Environmental Biology of Fishes **62:** 279–296, 2001.

Troglomorphic sculpins of the *Cottus carolinae* species group in Perry County, Missouri: distribution, external morphology, and conservation status

Brooks M. Burr[a], Ginny L. Adams[a], Jean K. Krejca[b], Regina J. Paul[a] & Melvin L. Warren, Jr.[c]
[a]*Department of Zoology and Center for Systematic Biology, Southern Illinois University, Carbondale, IL 62901-6501, U.S.A. (e-mail: burr@zoology.siu.edu)*
[b]*Section of Integrative Biology, University of Texas, Austin, TX 78712, U.S.A.*
[c]*Center for Bottomlund Hardwoods Research, U.S. Forest Service, Southern Research Stution, Oxford, MS 38655, U.S.A.*

Received 3 April 2000 Accepted 15 January 2001

Key words: Cottidae, grotto sculpins, cave distribution, density estimate, cave adaptation

Synopsis

The existence of cavernicolous sculpin (here allocated to *Cottus carolinae*, banded sculpin, and referred to as grotto sculpin), in the karst regions of Perry County, Missouri, first came to our attention in 1991. Examination of 35 caves in Missouri, 96 in Illinois, 17 in Tennessee, two in Indiana, and 11 in Arkansas revealed that banded sculpin are common in cave habitats; however, grotto sculpin are limited to two karst areas of Perry County, Missouri, where they are known from only six cave systems. These caves and their streams are extensive and apparently provide a unique habitat compared to other karst systems; this may be a critical factor in the present restricted distribution of the grotto sculpin. Grotto sculpin occupy pools and riffles of cave streams, and occur over a variety of substrates, from sediment to breakdown. Density estimates in Mystery and Running Bull caves were 0.29 and 0.63 individuals m^{-2}, respectively. Grotto sculpin have small eyes (1–6% SL vs. 6610% SL in epigean samples), significantly reduced pigmentation (including nearly complete loss of dorsal saddles), a reduction in pelvic fin ray number (from 4 + 4 elements to often 4 + 3 , or 3 + 3), and enlarged cephalic lateralis pores (e.g., mandibular pores of cavernicolous samples are 2-3 times those of epigean stream samples). Multivariate analyses of body shape revealed statistically significant separation of epigean and hypogean samples, with eye size highly variable, but smallest in the Running Bull Cave population. We interpret these results as representative of losses associated with long-term cave habitation. Caves of Perry County provide ample habitat for grotto sculpin, but because the caves are located downgradient of the city of Perryville and an intensively farmed landscape, point and non-point source pollution threaten their continued existence. Escape of farm-pond fishes through the extensive sinkhole network in Perry County has increased potential predation pressure on grotto sculpin by channel catfish, *Ictalurus punctatus*, and other species normally excluded from cave environments.

Introduction

> *'A certain predisposition in habit and structure must he present to enable a species to dispense with light and to live in caves.'*
>
> Carl H. Eigenmann (1909, p. 9)

The karst terrain and aquifers of North America provide habitats for some of the most spectacular examples of hypogean fishes found anywhere in the world including, an endemic family of cavefishes (Amblyopsidae) and troglobitic ictalurid catfishes from deep artesian waters of Texas and caves of Mexico (Page & Burr 1991, Walsh & Gilbert 1995). New hypogean species continue to be discovered and described (e.g., Walsh & Gilbert 1995) from the North American continent.

Fish species that bridge existence between epigean and hypogean habitats are relatively few in the New World. These include the most thoroughly investigated case of any epigean-hypogean species to date, the Mexican tetra, *Astyanax fasciatus* (Characidae) of Atlantic Slope karst in Mexico (Mitchell *et* al. 1977). Less studied is the cavernicolous population of Mexican molly, *Poecilia mexicana* (Gordon & Rosen 1962) from Tabasco, Mexico. Sculpins (Cottidae: *Cottus*), benthic cool-water fishes, are known to frequent springs, cave outflows, and the twilight and aphotic zones of caves in the karst topography of eastern United States (Mohr 1950, Cooper 1978. Williams & Howell 1979, Poly & Boucher 1996). These quasi cave-dwelling sculpin do not exhibit physical characteristics reported for typical stygobites (i.e., loss of pigmentation, reduced eyes, and enlarged lateral line receptors). Even an albinistic sculpin, *Cottus carolinae*, described from a single individual in a West Virginia cave, had normal eye size albeit unusual morphology (Williams & Howell 1979).

Periodic study of the fish fauna in Perry County caves and surface streams over the past 10 years has revealed unique populations of sculpin (allocated here to *Cottus carolinae*) comprised of reproducing, subterranean individuals exhibiting phenotypic variability in structural and physiological features normally restricted to fish populations inhabiting either epigean or hypogean environments.

Here we document the geographic distribution of the cave-inhabiting sculpins, provide population density data for two caves located 6 km apart, and describe the external morphology of the cave-inhabiting sculpins and their epigean relatives. We also document local environmental threats to the groundwater in Perry County. The cave populations may not represent a species different from C. *carolinae* but our observations establish their uniqueness and vulnerability to extinction from various groundwater pollution problems. To facilitate discussion we use the common name grotto sculpin for the unique cave-inhabiting sculpins.

Materials and methods

Study area

Perry County, Missouri, is located in the karst region of the Salem Plateau in the northeast Ozarks (N 37’ 35′ to 37° 55′, W 90” 05’ to 89” 45’). Perry County contains several large caves and one of the largest concentrations of caves in North America (about 650 identified caves, Elliott 2000), which are generally found in limestones and dolomites of Ordovician age. Cinque Hommes Creek, which drains into the nearby Mississippi River, is the resurgence stream for many caves in the area (Figure 1). Some caves in Perry County contain grotto sculpin that have access to a source pool of epigean sculpin. Hydrological information is insufficient to infer whether sculpin move between epigean and cave populations. Mean temperatures of caves and their associated streams in the region are about 13°C, and they contain permanent, slow-flowing streams over rubble and silt substrates.

Distribution

We undertook cave exploration in five states to determine the geographic range of the grotto sculpin. Our primary exploration focused on nine karst regions within Perry County, Missouri (House 1976), where grotto sculpin were known to occur (Figure 2). We used standard caving equipment (Rea 1987), specialized gear (e.g., wetsuits, ropes, and vertical gear), and vertical techniques (Padgett & Smith 1987) as conditions dictated.

We searched for sculpin in cave streams by walking slowly upstream in shallow water, catching fish by hand or small dip nets, and temporarily holding them in jars and buckets. We used a portable aerator, thermometer, ruler, and camera to aid in documentation of habitat and fish. We collected surface sculpin using standard seining techniques (Jenkins & Burkhead 1994).

Population density estimates and habitat

We estimated sculpin density in Mystery and Running Bull caves in October 1991 and 1993 when low water levels afforded high water clarity. We measured the lengths and widths of shallows and riffles with a Keson meter tape and took 10 random depths with a meter stick within each macrohabitat. We recorded current speed (nearest 1 m sec^{-1}) by floating a neutrally buoyant object over a measured distance. At fifteen sites in each of both Mystery and Running Bull caves we surveyed a total of 180 and 10 1 m of stream, respectively. Within a given macrohabitat, we turned over all rocks and counted sculpin. We qualitatively noted eye size (small vs. large [comparison shown in Figures 4, 5]) and size class (juvenile [<40 mm TL] or adult

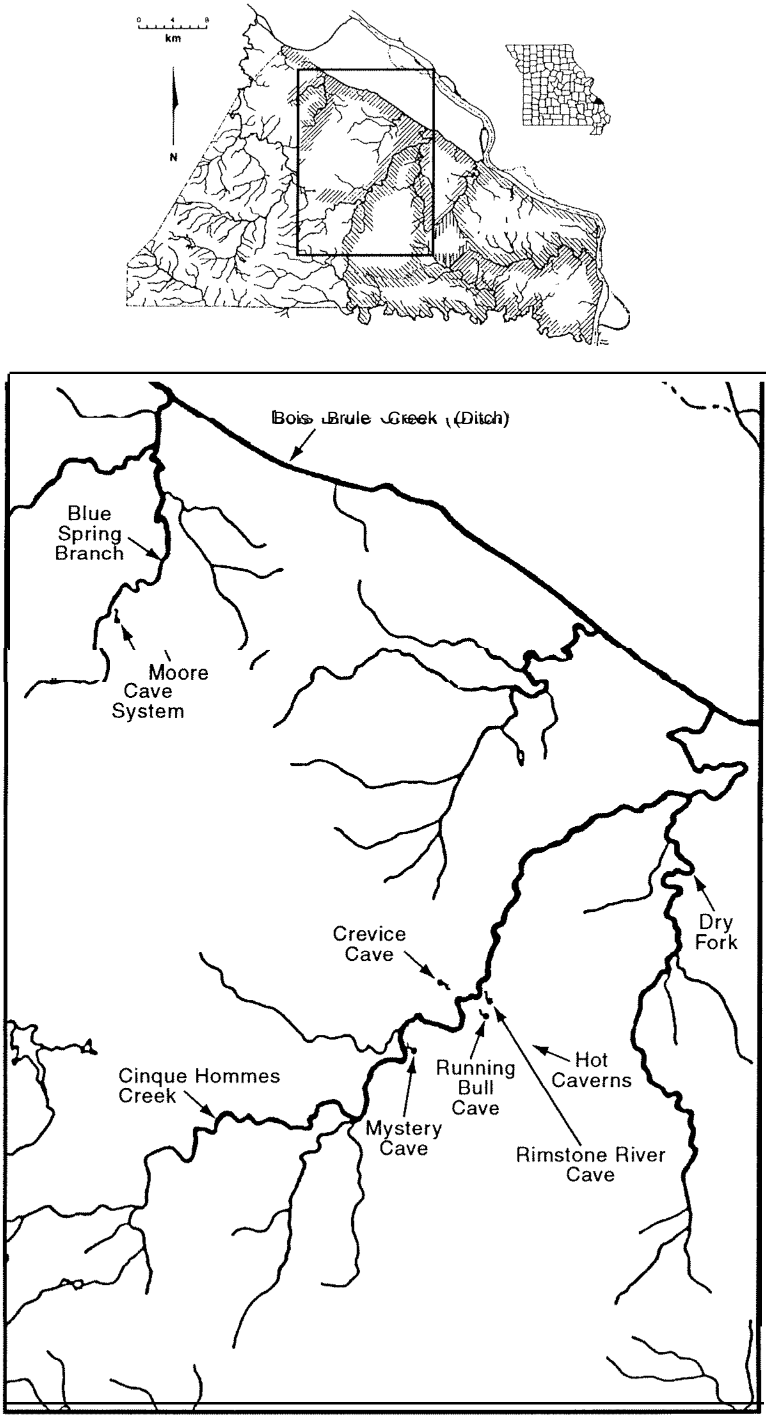

Figure 1. Drainage relations of central Perry County, Missouri

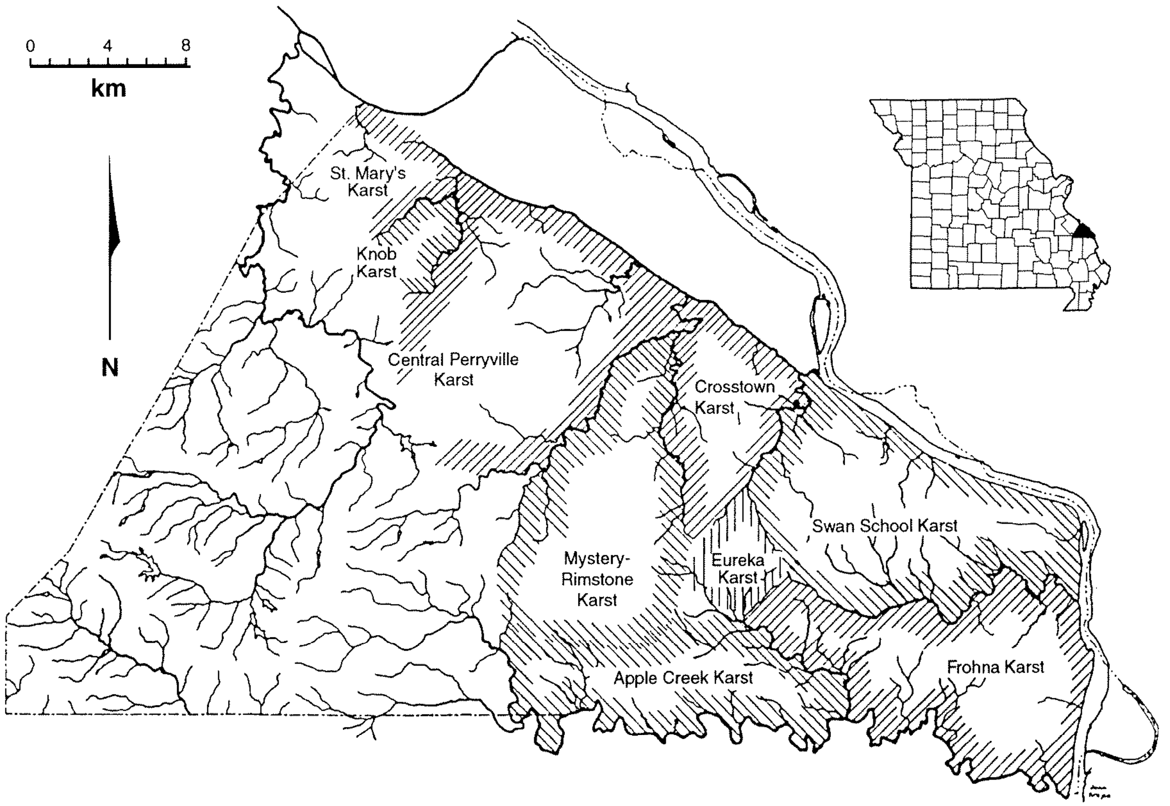

Figure 2. Karst regions of Perry County, Missouri, as defined by House (1976).

[>45 mm TL]) for each specimen. We based extrapolation of' total numbers of grotto sculpin on conservative estimates of available stream passage within each cave.

External morphology

We made seven measurements (eye length, eye depth, head depth in line with jaw tips, head depth in line with edge of preopercle spine, length of longest preopercle spine, length of pelvic fin, interorbital length) on the left side of 84 specimens using dial calipers (nearest 0.01 mm) following methods in Hubbs & Lagler (1974). An additional 24 angular distances were taken from one homologous landmark to another (Figure 3) following truss geometric protocol (Strauss & Bookstein 1982). We subjected the distance values to multivariate analyses (i.e., principal components, canonical variates, discriminant function) to elucidate phenotypic differences among populations. We then subjected each of the highest loading components from the principal component analyses to bivariate regression analyses with standard length serving as the independent variable. We conducted univariate and multivariate analyses on the Southern Illinois University at Carbondale (SIUC) mainframe computer using SAS (1985) and a Macintosh personal computer using programs in Statistica (Statsoft 1994).

Comparisons of nine meristic features between hypogean and epigean samples were nonsignificant. We retained one feature, number of pelvic fin rays, for further analysis. We counted all rays from both the right and left pelvic fins. We examined size of cephalic lateralis pores using a Wild binocular microscope fitted with an eyepiece micrometer. We measured the longest axis for length and width for each of pores 3, 4, and 6 on the mandibular canal, using the chin pore as pore number 1. We followed general terminology and illustrations of head pore arrangements from Robins & Miller (1957). We vouchered all specimens used in the analyses (Table 1) in the Fluid Vertebrate Collection of SIUC, where they are stored either in 70% ethanol or as frozen carcasses.

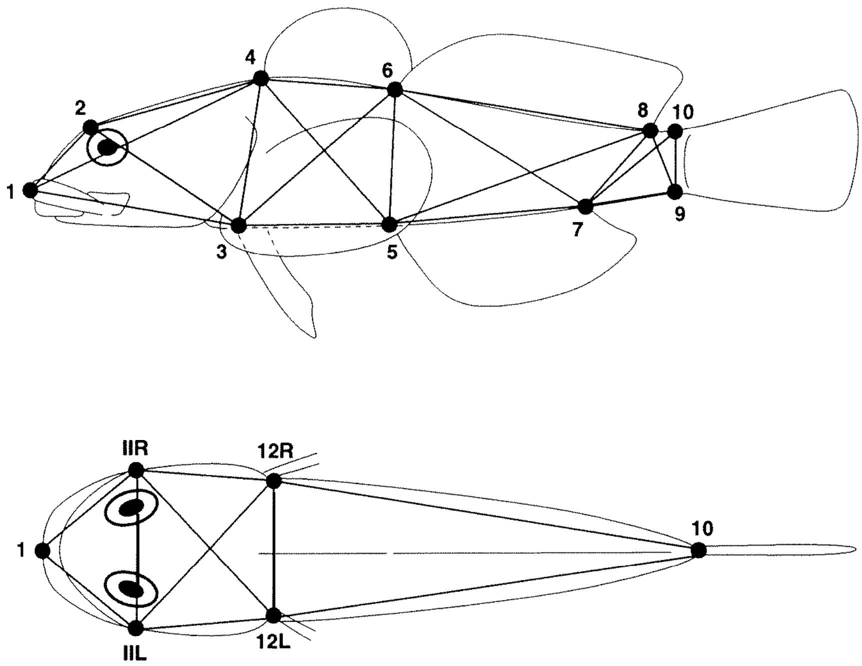

Figure 3. Truss geometric design for a sculpin (modified from Strauss & Bookstein 1982).

Table I. General location, macro-environment, sample size (N), and SIUC catalog number of specimens of C. *carolinae* **used** for counts and measurements. All specimens are from southeast Missouri; all resurgence stream and hypogean samples are from Perry County.

Location	Macro-environment	N	Catalog number
Black River, Reynolds Co.	Epigean stream	4	SIUC 18702
Big Piney River, Texas Co.	Epigean stream	3	SIUC 3216
Big Creek, Wayne Co.	Epigean stream	5	SIUC 3252
Big Creek, Wayne Co.	Epigean stream	4	SIUC 3158
Cinque Hommes Cr.	Resurgence stream	20	SIUC 19279
Mystery Cave	Hypogcan stream	20	SIUC 1x923
Rimstone River Cave	Hypogean stream	23	SIUC 19839
Running Bull Cave	Hypogean stream	7	SIUC 21260
Running Bull Cave	Hypogcan stream	12	SIUC 37892
Crevice Cave	Hypogean stream	2	SIUC 21261
Hot Caverns	Hypogean stream	4	SIUC 21258
Tom Moore Cave	Hypogean stream	9	SIUC 20916

Results

Distribution

Grotto sculpin are restricted to two karst areas of Perry County: Central Perryville Karst and Mystery-Rimstone Karst (Figure 2). Only six of27 cave streams sampled in six karst regions contained grotto sculpin. In the Central Perryville Karst, viable populations occur in the Moore Cave system and Crevice Cave (Figure 1). In the Mystery-Rimstone Karst, Hot Caverns, Mystery Cave, Rimstone River Cave, and Running Bull Cave (Figure 1) all harbor relatively large populations of grotto sculpin. Grotto sculpin also may occur in a few other caves that are hydrologically connected to these known sites but were not accessible for searches.

Results of specific distribution data arc divided between the two known karst areas of occurrence.

In the Central Perryville Karst, the Moore Cave system contains 3 1.18 km of mapped passage; we have observed grotto sculpin in the main cave stream from its source (known as Tom Moore) to the Siphon area downstream (Berome Moore), a distance of 3.45 km. Crevice Cave, with 45.47 km of mapped passage, is comprised of four major cave streams; grotto sculpin occur in the major southcast trending Eternity Passage. In the Mystery-Rimstone Karst, the main trunk of Rimstone River Cave contains 8 km of underground stream, referred to as the Nile, and sculpin were collected over a distance of 604 m beginning at the Flaming River entrance. Hot Caverns, a closely associated cave, contains a tributary of the Nile River, referred to as the Labyrinth Stream. Sculpin were seen or collected in this section of the Nile and Labyrinth streams. Running Bull Cave has 374 m of mapped passage; sculpin were found from between the first crawlway downstream of the entrance for a distance of about 252 m. Mystery Cave has 27.35 km of surveyed passage. Grotto sculpin were found in Main Stream for about 5 km downstream of the gated sinkhole entrance.

Of 12 surface stream sites sampled, we found epigean sculpin in Blue Spring Branch, Cinque Hommes Creek, and Dry Fork. Blue Spring is the principal resurgence stream for caves and sculpin in the Moore Cave system. Cinque Hommes Creek is the principal resurgence stream for caves in the Mystery-Rimstone Karst and Crevice Cave in the Central Perryville Karst that harbor grotto sculpin (Figure I).

Our field searches of 153 additional caves verified that sculpin frequent caves, but cavernicolous features were lacking in every case except populations discussed above from Perry County, Missouri. Field searches by us and colleagues revealed sculpin identified as C. *carolinae* in 25 caves in seven states (Table 2), but sculpin were not found in 11 Arkansas caves, 94 Illinois caves and karst springs, one Indiana cave, 28 Missouri caves, and 15 Tennessee caves and karst springs.

Population density estimates and habitat

We found 60 grotto sculpin in Mystery Cave (15 sites, 180 m of stream) at a mean density of 0.29 individuals m^{-2} (SE = 0.087). Of these, 24 had small eyes and 36 had larger eyes but not as large as those ofepigean samples taken outside of Perry County (Figure 4, Table 3). Sampled sites (mean reach of 12 m) averaged about 1 sculpin per 3 m. We found no correlation between eye size and pool length, pool width, pool area, and volume of site ($p > 0.05$). Total number ofsculpin and depth of water were correlated positively ($r = 0.39$, $p < 0.05$). Assuming that at least 8 km of stream is available, and that sculpin are distributed uniformly with no barriers to dispersal, we estimate 2667 sculpin occur in Mystery Cave.

In Running Bull Cave (15 sites, 101 m of stream), mean density of grotto sculpin was 0.63 individuals m^{-2} (SE = 0.19 1). We counted 51 sculpin, 43 of which had small eyes and eight had larger eyes. Sampled sites (mean reach of 6.8 m) averaged about 1 sculpin per2 m. We observed six other sculpin in areas where measurements were not made. We estimate at least 1SO sculpin occur in the 300m of stream available in Running Bull Cave.

Grotto sculpin frequent sustained, modest flow of pools and riffles with little reliance on physical cover or formation of large aggregations. The main streams in caves harboring grotto sculpin averaged 1.1-3.3 m in width and between 4 and 33 cm depth during autumn low-water levels. Sculpin occupy pools and riffles; mean current speed of riffles was 0.05 m sec^{-1}. They occur over a variety of substrates including silt, gravel, cobble, breakdown, and bedrock, most frequently over a rocky bottom, and are just as likely to be in open water as hidden under rocks. Most adults occur singly but occasionally as many as three individuals are found together, only a few centimeters apart.

Perry County has hundreds of sinkholes and numerous farm ponds that may unexpectedly drain through a sinkhole transferring the pond water, flora, and fauna into a cave. We have found epigean farm-pond fishes in all of the caves where cavernicolous sculpin occur including an unsuitably large population of channel catfish, *Ictalurus punctatus*, in Mystery Cave. Other fishes found with sculpin in Perry County caves include common carp *Cyprinus carpio*, fathead minnow *Pimephales promelas*, yellow bullhead *Ameiurus natalis*, green sunfish *Lepomis cyanellus*, and bluegill *Lepomis macrochirus*. All of these species are potential predators on eggs and young of sculpin. The two catfish species are the most significant potential predators of adult sculpin.

Identification and taxonomy

Wc recognize four phenotypes of sculpin in southeastern Missouri, two epigeun stream phenotypes and the

Table 2. Caves where sculpin (*Cottus carolinae*) have been found, but where specimens do not exhibit phenotypic features of cave adaptation (i.e., small eyes, depigmentation, reduced meristics, enlarged cephalic canal pores).

State Cave, County	Date	Source	Voucher
Illinois			
Fogelpole Cave, Monroe Co.	1 Jul Y2	J. Krejca. S. Taylor	SIUC 21305
Crystal Cave. Hardin Co.	7 Aug Y1	J. Krejca	None
Indiana			
Seiberts Well, Crawford Co.		H. H. Hobbs III, J. Lewis	None
Batey's Cave. Green Co.		H. H. Hobbs III	None
Binkley's Cave, Harrison Co.		H. H. Hobbs 111	None
Connerley's Cave, Lawrence Co.		H. H. Hobbs III	None
Donnehue's Cave, Lawrence Co.		H. H. Hobbs III	None
Popcorn Spring Cave, Lawrence Co.		H. H. Hobbs III	None
Sullivan's Cave, Lawrence Co.	29 Oct 92	H. H. Hobbs III. J. Krejca	None
Boone Cave, Owen Co.		H. H. Hobbs III	None
3 small caves, Washington Co.		H. H. Hobbs III	None
Fredericksburg Cave. Washington Co.		J. Lewis	None
Kentucky			
Cool Spring Cave, Barren Co.		H. H. Hobbs III	None
Mammoth Cave, Echo & Mystic R. Edmonson Co.		J. Lewis, M. Sutton	None
Missouri			
Saranac Spring Cave, Crawford Co.	25 Sep Y2	J. Krejca, S. Taylor	None
Blowing Spring Cave, Laclcde Co.	15 Oct 93	J. Krejca, S. Taylor	SIUC 23158
Lewis Cave, Ripley Co.	2X Jan 67	R. A. Brandon	SIUC 18948
Cropper Cave, Oregon Co.		M. Sutton, J. Gardner	None
Powder Mill Creek Cave Shannon Co.		M. Sutton. S. Et-vine	None
Tennessee			
Yates Cave, Robertson Co.	24 Apr 94	J. Krejca, S. Taylor	None
Virginia			
Butler Cave, Bath Co.		H. H. Hobbs 111	None
Tawneys Cave. Giles Co.		H. H. Hobbs 111	None
West Virginia			
Buckeye Creek Cave Greenbriar Co.	several dates	H. H. Hobbs 111	None

grotto sculpin represented by two hypogean phenotypes. We distinguish phenotypes based on relative eye size, degree of pigmentation, relative cephalic canal pore size, and pelvic fin-ray number. One epigean stream phenotype (Figures 5a,b) ranges outside of Perry County and the Cinque Hommes Creek system and is typical of the midlands race of *C. carolinae* as described by Robins (1954). The other epigean stream phenotype occurs in Cinyue Hommes Creek, the major stream of resurgence for several caves, and is distinguished by reduced pelvic fin-ray number (52% with 3 + 3 or 3 + 4 rays), moderate eye size (4–6% of SL), moderate cephalic canal pore size (Table 4), and dark pigmentation similar to midlands race sculpin. The two hypogean phenotypes co-occur in deep cave habitats of Perry County, Missouri. One hypogean phenotype is depigmented with reduced pelvic fin-ray numbers (47-75% with 3 + 3 or 3 + 4 rays) and enlarged cephalic canal pores, but eye size (5–6% of SL) is nearly equivalent to the epigean phenotypes (Figures 4b,f). The second hypogean phenotype is also depigmented (Figures 5c,d), has enlarged cephalic canal pores and reduced pelvic fin-ray numbers, but eye size is tiny (1–4% of SL) relative to other phenotypes (Figures 4a,e). We allocate these four phenotypes to the banded sculpin, *Cottus carolinae*, using the key characteristics and descriptive features outlined by Pflieger (1997) for Missouri sculpin and Page & Burr (199 1) for North American sculpins. The two features shared by all four populations include: (I) usually a complete lateral line ending near the base of the caudal fin, and (2) dorsal fins usually not connected.

External morphology

Trenchant features characterize individuals of grotto sculpin when compared to the wide-ranging midlands race of *Cottus carolinae carolinae* described in detail by Robins (1954). Further, sculpin from Cinque Hommes Creek (a resurgence stream), although a surface population, also have a unique set of morphological traits (see above) compared to other surface populations outside Perry County. Here we describe in detail the external features of grotto and resurgence stream sculpin as a basis for comparison in the Discussion to the midlands race in surface streams.

Size. Mean standard length (SL) of grotto sculpin was 59.98 mm (N = 8, SD = 20.53) for Tom Moore Cave; 68.18 mm (N = 4, SD = 13.57) for Hot Caverns; 70.13 mm (N = 20, SD = 11.33) for Mystery Cave; 69.83 mm (N = 21, SD = 13.51) for Rimstone River Cave; and 68.18 mm (N = 15, SD = 9.50) for Cinque Hommes Creek. The largest grotto sculpin was 104 mm SL from Mystery Cave. Specimens used for comparison from outside Perry County averaged 60.23 mm (N = 16, SD = 17.24). We detected no significant difference (p = 0.12) in mean SL among any of the populations studied.

Pigmentation and skin prickling. Grotto sculpin have dorsal, caudal, and pectoral fins and distal edge of anal fin lightly spotted with melanophores, forming weak bands to slightly tessellated; pelvic tin unpatterned; margin of first dorsal fin sometimes pale yellow, not edged with orange. Ground color light tan to bleached tan; throat, belly, and lower caudal peduncle unpigmented. Three saddles on back reduced to vestigial spotted blotches, not extending downward and forward on side. Lower sides without reticulations. Two dark bars on cheek reduced in size and intensity of pigmentation. Small dark blotch near middlc of pectoral fin base absent. Chin only slightly mottled to unmottled. We did not stain skin prickles or examine their shape, but grotto sculpin appear to be nearly devoid of prickles. Individuals from the resurgence stream (Cinque Hommes Creek) have pigmentation similar to that described for epigean stream populations (Robins 1954).

Lateral line system. Lateral line usually complete to caudal base (three specimens with lateral line ending under posterior edge of second dorsal fin). Lateral line pores in the resurgence stream population (Cinque Hommes Creek) 32-36, modally 35; preoperculomandibular pores 10-l 2, modally 1 l; canals never fused anteriorly into a median chin pore; infraorbital pores 8-9, modally 9. Lateral line pores in cavernicolous individuals (Mystery Cave) 32-36, modally 33; preoperculomandibular pores 10–1 l, modally l 1; canals often fused anteriorly into a median chin pore (e.g., 10 of 20 specimens from Rimstone River Cave); postmaxillary (or postmandibular) pore occurs at pore 5, missing in only 3 of 32 specimens examined; infraorbital pores 7-10, modally 9. Canal pore sizes large, especially those on the head (i.e., mandibular pores 3, 4, and 6) (Table 4).

Morphometry. Body deepest under first dorsal fin, tapering sharply in either direction; venter flat; caudal peduncle slender (N = 68), comprising 6.4% (±0.47% SE) to 7.6% (±0.77% SE) of SL. Eye length variable, ranging from eyes reduced to pin-holes to near eye length of epigean fish, comprising 1.2–6.5% of SL. Greatly reduced eye size is frequent (e.g., 24 of 60 individuals, Mystery Cave); however, the ratio of large-eyed and reduced-eyed individuals was not significantly different from 1 : 1 (adjusted G-test, G = 2.40, p = 0.5). Preopercular spines present, blunt and reduced in length. Dorsal fins slightly connected or entirely separate. Caudal fin truncate or subtruncate (sensu Jenkins & Burkhead 1994).

All cavernicolous sculpin and the samples from Cinque Hommes Creek have smaller eyes than those from epigean stream samples regardless of length of

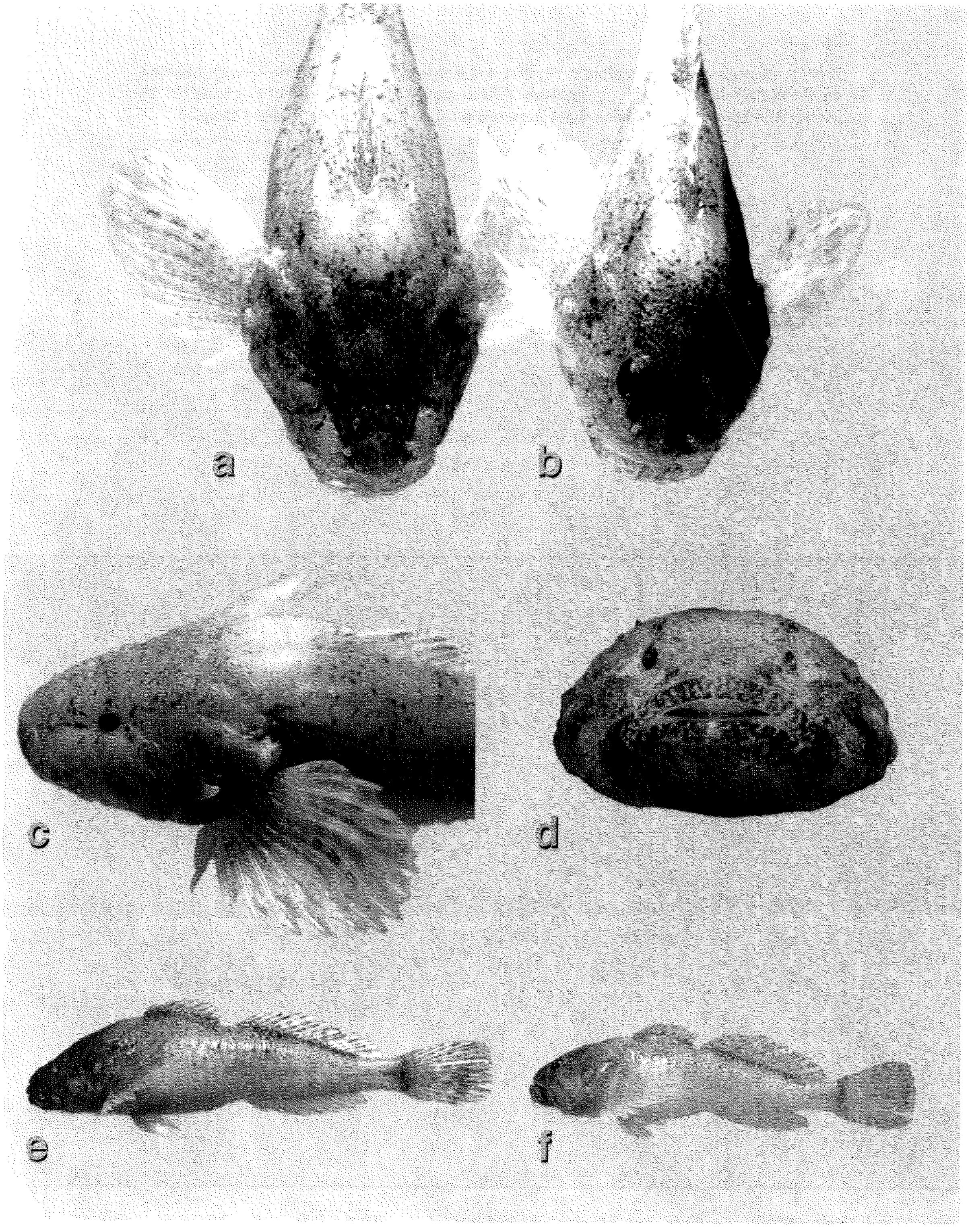

Figure 4. a and b: Dorsal view of tiny-eyed and larger-eyed grotto sculpin co-occurring in Mystery Cave, Perry County, Missouri, 1 Oct 1991. c: Close-up view of tiny-eyed individual from Mystery Cave, same data. Photos by J.J. Swayne. d: Frontal view of tiny-eyed individual from Mystery Cave showing enlarged mandibular pores. Photo by C.A. Lee. e and f: Lateral view of tiny-eyed and larger-eyed grotto sculpin co-occurring in Mystery Cave. Perry County, Missouri. 1 Oct 1991. Photos by B.M. Burr.

Table 3. Numbers of grotto sculpin in Mystery and Running Bull Caves, Perry County, Missouri, on 22 Oct 1991 and 11 Oct 1993, respectively. Fifteen sites representing a total of 179.5 and 101.2 m of stream in Mystery and Running Bull Caves, respectively, were characterized and sampled.

	Length (m) of pool/run	Width (m) of pool/run	Depth (cm) of pool/run	Number of sculpins	
				large eye	small eye
Mystery Cave					
Mean	12.0	1.9	15.2	2.4	1.6
Range	3.5-22.8	1.3–3.3	6.5-25.1	o-5	0–4
Total				36	24
				60	
Running Bull Cave					
Mean	6.8	1.3	11.3	0.5	2.9
Range	2.4–15.5	0.5–2.5	4.0-21.8	O-3	O-7
Total				8	43
				51	

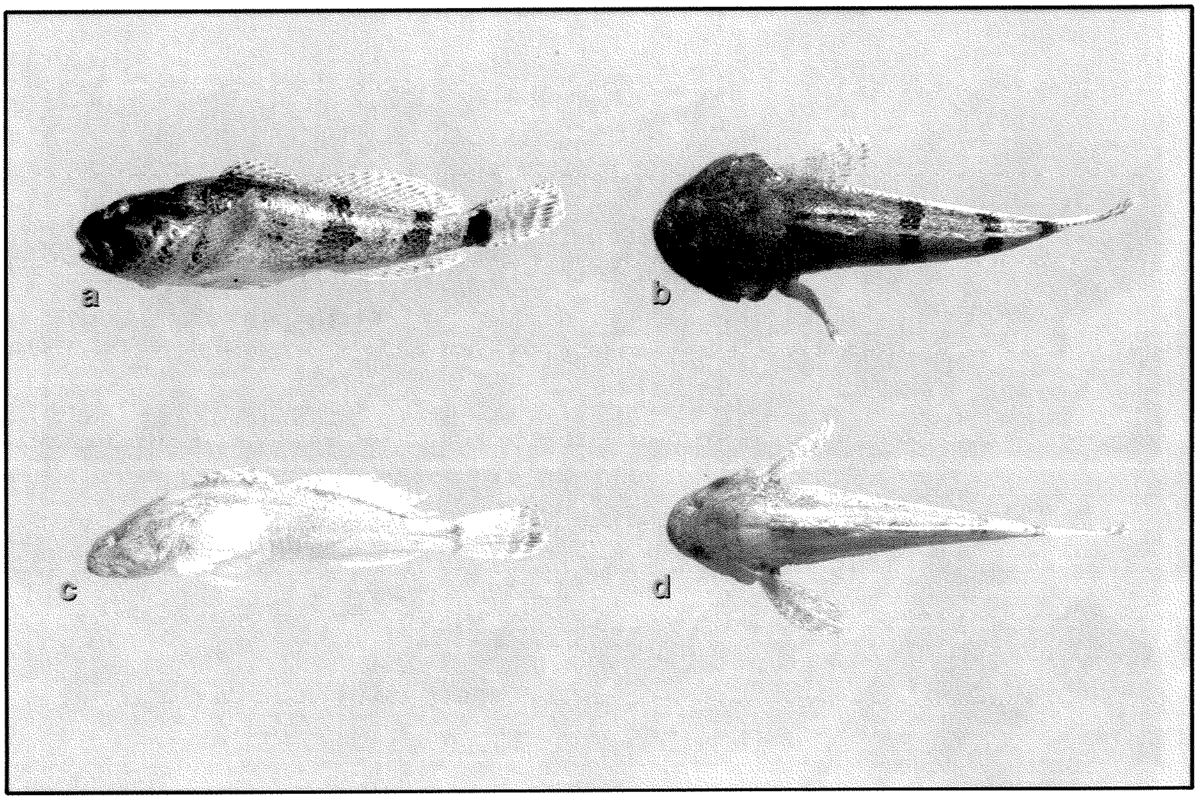

Figure 5. a: Lateral view of C. *carolinae* from Blowing Springs Cave (78 mm SL), Laclede County, Missouri, 15 Oct 1993 showing coloration and features of epigean stream sculpin. b: Dorsal view of Blowing Springs Cave specimen showing dark saddles across back and normal eye size. c: Lateral view of grotto sculpin (7.1 mm SL) showing tiny eyes and reduction in pigmentation, Mystery Cave, Perry County, Missouri, 1 Oct 1991. d: Dorsal view of Mystery Cave grotto sculpin showing lack of saddles and shrunken cyc sockets. The Blowing Springs Cave individual was found past the twilight zone of the cave. Photos by B.M. Burr.

Table 4. Mean size of cephalic lateralis canal pores in C. *carolinae* complex as determined by straight line measurements of greatest length (L) and width (W) of pore. Pores measured are those from the mandibular canal, numbers 3, 4, and 6, using the chin pore as pore number 1 (N = 8, each population). Units are micrometers rounded to the nearest whole number.

Location	L, pore 3 (± SE)	W, pore 3 (± SE)	L, pore 4 (± SE)	W, pore 4 (± SE)	L, pore 6 (± SE)	W, pore 6 (± SE)
Mystery Cave	819 (94)	442 (96)	640 (71)	390 (64)	515 (34)	299 (49)
Rimstone R. Cave	975 (70)	485 (33)	X25 (78)	450 (51)	540 (59)	408 (47)
Cinque Hommes Cr.	600 (28)	360 (25)	538 (68)	305 (26)	415 (39)	268 (22)
Epigean streams outside Perry Co.	298 (42)	205 (29)	260 (56)	185 (32)	240 (24)	168 (12)

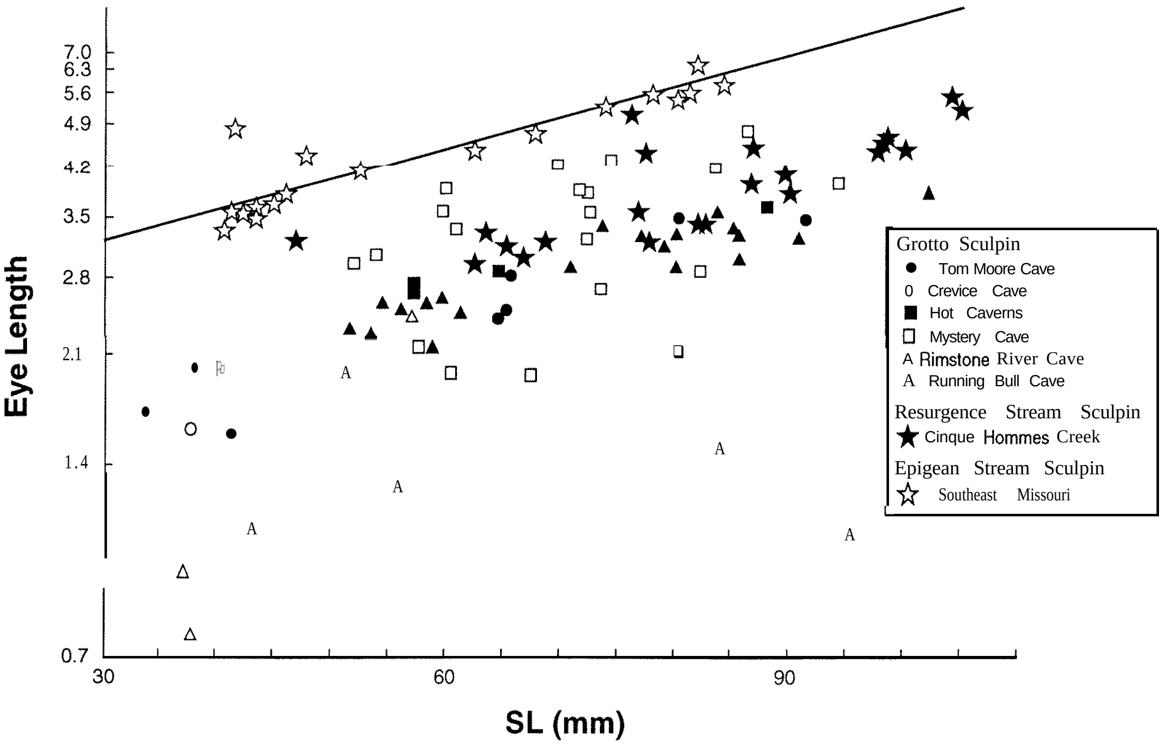

Figure 6. Relationship between eye length and standard length in six populations of grotto sculpin, one resurgence stream population, and epigean stream samples from southeast Missouri, outside Perry County. The regression line is fitted to individuals representing typical epigean populations of *Cottus carolinae* from southeast Missouri.

sculpin (Figure 6). In a principal component analysis, eye length loaded highest on PC axis 2 and separated populations in Running Bull Cave (smallest eyes) from several caves (intermediate in eye lengths) and epigean surface stream samples (largest eyes). Mystery Cave contained two distinct phenotypes, one with small eyes and one with eye sizes approaching those on the surface (Figure 7). Discriminant function analysis revealed all populations, except Rimstone River Cave and Hot Caverns which share drainage systems, were significantly different ($p < 0.05$, squared Mahalanobis distances, Table 5). Only three of 84 individuals were misclassified based on a discriminant function classification matrix. Two of the three individuals were from Hot Caverns and were misclassified with Rimstone River Cave individuals. Canonical variates analysis separated samples in Mystery Cave from those in other caves and epigean samples from southeast Missouri, on the basis of head size and shape and body width (Figure 8, Table 6).

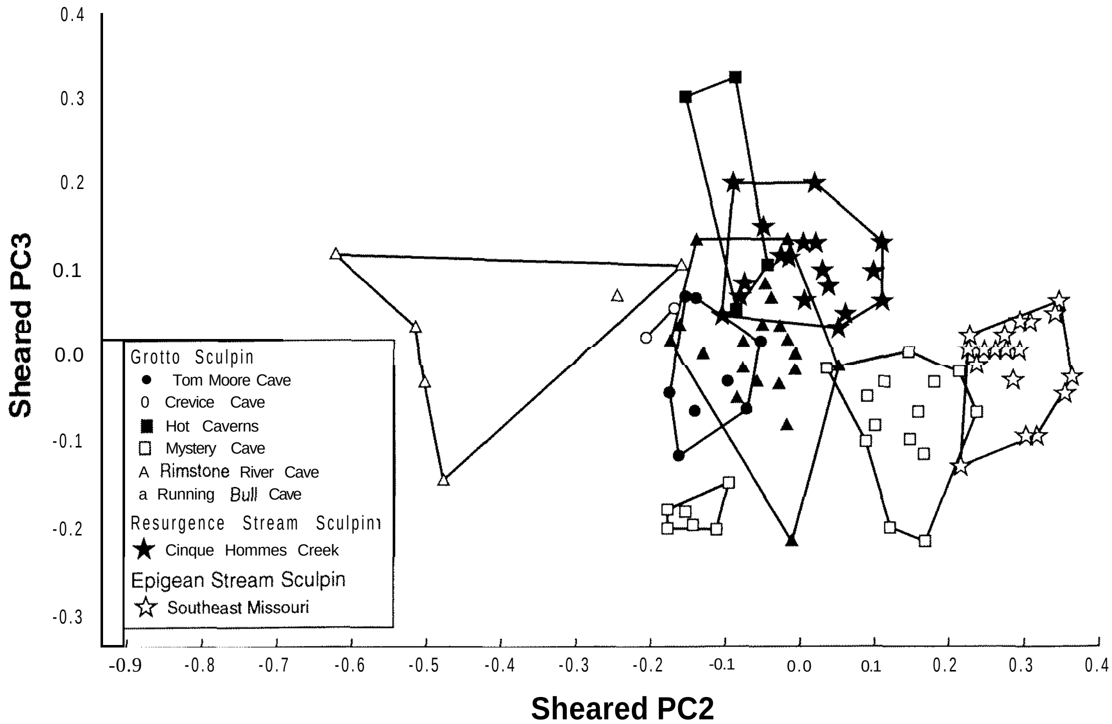

Figure 7. Scores of sculpin samples from six cave streams, a resurgence stream, and epigean surface streams on sheared principal component axes 2 and 3 for 3 l morphometric variables. Note that grotto sculpin from Mystery Cave exhibit two distinct phenotypes based on relative eye size.

Table 5. Matrix of squared Mahalanobis distances between group centroids of a discriminant function analysis based on 24 morphometric variables. Six populations representing the four phenotypes of the *C. carolinae* complex in southeastern Missouri were examined.

Location	Tom Moore	Hot Caverns	Mystery Cave	Rimstone River Cave	Cinque Hommes Cr.
Tom Moore Cave					
Hot Caverns	33.75				
Mystery Cave	33.90	26.35			
Rimstone River Cave	27.36	11.29	20.22		
Cinque Hommes Cr.	31.93	14.46	28.17	8.75	
Southeast Missouri	41.95	22.22	9.65	21.01	35.68

Meristic features. The resurgence stream population (Cinque Hommes Creek) had 7 (25%) or 8 (75%) dorsal tin spines, 17 (18%) or 18 (82%) dorsal fin rays; 12 (8%), 13 (83%), or 14 (8%) anal fin rays; pectoral rays were 14 + 15 (I), 15 + 1.5 (1), 16 + 16 (8), and 16 + 17 (1); branched caudal fin rays were consistently 9. Grotto sculpin from Mystery Cave had 7 (SO%), 8 (45%), or 9 (5%) dorsal fin spines, 16 (15%), 17 (65%), or 18 (20%) dorsal fin rays; 12 (25%), 13 (60%), or 14 (15%) anal fin rays; pectoral rays were 14 + 1.5 (1), 15 + 15 (4), 1.5 + 16 (3), 16 + 16 (9), 16 + 17 (2), and 17 + 17 (1); branched caudal fin rays were 8 (1), 9 (18), or 10 (1). A reduction in pelvic fin ray number from 4 + 4 to 3 + 4 or 3 + 3 occurs in

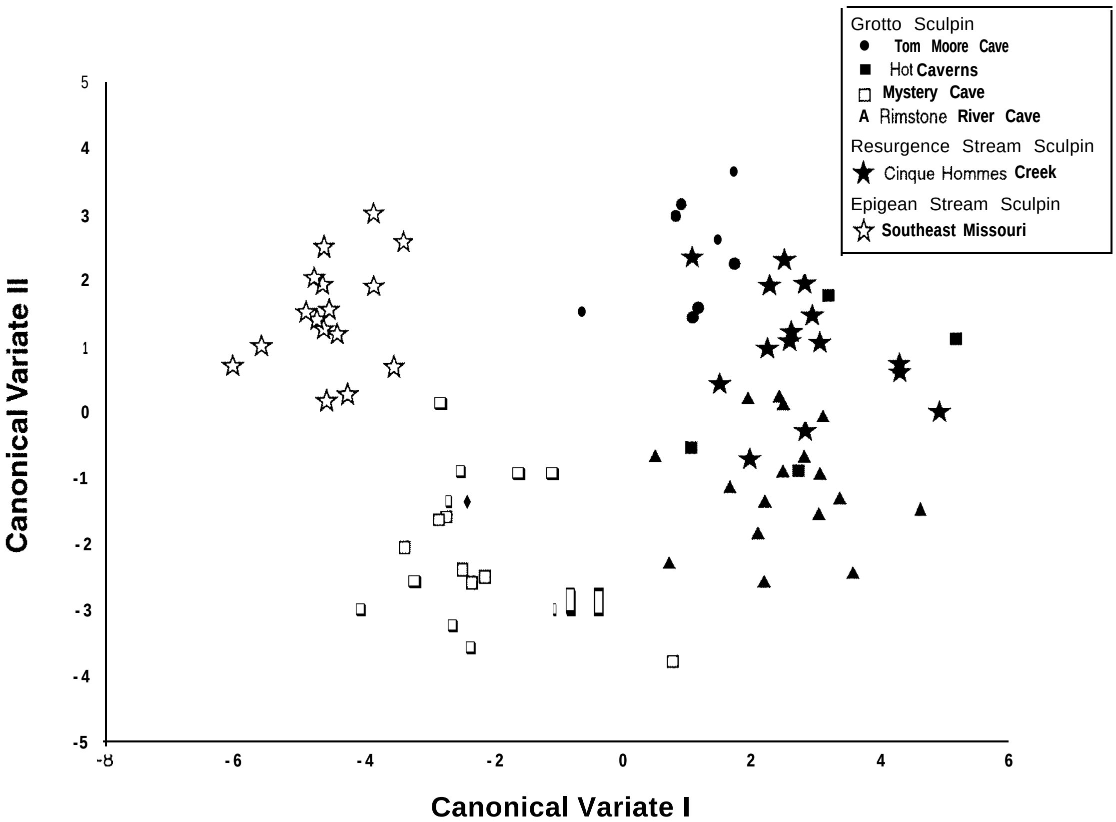

Figure 8. Scores of sculpin samples from four cave streams, a resurgence stream, and epigean surface streams on canonical variate axes I and II for 24 morphometric variables.

about 50% of grotto sculpin and the resurgence stream population (Table 7).

Discussion

Distribution

Grotto sculpin are limited in distribution to six major caves or cave systems in central Perry County, Missouri. A similar karst topography in terms of presumed age and structure occurs just east of Perry County across the Mississippi River in Monroe County, Illinois (Bretz & Harris 196 1). Some caves in Monroe County have enough permanent water to support populations of grotto sculpin, but we have found only one adult (120 mm SL) sculpin, in Fogelpole Cave, Monroe County, moderately depigmented, but otherwise with epigean characteristics.

We have never captured grotto sculpin in surface streams and suspect dispersal to epigean waters is low to non-existent. Habitat for the grotto sculpin also may be limited. Perry County has the four longest caves in the state, each exceeding 16 km in length, and containing sizable stream passages (Unklesbay & Vineyard 1992). Grotto sculpin have been found in all four of these major caves. These caves, formed beneath a sinkhole plain that provides substantial organic input, may be the only habitats that provide enough food and sustained flow to support sculpin populations. Inventories of the invertebrate cave fauna in this area indicated an abundance of isopods, amphipods, flatworms, and snails (Peck & Lewis 1978).

Population density estimates and habitat

Our population density estimates for grotto sculpin (0.3-0.6 individuals m^{-2}) are comparable to those for a surface population of C. *carolinae* in Little River, eastern Tennessee (0.4-0.9 sculpin m^{-2}) (Greenberg &

Table 6. Standardized canonical variate coefficients for 24 morphometric variables taken from six populations representing the four phenotypes of the *C. carolinae* complex in southeastern Missouri.

Variable	Canonical variate 1	Canonical variate II	Canocical variate 111
1. Lip to Coronal Pore (l-2)	0.84892	0.46649	-0.05148
2. Lip to Pelvic Fin Ins. (l-3)	-0.69874	0.04699	0.64716
3. Lip to 1 st Dorsal Fin Spine (14)	0.79944	2.05690	-0.35249
4. Coronal Pore to Dl Spine (2-4)	0.07899	-0.16379	-0.4666X
5. Coronal Pore to Pelvic Fin Ins. (2-3)	2.23308	-0.54269	-0.64243
6. Dl Spine to Pelvic Fin Insertion (4-3)	-0.51647	- 1.42064	- 1.66432
‘7. Dl Spine to Base Anal Spine (4-S)	0.08928	2.15818	2.97467
8. Length of D 1 Fin (4-6)	0.41621	-0.32719	0.71057
9. Base length of D2 fin (6-X)	-0.21571	-2.69564	-2.57967
10. D2A to Front of Anal Fin (6-S)	1.45704	1.61546	-0.46662
11. Front of D2 Fin to Front Anal Fin (6-3)	- 1.85940	-2.1 X929	1.55320
12. Front of D2 Fin to Back of Anal Fin (6-7)	-0.16915	2.11797	0.33814
13. Least Depth Caudal Peduncle (1 O-9)	-0.08950	- 1.58638	-0.91326
14. Lower Edge Caudal Peduncle (7-9)	-0.0608 1	-0.46937	-0.24219
IS. Posterior Anal Fin to D2P (7-X)	-0.03114	-0.48110	0.27565
16. Base length of anal fin (S-7)	0.52984	0.075 12	0.16596
17. Anterior Anal to D2P (S-X)	1.36360	0.05764	-0.30940
18. P2 to Anterior Anal (3-S)	-0.12309	0.44399	-0.15687
19. Head Width at Jaw	0.72743	0.91085	-0.63998
20. Body Width at Pectoral Fin	-0.1274X	-0.86602	0.91807
21. Eye Length	-0.85656	I.75491	-0.30613
22. Eye Depth	-0.33876	-0.86974	-0.16707
23. Head Depth at Jaw	-0.89976	-0.01912	0.57359
24. Head Depth at Pelvic Spine	-1.41893	-0.18526	0.35479
Eigenvalue	9.08743	2.58380	2.36978

Table 7. Frequency of pelvic fin ray number (left and right fins) in sculpins of the C. *carolinae* species group, including those of' the midlands race (from Robins 1954), and Perry County, Missouri, epigean and hypogean populations.

Population	Pelvic ray number 3 + 3	3 + 4	4 + 4	Total	% with reduced rays
C. carolinae (epigean streams) Midlands Race		2	1459	1461	0.1
C. carolinae (resurgence stream) Cinque Hommes Creek	9	9	16	34	52
Grotto sculpin, *C. carolinae* (hypogean streams)					
Tom Moore Cave	6	8	6	20	75
Hot Caverns	1	1	2	4	50
Mystery Cave	10	5	15	30	50
Rimstone River Cave	6	10	15	31	52
Running Bull Cave	3	6	10	19	47

Holtzman 1987). These population densities are much greater than typical densities of amblyopsid cavefishes (0.005-0.15 fish m^{-2}; Helfman et al. 1997), suggesting caves in Perry County are richer in nutrients than caves occupied by amblyopsids. We speculate, however, that grotto sculpin populations are relatively unstable because catastrophic events (e.g., food shortage, drought) could result in short-term depletion or local exlirpation.

Identification and taxonomy

Prior to more recent (e.g., Mitchell et al. 1977) studies of populations of fishes that bridge the gap between the epigean-hypogean environment, it was common practice to emphasize the phenotypic distinctiveness of cave fishes, many of which were placed in new genera. For example, Hubbs & Innes (1936) described a new genus and species, *Anoptichthys jordani*, for the cave-inhabiting population of the Mexican tetra, now referred to as *Astyanax fasciatus.* In that case, the parental epigean stock and hypogean populations introgressed at some locations (Avise & Selander 1972, Romero 1983). Because reductions in pigmentation, eye size, and other features are commonplace among stygobites, including the grotto sculpin studied here, most modern workers apply the zoological name of the epigean relative to its presumed cave descendants. Unfortunately, without a unique taxonomic name, an unusual biological phenomenon (i.e., cave adaptation or phenotypic plasticity) is buried under a single name, and as a consequence, only specialists are aware of the distinctive populations in caves. Moreover, when cave populations are treated nomenclaturally the same as epigean relatives, these unique animals may not be considered for actions needed to conserve them.

External morphology

Sculpin in Perry County represent three fundamental phenotypes: (1) epigean resurgence stream sculpin with essentially normal surface pigmentation, moderate eye size, moderate cephalic pore size, and a reduced pelvic fin-ray count; and (2) two hypogean sculpin phenotypes differing in eye size but sharing reductions in pigmentation and pelvic fin ray number, and enlarged cephalic canal pores. Sculpin outside of Perry County are typical epigean examples of the midlands race assigned to *Cottus carolinae* (Robins 1954). In contrast to the grotto sculpin described here, midlands race individuals have all fins spotted with dark brown, forming strong tessellations. The margin of the first dorsal fin is edged with orange-red, and the ground color is mottled dusky brown or rusty brown. Three dark brown and conspicuous saddles cross the back extending downward, usually well below the lateral line. The lower sides are reticulated, and there are usually two dark bars on the cheek and a small dark blotch near the middle of the pectoral fin base. The chin is usually darkly mottled. Hypogean sculpin from Mystery Cave retained their depigmented appearance even after several weeks in the laboratory in a 375-l aquarium with a 12L : 12D photoperiod.

Similar to Perry County sculpin, midlands race sculpin usually have a complete lateral line, with from 28 to 37 pores (N = 572), modally 33. Robins (1954) reported 11 preoperculomandibular pores and 9 infraorbital pores for midlands race sculpin, the modal numbers for Perry County sculpin, respectively. Cephalic lateralis pore size is larger in hypogean and resurgence stream sculpin when compared to sculpin outside Perry County (Table 4). In fact, mandibular pores of grotto sculpin are readily visible with the unaided eye and, on the average, are one to three times the size of those from epigean stream samples outside Perry County.

General body shape of midlands race sculpin is similar to sculpin in Perry County. Caudal peduncle depth (N = 22) was 8.2% (±0.61% SE) of SL in midlands race individuals (Robins 1954) and is not significantly ($p = 0.7$, $F_{5,77} = 3.20$) different from any Perry County population (ANCOVA, all assumptions met). Mean eye length in midlands race sculpin was 7.7% (±0.30% SE) of SL (range 6–10%), significantly ($p < 0.001$, $F_{5,77} = 29.98$) higher than the 2-4 % of SL found in grotto sculpin; additionally epigean sculpin from southeastern Missouri outside of Perry County were significantly ($p < 0.01$) different in eye length (7.7% [±0.19% SE] of SL, range 6-9%) from all Perry County populations (ANCOVA, all assumptions met). Preopercular spines appear sharper in epigean samples compared to those in caves, but we are unable to convincingly quantify this character. We initially observed a significant gap between the dorsal fins in samples from Perry County caves, but this feature is highly variable in midlands race fish and is not consistently different when samples are compared. We found no quantifiable difference in caudal fin shape among samples from caves, the resurgence stream, or the midlands race.

Multivariate analyses of shape quantitatively demonstrated differences we observed by eye. That is, head shape (affected by eye size and presumed differences in brain and optic lobe morphology) and eye size were often significantly discriminating features. Measures of body width were also discriminators, but those differences may be related to condition of cave fishes (i.e., subjected to limited food resources) relative to epigean populations. One meristic feature, pelvic finray number, distinguishes Perry County samples from those of the midlands race. In about 50% of Perry County sculpin, the pelvic fin ray count is 3 + 4 or 3 + 3 (both sides) compared to a count of 4 + 4 in 1459 individuals of epigean midlands race C. *carolinae* (Table 7; Robins 1954 in part) and 60 specimens from Buckeye Creek Cave, West Virginia (Williams & Howell 1979). Reduction or loss of pelvic fins is common in cavernicolous fishes (Woods & Inger 19.57, Cooper & Kuehne 1974).

The occurrence of *C. carolinae* in subterranean waters is well known (Poly & Boucher 1996), and we have documented such occupation in about 25 caves from several states with known karst environments (Table 2). None of these sculpin show evidence of cave adaptation and none are known to be permanent cave residents; it might, however, be expected because some cave-inhabiting sculpin occur several kilometers from a known surface stream source. An unusual albino specimen reported from Buckeye Creek Cave, Greenbriar County, West Virginia (Williams & Howell 1979), is an exception warranting further searches and study. We emphasize that only a single specimen is known and attempts to document a 'population' by numerous independent investigators were unsuccessful. The albino sculpin (67mm SL) has a frenum, enlarged cephalic lateralis pores (not quantified), fused postmandibular pores, and only 6 dorsal spines and 3 + 3 pelvic rays. None of the grotto sculpin from Perry County have a frenum or 6 dorsal spines (N = 40), but they do have enlarged cephalic pores. Fusion of median chin pores into a single large pore occurs locally in high frequency (10 of 20 specimens in Rimstone River Cave), complete loss of the postmandibular pore occurs in low frequency (3 of 32 specimens). Thus, three common features of cave adaptation are shared between grotto sculpin and the single albino specimen, namely reduced pigmentation, reduced pelvic fin-ray number, and enlarged cephalic canal pores. Clearly, the discovery of a permanent residential population of cave-adapted sculpin in Buckeye Creek Cave would be highly significant for further comparisons to grotto sculpin.

Grotto sculpin were somewhat smaller (means ranged from 60 to 70 mm SL) than typical adult sizes (75-l 10 mm SL) in Virginia populations of epigean C. *carolinae* (Jenkins & Burkhead 1994). The largest known Virginia specimen is 144 mm SL (185 mm total length). Our largest cave-inhabiting specimen was 104 mm SL; the single albino sculpin from Buckeye Creek Cave was 67 mm SL (Williams & Howell 1979). We are unaware of primary literature addressing the significance of adult size in cave-adapted fishes, but we predict a strong positive correlation between adult size and those caves that are richest in nutrient volume and varied food resources.

Conservation status

Prior to our work, cave adaptation was unknown in cottid fishes (Groombridge 1992). Some 90 species of fishes in 14 families are subterranean residents (Helfman et al. 1997), and show evidence of cave adaptation (i.e., trends toward eyelessness, lack of pigment, enlarged lateral line receptors, and low metabolic rates). Troglomorphic populations of sculpin may be jeopardized from a conservation standpoint because of their limited distribution (total range less than 260 km^2) and the impact of human activity on the surface. Caves are located downgradient of the city of Perryville and dye trace studies of water movement in the Central Perryville Karst indicated that urban runoff from Perryville and vicinity enters the cave systems. Ammonia, nitrite + nitrate, chloride, and potassium, primarily resulting from cultivation on the surface, were detected within caves at levels high enough to be detrimental to aquatic life (Vandike 1985). For example, we recently observed a mass mortality of grotto sculpin in Running Bull Cave, presumably resulting from point source pollution similar to that described above.

Aquifers in karst terrains are exceptionally sensitive and have higher potential for contamination than other aquifers (Helling 1986, Field 1989). Sinkholes, a prominent feature of karst terrain, allow sediments and chemicals to be directly injected into the groundwater without the filtration associated with non-karst regions (Mitchem et al. 1988, Field 1989). No less than one-half of sinkholes in Perry County contain anthropomorphic refuse, ranging from household cleansers and sewage to used pesticide and herbicide containers. In these places contaminants can amass in the soil and epikarst zone during dry periods to be recharged into the cave water later (Williams 1985, Hobbs & Smart', Smart & Friederich 1987, Field 1989).

Spear (1995), a representative of the U.S. Fish and Wildlife Service, considered that distinctness and significance would be the primary qualities addressed in defining a unique population segment of any species before ultimately protecting it under the U.S. Endangered Species Act. Grotto sculpin clearly meet several of the criteria (sensu Spear 1995) for distinctness and significance, as judged from the unique morphology and the unusual ecological settings. Because grotto sculpin hold the unique position as the only representatives of cottids showing cave adaptation we 'argue for their recognition, if not as a distinct taxonomic species, then at least as a distinct population segment or a biologically isolated unit. We recommend that the U.S. Fish and Wildlife Service

Hobbs, S. L. & P. L. Smart. 1986. Characterization of carbonate aquifers: a conceptual base. International Congress of Speleology (Barcelona, Spain), Communications 9: 43-46.

consider the grotto sculpin for listing under the U.S. Endangered Species Act.

Cooperative agreements with local landowners to maintain access to caves and to reduce contamination and sedimentation of the cave streams are needed. Communication with and assistance from organized speleological clubs and societies should be maintained because of their concern and knowledge of karst systems and groundwater. Educating the citizens of Perry County would increase understanding and appreciation for how karst systems function and might ultimately reduce contamination of groundwater. Finally, all fish predators (e.g., channel catfish) introduced into Perry County caves either intentionally or accidentally should be systematically captured and removed from the cave environment.

Future directions

We have studies underway comparing grotto sculpin to epigean relatives in aspects of physiology, brain morphology, life history traits, and reproductive biology. Additionally, we are continuing analyses of body shape, the lateral line system, pigmentation, and skin prickling. We will use frozen and ethanol-stored samples for an examination of population genetics, assessment of speciation models, and relationships of caves and karst history. Additional population density estimates are needed and a long-term monitoring program established and supported by the State of Missouri. We are searching for undiscovered populations of grotto sculpin in Perry County and in the similar karst topography of Monroe County, Illinois.

Acknowledgements

We are immensely grateful to S.R. Adams, C. Lee, M.L. Mugele, P.L. Moss, J.J. Swayne, S.J. Taylor, and R. Young for countless hours of assistance in difficult subterranean environments and their photographic expertise under less than ideal conditions. The SIUC Little Egypt Grotto granted permission to conduct field work in Mystery Cave and assisted with gaining landowner permission to explore other caves. The Missouri Speleological Survey (MSS Inc., c/o Missouri Geological Survey, P.O. Box 250, Rolla, Missouri) provided cave locations which are property of the MSS. Wc are most appreciative to our home institutions for providing space, secretarial assistance, curatorial support, and adequate time to pursue the captivating study of fishes. Aspects of this research were supported by grants to GLA from the Raney Fund and the Explorers Club.

References cited

Avise, J.C. & R.K. Selander. 1972. Evolutionary genetics of cave-dwelling fishes of the genus *Astyanax*. Evolution 26: 1-9.

Bretz, J.H. & S.E. Harris, Jr. 1961. Caves of Illinois. 111. State Geol. Surv. Rep. Invest. (215): I-87.

Cooper, J.E. 1978. American cave fishes and salamanders. Nat. Speleol. Soc. Bull. 40: 89.

Cooper, J.E. & R.A. Kuehne. 1974. *Speoplatyrhinus poulsoni*, a new genus and species of subterranean fish from Alabama. Copeia 1974: 486–493.

Eigenmann, C.H. 1909. Cave vertebrates of America. Carnegie Institution of Washington, Washington, D.C. 241 pp.

Elliott, W.R. 2000. Below Missouri karst. Miss. Conserv. 61: 4–7.

Field, M.S. 1989. The vulnerability of karst aquifers to chemical contamination. pp. 130–142. *In:* J.E. Moore, A.A. Zaporozec, S.C. Scallany & T.C. Varney (ed.) Proceedings of the International Conference on Recent Advances in Ground-Water Hydrology, American Institute of Hydrology, Minneapolis.

Gordon, M.S. & D.E. Rosen. 1962. A cavernicolous form of the poeciliid fish *Poecilia sphenops* from Tabasco, Mexico. Copeia 1962: X50-368.

Greenberg, L.A. & D.A. Holtzman. 1987. Microhabitat utilization, feeding periodicity, home range and population size of the banded sculpin, *Cottus carolinae*. Copeia 1987: 19–25.

Groombridge, B. (ed.) 1992. Global biodiversity, first edition. Chapman & Hall, London. 585 pp.

Helfman, G.S., B.B. Collette & D.E. Facey. 1997. The diversity of fishes. Blackwell Science, Inc., Malden. 528 pp.

Helling, C.S. 1986. Agricultural pesticides and ground water quality. pp. 161–175. *In:* Agricultural Impacts on Ground Water (Omaha, Nebraska), Proc. Nat. Water Well Assoc., Dublin, Ohio.

Hubbs, C.L. & W.T. Innes. 1936. The first known blind fish of the family Characidae: a new genus from Mexico. Occas. Pap. Mus. Zool. Univ. Mich. (342): 1-7.

Hubbs, C.L. & K.F. Lagler. 1974. Fishes of the Great Lakes region. University of Michigan Press, Ann Arbor. 213 pp.

House, S. 1976. Karst and cave distribution in Perry County. Southeast. Caver 2: 13-1 4.

Jenkins. R.E. & N.M. Burkhead. 1994. Freshwater fishes of Virginia. American Fisheries Society, Bethesda. 1079 pp.

Libra, R.D., G.R. Hallberg, B.E. Hoyer & L.G. Johnson. 1986. Agricultural impacts on ground water quality: the Big Spring basin study. Iowa. pp. 253–273. *In:* Agricultural Impacts on Ground Water (Omaha, Nebraska), Proc. Nat. Water Well Assoc., Dublin, Ohio.

Mitchell, R.W., W.H. Russell & W.R. Elliott. 1977. Mexican eyeless characin fishes, genus *Astyanax*: environment, distribution, and evolution. Spec. Publ. Mus. Texas Tech. Univ. (12): 1-89.

Mitchem, P.S., G.R. Hallberg, B.E. Hoyer & R.D. Libra. 1988. Ground-water contamination and land management in the karst areaofnortheastern Iowa. pp. 442–458. *In:* D.M. Neilsen & A.I. Johnson (ed.) Ground-Water Contamination: Field Methods, American Society for Testing and Materials Special Technical Publication 963, ASTM, Philadelphia.

Mohr, C.E. 1950. Ozark cave life. Nat. Speleol. Soc. Bull. 12: 3-l 1

Padgett, A. & B. Smith. 1987. On rope. North American vertical rope techniques. National Speleological Society, Huntsville. 341 pp.

Page, L.M. & B.M. Burr. 1991. Field guide to freshwater fishes, North America north of Mexico. Houghton Mifflin Co., Boston. 432 pp.

Peck, S.B. & J.J. Lewis. 1978. Zoogeography and evolution of the subterranean invertebrate fauna of Illinois and southeastern Missouri. Nat. Speleol. Soc. Bull. 40: 39-63.

Pflieger, W.L. 1997. The fishes of Missouri, revised edition. Missouri Department of Conservation, Jefferson City. 372 pp.

Poly, W.J. & C.E. Boucher. 1996. Nontroglobitic fishes in caves: their abnormalities, ecological classification and importance. Amer. Midl. Nat. 136: 187–198.

Rea, T. (ed.). 1987. Caving basics, revised edition. National Speleological Society, Huntsville. 128 pp.

Robins, CR. 1954. A taxonomic revision of the *Cottus bairdi* and *Cottus carolinae* species groups in eastern North America (Pisces, Cottidae). Doctoral Dissertation, Cornell University, Ithaca. 248 pp.

Robins, CR. & R.R. Miller. 1957. Classification, variation, and distribution of the sculpins, genus *Cottus*, inhabiting Pacific slope waters in California and southern Oregon, with a key to the species. Calif. Fish Game 43: 2 13–233.

Romero, A. 1983. Introgressive hybridization in the *Astyanax fasciatus* (Pisces: Characidae) population at La Cueva Chica. Nat. Speleol. Soc. Bull. 45: 81–85.

SAS Institute, Inc. 1985. SAS user's guide: statistics. Statistical Analysis Systems Institute, Inc., Cary. 584 pp.

Smart, P.L. & H. Friederich. 1987. Water movement and storage in the unsaturated zone of a Carboniferous limestone aquifer, Mendip Hills, England. pp. 59–87. *In:* Environmental Problems in Karst Terranes and Their Solutions (Bowling Green, Kentucky), Proceedings, National Water Well Association, Dublin, Ohio.

Smart, P.L. & S.L. Hobbs. 1986. Characterization of carbonate aquifers: a conceptual base. pp. l-14. *In:* Environmental Problems in Karst Terranes and Their Solutions (Bowling Green, Kentucky), Proceedings, National Water Well Association, Dublin, Ohio.

Spear, M. 1995. Considerations in defining the concept of a distinct population segment of any species of vertebrate fish or wildlife. pp. 423-424. In: J.L. Nielsen (ed.) Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation, American Fisheries Society Symposium 17, Bethesda.

Statsoft. 1994. Statisticafor the Macintosh, volumes l-3. Statsoft, Inc., Tulsa. 1062 pp.

Strauss, R.E. & EL. Bookstein. 1982. The truss: body form reconstructions in morphometrics. Syst. Zool. 31: 113-l 35.

Unklesbay, A.G. & J.D. Vineyard. 1992. Missouri geology: three billion years of volcanoes, seas, sediments, and erosion. University of Missouri Press, Columbia. 189 pp.

Vandike, J.E. 1985. Movement of shallow groundwater in the Perryville Karst area, southeastern Missouri. Water Res. Rept. (40): 1-56.

Walsh, S.J. & C.R. Gilbert. 1995. New species of troglobitic catfish of the genus *Prietella* (Siluriformes: Ictaluridae) from northeastern Mexico. Copeia 1995: 850–861.

Williams, P.W. 1985. Subcutaneous hydrology and the development of doline and cockpit karst. Z. Geomorph. 29: 463–482.

Williams, J.D. & W.M. Howell. 1979. An albino sculpin from a cave in the New River drainage of West Virginia (Pisces: Cottidae). Brimleyana 1: 141-146.

Woods, L.P. & R.F. Inger. 1957. The cave, spring, and swamp fishes of the family Amblyopsidae of central and eastern United States. Amer. Midl. Nat. 58: 232–256.