MACROINVERTEBRATE DIVERSITY AND FOOD WEB DYNAMICS IN A GUANO SUBSIDIZED CAVE ECOSYSTEM: BRACKEN BAT CAVE

THESIS

Presented to the Graduate Council of
Texas State University-San Marcos
in Partial Fulfillment
of the Requirements

for the Degree

Master of SCIENCE

by

Goniela Iskali, B.S.

San Marcos, Texas
May 2011

MACROINVERTEBRATE DIVERSITY AND FOOD WEB DYNAMICS

IN A GUANO SUBSIDIZED CAVE ECOSYSTEM:

BRACKEN BAT CAVE

Committee Members Approved:

_____

Yixin Zhang

_____

Weston Nowlin

_____

Benjamin Schwartz

Approved:

_____

J. Micheal Willoughby
Dean of Graduate College

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplicate Permission

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Yixin Zhang, and committee members, Dr. Benjamin Schwartz and Dr. Weston Nowlin, for all their hard work and helpful suggestions that made this project successful. I would also like to thank Bat Conservation International, especially Fran Hutchins and Jim Kennedy, for providing access to the cave, helping me collect samples, and answering my endless questions. I could not have completed this project without the help of my lab mates and lab technicians: Megan Lindsey, Mario Sullivan, Trey Nobles, Val Yerby, Sam Peterson, Jessica Callery, Natasha Lehr, and Ryan Stewart. Lastly, I would like thank all the field volunteers who made this project possible. Funding for this project was provided by the National Speleological Society.

This manuscript was submitted to the committee on March 27th, 2010.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

MACROINVERTEBRATE DIVERSITY AND FOOD WEB DYNAMICS
IN A GUANO SUBSIDIZED CAVE ECOSYSTEM:
BRACKEN BAT CAVE

by

Goniela Iskali, B.S.

Texas State University-San Marcos

May 2011

SUPERVISING PROFESSOR: YIXIN ZHANG

Caves are generally oligotrophic ecosystems and highly limited by energy sources due to the lack of primary productivity within the system. Therefore cave ecosystems rely almost entirely upon allochthonous energy supplies originating from surface ecosystems. Bracken Bat Cave is unique in terms of the magnitude of allochthonous energy resource input in the form of guano, which is provided by the largest bat community in the world (a colony of more than 20 million Mexican free-tail bats (*Tadarida brasiliensis*)). For this reason it is important to gain an understanding of food web dynamics within Bracken Bat Cave. The objectives of this study are: 1) provide baseline biological information of the macroinvertebrate taxa richness and abundance of the cave, 2) quantifying the importance of other environmental factors such as distance from entrances, depth within the guano

substrate of the cave floor, or seasonality on macroinvertebrate abundance and richness, 3) investigate how anthropogenic disturbances affect macroinvertebrate abundance and richness, 4) examine the key nutrient relationships in this ecosystem, the nutrient properties of the guano subsidy and its effect on the macroinvertebrate populations, and 5) investigate the food web interactions of the cave macroinvertebrates through stable isotope analysis. Our results indicate that seasonality does not have a major effect on macroinvertebrate abundance and richness, despite the fact that guano deposition significantly varied between months. An estimated dry weight total of 50,521.72 kg of guano were deposited in the cave (3078 m^2) August 2009-August 2010, with the highest guano deposition rate being recorded in September and the lowest deposition rate in January-March. On the other hand, macroinvertebrate abundance and richness were significantly correlated with guano depth and distance from the entrance. In addition, disturbances such as large amounts of guano removal led to an increase in macroinvertebrate abundance and richness. Nutrient analyses indicated that guano nutrient properties remained constant with respect to seasonality and guano depth, with the exception of carbon content, which decreased with guano depth. Lastly, the stable isotope analyses suggested that guano has a high range of variability in carbon and nitrogen stable isotope ratios. More specifically, $\delta^{13}C$ only varied with respect to sample type (guano, hair, macroinvertebrates) and $\delta^{15}N$ varied with respect to sample type and guano depth. Our findings thus describe Bracken Bat Cave as a relatively stable ecosystem that experiences few fluctuations with seasonality, and can withstand the effects of disturbances due to the large amount of guano subsidy found in the cave. The information gained from our study increases our knowledge of such cave ecosystems and

has ecological implications on the conservation of such a distinctive ecosystem and bat community.

CHAPTER I

INTRODUCTION

Caves are usually oligotrophic ecosystems and limited by energy resource availability (Culver 1982, 1985). Most caves are also characterized by other unique conditions such as stable temperatures, stable but high humidity (over 80%) and the absence of light. Troglobites or obligate cave organisms have thus developed adaptive strategies to withstand energy deprivation and the dark conditions of caves (Culver 1982). Some of these adaptations include loss of eyesight and body pigmentation, elongation of sensory organs, low reproductive rates, and longer life spans in comparison to their above-the-ground counterparts or related organisms (Culver 1982, Culver et al. 2000). In addition, subterranean cave ecosystems often have a lower abundance and diversity of organisms when compared with surface ecosystems (Holsinger 1998). The unique conditions of cave ecosystems have created special niches and led to specific adaptations for the organisms occupying these habitats. However, the adaptations of cave biota to these specific conditions have also exposed them to vulnerabilities when conditions are altered. This consequently can diminish cave organisms and biodiversity (Culver 1982, Biswas 2010).

Although the fragility and vulnerability of subterranean ecosystems are widely acknowledged, the impacts of human disturbances on cave ecosystems are poorly understood when compared with surface ecosystems (Dickson 1979, Wood et al. 2008).

There is limited data available that summarizes the magnitude and possible lasting effect of anthropogenic disturbances on subterranean environments, despite the increase of disturbances (Hancock 2002, Wood et al. 2008). This is unfortunate because subterranean ecosystems may be more sensitive than surface ecosystems, due to their relatively short food-chains and low diversity of food resources (Marmonier 1993, Taylor & Webb 2008). Subterranean and surface ecosystems can also react differently, even if affected by the same disturbance, because of the responses and the adaptations of organisms to resist disturbances are different in the two ecosystem types (Wood et al. 2008). Wood et al. (2008) also concluded that the degree of the initial disturbance effects, and recovery time after a disturbance, are just two of the many factors that can differ between subterranean and surface ecosystems. Lastly, subterranean ecosystems are monitored less frequently than surface ecosystems and therefore the long term effects of disturbances can go unnoticed (Wood et al. 2008). The combination of these characteristics of subterranean or cave ecosystems potentially make them some of the most threatened types of ecosystems.

The Edwards Plateau eco-region and its associated aquifers in central Texas has been recognized as a biodiversity hotspot for troglobitic organisms, and it ranks first in the US in diversity in terms of stygian (aquatic cave-adapted) organisms (Culver 2000). The karst aquifers of the Edwards Plateau are the source of thousands of springs (Brune 1981) and are also home to approximately 90 endemic animal species, including subterranean and surface-dwelling invertebrates, salamanders, and several fish species (Bowles & Arsuffi 1993). Further investigations on the distribution and range of these species and their sensitivity to disturbances are required in order to assess the full effect of urban disturbances upon them. This ecoregion, however, is experiencing widespread

challenges from increased anthropogenic disturbances. Urbanization is currently recognized as one of the major threats to the Edwards Plateau aquifer region and the expansion of urban areas has numerous potential effects on the surrounding karst and cave ecosystems. Urbanization can affect local cave ecosystems negatively through other factors such as the decrease of water quality and quantity, general habitat pollution and increase of erosion (TPWD 2005). Conservation of these subterranean ecosystems is only possible through our understanding of their ecosystem functioning and community structure.

Nevertheless, not all cave ecosystems are energy deprived. Some cave ecosystems rely on allochthonous energy inputs from cricket and colonial bat guano (Fenolio et al. 2006, Ferreira et al. 2007). Caves of this type, including Bracken Bat Cave, the focus of this study, can support large populations of bats or crickets and therefore receive a large quantity of allochthonous energy in the form of guano. The guano, in turn, acts as the resource base of ecosystem food webs and support relatively large populations of invertebrates, fungi, bacteria and other microorganisms (Ferreira et al. 2007). Ferreira et al. (2007) for example, found that cave organisms had specifically adapted to a reliable resource of bat guano in a Brazilian cave and had become dependent upon the microenvironment and food resource provided by the guano. Due to this direct dependence of cave organisms upon guano, and the uniqueness of these types of cave ecosystems, food web patterns of high energy caves can be as unique as their circumstances. Additionally, caves with high quantities of guano have historically experienced anthropogenic disturbance in the form of guano removal. The use of guano as fertilizer or to extract saltpeter has commonly occurred in the south-central area of

Texas for centuries (Hutchinson 1950). More specifically, guano has been historically removed for commercial purposes from Bracken Bat Cave on a bi-yearly since the early 1990s (Hutchins *pers. conv.).* However, there has been limited research conducted on these types of caves and the potential disturbances that may affect them. Thus the goals of this study are to investigate the food web structure and dynamics of invertebrates in Bracken Bat Cave by testing the following hypotheses: 1) Cave invertebrate abundance and richness will fluctuate with seasonality as guano quantity varies due to the changes in bat population densities. There is a significant difference in population densities between the summer-roosting bat populations and the overwintering bat populations of Bracken Bat Cave. 2) Cave invertebrate abundance and richness will vary with distance from entrances and depth within the guano substrate, as both of these factors can modify physical habitat characteristics or create a gradient of abiotic conditions. This gradient in abiotic conditions will thus create different niches for organisms and/or create a gradient of abundance of macroinvertebrates. Cave entrances also provide an opportunity for surface organisms to enter the cave, either accidentally or intentionally, and this may affect macroinvertebrate community assemblages in the cave. 3) Cave invertebrate abundance and richness will be significantly affected by anthropogenic disturbances such as commercial guano removal. We predict that the removal of guano will reduce the amount of available subsidy and thus negatively affect macroinvertebrate abundance and richness. 4) Nutrient quality (carbon: nitrogen: phosphorous ratios) of guano will vary with respect to seasonality and guano depth. We predict that changes in micro-climate conditions of the cave due to seasonality and the age of the guano will translate to changes in the guano nutrient content. Consequently we predict that guano with higher

nutrient content will positively correlate with macroinvertebrate abundance and richness and. 5) Carbon stable isotope ratios will not significantly differ between the guano and cave macroinvertebrates because we predict that this ecosystem guano is the base of the food web, and thus succeeding trophic levels should retain the same carbon stable isotope signature as the guano. This is because the ratio of the carbon isotopes should not change significantly as carbon moves through the food web (Roudnick & Winterbourn 1986, Peterson & Fry 1987, Post 2002). On the other hand, we expect that the nitrogen stable isotope ratios will change significantly between the guano and cave macroinvertebrates, signifying the various trophic levels of the food web. Research shows that an enrichment by 3-4‰ of nitrogen stable isotopes occurs with each increasing trophic level (DeNiro & Epstein 1981, Peterson & Fry 1987, Post 2002).

CHAPTER II

METHODS

This study was comprised of two parts spanning a two year period. The goals for the first part of the project were to describe macroinvertebrate species composition in Bracken Bat Cave and understand the factors that drive their population density variations. We used macroinvertebrate abundance and richness as indices of the macroinvertebrate population compositions. Abundance was defined as the total number of macroinvertebrates per sample and richness was defined as the total number of different taxa per sample. The second part of the project examined the effects of guano quality or nutrient content on cave organisms. The second chapter of this study also used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in order to understand trophic positions and food web interactions of the main invertebrate species at Bracken Bat Cave.

Study Site

Among the caves in the Edwards Plateau is Bracken Bat Cave, located near Garden Ridge, Texas. This cave provides a seasonal habitat for an estimated 20 million bats, mainly Mexican Free-tailed bats (*Tadarida brasiliensis*). Bracken Bat Cave is recognized as the cave that hosts the largest known population of bats in the world (Bat Conservation International 2009). Large chambers and low water availability make Bracken Bat Cave an ideal habitat and hibernacula for the bats (Glover & Altringham 2008). These large bat populations occupy the cave for 9 months each year and consume large numbers of airborne insects across a variety of landscapes from limestone hills to agriculture lands (McWilliams 2005, Bat Conservation International 2009).

As a result of the large consumption of insects, the Mexican Free-tailed bats (*Tadarida brasiliensis*) in Bracken Bat Cave also provide a large amount of guano to the cave, supporting the food web within this cave ecosystem (Bat Conservation International 2009). An unusually high abundance of invertebrates cover the cave floor at varying densities throughout the cave. The high quantity of guano has also created a unique but harsh environment for any non-cave adapted organisms. High levels of carbon dioxide, ammonia, and guano particulates in the air have created a toxic environment for non-adapted cave organisms. The combination of these unique biotic and abiotic conditions in this ecosystem, make Bracken Bat Cave a natural phenomenon that is ideal for study and exploration.

Guano Deposit Survey (September 2009-August 2010)

Guano is the base food-resource of the food web in Bracken Bat Cave. For this reason, determining the quantity of the guano that is being deposited is essential to understanding this ecosystem. Six raised plastic containers (0.34 m × 0.21 m × 0.12 m), two per zone, were randomly placed in the cave and collected each month (September 2009-August 2010). Therefore we assessed the quantity and seasonal variation of the guano being deposited by performing the monthly guano collections. I then dried the guano at 60º C for 72 hours and weighed each sample to the nearest 0.1 g.

Macroinvertebrate Abundance and Richness Dynamics

To monitor macroinvertebrate population differences due to proximity from a cave entrances, the cave was divided into three regions of relatively equal area at increasing distance from the natural entrance: entrance zone, twilight zone, and dark zone. However, a smaller, artificial ceiling entrance also exists in the third zone of the

cave (Figure 1). Each region was approximately 45 m in length. Regions were used for analyzing richness and abundance of invertebrate taxa encountered with respect to distance from the entrances. I collected three core samples from each region on a monthly basis for 5 months (August-December 2009). This was accomplished by randomly inserting a PVC pipe (0.08m diameter × 0.91m height) of 0.08 m diameter into the cave floor and determining the taxa richness and abundance of organisms present between the surface and 0.91 meters of guano depth. The random locations were selected by dividing the cave map into relatively equal area quadrants, numbering the quadrants per each cave zone and then generating random numbers that corresponded to the numbered quadrants.

The method for extracting stratified guano for this study is one that was used by Altenbach and Petit (1972) in order to study the effects of pollution and heavy metals on the environment through studying yearly guano deposits. The methodology involved inserting a pipe into the guano, digging a trench around it in order to place a stopper at the bottom of the pipe and then extracting the pipe (Figure 2). This method was modified slightly by using all plastic seals and dividers to contain the guano, rather than paraffin or cardboard seals which were originally used in this method. More specifically, I inserted 3 plastic dividers into the PVC pipe in order to divide the guano into four layers and prevent guano and organism displacement during the extraction. Each divider was placed through the pipe at 0.15 m increments. The division of the layers and length of the pipe used was determined through preliminary data collection, where 0.91 meter depth of guano was examined using the same pipe-trench method described above. Preliminary data collection illustrated that guano layers were significantly different in texture and color approximately every 0.15 m in depth. These layers represent yearly deposits of

guano (Wurster et al. 2007). Consequently, the guano mixture was consistently darker and soil-like after approximately 0.69 m. For this reason I decided to examine the first four apparently distinctive layers of guano (0.15 m each) to see if differences in guano layer composition yield differences in invertebrate distributions.

January-March 2010, 47 tons of guano was removed from all areas of the cave for commercial purposes by "Gardenville Nursery Fertilizer". Due to the relatively large scale of this disturbance, in December 2010 we ceased sampling using the aforementioned method of guano core sampling. Instead, we continued to core sample only the top layer of the guano, up until 0.15 m of depth, in order to monitor macroinvertebrate abundance and richness after the disturbance. We accomplished this by comparing the top layers of guano sampling extracted August-December 2009 to the top layers of guano extracted after the disturbance, March-May 2010.

The samples contained within each compartment of the core samples were placed into zippered plastic bags until they arrived in the lab. Upon arrival in the lab, samples were placed in the freezer to halt the biological activity and decomposition until the processing of the samples. Macroinvertebrates were separated from the guano mixture using a 1 mm sieve, picked and placed in 70% ethanol for preservation and later identified to the lowest taxonomic level possible (typically family or genus level). In other words, organisms smaller than 1 mm were ignored during this type of sampling.

In addition to core sampling, we also employed pitfall trap sampling to measure macroinvertebrate abundance and richness. The purpose of the pitfall traps was to account for all sizes of macroinvertebrates because species smaller than 1mm were ignored during core sampling. Furthermore, certain macroinvertebrate species might not

be detected during core sample surveying since the core samples focus on collecting only ground-dwelling organisms from relatively portions of guano, when compared to the entire quantity of guano found in the cave. In other words, the pit fall traps were used to supplement the invertebrate core-sampling method and ensure that our samples contained a more comprehensive representation of the cave organisms. Three pitfall traps (6 cm diameter) were installed in each zone of the cave for 48 hours every 3 month period (November 2009, April 2010, July 2010).The pitfall trap containers were filled with 50% water and 50% ethanol (70%). Ethanol has been shown to be an effective pitfall trap preservative; however we mixed it with water to reduce evaporation (Aristophanous 2010). Afterwards, pitfall trap specimens were preserved in 70% ethanol and identified to the lowest possible taxonomic level. During macroinvertebrate identification, the samples were sub-sampled to ¼ for macroinvertebrates larger than 1 mm and 1/12 for macroinvertebrates smaller than 1 mm. Sub-sampling was conducted because of the large number of macroinvertebrates contained in each pitfall trap sample.

Carbon, Nitrogen and Phosphorous Nutrient Content in Guano, Bat Hair and Macroinvertebrates

Guano collected during core sampling were examined with respect to nutrient content. To accomplish this, 36 samples (9 replicates × 2 seasons (summer vs. winter differences in bat populations) × 2 guano depth layers (top vs. bottom) were analyzed in order to determine the content of carbon, nitrogen, and phosphorous in guano samples from different depths and seasons. Preliminary data analyses of macroinvertebrate densities suggested that macroinvertebrates found in core samples are not affected by the proximity of distance from entrance. Therefore we did not determine quality with respect

to this variable. In addition to determining guano nutrient quality, I also analyzed the nutrient quality of macroinvertebrates (10 replicates) and bat hair (10 replicates) to further examine the cave nutrient cycles.

To determine carbon and nitrogen content, samples were processed using the Flash EA 1112 Elemental analyzer (Thermo Fisher Scientific 2011). Samples were analyzed for phosphorous content by the Soil Testing Services at the University of Missouri. The samples were dry-ashed using an adaptation of AOAC 985.01 (Isaac 2009) and phosphorous content was determined through inductively coupled plasma-atomic emission spectroscopy (US EPA 1992).

Stable Isotope Analysis

In addition to determining the types, abundances, and distribution of macroinvertebrates found in Bracken Bat Cave, stable isotope analysis was conducted to determine the trophic positions and to confirm that guano is the base of the food web of this ecosystem. For this reason, guano, bat hair, and different taxa of macroinvertebrates were analyzed for ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Carbon isotope analysis can determine if guano is the base of the food web in the cave ecosystem. If all analyzed samples display similar values of carbon isotopes as the guano, then this is evidence that guano is the carbon source. This is because the ratio of the carbon isotopes should not change significantly as carbon moves through the food web (Roudnick & Winterbourn 1986, Peterson & Fry 1987, Post 2002). Nitrogen fractionation, on the other hand, can determine the trophic position of the analyzed macroinvertebrate taxa. The ratio of nitrogen isotopes is typically enriched by 3-4‰ with each increasing trophic level (DeNiro & Epstein 1981, Peterson & Fry 1987, Post 2002).

The samples analyzed included 36 bat guano (2 seasons × 2 guano layers × 9 replicates), 3 bat hair samples, and samples of them most abundance taxa or invertebrates found in the cave, which included 9 Dermestidae adult , 11 Dermestidae larvae, 12 Tenebrionidae adult, 12 Tenebrionidae larvae, and 8 mite samples. These samples were processed in the Stable Isotope Facility located in the University of California at Davis. This laboratory analyzes solid materials for $\delta^{13}C$ and $\delta^{15}N$ isotopes using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. The obtained delta C and N values are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively (UC Stable Isotope Facility 2010).

Statistical Analyses

An unbalanced ANOVA was used to estimate if there are any significant differences between the guano tray deposits, where weight of the samples was the response variable and distance from the entrances and seasonality were the two predicting factors. To investigate the variability from the core samples data with respect to distance from the entrances or cave zone, guano depth, and seasonality, we employed two linear mixed-effects (LME) models. Seasonality was the random predictor variable, while cave zone and guano depth were the fixed predictor variables in the models. The guano depth data was organized to compare the two top layers of guano with the two bottom layers of guano. Macroinvertebrate abundance was the response variable in one of these regression models, and macroinvertebrate richness was the response variable in the other model. The abundance data was $\log_{10}(n+1)$ transformed in order to meet the normality and homoscedasticity assumptions. Core samples collected after the commercial guano

removal were analyzed using 2 unbalanced ANOVAs. Macroinvertebrate abundance and richness were the response variables of these ANOVAs, while seasonality and cave zone were the predicting factors for both ANOVAs. Since only the top layers of guano were collected post-guano removal, only top layers of pre-guano removal core samples were used in this analysis in order to have an unbiased comparison. The pitfall traps were analyzed in the same manner, using unbalanced ANOVAs. The macroinvertebrate abundance data from these samples was natural log (ln) transformed in order to achieve normality and homoscedasticity.

In addition, we used two-way balanced ANOVAs to compare the carbon, nitrogen and phosphorous content in the samples. We used one ANOVA for each nutrient (carbon, nitrogen and phosphorous) where seasonality and guano depth were used as factors for each of the tests. We also used unbalanced ANOVAs to determine if there are significant differences in the nutrient content (carbon, nitrogen and phosphorous) of the guano, hair and macroinvertebrate samples, while also taking into consideration seasonality.

In order to compare the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes ratios between the guano and various types of macroinvertebrates, we used LME models to determine if there are any differences between the different types of samples. Sample type was the random predictor variable, while seasonality and guano depth were the fixed predictor variables. We also created a bi-plot where carbon ($\delta^{13}C$) values were listed in the x-axis and nitrogen ($\delta^{15}N$) values were listed in the y-axis for each sample, in order to visually assess the trophic positions of the analyzed macroinvertebrates. Lastly, unbalanced ANOVAs were conducted to compare the differences in stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) with respect to life stages (larvae vs. adult) and species. All the aforementioned

analyses were performed using the statistical program R (R Development Core Team 2005).

CHAPTER IV

RESULTS

Guano Deposit Comparison

Dry mass of the guano deposition samples varied significantly with seasonality ($F_{1,27}$ = 16.87, P< 0.001*) (Fig. 7), but did not differ with respect to distance from the entrances, when comparing the three cave zones ($F_{2,27}$ = 2.68, P= 0.09) . There was also a significant interaction between the two factors ($F_{2,27}$ = 4.61, P= 0.02*).

September 2009 displayed the highest amount of guano deposition of (Mean+/-SE) 3.68 +/- 0.20kg/m^2, compared to the yearly average of 1.49 +/- 0.32 (Mean+/-SE) kg/m^2. When taking the total area estimate of Bracken Bat Cave into account (3078 m^2), we estimated that the amount of guano deposition into the cave per month ranged from 11,336.81 +/- 636.59 kg (September) – 65.95 +/- 49.45 kg (January-March). The average guano deposition in Bracken Bat cave was approximated to be 4,210.14 +/-988.96 kg/month and the total estimated guano deposition in the cave was 50,521.72 +/-11,867.51 kg in August 2009-August 2010.

Macroinvertebrate Population Trends

In the 9 months of collecting core samples at Bracken Bat Cave, 4510 macroinvertebrates were collected and identified. These invertebrates were classified into 4 orders (Coleoptera, Pseudoscorpionida, Dermaptera, Hymenoptera), and 7 families (Table 1). Coleoptera was by far the most abundant order since it contained 99.27 % of the individuals observed belonging to this order. The most abundant families of

Coleoptera were by far the Dermestidae and Tenebrionidae families, representing 98.55 % of the total number of invertebrates observed in core samples.

Linear mixed-effect (LME) model analyses revealed that monthly seasonality only explained 28% of the variation in macroinvertebrate abundance and 2.4% of the variation in macroinvertebrate richness from core samples, collected August-December 2009. The highest abundance (Mean+/-SE= 59.0+/-15.08) and richness (Mean+/-SE= 2.22+/-0.09) were encountered during the month of September, while the months of August, October, November and December displayed similar values in macroinvertebrate abundance and richness (Fig. 3). In addition, distance from entrances or cave zone did not significantly affect macroinvertebrate abundance ($F_{1, 115}$= 1.66, P= 0.19) and richness ($F_{1,117}$= 0.17, P= 0.67). Zone 2 or the middle of the cave displayed the highest abundance (Mean+/-SE= 30.51+/-0.78) and richness (Mean+/-SE= 1.82+/-0.02), however this was not significantly higher than zone 1 and 3. Lastly, guano depth did show a significant correlation with abundance ($F_{1, 117}$= 8.56, P= 0.004*) but not richness ($F_{1, 117}$= 3.46, P= 0.07) (Fig. 4). The top two layers of guano displayed significantly higher abundance (Mean+/-SE= 22.54+/-0.46) when compared to the bottom two layers of guano (Mean+/-SE= 14.73+/-0.65). However, richness was slightly but not significantly higher in the top layers of guano (Mean+/-SE= 1.80+/-0.01) vs. the bottom layers of guano (Mean+/-SE= 1.57+/-0.02).

On the other hand, the data analysis shows that abundance ($F_{1,63}$= 6.87, P= 0.01*) and richness ($F_{1,63}$ = 6.81, P= 0.01*) were significantly higher after the guano removal (Fig. 5). Sample abundance prior to the guano removal was (Mean+/-SE) 23.16+/-4.32 and (Mean+/-SE) 76.27+/-27.02 after the removal. On the other hand, sample richness

prior to the removal was (Mean+/-SE) 1.89+/-0.12 and (Mean+/-SE) 2.42+/-0.47 after the removal. This increase in species richness does not indicate that new species were observed post-removal, but rather that mean richness per sample increased after the guano removal.

The abundance of organisms found during pitfall trap sampling was insignificantly correlated to seasonality ($F_{2,13}$= 3.17, P= 0.07) and cave zones ($F_{2,13}$= 3.16, P= 0.07). The highest abundance of macroinvertebrates was collected during the month of July (Mean+/-SE= 56722.75 +/- 27137.42), compared to the average seasonality abundance (Mean+/-SE= 29451.32 +/- 14658.76). In addition, the highest abundance of macroinvertebrates was collected in zone 3 (Mean+/-SE= 55069.63+/- 27416.79), compared to the average zone abundance (Mean+/-SE= 32003.61+/- 16745.21). In this data analysis we also observed a significant interaction between cave zone and seasonality ($F_{4,13}$= 4.17, P= 0.02*).

On the other hand, we found a significant correlation between organism richness and cave zones ($F_{1,13}$= 8.91, P= 0.004*) (Fig. 6), but not seasonality ($F_{1,13}$= 3.48, P= 0.06). Zone 2 (Mean+/-SE= 4.75 +/- 0.37), or the middle zone of the cave, displayed significantly lower macroinvertebrate richness than zone 1 (Mean+/-SE= 6.50 +/- 0.56) and zone 3 (Mean+/-SE= 6.63 +/- 0.53). Additionally, the highest macroinvertebrate richness was observed in April 2010 (Mean+/-SE= 6.40 +/- 0.98), compared to the seasonal average (Mean+/-SE= 5.97 +/- 0.64). A significant interaction was also observed between cave zone and seasonality in this analysis ($F_{1,13}$= 3.89, P= 0.03*).

Guano Nutrient Content and Nutrient Flow in Cave Ecosystem

Carbon concentrations (moles/g) significantly differed between layers of guano depth ($F_{1,32}$= 7.04, P= 0.01*) but did not differ with seasonality ($F_{1,32}$= 1.40, P= 0.25). Moles of carbon/g were significantly higher in the top layer of guano (Mean+/-SE= 0.0348+/-8.19x10^{-5} mol/g) versus the bottom layer (Mean+/-SE= 0.031+/-3.27x10^{-4}). The mean overall carbon content found in the samples was 0.033+/-0.0008 mol/g. On the other hand, nitrogen (moles) did not differ significantly differ with respect to guano depth layers ($F_{1,32}$= 0.02, P= 0.9) and seasonality ($F_{1,32}$= 2.41, P= 0.13). The mean nitrogen content estimated from the samples was 0.0086+/-0.0002 mol/g. Similarly, phosphorous (moles) did not significantly differ with respect to guano depth layers ($F_{1,32}$= 0.12, P= 0.74) or seasonality ($F_{1,32}$= 1.33, P= 0.26) (Fig. 8). The mean phosphorous content estimated from the samples was 0.00053+/-2.57x10^{-5} mol/g. Additionally, we also analyzed samples of guano, bat hair, and macroinvertebrates in order to compare carbon: nitrogen(C: N), carbon: phosphorous(C: P), and nitrogen: phosphorous (N: P) ratios between these different types of samples, in order to quntify the nutrient values of the biotic components in Bracken Bat Cave. The ANOVA analysis conducted for this comparison revealed that there were no significant differences due to the type of sample in C: N ($F_{1,30}$= 0.0001, P= 0.99), C: P ($F_{1,30}$= 0.16, P= 0.69) or N: P ($F_{1,30}$= 0.07, P= 0.79) ratios.

Stable Isotope Analyses

Overall we also observed a relatively large variation in the δ^{13}C (-24.08 to -17.43 ‰) and δ^{15}N (9.32 to 17.43 ‰) signatures of guano. Fig. 10 shows this variation while also comparing the variation in stable isotope signatures of guano with respect to guano depth. The linear mixed effects (LME) analysis, which analyzed how carbon isotope ratios

($\delta^{13}C$) were correlated with the type of sample (guano, hair, species and life stage of organisms), seasonality and guano depth, revealed that there was a large variation in $\delta^{13}C$ with respect to the type of sample (Fig. 9). This variation accounted for 42.08 % of the total variance in the samples. ^{13}C δ signatures differed with species ($F_{1,40}$= 19.26, P= 8.093e-05*) and life stage ($F_{1,40}$ =5.02, P=0.03*). Additionally, there was not a significant correlation between $\delta^{13}C$ and seasonality ($F_{1,77}$= 1.74, P= 0.19) or guano depth ($F_{1,77}$= 0.04, P= 0.84).

The LME analysis used to analyze how nitrogen isotope ratios ($\delta^{15}N$) were correlated with the type of sample (guano, hair or species and life stage of organisms), seasonality, and guano depth, suggested that there was a substantial variation in $\delta^{15}N$ between different types of samples (Fig. 9). This variation accounted for 35.67 % of the total variance of this analysis. We particularly noted that guano was evenly distributed throughout the nitrogen stable isotope range, while the species of macroinvertebrates analyzed seem to be separated into two distinct groups. Mites comprise one of the groups and the other macroinvertebrate species (*Dermestes sp.* and *Alphitobius sp.)* comprise the other. These two groups are separated on average by about 3.5 ‰ with respect to nitrogen stable isotope ratios. ^{15}N differed with species ($F_{1,40}$ = 18.26, P= 0.0001 *) but not life stage ($F_{1,40}$ = 2.85, P=0.10). In addition, the depth of guano significantly affected $\delta^{15}N$ ($F_{1,77}$= 2.21, P= 0.03*), while seasonality did not have the same effect ($F_{1,77}$= 0.53, P= 0.60). More specifically, top layers of guano had a lower $\delta^{15}N$ signature (Mean+/-SE= 13.16+/-0.55 ‰) than the bottom layers of guano (Mean+/-SE= 14.46+/-0.59 ‰) suggesting that the ratio of the $\delta^{15}N$ to $\delta^{13}N$ isotopes was higher in the lower layers of guano

CHAPTER V

DISCUSSION

The effects of guano subsidy input on macroinvertebrate abundance and richness with respect to seasonality, distance from the entrances and guano depth

Communities in caves are profoundly impacted by the resource subsidies that they receive. The types of resources that enter caves vary in terms of regularity, duration and usability (Schneider et al. in press). The most prevalent resource input to caves are decaying leaf and woody debris that has fallen or washed into caves, and animal carcasses that fall into pit entrances or get lost within the cave (Culver 1982, Poulson 2005, Schneider et al. in press). However, fecal matter deposited by crickets and bats also represents an important, nutrient-rich energy resource for oligotrophic cave ecosystems (Poulson 2005, Ferreira et al. 2007, Fagan et al. 2007). Our results indicate that the conditions created by the deposition of bat guano significantly affected the macroinvertebrate abundance and richness of Bracken Bat Cave. More specifically, we found that macroinvertebrate abundance differed with respect to the depth within the guano substrate. Results from our pitfall trap sampling also indicated that macroinvertebrate richness was significantly correlated with the distance of the cave areas from the entrances. However, our findings indicate that seasonality or monthly variations do not have a significant effect on macroinvertebrate abundance and richness.

The Bracken Bat Cave ecosystem is defined by the amount of guano subsidy input. Guano input is significantly varied with seasonality (due to fluctuations in bat population densities) as demonstrated by our guano deposit samples. Nevertheless, these changes do not seem to translate into changes in macroinvertebrate population abundance and richness, as indicated by our guano core sampling and pitfall trap sampling. This is contrary to other related studies that have shown that cave organisms will respond to the fluctuations of nutrient supplies delivered to the cave (Humphreys 1991, Poulson 2005). However, the reason behind our findings could be due to the fact that there is a constant and plentiful amount of guano available throughout the year. Bracken Bat Cave is inhabited by the largest bat population in the world (*Tadarida brasiliensis*) during March-October and, in addition, is occupied by overwintering populations of northern bat colonies for the remainder of the year (Bat Conservation International 2009). Even though the densities of the summer vs. wintering populations of bats vary, there is still a continuous supply of guano in the cave throughout the year and in some places the guano is 20 meters deep (Hutchins *pers. interview* 2011). In addition, our nutrient analyses demonstrated that guano contents of carbon, nitrogen and phosphorous do not differ with respect to seasonality. Therefore, the lack of fluctuations in macroinvertebrate abundance and richness with respect to seasonality can be explained by large quantity and relatively equal quality of guano deposited and found in the cave, as indicated by the nutrient analyses of guano.

My study also shows that the rates of guano distribution are relatively even throughout the cave. I believe that this is the reason why we did not see significant differences in the guano core sample macroinvertebrate abundance and richness, when

comparing the three cave zones. However, I did observe a significant difference in macroinvertebrate richness, but not abundance, from the pitfall trap samples when comparing the three cave zones. This difference could be attributed to the fact that the two cave zones with the highest macroinvertebrate richness were near entrances, and thus had a higher chance of containing organisms which had entered the cave via the two entrances. These findings are in agreement with other studies that have examined macroinvertebrate richness with respect to the distance from the entrance (Ferreira and Martins 1998, Ferreira et al. 2007). For example, Ferreira et al. (2007) observed a reduction in diversity with increasing distance from the cave entrances in a Brazilian cave that is governed by bat guano subsidy inputs, similar to Bracken Bat Cave.

It is not surprising that this difference in macroinvertebrate richness was detected from pitfall trap sampling but not guano core sampling. Pitfall trap sampling allow for a larger time frame, and presumably larger cave area of sampling. Therefore, there would be a higher probability of capturing rare organisms through pitfall trap sampling rather than guano core sampling. Because of processing methods, guano core sampling also discriminated against smaller invertebrates (less than 1 mm), which could also explain why differences in macroinvertebrate richness may have not been detected in these types of samples. Furthermore, we observed significant interactions between the seasonality and cave zone factors when examining the rate of guano deposition and when analyzing how macroinvertebrate abundance and richness varies in the pitfall traps. This suggests that the rate of deposition may not be distributed equally in all cave zones in all the examined months, and this may explain the interactions observed from the pitfall traps if macroinvertebrates respond to the guano deposition.

Lastly, analysis of guano core samples indicated that there was a significant difference in macroinvertebrate abundance but not richness when comparing the different layers of guano depth. This was expected because abiotic conditions such as oxygen concentrations, moisture, temperature (Sanders 1981), and biotic conditions such as microbial activities vary with soil depth (Feirer et al. 2003). Thus I expected the abundance of macroinvertebrates to vary as well. My results are also supported by the nutrient analyses, which indicated that guano in the top layers had significantly higher levels of carbon, but not nitrogen and phosphorous, when compared to the bottom layers of guano. In addition, perhaps greater differences in macroinvertebrate distribution with respect to soil depth may be detected if a higher gradient of guano depth was analyzed. My study focused on less than 1 m of guano depth, however it would be interesting to monitor macroinvertebrate community variations that might occur across even greater guano depths.

In summary, we presume that the abundant quantity, equal-area distribution, and equal nutrient composition of guano (with respect to cave seasonality) have created a fairly stable ecosystem in Bracken Bat Cave in terms of resource availability. This ecosystem thus experiences relatively few fluctuations in macroinvertebrate abundance and richness with respect to seasonality, the guano depth we examined, and distances from the cave entrances. However, there is some evidence from pitfall trap sampling that suggests that cave entrances may play in role in the introduction of macroinvertebrates to the cave ecosystem.

<u>The effects of guano removal or disturbance on macroinvertebrate abundance and richness</u>

Disturbance has been shown to critically affect biodiversity (Huston 1994, Hooper et al. 2005, Banitz et al. 2008). The disturbance type, temporal and spatial variability, duration, and boundary of the disturbance are some characteristics of disturbance that can influence the response of an ecosystem to a disturbance (Loehle 2000, Ohsawa et al. 2002, Crawley 2004, McIntire 2004, Banitz 2008). In addition to the disturbance regime, the characteristics of the ecosystem and the type of species that inhabit it also shape the response of that ecosystem to a disturbance (Wood et al. 2008, Banitz et al. 2008).

The removal of the guano from Bracken Bat Cave represents a chronic and considerable disturbance. The guano is generally removed bi-yearly and sold as fertilizer. Our findings indicate that abundance and richness of macroinvertebrates per sample significantly increased after the guano removal (Fig. 5). These increases could have resulted because of the guano removal. We speculate the reduction of abundant macroinvertebrate populations could have led to a rise in the populations of less abundant macroinvertebrates. However, these increases in abundance and richness after the disturbance may also reflect seasonal variations in the macroinvertebrate populations and their responses to guano subsidy inputs. This is because samples collected after the guano removal coincided with the re-occupation of the cave by the Mexican free-tailed bat populations and thus a higher deposition rate of guano. Therefore, additional sampling would need to be completed before and after yearly guano removals in order to further support our speculation that fluctuations in macroinvertebrate populations occur due to disturbance effects.

Stable Isotopes: Determining food web structure and function

The stable isotope analysis indicates that there was a wide range of carbon isotope signatures in the guano samples (Fig. 9). This can be explained by the fact that Mexican Free-tailed bats (*Tadarida brasiliensis*), the predominant bat species of Bracken Bat Cave, has been documented to have a very diverse diet (Whitaker et al. 1996, Lee & McCracken 2005, McWilliams 2005). In fact, Lee and McCracken (2005) documented the highest diet diversity of any bat species in *T. brasiliensis* while sampling various Texas caves (including Bracken Bat Cave). This high dietary variation can be accounted for by 1) the large foraging dispersal of this species, which may cover over 400 km^2 (Williams et al. 1973) and can occur from a few meters to over 1,200 meters above the ground (Griffin & Thompson 1982, Caire et al. 1984, McCracken et al. 1997); 2) the large temporal duration of their foraging activity, which extends from late afternoon until past dawn and thus the bats encounter both diurnal and nocturnal insects (Lee and McCracken 2001). In other words, our results (supported by the aforementioned studies) suggest that *T. brasiliensis* exhibits high dietary variability. This large variability in diet may translate in a higher probability of the bats feeding from a variety of carbon pools and result in the high range of carbon isotope signatures documented in the guano samples. This large variation in turn leads to a high range of carbon isotope signatures in the macroinvertebrate species we sampled, given that we presume that guano is the base of the Bracken Bat Cave food web. This is because carbon stable isotope ratios do not significantly fractionate with increasing trophic levels (Roudnick & Winterbourn 1986, Peterson & Fry 1987, Post 2002).The bat hair samples analyzed also fall within the range of the guano carbon isotope signatures. However only 3 sample of bat hair were analyzed and therefore we cannot assess if bat hair would have the same range of variability as the

guano. In addition, we did not find a significant variation in carbon isotope signatures with respect to seasonality or guano depth, which suggests that the stable isotope variability in the guano is fairly constant through the months and years, given that guano depth represents guano deposits over the years.

Nitrogen isotope signatures also exhibited a high variation with respect to the type of sample we assessed (guano, bat hair and macroinvertebrates) (Fig. 9). The variability in the nitrogen isotope signatures of the guano samples was expected due to the high variability in the diets of Mexican Free-tailed bats (*Tadarida brasiliensis*) (Lee & McCracken 2005, McWilliams 2005). Similarly, the variability in nitrogen isotope signatures between the guano and the species of macroinvertebrates samples was also expected because it reflects the trophic levels of this ecosystem (DeNiro & Epstein 1981, Peterson & Fry 1987, Post 2002). While guano samples were distributed throughout the range of nitrogen isotope values we encountered, it seems that there is a distinct trophic level separation between the mite species versus the *Dermestes* sp. and *Alphitobius* sp. My findings indicate that mites occupy a lower trophic level than the *Dermestes* sp. and *Alphitobius* sp., which seem to occupy similar trophic levels in all life stages (adult vs. larvae).

In addition, nitrogen stable isotope samples from the top layers of guano contained a larger proportion of the light isotope than the bottom layers of guano (Fig. 10). This is because the ammonification or evaporation of ammonia (NH_3) from the guano favors the isotopically lighter nitrogenous compounds (Nadelhoffer & Fry 1994). Thus the bottom layers of guano, which have experience ammonification for longer periods of time, exhibited heavier isotope ratios. Lastly, nitrogen isotope samples did not

vary with respect to seasonality. This was expected because nitrogen isotope signatures reflect the trophic position of species and thus we did not expect the trophic positions to vary with seasonality.

Conclusions and Conservation Implications

This study sought to provide a description of the community and ecosystem dynamics of an understudied ecosystem: Bracken Bat Cave. Even though other studies have focused on researching the bat populations of Bracken Bat Cave, this was the first study to focus on the macroinvertebrate community of this cave, and started to examine the abiotic and biotic fluxes of this ecosystem as a whole. More specifically, this study aimed to determine how physical characteristics of the ecosystem (distance from entrance and guano depth), seasonality, and disturbances affect the macroinvertebrate community at Bracken Bat Cave, while also examining the nutrient and stable isotope characteristics of this system. My findings indicate that the macroinvertebrate communities of Bracken Bat Cave do not experience large fluxes in abundance or richness with respect to seasonality but fluctuate with respect to distance from the cave entrances and guano depth. In addition, this study implies that the macroinvertebrate community is resistant towards disturbances, as our monitoring after the guano removal indicates that the macroinvertebrate community was not negatively affected by this disturbance. Additionally, guano nutrient properties remain constant with respect to seasonality and guano depth, with the exception of carbon, which decreased with guano depth. Finally, the stable isotope analysis suggested that guano has a high range of variability in carbon and nitrogen stable isotope ratios. The variability in carbon stable isotope ratios is transmitted to the macroinvertebrates occupying the ecosystem and consuming the guano

because carbon fractionation is negligible with increasing trophic levels (Roudnick & Winterbourn 1986, Peterson & Fry 1987, Post 2002). On the other hand, the variability in nitrogen stable isotope ratios helped us analyze the trophic levels and dynamics of this ecosystem because nitrogen isotopes are typically enriched by 3-4 ‰ with each increasing trophic level (DeNiro & Epstein 1981, Peterson & Fry 1987, Post 2002). This study thus represents a preliminary but comprehensive view of the Bracken Bat Cave ecosystem. The information gained from this study can improve our understanding of the Bracken Bat Cave ecosystem and aid managers in developing management plans to conserve this unique ecosystem. The conservation of the Bracken Bat Cave ecosystem and its bat population can also have notable economical implications for the surrounding area. Large populations of Mexican-free tailed bats play an important role in controlling agricultural pest of south-central Texas (Cleveland et al. 2006, Federico et al. 2008). Cleveland et al. (2006) estimated that pest-control services provided by Mexican free tailed bats have an average to an annual value of $741,000 per year, with a range of $121,000-$1, 725, 000 per year in the south-central Texas area.

Lastly, our understanding of Bracken Bat Cave can also aid in the understanding other cave ecosystems, which can lead to their conservation. Concern over cave ecosystems and the cave-limited species that inhabit them has escalated over the last two decades (Schneider et al. in press). This concern has especially heightened in the last few years since the discovery of White-Nose Syndrome, a disease that is killing thousands of bats in the United States (Belhart 2009, Aley 2010). The disappearance of bats from caves will likely have a drastic negative effect on the invertebrate species that rely on the bat guano. Studies indicate that the disruption of the flow of organic matter into the caves

can endanger cave species (Culver et al. 2000). Therefore understanding the role of bats in caves such as Bracken Bat Cave can help us understand the effects of White-Nose Syndrome on the biodiversity and ecosystem processes of the affected caves. Additionally, disturbances outside of the cave ecosystem can also disrupt in-cave ecosystems. For example, changes in water availability (Wood et al. 2008) and vegetation structures (Taylor et al. 2005) can have dramatic effects on cave ecosystems and the organisms that comprise them. Despite the recent advances in cave research, cave ecosystems are still relatively understudied (Marmonier 1993, Elliott 2000, Hancock et al. 2005). For this reason, our understanding of cave ecosystems and the in-cave and out-of the cave processes that govern them is imperative for their conservation.

Table 1. Summary of the lower possible classification of macroinvertebrates found in Bracken Bat Cave through core sampling and pitfall traps methods (August 2009-July 2009).

Order	Family	Genus	Species
Acarina			
Araneae			
Coleoptera	Dermestidae	*Dermestes*	*carnivorus*
Coleoptera	Histeridae	*Carcinops*	*pumilio*
Coleoptera	Histeridae	*Euspilotus*	sp. 1
Coleoptera	Histeridae	*Euspilotus*	sp. 2
Coleoptera	Tenebrionidae	*Alphitobius*	*diaperinus*
Hemiptera			
Dermaptera			
Diptera			
Pseudoscorpionida	Chernetidae		
Siphonaptera	Ischnopsyllidae		

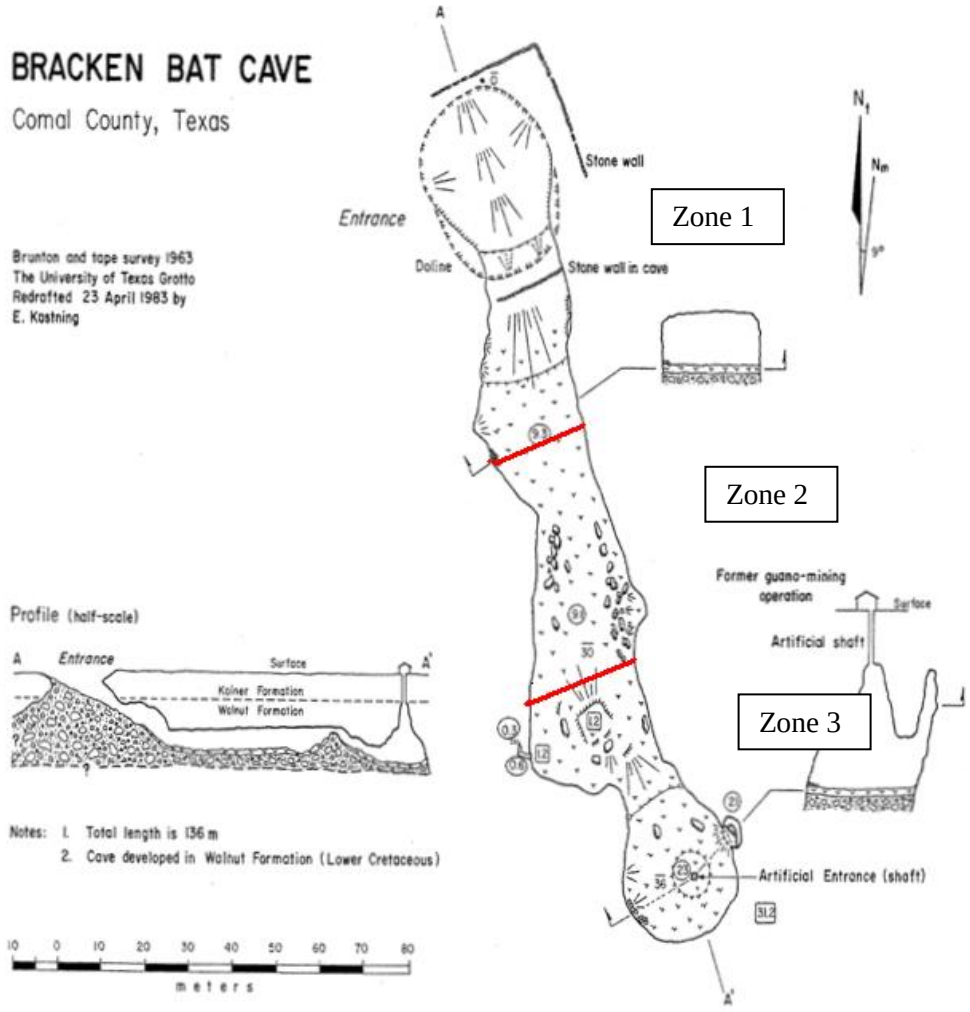

Fig. 1. Map of Bracken Bat Cave, Garden Ridge, Texas (modified from Kastning, 1963). The map includes the division of the cave into cave zones (each 45 m in length).

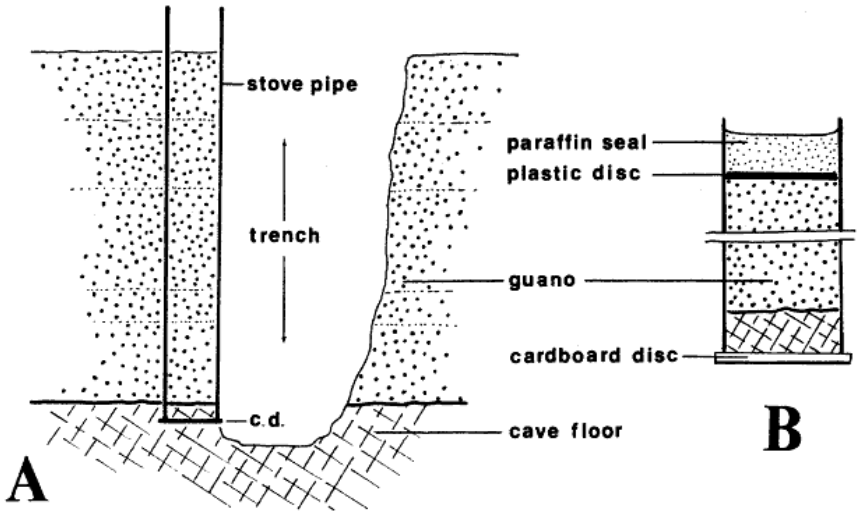

Fig. 2. Pipe-trench method modified to extract layers of guano. The modifications included omitting the paraffin seal and cardboard disk and replacing them with plastic seals and dividers (Altenbach & Petit, 1972).

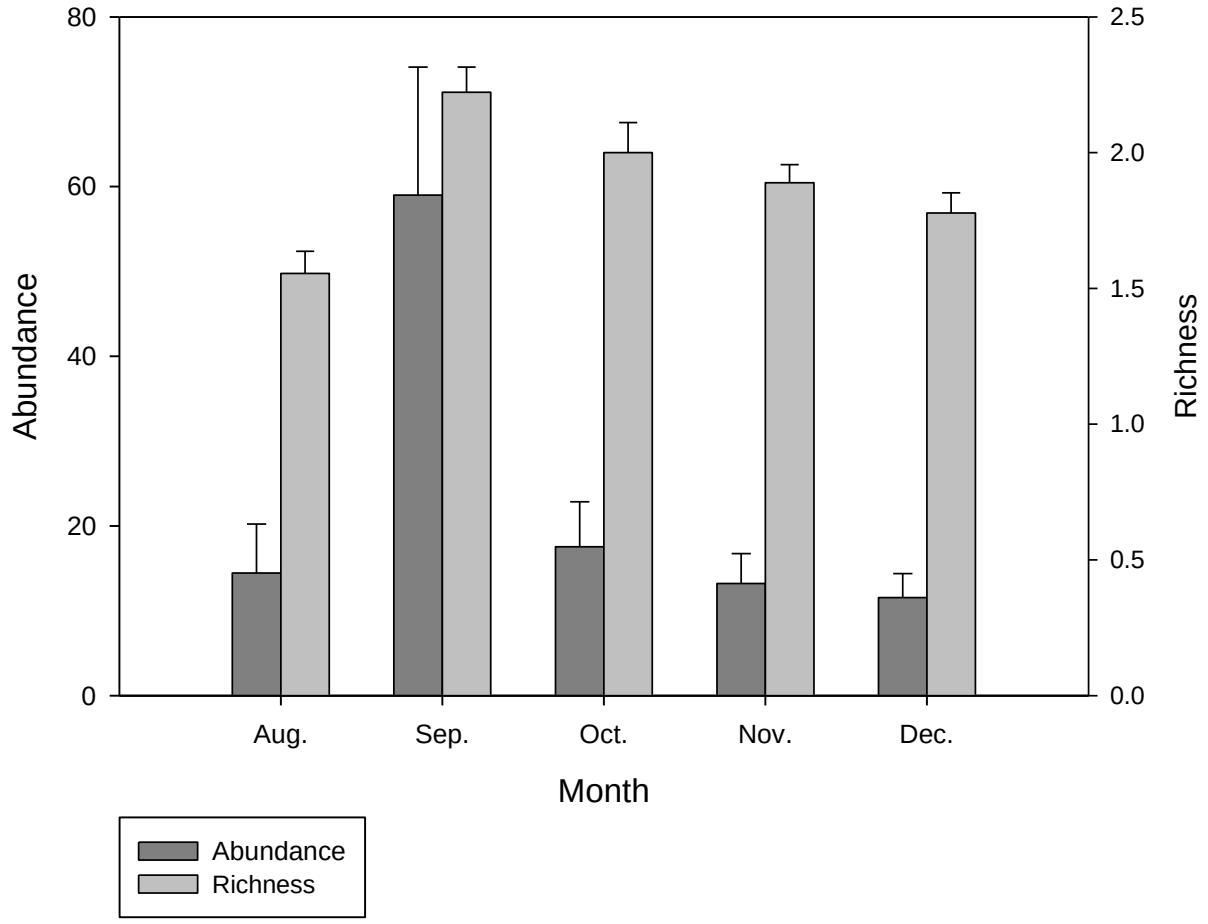

Fig. 3. Monthly macroinvertebrate abundance and richness +/- SE of Bracken Bat Cave encountered in core samples, with respect to seasonality (August-December 2009). Variation is not significantly different between months.

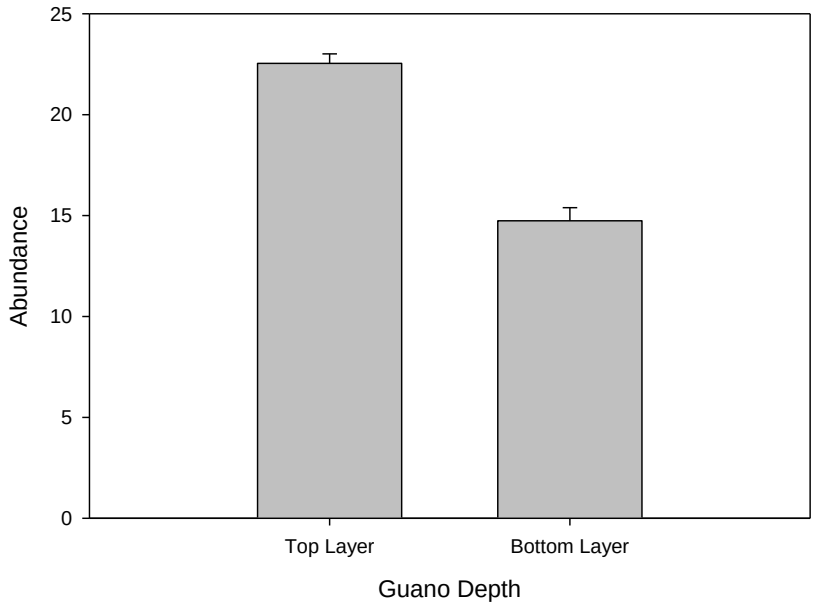

Fig. 4. Mean macroinvertebrate abundance +/- SE of Bracken Bat Cave with respect to guano depth encountered in the core samples. The top layer of guano (0-0.45m) displayed significantly higher macroinvertebrate abundance than the bottom layer of guano (0.45-0.91m) (($F_{1, 117}$= 8.56, P= 0.004*).

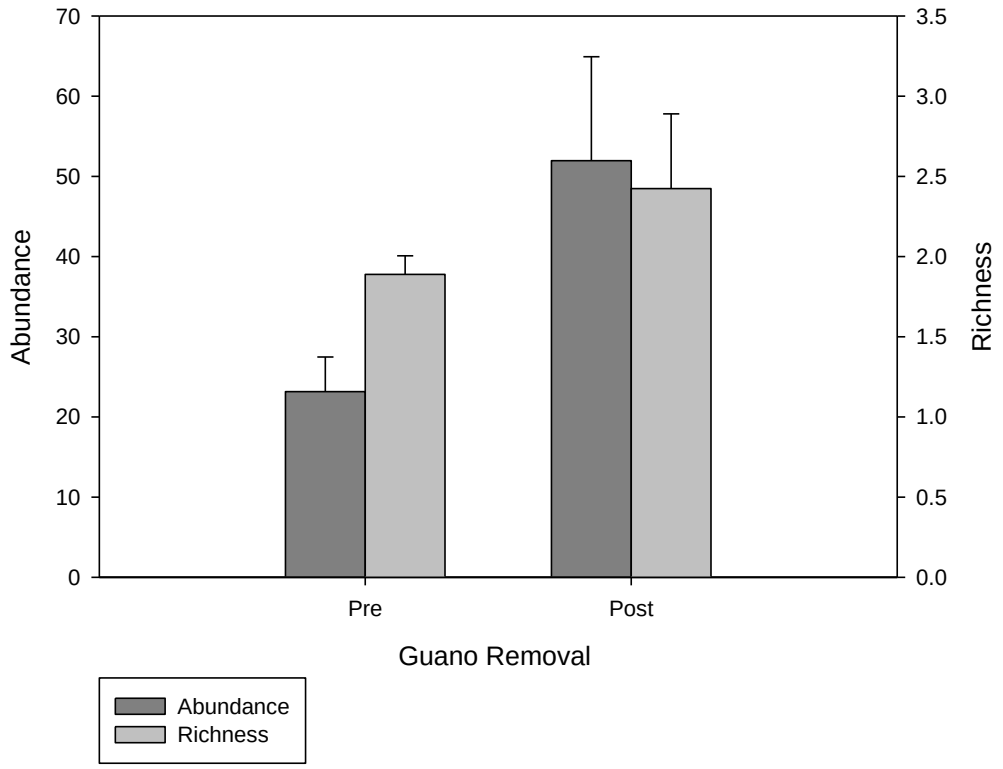

Fig. 5. Macroinvertebrate abundance and richness +/- SE of near surface guano depth (surface to 0.23m) in Bracken Bat Cave found pre (September-December 2009) and post guano removal (March-May 2010). Abundance ($F_{1,63}$= 7.44, P=0.008*) and richness ($F_{1,63}$ = 7.06, P=0.009*) were significantly higher after the guano removal.

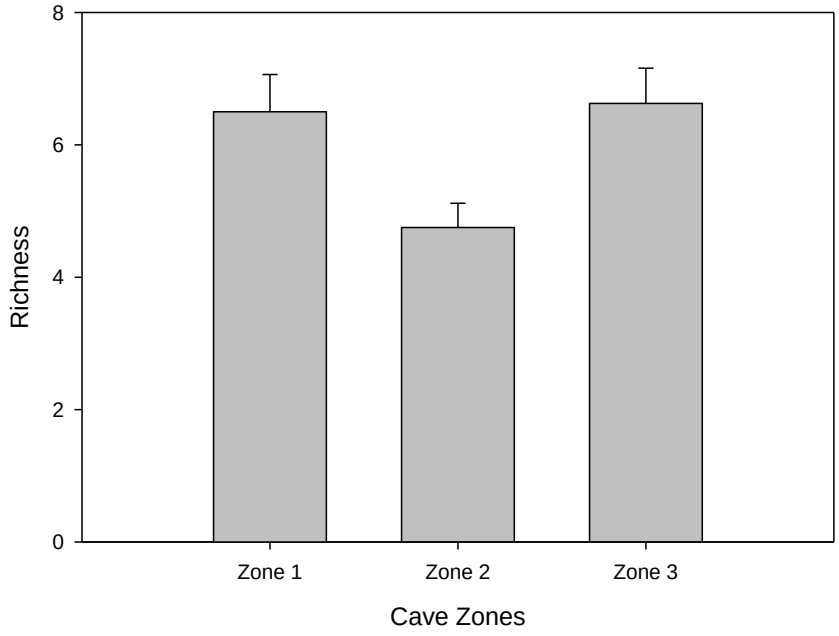

Fig. 6. Comparison of mean macroinvertebrate richness +/- SE found between the cave zones during pitfall trap sampling. Richness was significantly different between the three cave zones since zone 2 had a significantly lower richness when compared to zone 1 and 3 ($F_{1,13}$= 8.91, P=0.004*).

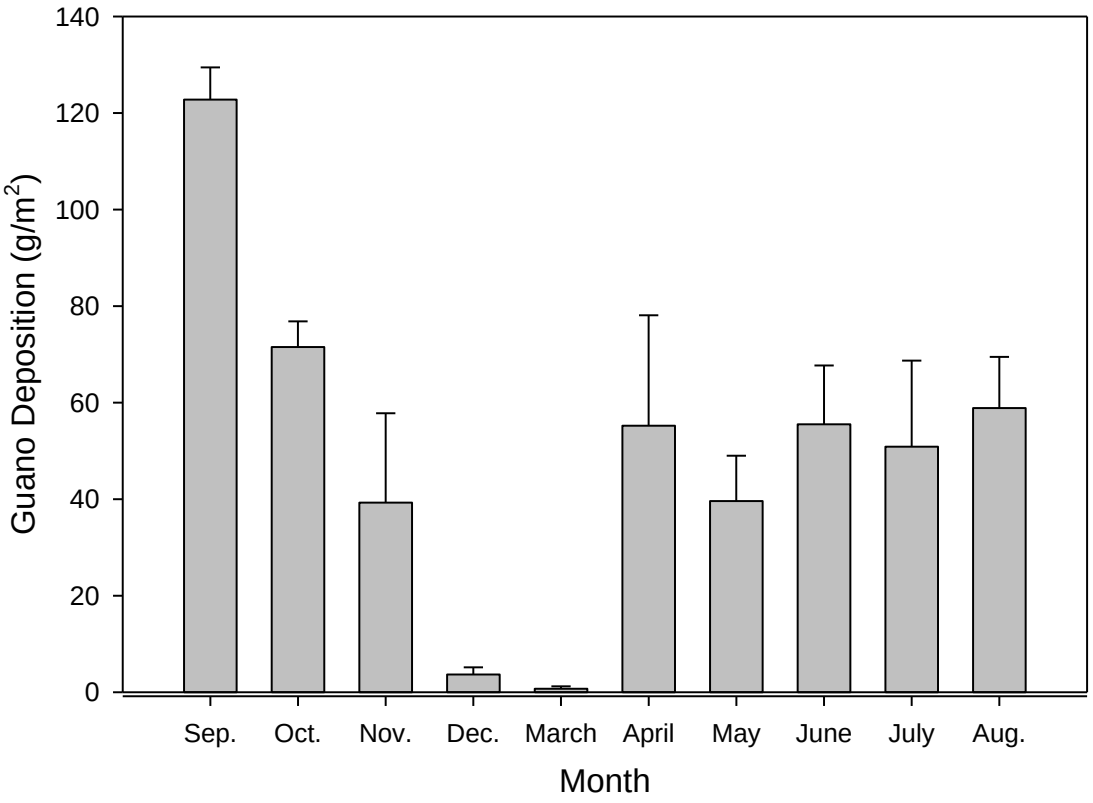

Fig. 7. Mean weight +/- SE of guano deposition (g/m^2) per month (September 2009-August 2010). The variation in guano deposition observed throughout the months was significantly different ($F_{1,27} = 16.87$, $P<0.001$*), depending on the variation of the migratory bat populations.

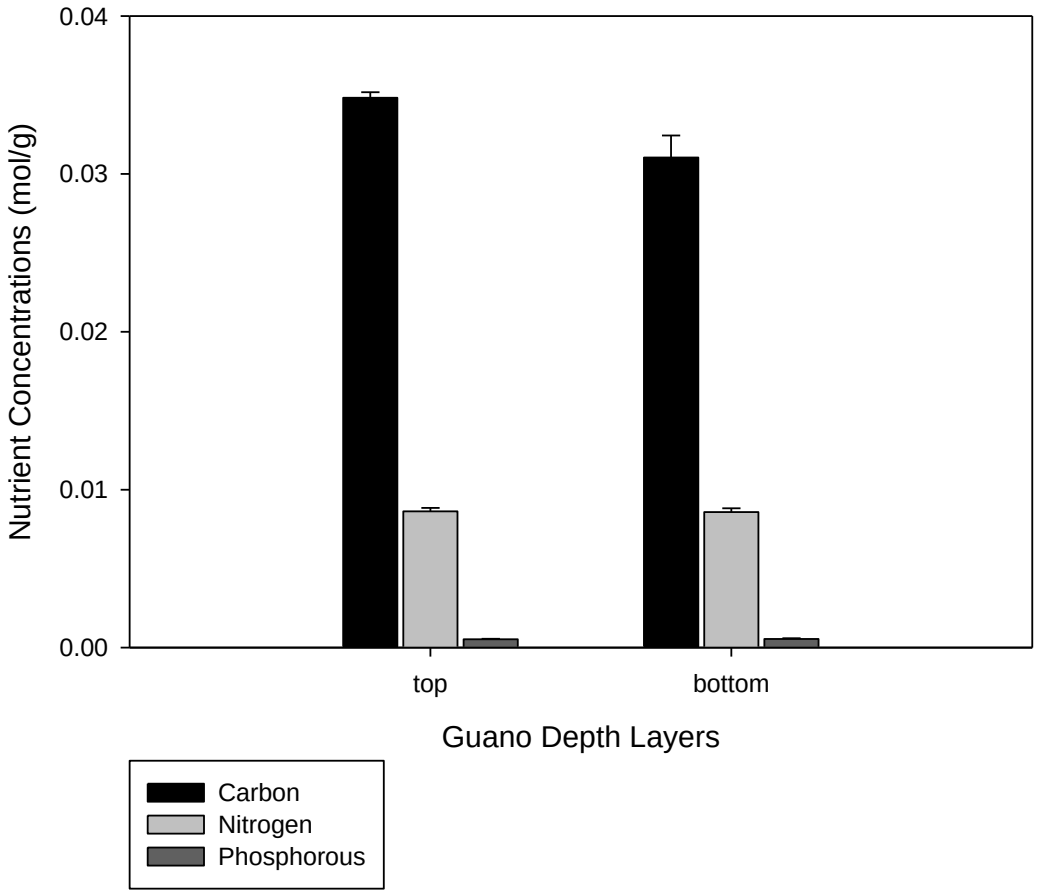

Fig. 8. Carbon, nitrogen and phosphorous concentrations (mol/g) compared between top (0-0.46m) and bottom (0.46-0.91m) guano depth layers. Carbon content (mol/g) was the only nutrient out of the three nutrients compared that varied with respect to guano depth (($F_{1,32}$=7.04, p-value=0.01*).

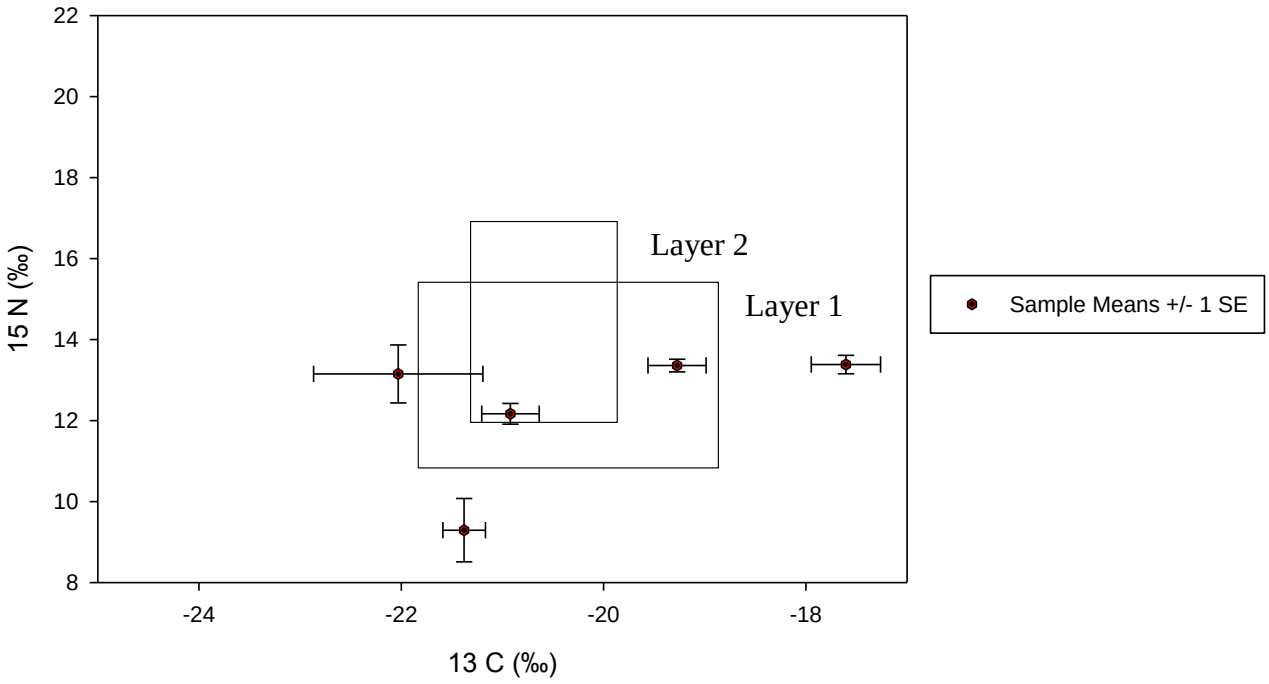

Fig. 9. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (‰) of bat guano, bat hair and various species and life stages of the most abundance Bracken Bat cave invertebrates. Boxes represent the means +/- 1 SD for each guano layer. Means +/- SE of the various types (species and life stages) of the organisms are also plotted with bi-directional standard error bars. Both $\delta^{13}C$ and $\delta^{15}N$ displayed a large variation with respect to the type of sample.

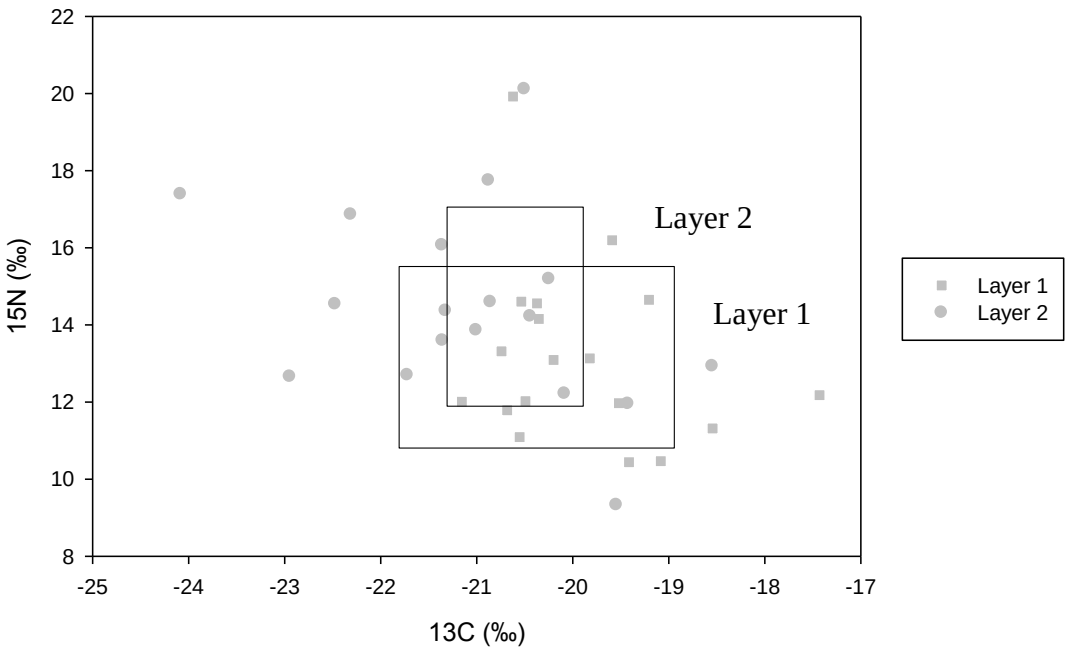

Fig. 10. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures (‰) of bat guano with respect to guano depth layer, which is significantly correlated to $\delta^{15}N$ ($F_{1,77}$=2.21, p-value=0.03*). Boxes represent the means +/- 1 SD for each guano layer.Bottom layers of guano (0.45-0.91m) displayed a higher ratio of $\delta^{15}N$ when compared to the top layers of guano (0-0.45m). No trends were detected in $\delta^{13}C$ ratios with respect to guano depth.

LITERATURE CITED

Aley, T. (2010) Management strategies for responding to White Nose Syndrome in bats. National Speleological Society News February 2010: 10-14.

Altenbach, S.J. & Petit, M.G. (1972) Stratification of guano deposits of the Free-Tailed Bat, *Tadarida brasiliensis*. Journal of Mammalogy 53: 890–893.

Aristophanous, Marios (2010) Does your preservative preserve? A comparison of the efficacy of some pitfall trap solutions in preserving the internal reproductive organs of dung beetles. Zookeys 34: 1-16.

Banitz, Thomas, Huth, Andreas, Grimm Volker & Johst, Karin (2008) Clumped versus scattered: how does the spatial correlation of disturbance events affect biodiversity? Theoretical Ecology 1: 231-240.

Bat Conservation International (2009) Bracken Bat Cave. Retrieved on March 10, 2009 from http://www.batcon.org/index.php/education/find-bat-locations/bracken-bat cave/subcategory.html?layout=subcategory.

Bat Conservation International (2011) Cave Myotis. Retrieved on December 16, 2011 from http://www.batcon.org/index.php/all-about-bats/species profiles .html?task=detail&species=2405&country=43&state=all&family=all&start=25.

Belhart, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R.,Gargas, A., Nivar, R., Okoniaswki, J.C., Rudd & R.J., Stone, W.B. (2009) Bat White-Nose Syndrome: An emerging pathogen? Science 323: 227.

Biswas, Jayant (2010) Kostsumar Cave biodiversity: A review of cavernicoles and their troglobitic traits. Biodiversity Conservation 19: 275-289.

Bowles, D.E. & Arsuffi T.L. (1993) Karst aquatic ecosystems of the Edwards Plateau region of central Texas, USA: A consideration of their importance, threats to their existence, and efforts for their conservation. Aquatic Conservation: Freshwater and Marine Ecosystems 3: 317–329.

Brune, G. (1981) Springs of Texas, Vol. 1. Branch-Smith, FortWorth, Texas.

Caire, W., Smith, J.F., McGuire, S. & Royce, M.A. (1984) Early foraging behavior of insectivorous bats in western Oklahoma. Journal of Mammalogy 65: 319-324.

Cleveland, Cutler J., Betke, Margrit, Federico, Paula, Frank, Jeff D., Hallam, Thomas G., Horn, Jason, Lopez, Juan D. Jr. , McCracken, Gary F., Medellin, Rodrigo A., Moreno-Valdez, Arnulfo, Sansone, Chris G., Westbrook, John K. & Kunz, Thomas H. (2006) Economic value of the pest control service provided by Brazilian free-tailed bats in south-central Texas. Frontiers in Ecology and the Environment 5: 238–243.

Crawley, M.J. (2004) Timing of disturbance and coexistence in a species-rich ruderal plant community. Ecology 85: 3277–3288.

Connell, J.H. (1978) Diversity in tropical rain forests and coral reefs. Science 199: 1302–1310.

Culver, D.C. (1982) Cave life: evolution and ecology. Harvard University Press, Cambridge, Massachusetts, USA.

Culver, D.C. (1985) Trophic relationships in aquatic cave environments. Stygologia 1: 143–53.

Culver, David C., Master, Lawrence L., Christman, Mary C., & Hobbs, Horton H. (2000) Obligate cave fauna of the 48 contiguous United States. Conservation Biology 14: 386-401.

DeNiro, Michael J. & Epstein, Samuel (1978) Influence on the diet and distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 42: 495-506.

Dickson, G.W., Briese, L.A., & Giesy, G.P. (1979) Tissue metal concentration in two crayfish species cohabiting a Tennessee cave stream. Oecologia 44: 8-12.

Elliott W.R. (2000) Conservation of the North American cave and karst biota. In Subterranean Ecosystem: Ecosystems of the World. 30, Wilkins H, Culver DC, Humphries WF (eds). Elsevier Science: Amsterdam, The Netherlands: 665–689.

Fagan, W.F., Lutscher, F. & Schneider, K. (2007) Population and community consequences of spatial subsidies derived from central-place foraging. The American Naturalist 170: 902-915.

Federico, Paula, Hallam, Thomas G., McCracken, Gary F., Purucker, S. Thomas, Grant, William E., Correa-Sandoval, A. Nelly, Westbrook, John K., Medellín, Rodrigo A., Cleveland, Cutler J., Sansone, Chris G., López, Juan D. Jr., Betke, Margrit, Moreno-Valdez, Arnulfo & Kunz, Thomas H. (2008) Brazilian Free-Tailed Bats as Insect Pest Regulators in Transgenic and Conventional Cotton Crops. Ecological Applications 18: 826-837.

Fenolio D.B., Graening G.O., Collier B.A. & Stout J.F. (2006) Coprophagy in a cave-adapted salamander; the importance of bat guano examined through nutritional and stable isotope analyses. Proceedings of the Royal Society B-Biological Sciences 273: 439-443.

Ferreira, R.L. & Martins, R.P. (1998) Diversity and distribution of spiders associated with bat guano piles in Morrinho Cave (Bahia State, Brazil). Diversity and Distribution 4: 235-241.

Ferreira, R.L., Prous, X. & Martins, R.P. (2007) Structure of bat guano communities in a dry Brazilian cave. Tropical Zoology 20: 55-74.

Fierer, Noah, Schmiel, Joshua P., & Holden, Patricia A. 2003. Variations in microbial community composition through two soil depth profiles. Soil Biology and Biochemistry 35: 167-176.

Glover, Anita M., & Altringham, John D. (2008) Cave selection and use by swarming bat species. Biological Conservation 141: 1493-1504.

Griffin, D.R. & Thompson, D. (1982) High altitude echolocation of insects by bats. Behavioral Ecology and Sociobiology 10: 303-306.

Hancock, P.J. (2002) Human Impacts on the Stream-groundwater Exchange Zone. Environmental Management 29: 763-781.

Hancock P.J., Boulton A.J., Humphreys W.F. (2005) Aquifers and hyporheic zones: towards
an ecological understanding of groundwater. Hydrogeology Journal 13: 98–111.

Holsinger, J.R. 1998. Troglobites: the evolution of cave-dwelling organisms. American Scientist 76: 146–153.

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S. et al. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75: 3–35.

Humphreys, W.F. (1991) Experimental re-establishment of pulse-drive populations in a terrestrial troglobite community. The Journal of Animal Ecology 60: 609-623.

Huston, M.A. (1994) Biological diversity. Cambridge University Press, Cambridge.

Hutchins, Fran. Personal Interview. February 2011.

Hutchinson, George Evelyn (1950) Survey of Existing Knowledge of Biochemistry: The Biochemistry of Vertebrate Excretion. Bulletion of the American Museum of Natural History 96.

Isaac, R.A. (2009) AOAC Method 985.01. Metals and other elements in plants. In: Official methods of analysis of AOAC international, 16th ed., P.Cunniff (ed.), Gaithersburg, Md: AOAC International.

Kastning, E. (1963) Bracken Bat Cave map, Comal County, Texas. The University of Texas Grotto.

Lee, Ya-Fu & McCracken, Gary F. (2005) Dietary variation of Brazilian Free-Tailed bats links to migratory populations of pest insects. Journal of Mammalogy 86: 67-76.

Lee, Ya-Fu & McCracken, Gary F. (2001) Timing and variation in the emergence and return of a large colony of Mexican free-tailed bats *Tadarida brasiliensis* (Molossidae). Ecoscience 9: 306-313.

Loehle, C. (2000) Strategy space and the disturbance spectrum: a life history model for tree species coexistence. American Naturalist 156: 14–33.

Marmonier, P., Vervier P., Gibert J.,& Dole-Olivier, M.J. (1993) Biodiversity in ground waters. Trends in Ecology and Evolution 8: 392–394.

McCracken, G.F., Lee, Y.F., Westbrook, J.K., Balsley, B.B. & Jensen, M.L. (1997) High altitude foraging by Mexican free-tailed bats: vertical profiling using kites and hot air balloons. Bat Research News 38: 117.

McIntire, E.J.B. (2004) Understanding natural disturbance boundary formation using spatial data and path analysis. Ecology 85: 1933–1943.

McWilliams, Lisa A. (2005) Variation in diet of the Mexican Free-Tailed bat (*Tadarida brasiliensis mexicana*). Journal of Mammalogy 86: 599-605.

Nadelhoffer, K.J. & Fry, B. (1994) Nitrogen isotopes studies in forest ecosystems. In: Stable isotopes in ecology and environmental science (Eds K Lajtha & R.H. Michener): 22-44. Blackwell Scientific, Oxford.

Ohsawa, K., Kawasaki, K., Takasu, F. & Shigesada, N. (2002) Recurrent habitat disturbance and species diversity in a multiple competitive species system. Journal of Theoretical Biology 216: 123–138.

Persson, Lennart (1999) Trophic cascades: Abiding heterogeneity and the trophic level concept at the end of the road. Oikos 85: 385-397.

Peterson, Bruce J. & Fry, Brian (1987) Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18: 293-320.

Polis, Gary A. & Strong, Donald R. (1996) Food web complexity and community dynamics. The American Naturalist 147: 813-846.

Post, David M. (2002) Using stable isotopes to estimate trophic position: Models, methods and assumptions. Ecology 83: 703-718.

Poulson, T.L. (2005). Food Sources. Pages 255-264 in D.C. Culver and W.B. White, editors. Encyclopedia of caves. Ensevier, Burlington.

R Development Core Team., 2005. R: A Language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Roudnick, J.S. & Winterbourn, M.J. (1986) Stable carbon isotopes and carbon flow in ecosystems. Bioscience 36: 171-177.

Sanders, John E. (1981) Principles of Physical Geology. John Wiley and Sons, Inc. New York.

Schneider, K., Christman, M.C. & Fagan, W.F. (in press) The influence of resource subsidies on cave invertebrates: results from an ecosystem-level manipulation experiment. Ecology.

Taylor, Steven J., & Webb, Donald W. (2008) The Fragile Fauna of Illinois Caves. Retrieved on January 10, 2009 from http://dnr.state.il.us/lands/landmgt/PARKS/R4/caverns/CAVE.HTM.

Taylor, S.J., Krejca, J.K. & Denight, M.L. (2005) Foraging range and habitat use of *Ceuthophilus secretus* (Orthoptera: Rhaphidophoridae), a key toglozene in central Texas cave communities. American Midland Naturalist 154: 97-114.

Texas Parks and Wildlife Department. (2005) Texas comprehensive wildlife conservation strategy 2005-2010. Retrieved on January 02, 2009 from http://www.tpwd.state.tx.us/publications/pwdpubs/pwd_pl_w7000_1187a/.

Thermo Fisher Scientific. Flash EA 1112 NC Analyzers. Retrieved on January 10, 2011. http://www.ceinstruments.co.uk/Flash.htm.

UC Davis Stable Isotope Facility. ^{13}C and ^{15}N Analysis of Solids by EA-IRMS. Retrieved on January 21, 2010. http://stableisotopefacility.ucdavis.edu/13cand15n.html.

United States Environmental Protection Agency (US EPA) Method 6010A. (1992a) Inductively coupled plasma-atomic emission spectroscopy. In: Test methods for evaluation solid waste, physical/chemical methods. SW-846, 3rd Edition.

Whitaker, John O., Neefus, Christopher, Kunz, Thomas H. (1996) Dietary variation in the Mexican Free-Tailed bat (*Tadarida brasiliensis mexicana*). Journal of Mammalogy 77: 716-724.

Williams, T.C., Ireland, L.C. & Williams, J.M. (1973) High altitude flights of the free-tailed bat (*Tadarida brasiliensis*). Journal of Mammalogy 54: 807-821.

Wood, P.J., Gunn, J., & Rundle, S.D. (2008) Response of benthic cave invertebrates to organic pollution events. Aquatic Conservation-Marine and Freshwater Ecosystems: 909-922.

Zhang, Yixin, Richardson, John S., Negishi, Junjiro N. 2004. Detritus processing, ecosystem engineering and benthic diversity: a test of predator–omnivore interference. Journal of Animal Ecology 73: 756–766.

VITA

Goniela Iskali was born on April 20th, 1985 in Korce, Albania. She graduated from the University of Illinois in Urbana-Champaign in December 2006. After graduation, Goniela pursued a variety of internships and jobs with the National Park Service in Pinnacles NM, Oregon Caves NM, Timpanogos Caves NM and Sequoia and Kings Canyon NP. These experiences reaffirmed her passion to study caves and cave biota. Therefore in January 2009, Goniela enrolled as a graduate student at Texas State University-San Marcos with the intention to study cave biodiversity.

Permanent Address: 36 N. Martha St.

Lombard, IL. 60148

This thesis was typed by Goniela Iskali.