FINAL REPORT

SECTION 6

ENDANGERED SPECIES ACT

FEDERAL AID PROJECT E-5

SURVEY AND SPECIES DETERMINATION OF CAVE CRAYFISH IN OKLAHOMA

JUNE 1, 1989 - MAY 31, 1990

CAVE CRAYFISH FROM OKLAHOMA

OBJECTIVES

This study was undertaken to determine the identification, distribution and abundance of troglobitic crayfish in the Ozark Plateau region of Oklahoma. Specific objectives were:

1. To survey sites with historical records of cave crayfish in the Ozarks of Oklahoma, and determine the current status of cave crayfish at those sites.

2. To survey other potential sites for cave crayfish for which no records of occurrence exist.

3. To determine which species are in which caves using tissue electrophoresis to clarify genetic similarity of populations.

Information from this study is expected to be useful in making decisions on listing and protection needs for cave crayfish in the Ozark Plateau of Oklahoma, Missouri and Arkansas.

BACKGROUND

Historical Distribution and Status:

Cave crayfish are troglobitic, entirely dependent upon a subterranean existence. While a divergent group taxonomically, they possess convergent characteristics of deceased pigmentation and claw musculature. They are represented in the Americas by species in eight families, which also have epigean species (Hobbs et. al. 1977). Four species have been named from the western part of the Ozark Plateau, which includes Oklahoma, Arkansas and Missouri. All are members of the subgenus Jugicambarus in the genus Cambarus (Hobbs and Brown 1987). These are considered to have derived from an epigean parental stock in the southern Appalachian Mountains (Hobbs et. al. 1977, Hobbs and Barr 1960). The only troglobitic species from the subgenus Jugicambarus that is known to occur outside of the Ozark Plateau is Cambarus cryptodytes from the panhandle of northern Florida (Hobbs and Barr 1960). The species Cambarus hubricti is a troglobitic species of a different subgenus that occurs in the eastern part of the Ozark Plateau (Gardner 1986, Hobbs et. al. 1977, Hobbs and Barr 1960). None of the troglobitic species from the Ozarks are known to co-occur.

The main morphological feature used to distinguish the troglobitic species of Jugicambarus is the gonopod (first pleopod) of males in reproductive condition, called first form males (in this report abbreviated Male-I). While non-sexual characters can be used for tentative identifications, confirmed identifications must be based on the gonopods of Male-Is (Hobbs et. al. 1977, Hobbs 1976 and personal communication).

CAVE CRAYFISH FROM OKLAHOMA

The status of cave crayfish in Oklahoma is particularly problematic, principally because adequate data have not been collected. Of the four species of the subgenus Jugicambarus described from the Ozark Plateau, two are thought to occur in Oklahoma. Cambarus setosus Faxon is known from eight counties in southwestern Missouri (Figg and Lister 1990). Prior to the mid 1970s, it was thought that C. setosus probably occurred in Oklahoma (Black 1971, Creaser and Ortenburger 1933, Williams 1954). On the basis of specimens that were not Male-I from Twin Cave and "Spring Creek, 5 mi. S. of Locust Grove" in Oklahoma, Hobbs and Barr (1960) tentatively called populations from those caves C. setosus. Cambarus tartarus Hobbs and Cooper was described from type specimens from a single cave in Oklahoma (Hobbs and Cooper 1972). Without having specimens to examine from three other Oklahoma caves (Jail, Rodman, and Star), the cave crayfish from those caves were tentatively assigned to C. tartarus (Hobbs et. al. 1977). Cambarus aculabrum Hobbs and Brown has been described from two caves in Arkansas, both of which are close to the Oklahoma state line (Hobbs and Brown 1987). Cambarus zophonastes Hobbs and Bedinger is known from a single cave in Stone County, Arkansas (Hobbs and Bedinger 1964), considerably to the east of Oklahoma and all other known sites for species of Jugicambarus (Figg and Lister 1990, Hobbs and Brown 1987, Hobbs et. al 1977).

Prior to this study, cave surveys in the Ozark Plateau of Oklahoma had not focused on status assessment of cave crayfish, so records have been scattered and incomplete.

Present Legal or Other Formal Status:

A. National:

Cambarus setosus:	none
Cambarus tartarus:	Candidate 2, petitioned in 1986 for listing as endangered (Brown 1986).
Cambarus aculabrum:	Candidate 2, petitioned for listing as endangered.
Cambarus zophonastes:	Endangered, Federal Register for April 7, 1987.

B. State of Oklahoma: no status

Habitat:

As troglobites, cave crayfish are obligate inhabitants of subterranean aquatic systems. In the Ozark Plateau of Oklahoma, some caves have sizeable perennial streams that follow the main course of the cave for its length; some caves have perennial streams and/or

pools in only a part of the cave; and some caves have ephemeral streams and/or pools that have water only when water levels are raised by rains. Based on a few records on microhabitat use in Oklahoma caves, it appears that the cave crayfish are found more often in pools and lower gradient parts of streams. In all aquatic caves, water levels and gradient fluctuate with flood conditions. In most caves and subterranean aquatic systems, the lack of primary producers results in dependency of primary consumers and detritivores on the import of organic material from surface areas. Thus, though caves in Oklahoma may not have cave crayfish at all times, they may be important habitat with respect to sources for nutrient inputs.

METHODS

Study Area:

The study area was in that portion of the Ozark Plateau of Oklahoma in which aquatic caves are known to occur. This includes parts of Delaware, Mayes and Ottawa counties, principally where the surface geology is limestone and chert (Figures 1 and 2).

Collection of Historical Records:

Personal communication with Dr. Arthur Brown, Dr. Jeffery Black and Mr. William Puckette, who have seen cave crayfish in Oklahoma caves, indicated that all known specimens deposited in a museum are in the collection of the U. S. National Museum. I visited that collection in March of 1990 and examined all specimens of cave crayfish from Oklahoma. In addition, published and unpublished records were obtained and reviewed. Historical records also were obtained for the Ozark cavefish, Amblyopsis rosae, on the assumption that habitat suitable for a troglobitic fish might be suitable for a troglobitic crayfish.

Field Surveys:

Caves selected for field survey work included those with reported records of cave crayfish and cavefish and those aquatic caves in close proximity to caves with historical records. The rationale was that caves in proximity were most likely to be in the same hydrological system as caves with historical records. Aquatic caves with no records of cave crayfish or cavefish were not visited if they had had frequent checks by W. L. Puckette during winter bat surveys.

Field surveys of caves were conducted from September 30, 1989 to May 26, 1990. All

COUNTY DISTRIBUTION IN OKLAHOMA FOR CAVE CRAYFISH (Cambarus sp.)

Figure 1

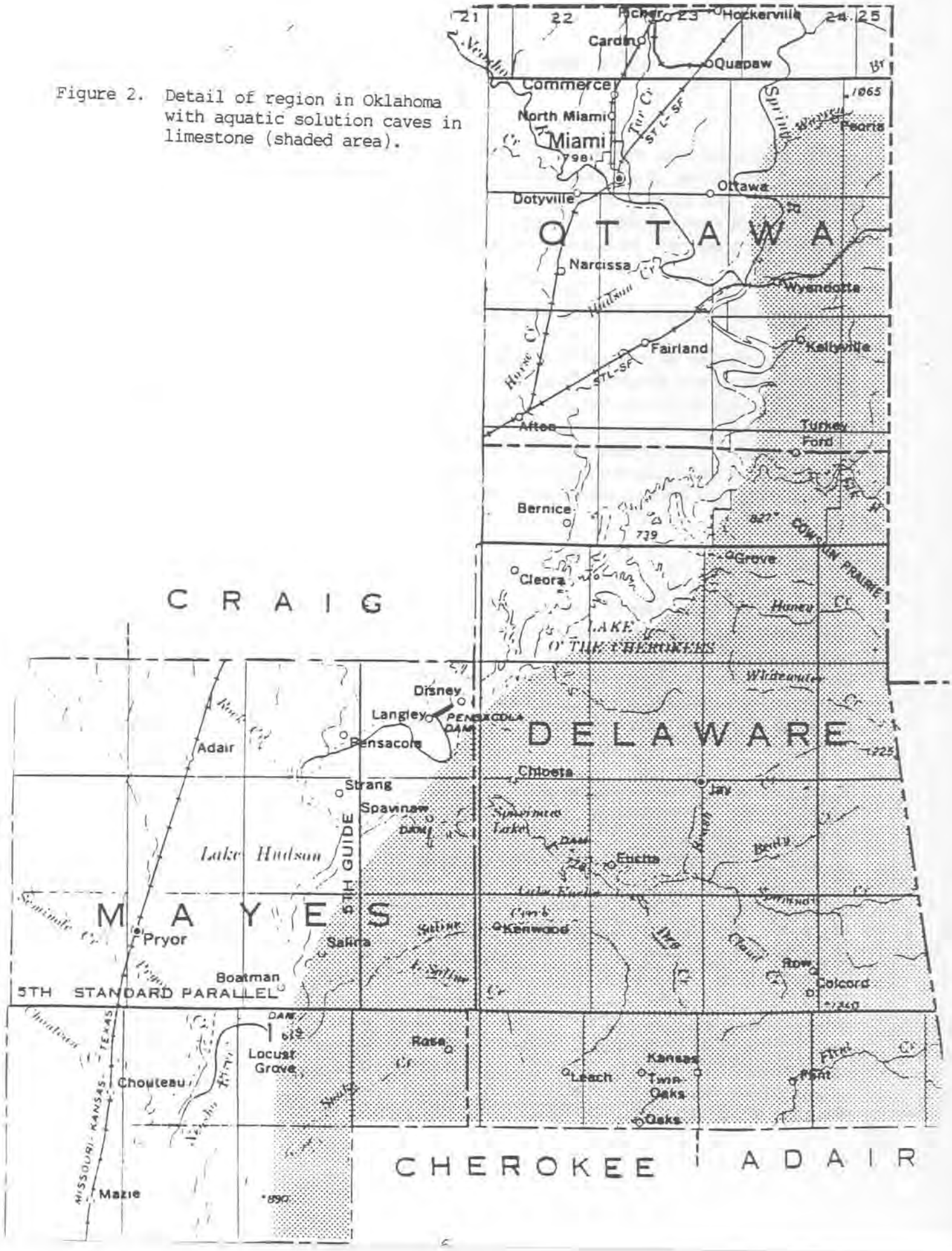

Figure 2. Detail of region in Oklahoma with aquatic solution caves in limestone (shaded area).

surveys were conducted with W. L. Puckette who is most familiar with caves in the Ozarks of Oklahoma. Accessible ephemeral and perennial aquatic areas of each cave were visited. Two caves were visited two times because water levels were extremely low during the first visit and did not afford adequate assessment of crayfish use of those caves. A third cave was revisited to collect a male for morphometric examination.

Electrophoretic Analysis of Tissue Proteins:

When cave crayfish were found in a cave, a claw (usually the left claw) was removed from individuals that possessed two claws if the individuals appeared healthy. Samples were sent on dry ice to the Missouri Department of Conservation for electrophoretic analysis of the claw muscle tissue. Dr. Jeffery Koppelman performed the analyses along with samples of cave crayfish from Ozark Plateau caves in Missouri. We were unsuccessful in obtaining samples from Arkansas caves, principally because the people in that state who are familiar with Ozark caves were not available to do the work.

RESULTS

Because of the sensitivity of caves of the Ozark Plateau and extreme pressures for human visitation use, the precise location of caves we visited is not presented in this report. Instead, by agreement with the U. S. Fish & Wildlife Service (Steven Hensley, Ecological Services Office, Tulsa, Oklahoma, personal communication), the location of known caves is given to the nearest four sections in Appendix I. Caves are identified by the same code system that has been used in studies of endangered bats in caves in the study area.

A total of 16 caves were located and surveyed, one spring was visited, and we were unable to locate one cave for which an historical record exists. A summary of the results of both the historical record and field surveys is given in Table 1. The results are detailed in Appendix I. Cave crayfish were found in four caves, coded as DL-38, DL-39, DL-74 and DL-91 (Table 2 and Appendix I). Morphological measurements taken on live individuals were not an adequate means of distinguishing the species (Table 2 and H. H. Hobbs, Jr., personal communication). Males in reproductive condition (Male-I) were distinguished from non-reproductive males (Male-II) and examined for species determination. One Male-I was captured, examined and released in each of caves DL-38, DL-74, and DL-91. Although the gonopods were viewed under a 10X hand lens, a positive determination of species could not be made. On the basis of published descriptions, Cambarus setosus can be distinguished from C. tartarus by a notch on the distal end of the central projection of the gonopod; the notch is lacking in C. setosus. No notch was seen on the gonopod of the Male-Is from DL-38, DL-74 or DL-91. Because of the apparent low density of these cave populations, no voucher specimens

Table 1. Summary of historical records and results of 1989-90 field surveys for cave crayfish in the Ozark Plateau of Oklahoma.

Cave code	Site no.	Record of cavefish?	Pre-study record of cave crayfish?	No. of crayfish seen per visit in this study?	Probability cave crayfish occur
DL-91	1	yes	yes	13	occur
DL-38	2	yes	yes	4	occur
DL-21	3	yes	no	0	not probable
DL-22	4	no	no	0	not probable
DL-146	5	no	no	0	not probable
DL-39	6	no	yes	6	occur
DL-49 sump	7	no	no	0	not probable
DL-49 #1	8	no	no?*	0	possible intermittent use
DL-49 #2	9	yes	no?*	0	possible intermittent use
DL-49 #3	10	no	yes	0	probable intermittent use
DL-84	11	no	no	0	not probable
DL-74	12	no	yes	17	occur
DL-16	13	no	no	0	not probable
DL-64	14	no	yes	0	possible intermittent use
DL-61	15	no	no	0	not probable
OT-19	16	yes	no	0	unknown
Spring Creek	-	no	yes	not found	historical site
Locust Grove Spring	17	no	no**	0	possibly flushed out from spring
DL-19	-	no	no?*	not surveyed	not probable

* Looney (1975) reported cave crayfish from this cave, but without documentation.

** J.H. Black (personal communication) suggested that this may be the actual site reported as Spring Ck.

Table 2. Measurements (mm) of Cave Crayfish (Cambarus spp.) captured and released in Oklahoma caves.

Cave	Site No.	Indiv. No.	Sex	Areola L	Areola W	Carapace L	Carapace W	Chelae L	Chelae W
DL-91	1	1	F	11.2	3.2	25.1	10.5	11.3	5.5
		2	F	10.1	2.8	19.5	9.4	8.1	4.2
		3	F	8.0	1.3	15.4	6.4	6.0	2.3
		4	M-II	9.4	2.5	17.2	8.1	6.0	3.3
		5	F	8.6	1.2	16.3	7.1	6.0	2.9
		6	F	7.4	1.4	14.0	6.0		
		7	M-I	7.8	2.1	16.3	9.4	7.4	3.8
		8	F	10.2	2.2	20.5	8.0	6.0	4.6
		9	F	10.4	3.6	15.6	7.9	5.7	2.8
		10	M-II	9.1	1.2	18.1	6.9	4.7	2.6
		11	F	9.5	2.0	18.3	6.9	5.3	1.5
DL-38	2	1	M-I	5.2	1.0	11.5	5.2	4.5	2.4
		2	F	7.0	1.3	12.1	5.5	3.9	1.5
		3	M-II	8.5	1.6	16.5	6.9	6.0	3.4
DL-74	12	1	F	8.2	1.1	21.2	9.1		
		2	F	8.5	1.6	21.8	9.0	8.1	4.6
		3	M-II	8.3	1.3	17.0	7.0	6.2	3.0
		4	F	8.3	1.3	17.7	7.4	6.2	3.2
		5	F	5.9	.9	13.6	4.9	5.3	2.6
		6	M-II	5.1	.9	11.3	4.2	4.1	1.4
		7	F	11.6	1.4	23.4	11.3	11.0	7.2
		8	F	7.7	1.2	16.5	7.5	5.7	2.8
		9	F	11.9	3.0	24.6	10.6	10.1	4.8
		10	M-I	8.0	1.4	18.6	7.3	4.9	2.2
		11	M-II	6.7	1.1	14.1	6.4	3.4	1.2
DL-39	6	1	F	11.1	1.5	23.9	10.5	8.5	5.3
		2	F	11.5	1.6	24.7	11.9	9.2	6.1
		3	F	11.5	.9	23.5	10.4	9.4	5.1
		4	M-II	8.5	.6	18.7	8.0	6.8	3.4
		5	F	9.5	1.3	19.7	9.2	7.3	4.3
		6	F	10.1	.9	19.4	8.2	6.9	3.7

were collected in this study. On the basis of the gonopods of two Male-I individuals from DL-91 (U. S. National Museum # 133842 & 148793), H. H. Hobbs, Jr. assigned this species to C. setosus. No Male-I vouchers exist for caves DL-38 or DL-74. A second visit was made to DL-38 to obtain a voucher. No Male-I was captured, but a Male-II was taken and is being held alive until it molts to Male-I condition. Cave crayfish from those three caves had more robust chelae than the cave crayfish from DL-39. Though no Male-I was found in DL-39 during our field surveys, the species from that cave has been confirmed by H. H. Hobbs, Jr. to be C. tartarus, based on type specimens in the U. S. National Museum (see Appendix I).

Crayfish were not found during either of two visits to caves DL-49 #3 and DL-64, though historical records of occurrence exist for both. In each of those caves, human access to water is limited, especially during dry weather (such as we had in the fall of 1989) when water levels are low. Those caves and their associated subterranean streams probably are used by cave crayfish, but detection of crayfish presence is limited by access to water. In both caves, there was evidence of extensive flooding with mud and debris washed into them during the spring rains of 1990. For this reason, DL-49 #3 may be an important nutrient input source for the cave crayfish. However, cave DL-64 is at the upstream end of a series of resurgences in the stream. It appears most likely that troglobites would be restricted to subterranean areas upstream from the cave, while nutrients flushed into the cave would be transported downstream. The historic crayfish record from DL-64 may represent an individual being flushed out of its usual habitat upstream. Cave DL-19 was mentioned in a popular article by Looney (1975) as having had a report of a cave crayfish, but we found no such reports and no specimens. W. L. Puckette (personal communication) has visited the cave three times and not found troglobites present. The cave does not appear to be good habitat because of its tendency to flush (Appendix I).

Attempts to locate an historical cave site, Spring Creek 5 mi. south of Locust Grove in Mayes County, were unsuccessful. Four long-time landowners along Spring Creek had not heard of a cave in that area, and a search of cliffs along the bank did not reveal any. The specimen collected from the site (U. S. National Museum, H. H. Hobbs, Jr. # 5-2150-1) was an immature female, which H. H. Hobbs, Jr. tentatively assigned to C. setosus (Hobbs et. al. 1977, personal communication). Because that area is disjunct from all other caves in Oklahoma that have confirmed or reported occurrences of cave crayfish, it would be most interesting to learn the identity of the cave crayfish from that cave.

The results of the electrophoretic analyses are given in a report by Koppelman (1990, Appendix II). Caves DL-38, Dl-74 and DL-91 (Oklahoma sites 1, 2 and 12 in the report) show close genetic relatedness. While electrophoretically they are most similar to C. aculabrum, their similarity distance from it is quite large (0.201). Morphometrically, the Male-I gonopod is more similar to C. setosus.

Electrophoretically, C. tartarus was not distinguishable from C. setosus from southern Missouri, though morphologically they can be readily distinguished on the basis of Male-I gonopods.

All of the caves visited are in the Neosho River (Grand Lake) drainage. Delaware County caves with cave crayfish records are in relatively close proximity to one another. Caves DL-74 and DL-91 are in the same surface tributary drainage to Grand Lake and close to the surface tributary in which DL-38 is located. Caves DL-39 and DL-49 are in nearby small drainages that are tributaries to a larger tributary to Grand Lake, and DL-64 is in a more distant tributary to that same larger tributary. Spring Creek, 5 mi. south of Locust Grove flows into Grand Lake farther south. Based on proximity of caves and surface waters, cave crayfish in DL-38, DL-74 and DL-91 would be most likely to be closely related. Crayfish in caves DL-39, DL-49 and probably DL-64 would be expected to be more closely related to one another than to other populations, and the Spring Creek cave crayfish population is most isolated.

DISCUSSION

Cambarus tartarus appears to be a distinct species, based on its distinct morphology Hobbs and Cooper 1972). Its high electrophoretic similarity to Cambarus setosus from southern Missouri indicates that it is recently derived from that species. Although DL-39 is the only cave from which it has been confirmed, based on proximity of surface drainages, it is possible that the cave crayfish associated with DL-49 and DL-64 are C. tartarus as well.

Although C. aculabrum was not an original focus of this study, it is of interest because of the results of the electrophoretic analyses and the proximity of the species to Oklahoma. Cave crayfish from DL-38, DL-74 and DL-91 are genetically similar to one another, based on similarity distance of the tissue proteins. Cambarus aculabrum is their nearest relative, but with a rather large similarity distance (0.20). Those four samples (three from Delaware County plus C. aculabrum) then cluster with C. setosus from southern Missouri at only a slightly greater similarity distance (0.25). Based on its distinct morphology described in its species account (Hobbs and Brown 1987) and its genetic uniqueness revealed by Koppelman (1990), a species designation is entirely appropriate for C. aculabrum.

A final species determination for the three similar Delaware County cave crayfish populations (DL-38, DL-74 and DL-91) must await further study. Electrophoretically, they appear distinct from all other species with their nearest relative being C. aculabrum. Initial results indicate that morphologically they more closely resemble C. setosus. Based on morphology of the gonopods from two Male-I individuals, H. H. Hobbs, Jr. assigned the population in DL-91 to C. setosus. Although my examination

of Male-I gonopods from DL-38 and DL-74 indicate that they have a C. setosus-type of morphology, voucher specimens need to be collected from those caves and examined under a microscope. (Toward that end, a Male-II from DL-38 is being held in captivity until it molts to Male-I condition.)

CONCLUSIONS

The conclusions that can be drawn from this study are summarized in Table 3. Cave crayfish occur in only a few caves in Oklahoma with four caves having persistent long-term use by individuals. Two other caves appear to be used intermittently, and a third cave could not be located. Of the four caves found to have persistent use, one is the only documented site for C. tartarus. The other three caves appear to have a second species initially identified as C. setosus. The electrophoretic studies that were conducted indicate that cave crayfish from these three caves are genetically unique from C. setosus in southern Missouri. Additional morphometric study is needed before a species determination can be made.

RECOMMENDATIONS

1. Cambarus tartarus should be listed as an endangered species on the basis of its morphologic distinctness and its rarity.

2. Cambarus aculabrum should be accepted as a distinct species, based on both its morphologic and genetic distinctness. It is an extremely rare species, known only from two caves, and it is dependent on water from the Ozark karst hydrologic system that is vulnerable to rapid pollution. Based on its extreme rarity and the vulnerability of the water system to pollution, this species should be listed as endangered.

3. Voucher Male-I specimens need to be collected from DL-38 and DL-74 in order to firmly establish the identity of cave crayfish populations from those caves and DL-91. If morphological differences are found between those populations as a group and C. setosus, they may merit designation as a distinct species, since genetically they are more closely aligned with C. aculabrum than with C. setosus.

4. Caves DL-49 and DL-64 should be revisited immediately following a rainy season that causes water levels to rise significantly. Cave crayfish appear more likely to be found during high water intervals.

Table 3. Summary of current status of species determinations for cave crayfish (Cambarus spp.) in Oklahoma.

Cave Code	Site No.	Species determination based on Morphology	Species determination based on Electrophoresis	additional work needed
DL-91	1	C. setosus	new species	Confirm morph. identification.
DL-38	2	C. setosus?	new species	Collect Male-I for morph. identification.
DL-74	12	C. setosus?	new species	Collect Male-I for morph. identification.
Spring Creek collect	-	C. setosus?	-	Find cave; collect Male-I for morph. & electro. identification.
DL-39	6	C. tartarus	C. setosus	List as endangered.
DL-49 cave	10	-*	-	Continue to check for individuals.
DL-64 cave	14	-**	-	Continue to check for individuals.

*Tentatively assigned to C. tartarus based on proximity to DL-39.
**Tentatively assigned to C. tartarus based on shared surface drainage with DL-39.

5. Caves DL-21 and OT-19 should be surveyed for cave crayfish during routine surveys for other cave species such as Myotis grisescens and Amblyopsis rosae. Along with DL-64 (see Recommendation 4), they are caves with records of A. rosae, so habitat for cave crayfish appears to exist in them.

6. Efforts should continue to locate the cave on Spring Creek in Mayes County and to identify the cave crayfish inhabiting it.

7. Hydrologic and pollution studies of the subterranean regions around caves DL-74 and DL-39 need to be conducted. Both caves have substantial numbers of cave crayfish, which are vulnerable to changes in water quality and quantity. Fortunately, hydrologic and pollution studies are underway for caves DL-91 and DL-38 as part of recovery work with Amblyopsis rosae.

8. Protection efforts for C. tartarus in DL-39 should continue since this is the only cave in the world documented to have that species. Even if cave crayfish from DL-49 and DL-64 are found to be C. tartarus, the largest population remains in DL-39.

9. If the species in DL-38, DL-74 and DL-91 is determined to be a unique taxa, those caves would merit strong protection as well. Because DL-38 and DL-91 harbor the cavefish in addition to cave crayfish, protection of those caves would be expected to be forthcoming as part of recovery efforts for that species.

10. The electrophoretic studies of troglobites in the Jugicambarus group needs to be completed. In particular, C. zophonastes from Hell Creek cave and C. aculabrum from Logan cave in Arkansas need to be sampled. Although the two known sites for C. aculabrum are on surface drainages that flow into the Neosho River system, Bear Hollow Cave surface drainage flows into a tributary of the Elk River to the north in Missouri, while Logan Cave surface drainage is into the Illinois River far to the south. Thus, it is possible that the subsurface waters may no longer be connected between these caves. Because C. zophonastes is found far to the east of all other known species of Jugicambarus, it would be expected to show greater genetic distance from the other species. One C. setosus site in extreme southwestern Missouri, south of Neosho, has not been sampled electrophoretically. Because of its proximity to Oklahoma caves, it merits sampling to determine its genetic relatedness to the C. setosus-types in Oklahoma.

11. Cave crayfish in the Ozark Plateau have not been studied in any depth. In order to know protection needs for these species, we need to know the nature and degree of their dependency on caves as well as basic population parameters. Toward this end, a method of following individuals over time through mark-recapture or other techniques should be worked out and initiated.

Southern Missouri caves that have relatively large populations of the more abundant species, C. setosus, probably is the best area to initiate this work. However, if electrophoretic studies continue, marking individuals from which claw samples are taken would allow a test of marking techniques concomitant an assessment of the impact of claw removal on individuals.

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Appendix I. Sites surveyed for crayfish in Oklahoma in 1989 and 1990. Results of field and historical record surveys are included. All caves that were located are reported to the nearest four sections (4 square miles).

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus setosus?

Common Name: Bristly cave crayfish

Federal Status: none

State Status: none

State: none

County: Delaware

Cave Code: DL-91 **Site Number:** 1

USGS Quadrangle map: Chloeta

Site Location to Nearest 4 Sections: T23NR22E Secs. 13,18,19,24

Date	Data	Source
30 Sep 89	13: including 8 female, 2 male-II, 1 male-I	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH74)
1987	2 seen	W.L. Puckette, personal communication
17 Aug 75	1 male-I	Specimen 148793, U.S. National Museum, coll. C. Rudolph
12 May 72	1 male-I	Specimen 133842, U.S. National Museum, coll. H.H. Hobbs III
18 Jul 71	1 male-II	Specimen 132575, U.S. National Museum, coll. J. Black
27 Feb 51	2: 1 imm. female, 1 ad. female	Specimens in H. Hobbs coll. #122350-1, U.S. National Museum, coll. A. Blair

Site Description: A large solution cave in limestone. Crayfish have been documented from the main (larger) pool and from the stream that is crossed to get to the main pool. A stream access point near the entrance and a second,

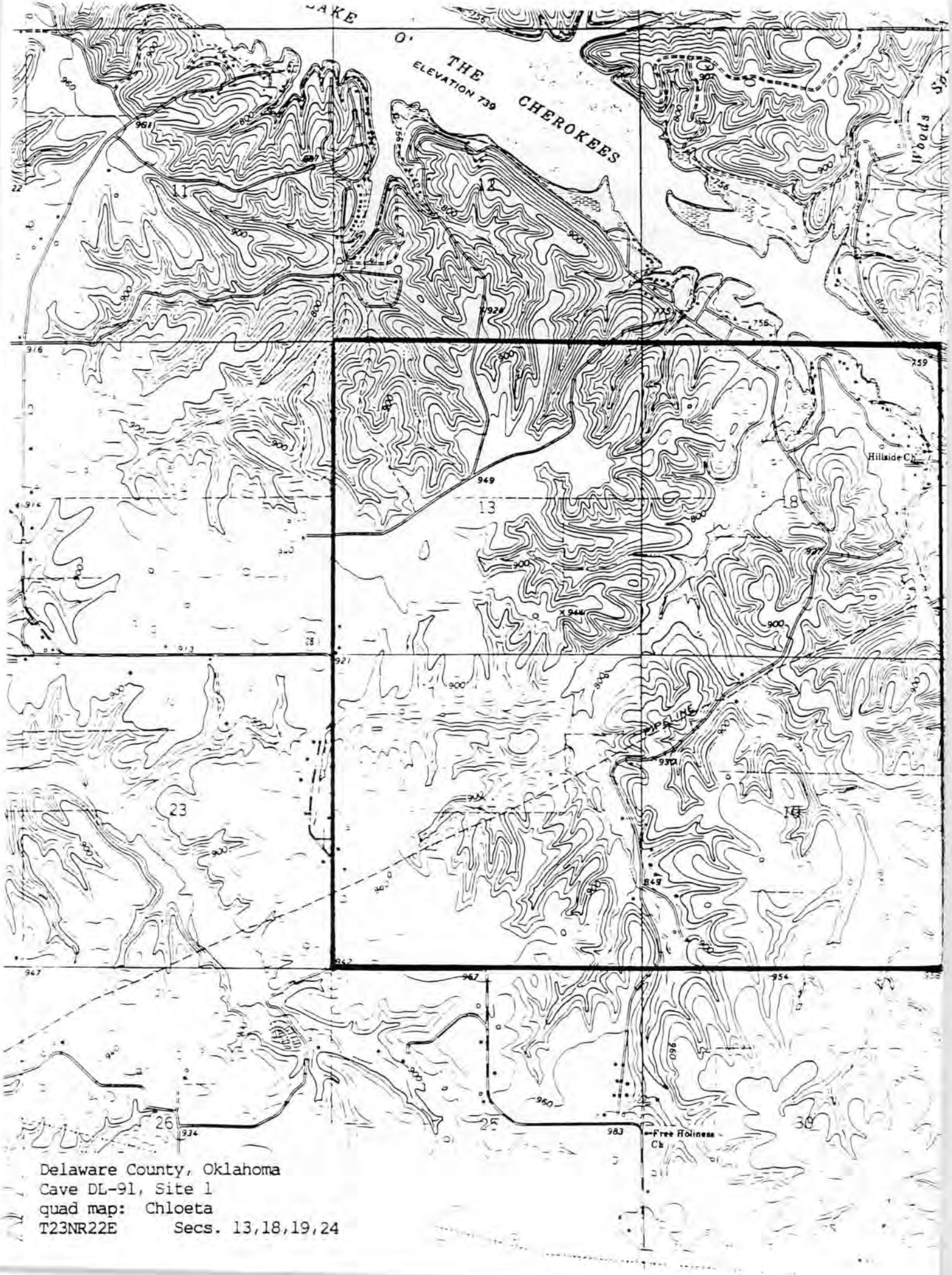
smaller pool did not have crayfish.

Threats: Water pollution from external sources could be a threat in the future.

Amblyopsis rosae Known from Site? yes

Comments: On 8 Mar 90, H.H. Hobbs, Jr. reconfirmed the identification of Specimen 133842 as C. setosus. The specimens collected in 1951 were from "cave between Spavinaw and Jay" which is presumed to be Twin Cave.

Electrophoretic analysis by Koppelman (1990) suggests that this is a new species most closely aligned with C. aculabrum.



Delaware County, Oklahoma
Cave DL-91, Site 1
quad map: Chloeta
T23NR22E Secs. 13,18,19,24

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus setosus?

Common Name: Bristly cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-38 **Site Number:** 2

USGS Quadrangle Map: Chloeta

Site Location to Nearest 4 Sections: T23NR22E Secs. 16,17,20,21

Date	Data	Source
26 May 90	4 seen: includes 1 female, 1 male-II	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F90MEH03)
22 Nov 89	1 seen	Aley (1989)
5 Oct 89	2 seen	R. Hamilton, The Nature Conservancy, personal communication
30 Sep 89	4 seen: includes 1 female, 1 male-II, 1 male-I	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH75)
20 Aug 76	1 female	Specimen 148794, U.S. National Museum, coll. C. Rudolph

Site Description: A solution cave in limestone. Crayfish were seen during this survey only in the single pool in the cave. A stream is present, but little of it can be accessed for survey.

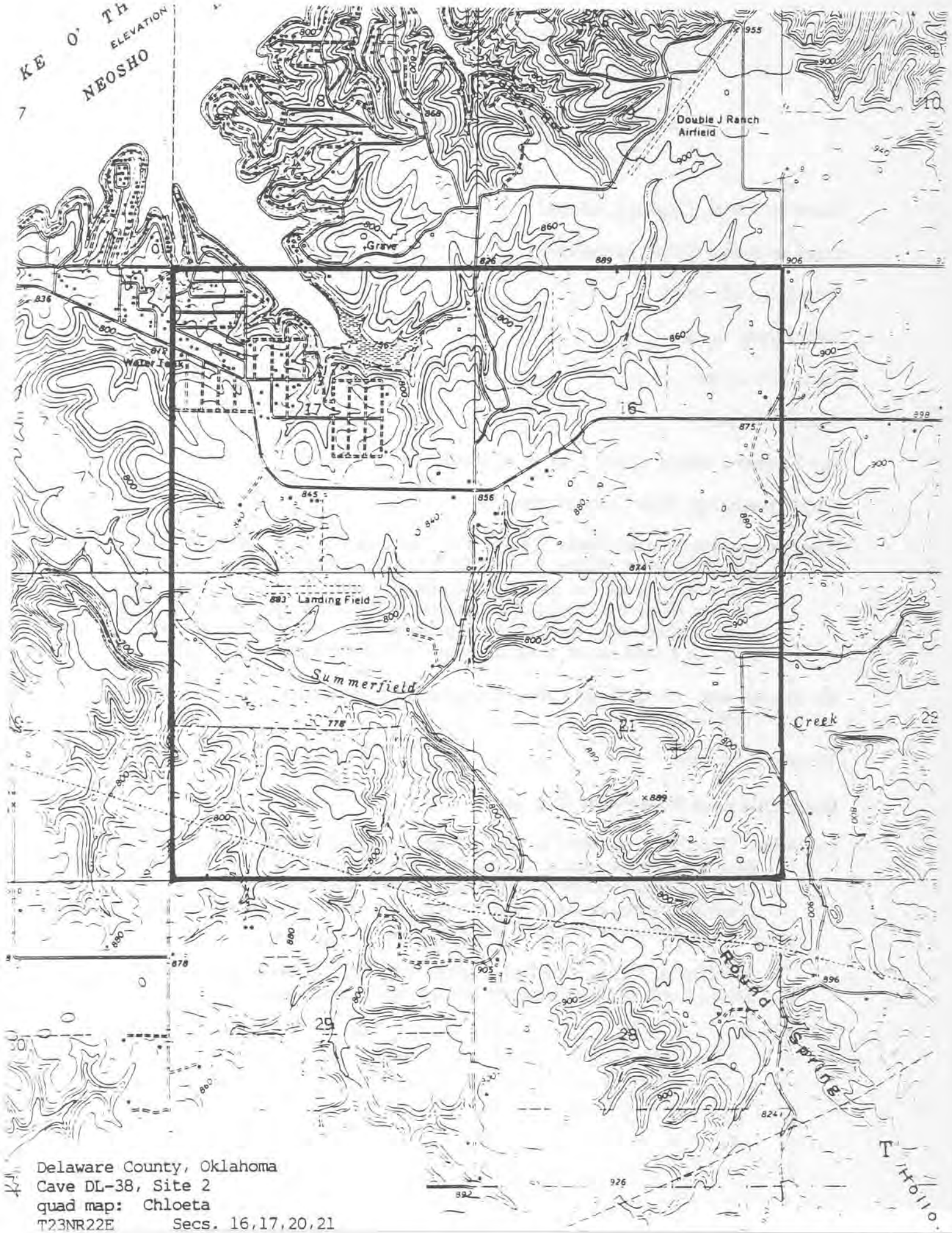
Threats: Depletion of nutrient supply from bat guano may be a threat. Historically,

the cave was a maternity site for Myotis grisescens (gray bats) which brought nutrients into the cave through guano. Water pollution from external sources could be a threat in the future. Hydrology of the cave could be altered by rises in the level of Grand Lake.

Amblyopsis rosae Known from Site? yes

Comments: H.H. Hobbs, Jr. tentatively assigned specimen 148794 to C. setosus, though previously (Hobbs et al 1977) it was assigned to C. tartarus with no specimens having been examined. A male-II was taken alive by Mehlhop-Cifelli on 26 May 90 to hold until it molts to male-I and can be identified morphologically.

Electrophoretic analysis by Koppleman (1990) suggests that this is a new species most closely aligned with C. aculabrum.



Delaware County, Oklahoma
Cave DL-38, Site 2
quad map: Chloeta
T23NR22E Secs. 16,17,20,21

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus setosus?

Common Name: Bristly cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Mayes

Site Location: Spring Creek, 5 mi. S. of Locust Grove. T19NR20E Sec. 9, 10

USGS Quadrangle Map: Locust Grove

Dates Surveyed & Source of Data: 27 Feb 51: 1 immature female was collected by A.P. Blair (H.H. Hobbs, Jr. #5-2150-1, U.S. National Museum). No records of a cave or troglobite have been made since that time. Contact was made October 89 with four long-term residents of the south slope of Spring Creek. None of them had heard of a cave being present. No cave crayfish were found in a field search along the slopes of the creek 26 October 1989.

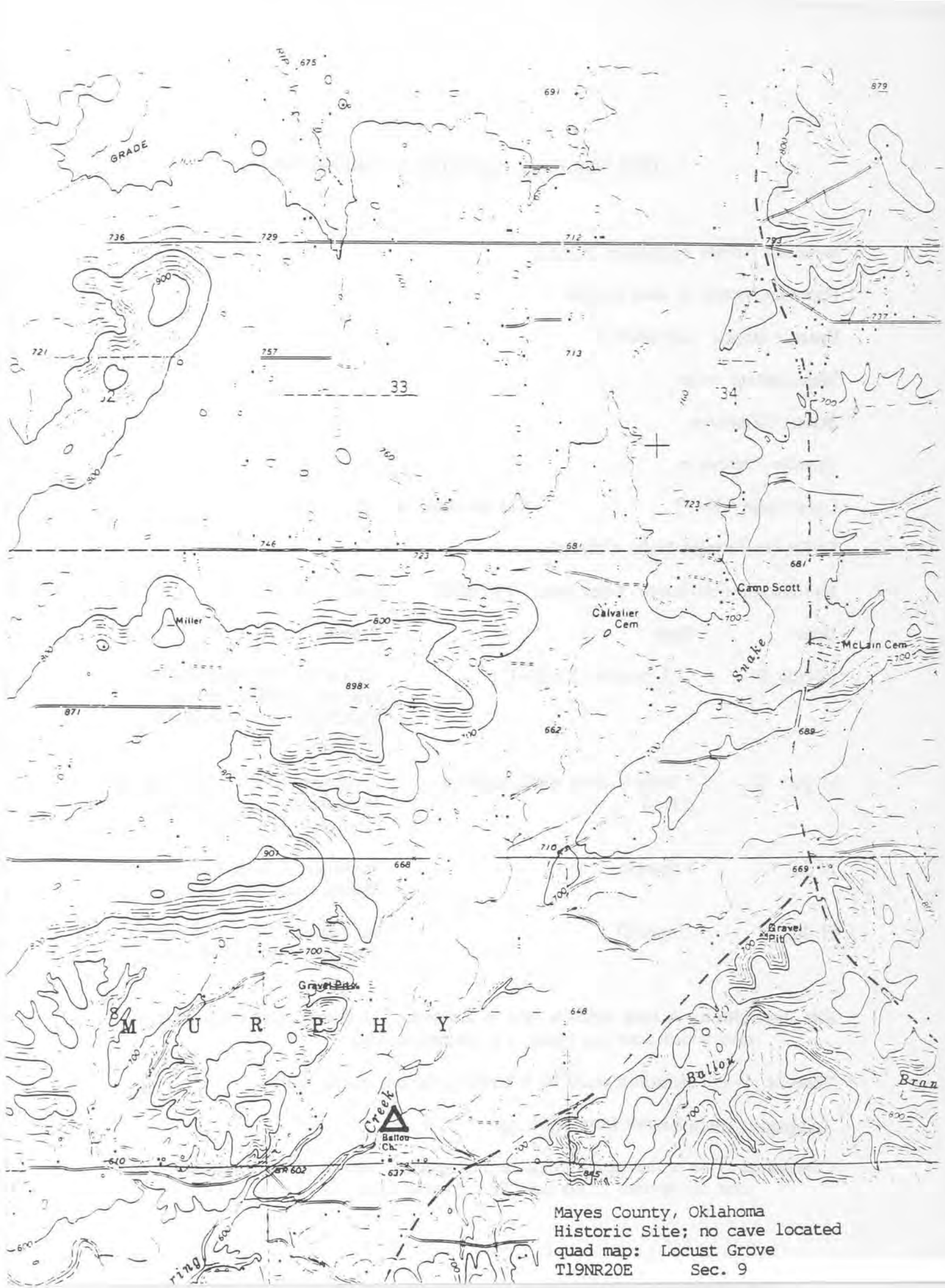
Site Description: Along Spring Creek, limestone occurs intermittently, mostly on the North side.

Threats: Unknown

Amblyopsis rosae Known from Site? no

Comments: J.H. Black (personal communication) suggested that the source could have been a well-known spring 0.5 mi. E. of Locust Grove (Site 17). The spring has been closed except for a pipe from which the water flows. If a cave crayfish occurred in the spring, it would have been ejected from more suitable habitat underground and upstream.

H.H.Hobbs, Jr. has tentatively assigned this to Cambarus setosus, but a Male-I needs to be collected to confirm the identification.



Mayes County, Oklahoma
Historic Site; no cave located
quad map: Locust Grove
T19NR20E Sec. 9

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus tartarus

Common Name: A cave crayfish

Federal Status: Candidate 2

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-39 **Site Number:** 6

USGS Quadrangle Map: Colcord

Site Location to Nearest 4 Sections: T21NR24E Secs. 1,2,11,12

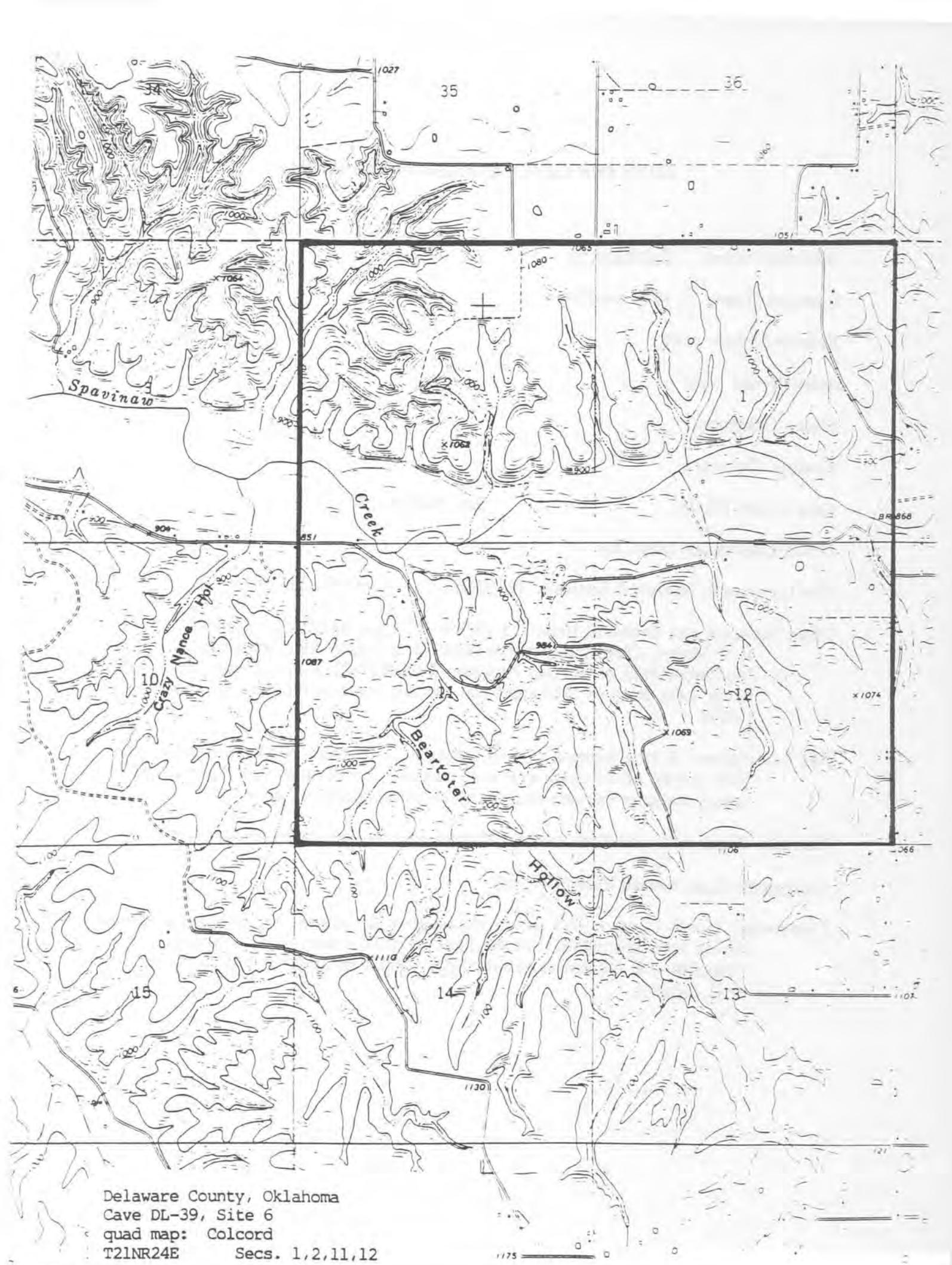
Date	Data	Source
12 Nov 89	6: 5 females, 1 male-II	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and others (F89MEH79)
11 Apr 71	1 male-II, held until molted to male-I	Specimen 131951, U.S. National Museum, coll. J.H. Black. (molted 7 May 1971)
11 Jul 70	1 female	Specimen 131411, U.S. National Museum, coll. J.H. Black
11 Jul 70	1 male-II	Specimen 132754, U.S. National Museum, coll. J.H. Black

Site Description: A long solution cave in limestone. A stream runs the length of the cave which does not occur in a resurgence area.

Threats: Water pollution could be a serious problem in the future.

Amblyopsis rosae Known from Site? no

Comments: This is the type locality for this species. Brown (1986) indicated he has seen the species in the cave, but gave no data.



Delaware County, Oklahoma
Cave DL-39, Site 6
quad map: Colcord
T21NR24E Secs. 1,2,11,12

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-74 **Site Number:** 12

USGS Quadrangle map: Jay

Site Location to Nearest 4 Sections: T23NR23E Secs. 21,22,27,28

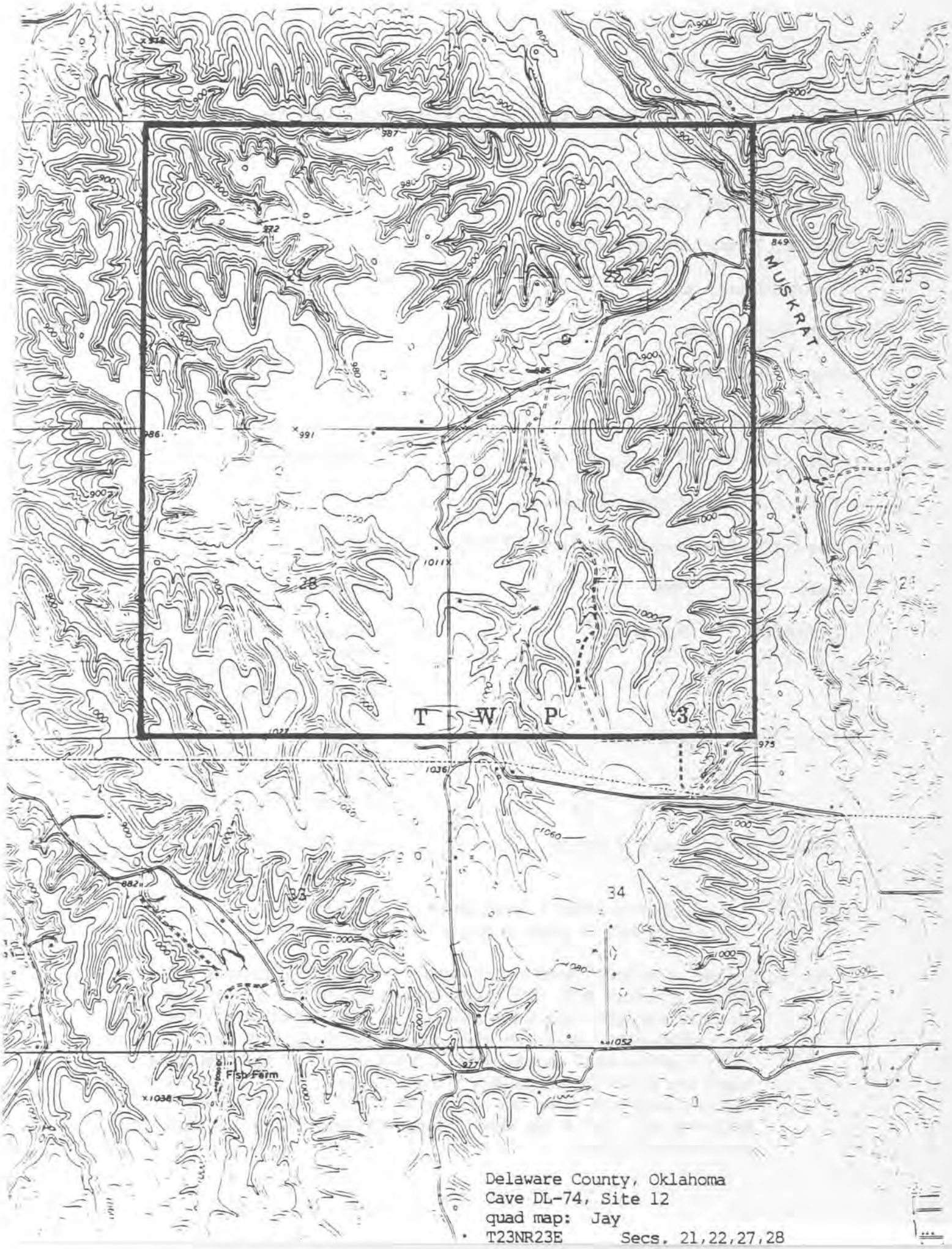
Dates Surveyed and Source of Data: 10/28/89: 17 seen, including 7 females, 3 Male-II, 1 Male-I, Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli & William Puckette (F89MEH82); Puckette knew of crayfish in the cave prior to 1989 (personal communication); reported by Black (1971)

Site Description: A long solution cave in limestone. Six small pools were found in 1989 survey, all of which had cave crayfish. Loose rock slabs in the crawl spaces mandate extreme caution by humans visiting the cave.

Threats: Water pollution from external sources may be a problem in the future.

Amblyopsis rosae Known from Site? no

Comments: Hobbs et. al. (1977) tentatively assigned the species to C. tartarus without having seen specimens. On the basis of visual observation of male-I gonopods and robustness of the chelae, Mehlhop-Cifelli would not assign it to C. tartarus.



Delaware County, Oklahoma
Cave DL-74, Site 12
quad map: Jay
T23NR23E Secs. 21,22,27,28

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-49 - sump, #1, #2, #3 **Site Number:** 7, 8, 9, 10

USGS Quadrangle Map: Colcord

Site Location to Nearest 4 Sections: T21NR24E Secs. 1,6,7,12

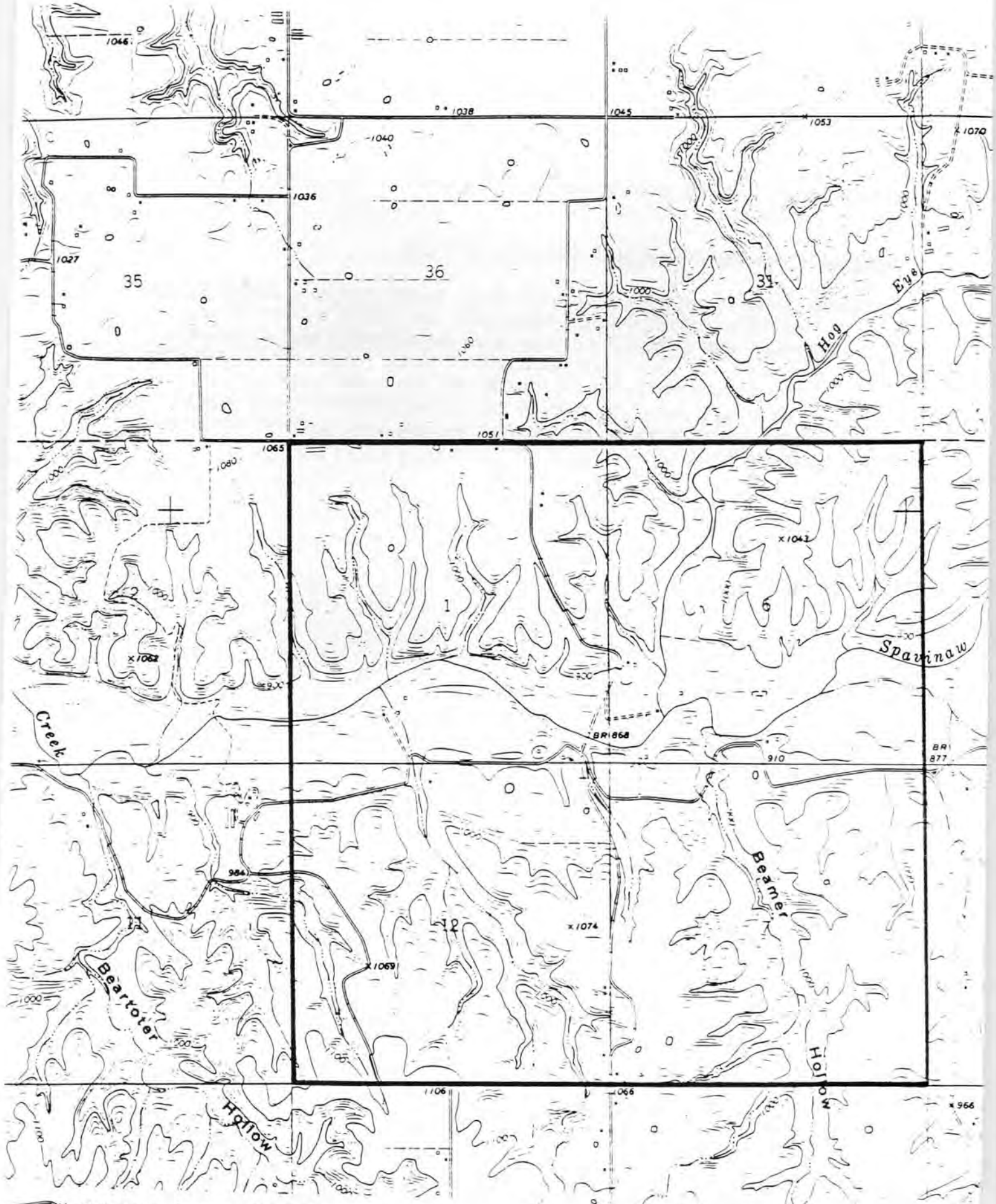
Date	Data	Source
25 May 90	none seen	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F90MEH01)
23 Oct 89	none seen	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH80)
1983	1 seen in site 10	W.L. Puckette and J. Stewart (personal communication)
1975	Reported in the 3 caves (sites 8-10) with no detail in article	Looney (1975)

Site Description: A series of small mud bottomed solution caves running east-west along a limestone cliff. The easternmost cave, site 7, is a small sump where no water was seen. Site 8 has about 2m of visual access to a stream. Site 9 has a small sump within it in which Amblyopsis rosae were seen in the past. There is also a small stream. Site 10 has a small pool near the entrance which was dry 10/23/89, but wet 5/26/90. Also, it has a muddy crawl area that is usually with water, but was dry 10/23/89. These caves flooded to a height of more than 4 feet during spring 1990 rains, but waters appear to have receded quickly.

Threats: Water pollution could be a threat in the future, particularly from increasing pig and chicken farming.

Amblyopsis rosae Known from Site? yes, from site 9 only

Comments: Based on proximity to cave DL-39, the species may be Cambarus tartarus. Puckette and Mehlhop-Cifelli believe that cave crayfish use site 10 and probably sites 8 and 9 when water levels are sufficiently high in the caves. The caves may act as a significant nutrient source for the crayfish and other subterranean species when mud washes into the caves during flooding. There is some use of the caves by bats, including sign of small groups of Myotis grisescens, indicating that bat guano may be a significant source of nutrients as well.



Delaware County, Oklahoma
Cave DL-49, Site 7,8,9,10
quad map: Colcord
T21NR24E Secs. 1,6,7,12

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-64 **Site Number:** 14

USGS Quadrangle Map: Jay

Site Location to Nearest 4 Sections: T22NR23E Secs. 7,12,13,18

Date	Data	Source
26 May 90	none	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F90MEH02)
28 Oct 89	none	Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH84)
1968	reported present	Black (1971), W. L. Puckette (personal communication)

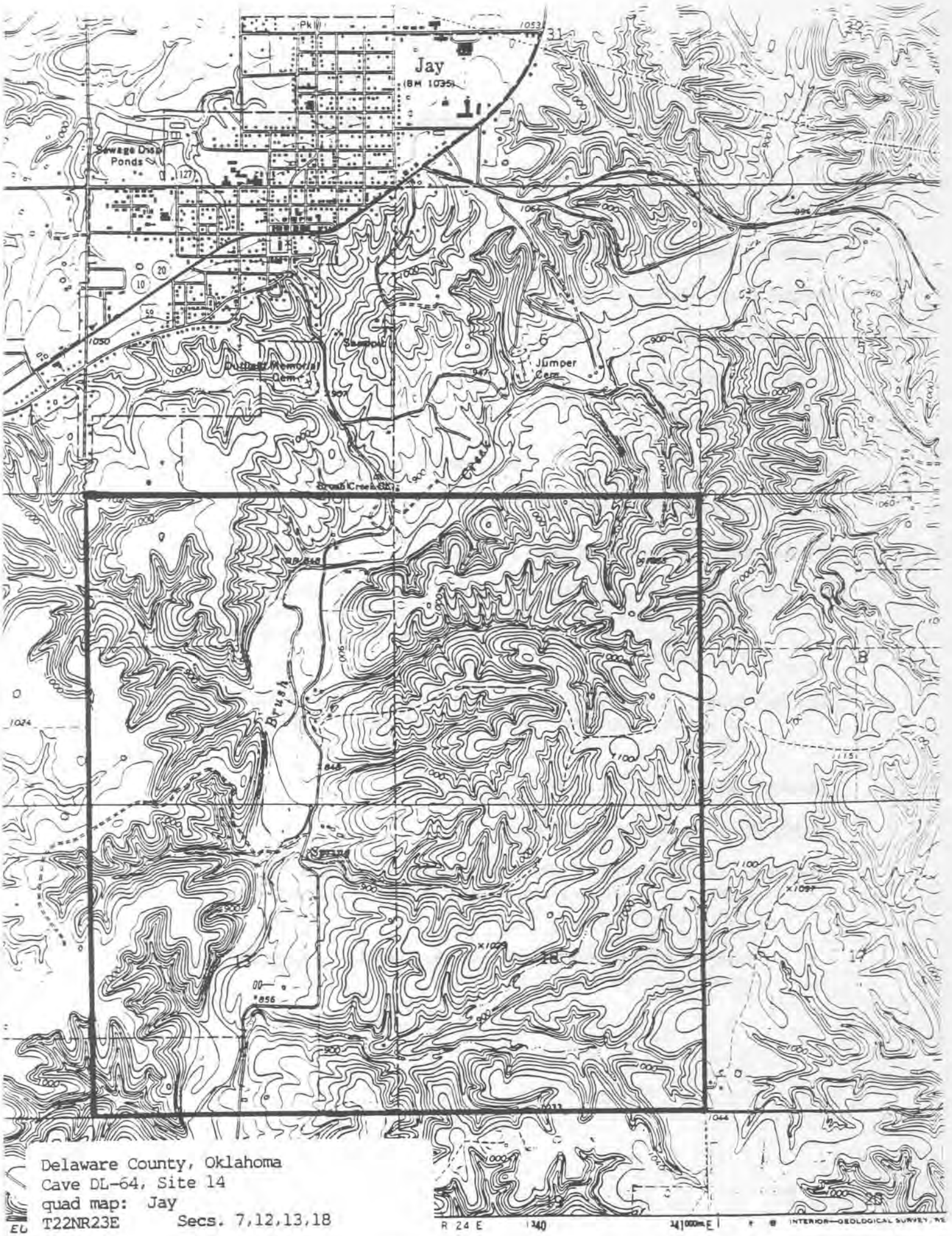
Site Description: A limestone solution cave just above an upper resurgence area to the underground stream. Troglobitic species, therefore, would be expected upstream rather than downstream from the cave. There is a moderately swift flowing stream only 3m length of which can be accessed. A moderate-sized pool with stream flow through it was very low 10/28/89, but about 3 feet and rising during rain 5/26/90. Apparently, water levels in it rise and fall rapidly. A second very small pool was dry 10/28/89 and held little water 5/26/90.

Threats: Water pollution could be a problem

Amblyopsis rosae Known from Site? no

Comments: High water level fluctuations and current flows may not be conducive to frequent use by cave crayfish.

Hobbs et al (1977) tentatively assigned the species to C. tartarus without examination of specimens. Because this cave is in the same major surface drainage as DL-39, that tentative assignment is appropriate.



Delaware County, Oklahoma
Cave DL-64, Site 14
quad map: Jay
T22NR23E Secs. 7,12,13,18

SITES FOR CAVE CRAYFISH IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-19 **Site Number:** site not surveyed

USGS Quadrangle Map: Colcord

Site Location to Nearest 4 Sections: T21NR24E Secs. 2,3,10,11

Dates Surveyed and Source of Data: W.L. Puckette has surveyed the cave 3 times and not seen a cave crayfish. Mary Looney (1975) mentioned that cave crayfish had been reported from the cave, but no records have been located to substantiate it.

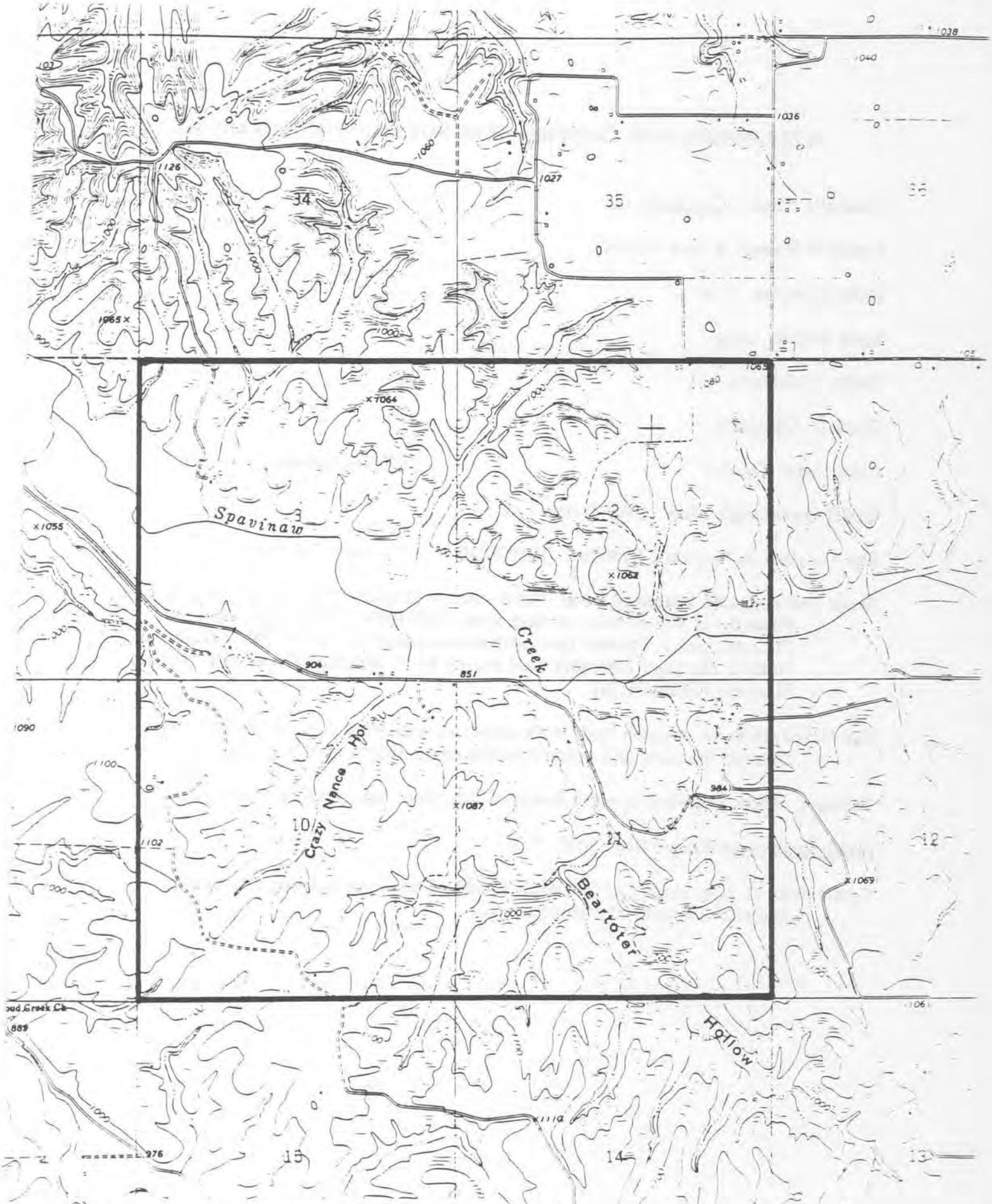
Site Description: A narrow solution cave in limestone. A perennial stream runs along the length of the cave. Water flushes through the cave without pool formation.

Threats: Water pollution could be a problem.

Amblyopsis rosae Known from Site? no

Comments: According to W.L. Puckette, the cave probably is not a suitable long term habitat for troglobites because they would be flushed from the cave during high water periods.

If cave crayfish occur in this cave, they may be C. tartarus based on proximity to DL-39.



Delaware County, Oklahoma
Cave DL-19
quad map: Colcord
T21NR24E Secs. 2,3,10,11

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-21 **Site Number:** 3

USGS Quadrangle Map: Lake Eucha East

Site Location to Nearest 4 Sections: T21NR23E Secs. 7,8,17,18

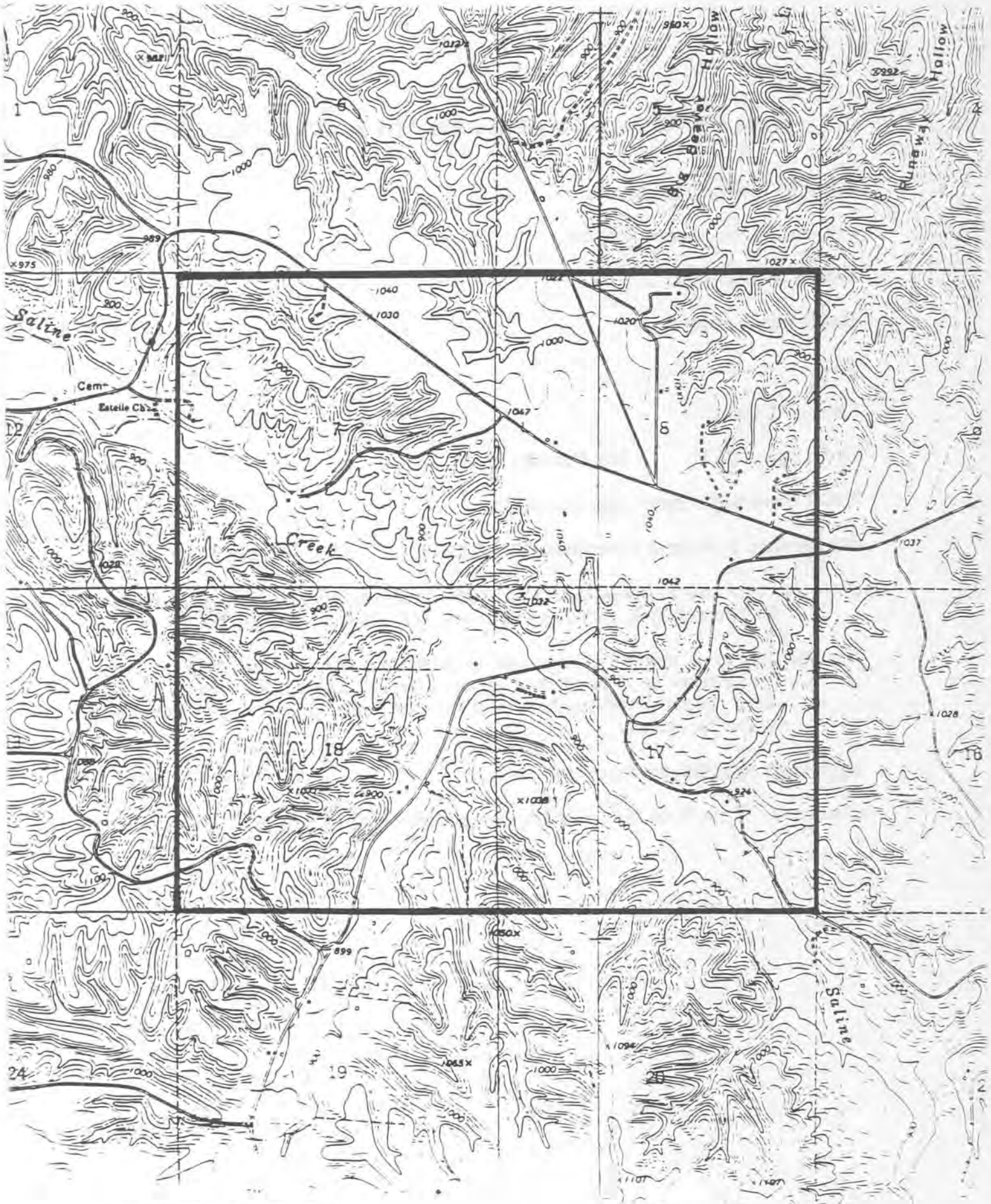
Dates Surveyed and Source of Data: None seen by Black (1971). None seen by W.L. Puckette in winter visits to cave from 1980-1989. 1983: none seen by Puckette and J. Stewart (personal communication). 5 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and William Puckette (F89MEH76)

Site Description: A solution cave in limestone of moderate size. A stream meanders through the cave and permits access along approximately 50 m.

Threats: Water pollution is not a threat at this time, but could be in the future.

Amblyopsis rosae Known from Site? Yes

Comments: A long history of biological survey of this cave has not revealed any evidence of cave crayfish presence.



Delaware County, Oklahoma
Cave DL-21, Site 3
quad map: Lake Eucha East
T21NR23E Secs. 7,8,17,18

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-22 **Site Number:** 4

USGS Quadrangle Map: Lake Eucha East

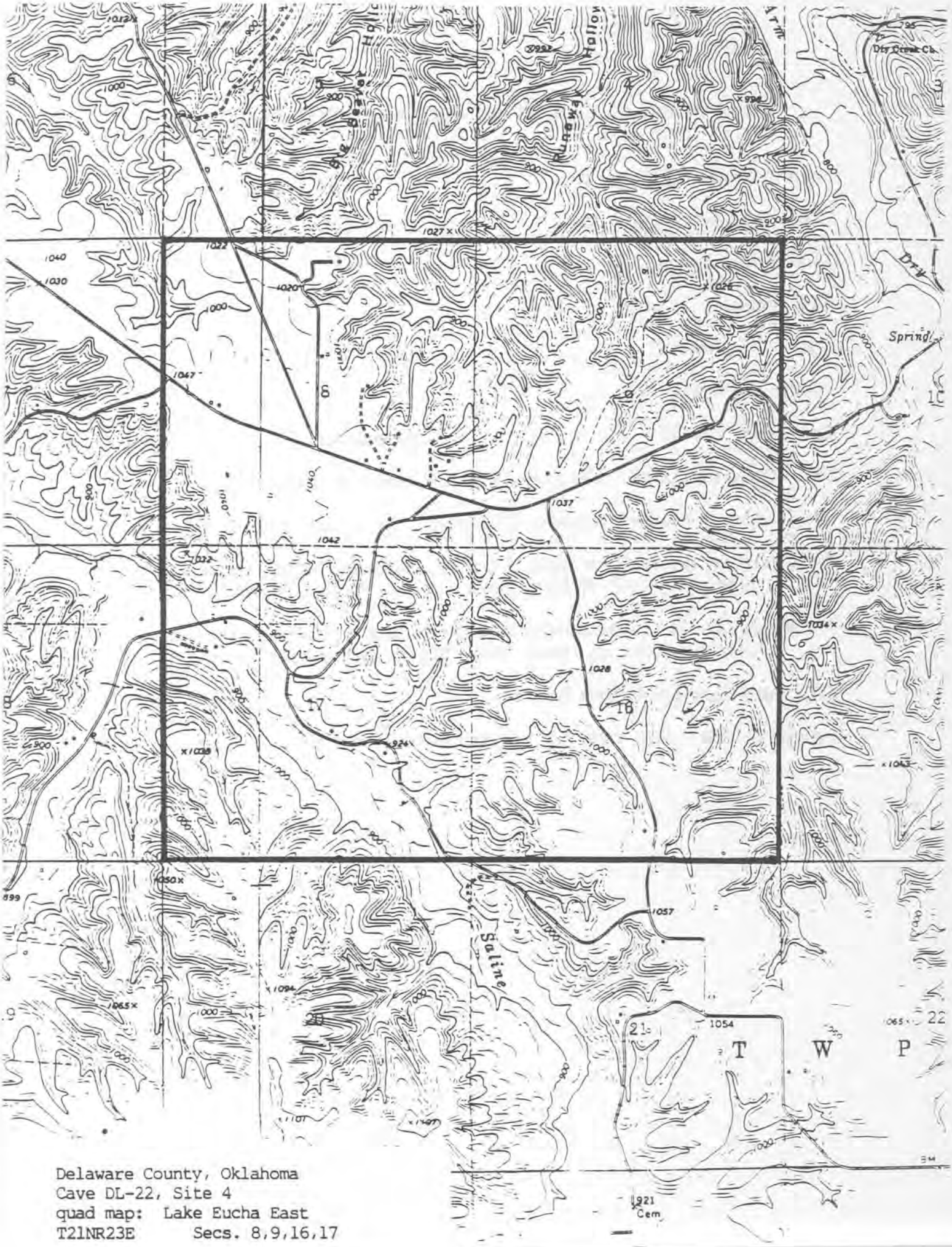
Site Location to Nearest 4 Sections: T21NR23E Secs. 8,9,16,17

Dates Surveyed and Source of Data: 5 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH77)

Site Description: A shallow solution cave in limestone. Water covers the cave from the entrance to as far back as can be accessed, which is just beyond the light zone. Water covers the bottom of the cave from the entrance to as far back as can be accessed.

Amblyopsis rosae Known from Site? no

Comments: There is no history of troglobitic species from this cave.



Delaware County, Oklahoma
Cave DL-22, Site 4
quad map: Lake Eucha East
T21NR23E Secs. 8,9,16,17

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-146 **Site Number:** 5

USGS Quadrangle Map: Lake Eucha West and Lake Eucha East

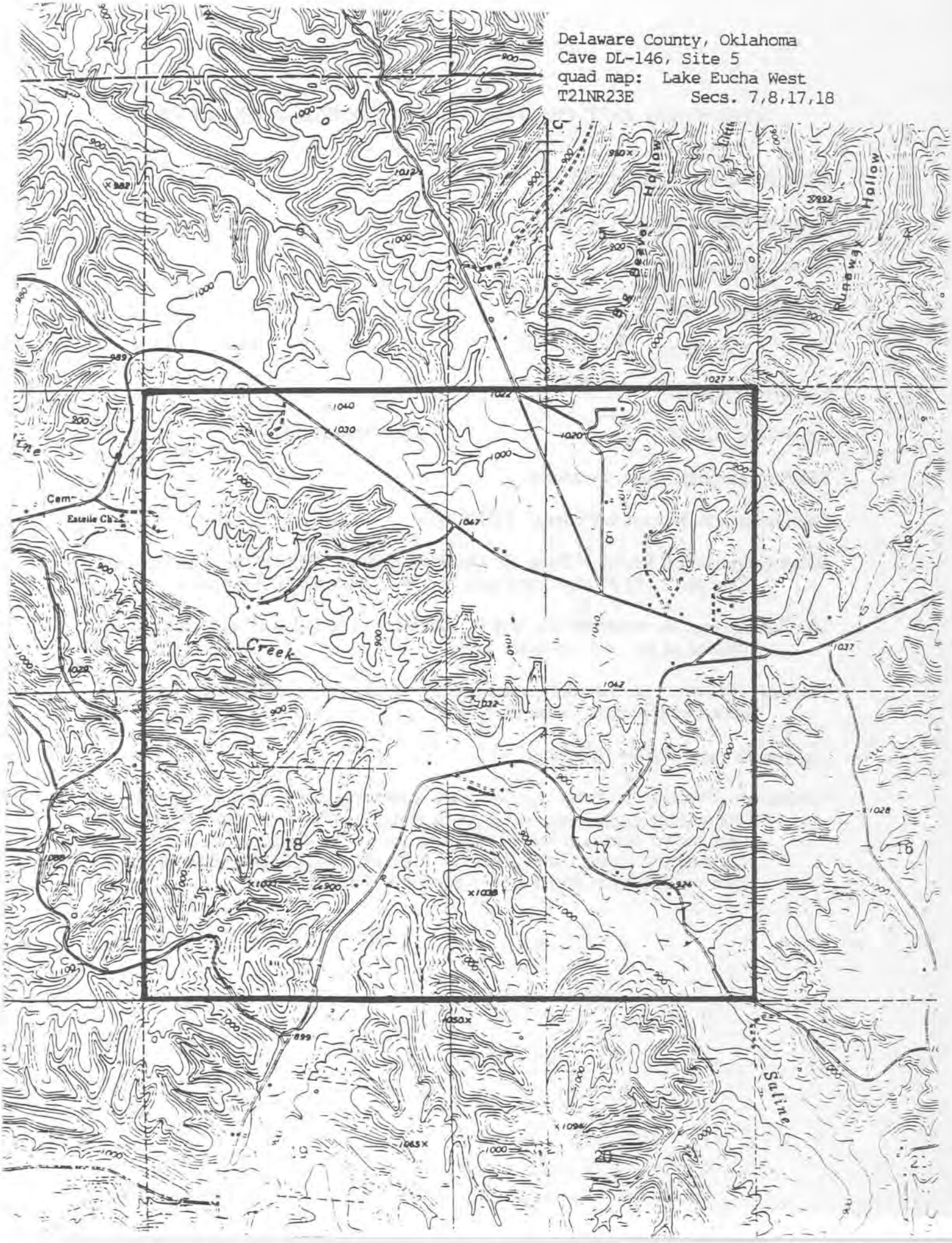
Site Location to Nearest 4 Sections: T21NR23E Secs. 7,8,17,18

Dates Surveyed and Source of Data: 5 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH78)

Site Description: A shallow solution cave in limestone with no apparent access beyond the dim light zone. Water fills the cave bottom.

Amblyopsis rosae Known from Site? no

Comments: There is no history of troglobitic species from this cave.


Delaware County, Oklahoma
Cave DL-146, Site 5
quad map: Lake Eucha West
T21NR23E Secs. 7,8,17,18
Cem.
Estella Ch.
Creek
Saline
18
17
16
19
20
21

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-84 **Site Number:** 11

USGS Quadrangle Map: Sycamore

Site Location to Nearest 4 Sections: T23NR24E Secs. 11,12,13,14

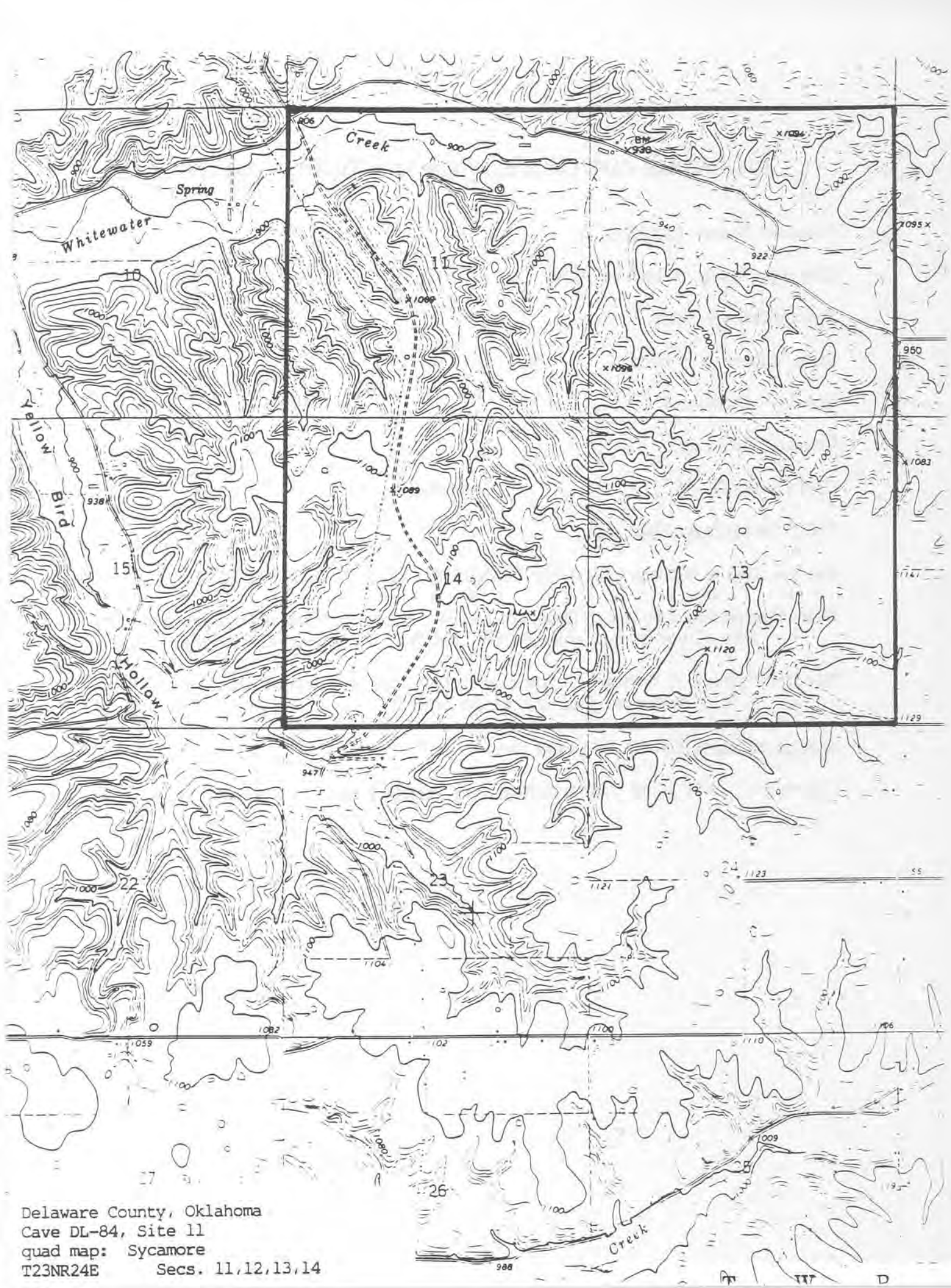
Dates Surveyed and Source of Data: 26 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH81)

Site Description: A moderate size cave in limestone. There is a small stream that is crossed to get to a moderate pool.

Threats: Flocculent on the pool surface indicates water pollution, probably from the cave owners house above the cave.

Amblyopsis rosae Known from Site? no

Comments: This cave probably is in the same major drainage (Sycamore Creek) where Amblyopsis rosae was exposed during a construction project. It is the only known aquatic cave of noteworthy size in the drainage. S. Bozeman (Central Oklahoma Grotto, personal communication) knows of no records of cave crayfish from this cave, nor does W.L. Puckette.



Delaware County, Oklahoma
Cave DL-84, Site 11
quad map: Sycamore
T23NR24E Secs. 11,12,13,14

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-16 **Site Number:** 13

USGS Quadrangle Map: Jay

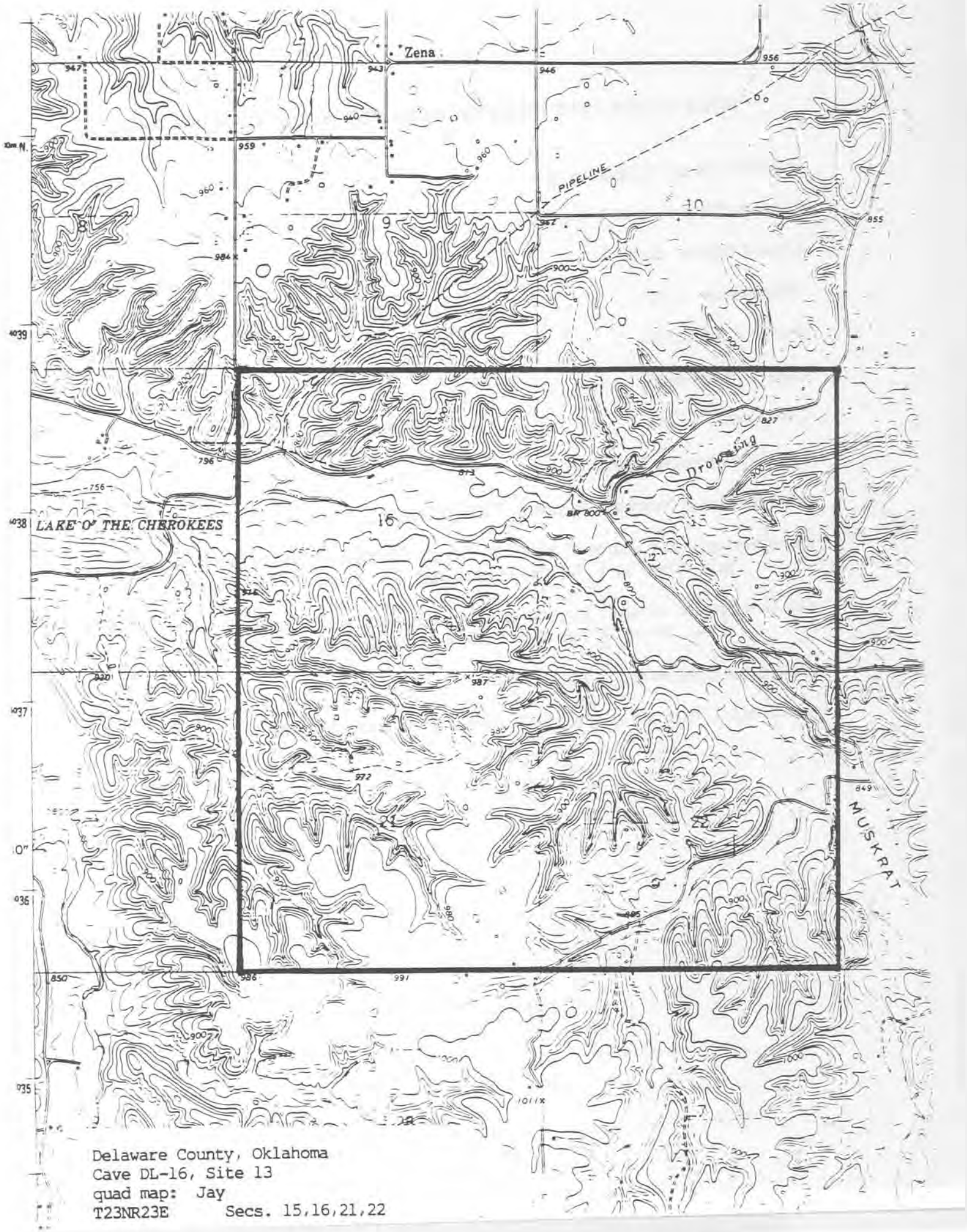
Site Location to Nearest 4 Sections: T23NR23E Secs. 15,16,21,22

Dates Surveyed and Source of Data: 28 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH83)

Site Description: A shallow solution cave in limestone with several entrances. There is little dark zone accessible in the cave.

Amblyopsis rosae **Known from Site?** no

Comments: There is no history of troglobitic species from this cave.



Delaware County, Oklahoma
Cave DL-16, Site 13
quad map: Jay
T23NR23E Secs. 15,16,21,22

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Delaware

Cave Code: DL-61 **Site Number:** 15

USGS Quadrangle Map: Jay

Site Location to Nearest 4 Sections: T22NR23E Secs. 11,12,13,14

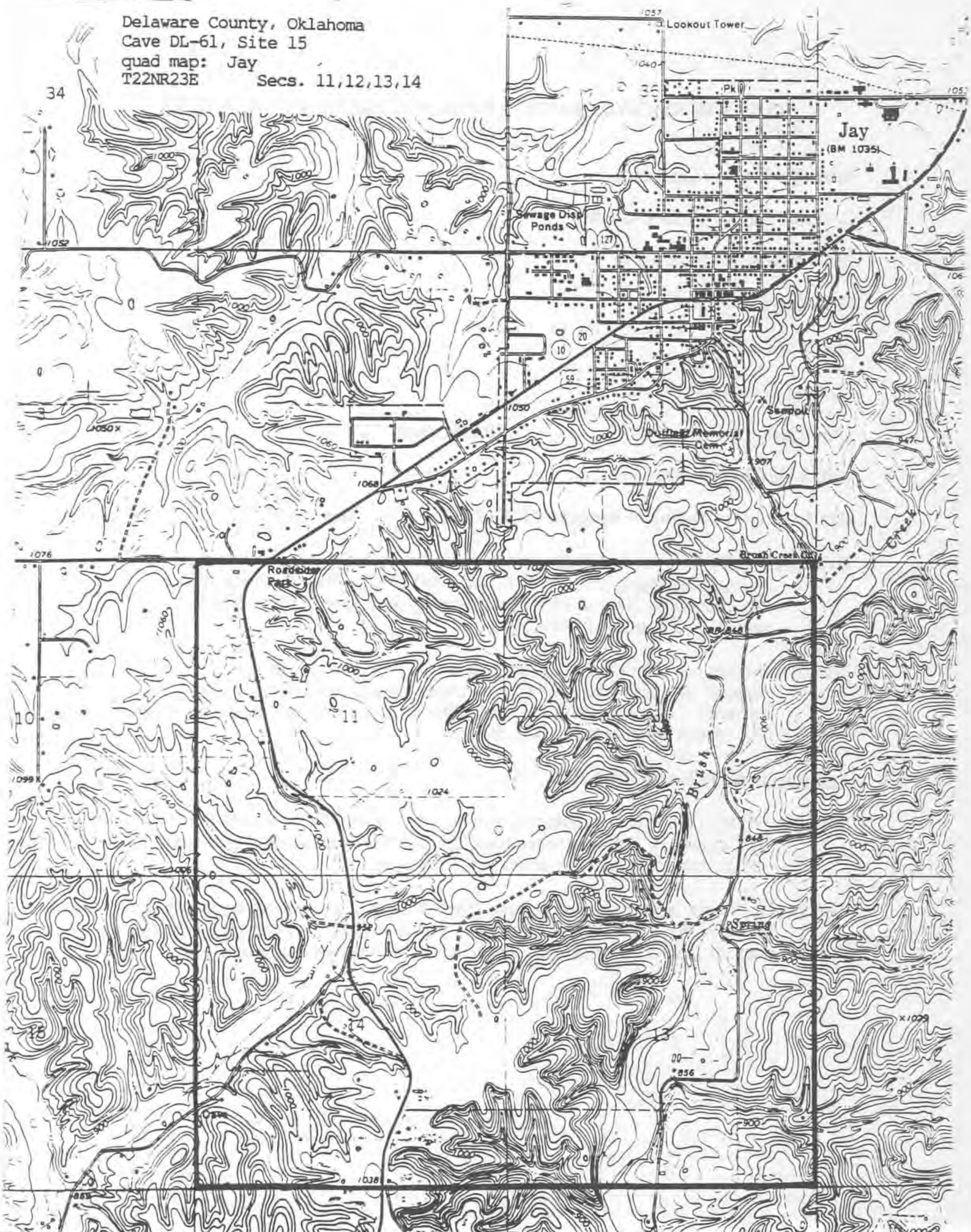
Dates Surveyed and Source of Data: 28 Oct 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH85)

Site Description: A small solution cave in limestone. A dry gravel stream bed was visible, but could not be accessed without digging.

Amblyopsis rosae Known from Site? no

Comments: There are no records of cave crayfish from this site.

Delaware County, Oklahoma
Cave DL-61, Site 15
quad map: Jay
T22NR23E Secs. 11,12,13,14



SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: A cave crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Ottawa

Cave Code: OT-19 **Site Number:** 16

USGS Quadrangle Map: Peoria

Site Location to Nearest 4 Sections: T28NR24E Secs. 14,15,22,23

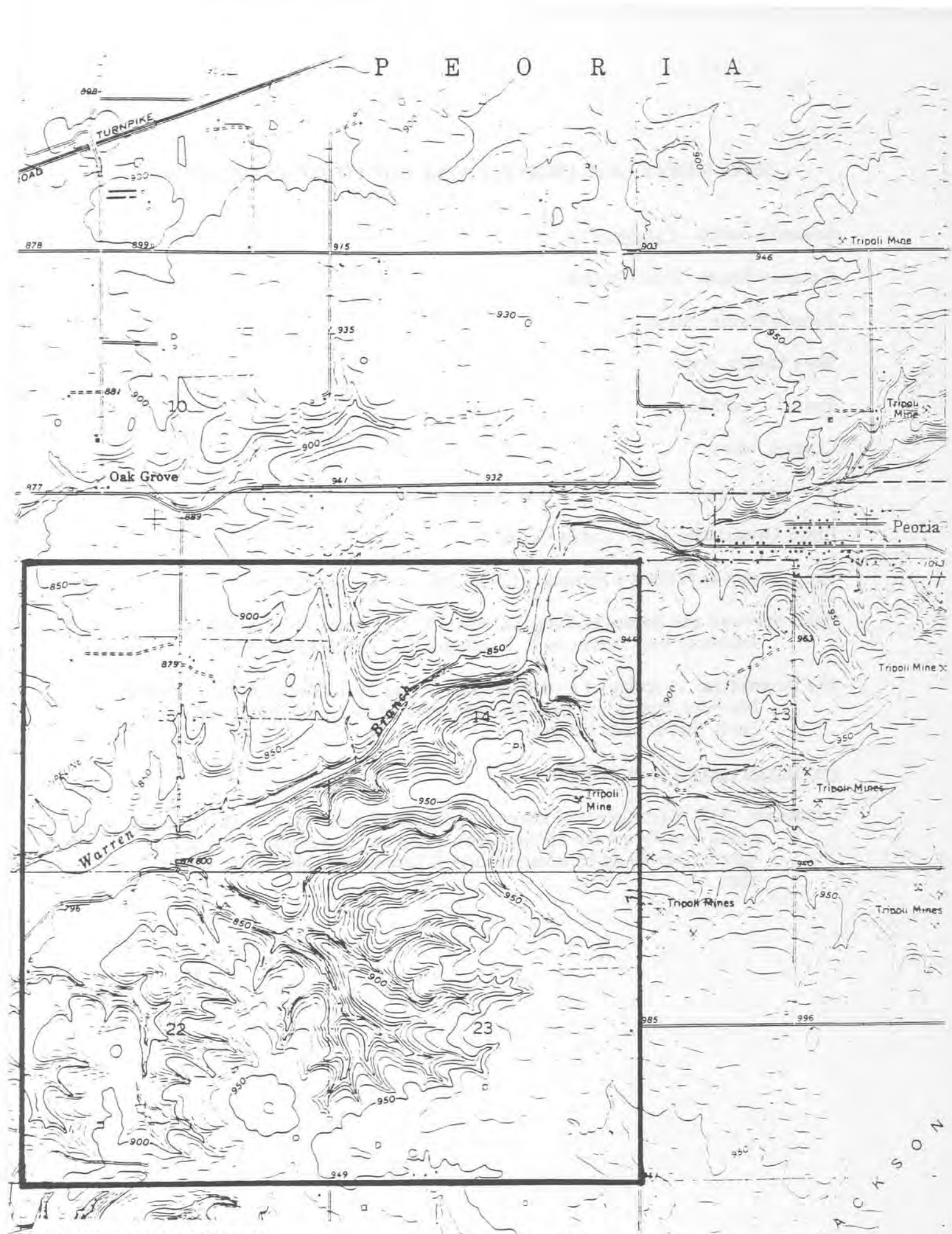
Dates Surveyed and Source of Data: None recorded as being seen during surveys for Amblyopsis rosae in 1954, 1966 and 1980-83 (Mayden & Cross 1983). 7 Dec 89: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli and W.L. Puckette (F89MEH84)

Site Description: A solution cave in limestone. A rock and gravel stream runs through the cave and goes slightly under ground just inside the cave entrance. Access is easy the first 100m or more into the cave, but much of the stream flows beneath a rock shelf.

Amblyopsis rosae Known from Site? yes

Threats: Water pollution is a potential threat. An impoundment below the cave does not appear to threaten water back up into the cave. However, if back up did occur, predatory fish could enter the cave.

Comments: There is no evidence of cave crayfish from this cave, although the habitat appears suitable. Any troglobites in this cave may be getting flushed by the stream from more populous and inaccessible reaches upstream.



Ottawa County, Oklahoma
Cave OT-19, Site 16
quad map: Peoria
T28NR24E Secs. 14,15,22,23

SITES WHERE CAVE CRAYFISH WERE NOT FOUND IN OKLAHOMA

Scientific Name: Cambarus sp.

Common Name: Cave Crayfish

Federal Status: none

State Status: none

State: Oklahoma

County: Mayes

Cave Code: none **Site Number:** 17

USGS Quadrangle Map: Locust Grove

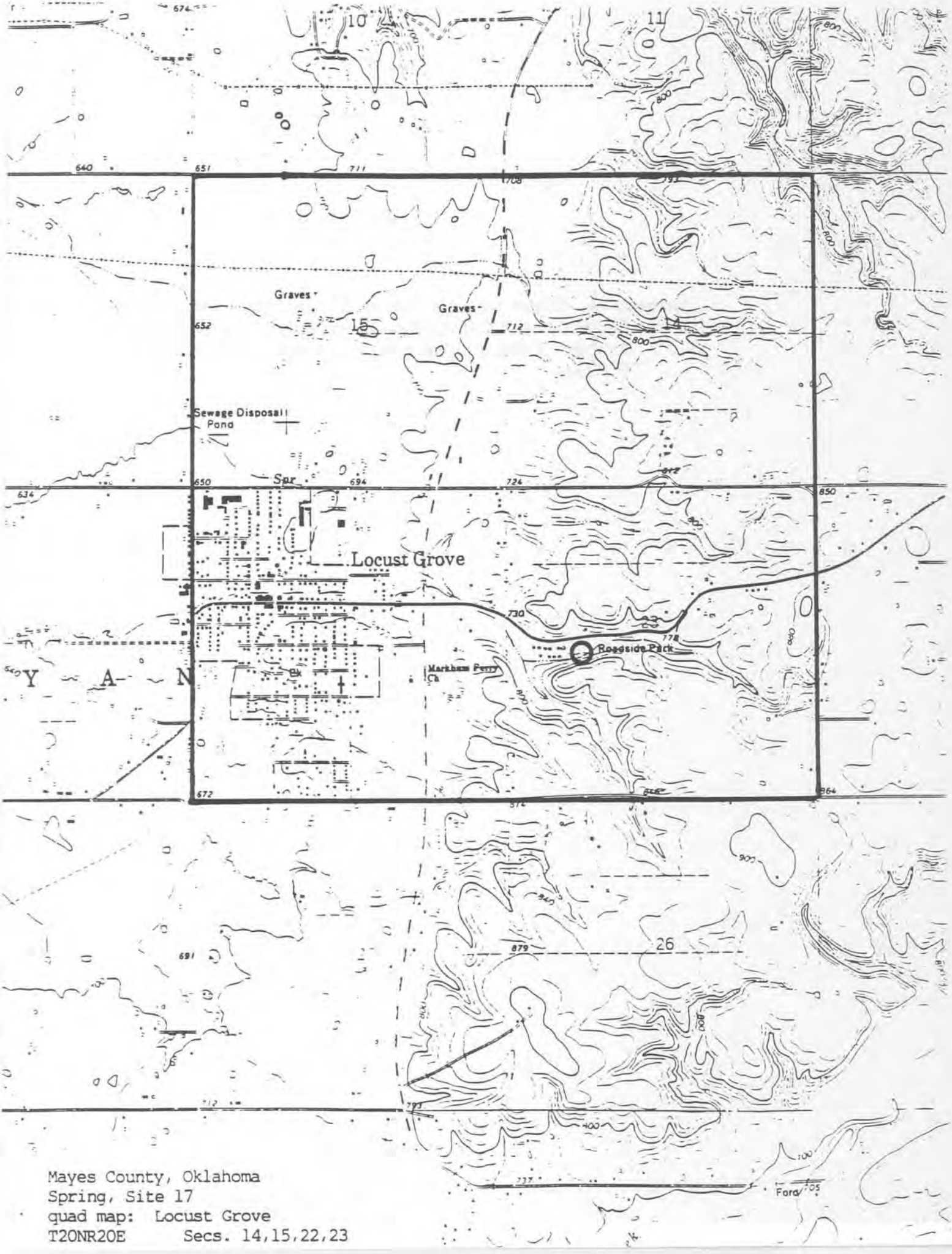
Site Location to Nearest 4 Sections: T20NR20E Sec. 23

Dates Surveyed and Source of Data: 26 May 90: Oklahoma Natural Heritage Inventory field survey by P. Mehlhop-Cifelli (F90MEH04)

Site Description: A spring in a roadside park 0.5 mi. E. of Locust Grove. The spring has been capped. A pipe has been inserted to let the spring water run freely into a small drainage.

Amblyopsis rosae Known from Site? no

Comments: J.H. Black (personal communication) suggested that this might be the actual locality for the Spring Creek specimen. If cavefish occur in this system, they could be observed only if flushed from the spring through the pipe.



Mayes County, Oklahoma
Spring, Site 17
quad map: Locust Grove
T20NR20E Secs. 14,15,22,23

Appendix II. Report from Dr. Jeffrey Koppelman of the Missouri Department of Conservation on results of electrophoretic analyses of tissue proteins from cave crayfish in the Ozark Plateau.

A Biochemical Genetic Analysis of Troglobitic Crayfish (Cambarus spp.) in Missouri, Oklahoma and Arkansas

A Report To

Missouri Department of Conservation

Oklahoma Natural Heritage Inventory

Arkansas Game and Fish Commission

By

Jeffrey B. Koppelman
Fisheries Research Biologist

April, 1990

Background

This research study was a preliminary investigation of the feasibility of using electrophoretic techniques such as those currently in use in the Fish Genetics Laboratory to examine systematics of cave crayfish (Decapoda: Cambarus spp.) in the Ozarks region. Five species of troglobitic Cambarus have been reported from this region: C. aculabrum, C. hubrichti, C. setosus, and C. tartarus and C. zophonastes. Morphological identification of these crayfish is difficult because Form I males are needed, and these may not be available in the small samples that can be obtained at a specific site at any one time. Biochemical genetic analysis has the potential advantage of providing a large amount of information from a few individuals from different populations since there is little gene exchange between these populations. Past investigations have revealed that epigean crayfish species have relatively low levels of heterozygosity (Nemeth and Tracey 1979; Brown 1981). The study objective was to determine whether electrophoresis can be used to distinguish between different species and to assess intraspecific genetic variability.

Methods

Because individual cave populations are relatively small, it was decided that chela muscle would be used for isozymic analysis to avoid population reductions. Although population genetic variation estimates are strongly enhanced by increased sample sizes, greater strength is gained through increased numbers of gene loci. A total of 48 individuals were captured from 20 different cave sites in Missouri, Oklahoma, and Arkansas during fall and winter, 1989, representing four of the five species in the tri-state region (Table 1). Specimens of C. zophonastes, known only from a single site in Arkansas, were not collected. The left chela was removed from each collected crayfish, which was then released. Cave crayfish missing a

left chela were observed in subsequent visits to some sites, indicating that they survived this method of tissue sampling.

All chelae were held on dry ice and placed in an ultra-low freezer (-80°C). To process the tissues for analysis, each whole claw, including the chitinous exoskeleton, was crushed, mixed with a buffer, homogenized, and centrifuged for 30 minutes at 12,000 x g. Samples were electrophoresed and subjected to histochemical staining procedures (Philipp and Whitt 1979; Koppelman and Philipp 1986). The resulting phenotypic banding patterns were used to directly infer genotype, and the resultant individual allelic information was statistically analyzed using the BIOSYS-1 program of Swofford and Selander (1981).

Results

Approximately 30 specific enzyme and general protein staining techniques were used to reveal gene products present in cave crayfish chelae muscle tissue, and 20 gene loci could be reliably scored using these methods. Thirteen loci were polymorphic (Table 2); the maximum number of alleles observed at a single locus was three (Table 2). However, only four populations had any heterozygosity: Fauna Cave, Sequiota Cave, Smallin's Cave, and Oklahoma Site 1. The actual amount of heterozygosity may have been greater, since an average of only 2.4 individuals were sampled per site. Of these four populations, Sequiota Cave had the greatest amount of direct-count heterozygosity (Table 3).

Contingency chi-square analyses confirm ($P<10^{-5}$) that these samples do not represent one large, randomly breeding population. Differentiation levels for the cave crayfish populations were very high for all polymorphic loci (mean $F_{DT}=0.963$). In term's of Nei's gene diversity analysis, 3.7% of the total gene diversity is included in the within-deme component; the among-deme diversity represents 96.3% of the total. Examining C. setosus together, mean $F_{DT}=0.924$.

Rogers distance measure clusters three main groups of populations at or above the 0.249 level (Figure 1). All three C. hubrichti populations are very similar (D=0.00) and they are very different from the other populations (D=0.423). C. aculabrum and Sites 1, 2 and 12 comprise a second cluster, although there is a moderate amount of differentiation (D=0.201) between C. aculabrum and these three Oklahoma sites. Sequiota, Smallin's, Fauna, and Fulbright caves were different from each other but they were clustered together separate from the remaining C. setosus populations and the single C. tartarus sample site, all of which were virtually identical (D=0.00).

Discussion

Several loci in addition to those reported could possibly be resolved for inclusion in future analyses. Thirteen polymorphic loci out of twenty is average among congeners. The low average number of samples per collection site does not allow for accurate representation of heterozygosity within each population. Under these conditions, it is unusual to see 15% polymorphic loci in population samples as small as those for Sequiota and Smallin's caves. It is even more interesting that these two caves are proximal in Greene County, Missouri.

It was expected that contingency chi-square analysis would confirm that all of these populations were not one large, randomly breeding population due to already confirmed multiple taxa. It is also not that unusual to observe the level of differential fixation found for a species complex represented by such small sample sizes. Fixation within C. setosus was similarly high, probably resulting from small sample sizes.

C. hubrichti represents the only troglobitic species in the subgenus Erebicambarus, and it was correspondingly the most genetically distinct species evaluated. The most significant

finding was different clustering of three groups of C. setosus, and the genetic relatedness of one of these groups to the C. tartarus population sample. C. aculabrum and Oklahoma C. setosus clustered together but at a distance level great enough to indicate that the two groups are genetically distinct. The remaining C. setosus were divided with a notable east to west split. Fantastic Caverns, however, was more proximal to the east group but clustered with the west group. The presence of variation in all of the eastern populations and an absence of variation in the western group, including Fantastic Caverns, accounted for the pairwise distance comparison separation. However, failing to detect variation in the western group, a likely outcome of small sample size, would not necessarily account for the three variable populations clustering together. Therefore, there is reason to conclude that there is some level of "uniqueness" in populations of C. setosus from the Greene County, Missouri, area. There is likewise something unique about Oklahoma C. setosus populations, producing a phenetic relationship intermediate between the western Missouri conspecifics and C. aculabrum.

Recommendations

There is ample evidence that the use of starch gel electrophoresis can provide the necessary resolution to examine intra- and interspecific genetic variation in troglobitic crayfish, provided that either the number of caves or the number of individuals sampled from each cave are increased. While heterozygosity is undoubtedly underestimated to some degree with small population sample sizes, the phenomenon of low heterozygosity among decapod crustacea has been well documented (Nemeth and Tracey 1979; Brown 1981). Therefore, variablility levels may be very low in cave crayfish populations. The fact that most geographically similar populations also clustered closely supports continued electrophoresis efforts.

Future research should be designed to accomplish two objectives. First, an accurate genetic evaluation of Ozarks representatives of both subgenera involved, Erebicambarus and Jugicambarus, is necessary to put into perspective the amount of differentiation observed between C. hubrichti and the other troglobitic species, which belong to the subgenus Jugicambarus. Analysis of additional C. hubrichti, as well as analysis of two epigean consubgeners, C. maculatus and C. hubbsi, would be required. C. zophonastes, known only from one location in Stone County, Arkansas, C. aculabrum from a second site in Benton County, Arkansas, additional C. tartartus from the only known site in Delaware County, Oklahoma, and additional C. setosus from both the Springfield, Missouri, and southwestern Missouri areas should be genetically evaluated.

The second objective should be to determine the extent of genetic differences between cave crayfish from the central region of Delaware County, Oklahoma, including Sites 1, 2 and 12, and other C. setosus in adjacent regions. C. aculabrum from both known sites in Arkansas should be sampled for comparison. It is possible that there is a separate taxon of cave crayfish distributed in this range in Oklahoma, or a continuous distribution of allele frequencies may exist between these taxa, indicating subspecific status.

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Table 1. **Cave crayfish (Cambarus spp.) populations, county, state, species and the number of individuals sampled.[a]**

	Population	County	State	Species	N
1.	Lewis Cave	Carter	Missouri	hubrichti	2
2.	Medlock Cave	Shannon	Missouri	hubrichti	1
3.	Deadman Cave	Oregon	Missouri	hubrichti	2
4.	Bear Hollow Cave	Benton	Arkansas	aculabrum	2
5.	Fantastic Caverns	Greene	Missouri	setosus	2
6.	Fulbright Spring Cave	Greene	Missouri	setosus	1
7.	Fauna Cave	Greene	Missouri	setosus	1
8.	Sequiota Cave	Greene	Missouri	setosus	3
9.	Smallin's Cave	Christian	Missouri	setosus	3
10.	Coolbrook Cave	Jasper	Missouri	setosus	1
11.	Kellhofer's Cave	Jasper	Missouri	setosus	5
12.	Wilson's Cave	Jasper	Missouri	setosus	1
13.	Elm Spring	Newton	Missouri	setosus	1
14.	Hearrell Spring	Newton	Missouri	setosus	1
15.	Hayes Spring Cave	Stone	Missouri	setosus	2
16.	Carrico Cave	Dade	Missouri	setosus	1
17.	Site 1	Delaware	Oklahoma	setosus	7
18.	Site 2	Delaware	Oklahoma	setosus	2
19.	Site 6	Delaware	Oklahoma	tartarus	6
20.	Site 12	Delaware	Oklahoma	setosus	4

[a] Population numbers correspond to those in Table 2.

Table 2. **Allele frequencies at each polymorphic locus surveyed. Population numbers correspond to those in Table 1.**

	Population																			
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
AAT-M																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	1.000	1.000	1.000	.000	1.000	.000	.500	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
3	.000	.000	.000	.000	.000	1.000	.500	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
AAT-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	.000	.000	1.000	1.000	1.000	.333	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADA-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ADK-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
ALD-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
FDP-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	.000	.000	.000	.000	.667	.333	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.333	.667	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GAP-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 2. (Continued)

GPI-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	1.000	.333	1.000	.000	.000	.000	.000	.000	.000	.000	.071	.000	.000	.000
2	.000	.000	.000	1.000	1.000	1.000	.000	.667	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.929	1.000	1.000	1.000
IDH-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	.000	.333	.167	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	.000	1.000	1.000	1.000	.667	.833	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	.000
3	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	.000	1.000
IDH-B																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	.000
3	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	.000	1.000
LDH-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MDH-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH-B																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MEN-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	.000
2	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	.000	1.000
PGD-A																				
(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	1.000	.000
3	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	.000	1.000

Table 2. (Continued)

PGM-A

(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

PRO-A

(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

PRO-B

(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

PRO-C

(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	.000	.000	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
3	.000	.000	.000	1.000	1.000	.000	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

SOD-A

(N)	2	1	2	2	2	1	1	3	3	1	5	1	1	1	2	1	7	2	6	4
1	.000	.000	.000	.000	.000	.000	.000'	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
2	.000	.000	.000	1.000	1.000	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
3	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Table 3. **Genetic variability at 20 gene loci in cave crayfish. A locus is considered polymorphic if more than one allele was detected. Mean heterozygosity is determined by direct count method.**

	Population	Sample Size	% Loci Polymorphic	Mean Heterozygosity
1.	Lewis Cave	2	0.0	0.000
2.	Medlock Cave	1	0.0	0.000
3.	Deadman Cave	2	0.0	0.000
4.	Bear Hollow Cave	2	0.0	0.000
5.	Fantastic Caverns	2	0.0	0.000
6.	Fulbright Spring Cave	1	0.0	0.000
7.	Fauna Cave	1	5.0	0.050
8.	Sequiota Cave	3	15.0	0.033
9.	Smallin's Cave	3	15.0	0.017
10.	Coolbrook Cave	1	0.0	0.000
11.	Kellhofer's Cave	5	0.0	0.000
12.	Wilson's Cave	1	0.0	0.000
13.	Elm Spring	1	0.0	0.000
14.	Hearrell Spring	1	0.0	0.000
15.	Hayes Spring Cave	2	0.0	0.000
16.	Carrico Cave	1	0.0	0.000
17.	Site 1	7	5.0	0.007
18.	Site 2	2	0.0	0.000
19.	Site 6	6	0.0	0.000
20.	Site 12	4	0.0	0.000

Figure 1. Unweighted pair group cluster analysis of genetic distance (D) values (Rogers 1972), based on all loci surveyed.