave. Stream water in Stemler Cave contained higher concentrations of organic carbon, potassium, silica, chloride, fluoride, sulfate, iron and manganese than Illinois Caverns. Perhaps most importantly, dissolved oxygen (DO) concentrations in Stemler Cave were, during periods of low flow, substantially lower than in Illinois Caverns. Based on land use, there are probably at least eight times more private septic systems in the Stemler Cave ground-water basin than in the Illinois Caverns ground-water basin. Low DO concentrations were likely the result of microbial breakdown of soil organic matter and wastewater treatment system effluent, and the oxidation of pyrite in bedrock. The near-hypoxic DO in Stemler Cave that occurred during low-flow conditions, and, we speculate, a limited range of* G. acherondytes *within the Stemler Cave ground-water basin due to a metabolic advantage of the stygophilic aquatic invertebrates over the stygobitic* G. acherodytes*, resulted in the apparent loss of* G. acherondytes *from Stemler Cave.*

Introduction

Gammarus acherondytes in Illinois Caves

The cave streams of Illinois' sinkhole plain comprise the only habitat of the Illinois Cave amphipod (*Gammarus acherondytes*) (Fig. 1), a federally endangered species (U.S. Fish and Wildlife Service, 2002). The sinkhole plain is a hydrologically connected sequence of karstified limestone that constitutes an extensive aquifer in southwestern Illinois (Fig. 2). Each cave within the sinkhole plain lies within or is the focal point of a ground-water basin (analogous to a watershed). Because of the rapid movement of ground water through conduits within the subsurface, ground water in karst aquifers undergoes little natural cleansing (White, 1988). Contaminants from row-crop agriculture, livestock waste, urban runoff, and wastewater treatment systems are easily transferred to the subsurface where they may have adverse effects on the quality of the aquatic environment and its aquatic biota (USFWS, 2002).

In this investigation, samples were collected from two reaches of the main cave stream in Illinois Caverns and the cave stream in Stemler Cave (Fig. 2). Based on a survey conducted along nine transects in Illinois Caverns in 2000, the upper reach of the stream in Illinois Caverns (upstream and just downstream of the main entrance) supports a viable/stable population of *G. acherondytes*, but the amphipod was absent in lower reaches of the cave stream (up to the "T-Junction;" a map of the cave may be found in Panno *et al.*, 2004). Its absence was assumed to be related to a poorer water quality in these reaches (Lewis, 2000). A "viable/stable population" is defined as density of *G. acherondytes* of approximately ten individuals per m^2 (Lewis, 2003; Venarsky 2005). In addition, Lewis (2003) found a correlation between the increase in biofilms and filamentous microbial growth on cave stream gravels (these filaments bridged gaps between the gravels in the downstream parts of Illinois Caverns) and the decrease in population of *G. acherondytes* relative to the upstream areas where the amphipod was found. However, during our investigation, *G. acherondytes* was found in about equal densities in both the upstream and downstream portions of Illinois Caverns. Conversely, *G. acherondytes* is reported to have been completely extirpated from Stemler Cave (Fig. 2). *Gammarus acherondytes* was reported to be present in Stemler Cave based on an examination of samples collected in 1965 (Peck and Lewis, 1978), but more recent surveys (Peck and Lewis, 1978; Webb, 1995; Taylor and Webb, 2000; Lewis *et al.*, 2003) and our investigation failed to locate any individuals.

The objective of this investigation was to compare water-quality parameters between Stemler Cave and Illinois Caverns

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Figure 1. Photograph of *G. acherondytes* collected from Illinois Caverns (photograph by Frank Wilhelm). Scale bar is 1 mm.

to determine if differences in water quality between the two cave streams could account for the presence of *G. acherondytes* in Illinois Caverns and its reported absence from Stemler Cave. Because the geology and hydrology of the two ground-water basins are similar (Panno *et al.* in press a, b), any differences in the chemical compositions of the two cave streams are most likely due to differences in land use between the ground-water basins. Specifically, changes in land use within the Stemler Cave ground-water basin between the 1960s and the 1990s may have resulted in changes in cave-water quality that could not be tolerated by *G. acherondytes*, resulting in its apparent extirpation.

Geology and Hydrology

The two caves lie within Illinois' sinkhole plain in the southwestern part of the state. Bedrock consists of calcite-rich, Mississippian-age limestone that either crops out or is covered by up to 15 m of glacial till and loess (Herzog *et al.*, 1994). The bedrock surface is predominantly St. Louis Limestone, with relatively thin occurrences of the overlying Ste. Genevieve Limestone. Karst and cave formation are controlled by the lithology, poor primary porosity coupled with well-developed secondary porosity (*i.e.*, near vertical fractures and dilated bedding planes). The majority of caves in the study area, including Illinois Caverns and Stemler Cave, are branchwork type caves that formed along bedding planes within the St. Louis Limestone (Panno *et al.*, 2004).

Illinois Caverns is located near the center of the sinkhole plain and contains about 9.6 km of explored passage (Panno *et al.*, 2004). Stemler Cave is located 29 km to the north of Illinois Caverns and is shorter, with only about 1.8 km of explored passage. Both caves contain perennial streams, and the stream beds contain bedrock, cobble, gravel, sand and silt substrates.

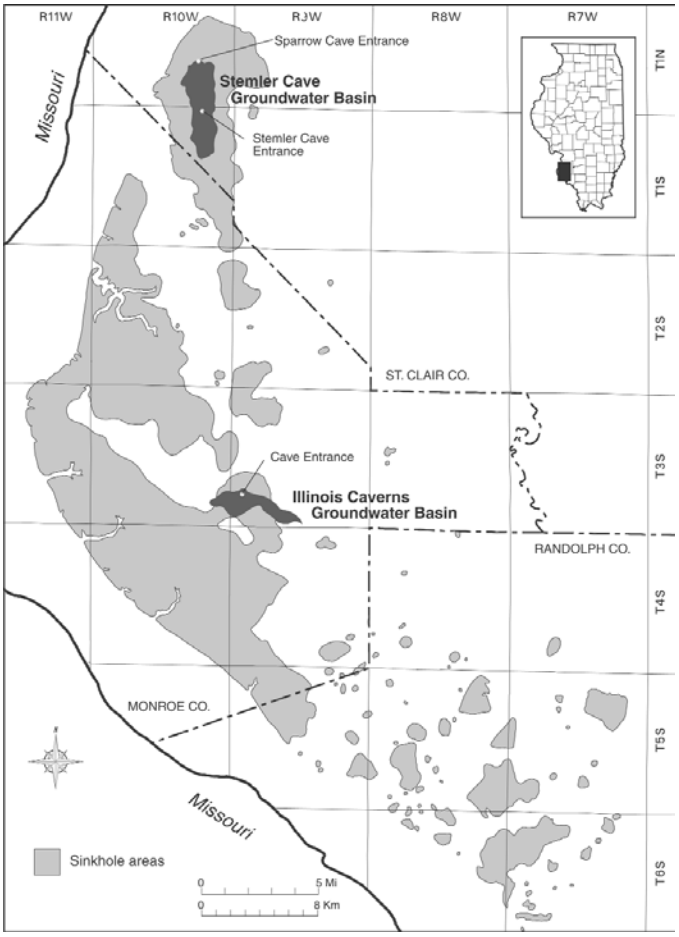

Figure 2. Map showing the locations of Illinois Caverns and Stemler Cave in southwestern Illinois' sinkhole plain (modified from Panno *et al.*, 1997). The black shapes near the cave entrances represent the groundwater basins for each cave (basin boundaries modified from Aley *et al.* 2000).

Land Use Analysis

Land use in both the Illinois Caverns and Stemler Cave ground-water basins is dominated by agriculture (Table 1). The main difference between the two basins is the amount of urban land. The Stemler Cave Ground-Water Basin has a higher percentage of urban land (4.1%) than the Illinois Caverns basin (0.53%). Urbanization of the Stemler Cave basin has primarily occurred in the last two decades, and recently there has been a large increase in the number of permits to build single family houses in the basin (D. Newman, Illinois Nature Preserves Commission, personal communication, 2004). Municipal sewerage systems are not present in either basin, and all households use on-site wastewater-treatment systems. Many of these systems discharge within the drainage area of a sinkhole (Panno *et al.*, 1997). An eight-fold increase in urban land use could mean a similar increase in the number of private septic systems in the Stemler Cave ground-water basin. In addition,

Table 1. The percent land use in the Illinois Caverns and Stemler Cave ground-water basins based on maps by Aley *et al.* (2000); land-use estimates were made using GIS techniques.

Land Use	Illinois Caverns ($6.03\ km^2$)	Stemler Cave ($19.1\ km^2$)
Row crop agriculture	62.8	57.7
Rural grasslands and misc.	17.0	17.5
Urban	0.53	4.05
Wooded	17.5	15.8
Waterways, wetlands, *etc.*	2.21	5.02

intensified construction activities in the region could have increased the sediment load of runoff; sedimentation associated with such development could have adverse affects on the cave biota (USFWS 2002).

Methods

To investigate cave-water quality, 19 stream-water samples were collected from Illinois Caverns and seven from Stemler Cave. Nine of the samples in Illinois Caverns were collected about 50 m upstream from the main entrance, and 10 were collected from a location approximately 800 m downstream from the entrance, in a reach reported by Lewis (2000) to be devoid of *G. acherondytes*. Ground-water samples were collected monthly to bi-monthly between May 2003 and March 2004. Because of our findings, four months into the investigation we began sampling Stemler Cave where the Illinois Cave amphipod was reported to have been extirpated about 25 years earlier. Cave-stream samples from Stemler Cave were collected at its entrance (a sinkhole intersecting the downstream reach of the subsurface stream) monthly to bi-monthly between September 2003 and May 2004. Because of this adjustment in our sampling efforts, ground-water samples from April through August 2003 collected from the two caves were not time synchronous.

Ground-water samples were collected just before and soon after agrichemical application for five out of eight sampling events. Springtime applications of synthetic fertilizer (estimated to be 50% of the annual application) and herbicides generally occur between mid-April and mid-May; fall application (the other 50%) generally occurs in November (P. Kremmell, State Extension Agent, Southern Illinois University, Edwardsville, personal communication, 2000). Samples were collected during periods of both high and low stream discharges. In general, grab samples were collected, but nine of the samples from Illinois Caverns and 1 from Stemler Cave were collected using automatic samplers (ISCO®) in May, June, September, December 2003 and March 2004. These were composite samples that were mixtures of samples collected daily for 24 days in order to have integrated ground-water samples that included contaminants carried into the cave during and following large rainfall events. Some of the water sampling events were coordinated with timed area searches and bait trapping of invertebrates at the same ground-water sampling locations in both caves. However, the tasks were separated by several days so that sediments suspended by invertebrate counts did not affect subsequent water sampling. Invertebrates were counted at Illinois Caverns on two occasions; one event was conducted during high-flow conditions (May 2003) and the other at low flow (September 2003). Invertebrates were counted in Stemler Cave on one occasion under high-flow conditions (May 2004). An inspection of Sparrow Creek Cave, the distal end of the Stemler Cave ground-water basin, was conducted in March 2004. Because of the relatively deep water in this part of the cave, a dip net and aquarium net were used to examine sediment whenever possible. When found, all adult amphipods were field identified with the aid of a dissecting microscope, counted, and released.

Temperature, pH, Eh, dissolved oxygen (DO) and specific conductance (SpC) were measured using standard field techniques and meters with temperature compensation. Stream discharge was measured using a stream velocity gage along measured cross sections of the cave streams in non-turbulent stream segments (*e.g.*, Panno *et al.*, 2003). All water samples were analyzed for major cations and anions; ammonium-nitrogen (NH_4-N); total Kjeldhal nitrogen (TKN); herbicides used on fields in the study area including atrazine, bentazon, chlorimuron, cyanazine, glyphosate, imazaquin, imazethapyr, metolachlor, sethoxydim and trifluralin (Panno *et al.*, 1996); total organic carbon (TOC); and caffeine. The detection limits for many of these analyses are available on Table 2; the detection limit for caffeine is 0.15 μg L^{-1}.

Samples for cations, anions and isotopic analyses were filtered through 0.45-μm high-capacity filters and stored in polyethylene bottles. Cation samples were acidified in the field

Table 2. Minimum, median, and maximum values of chemical and physical parameters measured in two locations in Illinois Caverns (n = 19) and Stemler Cave (n = 7). Results are reported in mg L^{-1} unless otherwise noted. Detection limits, where applicable, are reported as less than values.

	Illinois Caverns			Stemler Cave		
Parameters	Minimum	Median	Maximum	Minimum	Median	Maximum
Stream discharge (L s^{-1})	1.8	19	57	18	93	>169
Temperature (°C)	13.6	13.8	14.6	10.7	13.5	14.3
pH (pH units)	7.0	7.8	8.4	7.2	7.6	7.8
DO (mg L^{-1})	6.0	7.9	8.8	2.8	4.0	9.8
O_2 Saturation (%)	58.8	74.5	85.4	24.4	39.6	92.5
SpC (μS cm^{-1})	250	537	622	184	620	753
Alkalinity ($CaCO_3$)	84	226	252	72	245	281
Na	5.60	22.0	33.5	2.06	22.6	29.8
K	<5	<5	13	3	8	14
Ca	36.6	78.7	103	22.1	95.0	105
Mg	5.58	10.3	14.7	4.00	15.5	18.5
Ba	0.07	0.08	0.09	0.05	0.08	0.87
B	<0.01	<0.01	0.04	<0.01	<0.02	0.06
SiO_2	14.6	17.2	21.6	5.91	19.3	20.4
SO_4	13.1	21.8	28.7	10.7	43.6	66.4
Cl	8.1	15.6	19.8	4.3	22	34.2
F	<0.04	0.1	0.3	<0.2	0.3	0.4
Fe	<0.01	<0.01	0.04	<0.01	0.01	0.05
Mn	<0.001	0.01	0.04	<0.001	0.03	0.07
Sr	0.11	0.17	0.20	0.07	0.21	0.23
NO_3-N	4.99	7.04	8.43	0.60	4.30	4.74
NH_4-N	0.02	0.04	0.12	<0.01	0.06	0.28
TKN	0.07	1.02	3.81	0.02	1.66	3.38
PO_4-P	<0.01	<0.01	0.30	<0.01	0.01	0.70
TOC	0.9	1.6	8.2	2.4	3.2	10.2
Atrazine (μg L^{-1})	<0.05	0.79	4.79	<0.05	<0.05	0.38
Metolachlor (μg L^{-1})	<0.02	0.12	1.54	<0.02	0.06	0.42
Trifuralin (μg L^{-1})	<0.01	0.02	0.05	<0.01	<0.01	0.01

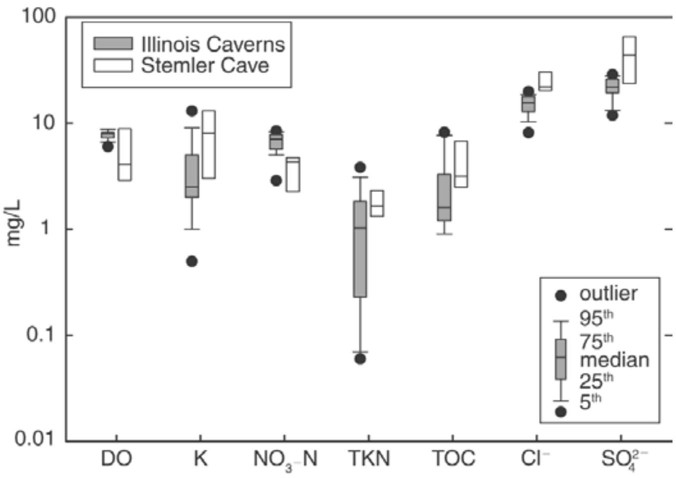

Figure 3. Box and whisker plots comparing stream water quality in Illinois Caverns (n = 19) and Stemler Cave (n = 7). Whiskers are missing from the Stemler Cave data because there were an inadequate number of samples to calculate the 5th and 95th percentiles.

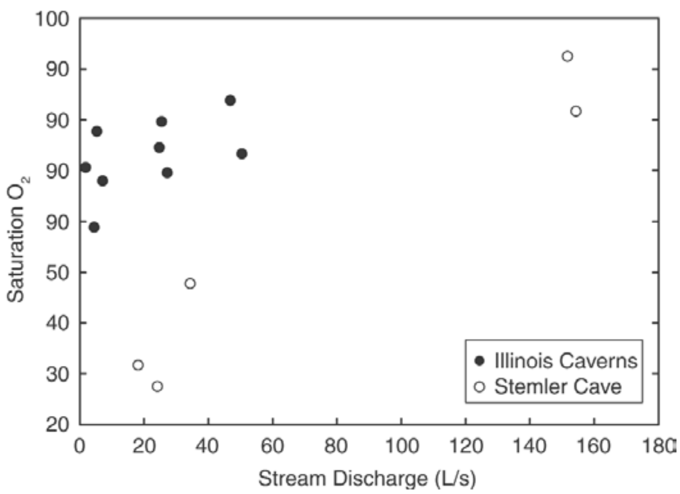

Figure 4. Percent O_2 saturation in cave streams as a function of stream discharge in Illinois Caverns and Stemler Cave. Stemler Cave exhibited a very low % O_2 saturation at low flow.

with ultra-pure nitric acid to a pH of < 2. All samples were transported to the laboratory in ice-filled coolers and kept refrigerated at approximately 4° C until analysis.

Cation concentrations were determined with an inductively coupled argon plasma spectrometer, and anions were determined by ion chromatography as described in Panno *et al.* (2001). TKN and NH_4-N were determined colorimetrically as described in Panno *et al.* (2006). Organic N was operationally defined as the difference between TKN and NH_4-N. Herbicides were determined with gas chromatography-mass spectrometry techniques using USEPA Method 508 (USEPA, 1988).

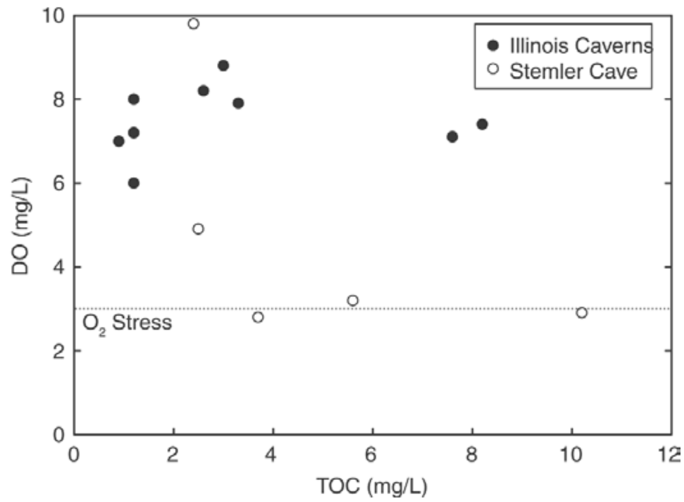

Figure 5. DO *vs* TOC concentrations of Illinois Caverns and Stemler Cave stream-water samples. Stemler Cave water exhibits a relationship with TOC not seen in Illinois Caverns water.

Caffeine was determined using high performance liquid chromatography. TOC was determined using a carbon analyzer.

Optical brighteners were measured during one sampling episode. The presence of optical brighteners in water is a strong indication of the presence of septic effluent (Alhajjar *et al.*, 1990). White cotton fabric devoid of optical brighteners was placed in both cave streams in February 2004 and retrieved the following week. Samples were analyzed with a spectrofluorophotometer using a technique described by Aley (1985).

Results and Discussion

Chemical Composition of Cave Ground water

There were no statistically significant differences in the chemical composition of the cave-stream water in the two sample locations within Illinois Caverns, and the composition was within the normal range for karst ground water in this region (Panno *et al.*, 1996). Thus, we combined the results from both locations within Illinois Caverns when comparing them to Stemler Cave. The range and median of parameter concentrations from each sampling site were used as descriptors; mean values were not used because they are easily biased by extreme values.

There were differences in stream water quality between Stemler Cave and Illinois Caverns (Table 2; Fig. 3). The results of this investigation showed that Stemler Cave water generally had higher concentrations of TOC, potassium (K^+), chloride (Cl^-), sulfate (SO_4^{2-}) and manganese (Mn), and lower pH and NO_3-N concentrations than Illinois Caverns. Phosphate (PO_4-P) was detected in four of seven Stemler Cave samples but only two of sixteen Illinois Caverns samples. The differences were statistically significant for K^+, SO_4^{2-}, Cl^-, and NO_3-N, as

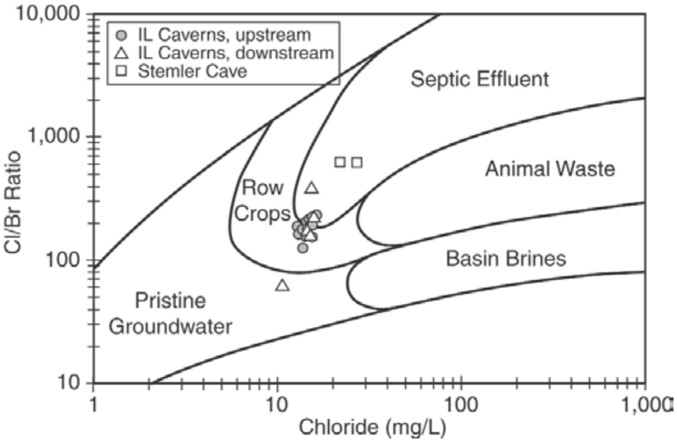

Figure 6. Cl/Br *vs* Cl^- concentrations (domains from Panno *et al.*, 2006) of Illinois Caverns and Stemler Cave stream-water samples revealed that groundwater in both caves is affected by both row crop agriculture and septic effluent. Data were from previous sampling efforts conducted by Panno et al. (ISGS, unpublished data).

determined using the rank sum test, but not for the other parameters. These results are consistent with those of Panno *et al.* (2001) who had time-synchronous, seasonally-collected samples from Illinois Caverns and the resurgence spring of Stemler Cave. In their study, Panno *et al.* (2001) also observed higher concentrations of iron (Fe), silica (SiO_2), and fluoride (F^-) in ground water from the Stemler Cave system. In this present investigation, stream water in Stemler Cave also had a wider range with both the lower and upper extremes of DO concentrations than that in Illinois Caverns stream water. Although based on limited data, DO concentrations in the cave streams appeared to be influenced by discharge; at high-flow rates, DO concentrations (converted to % saturation O_2, to take water temperature into account) were elevated, especially in Stemler Cave. At low flow rates, % O_2 saturation in Stemler Cave was much lower than in Illinois Caverns (Fig. 4).

The lower pH, DO, and NO_3-N, and elevated Fe, Mn and TOC concentrations in Stemler Cave waters suggest a more organic-rich soil-water component entering the Stemler Cave stream than that of Illinois Caverns (Panno *et al.*, 2001). Dissolution of soil organic matter (SOM), and human/animal waste can increase TOC concentrations; the water samples from Stemler Cave typically had a pale-yellow color (during low-flow conditions) suggesting the presence of elevated concentrations of organic acids. Reduction of SOM and human/animal waste consumes O_2 and generates CO_2, which lowers the pH due to formation of carbonic acid (H_2CO_3). Low-DO concentrations coincided with TOC concentrations greater than about 3 mg L^{-1} in Stemler Cave. However, DO in Illinois Caverns stream water didn't show this relationship with TOC (Fig. 5). Alkalinity, which is a proxy for dissolved CO_2 concentrations, was slightly higher in samples from Stemler Cave than Illinois Caverns. Thus, if water recharging Stemler Cave remains in contact with soil longer than in the Illinois Caverns basin and receives more animal/human waste, it should be more reducing with a lower pH and higher organic carbon.

The stream-water chemistry in Stemler Cave appears to be influenced by two types of water: (1) soil water seeping into the ground water that feeds the cave, and (2) nutrient-enriched wastewater effluent or animal waste discharging and/or being washed into sinkholes directly connected to the karst aquifer. The water chemistry is also influenced by reaction with the limestone bedrock; the water is generally close to saturation with respect to calcite, and the likely source of elevated concentrations of SO_4^{2-} in Stemler Cave water (median concentration two times greater than Illinois Caverns) may be the oxidation of pyrite in the bedrock limestone (Panno *et al.*, 2001; Hackley *et al.*, in press). Elevated Fe concentrations and lower pH in Stemler Cave water, relative to Illinois Caverns, further supports this hypothesis. Oxidation of pyrite within the limestone could substantially contribute to the reduction of DO in ground water feeding Stemler Cave. The relatively elevated Cl^-, F^- and K^+ concentrations and the more frequent occurrence of PO_4-P above its detection limit in Stemler Cave cannot be explained by soil-water contributions or water-rock interactions alone, and are more likely the result of surface-borne contamination. Potential sources of Cl^-, F^-, K^-, and PO_4-P include human wastewater, animal waste, and fertilizer (KCl) (Panno *et al.*, 2002, 2006). Potassium and PO_4-P are generally immobile in the soil zone, being adsorbed by clay particles and organic compounds. Without a geologic or anthropogenic source, they are typically below detection or at low concentrations in subsurface waters. Chloride and K^+ were elevated in Stemler Cave throughout the year, except during a major recharge event when the Cl^- concentration was only 4.3 mg L^{-1}. This suggests a continuous source that generally bypasses the soil zone, where K^+ and PO_4-P would likely have been removed. The most likely source of Cl^-, F^-, K^+, and PO_4-P is effluent from on-site wastewater treatment systems, many of which typically discharge directly into sinkholes in this area (Panno *et al.*, 1997).

To test the hypothesis of the presence of septic effluent, we looked at caffeine, optical brighteners and Cl/Br ratios. Caffeine was detected in only one sample, in the downstream location of Illinois Caverns in September 2003. Optical brighteners were measured on only one occasion (March 2004) and were detected in Stemler Cave but not Illinois Caverns. These two compounds are indicators of human wastewater, and their detection is evidence of effluent from domestic wastewater treatment systems in both caves. Caffeine appears to be only episodically present at concentrations great enough to be detected by the technique used in this investigation. A new version of a Cl/Br *vs* Cl^- scatter diagram (developed by Panno *et al.*, 2006) including groundwater samples from both caves (only a few samples had Br^- concentrations above detection limits) provides further evidence of the presence of septic effluent in Stemler Cave and, to a lesser extent, Illinois Caverns (Fig. 6). The relatively low Cl/Br ratio precludes road salt from being the source of Cl^- (Panno *et al.*, 2006). The infiltration of septic effluent into the shallow karst aquifer is also consistent with the lack of seasonal trends for Cl^- and K^+, because effluent would be introduced into the hydrologic system year round. Elevated TOC and lower DO in Stemler Cave may also be due in part to a greater influx of septic effluent. Boron, another element associated with wastewater, was detected (> 0.01 mg L^{-1}) in about 5% of Illinois Caverns samples, whereas, it was detected in about 38% of the Stemler Cave samples. Livestock are present, but uncommon within the ground-water basins. Contamination of both Illinois Caverns and Stemler Cave by human/animal waste has been reported previously; enteric bacteria, sometimes at very high levels, are almost always detected when these cave streams have been tested (Panno *et al.*, 1996; Taylor *et al.*, 2000).

Herbicides have been shown to have adverse impacts on aquatic biota (U.S. Fish and Wildlife Service, 2002), but the fact that herbicides were detected in both Illinois Caverns and Stemler Cave, and at higher concentrations in Illinois Caverns, suggests that they alone are not responsible for the extirpation of *G. acherondytes* from Stemler Cave. The herbicides further suggest that there was a stronger agricultural influence within the Illinois Caverns ground-water basin.

CAVE BIOTA

Gammarus acherondytes was found in both upstream and downstream locations in Illinois Caverns in surveys undertaken in May 2003 and September 2003. Populations were roughly equal at both locations in spite of previous extirpation in the downstream reaches. *Gammarus acherondytes* was not detected in Stemler Cave in a survey during May 2004 or during reconnaissance trips in November 2003 and March 2004 to Sparrow Creek Cave, the resurgence of the Stemler Cave ground-water basin, located several km to the north (Fig. 2). The presence of biofilms and total absence of cave biota within Sparrow Creek Cave suggests the possible discharge of animal and/or human waste prior to the survey. No similar biofilms were observed in Illinois Caverns during or prior to this investigation. In July 2000, Lewis (2003) conducted a survey in Spider Cave, a small cave located about 1 km to the north of the entrance to Illinois Caverns. Within the cave system, there was a strong, putrid odor and evidence of sewage and/or animal waste. Lewis (2003) reported that a putrid, microbial mat covered all surfaces and was present in interstices within the cave; the microbial mat and odor were gone by the following September 2000. Subsequent work by F. Wilhelm at Spider Cave in September 2003 revealed no additional microbial mats or odor and *G. acherondytes* had returned. High levels of bacterial contamination associated with human and livestock waste have been shown to have impacted cave communities elsewhere in the eastern United States (Poulson, 1991; Simon and Buikema, 1997). In Virginia, *Caecidotea recurvata* (Steeves), an asellid isopod, had a higher tolerance for waters negatively influenced by septic effluent than did the gammarid amphipod *Stygobromus mackini* Hubricht. Elsewhere in Virginia, Culver *et al.* (1992) attributed the extirpation of the stygobitic crangonyctid amphipod *Crangonyx antennatus* Packard and the asellid isopods *C. recurvata* and *Lirceus usdagalun* Holsinger and Bowman to increased flux of organic pollutants in the karst system. These observations suggest that discharge or dumping of sewage and/or animal waste in the sinkhole plain may occur and could be responsible for the apparent extirpation of *G. acherondytes* from cave streams.

CONSEQUENCES TO CAVE BIOTA

Probably the most important difference that we observed in the chemical composition between the two cave streams is the periodically low DO concentrations in Stemler Cave. Differences in other chemical parameters do not appear to vary sufficiently to account for the apparent absence of *G. acherondytes* from Stemler Cave and its continued presence in Illinois Caverns. Low-DO concentrations are known to be deleterious to aquatic organisms; average DO concentrations less than 3 mg L^{-1} are stressful for aquatic organisms, and concentrations less than 2 mg L^{-1} are defined as hypoxic (SCDNR, 2004). The lowest measured DO concentration in Stemler Cave was 2.8 mg L^{-1}, and it is possible that even lower concentrations can occur. We conclude that of all the chemical data we examined, low DO is the most likely cause contributing to the apparent loss of *G. acherondytes* from Stemler Cave.

Even though we have a limited number of samples, the DO concentrations in Stemler Cave stream water were depressed (and possibly hypoxic) during periods of low flow. The loss of all biota at the resurgence of Stemler Cave (Sparrow Creek Cave) and observations of the formation of biofilm on rocks and walls of the cave suggest that the aquatic biota of Stemler Cave are subjected to periodic low oxygen stress. This likely resulted from periodic and some continuous influx of human and/or animal waste. Such a reduction of DO is often responsible for the decline of aquatic biota in waters receiving organic waste.

Taylor *et al.* (2003) suggested that cave facultative amphipods such as *Gammarus troglophilus* could out-compete (through numerical displacement) *G. acherondytes* because of metabolic advantages under heightened-food conditions in caves. Although the lower metabolic rate of *G. acherondytes* would have an advantage in a "food-poor hypogean environment" such as a near pristine cave, urbanization and the influx of abundant organic matter from anthropogenic sources (*e.g.*, septic effluent) would favor the proliferation of *G. troglophilus* (Wilhelm *et al.*, 2006). The influx of organic materials into the cave streams would increase in severity with greater anthropogenic surface activities (*e.g.*, Panno *et al.*, 1996, Simon and Buikema 1997). We speculate that because of the metabolic advantage of the stygophilic biota, the stygobitic *G. acheroudytes* may not have had as wide a range in the Stemler Cave ground-water basin as did the other aquatic invertebrates, thereby allowing the other aquatic invertebrates (but not *G.*

acherondytes) to recolonize Stemler Cave following periods of low DO in the cave stream.

The recent (since 2000) repopulation of downstream reaches in Illinois Caverns by *G. acherondytes* suggests either the quality of water recharging the cave has improved or that the distribution of *G. acherondytes* is sufficiently widespread in the cave to allow repopulation following low oxygen episodes in parts of the stream. This also suggests that the timing of population surveys (*i.e.*, amount of time following hypoxic episodes), is critical to accurately determining the abundance of invertebrate populations.

Conclusions

During this investigation, *G. acherondytes* was found in both upstream and downstream locations in Illinois Caverns, contrary to previous surveys when it was nearly absent from downstream reaches. This can be attributed to timing of surveys, improved water quality, or, more likely, periodic loss and repopulation of the amphipod in the downstream reaches of Illinois Caverns. Multiple surveys over the past 40 years support a conclusion that *G. acherondytes* has been extirpated from Stemler Cave; however, it is possible that the amphipod may exist in small tributaries within the ground-water basin and have the potential to repopulate the cave stream if the water quality improves. Stemler Cave stream-water samples had higher concentrations of TOC, K^+, Cl^-, F^-, SO_4^{2-}, SiO_2, Fe and Mn, and lower NO_3-N and pH than those from Illinois Caverns, and most importantly experienced substantially lower DO concentrations. The dominant influence on Illinois Caverns stream water appears to be agricultural. Whereas, Stemler Cave stream water appears to be influenced by two distinct sources: one with a large soil water component and the other with a human wastewater/animal waste component, the organic matter of which could contribute to low-DO concentrations. This characterization is further supported by the fact that the percentage of urban land use in the Stemler Cave ground-water basin is about eight times greater that in the ground-water basin of Illinois Caverns.

The low DO in Stemler Cave apparently occurs only during low-flow conditions, causing the cave stream aquatic invertebrates to periodically (seasonally?) experience low-oxygen conditions and increased stress. The presence of optical brightener, wastewater microbial taxa, and the results of Cl/Br *vs* Cl^- scatter diagrams of Illinois Caverns and Stemler Cave water suggest that effluent from wastewater-treatment systems is also entering their ground-water basins, most likely through discharge into sinkholes. This situation would make organics (a heightened-food condition) much more abundant within the caves. Based on land-use data, the ground-water basin of Stemler Cave could contain at least eight times more private septic systems that that of Illinois Caverns. Dissolved oxygen is probably lost not only from degradation of human waste, but also from degradation of SOM, as well as pyrite oxidation. We speculate that because of metabolic differences between *G. acherondytes* and other aquatic invertebrates, *G. acherondytes* may not have had as wide a range in the Stemler Cave ground-water basin as the other aquatic invertebrates. This situation would allow them, but not *G. acherondytes*, to recolonize the downstream reaches of the cave following periods of low DO. The fact that cave facultative amphipods have a metabolic advantage over *G. acherondytes* under heightened-food conditions probably led to its displacement and apparent extirpation from Stemler Cave.

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