Calcium as a limiting resource to insectivorous bats: can water holes provide a supplemental mineral source?

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Abstract
Data are presented on a bat assemblage captured among 10 water holes in Colorado over 5 years. The assemblage consists of Myotis ciliolabrum, M. evotis, M. lucifugus, M. thysanodes, M. volans, Eptesicus fuscus, Lasionycteris noctivagans and Corynorhinus townsendii. Results show that reproductive females and juveniles are captured in higher frequencies at water holes containing higher water hardness and that water hardness correlates highly significantly with dissolved calcium content. Also presented are laboratory test data on the stomach volume of Eptesicus fuscus that provide a model for understanding the effect of dissolved calcium content in water as a significant resource. These data indicate that water holes provide supplemental sources of calcium for bats not provided by diet.

Key words: calcium, bats, Colorado, abiotic factors, Vespertilionidae, Myotis

INTRODUCTION
Preferential resource use (i.e. selection) by animals has been documented in many taxa (Johnson, 1980; Dunn & Braun, 1986; Edge et al., 1987; Ofetedal, 1991; Manly, McDonald & Thomas, 1993; Post, 1993; Wilson & Comet, 1993; Murphy, 1994; Arthur et al., 1996; Gorgulu et al., 1996; Kennish & Williams, 1997; Millsopraugh et al., 1998; Ferguson, Nelson & Gous, 1999; Illius et al., 1999). For example, black bears Ursus americanus preferred high carbohydrate and low-terpene forage (Kimball et al., 1998), and hispid cotton rats Sigmodon hispidus preferred quadrants containing the highest levels of supplemental protein, even though cover was low, thereby increasing the risk of predation (Eshelman & Cameron, 1996).

For insectivorous bats, the high metabolic rates of flight require large amounts of food (for review see Altringham, 1996). As a result of conflicting study results, the question of whether bats select specific insect species as prey or consume simply those species occurring in the greatest abundances remains unclear (Buchler, 1976; Fenton & Morris, 1976; Anthony & Kunz, 1977; Studier, Viele & Sevick, 1991; Fenton, 1995).

Several studies have demonstrated that dietary composition does vary intraspecifically across relative age-classes of bats (Rolseth, Koehler & Barclay, 1994; Adams, 1996, 1997, 2000) and between sexes (Husar, 1976), supporting the dietary-choice hypothesis. Definitive tests to determine whether or not insectivorous bats choose prey species based upon nutrient content have not been performed. It seems reasonable to presume that, as in other animals, nutrient requirements of bats would probably vary according to age and sex (for review see Adams, 2000). Of particular interest to our study, reproductive females require large amounts of calcium during gestation and lactation. Increased demand for calcium during pregnancy and lactation caused depletion of long-bone mass by as much as 50% in little brown bats M. lucifugus (Kwiecinski, Krook & Wimsatt, 1987). Calcium removal from bone in reproductive females is thought to facilitate skeletal growth of gestating foetuses and to be used in synthesizing milk for suckling young. By measuring accretion of calcium in juvenile cave bats Myotis velifer and Brazilian free-tailed bats Tadarida brasiliensis, Studier & Kunz (1995) concluded that calcium is a limiting nutritional factor of milk. Perhaps even more compelling, dietary calcium limitations may have ultimately resulted in low reproductive rates and restricted litter sizes of bats over evolutionary time (Barclay, 1994, 1995; Bernard & Davison, 1996).

Evidence for a calcium shortfall in the diets of insectivorous bats is mounting (Studier, Viele et al., 1991; Studier & Sevick, 1992). For big brown bats Eptesicus fuscus living in southern Michigan and ingesting large
numbers of June beetles (Scarabeaeidae), calcium intake was estimated to be 10 times below the required levels (Keeler & Studier, 1992). Using the calculations of Keeler & Studier (1992), Barclay (1995) estimated that female E. fuscus would have to consume two to four times their body mass in insects to meet calcium demands solely through diet. Similarly, using an energy assimilation rate of 75% derived by Kurta, Bell et al. (1989), Barclay (1995) estimated that female E. fuscus would need to consume 3.5 to seven times their required energy (Kurta, Kunz & Nagy, 1990) to gain sufficient calcium. Thus, although energy in the form of prey is relatively abundant to insectivorous bats, calcium for skeletal maintenance in females and for growth in juveniles is less available and potentially limiting. Calcium is thought to be limited in the diets of frugivorous, some carnivorous, and nectarivorous bats as well (Studier, Sevick, Ridley et al., 1994; Studier, Sevick & Wilson, 1994; Studier, Sevick, Wilson & Brooke, 1995).

Considering that calcium is indicated to be an essential limiting factor in the diet of bats, one would predict that females and volant juveniles would seek accessible sources of calcium in the environment. Unlike birds that search the ground for calcium-rich items such as bone fragments and eggshells, bats must find other supplemental sources in the environment (Barclay, 1995). Common bent-wing bats Miniopterus schreibersii in Australia visit mineral-rich waterholes formed by seepage permeating through limestone and also lick the surface of limestone rocks, presumably to ingest minerals (Codd, Clark & Sanderson, 1999). Observations such as these suggest that bats do seek out supplemental sources of calcium independent of daily food intake, and that some water resources may provide such a source.

In this study, visitation patterns at water holes by adult female and juvenile bats were quantified relative to water hardness and calcium content of water. Laboratory tests of stomach volumes in E. fuscus were used to derive a model that was useful for quantifying the potential relative gains derived from drinking water with higher levels of dissolved calcium.

THE ASSEMBLAGE AND ITS STRUCTURE

Our study assemblage consisted of 10 species, all of which are insectivores (Armstrong, Adams & Freeman, 1994). Nine of these species belong to the family Vespertilionidae, and one to the family Molossidae. Four species are fast-fliers with low manoeuvrability and are open-area foragers (hoary bat Lasiurus cinereus, silver haired bat Lasionycteris noctivagans, big brown bat E. fuscus, and the molossid, Brazilian free-tailed bat Tadarida brasiliensis; Adams & Thibault, 1999). Three species fly at moderate speeds and forage along forest edges (western small-footed myotis Myotis ciliolabrum, little brown bat Myotis lucifugus, and the long-legged myotis Myotis volans). Three species are slow-speed flyers that forage within cluttered forest (long-eared myotis Myotis evotis, fringed myotis Myotis thysanodes, and Townsend’s big-eared bat Corynorhinus townsendii). Of these, M. evotis and C. townsendii are specialized as gleaners, using slow manoeuvrable flight to pick insects from the surface of vegetation (Armstrong et al., 1994).

MATERIALS AND METHODS

Study site

The study site encompassed 84 km² along the Front Range of Colorado, immediately west of Boulder, Colorado. The east to west elevation gradient was 539 m over a 6-km transect and comprised 2 vegetation zones, Ponderosa pine Pinus ponderosa and Douglas fir Pseudotsuga menziesii (Armstrong, 1972; Fitzgerald, Meaney & Armstrong, 1994). At lower elevations, open P. ponderosa habitat dominated, whereas above 1980 m, a relatively closed forest of P. menziesii and juniper Juniperus monosperma occurred. Along the transition line between these 2 habitats, P. ponderosa and P. menziesii interdigitated in a complex manner (Armstrong, 1972). Throughout the elevation gradient at the field site, riparian habitats consisted predominately of plains cottonwood Populus sargentii and mountain willow Salix monicola that paralleled streams (Fitzgerald et al., 1994). Some of the water holes where bats were captured were small stream sites surrounded by medium to high vegetative clutter. Other sites were located in open P. ponderosa habitat, and had little vegetation clutter in proximity to water holes.

Trapping protocol

All bats were captured using mist nets (Avinet, Inc.) erected at 10 water holes in Boulder County, Colorado, from May to August 1996–2000. To lessen the chances of sampling bias at different-sized water holes, an array of net sizes (7, 12 and 20 m) was used provide the approximate same relative coverage of approaching flight paths at all sites. Nets were set so that 1 crossed the middle of the water hole, whereas the other was positioned along 1 complete edge of the water hole perpendicular to the middle net. Captured bats were weighed, sexed, identified to species, marked with numbered splitting, 3-mm forearm bands (Hughes Ltd, England) and released.

Measurements of water hardness and calcium content

Water was collected from 10 water holes on 22 June (6 sites) and 23 June (4 sites) and again on 24 July (only 9 sites were sampled because 1 water hole went dry). Surface-water sampling was conducted using the dip-sampling method, which involved filling containers held just below the surface of the water (Greenberg, Rand &
Laboratory test of stomach capacity

To determine if the levels of calcium gained from drinking at calcium-rich water holes would account for a gain in body calcium levels, stomach volume was quantified for 10 thawed specimens of *E. fuscus* (7 males, 3 females). These data, in combination with data on calcium content of the water holes, allowed us to estimate how much calcium a bat would gain by drinking a specific volume of water. All specimens were procured from the South Dakota Department of Health during autumn 2000. Each stomach was observed before the procedure was attempted and all were empty of food. Three specimens were discarded before testing because of decomposition of the gut wall. For the 7 test animals, the abdominal wall was opened along the midline in 6 individuals, exposing the stomach, and a 20-gauge Teflon catheter (Longdwel catheter-needle, Becton & Dickinson & Co., Rutherford, NJ) was inserted through the duodenum into the gastric lumen. Water was slowly injected into the stomach using a graduated 10 ml syringe. The overall dimensions of the stomach (cardia-pylorus length, and lesser–greater curvature depth) were measured to the nearest 0.1 mm with a small ruler placed behind the stomach during the procedure, with the last measurement taken as the stomach began to rupture. Oesophageal sphincters prevented water from spilling into the oesophagus, and the catheter was held firmly within the duodenum to prevent backflow into the small intestine. A single animal was prepared in a different manner to ascertain the stomach volume with an intact abdominal wall. In this case, a 20-gauge Teflon catheter was inserted into the oesophagus through the mouth. After clamping both ends of the digestive tract, the abdominal wall was opened carefully to expose the swollen stomach for measurement.

Statistics

To adjust for different number of sampling nights across sites, an ‘index’ was calculated that represents the frequency of captures of adult females and juveniles at each site (no. of females + no. of juveniles/total captures). Multiple regression analysis was run on index data vs water hardness (mg/l) and on dissolved calcium (mg/l) vs water hardness. Principle component analysis determined multivariate relationships among nutrients across water holes.

### RESULTS

**Capture data**

A total of 1398 bats of 10 species was captured during the 5 years of the study. Of the 10 species captured, the five *Myotis* species and *E. fuscus* comprised 97.2% of all captures: *Myotis ciliolabrum* (*n* = 102), *M. evotis* (164), *M. lucifugus* (446), *M. thysanodes* (157), *M. volans* (129), and *Eptesicus fuscus* (361). *Lasiurus cinereus* (18), *Lasionycteris noctivagans* (9), *Corynorhinus townsendii* (12) and *Tadarida brasiliensis* (1) combined for 2.8% of all captures. Because *Myotis* spp. and *E. fuscus* accounted for 97% of all captures, statistical analyses were restricted to these groups.

**Water analysis**

There was a significantly positive correlation ($r^2 = 0.47$, $r = 0.69$, $P = 0.02$) between the frequency of reproductive females and juveniles of *Myotis* spp. and *E. fuscus* captured at water holes and water hardness (Fig. 1a). A significantly positive correlation ($r^2 = 0.97$, $r = 0.99$, $P < 0.00$) was also found between water hardness and dissolved calcium content of water (Fig. 1b).

**Calcium profiles among water holes**

The amount of dissolved calcium found per site was highly variable, but within-site calcium levels varied little between June and July. Highest calcium levels were consistently found at Gregory Canyon, Long Canyon, NIST and Lindsay Pond sites. In addition, some water holes contained higher amounts of sodium, iron, potassium and magnesium than others. Principal component analysis of mineral content among the sites (Table 1) showed that factor 1 contained the majority of variation in the sample (56.6%), and the factor loadings indicate that calcium was one of the important

### Table 1. PC analysis of mineral attributes of 10 waterholes showing highest eigenvalue for Factor 1 on which calcium loaded as the highest value, followed closely by magnesium. Levels of iron and potassium tended to be inversely proportional to calcium levels

<table>
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<th>Factor</th>
<th>Eigenvalue</th>
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<th>Cumulative percentage</th>
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<tr>
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<td>2.829</td>
<td>56.59</td>
<td>56.59</td>
</tr>
<tr>
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<td>1.774</td>
<td>35.47</td>
<td>92.06</td>
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</table>

Factor loadings

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<th>Factor 2</th>
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</thead>
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distinguishing variables among sites, followed closely by magnesium. Concentrations of iron and potassium were inversely proportional to calcium levels at most water holes (Table 1).

Stomach volume tests

To understand if the level of calcium present in water holes can provide a meaningful resource for adult bats, the average stomach volume of seven *E. fuscus* was quantified. The stomach walls of the thawed specimens ruptured at a point between 3 and 4.5 ml of water with cardia-pylorus lengths ranging between 28 and 30 mm. For the specimen with its abdominal wall left intact, c. 4–4.5 ml of water were observed in the stomach (30 × 18 mm), and the remainder filled the rest of the gut tube. Given the ‘absolute volume’ of the stomach of *E. fuscus*, the average volume of an intact individual was determined to be 3 ml.

DISCUSSION

Although much work has quantified the dietary affinities and habitat use of bats (see Walsh et al., 1996 for review), few investigations provide data on use of water resources (Commissaris, 1961; Cockrum & Cross, 1964; Fenton et al., 1980; Adams & Simmons, 2002). Capture data from the 10 water holes show that females and juveniles are captured in higher frequency at sites having highest water hardness, which correlates strongly with dissolved calcium content. Calcium and magnesium are known to cause water hardness (Cole, 1983), and these covaried in our samples (Table 1). Reproductive females and juveniles, which are known to require high levels of calcium during the breeding season, were strongly correlated with water hardness. These data suggest that bats can discern the level of water hardness, perhaps by taste, and therefore gain calcium by visiting such sites. Although magnesium is also known to cause water hardness, there are no data showing that bats are magnesium deficient, thus, it is unlikely that this is the important element gained by calcium deficient bats visiting calcium-rich sites. However, it is certainly conceivable that bats visiting water holes that contain hard water solely as a result of the magnesium content are being deceived.

Radio-telemetry data on two lactating females provided limited independent data that females seek out water holes which contain high calcium levels. Because reproductive females, and probably weaned juveniles, are calcium deprived (Barclay, 1995), one would predict that they would seek alternative sources of calcium in the environment that would help fulfill their daily demands. Females and young that ingest water with high calcium loads may supplement their dietary calcium intake.

In addition, the amount of calcium present in some water holes seems to be significant enough to be an important resource for bats. Our data for *E. fuscus* show that a full stomach volume of water averages 3 ml. At some of our sites, calcium concentrations reach 29 mg/l or 0.029 mg/ml and, therefore, a bat drinking 3 ml of this water will ingest 0.087 mg of calcium. In a study conducted by Keeler & Studier (1992), *E. fuscus* ingested 1.25 mg/day and egested 0.08 mg/day of calcium. Thus, if an *E. fuscus* individual visited one of the calcium-rich sites and consumed 3 ml of water throughout the night, it would minimally replenish the amount of calcium egested that day. Combining data from our study on *E. fuscus* stomach volumes with published data on calcium requirements for this species (Studier & Sevick, 1992), a single stomach full of water from the highest calcium sites would provide 7% of the daily minimum requirement (1.25 mg).
Although individuals would probably have food in their stomachs, leaving less volume available for water intake at any given visit, it seems reasonable to predict that an individual will ingest a stomach-full of water throughout the night to replace, in some cases, up to 30% of the water-loss related body mass during diurnal roosting (Kurta, Kunz et al., 1990; Webb, 1995). Although insects provide a more significant calcium source than does water, visiting high-calcium water holes would provide bats with additional calcium in concert with needed water intake. Similar gains of calcium, as estimated for *E. fuscus* in our model, would be expected for *Myotis* and other species scaled to body size.

Despite growing interest in the role of calcium as a limiting factor in the lives of bats and the recent discovery that plant-visiting bats feed on calcium-rich foliage in addition to energy-rich fruit and nectar (Kunz & Ingalls, 1994), this study represents one of the first quantifications of female and juvenile bats occurring in higher numbers at calcium-rich water holes. Although insectivorous bats clearly attain much of their minimum daily requirements of calcium from food, calcium-rich water holes would supplement deficits in reproductive females and juveniles. Field experimental analyses of these patterns is the obvious next phase and should lead to a greater understanding of the nutrient ecology of bats.

Acknowledgements

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REFERENCES


