DETERMINING TAPHONOMIC CONTROLS AND RATES OF DECAY IN CAVE ENVIRONMENTS USING MICROCOSMS

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ABSTRACT

Caves are important sites of fossil preservation, especially for Quaternary vertebrates. Taphonomic processes operating in caves are not well understood and have never been experimentally examined. This study focuses on the potential role of bat guano, which impacts environmental biogeochemistry and serves as the base of the food chain in cave ecosystems. The presence or absence of guano is expected to be a major control of preservation potential. The oldest bats are Eocene and so bat guano likely influenced cave preservation only during the Cenozoic. This is a probable megabias of the cave fossil record. Microcosm experiments were used to determine the impact of guano presence and composition, moisture, temperature, and time on preservation potential of small mammal bones, leaves, and crickets. Guano came from insectivorous and frugivorous bats. The guano of insectivorous bats has an acidic pH, while the guano of frugivorous bats is close to neutral. Lab studies were supplemented with field experiments at Crumps Cave, Kentucky. Leaves and crickets were better preserved in the guano of insectivorous bats, while bones showed recrystallization after burial. Leaves and crickets buried in the guano of frugivorous bats were quickly colonized by fungi and mostly destroyed, while

only a few bones showed signs of fungi and degradation. Microcosms with a higher moisture content showed greater degradation, while time and temperature had less of an effect. Bones buried in both types of guano decayed much more rapidly than in sand. Bones buried in situ in cave sediments showed little degradation over three months. These results provide insight into the variable impact of environmental conditions on the taphonomy of Quaternary vertebrates and plants.

INTRODUCTION

Caves are important sites of fossil preservation, especially for Quaternary vertebrates throughout the world (Behrensmeyer et al. 1992; Noto 2011; Plotnick et al. 2014). For example, of 84 Quaternary fossil sites in Virginia in the Paleobiology Database (paleodb.org), 47 are in currently open caves. Caves are common sites of preservation for small animals that are unlikely to be preserved outside of that environment (Behrensmeyer et al. 1992) and are often the only localities to preserve plant and animal fossils from terrestrial highland environments (Behrensmeyer et al. 1992; Noto 2011). Over 50,000 caves have been discovered in the United States alone (Barton and Jurado 2007), suggesting the potential for additional fossil finds in these settings. It is therefore critical to understand taphonomic processes operating in these environments.

Taphonomic processes in caves have not been well studied. The majority of taphonomic studies in caves examine only vertebrates (Andrews 1990; Kowalski 1995; Grandal-d'Anglade and López-Gonzalez 1998; Robu 2016) and have focused mostly on biostratinomy and predator behavior (de Ruiter and Berger 2000; Terry 2010a) rather than on how the physical cave environment affects diagenesis and preservation potential of buried materials. Studies of large Plio-Pleistocene mammals such as cave bears,

leopards, and ground sloths are commonly found in literature on preservation in caves. Grandal-d'Anglade (1998) and Robu (2016) carried out studies of fossil cave bear population structure, examining age and sex distribution with a minor focus on the taphonomy of the bones. Both authors found that population structure and preservation quality differed among the caves studied. In a study of a modern cave in South Africa, de Ruiter and Berger (2000) found evidence supporting the hypothesis that leopards have used caves as feeding dens throughout the Plio-Pleistocene, creating accumulations of bone from large mammals such as zebra, caracal, and antelope.

Andrews (1990) examined the preservation of small mammals in cave environments, focusing on modifications to the bones and assemblages related to the predatory behavior of owls and other raptors. Similarly, Terry (2010a and 2010b) carried out multiple live-dead agreement studies in caves to examine how well small mammal bone assemblages produced by predators match the current populations in the surrounding areas. These reports have shown that the agreement between the death assemblages and current living communities is high (Terry 2010b, 2010a).

Experimental methods have been extensively used to examine early stages of taphonomic change in marine systems (Briggs and Kear 1994; Briggs 1995; Kidwell and Baumiller 1990). While many think of fossilization on the scale of millions of years, the probability that after death an organism will become fossilized can be determined rather quickly based on variations in rates of decay, dissolution, and disintegration which can all be investigated using relatively short term laboratory experiments (Briggs 1995). If organic remains don't persist beyond these early stages of alteration there is nothing left

to fossilize and for remains that do survive, any early alterations will be passed on to the next stage of the fossilization process (Behrensmeyer et al. 2000).

Bats, and their guano, are currently important components of many cave ecosystems and bat remains have been found associated with guano in Mammoth Cave (Widga and Colburn 2015, Colburn 2005). In this paper we experimentally examine the potential impact of bat guano on vertebrate, insect, and plant preservation in cave environments and discuss its possible role as a key taphonomic megabias in these settings.

Guano

Guano is a term used to refer to accumulations of excrement produced by a number of different animals, such as bats, birds, and seals. Many modern caves provide habitats for bats (Santana et al. 2011), which produce copious amounts of guano. In nutrient limited cave environments, bat guano often serves as the base of the food chain and the largest source of energy and nutrients for many organisms to survive on (Emerson and Roark 2007).

Active summer bat colonies are known to produce large amounts of guano, with rates of accumulation estimated from 2 to 10 cm/yr (Hutchinson 1950), making guano a significant modifier of the cave environment. Bat colonies and the guano piles they produce have been shown to increase the air temperature in caves by up to 10° C, with the largest temperature increases in the highest parts of the cave ceiling and the fresh upper layers of guano piles (Harris 1970). Accumulations of guano can thus impact the cave environment by introducing important nutrients and altering pH, temperature, and

biogeochemistry. Since bat guano impacts environmental chemistry and often serves as the base of the food chain in cave ecosystems (Emerson and Roark 2007), we hypothesize that its presence or absence should be a major control on fossil preservation potential. This impact has not previously been examined experimentally.

The composition and chemistry of bat guano depends on the diet of the bat producing it. Most species of bats are either insectivorous or frugivorous with a small percentage classified as sanguinivorous, carnivorous, or nectarivorous (Nowak 1994); in this study, we focus only on the guano of insectivorous and frugivorous bats. Organic matter (OM) and carbon content of guano from both insectivorous and frugivorous bats does not differ significantly, with OM ranging from 53 to 60% by weight in guano of insectivorous bats compared to an average of 63% by weight in guano from frugivorous bats (Shahack-Gross et al. 2004; Emerson and Roark 2007). Fresh guano from insectivorous bats is slightly acidic and contains large amounts of phosphate (25 to 57% by weight) and nitrogen (8 to 12%) (Hutchinson 1950; Shahack-Gross et al. 2004; Emerson and Roark 2007). Over time as the guano ages and decomposes it can become more acidic with a pH of approximately 4 (Moulds 2004). In contrast, the guano of frugivorous bats contains less nitrogen at approximately 2% and has a neutral to alkaline pH (Shahack-Gross et al. 2004; Emerson and Roark 2007).

Bat guano is expected to impact preservation potential in several ways. Guano piles are one of the few nutrient sources in many cave environments, making the areas in and around the pile very active. As such, guano piles experience extensive bioturbation (Bird et al. 2007). As has been shown in marine environments, this should lower preservation potential by increasing rates of decomposition and recycling. The increase in

121 temperature of guano piles relative to the cave environment should also negatively impact
122 preservation potential by increasing decomposition rates. If a guano pile is large enough,
123 there may be areas below the taphonomically active zone (TAZ) that provide an anoxic
124 environment, which would generally favor preservation of all types of organic matter (but
125 this has not been discussed in literature to best of our knowledge).
126 The acidic pH of the guano of insectivorous bats should promote dissolution of
127 bone over time (Shahack-Gross et al. 2004). Since active summer bat colonies can
128 produce large amounts of guano and thus quickly bury organisms that fall in the piles, it
129 is unlikely that any small mammals will be preserved (Kowalski 1995). The high
130 phosphorous content of guano from insectivorous bats may induce precipitation of
131 phosphorous rich minerals, such as brushite, hannayite, whitlockite, and newberyite on
132 the bone and in the guano (Bridge 1973; Karkanas et al. 2002). In contrast to its effect on
133 bone, the acidic pH of fresh insectivorous bat guano should favor the preservation of
134 cellulose and chitin (Greenwood 1991; Kielak et al. 2013). Degradation of these
135 compounds in low pH environments may be partially limited by a reduction in the
136 diversity of the fungal and bacterial decomposer communities (Miller et al. 1993).
137 In contrast to that of insectivorous bats, frugivorous bat guano contains less
138 nitrogen and has a neutral to alkaline pH. Lower levels of nitrogen could limit growth of
139 bacteria, which may reduce decomposition and alteration of the materials buried in guano
140 (Shahack-Gross et al. 2004; Emerson and Roark 2007). It is expected that a neutral to
141 alkaline pH should not contribute to the dissolution of bone. Thus, a bone buried in guano
142 from frugivorous bats is expected to have a higher preservation potential compared to a
143 bone buried in the guano of insectivorous bats. Since higher pH has been associated with

increased chitin degradation (Kielak et al. 2013), plant and insect remains composed of cellulose and chitin may experience lower preservation potential in frugivorous guano.

The specific impact of guano on taphonomy in caves thus should depend on the material (bone, insect, or plant remains) and the type of guano (Emerson and Roark 2007). As a result, it can be hypothesized that the different environmental chemistry of guano piles produced by frugivorous and insectivorous bats should affect taphonomic processes differently.

The aim of this study is to determine the effect of presence and type of guano (insectivorous vs. frugivorous) on the preservation potential of plant, cricket, and small mammal remains by simulating a guano rich cave environment using microcosms. The use of small microcosms will also allow for examination of the effects of moisture and temperature on preservation in guano. A field study where bones and teeth were buried in cave sediment at different distances within the cave was conducted for comparison.

MATERIALS AND METHODS

Microcosm Design and Preparation

Twelve microcosm environments were created to test the effects of temperature, moisture (wet vs. dry), time, and the presence and composition of bat guano on the rate of decay of small mammal bones and teeth, insects, and plant remains (Table I). Individual microcosms were contained in 30 ml glass vials with polypropylene caps (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Vials were sterilized by autoclaving. Plastic caps from the vials could not be autoclaved and were acid washed instead. After autoclaving, vials were wrapped in aluminum foil to prevent light exposure. Each microcosm was

168 filled with 20 cc of autoclaved sand, guano from frugivorous bats, or guano from
169 insectivorous bats. The amount of guano was measured by volume, instead of weight,
170 because the insectivorous Mexican bat guano was much less dense than the guano from
171 the Jamaican frugivorous bats. Two types of guano were used: guano from insectivorous
172 Mexican bats with an N-P-K ratio of 10-1-1 and guano from Jamaican frugivorous bats
173 with an N-P-K ratio of 0-10-0 (Sunleaves Garden Products, Bloomington, IN, USA). The
174 Jamaican, frugivorous bat guano arrived moist but was allowed to dry at room
175 temperature in the lab for approximately three days before use. After sterilization, the
176 control sand was also moist and allowed to dry in a foil-covered beaker for approximately
177 the same amount of time. Each microcosm setup had a wet and dry variant; to prepare the
178 wet microcosms 5 mL of autoclaved milliQ water was added to the 20 cc of material in
179 the vial. Each microcosm treatment was replicated to test the effect of temperature on
180 preservation potential. Microcosms were either kept at room temperature, 26.4°C, or
181 stored at 11°C to simulate the average temperature of cave environments in the Midwest
182 region. Replicates of each microcosm treatment were prepared so that the bone, insect,
183 and plant materials could each be placed in individual vials.
184 Materials added to the microcosms included: small mammal bones and teeth
185 (mainly from voles) extracted from owl pellets (Ward's Science, Rochester, NY, USA),
186 the legs and wings of freshly killed crickets *Acheta domesticus* (obtained live from a
187 retail pet store and killed in the freezer overnight), leaves from a deciduous tree *Gleditsia*
188 *triacanthos* (common name: thornless honeylocust), and needles from a gymnosperm
189 *Picea pungens* (common name: blue spruce) obtained from the east campus of UIC.
190 Before emplacement, all materials were photographed using a Leica LED5000 SLI

191 microscope (Leica Microsystems, Buffalo Grove, IL, USA) and weighed on analytical
192 balance. Microcosms were sealed and placed in their respective storage areas for periods
193 of one-week, one month, two months, or three months. Four replicates were created so
194 that each treatment and material combination was allowed to run for the entire time
195 period without being disturbed. The total number of microcosms, including replicates for
196 moisture, temperature, guano, added substrates, and controls was 139.

197 Microcosm Examination
198
199 Microcosm incubations were halted after one-week, one month, two months, or
200 three months, and materials were removed and lightly brushed off with a paintbrush if
201 any large clumps of sand or guano remained attached. The general state of the
202 microcosms and materials was recorded and all materials were photographed again in
203 approximately the same orientation, using the same microscope and magnification. The
204 condition of materials was scored based on presence of fungi, breakage, recrystallization,
205 staining, and whether the material was totally unrecognizable when recovered. Materials
206 were allowed to dry overnight and then weighed on the same scale used for initial
207 measurements. Materials were then placed in labeled plastic vials and stored at -20°C.
208 Half of the remaining contents of the microcosms were stored in the microcosm vial at -
209 20°C, while the other half was used for pH measurements.

210 pH Measurements
211
212 To establish the average starting pH of both types of guano, three samples were
213 taken from each bag of guano. In a 20 mL glass beaker, 5 mL of milliQ H_2O was added
214 to 2.5 g of guano to create a slurry. The slurry was stirred for 15 seconds then allowed to
215 sit for 30 minutes before pH measurements were taken. Measurements were taken at

26.4°C using an Oakton PH/mV/Temperature Meter Series WD-35615 (OAKTON Instruments, Vernon Hills, IL, USA) that was calibrated using buffers of pH 4, 7, and 10 prior to taking each set of measurements. After microcosm incubations concluded, the bone, insect, or plant remains were removed and half of the remaining guano or sand was used to measure pH in the same manner as described above.

Scanning Electron Microscopy of Materials

After initial examination, materials of interest on some samples were photographed at higher magnification using a Hitachi S-3000N Variable Pressure Scanning Electron Microscope (SEM; Hitachi High-Technologies Corporation, Tokyo, Japan) at the UIC Electron Microscopy Service. Samples were attached to aluminum stubs using silver paint and carbon tape (Electron Microscopy Sciences, Hatfield, PA, USA). Bones and teeth were not sputter-coated so that the elemental composition of crystals on the surface could be identified after imaging. Images were acquired using the secondary electron detector at a voltage of 8-15 kV. Weight percent of elements in crystals on the bone surfaces were determined using an Oxford INCA X-ray energy dispersive spectrometer (XEDS; Oxford Instruments, Tubney Woods, Abingdon, Oxfordshire, UK) equipped on the SEM.

Field Experiment

A field experiment was carried out in conjunction with the laboratory microcosm experiments to determine the effect of burial in wet cave sediment and at varying distances within the cave on preservation potential. Crump's Cave Preserve in Smith's Grove, KY was chosen as the site of the field study based on several factors. First, it is a wet cave inhabited by a small colony of insectivorous bats. Second, Crump's Cave was

241 already equipped with multiple monitoring stations for environmental parameters such as
242 humidity, temperature, bat entrance/exit flights, and water sampling. Third, the Hoffman
243 Environmental Research Institute at Western Kentucky University maintains the cave
244 solely for research purposes limiting outside interference.

245 Cricket and plant material could not be buried in the Crump's Cave field site due
246 to concerns of introducing non-native species and pathogens. Bones and teeth used in the
247 field study were obtained from the same owl pellets used for the microcosms. All
248 materials were photographed and weighed before and after the experiment. Materials
249 were placed in handmade mesh bags to exclude scavengers and buried at varying
250 distances from the entrance of the cave.

251 Three sites were chosen within the cave (Fig. 1). Site 1 was located approximately
252 1.5 m from the entrance of the cave, along the left wall near a petroglyph of a dog. Site 2
253 was located along the right wall of the cave, approximately 90 m from the cave entrance
254 and 4.5 m off the main path. Site 3 was located along the left wall of the cave,
255 approximately 90 m from site 2, just beyond a small pond formed by water dripping into
256 the cave from above. At each site three mesh bags containing one small mammal bone,
257 tooth, skull and intact owl pellet were buried in separate holes (1a, 1b, 1c, 2a, 2b, etc.) at
258 an approximate depth of 10 cm. Soil moisture and pH measurements were taken near
259 each sub-site using a Kelway soil pH acidity and moisture meter (Kel Instruments
260 Company, Wyckoff, NJ, USA). Soil and air temperatures were measured using a digital
261 thermometer. Soil samples were taken from each sub site using a tubular soil sampler and
262 stored in separate soil sampling bags (LaMotte, Chestertown, MD, USA) in a cooler until
263 they could be stored at -20°C. Materials were buried on August 14th, 2014 and retrieved

on October 11th, 2014 at which time all aforementioned measurements were repeated. One bag was left buried at each site for long-term study; these will be retrieved at a future date.

RESULTS/DISCUSSION

Microcosm Experiment Results

Overall Trends.—Results from the one-week, one-month, two-month, and three-month microcosms are displayed in Supplemental Tables 1 through 4, respectively. Black cells in tables denote weight measurements that could not be obtained either because the materials were not recovered or were recovered in such poor condition that they could not be weighed. The condition of materials removed from the one-week, one-month, two-month, and three-month microcosms are displayed in Supplemental Tables 5 through 8, respectively. Conditions in microcosms (MC) 1-3 and 7-9 generally had no effect on the bones and teeth buried in them and were omitted in the one-week bone microcosms due to lack of supplies.

In general, dry microcosms generally showed little to no visual change in materials recovered from them. Most materials tested showed a decrease in mass, which can be seen in Figure 2 and in the %WC 1 and %WC 2 columns of Supplemental Tables 1 through 4. Temperature was not observed to have an effect on pH, percent weight change, or the visual condition of materials. Decomposition of materials did not directly correlate with the time spent in the microcosms. This could be related to the fact that microcosms were sealed and may have become anoxic during the experiments, which would decrease decomposition rates.

The average pH of bat guano before experiments was 6.68 for guano from insectivorous bats and 6.54 for guano from frugivorous bats. The average post-experiment pH for control microcosms with sand was 5.50, 5.66 for microcosms with insectivorous bat guano, and 6.57 for microcosms with frugivorous bat guano. The pH of insectivorous bat guano microcosms decreased over the course of the experiments, while the pH of sand and frugivorous bat guano microcosms remained stable. A two-sample t-test confirmed that the pre-experiment and post experiment pH of insectivorous bat guano were significantly different from one another (p-value 0.000, 23 df). A two-sample t-test was also used to compare pH measurements of the two guanos at the conclusion of the experiments. The test showed that the pH of both guanos was significantly different (p-value 0.000, 71 df). The frugivorous bat guano was not alkaline as originally expected but was less acidic/closer to neutral than the insectivorous bat guano.

Bone and Teeth Microcosm Results.—The majority of bones and teeth from microcosms showed a decrease in mass, with the exception of bones and teeth recovered from microcosms that contained wet guano from insectivorous bats (Microcosms B-6 and B-12), which consistently showed an increase in mass. This increase in mass resulted from recrystallization and crystal growth on the outer surfaces of the bones and teeth. A before and after view is shown in Figure 3. More detailed images of the crystallization are shown in Figure 4. Staining, which can be seen in Figures 3 and 4, was observed on bones and teeth recovered from microcosms that contained wet, insectivorous bat guano. This staining effect can be compared to staining observed on bat bones (Fig. 5) recovered from guano in the Chief City guano deposits in Mammoth Cave, KY dated at approximately >50,000 ybp. In contrast, bones and teeth buried in microcosms with dry,

311 insectivorous bat guano exhibited little to no physical change, no crystal growth, and
312 generally showed a slight decrease in mass of approximately 1% or no change in mass at
313 all. The reason for this slight loss of mass is unknown, but options include desiccation,
314 microbial degradation of collagen, or loss of matted hair or other material from the
315 surface.

316 *Cricket Microcosm Results.*—Microcosms that contained cricket legs and wings
317 had the most instances of unrecoverable materials across all time periods, making
318 crickets the most fragile out of all materials used in the microcosm experiments. Cricket
319 wings were recovered approximately 77% of the time compared to the 97.8% rate of
320 recovery for all other materials. In the three-month microcosms, 25% of the material was
321 unrecovered, with five out of twelve wings and one leg never recovered. All unrecovered
322 materials were originally buried in wet microcosms with varying materials (i.e. sand or
323 guano from frugivorous or insectivorous bats). With 25% of buried material missing after
324 only three months, crickets buried in wet guano piles may not survive long enough to be
325 fossilized.

326 For cricket legs and wings that were recovered, 93% showed a loss of mass,
327 indicating desiccation and degradation. The increase in mass observed in 7% of cricket
328 materials was the result of sand or guano particles that had attached to them and could not
329 be removed without destroying the materials. Fungi were often present on legs and wings
330 recovered from microcosms that contained wet sand or wet frugivorous bat guano (Fig. 6)
331 but were never observed on any materials in microcosms that contained insectivorous bat
332 guano. If fungi are a major factor in the degradation of cricket legs and wings, as they
333 were observed to be in this study, then they may actually have better preservation if

buried in insectivorous bat guano or other environments that inhibit the growth of fungi. This is contrary to what we expected for insect remains buried in guano from insectivorous bats, since chitinolytic activity was expected to be high. Cricket legs and wings recovered from microcosms that contained wet insectivorous bat guano were generally intact and in fair condition with the exception of two instances in the one-week microcosms (Fig 7). Fossils of highly sclerotized insects such as beetles and millipedes have been found in caves containing Quaternary fossil packrat middens in arid environments like the Chihuahuan Desert, Arizona, and Utah (Elias 1990, 1992). In assemblages from those studies, softer bodied insects such as crickets were underrepresented. Results from the cricket microcosms in this study demonstrate the fragility of crickets in our simulated guano rich cave environments. If these fragile organisms are to persist in caves, the environment should be arid and free from fungi similar to our dry microcosms containing guano from insectivorous bats.

Plant Microcosm Results.—Overall, plant material recovered from microcosms was in better condition than insect remains, which can be seen in Figure 8 and Supplemental Tables 5 through 8 as lower total scores for alteration. Plant material remained mostly intact, with the occasional disarticulation of the woody attachment at the end of the spruce needles. Fungi were regularly observed on plant material recovered from microcosms that contained wet frugivorous bat guano, but they were not destroyed to the same degree that cricket remains were (Fig. 8D). Plant material recovered from microcosms that contained wet insectivorous guano showed evidence of desiccation and varying degrees of staining (Fig. 9). Approximately 95% of recovered plant material showed a decrease in mass. The five observed instances of an increase in mass were the

result of visible particles of guano or sand attached to the materials. One leaf recovered from a microcosm that contained dry frugivorous bat guano showed no change in mass at all. The improved preservation potential of plant material compared to cricket remains is similar to what Elias (1990) observed in fossil packrat middens in caves of the Chihuahuan Desert.

Scanning Electron Microscopy and X-Ray Energy Dispersive Spectrometer Results.—SEM confirmed the presence of crystal growth on the surface of bones recovered from microcosms that contained wet, insectivorous bat guano (Fig. 10). Several morphologies were observed with many, but not all, crystals exhibiting a rhomboid shape as seen in Figure 10A and F. Results from the XEDS analysis of five crystals, from four different bones are shown in Table III and Figure 11. The crystals are a mix of calcium, magnesium, and phosphorous minerals. Based on the elemental weight percent, the crystals from B2-12 and B2-6a are likely calcite. If the small weight percent of carbon in the crystal analyzed from B2-6b is not considered, then Magnesium Whitlockite is a possibility. Whitlockite is found in bone and known to be associated with bat guano (Bridge 1973).

Field Experiment Results

Unlike the bones and teeth buried in microcosms containing wet, insectivorous bat guano, bones buried in Crump's Cave did not show evidence of recrystallization and/or crystal growth. In fact, the materials recovered from Crump's Cave showed little to no visual change. Two differences between the field site and microcosm experiments may account for this. First, the average soil pH in Crump's Cave was 6.5 (Table III) compared to an average pH of 5.6 in microcosms that contained wet insectivorous bat

guano. Second, bones in the microcosms were buried directly in bat guano while the bones in the field experiment were buried in cave sediment, not an actual guano pile. This lack of alteration compared to results observed for bones buried in wet insectivorous bat guano highlights how preservation in a guano pile differs from preservation in other areas of caves.

CONCLUSIONS

Degradation of all materials in all microcosm setups, except for bone and teeth, is evidenced by the decrease in mass. However, amount of mass lost did not directly correlate with length of time. All materials recovered from dry microcosms of both sand and guano exhibited little to no alteration, supporting the idea that arid caves boost preservation potential (Culver and White 2005).

In wet microcosms, preservation potential for plant and cricket material was highest in those that contained guano from insectivorous bats. By three months however, only 75% of cricket legs and wings in wet microcosms were recovered. The presence of fungi proved to be the most significant factor in the degradation of plant and cricket remains. Whether due to pH, microbial antifungal interactions, or other factors, fungi were excluded from microcosms that contained insectivorous bat guano, lowering degradation rates for cricket and plant remains. Preservation potential was lowest in the frugivorous bat guano where fungi were prevalent.

Preservation potential for bones and teeth was highest in microcosms that contained sand. Bones and teeth recovered from microcosms that contained guano from insectivorous bats exhibited recrystallization and crystal growth on the outer surfaces. A combination of recrystallization and authigenic crystal growth is proposed because a

404 positive weight change in practically all bones and teeth points to the addition of new
405 crystals, not simply the recrystallization of preexisting bone mineral. This introduces a
406 new complication for bones and teeth buried in the guano of insectivorous bats. If they do
407 not experience total dissolution, crystal growth on the bone surface may eventually
408 obscure fine details and prevent identification altogether if growth continues at a steady
409 rate. Our results support the idea that the pH of guano decreases as it ages (Moulds 2004).
410 If the decrease in pH of insectivorous bat guano seen over the course of the experiments
411 continues over longer timescales, preservation potential should decrease for buried bone.
412 Several instances of fungal attack on bone to the point of breakage were observed in
413 microcosms that contained guano from frugivorous bats. In the end, preservation
414 potential for bone was lower in both guanos than for bones buried in sand. This supports
415 the idea that bat guano has biased the fossil record of bones in caves where accumulation
416 of guano occurs. Whether crystallization or fungal attack led to lower preservation
417 potential was not determined over the three-month course of experiments.
418 Connections between the microcosm and field experiments were limited by two
419 factors. First, only bones and teeth could be buried due to concerns about introducing
420 non-native species and pathogens. Second, materials could not be buried directly in
421 guano piles due to restricted access to areas where bats roosted in Crump's Cave. Little to
422 no alteration, no fungi, and no crystallization were observed on bones recovered from
423 Crump's Cave. Compared to microcosm experiments, bones buried in cave sediment
424 were most similar to those buried in sand since practically no alteration was observed.
425 This lack of similarity between the microcosm field experiments highlights how different
426 guano piles are from the rest of the cave environment. Longer periods of burial in cave

sediment may be necessary in order to observe marked alteration. Although little alteration was observed, field studies of taphonomy in cave sediment are still of interest. The majority of space in caves around the world is not entirely occupied by bats and a greater understanding of taphonomic processes in those areas is still needed.

When present, guano plays a major role in the cave ecosystem, but its impact may be relatively recent. The oldest bats are Early Eocene (Simmons et al. 2008), which makes the possible preservational bias introduced by bat guano a Cenozoic phenomenon. If we are to understand preservation in caves and how it may have changed over time, the role of bat guano in the preservation of buried materials must be understood. In our experiments, bat guano did not universally decrease preservation potential for all materials. Cricket and plant remains buried in guano of frugivorous bats had lower preservation potential than similar materials buried in the guano of insectivorous bats, primarily due to the presence of fungi in frugivorous bat guano. Bone was the most robust material compared to cricket and plant remains but it did experience instances of significant alteration in both guanos compared to practically no alteration in sand and cave sediment. Based on this study, preservation potential for bone buried in either type of guano is lower than for bone buried in cave sediment or sand, preservation potentials for cricket and plant remains are highest in insectivorous bat guano, and all materials experience lower preservation potential in frugivorous bat guano. Ultimately, the evolution of bats and their production of guano may be a megabias against the preservation of bone in cave environments where guano accumulates. As such, the quality of the vertebrate fossil record of caves should have decreased since the early Cenozoic when bats evolved.

Further research on all topics discussed here is needed. Based on these results, future microcosm studies should incorporate longer periods of time and a wider range of whole and fragmented organisms. Additional microcosm studies examining the abiotic and biotic factors affecting preservation in bat guano are needed. Larger microcosms that could incorporate different guanos and the insect scavengers commonly found in guano piles would help determine to what degree scavengers in guano piles lower preservation potentials for plant and insect remains. Expansion of in situ field studies with a wider range of organisms buried in a greater variety of cave environments, cave sediments, and guano piles should also be considered.

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CITED LITERATURE

Andrews, P., 1990, Owls, Caves, and Fossils: University of Chicago Press.

Barton, H.A., and Jurado, V., 2007, What's up down there? Microbial diversity in caves:

Microbe, v. 2, p. 132–138.

Behrensmeyer, A.K., Damuth, J.D., DiMichele, W.A., and Potts, R., 1992, Terrestrial Ecosystems through Time: Evolutionary Paleoecology of Terrestrial Plants and Animals: University of Chicago Press, 62-64 p.

Behrensmeyer, A.K., Kidwell, S.M., and Gastaldo, R.A., 2000, Taphonomy and paleobiology: Paleobiology, v. 26, p. 103–147, doi: 10.1666/0094 8373(2000)26[103:TAP]2.0.CO;2.

Bird, M.I., Boobyer, E.M., Bryant, C., Lewis, H. a., Paz, V., and Stephens, W.E., 2007, A long record of environmental change from bat guano deposits in Makangit Cave, Palawan, Philippines: Earth and Environmental Science Transaction of the Royal Society of Edinburgh, v. 98, p. 59–69, doi: 10.1017/S1755691007000059.

Bridge, P.J., 1973, Guano Minerals from Murra-el-elevyn Cave Western Australia: Mineralogical Magazine, v. 39, p. 467–469, doi: 10.1180/minmag.1973.039.304.10.

Briggs, D.E.G., 1995, Experimental taphonomy: Palaios, v. 10, p. 539–550.

Briggs, D.E.G., and Kear, A.J., 1994, Decay and Mineralization of Shrimps: Palaios, v. 9, p. 431–456.

Colburn, M. L., 2005, Paleontological Inventory Project: Vertebrate Remains in Select Passages and Caves at Mammoth Cave National Park, Kentucky: Illinois State Museum Landscape History Program Technical Report 2005-1199-007, p. 1-316.

Culver, D., and White, W., 2005, Encyclopedia of caves: Elsevier Inc., p. 590-598.

Elias, S.A., 1990, Observations on the Taphonomy of Late Quaternary Insect Fossil Remains in Packrat Middens of the Chihuahuan Desert: Palaios, v. 5, p. 356–

363.

Elias, S.A., Mend, J.I., and Agenbro, L.D., 1992, Late quaternary arthropods from the Colorado Plateau, Arizona, and Utah: Great Basin Naturalist, v. 52, p. 59–67.

Emerson, J.K., and Roark, A.M., 2007, Composition of guano produced by frugivorous, sanguivorous, and insectivorous bats: Acta Chiropterologica, v. 9, p. 261–267, doi: 10.3161/1733-5329(2007)9[261:COGPBF]2.0.CO;2.

Grandal-d'Anglade, A., and López-Gonzalez, F., 1998, A population study on the Cave Bears (Ursus spelaeus Rosenmüller-Heinroth) from Galician caves, NW of Iberian Peninsula: Cadernos Lab. Xeolóxico de Laxe, v. 23, p. 215–224.

Greenwood, D., 1991, The taphonomy of plant macrofossils, *in* Donovan, S.K., ed., The Processes of Fossilization: Columbia University Press, p. 141–169.

Harris, J.A., 1970, Bat-guano cave environment.: Science (New York, N.Y.), v. 169, p. 1342–1343, doi: 10.1126/science.169.3952.1342-a.

Hutchinson, G.E., 1950, Survey of Contemporary Knowledge of Biogeochemistry: 3. The Biogeochemistry of Vertebrate Excretion: Bulletin American Museum of Natural History, v. 96.

Karkanas, P., Rigaud, J.-P., Simek, J.F., Albert, R.M., and Weiner, S., 2002, Ash Bones and Guano: a Study of the Minerals and Phytoliths: Journal of Archaeological Science, v. 29, p. 721–732, doi: 10.1006/jasc.2001.0742.

Kidwell, S., and Baumiller, T., 1990, Experimental disintegration of regular echinoids: roles of temperature, oxygen, and decay thresholds: Paleobiology, v. 16, p. 247–271.

518 Kielak, A.M., Cretoiu, M.S., Semenov, A. V., Sørensen, S.J., and Van Elsas, J.D., 2013,
519 Bacterial chitinolytic communities respond to chitin and pH alteration in soil:
520 Applied and Environmental Microbiology, v. 79, p. 263–272, doi:
521 10.1128/AEM.02546-12.

522 Kowalski, K., 1995, Taphonomy of bats (Chiroptera): Geobios, p. 251–256.

523 Miller, R.F., Voss-Foucart, M.F., Toussaint, C., and Jeuniaux, C., 1993, Chitin
524 preservation in Quaternary Coleoptera: preliminary results: Palaeogeography,
525 Palaeoclimatology, Palaeoecology, v. 103, p. 133–140, doi: 10.1016/0031-
526 0182(93)90139-A.

527 Moulds, T., 2004, Review of Australian cave guano ecosystems with a checklist of guano
528 invertebrates: Proceedings of the Linnean Society of New South Wales, v. 125, p. 1–
529 42.

530 Noto, C.R., 2011, Hierarchical Control of Terrestrial Vertebrate Taphonomy Over Space
531 and Time: Discussion of Mechanisms and Implications for Vertebrate Paleobiology,
532 *in* Taphonomy: Process and Bias Through Time: p. 287–336.

533 Nowak, R.M., 1994, Walker's Bats of the World: The Johns Hopkins University Press,
534 Baltimore, MD.

535 Plotnick, R.E., Kenig, F., and Scott, A., 2014, Using the voids to fill the gaps: caves,
536 time, and stratigraphy: Geological Society, London, Special Publications, v. 404.

537 Robu, M., 2016, Fossil population structure and mortality analysis of the cave bears from
538 Urşilor Cave , north-western Romania: Acta Palaeontologica Polonica, v. 61, p.
539 469–476, doi: 10.4202/app.00201.2015.

de Ruiter, D.J., and Berger, L.R., 2000, Leopards as Taphonomic Agents in Dolomitic Caves—Implications for Bone Accumulations in the Hominid-bearing Deposits of South Africa: Journal of Archaeological Science, v. 27, p. 665–684, doi: 10.1006/jasc.1999.0470.

Santana, S.E., Strait, S., and Dumont, E.R., 2011, The better to eat you with: functional correlates of tooth structure in bats.: Functional Ecology, v. 25, p. 839–847.

Shahack-Gross, R., Berna, F., Karkanas, P., and Weiner, S., 2004, Bat guano and preservation of archaeological remains in cave sites: Journal of Archaeological Science, v. 31, p. 1259–1272, doi: 10.1016/j.jas.2004.02.004.

Simmons, N.B., Seymour, K.L., Habersetzer, J., and Gunnell, G.F., 2008, Primitive Early Eocene bat from Wyoming and the evolution of flight and echolocation.: Nature, v. 451, p. 818–21, doi: 10.1038/nature06549.

Terry, R.C., 2010a, On raptors and rodents: testing the ecological fidelity and spatiotemporal resolution of cave death assemblages: Paleobiology, v. 36, p. 137–160, doi: 10.1666/0094-8373-36.1.137.

Terry, R.C., 2010b, The dead do not lie: using skeletal remains for rapid assessment of historical small-mammal community baselines: Proceedings of the Royal Society of London B: Biological Sciences, v. 277, p. 1193–1201, doi: 10.1098/rspb.2009.1984.

Widga, C., and Colburn, M., 2015, Paleontology and paleoecology of guano deposits in Mammoth Cave, Kentucky, USA: Quaternary Research, v. 83, p. 427–436.

FIGURE CAPTIONS

FIG. 1.—Map of Crump's Cave field sites. Star = site 1; diamond = site 2; triangle = site 3. Map courtesy of Pat Kambesis, Western Kentucky University.

FIG. 2. Histograms showing percent weight change of bone, insect, and plant remains from microcosm experiments.

FIG. 3.—Before and after view of the bone buried in MC B2-6 (wet insectivorous bat guano at 11°C) **A**) Bone before burial. **B)** Bone after recovery showing crystal growth/recrystallization on the surface.

FIG. 4.—Recrystallization and crystal growth observed on bones recovered from microcosms that contained wet insectivorous bat guano. Crystals are a mix of calcium, phosphorous, and magnesium minerals. **A-D)** Crystals observed on the bone recovered from MC B1-12. **E-F)** Crystal growth observed on bone from MC B1-6.

FIG. 5.—Staining observed on bat bones and teeth from the Chief City guano deposit in Mammoth Cave, KY. **A**) Single bat bone with possible crystal growth on surface. **B)** Multiple bones and two teeth showing varying degrees of staining.

FIG. 6.—**A-D)** Fungi observed on cricket legs and wings recovered from various microcosms that contained wet sand or wet frugivorous bat guano.

FIG. 7.—Condition of cricket legs recovered from microcosms containing wet, insectivorous bat guano. **A-B)** Usual condition of cricket legs and wings recovered. **C-D)** Unusual condition of cricket legs and wings recovered from one-week microcosms of the same conditions.

FIG. 8.—Before and after photos of thornless honeylocust leaves and blue spruce needles recovered from microcosms. Needles in these photographs are approximately 20 mm long. **A-B)** Wet sand microcosm. **C-D)** Wet frugivorous bat guano microcosm. **E-F)** Wet insectivorous bat guano.

FIG. 9.—Photographs of leaves and needles recovered from microcosms that contained wet insectivorous bat guano showing the varying degrees of staining. Degree of staining did not universally increase with time. **A)** Plant material recovered from MC P1-6. **B)** Material from P1-12. **C)** Material from P2-12.

FIG. 10.—SEM images of crystals observed on the surfaces of bones removed from microcosms that contained wet insectivorous bat guano. **A)** Crystal growth observed on the bone recovered from microcosm B1-6. **B)** Crystal growth on the bone from B2-6. **C-D)** Crystal growth on the bone from B2-12. **E)** Crystal growth on the bone from microcosm B3-12. **F)** Crystal growth on the tooth from microcosm B3-12.

FIG. 11.—SEM images of five crystals analyzed using XEDS. **A)** One crystal from bone B1-6. **B-C)** Crystals from B2-6, referred to as B2-6a and B2-6b in Table 2, respectively. **D)** Crystal from B2-12. **E)** Crystal from B3-12.

TABLE 1.— Microcosm Experimental Design. 30 ml vials containing either sand, frugivorous bat guano, or insectivorous bat guano. Microcosms were either dry or wet and kept at room temperature or in a refrigerator. Each vial contained either bone and teeth, cricket legs and wings, or leaves and needles. Four vials were used for each combination of environment and material. There were a total of 138 microcosms.

TABLE 2.— Elemental Weight % of Crystals Analyzed Using XEDS. Five crystals of varying morphology observed on bones recovered from microcosms that contained wet guano from insectivorous bats were analyzed using an X-Ray Energy Dispersive Spectrometer (XEDS) in an attempt to determine the mineralogy.

TABLE 3.— pH and Saturation Measurements at Crump's Cave Field Sites. Measurements of pH and percent saturation were taken at each subsite before materials (bones and owl pellets) were buried and again after they were retrieved two months later.