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*Submitted 7 July 2005. Accepted 11 January 2006.*
*Associate Editor was Joseph P. Shannon.*

# RANGE EXTENSION AND STATUS UPDATE OF THE ENDANGERED HELL CREEK CAVE CRAYFISH, *CAMBARUS ZOPHONASTES* (DECAPODA: CAMBARIDAE)

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ABSTRACT—The range of the endangered Hell Creek Cave crayfish (*Cambarus zophonastes*) is expanded to include a second population, determined by genetic analyses. This crayfish is still restricted to Stone County, Arkansas, and is known from only 14 individuals at Hell Creek Cave and 9 individuals at Nesbitt Spring Cave. Despite this range extension, *Cambarus zophonastes* remains vulnerable to extinction. Water quality sampling of Hell Creek Cave did not detect any major contamination, although numerous potential point and nonpoint source pollutants exist in the watershed. Habitat degradation remains an important threat to this species. Recovery plan implementation status and conservation activities were reviewed and recommendations made.

RESUMEN—La distribución geográfica del cangrejo en peligro de extinción *Cambarus zophonastes,* se incrementa al incluir una segunda población, determinada por análisis genético. Este cangrejo se limita a Stone County, Arkansas, y se conoce solamente de 14 individuos en Hell Creek Cave y 9 individuos en Nesbitt Spring Cave. A pesar de esta extensión geográfica, *Cambarus zophonastes* continúa vulnerable a la extinción. Muestras de la calidad del agua de Hell Creek Cave no detectaron contaminantes trascendentales, aunque numerosas fuentes potenciales localizadas y difusas de contaminación existen en la cuenca. La degradación del hábitat continúa siendo una amenaza importante a esta especie. El estatus de un plan de recuperación y actividades de conservación fueron revisadas y se hicieron recomendaciones.

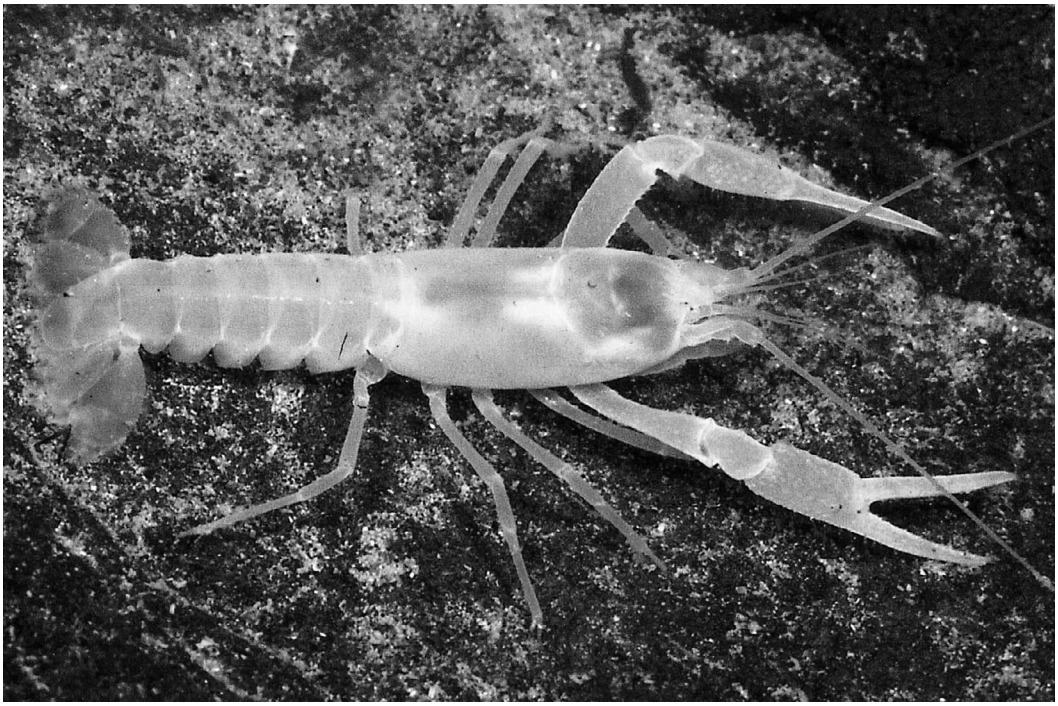

FIG. 1—First published photograph of the Hell Creek Cave crayfish (*Cambarus zophonastes*), Nesbitt Spring Cave, Stone County, Arkansas, by D. Fenolio.

As part of the multi-agency initiative to document the subterranean biodiversity of the Ozark Plateaus (the Ozark Subterranean Biodiversity Project), the status and distribution of the groundwater-adapted (stygobitic) crayfishes of this ecoregion were updated. *Cambarus zophonastes* is one of the rarest of all crayfishes, known only from its type locality (Fig. 1) and a maximum of 15 individuals. Because of its rarity and susceptibility to disturbance, it was designated endangered by the U.S. Fish and Wildlife Service (USFWS) (Stewart, 1987), critically endangered by the International Union for the Conservation of Nature and Natural Resources (2003), and critically imperiled by the Natural Heritage Network (NatureServe, 2004). The *Cambarus zophonastes* Recovery Plan cited limiting factors as destruction of habitat, scientific collection, disturbance by amateur cavers, and lack of reproduction (USFWS, 1988). The recovery plan also detailed specific objectives needed for the recovery and delisting of the species, and this report summarizes the status of these objectives to date. The following recovery plan recovery objectives were implemented in this study: surveys for additional populations of Hell Creek Cave crayfish (objective 2); regular censuses of each population (objective 3); and analyses of habitat quality (objective 4).

The type locality (Hell Creek Cave, Stone County, Arkansas) is a phreatic conduit formed in limestone of the Plattin Formation, Ordovician Period. This karst system pirates surface water from the Hell Creek watershed, collects it in a dendritic conduit system, and discharges the groundwater to springs downstream, where Hell Creek resurfaces and eventually joins the White River. At least 3,150 m of non-submerged passage have been mapped (D. Taylor, Association for Arkansas Cave Studies, Incorporated [AACS], pers. comm., 2002). A second entrance, created in the early 1900s by boring for mineral exploration, acts as a funnel, trapping and dropping a diverse array of epigean fauna into the base of the shaft near the terminal sump. Beyond this sump, only 300 m of passage have been mapped and censused, and extensive habitat is predicted to occur upstream, but will require more technical diving

and logistical support (J. Disler, pers. comm., 2000).

The first published records of *C. zophonastes* in Hell Creek Cave were the collection of 5 specimens in 1961 by Hobbs and Bedinger (1964), and 2 more by Hobbs and colleagues in 1972 (Smith, 1984, unpublished report to USFWS). On 23 August 1983, USFWS sponsored the first census, and Arkansas Natural Heritage Commission (ANHC) employees counted 15 crayfish with the help of cave diver T. Ernst, who used self-contained underwater breathing apparatus (SCUBA) in the submerged passages of Hell Creek Cave (Smith, 1984, unpublished report to USFWS). Another census occurred on 5 October 1990, when S. Snyder counted 13 crayfish (ANHC Natural Heritage Database, C. Osborne, pers. comm., 2001). The latest census of *C. zophonastes* (objective 3.1) was performed using headlamps and dive lights underwater; 1 to 3 surveyors moved slowly upstream, counting crayfish in all accessible aquatic habitats. For submerged passages, certified cave divers were employed after receiving biological training. This census of *C. zophonastes* was performed during 2 site visits because of the difficulty involved in transporting SCUBA throughout the subterranean habitat. On 16 September 2000, we assisted J. Disler (Mid-Ozark Sump Team) in transporting SCUBA into the first cave pool (''Dipping Vat''), where Disler sighted 2 crayfish. We then proceeded upstream to the first sump (the terminus for non-divers) and counted 4 more in the non-submerged passages. On 8 April 2001, members of AACS assisted the Mid-Ozark Sump Team in transporting SCUBA to the first sump to continue the census. Disler swam upstream of the first sump approximately 300 m into unmapped passage and counted 8 additional crayfish, completing the census with a tally of 14 individuals. Another partial census on 20 February 2003 by MES, BKW, D. Fletcher, and D. Kampwerth detected 6 individuals.

Recovery objective 2 identifies the need to survey additional caves for the possible presence of the Hell Creek Cave crayfish. We surveyed an additional 30 cave streams in Stone County (Graening et al., 2001, unpublished report to Arkansas Game and Fish Commission and ANHC; Graening et al., 2004). We found stygobitic crayfish, determined from morphological characters to be members of the genus *Cambarus,* in only 2 locations: Cave River Cave and Nesbitt Spring Cave. Because of their proximity to Hell Creek Cave, we suspected that these crayfish might be additional populations of the *C. zophonastes.* Cave River Cave was censused on 24 November 2002; however, the landowner denied permission to use SCUBA or to collect any crayfish. We sighted a single crayfish in Cave River Cave and 5 others in the upstream karst window named Flitterin' Pit. Future, non-lethal genetic sampling of this population will be attempted for specific determination.

In 1992, during the first SCUBA exploration of Nesbitt Spring Cave, ''dozens'' of stygobitic crayfish were reported (J. Disler and J. Fant, pers. comm., 2001). On 30 March 2002, we censused the non-submerged passages of Nesbitt Spring Cave, saw 2 crayfish, and collected one of them (male Form I) for specific determination. On 5 March 2005, we censused the entire known habitat with the assistance of the Ozark Cave Diving Alliance. Cave diver S. Wallace sighted 9 stygobitic crayfish between the second and third sumps, and carefully captured 6 live crayfish using a suction device, transported the specimens back to D. Kampwerth (USFWS), who then removed a pereopod from each individual. Five of the crayfish were released back into the stream and one was preserved as a voucher specimen. The tissue samples were stored in a preservative of 75% ethanol and 10% 0.5M EDTA. An approximately 502-base-pair (bp) region of the 16S ribosomal DNA (rDNA) from the mitochondrial genome was amplified and sequenced (ABI 3700 sequencer with M13 primers). Pairwise percent sequence differences between specimens from the type locality and Nesbitt Spring Cave ranged from 0 to 1.83% for the same 492-bp fragment. The greatest differences (9 bp) were seen between the Hell Creek Cave specimen and 2 of the Nesbitt Spring Cave samples; however, within Nesbitt Spring Cave, base differences ranged from 0 to 7, so there was a continuum of variation among all the samples. Therefore, the Nesbitt Spring Cave site is considered to represent an additional site for *C. zophonastes.* Voucher specimens will be deposited in the National Museum of Natural History, Smithsonian Institution.

Recovery objective 4.1 is the establishment

of baseline data on water quality. Grab samples were collected at Hell Creek Cave at the Dipping Vat on 20 February 2000 and 7 April 2001 at base-flow conditions. Sampling techniques and analytical procedures followed U.S. Environmental Protection Agency standard methods; appropriate quality assurance and quality control measures were taken. Metal analyses were performed by the University of Arkansas at Fayetteville Central Analytical Laboratory and all others by the University of Arkansas at Fayetteville Arkansas Water Resources Center Water Quality Laboratory. The subterranean stream had the following mean physical and chemical characteristics: pH of 6.5 ± 0.5 units; temperature of 14.5 ± 0.5°C; turbidity of 1 ± 0.5 NTU; conductivity of 265 ± 1 μS/cm; water hardness of 128 mg/L, with a mean concentration (mg/L) of ions of 49.3 for calcium, 1.2 for magnesium, 4.2 for chloride, 0.01 for fluoride, 3.0 for sodium, 0.5 for potassium, 0.1 for fluoride, and 3.2 for sulfate. The following nutrient parameters were sampled and are reported as the mean value: total organic carbon 0.5 mg/L; total Kjeldahl nitrogen < 0.05 mg/L; nitrate-nitrogen 0.7 mg/L, ammonia-nitrogen < 0.01 mg/L; total phosphorous 0.08 mg/L; ortho-phosphate 0.023 mg/L; total coliform density of 1,000 colony forming units/100 mL; and *Escherichia coli* density of 50 colony forming units/100 mL. Metals and other dissolved elements were sampled, and the following were detected, expressed as a mean concentration (μg/L): barium 15.1, boron 2.0, copper 1.3, iron 7.0, and zinc 7.5. Aluminum, antimony, arsenic, beryllium, cadmium, cobalt, chromium, lead, manganese, molybdenum, nickel, selenium, and vanadium were not detected at a detection limit of 1.0 μg/L. These water quality parameters did not exceed Arkansas State Water Quality Standards (Arkansas Pollution Control and Ecology Commission, 1998) and were comparable to regional levels reported by National Water Quality Assessment Program (Petersen et al., 1998). Future, periodic environmental quality sampling of both habitats (objective 4.2) will be coordinated by ANHC and USFWS.

Protection of Hell Creek Cave and its recharge zone (objective 1) is being implemented. The Hell Creek Cave groundwater recharge area was defined (objective 1.2) by Aley and Aley (1985, unpublished report to ANHC and The Nature Conservancy) as a 906-ha zone. The groundwater recharge zone of Nesbitt Spring Cave should be delineated as soon as possible. Lands adjacent to Hell Creek Cave are protected within the 65-ha Hell Creek Natural Area, and ANHC is attempting to expand their natural area by acquisition of additional acreage in the recharge zone (recovery objectives 1.1 and 1.3). A site conservation and management plan has been prepared jointly by The Nature Conservancy and ANHC. Installation of cave gates, fencing, and monitoring has secured the entrances to Hell Creek Cave, although trespass and vandalism continue to occur. No conservation activities have occurred at Nesbitt Spring Cave or within the Rocky Bayou watershed, but the Nesbitt Family remains dedicated to the protection of the groundwater resource. Recovery objective 1.5 specifies the implementation of an outreach and education program, which should begin immediately. The most efficient approach would be to coordinate with the cave education program of the U.S. Forest Service at nearby Blanchard Springs Caverns. Outreach efforts also should focus on watershed awareness.

Although its range is now extended, the Hell Creek Cave crayfish remains vulnerable to extinction. The recovery plan cited lack of reproduction as a threat; however, no data on population dynamics exist to evaluate this threat. Another threat listed in the recovery plan is over-collection. While this was a significant mortality factor at the time (7 sacrificed out of the observed maximum of 15 individuals), scientific collection has ceased and no amateur collection is known. The 2 other threats cited in the recovery plan—recreational caving impacts and habitat destruction—continue to be potential factors threatening the viability of this species. Inadvertent trampling of stygobitic crayfish by cavers has been documented in another Ozark cave (Graening et al., 2006), so pedestrian traffic in the streambed of both caves should be minimized regardless of reason for visitation. Aley and Aley (1985, unpublished report to ANHC and The Nature Conservancy) conducted a threat analysis of the Hell Creek Cave recharge zone and documented several potential pollution sources, including illegal refuse dumping areas, salvage yards, and malfunctioning septic systems. Other potential habitat stressors include the dis-

charging of urban storm-water or treated municipal sewage from the town of Mountain View, or hazardous material releases from major transportation corridors in the vicinity (Arkansas State Highways 9 and 14). Even though water quality sampling during this study did not detect excessive nutrient, bacteriological, or metal contaminants, source-water protection programs should be implemented for both the Hell Creek watershed and the Rocky Bayou watershed. Additional legal protection for these habitats could be provided by designating these waterbodies as ''Extraordinary Resource Water Body'' by the Arkansas Department of Environmental Quality and by designating both recharge zones as critical habitat by the USFWS.

Funding for this study was provided by the Arkansas Game and Fish Commission Nongame Aquatics Program through USFWS ESA Section 6 funding, Ozark Karst Program of The Nature Conservancy, and ANHC. Collections were made under permits from the USFWS (numbers PRT-834518, TE834518-2, TE834518-1, and TE834518-3), ANHC Permit S-NHCC-99-005, and Arkansas Game and Fish Commission Permits 1082 and 1476. This study could not have been accomplished without the assistance of many colleagues, including: H. Hobbs, III for his taxonomic expertise; mapping and logistical support by AACS; underwater censuses by M. Budde and other members of the Ozark Cave Diving Alliance, and J. Disler and the Mid-Ozark Sump Team; and fieldwork by biology graduate students of the University of Arkansas at Fayetteville and D. Fenolio (University of Oklahoma). Thanks also to J. Manges (Center for Gene Therapy, Tulane University) and C. Dillman (Department of Biology, St. Louis University) for assistance with the genetic confirmations. The Nesbitt Family, the McCain Family, and the Church of Janus Karst graciously permitted access to their lands for study purposes, and they continue to value and protect their cave resources.

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*Submitted 29 March 2005. Accepted 11 January 2006. Associate Editor was Steven Goldsmith.*