SEASONAL MOVEMENT PATTERNS OF PICKEREL FROGS (*RANA PALUSTRIS*) IN AN OZARK CAVE AND TROPHIC IMPLICATIONS SUPPORTED BY STABLE ISOTOPE EVIDENCE

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ABSTRACT—We monitored a population of pickerel frogs (*Rana palustris*) in an Ozark cave over a 2-year period. Frogs were found from August to April with densities peaking from November to December. Although densities were quite high in these peak months, stomach content and stable isotope analyses reveal that *R. palustris* does not play a significant role as a predator in this subterranean system. These results suggest that the caves are being used as thermal refugia during the coldest months of the year.

RESUMEN—Observamos durante un periodo de dos años una población de *Rana palustris* en una cueva en los Ozarks. Se detectaron ranas de agosto y abril con densidades picos en noviembre y diciembre. Aunque las densidades fueron muy altas en estos dos meses, los contenidos estomacales y análisis de isótopos estables revelan que *R. palustris* no juega un papel significativo como depredador en este sistema subterráneo. Estos resultados sugieren que las cuevas son usadas como refugios térmicos durante los meses más fríos del año.

The North American continent is the richest in obligate cave-dwelling (troglobitic) salamanders with approximately ten described species, and possibly as many undescribed species, particularly from the Edward's Plateau, Texas. However, no troglobitic anurans are known from this continent nor from temperate regions in general (Resetarits and Aldridge, 1988). Of the Nearctic anurans that inhabit caves opportunistically (trogloxenes), frogs of the genus *Rana* are the most common (Barr, 1953; Prather and Briggler, 2001), especially the pickerel frog, *Rana palustris* (Myers, 1958; Schaaf and Smith, 1970; Black, 1971; McDaniel and Gardener, 1977; Resetarits 1986; Resetarits and Aldridge, 1988; Black and Sievert, 1989; Trauth et al., 2004). Seasonal use of caves by pickerel frogs has been attributed to avoidance of summer heat and drought, and associated desiccation (Barr, 1953; Prather and Briggler, 2001), as well as avoidance of winter frost and related mortality (Resetarits, 1986; Resetarits and Aldridge, 1988). Pickerel frogs are so prevalent in Ozark caves that researchers originally hypothesized that their life cycle might be completed underground (Brown, 1984). Hypotheses suggested that some demes might be evolving towards a body form specialized for subterranean existence (troglomorphy), although no evidence for modification of body form or of decreased reproductive output has been demonstrated in *R. palustris* (Resetarits and Aldridge, 1988). However, populations of *R. palustris* could impact true troglobitic species through predation or competition. We investigated the potential seasonal use and trophic impact of a deme of pickerel frogs in an Ozark cave stream using a combination of field observations, stomach content analyses, and stable isotope assays.

We examined a population of pickerel frog in January-Stansberry Cave, Delaware County, Oklahoma, located 6 km north of the town of Colcord. January-Stansberry Cave is a typical Ozark cave formed from the dissolution of fractures in Mississippian-aged, cherty limestone bedrock of the Boone Formation. The study area was defined as a 440-m section of the cave system (total mapped passage is approximately 1,800 m), beginning with the cave mouth, where the subterranean stream ''January River'' resurges, and ending in the ''Moonshine Room''. The average passage dimensions are 5 m wide and 2 m tall. Terrestrial habitats within the cave include mud banks, cobble, bedrock, ceiling breakdown, precipitating formations (speleothems), and bat guano piles (ranging from 3 to 7 m in diameter and 0.1 m to 2 m in depth). A maternity population of approximately 15,000 gray bats (*Myotis grisescens*) inhabits the cave from April to October (pers. comm., S. Hensley, United States Fish and Wildlife Service). January River has an average depth of 1.0 m, but some pools are as deep as 2 m and riffles as shallow as 2 cm. The predominant substrate is chert cobble, but other substrate materials include clastic sediment and bedrock. Outside of the cave, January River flows 300 m as a surface stream until it joins Spavinaw Creek, a tributary of the Neosho River.

From July 2001 to October 2003, we performed monthly ocular censuses of pickerel frogs by using bright dive lights and helmet-mounted lamps; behavioral observations of frogs were recorded on diving slates. We grouped population counts by season (Winter: January, February, March; Spring: April, May, June; Summer: July, August, September; and Fall: October, November, December) and also grouped by cold period (winter and fall) and hot period (spring and summer). We employed a Chi-square test to test the null hypothesis that counts of pickerel frogs were evenly distributed by season and by period.

At the end of the study, we collected 32 adult pickerel frogs from within the cave and immediately preserved them for stomach content analysis following McDiarmid (1994). We had also conducted inventories in the cave ecosystem over 3 years and were confident that we could identify all common members of the fauna in the cave that could serve as potential food items for the frogs. We then dissected the stomachs of the frog and examined the contents under a stereomicroscope, identifying items to the lowest possible taxon; all contents were stored in glass vials in 95% ethanol. All specimens of frogs were deposited in the Sam Noble Oklahoma Museum of Natural History (catalog number OMNH 39837-68).

We employed natural abundance stable isotope analyses to elucidate the role of the pickerel frogs in the trophic web of January-Stansberry Cave. Isotope ratio mass spectrometry can detect small (one part per thousand) but predictable changes in ratios of carbon and nitrogen stable isotopes as organisms are assimilated into subsequent trophic levels (Gearing, 1991). An organism can be linked to its diet by the similarity of stable carbon isotope ratios ($^{13}C/^{12}C$), and the trophic position of an organism can be inferred by the characteristic enrichment of the stable nitrogen isotope ($^{15}N/^{14}N$) of 3.5‰ per trophic level (DeNiro and Epstein, 1981). In January 2003, we collected the following samples in triplicate as described in Graening and Brown (2003): leg muscle tissue of pickerel frogs, *M. grisescens* guano (feces), cave stream sediment; abdominal muscle of ringed crayfish (*Orconectes neglectus*), whole bodies of grotto salamander larvae (*Eurycea spelaeus*), composite samples of whole troglobitic amphipods (*Stygobromus*) and isopods (*Caecidotea*), and one sample each of the whole body of cave salamander (*Eurycea lucifuga*) and dark-sided salamander (*E. longicauda melanopleura*). We collected animals under Oklahoma Department of Wildlife Conservation special license number 3086 and University of Oklahoma Animal Care and Use Committee assurance number A3240-01. We collected all samples in sterile glass vials with Teflon lids and immediately froze them for transport to the university lab. We then pulverized, freeze-dried, and sieved the samples through a number 30-mesh screen and had them analyzed at the Stable Isotope Ratio Facility for Environmental Research, University of Utah, Salt Lake City, using primary standards (Lajtha and Michener, 1994); analytical variability averaged 0.1‰.

Results of the monthly population censuses are presented in Fig. 1. Statistical analysis of the data revealed that counts of pickerel frogs were significantly different by season, with fall having the highest count and winter the second highest (Pearson $\chi^2 = 415.62$, $df = 3$, $P < 0.0001$). Pickerel frog counts also were significantly different by period, with the cold period (winter and spring) having higher counts (Pearson $\chi^2 = 232.67$, $df = 1$, $P < 0.0001$). Our field observations did not document any feeding activity by pickerel frogs. Of the 32 pickerel frogs collected for stomach content analysis, 14 had empty stomachs. We recovered stomach

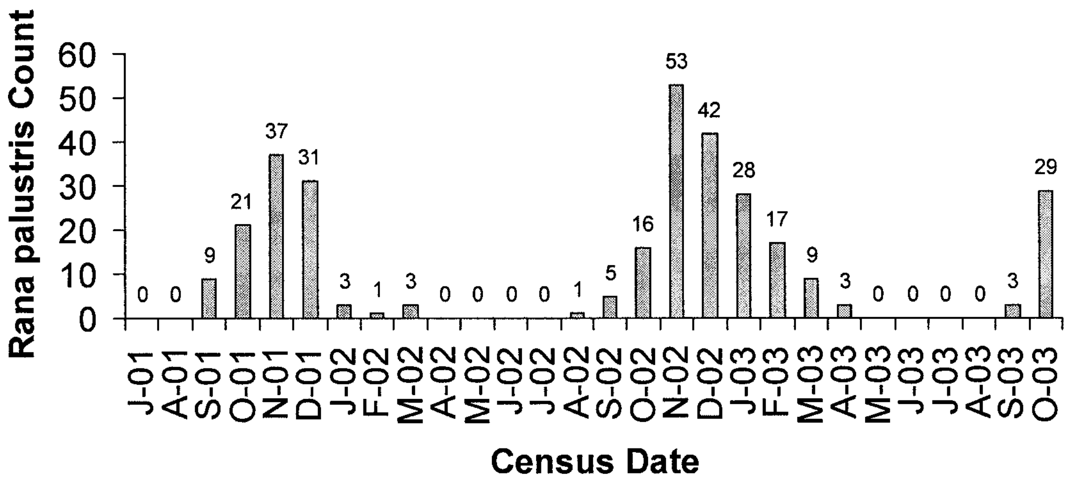

FIG. 1—Monthly ocular censuses of pickerel frogs (*Rana palustris*) in January-Stansberry Cave, Oklahoma, from July 2001 to October 2003.

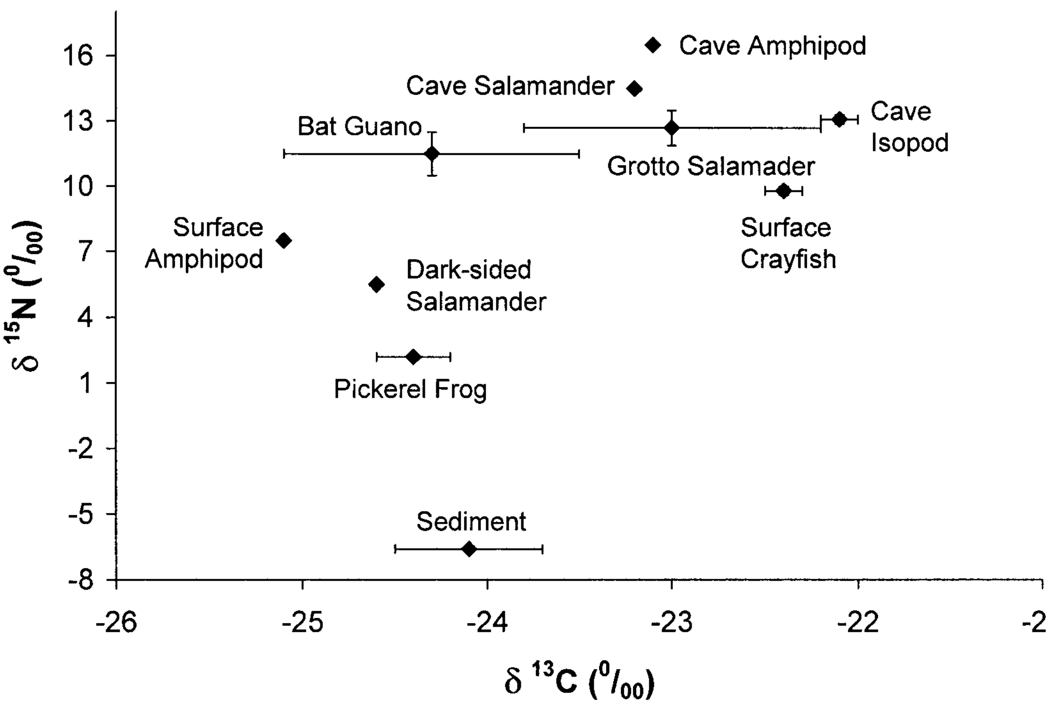

FIG. 2—Carbon and nitrogen stable isotope crossplot of pickerel frogs and their potential dietary items sampled on 12 January 2003 in January-Stansberry Cave, Oklahoma, replicated (n) as follows: cave sediment (n = 3); pickerel frogs (*Rana palustris,* n = 3); grotto salamander (*Eurycea spelaeus,* n = 4); cave salamander (*E. lucifuga,* n = 1); dark-sided salamander (*E. longicauda melanopleura,* n = 1); surface stream amphipods (*Gammarus,* n = 1); surface crayfish (*Orconectes neglectus,* n = 3); troglobitic amphipods (*Stygobromus,* n = 2); isopods (*Caecidotea,* n = 3), and bat guano (*Myotis grisescens* feces, n = 3). Error bars are 1 *SD*. Nitrogen content of 2 cave sediment samples was below detection levels.

TABLE 1—Other occurrences of pickerel frogs (*Rana palustris*) in Ozark caves during cold months (G. Graening and M. Slay, unpublished data).

State, county	Site	Date	Count
Arkansas			
Benton	Bear Hollow Cave	1 Oct 1999	1
		6 Nov 2000	1
Independence	Blevins' Cave	5 Oct 2002	1
	Dozen's Den Cave	22 Mar 2001	1
Izard	Mr. Griffin's Cave	22 Nov 2002	5
Marion	Blue Heaven Cave	23 Oct 2000	2
Newton	Fitton Spring Cave	5 Oct 2000	1
	Flowstone Façade Cave	5 Oct 2000	7
	Pretty Clean Cave	16 Mar 2002	1
	Tweet's Cave	26 Oct 2001	3
Stone	Flittering Pit	24 Nov 2002	5
	Gunner Cave	27 Jan 2001	1
	Martin Hollow Cave	14 Oct 2000	4
	Unnamed cave	31 Mar 2002	1
Oklahoma			
Ottawa	Schifleff Cave	6 Dec 2003	1
Delaware	Engelbrecht Cave	8 Dec 2003	1
Sequoyah	Cottonwood Cave	9 Dec 2003	1

contents in various states of digestion, making specific identification difficult. We were able to make the following taxonomic delineations: 6 Diptera (including 2 Heleomyzidae), 13 Arthropoda (including 5 Coleoptera), and 2 plant tissues.

Results of stable carbon and nitrogen isotope analyses, presented as a dual isotope crossplot (Fig. 2), graphically demonstrate that the isotopic signatures of the pickerel frogs sampled differ from the other animals inhabiting the cave. If pickerel frogs were consuming these potential prey items, such as crustaceans or salamanders, the position of the frogs in the crossplot should be higher (i.e., the nitrogen isotopic signature of the frog tissues should be enriched in ^{15}N).

Contrary to the most recent study of pickerel frogs in Ozark caves (Prather and Briggler, 2001), our study documented a population of pickerel frogs that utilized an Ozark cave during the winter season, and not summer. In a concurrent bioinventory of other caves in the Ozark Plateaus Ecoregion, we found pickerel frogs during fall and winter months in at least 16 other caves, summarized in Table 1. Resetarits (1986) reported that pickerel frogs were most abundant in caves in winter. We concluded that populations of pickerel frogs utilize caves in the Ozarks to avoid harsh conditions in summer or in winter, but not necessarily both, and local site conditions apparently dictate the period of use. The derivation of this apparent behavioral thermoregulation is not known, but Dowling (1956) and Resetarits (1986) hypothesized that it is a relictual behavior derived from the use of caves as refugia from climatic extremes of Pleistocene glacial advance and retreat.

The persistence of ranid populations in caves might have significant impact upon subterranean ecosystems, both in terms of energy flow and community composition. Temperate subterranean ecosystems are generally energy poor environments, and frogs might contribute energy via their feces and cadavers (Culver, 1982; Resetarits, 1986). More importantly, perhaps, is their potential role as predators. Bullfrogs (*Rana catesbeiana*) inhabiting caves have been reported to consume subterranean organisms, including troglobitic salamanders (*Gyrinophilus palleucus*) and crayfish (*Procambarus lucifugus*), and at least 3 species of bats (Lee, 1969; Kirkpatrick, 1982). Smith (1948) documented the stream isopod *Lirceus hoppinae* to be a regular prey item of the pickerel frog.

Yet, our analyses suggest that the pickerel frog population in January-Stansberry Cave was not preying upon the other trophic members in our study cave; nearly half of all frogs collected for the stomach content analysis had nothing in their stomachs at all. We believe that the pickerel frog is not a significant threat to Ozark subterranean communities, unlike the voracious bullfrog.

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