

# Coprophagy in a cave-adapted salamander; the importance of bat guano examined through nutritional and stable isotope analyses

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During a two year population ecology study in a cave environment, 15 *Eurycea* (= *Typhlotriton*) *spelaea* were observed ingesting bat guano. Furthermore, *E. spelaea* capture numbers increased significantly during the time that grey bats (*Myotis grisescens*) deposited fresh guano. We investigated the hypothesis that this behaviour was not incidental to the capture of invertebrate prey, but a diet switch to an energy-rich detritus in an oligotrophic environment. Stable isotope assays determined that guano may be assimilated into salamander muscle tissue, and nutritional analyses revealed that guano is a comparable food source to potential invertebrate prey items. This is the first report of coprophagy in a salamander and in any amphibian for reasons other than intestinal inoculation. Because many temperate subterranean environments are often energy poor and this limitation is thought to select for increased diet breadth, we predict that coprophagy may be common in subterranean vertebrates where it is not currently recognized.

**Keywords:** *Eurycea spelaea*; coprophagy; stable isotope analysis; subterranean food web; bat guano; omnivory

## 1. INTRODUCTION

Many temperate subterranean ecosystems are energy limited, and colonial bat guano has been reported to be the dominant energy resource in many cave ecosystems, influencing trophic dynamics, community structure and even physiological specialization (Harris 1970; Poulson 1972; Gnaspini & Trajano 2000). Densities of the cave-adapted salamander *Eurycea spelaea* (Bonett & Chippindale 2004) have been suspected to increase in the main rooms of some caves during summer months when grey bats (*Myotis grisescens*) utilize these caves as maternity roosts (Hendricks & Kezer 1958; Brandon 1971). Invertebrate communities associated with bat faeces (guano) increase in density or 'pulse' after the bats appear and deposit fresh guano (Poulson & Lavoie 2000). This invertebrate pulse provides a potentially important food resource for salamanders that are understood to be strictly carnivorous and that are living in an oligotrophic environment. For two years, we studied the community ecology of an oligotrophic cave habitat (January-Stansbury Cave, Delaware County, Oklahoma) to investigate the influence of bat guano on the community ecology and trophic dynamics of the system.

To our surprise, during the course of the study we observed 15 larval *E. spelaea* eating bat guano or regurgitating it upon capture. We initially considered the ingestion of bat guano by *E. spelaea* to be incidental to

capturing invertebrate prey, because consumption of non-food items is known in amphibians. For example, vegetation has been found in the digestive tracts of the carnivorous siren, *Siren lacertina* (Ultsch 1973). Sirens feed on small aquatic invertebrates by sucking them into their mouths, bringing debris in with the prey. We observed larval *E. spelaea* employing a suction mechanism to draw prey into their mouths, which would enhance the likelihood of unintentional ingestion of non-target items like a detritus or silt. Silt (i.e. cave stream sediment) was reported to be a major item in the diet of another subterranean salamander, the Florida blind cave salamander (*Haideotriton wallacei*) (Lee 1969), although another study argued that the ingested silt reflected failed feeding attempts rather than food (Peck 1973). A third cave-adapted salamander, the European olm (*Proteus anguinus*), has been reported to thrive on a diet of mud and associated microflora (Vandel & Bouillon 1959; Vandel 1964). More specifically to this study, Bogart (J. P. Bogart 1967, unpublished M.A. thesis) and Chippindale (2005) both suspected groundwater salamanders of guano feeding but did not have evidence to document the event. Our repeated observations of larval *E. spelaea* feeding on bat guano led us to investigate this behaviour as a hypothetically deliberate action and its potential role as a dietary supplement of *E. spelaea*, utilizing both stable isotope analyses and dietary metrics.

## 2. MATERIAL AND METHODS

January-Stansbury Cave is located 6 km north of the town of Colcord, and is a typical Ozark cave formed from the

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dissolution of fractures in Mississippian-aged, cherty limestone bedrock of the Boone Formation. The study area was limited to the first 440 m of the cave system (total mapped passage is approximately 1800 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 high. Terrestrial habitats within the cave include mud banks, cobble, bedrock, ceiling breakdown, precipitating formations (speleothems) and bat guano piles (ranging in diameter from 3 to 7 and 0.1 to 2 m in depth). A maternity population of approximately 15 000 grey bats (*M. grisescens*) inhabits the cave from late April to October (Fenolio *et al.* 2005). 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 others 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.

We visually surveyed *E. spelaea* once a month from September 2001 to October 2003. Visitation was limited by the United States Fish and Wildlife Service because of the federally protected *M. grisescens* and the presence of a state protected, cave-adapted crayfish *Cambarus tartarus*. Summer surveys were only conducted at night after the endangered bats had left the cave to forage. Headlamps and hand-held flashlights were used to survey for aquatic larval salamanders and terrestrial adults. Note that *E. spelaea* has a distinctly biphasic life cycle involving an aquatic larval period of 1–3 years (Brandon 1971; D. C. Rudolph 1980, unpublished Ph.D. dissertation) followed by metamorphosis into a terrestrial adult. Behavioural observations of *E. spelaea* were recorded on diving slates. We grouped population counts by season (winter—January, February, March; spring—April, May, June; summer—July, August, September; and autumn—October, November, December), and employed the Pearson chi-squared test to test the null hypothesis that salamander counts were evenly distributed by season and by period.

Stable isotope analyses are now widely used in trophic studies of freshwaters (Fry 1999) and cave stream ecosystems (Graening & Brown 2003). Because naturally occurring carbon ( $^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}$ ) isotopes persist and accumulate in food chains, the technique can be used to decipher both diet and trophic position of a given organism (Peterson & Fry 1987). 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 ( $^{13}\text{C}/^{12}\text{C}$ ), and the organism's trophic position can be inferred by the characteristic enrichment of the stable nitrogen isotope ( $^{15}\text{N}/^{14}\text{N}$ ) of 3.5‰ per trophic level (DeNiro & Epstein 1981). In January 2003, we collected multiple samples of each of the following materials from the study cave, as described in Graening & Brown (2003): *M. grisescens* guano (faeces), cave stream sediment; whole bodies of the larvae of *E. spelaea*; and composite samples of whole amphipods (*Gammarus minus*) from the system. Samples collected in December 1999 from a second, proximal study site, Logan Cave, were also analysed. Logan Cave (Benton County, Arkansas) occurs in the same geologic formation as January-Stansbury Cave and has a similar cave stream and maternity colony of grey bats. 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 No. 30-mesh screen. Both sample sets were processed using standard methods (France 1996) and analysed at the University of Utah Stable Isotope Ratio Facility for Ecological Research using primary standards (Lajtha & Michener 1994), with low analytical variability ( $\pm 0.1\%$ ), and reported in standard delta notation (McKinney *et al.* 1950).

For the nutritional analysis, we collected replicate samples of bat guano from different guano piles in January-Stansbury Cave as well as clastic sediment and whole amphipods (*G. minus*) from the cave stream. Samples were analysed at the University of Arkansas' Central Analytical Laboratory using a bomb calorimeter for caloric density, a gas chromatograph/Dumas combustion method for crude protein, and an acid digest and inductively coupled plasma/atomic absorption spectroscopy for mineral content.

### 3. RESULTS

Figure 1 presents the results of the monthly salamander surveys in relation to the presence or absence of the grey bats. Statistical analysis of the data revealed that the surveys were significantly different by season, with spring and summer (the grey bat maternity seasons) having the highest salamander counts (Pearson  $\chi^2_3 = 88.3846$ ,  $p < 0.0001$ ).

The results of the stable isotope analysis are presented in table 1 using standard delta notation (McKinney *et al.* 1950). Carbon isotopic signatures ( $^{13}\text{C}/^{12}\text{C}$ ) of *M. grisescens* guano in January-Stansbury Cave and Logan Cave were almost identical, and were very similar to those reported in other studies (Mitzutani *et al.* 1992). Carbon isotopic signatures of *E. spelaea* and guano were similar, suggesting that bat guano could be a dietary item of this salamander. Nitrogen stable isotope analysis reinforced this supposition, because the *E. spelaea* samples were also enriched in  $^{15}\text{N}$  compared to the *M. grisescens* guano in both caves, which places the salamander higher in trophic position.

Nutritional analyses of bat guano reveal that it contains nutrients roughly equivalent to those that would be found in a potential prey item in this ecosystem, amphipods (table 2). Bat guano's surprisingly high crude protein content (54%), caloric density ( $4124 \text{ cal g}^{-1}$ ) and essential mineral content (parts per thousand) exceeded those of gammarid amphipods, a suitable syntopic prey item of larval salamanders. Conversely, cave stream sediment, a far more abundant potential food source, had almost no detectable protein or caloric content.

### 4. DISCUSSION

Our study demonstrates that *E. spelaea* numbers increase significantly in the main caverns of the system when and where grey bats deposit fresh guano, and that salamander larvae ingest guano that falls into the cave stream. Terrestrial adults may also utilize guano as a food source because we observed salamanders directly upon guano piles next to the cave stream. Stable isotope analyses indicate that larval *E. spelaea* may assimilate this guano, and nutritional analyses imply that bat guano could sustain larval *E. spelaea* if normal prey, such as amphipods,

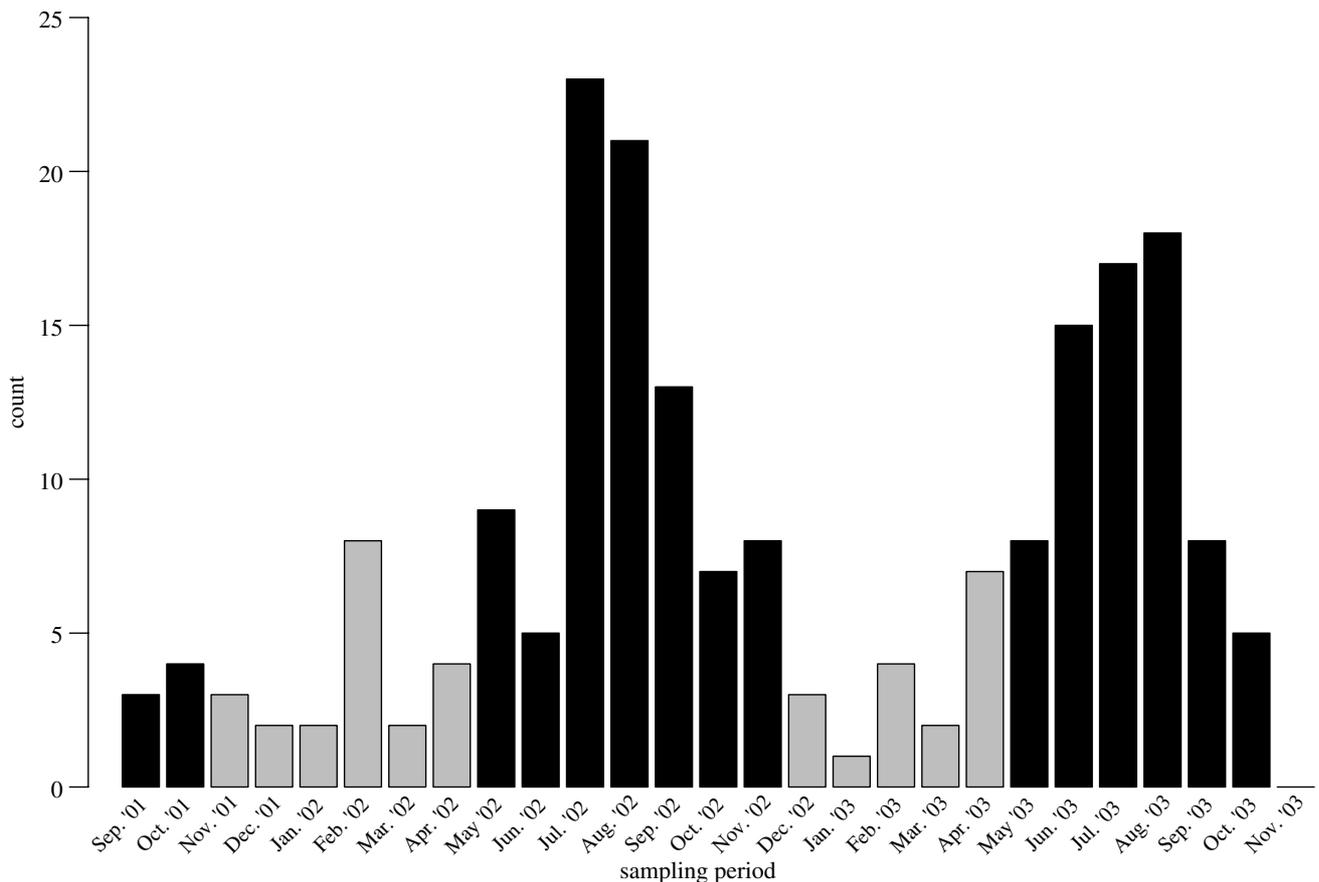


Figure 1. Numbers of *Eurycea spelaea* from September 2001 to October 2003. The black bars indicate the period of time in which the maternity colony of *Myotis grisescens* occupied the cave and deposited guano into the habitat.

were unavailable. To put the nutritional value of guano into perspective, we concurrently analysed a hamburger with the other samples. Bat guano had nearly twice the crude protein content and almost two-thirds of the calories as the sampled hamburger: McDonald's Corporation Big Mac sandwich contained 23% crude protein and 6139 cal g<sup>-1</sup> (dry matter basis). Unlike the faeces of many animals, bat guano is still rich in calories and nutrients. To meet the energetic demands of flight and reproduction, insectivorous bats such as *M. grisescens* have evolved extremely short digestive tracts and rapid food transit times (Mitzutani *et al.* 1992). The digestive efficiency of myotid bats is only 69–78%, resulting in the expulsion of unabsorbed nutrients in guano and urine (Webb *et al.* 1993; Stalinski 1994). Microbial biofilms that form on the guano may boost its nutritional value in much the same way that microbial conditioning of leaves increases their palatability and assimilation by detritivores (Allan 1995). Cummins (1974) used the analogy of enhancing a dry cracker with peanut butter. With regard to amphibians, the notion of deriving nutrition from bacteria is not unknown; Burke (1933) demonstrated that tadpoles could be reared to metamorphosis solely on bacteria.

Our study contradicts the general understanding that salamanders are strictly carnivorous. In fact, coprophagy may be common in subterranean vertebrates. Oligotrophy is thought to select for increased diet breadth in subterranean fauna (Culver 1982, 1994; Holyoak & Sachdev 1998). Other studies determined that Ozark cavefish (*Amblyopsis rosae*) and black bullhead catfish

(*Ameiurus melas*) feed on bat guano in Ozark caves (Poulson 1963; Black 1971), but the studies did not include nutritional and stable isotope analyses to demonstrate the nutritional benefit and habitual nature of the behaviour. Coprophagy has been reported in larval frogs, but for the purpose of inoculation of their intestines by beneficial microbes (Steinwascher 1978; Beebe 1991;

Table 1. Stable isotopic signatures of carbon (<sup>13</sup>C/<sup>12</sup>C) and nitrogen (<sup>15</sup>N/<sup>14</sup>N) of *Eurycea spelaea* muscle (*n*=5) and *Myotis grisescens* guano (*n*=4) in two similar Ozark cave stream ecosystems, January-Stansbury Cave (Delaware County, Oklahoma) and Logan Cave (Benton County, Arkansas), presented in standard delta notation ( $\delta$ ) on a per mil (‰) basis (Lajtha & Michener 1994).

sample type	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
<i>January-stansbury Cave</i>		
<i>E. spelaea</i> muscle # 1	14.3	-21.8
<i>E. spelaea</i> muscle # 2	13.9	-22.1
<i>E. spelaea</i> muscle # 3	11.0	-25.4
<i>E. spelaea</i> muscle # 4	11.5	-22.8
mean	12.7	-23.0
<i>M. grisescens</i> guano # 1	11.5	-24.6
<i>M. grisescens</i> guano # 2	13.2	-22.9
<i>M. grisescens</i> guano # 3	9.7	-25.4
mean	11.5	-24.3
<i>Logan Cave</i>		
<i>E. spelaea</i> muscle	11.0	-24.0
<i>M. grisescens</i> guano	9.5	-24.0

(*Ameiurus melas*) feed on bat guano in Ozark caves (Poulson 1963; Black 1971), but the studies did not include nutritional and stable isotope analyses to demonstrate the nutritional benefit and habitual nature of the behaviour. Coprophagy has been reported in larval frogs, but for the purpose of inoculation of their intestines by beneficial microbes (Steinwascher 1978; Beebe 1991;

Table 2. Nutritional analyses of potential dietary items of *Eurycea spelaeus*: *Myotis grisescens* guano, amphipods (*Gammarus minus*) and cave stream sediment. (Parameters measured (dry matter basis) are mean percentage dry matter content, mean caloric density (cal g<sup>-1</sup>), mean percentage crude protein, mean percentage crude fat and mineral content (mg kg<sup>-1</sup>). Nutritional data of a hamburger (McDonald's Corporation Big Mac sandwich) provided for comparison to human diet.)

	grey bat guano	stream amphipods	cave sediment	Big Mac hamburger
sample size ( <i>n</i> )	3	2	1	2
percentage of dry matter	13	14	77	55
calories (cal g <sup>-1</sup> )	4124	3600	<1	6139
percentage of protein	54	44	<1	23
percentage of fat	1	8	<1	33
percentage of ash	15	—	97	3
<i>minerals (mg kg<sup>-1</sup>)</i>				
calcium	27 552	119 221	6215	1223
copper	161	62	15	2
iron	1753	320	17 321	25
magnesium	2408	1356	669	216
manganese	169	62	2247	—
phosphorous	8951	7537	1011	1499
potassium	4792	11 409	1136	1988
zinc	400	80	55	29

Beebe & Wong 1992). To our knowledge, however, this is both the first reported instance of coprophagy by a salamander and also the first report of its practice by any amphibian for what we believe to be nutritional benefit. In general, the assumption that temperate subterranean vertebrates are strictly carnivores is incorrect, particularly in oligotrophic environments that contain an alternative food resource such as an energy-rich detritus. We predict that other vertebrates inhabiting subterranean environments, currently unknown to consume nutrient-rich detritus, will be found to have shifted their diet from strict carnivory to include coprophagy when faced with starvation in oligotrophic habitats.

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