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THE AQUATIC INVERTEBRATES OF

MYSTERY CAVE,

FORESTVILLE STATE PARK, MINNESOTA

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Abstract

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Aquatic invertebrates were sampled from lotic and lentic waters in Mystery Cave, Minnesota. Nine micro-crustaceans, 3 rotifers, and 25 insect taxa were collected from sites within the cave. Two species of Collembola not previously reported from Minnesota were collected from pools along a tour route. Some of the micro-crustaceans and rotifers had eggs attached, displaying evidence of reproducing populations. Adult mayflies, stoneflies, and chironomids were found on walls of cave passages; however, it is doubtful that reproduction of the aquatic insects is occurring in the cave. The insect fauna in the lotic waters of the cave likely originate as drift above a sink hole in a nearby surface stream, and represent loss from the surface waters. Introduction

Cave systems present unique challenges and opportunities to the fauna which inhabit them. Temperature fluctuations are usually minimal and climatic extremes are often dampened, but light is absent, and food resources are often lacking. Some organisms have become obligate cave dwellers (troglobites) and show various adaptations to the environment, some can live in caves as well as surface waters (troglophiles) while others are 'accidentals' and cannot complete their life cycles in the caves (Peck and Lewis 1977).

Larger animals and terrestrial invertebrates (cave crickets, spiders) are highly visible cave fauna. Aquatic organisms are more secretive and often less accessible. Thus, this fauna may

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Acknowledgements: I would like to thank Warren Netherton and the staff at Mystery Cave for expert assistance and making sure that I always was able to come <u>out</u> of the cave each time I went in. Thanks also to Dr. Kenneth Christiansen of Grinnell College Iowa for his expert help in the identification of the Collembola that were collected.

Funding for this project was approved by the Minnesota Legislature M.L. Chapter 254, Art. 1, Sec. 14, Subd. 3(1) as recommended by the Legislative Commission on Minnesota Resources from the Minnesota Future Resources Fund. often be overlooked. Fish, crayfish or amphipods are larger and easier to collect while oligochaetes, Chironomids or microcrustacea are microscopic and require specialized collection methods. Several recent studies (Kathman and Brinkhurst 1984, Peck 1988, Brussock et al. 1988) have begun to examine aquatic invertebrates in their surveys and to document micro- and macroinvertebrates found in cave waters.

Information on the invertebrates in Mystery Cave is limited to data from Peck and Christiansen (1990) in a report on cave fauna in a four state area of the Upper Mississippi Valley. However, their efforts were not focused on the aquatic fauna. After the Minnesota Department of Natural Resources purchased the cave and became responsible for management, DNR Parks personnel were contacted regarding information on the aquatic benthic organisms. Due to the sparse data, preliminary survey sampling was begun in 1989 on the aquatic invertebrates of the cave. The study was expanded in 1991 as part of a Legislative Commission on Minnesota Resources (LCMR) grant entitled "Mystery Cave Resource Evaluation". Invertebrates were collected from waters within various areas of the cave and two surface sites. The major purpose of the study was to begin documentation of the fauna within the cave and associated surface waters, and to aid interpretive efforts by Parks personnel.

Materials and Methods

Study Site

Mystery Cave is located in southeastern Minnesota (Fig. 1) and is a recent acquisition to the Minnesota State Parks system.

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It is the 32nd longest cave in the United States with over 20 km of mapped passages. Much of the waters in the cave originate in sinkholes in the bed of the South Branch Root River. The waters travel over 2.4 km underground, and reemerge at Seven Springs to the northeast of the cave. In addition to the lotic waters in the cave, there are many pools and lakes within the passages. Sample Methodology

Samples were collected from six sites (Table 1) from 1989 through 1992. A Wisconsin plankton net (80 um mesh) was towed through Blue Lake to collect micro-crustaceans. The same net was set in the Disappearing River for 48 hours as a drift net. Qualitative samples were taken by hand-picking organisms from rocks and substrate at various other sites within the cave. Emerging adult insects and exoskeletons were collected from passage walls. Specimens of Collembola were collected by Parks personnel along a tour route in the cave, and sent to Dr. K. Christiansen (Grinnell College, Iowa) for identification.

All samples were preserved in 80% ethanol and transported back to the laboratory. Drift samples were sorted under a dissecting microscope at 25x magnification after staining with Bierbrich Scarlet - Eosin B. Plankton net tows were examined in a Sedgewick-Rafter cell at 100x with a compound microscope. Microinvertebrates were mounted in polyvinyl lactophenol for identification.

Sampling of surface waters was conducted in the South Branch Root River above a large sinkhole which had been determined to be the major source of water for Coldwater Canyon, and at Seven

Table 1. Collection sites for aquatic macroinvertebrate sampling in Mystery Cave and associated surface waters, 1989 - 1993. (Qualitative = Hand pick from rocks and substrate)

Site

Sample date Sampler

Cave waters

Coldwater Canyon (Mystery I) Coldwater Canyon (Mystery I)	27 October 1989 31 January 1992	Qualitative Qualitative				
The Bath Tub (Mystery I)	31 January 1992	Qualitative				
Disappearing River (Mystery I)	27 October 1989	Drift Net				
Formation Creek (Mystery III) Formation Creek (Mystery III)	14 May 1991 25 February 1992	Qualitative Qualitative				
Blue Lake (Mystery II)	24 August 1989	Plankton net				
Pool along tour route (Mystery I)	26 July 1992	Qualitative				
Surface waters						
Seven Springs	24 August 1989	Qualitative				
Sinkhole (S. Branch Root River)	27 October 1989	Qualitative				

Springs, where waters from the cave reemerge. Organisms were collected with a kick net or by hand picking rocks and substrate. Samples were preserved in ethanol, sorted and identified under a dissecting microscope.

A small white sucker (<u>Catostomus commersoni</u>) was collected in The Bath Tub near Coldwater Canyon. The stomach was removed and the gut contents were mounted on slides and examined with a compound microscope to determine food consumption.

Results and Discussion

A total of 44 taxa (Table 2) were collected from the cave. Insects and Crustacea comprised the majority of all organisms collected (57% and 25%, respectively). Most of the Crustacea were cladocerans or copepods. Adults or exoskeletons of <u>Isoperla</u> sp. (Plecoptera), Heptageniidae (Ephemeroptera) and Chironomidae (Diptera) were collected from walls of the cave, and an unidentified Trichoptera pupae was collected attached to a rock in Formation Creek.

Many of the studies on subterranean waters in North America do not list the micro-crustacean fauna. The sample methods of most surveys (hand picking or coarse nets) are inadequate to collect this group. Dumont (1987) reported groundwater Cladocera collected in European studies. He classified both <u>Leydigia</u> <u>leydigi</u> and <u>Ilyocryptus sordidus</u> as stygobiont (troglobitic) or stygophilic (troglophilic) taxa. Brancelj and Sket (1990) listed Cladoceran species found in subterranean waters in Yugoslavia. In their study of the waters of the Postojna-Planina cave system, they listed <u>Chydorus sphaericus</u> and <u>Ilyocryptus sordidus</u> as

Table 2. Aquatic invertebrates collected in waters of Mystery Cave from August 1989 - June 1993. Pool = Pool along tour route CC = Coldwater Canyon; DR = Disappearing River; BT = The Bath Tub; FC = Formation Creek; BL = Blue Lake Numbers under locations indicate collection date

TAXA Pool CC DR BT FC BL 92 89 92 89 92 91 92 89 ROTIFERA Keratella cochlearis Х Keratella quadrata Х Kellicotia longispina Х Х Bdelloidea TARDIGRADA Х Dactylobiotus? sp. OLIGOCHAETA Naididae Pristina foreli? Х Tubificidae Х Vejdovskyella intermedia CRUSTACEA DECAPODA Orconectes virilus?? Х Х Orconectes propinguus?? CLADOCERA Х Acroperus harpae Alonella excisa? Х х Camptocercus rectirostris? Х Eubosmina sp. Х <u>Ilyocryptus sordidus</u> Leydiqia leydiqi Х COPEPODA Cyclopoida Acanthocyclops vernalis? Х Eucyclops speratus х Harpacticoida Phyllognathopus viguieri Х ARTHROPODA COLLEMBOLA Onchiurus obesus Х X Onchiurus reluctus DIPTERA Ceratopogonidae Х Tipulidae Х Dicranota sp. Simuliidae Simulium sp. Х Chironomidae Tanytarsini Tanytarsus coffmani? Х Chironomini Dicrotendipes notatus grp. Х Microtendipes pedallus grp. Х

14¢.

Table 2. (continued)

		TAXA	Pool	CC	DR	BT	FC	BL
	Orthogladiinae		92	89 92	89	92	91 92	2 89
	Grigotopus biginat		3		v	•		
	<u>Cricocopus picince</u>	<u>us</u> grp.		v	X			
		<u>11a</u> :		A				
	EPHEMEROPIERA							
	Heptageniidae	at		v				
	Stenonema mediopun	CLALUM		X ·			X	
	Stenonema vicarium	1		XX.		X	X X	
	<u>Stenonema</u> sp.			<u>х х</u>				
	Leptophiebildae							
	Leptophiebia sp.						Х	
	Caenidae							
	<u>Caenis</u> sp.						Х	
•	Ephemerellidae			•				
	Ephemerella needha	<u>mı</u> ?					х	
	Hexagenia limbata?		•			Х		
	Baetidae						X	
	PLECOPTERA		*					
	Perlidae			.*				
	<u>Paragnetina media</u>			x				
	Perlodidae		1999 - A.					
	<u>Isoperla signata</u>					Х	X	
	Taeniopterygidae							
	<u>Taeniopteryx</u> nival	is	·				Х	
	<u>Taeniopteryx</u> parvu	lum??				•	X	
	<u>Prostoia</u> ?? sp.						X	
	COLEOPTERA							
	Elmidae							
	<u>Stenelmis cremata</u>		-				Х	
	TRICOPTERA		. •			•		
	unidentified pupae	17、克爾蘭尔	$\chi_{\rm eff} = -\frac{1}{2} \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm eff} \right)^2 + \frac{1}{2} \left(\chi_{\rm eff} - \chi_{\rm$				X	н
MC	DLLUSCA							
	GASTROPODA							
	<u>Ferresia</u> sp.	·		X				
	Physa sp. (shell)	÷		,		х	Х	
	Helisoma sp. (shell	1) .					х	
	BIVALVIA							
	Sphaeriidae	•						
	<u>Sphaerium</u> ?? sp. (s)	hell)		,			х	

'facultative but permanent' inhabitants of the waters. Of the other micro-crustaceans collected form Mystery Cave, only the harpacticoid <u>Phyllognathopus viguieri</u> is reported as common in subterranean waters (Wilson and Yeatman 1959). This was the predominant micro-crustacean collected in drift net samples in the Disappearing River.

Some of the micro-crustaceans collected had eggs attached or in their carapace. This would indicate the strong possibility of reproducing populations in the waters in Mystery Cave. Some of these types of organisms exhibit parthenogenesis - the females produce viable eggs, which hatch into more females. Thus, the population can sustain itself at low levels. Food would not necessarily be limiting, as these animals feed on small organic particles, bacteria or smaller animals. Thus, while not necessarily troglobites, Mystery Cave may sustain populations of these micro-crustaceans in its waters.

Surprisingly, no amphipods or isopods were collected. These crustaceans are commonly found in cave surveys, and numerous species are listed from different caves. There are a number of possible reasons for this conspicuous absence. First, the limited sampling may simply have missed these organisms. Also, it may be that no representatives from these groups, have had sufficient time to establish populations in Mystery Cave. Finally, the flow of the water through the cave may account for this absence. Parts of the cave may be dry in winter (Coldwater Canyon), while spring melts and rains can create enormous volumes of water flashing through other areas, physically destroying

habitat or organisms.

Several specimens of Collembola (springtails) were collected along tour routes in small pools of water. These were identified as <u>Onchiurus obesus</u> and <u>Onchiurus reluctus</u>. Both are reported as cave forms, although <u>O. reluctus</u> is found occasionally on the surface (Christiansen 1982). Neither of these species had been previously reported from the state, representing new distributional records.

An unexpected number of aquatic insect larvae as well as emergent adults were collected from the cave. Nearly all of the insects collected are not normally found in caves, and can be considered accidentals (Peck and Lewis 1977). Other studies to report similar taxa include Peck (1988) who sampled invertebrate fauna from 35 caves and mines in Canada, and Kathman and Brinkhurst (1984) who collected benthic invertebrates from eight caves in Tennessee and Kentucky.

The likely source of the Mystery Cave insect fauna are sinkholes which occur in the South Branch Root River. All of the taxa found in the cave were also collected in kick net samples upstream of one such sinkhole (Table 3). Thus, drift entry from the river seems to be the logical source for these organisms. There is a substantial distance for these drifting invertebrates to travel (upwards of 200 m) before emerging into Coldwater Canyon in the cave. However, a dye trace study done on Mystery Cave (Foster et al. unpublished) suggested that transit time for waters coming in through a sinkhole was relatively short, with a total transit time through the entire cave of under 10 hours.

Table 3. Aquatic macroinvertebrates collected from surface waters near Mystery Cave in August and October 1989. Samples collected qualitatively with kick net, or hand picking from substrate. S. Springs = Seven Springs area, August 1989.

Sink Hole = Above large sink hole in the South Branch Root River, October 1989

TAXA	S. Springs	Sink Hole
EPHEMEROPTERA Heptageniidae <u>Stenacron</u> sp.	• •	Х
<u>Stenonema vicarium</u>	×	X
<u>Stenonema mediopunctatum</u>	verne X	X
<u>Stenonema femoratum</u>	X	
Baetidae	Y Y	•
Baetis intercalaris?		V ·
<u>Pseudocloeon</u> sp.	,	A
Jaonuahia an		Y
<u>Isonychia</u> sp.		A
Caenig en		x
<u>Lentophlebiidae</u>		x
PLECOPTERA		
Perlidae		
Paragnetina media		T A REAL
Taeniopterygidae		
Taeniopteyx nivalis?		X
Leuctridae??		X
TRICHOPTERA		-
Hydropsychidae		•
Cheumatopsyche sp.		. X
<u>Hydropsyche</u> sp.	X	•••
Helicopsychidae		
<u>Helicopsyche borealis</u>		X
Hydroptilidae		
<u>Hydroptila</u> sp.	X	•
Brachycentridae		
<u>Micrasema</u> ? sp.	_ X	
Psychomyidae Daughomuia an	v	
<u>PSychomyla</u> sp. Limnophilidae	~	
Neophylar sp	x	
Neophylax sp. Dychonsyche sp	X	• • • • • • • •
COLEOPTERA	41	
Elmidae	•	
Stenelmis sp	x	x .
Stenelmis cremata		X .
Optioservus sp.		X
Optioservus fastiditus		X
Dryopidae		
<u>Helichus</u> sp.	X	X
-		

Table 3. (continued)

TAXA

S. Springs

MEGALOPTERA Sialidae	
Sialis sp.	v
DIPTERA	A
Simuliidae	x
Tipulidae	A
<u>Tipula</u> sp.	x
Dicranota sp.	x
Chironomidae	41
<u>Corynoneura</u> sp.	x

Alexander (personal communication) indicated that the Disappearing River, Coldwater Canyon and Formation Creek are connected to the South Branch Root River by direct flow with travel times ranging from minutes to around one hour.

Adult insects and exoskeletons were collected from walls of the cave. Additionally, some of the larval stoneflies collected at Formation Creek (Taeniopteryx sp.) were in the final instar, with adult structures visible through the exoskeleton. This seems to indicate that some of the insects that drift into the cave complete their larval growth and emerge as adults. It is unknown whether these insects drift in at a critical larval stage and would complete their growth, or if the water temperatures and food are sufficient to allow completion of the life cycles. For example, the growth and maturation of Parganetina media appears to be dependent on higher water temperatures (20° C) than are commonly found in caves (Radomski, M.S. Thesis). However, Jameson (personal communication) reported warmer temperatures in Mystery Cave waters.

It is highly unlikely that these adults ever reproduce inside that cave, as light often is reported to play a role in reproducative swarming of some insects (Edmunds et al. 1976). One group which may be reproducing are the Chironomidae. Midges have been reported infesting water supply systems in North America, and some species are facultatively parthenogenic, which would enable continuation of the population in the cave.

Gut contents were examined from stonefly and mayfly larvae and from one small fish, a white sucker. <u>Stenonema</u> sp. gut

contents were amorphous detritus, while Paragnetina sp. nymphs contained one heptageniid nymph and several chironomid larvae. Heptageniidae are reported as deposit (sediment) feeders, detritivores, and scrapers (Unzicker and Carlson 1982). Harper and Stewart (1984) list <u>Paragnetina</u> as a predator (engulfer) with such prey items as Diptera, Trichoptera and Ephemeroptera. The stomach of the white sucker contained micro-crustaceans, chironomidae and other small invertebrates. The majority of the identifiable items (74%) were midge head capsules with microcrustaceans comprising the next largest group (16%). It is not known if the fish was actively feeding on these items, or picked them up while consuming detritus. However, the white sucker is a generalist in feeding. The abundance of chironimids and microcrustacea in sediment samples indicates an available food source for any such opportunistic feeder.

It would appear that simplistic food paths may exist in the cave waters. <u>Stenonema</u> nymphs and Chironomidae larvae consume detritus, most likely carried in from surface waters via the sinkhole. The stonefly nymphs then act in the predator capacity, consuming both of these detritivores. Micro-crustaceans feed on bacteria and small particles, and are fed on by insects and the few fish which wash in to the cave.

Sphaeriidae (fingernail clams) were observed in several areas of the cave. This group of molluscs may be making a home in these waters, as self-fertilization is reported as common in these clams. They can survive low flows and even exposure for extended periods of time.

Summary

It seems likely that the aquatic insect fauna in Mystery Cave represents a dead end channel from the community of the South Branch Root River. While drifting insects can enter the cave waters and potentially grow and emerge, reproducing populations are unlikely. At this time, only the microinvertebrates (Collembola, micro-crustaceans) appear to be a reproducing part of the Mystery Cave waters.

Recommendations

1. Further sampling is needed in other areas of the cave. In particular, an effort should be focused on the absence of isopods and amphipods. Any cavers exploring Mystery Cave passages should be instructed to carefully examine waters they find, turn over rocks and make careful searches for these organisms. Small plastic vials filled with preservative could be issued to any caving group. While these two groups may truly be lacking from Mystery Cave, it may be that more intensified searching will turn up representatives of these crustaceans.

Along the route where the Collembola were collected, an effort should be made to collect more specimens. The discovery of two species not previously recorded from the state is very interesting to invertebrate specialists. Dr. K. Christiansen could be contacted for appropriate sampling methods to use to try and expand on the species composition of this group in the cave.
More collections should be made in a regular systematic manner to examine the micro-crustacean community. Fine mesh nets could be used to sample drift, or funnel activity traps could be

set in standing waters. These may reveal additional taxa not collected in this study. Also, these organisms tend to have short life cycles. Thus, sampling needs to be done on perhaps a monthly basis, to fill in any gaps left in this study.

4. Any work in the cave (along trails, tour routes) must not be done without ensuring that sedimentation of the cave waters is eliminated. Any inflush of sediment may eliminate invertebrates from a specific area.

5. The waters for Mystery Cave originate as surface flow. Any impacts to the surface streams (sedimentation, eutrophication) will be transmitted to the cave waters and may have serious impacts on the cave fauna. Surface waters within the park should be protected from any potential negative impacts. On a larger scale, the idea of stream protection will require efforts to enlist the cooperation of private landowners. The Park should give consideration to negotiating easements for a protective buffer along the South Branch Root River upstream of the park. A small undisturbed buffer area along the stream would greatly reduce any sedimentation that may occur upstream. It may also ease impacts from pesticide/herbicide use in agricultural areas.

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