2019

# Seasonal Diet Variation in Thirteen-Lined Ground Squirrel (Ictidomys Tridecemlineatus) in Southernwestern Michigan

Austin Menzmer
*Andrews University*, menzmer@andrews.edu

## Recommended Citation

Menzmer, Austin, "Seasonal Diet Variation in Thirteen-Lined Ground Squirrel (Ictidomys Tridecemlineatus) in Southernwestern Michigan" (2019). *Master's Theses*. 134.
https://dx.doi.org/10.32597/theses/134
https://digitalcommons.andrews.edu/theses/134

# ABSTRACT

# SEASONAL DIET VARIATION IN THIRTEEN-LINED GROUND SQUIRRELS (*ICTIDOMYS TRIDECEMLINEATUS*) IN SOUTHWESTERN MICHIGAN

by

Austin James Menzmer

Chair: H. Thomas Goodwin

ABSTRACT OF GRADUATE STUDENT RESEARCH

Thesis

Andrews University

College of Arts and Sciences

# SEASONAL DIET VARIATION IN THIRTEEN-LINED GROUND SQUIRRELS (*ICTIDOMYS TRIDECEMLINEATUS*) IN SOUTHWESTERN MICHIGAN

Name of researcher: Austin James Menzmer
Name and degree of faculty chair: H. Thomas Goodwin, Ph.D.
Date completed: June 2019

Thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) are omnivorous ground squirrels that range from Texas to Alberta and from Michigan to Utah. The amount and type of both animal and plant matter in their diet may vary both geographically and seasonally. The present study was motivated by prior work, based on $\delta^{13}C$ analysis of incisor enamel of thirteen-lined ground squirrels from a colony in southwestern Michigan, suggesting that these animals underwent a pronounced, late-season shift in diet from predominantly C3 to C4 plants. However, this inferred dietary shift was not directly demonstrated. Here, I tracked diet of thirteen-lined ground squirrels in southwestern Michigan from June to October 2018 using fecal samples deposited by animals when live-trapped; squirrels were then released unharmed. In addition, I collected and identified voucher plant specimens at the field site.

Microhistological and isotopic analyses of fecal samples documented two primary seasonal shifts in diet. First, the microhistological data indicated a sharp decrease in arthropod consumption as summer turned to fall; this decrease correlated with the overall decrease in $\delta^{15}$N and 1/C:N (carbon:nitrogen; a measure of trophic position and degree of carnivory). These observations were consistent with a shift from a protein-rich early summer diet rich in arthropods to a fall diet with less protein. Secondly, the microhistological data demonstrated a dramatic late-season increase in consumption of grass seeds and glumes, which mirrored a sharp increase in $\delta^{13}$C. Both of these data sets indicated a shift from primarily C3-plants to a mixed or primarily C4-plant diet in August, with a C4-rich diet continuing into October. In summary, my study a) documented a previously unknown shift from high to low use of arthropods, a pattern that is contrary to findings of prior studies, and b) confirmed the late-season shift from C3- to C4-plants previously inferred from enamel isotope data.

Andrews University

College of Arts and Sciences

# SEASONAL DIET VARIATION IN THIRTEEN-LINED GROUND SQUIRRELS (*ICTIDOMYS TRIDECEMLINEATUS*) IN SOUTHWESTERN MICHIGAN

A Thesis

Presented in Partial Fulfillment

Of the Requirements for the Degree

Master of Sciences

by

Austin James Menzmer

June 2019

# SEASONAL DIET VARIATION IN THIRTEEN-LINED GROUND SQUIRRELS (*ICTIDOMYS TRIDECEMLINEATUS*) IN SOUTHWESTERN MICHIGAN

A thesis
presented in partial fulfillment
of the requirements for the degree
Master of Science

by

Austin James Menzmer

APPROVAL BY THE COMMITTEE:

H. Thomas Goodwin, Ph.D., Chair

Benjamin Navia, Ph.D.

Daniel Gonzalez-Socoloske, Ph.D.

Date approved

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# ACKNOWLEDGEMENTS

First of all, I would like to express my heartfelt thanks to Dr. Tom Goodwin for his continual support, encouragement, and words of advice during the last year and a half. Without him, this thesis would simply not be and I certainly would not have had the courage to attempt a project this large. I would also like to thank Dr. Daniel Gonzalez-Socoloske and Dr. Benjamin Navia for agreeing to be on my committee and the feedback they provided while in those roles. I would like to thank my parent institution, Andrews University, for awarding me a Grants-in-Aid-of-Research grant in 2018. This grant enabled me to have microhistological analysis conducted on my fecal samples, from which I learned the most fascinating discoveries. I want to thank Jeremy McLarty for his cheerful aid in field work, Dr. Shandelle Henson (AU Mathematics Department) for her help in mathematical analysis of my data, and Ralph Reitz of Fernwood Botanical Gardens for his aid in plant identification.

On a personal note, I would like to thank my family and fellow graduate students for continually cheering me on over the last two years, even when I did not believe in myself. Last but certainly not least, I want to thank Endi and Kristina Stojanovic, as well as the rest of the Young Adult class at Village Seventh-Day Adventist Church, for showing genuine interest in me and my project, rejoicing when I caught squirrels, and encouraging me to continue on this path of becoming a professor.

# Introduction

The diets of small herbivores such as squirrels and other rodents have been found to change with the seasons. For instance, Kincaid and Cameron (1982) found that the fulvous harvest mouse (*Reithrodontomys fulvescens*) consumed many insects in the spring, some in summer, and even fewer in the fall. However, overall plant consumption (monocots and dicots combined) steadily increased as the seasons progressed. In another case of rodent diet variation, Fagerstone *et al.* (1981) discovered that the black-tailed prairie dog (*Cynomys ludovicianus*) ate different grasses in different quantities each season. For example, *Agropyron* spp. of grasses were consumed most in May, with a steady decline in July and September (diet was sampled every other month). Mosquito grass (*Bouteloua gracilis*) was consumed at roughly equal levels in May and September, but July levels of consumption were almost twice as much as May/September values. Insect consumption was minimal through the seasons.

Studies of ground squirrels have shown that seasonal differences in diet can vary across years or locations. Leitner and Leitner (2017) found over their nine-year study (1988-1996) in Inyo County, California, that the Mohave ground squirrel (*Xerospermophilus mohavensis*) altered its diet according to how much rainfall had occurred the previous winter. Following a wet winter, spring diet of Mohave ground squirrels consisted of about 23% shrub leaves, but jumped to over 66% after a dry winter. Similarly, following a wet winter, herbaceous leaf material made up about 20% of the squirrels’ diet in summer; this number jumped to over 50% during a summer that followed a dry winter.

As an example of geographic variation and dietary plasticity in diet during the same season, Flake (1973) found in Colorado that thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) predominantly consumed scarlet globemallow (*Sphaeralcea coccinea*) in the spring and summer, whereas Whitaker (1972) in Indiana found that the species heavily consumed clover (*Trifolium*) leaves in that same timeframe.

These dietary differences may be either opportunistic or selective. Hobbie *et al.* (2017) found that arctic ground squirrels (*Urocitellus parryii*) at two locations in northern Alaska chose their diet based on availability, particularly with regards to forbs and ectomycorrhizal shrubs. Ritchie (1988), however, found that almost two-thirds of the Columbian ground squirrels (*Urocitellus columbianus*) he studied in Montana chose a diet that approximated maximum energy intake; this diet was not particularly influenced by the abundance of one or another specific food option. The other one-third of the squirrels appeared to make some non-optimal foraging decisions.

Diet variation can be tracked through a variety of methods: direct observation, stomach content analysis, and fecal sample analysis are among the top choices. The challenges of the former two methods have been well described in the literature. Leitner and Leitner (2017) noted the difficulty of actually tracking the activities of Mohave ground squirrels due to their ability to move cautiously and blend in well. Only during three of the nine years spent in the field were they able to visually track individual squirrels who had been accustomed to human presence. Vispo and Bakken (1993) remarked that car traffic or construction noise often startled the thirteen-lined ground squirrels they were studying in Indiana, and these disturbances caused the squirrels to be above ground for only 42.5 minutes of the 50-minute observation period. Additionally, the small size of plants and insects can heighten the difficulty of identification while they are being consumed in a matter of seconds. Stomach content analysis presents challenges as well. First off is deciding whether it is acceptable ethics to kill squirrels for research purposes; and if so, how many. Even if this method is chosen, Fitzpatrick (1923) and Flake (1973) pointed out the challenge of separating thoroughly chewed plant and animal matter, much less identifying the contents.

Fecal sample analysis is an attractive alternative to direct observation and stomach content analysis in that it reduces ethical dilemmas; fecal samples give a solid record of what was consumed and the animal is unlikely to be hurt by collection (Hwang *et al.* 2007). Bergstrom (2013), who studied rodents in Africa, further noted that both microhistological and isotopic analyses (sub-areas of fecal sample analysis) can be conducted on feces from the same individual, providing independent lines of evidence of diet.

Microhistological analysis involves dissecting the fecal samples and recording how often fragments of certain food items are found over the span of a specified number of scans underneath the microscope (Sparks and Malechek 1968). To aid in identification, these fecal sample fragments are then compared to an already-identified reference collection of food item fragments. Limitations of microhistological analysis include the difficulty of successfully identifying food items when they have been processed through the entire digestive system. Khanam *et al.* (2016) experienced this difficulty, noting that sometimes by this point there is not enough material remaining to identify. However, microhistological analysis has been successfully used to identify diet and diet variation in herbivorous rodents such as the Columbian (Ritchie 1988) and Townsend's ground squirrels (*Urocitellus townsendii*; Van Horne *et al.* 1998), as well as omnivorous rodents such as the fringe-tailed gerbil (*Gerbilliscus robustus*) and Percival's spiny mouse (*Acomys percivali*; Bergstrom 2013).

Isotopic analysis involves documenting the ratio of a rarer isotope of an element (i.e., $^{13}C$) to the more common isotope of that same element (i.e., $^{12}C$). The ratio is expressed as the difference from a standard and in parts per thousand (‰). The standardized ratio (δ) is calculated using the formula $((R_{sample} - R_{standard})/R_{standard}) \times 1{,}000$, where R represents the relevant isotope ratio. For example, $\delta^{13}C$ is calculated as $((^{13}C/^{12}C_{sample} - {}^{13}C/^{12}C_{standard})/{}^{13}C/^{12}C_{standard}) \times 1000$, where the standard is the Vienna Peedee Belemnite (VPDB; Ben-David and Flaherty 2012).

Fecal sample analysis, such as isotopic analysis, provides a "snapshot" of recent diet, due to the time it takes the body to engage in digestive processing - from a few hours to a few days, not months or years (Hwang *et al.* 2007; Sponheimer et *al.* 2003). The fecal isotope values closely approximate the isotope ratios of diet in larger herbivores, although there may be some fractionation between diet and fecal values in smaller herbivores (Hwang *et al.* 2007). Fractionation is when products and substrates in a chemical reaction vary in their isotope ratios; the rarer isotope (i.e., $^{13}C$) ends up creating stronger connections with either the product or substrate (Ben-David and Flaherty 2012). Thus, as the digestive process occurs, there is potential for fractionation of isotopic values between diet and fecal samples.

Two isotope systems of particular relevance to dietary inference are $\delta^{13}C$ and $\delta^{15}N$. The primary control on $\delta^{13}C$ in herbivorous terrestrial animals is the proportion of C3 or C4 plants in the diet (Ben-David and Flaherty 2012). In C3 plants, initial carbon fixation is catalyzed by the enzyme rubisco. This process strongly discriminates against $^{13}C$, resulting in $\delta^{13}C$ of about -27‰ in C3 plant tissues. In contrast, initial carbon fixation in C4 plants is catalyzed by PEP carboxylase, an enzyme with high affinity for $CO_2$. Because of this high affinity for $CO_2$, PEP carboxylase discriminates less strongly against $^{13}C$, resulting in $\delta^{13}C$ values of roughly -11 to -15‰ in C4 plant tissues (Ehleringer *et al.* 1991, Ben-David and Flaherty 2012). Thus, the relative properties of C3/C4 in the diet of consumers can be inferred from values of $\delta^{13}C$ in their tissues or feces (Ben-David and Flaherty 2012).

Most plants are C3, but C4 plants are present across diverse taxa, with the most abundant C4 groups being warm-season grasses (Xu *et al*. 2010). In general, C4 plants appear to be adapted to avoid water loss. When considering a swath of the Great Plains from Texas to Montana, Epstein *et al.* (1997) found the majority of C4 production to be in Texas, Oklahoma, and Kansas – areas that are typically much warmer for a longer time span than a northern state such as Montana. Even in the northern latitudes, the C3/C4 production switch can still be observed. For example, Ode *et al.* (1980) found that a prairie in South Dakota possessed primarily low $\delta^{13}C$ values (i.e., C3 plants) in the spring, switched to higher $\delta^{13}C$ (C4) values in the warmer summer season, but returned to lower $\delta^{13}C$ values in the fall. Thus, both geographic and seasonal patterns are evident in the relative abundance of C3 and C4 plants.

$\delta^{15}N$ is an isotope system that is more complexly controlled when compared to $\delta^{13}C$. For example, rooting depth, plant water-use efficiency, and microbial nitrification rates in soil all influence $\delta^{15}N$ in plant tissues, which in turn affects $\delta^{15}N$ of plant consumers (Ben-David and Flaherty 2012). Dietary quality – how well the food an animal eats matches its' nutritional needs – also impacts $\delta^{15}N$ (Bergstrom 2013). In addition, $\delta^{15}N$ may vary according to trophic position of the consumer (Ben-David and Flaherty 2012); the higher the trophic level an animal occupies, the higher its $\delta^{15}N$ values (Minagawa and Wada 1984). Thus, when studying omnivorous animals who may have different diet choices based on season, $\delta^{15}N$ values might illuminate when the animals are more carnivorous (elevated $\delta^{15}N$) and when they are more herbivorous (lower $\delta^{15}N$). However, Bergstrom (2013) failed to find a clear link between degree of carnivory and $\delta^{15}N$. Thus, the value of $\delta^{15}N$ for predicting trophic position may be limited.

Another potential measure of trophic position is C:N fecal sample ratios, which may reflect an animal's degree of carnivory. For example, a lower ratio (that is, a larger N number) may indicate higher protein content of foods consumed (other animals or high-quality plant forage), whereas a higher ratio may indicate foods with lower protein content (Bergstrom 2013).

This study focused on seasonal diet variation of the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) from a site in southwestern Michigan. This species is a diurnal squirrel (Fitzpatrick 1923) with an average lifespan of only a few years at maximum; Streubel and Fitzgerald (1978) reported that juvenile mortality rates can be upwards of 80-90%.  They are obligate hibernators and, depending on age and sex, will enter hibernation between July and November; emergence from hibernation typically occurs in March to April with males emerging before the females (Kisser and Goodwin 2012). Sexual maturity is reached at one year of age (Schwagmeyer 1984). Thirteen-lined ground squirrels range widely across central North America from Texas to Canada and Michigan to Utah (Streubel and Fitzgerald 1978). Their preferred habitat is the shortgrass prairie, though sometimes they will inhabit tallgrass prairie as well (Clark *et al*. 1992). As an omnivore (Whitaker 1972), the thirteen-lined ground squirrel fills the niche of a primary/secondary consumer. This species also serves as food to organisms higher on the food chain such as bull snakes or roadrunners (Streubel and Fitzgerald 1978).

Thirteen-lined ground squirrels are more omnivorous than most ground squirrels (Whitaker 1972). Prior studies using stomach-content analysis have documented how the diet of this species varies with season and location. For example, Fitzpatrick (1923) reported that thirteen-lined ground squirrels in Iowa consumed a higher proportion of insects in autumn compared to spring. Grasshoppers were found to be the primary insect of choice in autumn. Seeds from plants such as rag weed (*Ambrosia artemisifolia*) or hemp weed (*Cannabis* spp.) were consumed throughout the seasons, with clover being preferred when available. Flake (1973), studying thirteen-lined ground squirrel diet on a natural shortgrass prairie, showed consistent arthropod consumption over the seasons; some insect groups were consumed consistently, while others were more highly selected for in a certain season. He also reported that the ground squirrels preferred the fungus *Endogone* over other fungi and mosses during the late spring and early summer. Whitaker (1972), studying thirteen-lined ground squirrels in human-modified habitats in Indiana, found chickweed seeds and fruit to make up the key part of diet in the spring. Insects and clover played fairly equal dietary roles in the summer, and grasses and grasshoppers dominated in the fall. Despite differences in locations, all studies showed the squirrels consuming insects during the fall.

Prior work in the Goodwin lab suggested that thirteen-lined ground squirrels in southwestern Michigan (Rosehill Cemetery, Berrien Springs) underwent a sharp diet change in late summer from a predominantly C3 diet to a predominantly C4 diet. This inference was based on a prominent spike in the $\delta^{13}C$ of incisor enamel deposited in mid-August to mid-September, prior to entry into hibernation (Jang *et al.* 2012, Brassington *et al.* 2013). However, the identity of the C4 material was not determined; hypothesized sources included direct consumption of C4 plants, or consumption of insects who fed on C4 plants.

This study used microhistological and isotopic analyses of fecal samples collected June-October, 2018. The $\delta^{13}C$ analysis tested if the squirrels in Rosehill Cemetery in 2018 also underwent a late-season diet shift from predominantly C3 to predominantly C4 plants, as compared to diet shift previously inferred for the same colony based on $\delta^{13}C$ of incisor enamel; whereas the analysis of $\delta^{15}N$ and C:N sought to shed light on seasonal shifts in protein quality, likely reflecting degree of carnivory. Finally, the microhistological analysis sought to ascertain with greater precision which C3 and C4 plants the squirrels were eating, and the relative abundance of food types (including plants and arthropods), from June to October. This is the first non-lethal study of diet variation in this species.

## Materials and Methods

### Squirrel Capture and Handling

Squirrels were captured between 12 June and 18 October 2018 at Rosehill Cemetery in Berrien Springs, Michigan (N 41º 55.942, W 86º 20.459). The site is bordered on the east by the St. Joseph River; however, this study used the western portion of the cemetery. The most northern and southern thirds of cemetery used in this study were predominantly open fields with few gravestones and trees. The central third featured a number of trees and gravestones. The grass was mown approximately once a week; during this process squirrels remained underground. Disturbances from cars, and to a lesser extent the movement of cemetery patrons on foot, also occurred regularly.

Initially metal Sherman traps (LFA Folding Live Capture Rodent/Rat/Mouse Trap, H. B. Sherman, Tallahassee, Florida) were used, but few squirrels entered the traps. Thus, home-made metal mesh traps (18”x6”x6” in) with a design that increased chances of capturing a squirrel were used instead. A free-moving flap was hung at one end of the trap, and was directly above a ~ 4x4 inch opening in the bottom of the trap. This arrangement allowed a squirrel to enter the trap from its burrow below by pushing up the flap, but not return to its burrow as the flap closes behind it. The squirrel then was confined within the trap, which in some cases had an attached mesh bag (Fig. 1). A tray was slid underneath the trap and bag, to collect fecal pellets from the entrapped squirrel.

Once a squirrel was observed to enter a particular burrow, a trap was placed over the burrow with the opening of the trap directly over the burrow it entered. The trap was staked to the ground to keep it from moving. Traps were checked every 20-30 minutes for squirrel captures, or if the trap was vacant for 30-60 minutes, it was moved to a more active burrow. If capturing on a particularly hot day, captured squirrels were moved in their bags into the shade to prevent overheating.

Captured squirrels were handled and released within 30 minutes of initial capture. Each squirrel was sexed and weighed to the nearest gram with a hand-held digital scale. Additionally, GPS coordinates were recorded for each capture location. A Jiffy 593 ear tag (National Band & Tag Co.) was then attached with plyers to the left or right ear of the squirrel before release. At least two of the squirrels over the season lost ear tags (based on a torn ear), so these squirrels were retagged on a subsequent capture. After August 31, captured squirrels were not ear tagged due to lack of an assistant needed for the process.

Finally, fecal pellets were collected for each squirrel if deposited during capture and handling. The pellets were then transferred into vials, labeled, and frozen for later analyses.

Protocol for capture and handling was approved by the Andrews University IACUC. Permit for collection was obtained from the Michigan DNR.

## Preparing Samples for Analysis

Fecal samples were sent to two labs, the Micro Composition Laboratory (MCL) in Broomfield, Colorado for microhistological investigations, and the Stable Isotope Ratio Facility for Environmental Research (SIRFER) lab at the University of Utah (Salt Lake City, Utah) for carbon (C) and nitrogen (N) isotope analysis. Before any samples were sent to either lab, they were first dried for 8-10 hours in an oven at 70°C. Samples sent to MCL were placed whole in vials and labeled prior to shipment. Samples sent to SIRFER underwent further preparation prior to shipment. First, the samples were ground into fragments with mortar and pestle. Then 3-4 mg of each pulverized sample was funneled into a 1.5 ml microcentrifuge tube prior to being labeled and readied for transport. Unfortunately, a number of microcentrifuge tubes initially sent to SIRFER opened during transport, resulting in cross-contamination. Thus, a duplicate set of fecal samples was sent to SIRFER. Due to low stock of fecal samples, we were unable to provide duplicates for two squirrels in this second set. The number of samples per month sent for microhistological analysis ranged from 4-8; and the number of samples sent for isotope analysis ranged from 3-8 (Table 1). The number of samples sent to each lab was smaller in number than the total number of squirrels captured each month because of funding limitations.

## Laboratory Analyses

Microhistological analysis was conducted by the Micro Composition Laboratory following the protocol of Sparks and Malechek (1968). The fecal pellets were ground over a 1-mm mesh screen. The remaining pieces were mounted on a microscope slide using Hertwig’s and Hoyer’s solutions. After drying the slides in an oven at 60°C, the analyst scanned 20 distinct fields of view at 125-power magnification. When a potential food item was found, a reference collection was consulted to confirm the identification. Relative abundance of a given source was calculated as the number of fields with target food item / total number of scans (always 20).

Isotopic analysis, such as conducted at SIRFER, typically first involves homogenizing and weighing into tin cups (3x5x5 mm). The organic samples are then inserted into an analyzer where they are converted to gas through combustion or pyrolysis. Next, the gases ($N_2$ and $CO_2$) are separated and enter the mass spectrometer. After ionization, the gases are deflected based on mass through contact with a magnet. The amount of magnet-driven deflection is directly related to the isotope mass; relative abundance of each isotope species is then used to determine the standardized isotope ratios (Ben-David and Flaherty 2012).

## Plant Collection

Plant specimens were collected opportunistically from areas of the study where squirrels had been observed.  To facilitate identification, plants were collected when in flower or with seed heads. Specimens were then pressed and dried for 24-48 hours prior to mounting. Identification was completed with *Field Manual of Michigan Flora* (Voss and Reznicek 2012), and the occasional assistance of Ralph Reitz, botanist at Fernwood Botanical Gardens (Niles, Michigan).

## Graphical and Statistical Analyses

IBM SPSS Statistics 25 was used to visually display and test the statistical significance of relationships among relevant variables (mass, relative abundance of key food types, isotope and C:N values, date). Body mass (g) and standardized isotope ratios

($\delta^{13}$C, $\delta^{15}$N) were not transformed prior to graphical and statistical analyses. Relative abundance of food types was calculated as ($N_{food}/N_{total}$) x 100, where $N_{food}$ equals the number of microscope scans with the given food type and $N_{total}$ was the total microscope scans (always 20). To facilitate graphical comparisons across potential measures of degree of carnivory (relative dietary abundance of arthropods, $\delta^{15}$N, relative abundance of N compared to C), the C:N ratio was expressed as 1/C:N. Thus, as the relative abundance of N increased, the value of 1/C:N also increased.

# **Results**

## Squirrel captures

Fifty-three squirrel captures were made between June and October 2018. Between June and August, 41 squirrel captures occurred; 6 of these captures were known recaptures. Fecal samples were collected during 33 of the captures. Between September and October, 12 captures were made with 2 being known recaptures. Fecal samples were collected during 10 of these captures (Table 1). Out of the fecal samples sent to the labs for isotopic and microhistological analysis, these captures represented at least 25 individuals and 1 individual who was recaptured once.

All squirrels were captured within a 2.4-hectare plot within Rosehill Cemetery (Fig. 2). Most captures in June and July occurred within the middle and southern one-third of the study plot, whereas most captures in August and later occurred within the middle and northern one-third of the study plot. Thus, capture locations generally shifted to the north as the season progressed.

Overall, body masses of captured squirrels decreased and then increased as the season progressed (Fig. 3), possibly because of capturing mature adults early in the season, then the young juveniles, and finally the now more mature juveniles who would have reached their parents' weights. During July, juveniles were determined as those squirrels who weighed < 75 g; no juveniles were captured in June. In August and later, all captured squirrels except a notably heavy individual were interpreted to be juveniles (Fig. 3). Males were underrepresented early in the season (June-July) but overrepresented late in the season (August-October); the seasonal shift in frequency of males and females was significant ($X^2(1) = 10.43$, $p < 0.05$).

## Food items identified in study plot and fecal samples

Sixteen plant species were collected and identified in the study plot, representing 14 genera and 7 families (Table 2). Of note, two of the three C4 grasses - Indian goosegrass (*Eleusine indica*) and Malabar sprangetop (*Leptochloa fusca*) - were collected from the study plot in September. The other C4 grass, Bermuda grass (*Cynodon dactylon*), as well as all other plants were collected in June or July.

Fifteen categories of food were identified microhistologically in fecal samples (Table 3; see Appendix 1 for details). Members of the clover genus *Trifolium*, a C3 plant, were consumed by squirrels throughout the five-month study season, although species-level consumption of *Trifolium* shifted mid-season (Table 3). Other C3 plants, including two C3 grasses, bluejoint grass (*Calamagrostis canadensis)* and fescues (*Festuca* sp.), were documented in fecal pellets from June to August. In contrast, known C4 plants (sedges, *Leptochloa pretense*) were documented in fecal samples from August to October (Table 3).

Notable shifts in relative consumption were observed for two food categories. Arthropod cuticle was relatively abundant in fecal samples in June and July, but decreased sharply in abundance in August and onwards (Table 3; Fig. 4A). In contrast, grass seeds and glumes were absent in fecal samples from June, absent to present at low frequency in July, but abundant in most fecal samples from August and thereafter (Table 3; Fig. 5A).

## Relationships between diet shifts reflected in microhistological and isotopic data

The seasonal decrease in dietary abundance of arthropods documented microhistologically (Table 3; Fig. 4A) was accompanied by a significant, but less dramatic, reduction in $\delta^{15}N$ (Fig. 4B) as well as a decrease in percent N compared to percent C (1/C:N; Fig. 4C). Early-season individuals with elevated $\delta^{15}N$ and relatively high N content of feces were primarily adults, whereas late-season individuals with the lower $\delta^{15}N$ and N content were primarily juveniles (Fig. 4B-C). Both $\delta^{15}N$ and N content (1/C:N) were positively associated with relative dietary abundance of arthropods (Fig. 6A-B); the latter association was especially strong ($r^2=0.65$, $p<0.01$; Fig. 6B).

The seasonal increase in dietary abundance of grass seeds and glumes (Table 3; Fig. 5A) was paralleled by an equally sharp increase in $\delta^{13}C$ (Fig. 5B). Not surprisingly, $\delta^{13}C$ was strongly associated with relative dietary abundance of grass seeds and glumes ($r^2=0.88$, $p<0.01$, Fig. 7). The latter observation indicates that the grasses consumed heavily in August and later were mostly or exclusively C4 grasses.

Three individuals deserve additional comment. The first, captured 30 September 2018, was an adult with substantially greater body mass than other late season squirrels and interpreted to be the only late-season adult (Fig. 3). It was also the only late-season (September—October) individual that consumed no grass seeds and glumes (Fig. 5A) and that exhibited fecal $\delta^{13}C < -25.0$, indicating a C3 diet (Fig. 5B). The second individual was the only inter-month recapture of the season; it was first captured on 22 August 2018, and next on 28 September 2018. Between the two capture dates this individual gained on average 1.08 g/day (Fig. 3). Its $\delta^{15}N$ values closely followed the overall line of best fit (Fig. 4B). This individuals' 1/C:N and relative abundance of grass seeds and glumes values ran counter to the line of best fit (Figures 4C, 5A). The third individual was a female captured 18 October 2018, who had noticeably lower body mass (42 g) than other late season individuals interpreted to be juveniles (Fig. 3). This squirrel consumed a high quantity of grass seeds and glumes (Fig. 5A).

## Discussion

The thirteen-lined ground squirrels in this study showed two main seasonal shifts in their diet, as indicated by the microhistological data. First, the squirrels decreased their arthropod intake as the seasons progressed from summer to fall (Table 3; Fig. 4A). Secondly, there was an abrupt late-season shift (in August) in consumption of grass seeds and glumes that persisted through the rest of the study season; earlier in the season the squirrels ate very few grass seeds and glumes (Table 3; Fig. 5A).

The isotopic and C:N ratio data were congruent with the microhistological data. While not as prominent, there was a steady decrease in $\delta^{15}$N values (Fig. 4B) as well as a decrease in total N (Fig. 4C) as the season progressed, in both cases consistent with a reduction in consumption of arthropods through the study season. Similarly, there was also a late-season increase in $\delta^{13}$C values (Fig. 5B), mirroring closely the increase in consumption of grass seeds and glumes (Fig. 5A), indicating a shift from primarily C3- to mixed or C4-dominated diets during the study. These correlations suggest that a) the squirrels had a nitrogen-rich diet supplied by abundant arthropods early in the season, and that b) the grass seeds and glumes consumed late in the season were from C4 plants.

The dietary shift from predominantly C3 material to C4 material, implied by the microhistological data and confirmed by the isotopic data, aligns with expectations from previous work in the Goodwin lab. This expectation was based on a prominent spike in the $\delta^{13}$C of incisor enamel deposited in mid-August to mid-September, 2007, prior to entry into hibernation, a spike interpreted to indicate a transient shift from C3- to C4-based diets late in the active season (Jang *et al.* 2012, Brassington *et al.* 2013). This correspondence between the results presented here (from 2018) and those obtained from incisor enamel (from 2007; Jang *et al.* 2012, Brassington *et al.* 2013) confirm that the thirteen-lined ground squirrels at Rosehill Cemetery in southwestern Michigan do indeed undergo a late-season shift from C3 to C4 plant consumption and suggest that this shift occurred repeatedly.

The Michigan squirrels' arthropod consumption pattern, peaking in summer and decreasing in the fall, differs with findings of prior studies of thirteen-lined ground squirrel diet. For example, Flake (1973) found that thirteen-lined ground squirrels in Colorado ate almost twice as much animal matter in the late summer and early fall as in the spring. Grasshoppers were the insect of choice in late summer to early autumn. Late-season increase in grasshopper consumption was also demonstrated for thirteen-lined ground squirrels in Iowa (Fitzpatrick 1923) and Indiana (Whitaker 1972). Given that grasshoppers have some chitinous cuticle that should preserve in feces, the rarity or absence of arthropod cuticle in late-season feces at our site seems surprising. It is unclear whether insect populations were down during this time or if there were other factors involved. One factor might be the type of habitat studied. However, both Whitaker (1972) and this present study considered thirteen-lined ground squirrels in manmade habitats such as hayfields, golf courses, or cemeteries; thus, habitat type does seem to be an adequate explanation for the late season reduction in arthropod consumption.

The abrupt decrease in arthropod consumption from June to October was mirrored by a slight decrease in $\delta^{15}$N, consistent with the hypothesis that $\delta^{15}$N is enriched up the trophic level (Minagawa and Wada 1984). However, due to the multiple factors that influence $\delta^{15}$N, one must be cautious in directly linking $\delta^{15}$N with trophic position.

Bergstrom (2013) found that $\delta^{15}N$ did not mirror degree of carnivory. For example, he found that the most herbivorous rodent in his study, the African grass rat (*Arvicanthis niloticus*), had the highest $\delta^{15}N$ of all the species he studied whereas the fringe-tail gerbil (*Gerbilliscus robustus*), the most carnivorous rodent in the study, only had an intermediate $\delta^{15}N$ value. These findings indicated that at least in his study, $\delta^{15}N$ value did not track degree of carnivory.

The late-season increase in C4 plant consumption that we observed is consistent with the findings of Whitaker (1972). He found thirteen-lined ground squirrels to consume high quantities of chickweed seeds (a C3 plant) in the spring; but squirrels showed a dramatic late-season increase in consumption of seeds of crabgrass (*Digitaria* sp.), a C4 plant. However, this seasonal dietary shift from C3 to C4 plants was not observed in all studies. For example, Flake (1973) observed more or less consistent consumption of grasses and sedges (some C3, some C4) throughout the season.

It is unclear whether the dietary shifts documented in this study were opportunistic or selective. However, anecdotal evidence suggests that the late-season increase in grass seed and glume consumption may be selective for fattening in preparation for hibernation. For example, consider the late-season adult, which had the highest body mass (168 g) of all squirrels captured in our study (Fig. 3) and was the only late-season individual that consumed no grass seeds and glumes (Fig. 5A), perhaps suggesting that this individual had achieved needed pre-hibernation body mass and no longer needed to fatten. Although speculative, this interpretation is consistent with weight gain and dietary shift demonstrated by the juvenile captured in both August and September: this individual gained ~50 g in body mass between August 22 and September

28, becoming the second heaviest squirrel (121 g) late in the active season (Fig. 3); and it demonstrated a simultaneous, dramatic decrease in consumption of grass seeds and glumes (Fig. 5B). One of the most important jobs for a juvenile ground squirrel is to grow and fatten for the winter ahead (Morton *et al*. 1974). Perhaps these individuals had reached (the adult) or were approaching (the juvenile) a fattening threshold of sorts, and would receive no further benefit from continued consumption of calorie-rich grass seeds and glumes. Under this interpretation, both of these individuals would then be taking the selective approach to their diet.

As thirteen-lined ground squirrels are obligate hibernators that must achieve appropriate body mass prior to entry into hibernation, their dietary strategy in the later portion of the active season is undoubtedly under selection. One possible outcome of increased grass seed consumption during the late season is increased fat stores, which increase chances of a successful hibernation. Clearly, grass seeds provide high nutritional value which would increase fattening. In addition, prior studies indicate that thirteen-lined ground squirrels consume high quantities of arthropods late in the season, a food source that is also high in nutritional value (Fitzpatrick 1923, Whitaker 1972, Flake 1973). Thus, one of the results of this study – low to no consumption of arthropods late in the season – is puzzling in light of prior studies and the high nutritional value that arthropods offer. However, no studies at the present time have looked into this facet of the thirteen-lined ground squirrel life cycle. The study of why ground squirrels in one colony would apparently ignore a major food source that usually the species consumes in high quantities during the late active season is worthy of further investigation.

One piece of circumstantial evidence that this study shows regarding the interplay of food consumption and season is the low-weight female caught in late October (Fig. 3). Compared to the other individuals interpreted to be juveniles at this point in the season, this squirrel had a weight that was much lower than anticipated. Thus, two hypotheses develop: 1) that this squirrel was the sole survivor of a second, late summer litter; or 2) this is an extreme example of a squirrel exhibiting inadequate growth. Given that this individual consumed abundant grass seeds and glumes (relative abundance value of 75%), it seems doubtful that this squirrel was displaying inadequate growth. From these data, the second litter hypothesis seems more likely. If this is true, the present study would be the first to record this phenomenon in the northern portion of this species' range; previously, McCarley (1966) had only observed this behavior in Texas.

Limitations of this study include the constraint of only having summer and fall data; due to constraints of permitting, no spring data were collected in time to include in this thesis. Thus, when comparing my results with prior studies, we are unable to assess spring diets; future work will shed light on spring diets. However, the motivation for the present study was the previously documented late-season dietary shift inferred from $\delta^{13}C$ of tooth enamel, and my study spanned the appropriate time frame for determining the basis of this dietary shift.

An additional limitation to my study is that I only collected voucher plant specimens on a single date for each plant species recorded on the study site and did not record the relative abundance of each species. Thus, in analysis, I was unable to compare what was observed on the study plot each month to what was actually consumed based on microhistological data. Future work could fill in this gap.

In summary, prior research of $\delta^{13}C$ in incisor enamel had suggested that thirteen-lined ground squirrels in southwest Michigan exhibited a late-season spike in C4 plant consumption but did not identify the source of this dietary shift (Jang *et al.* 2012, Brassington *et al.* 2013). The research presented in this paper has confirmed this dietary shift through microhistological and $\delta^{13}C$ analysis: in August, squirrels began to heavily consume grass seeds and glumes, with $\delta^{13}C$ demonstrating that this late-season diet was enriched in C4 plants. In addition, this study demonstrated a dramatic, but previously unknown shift in arthropod consumption: from high consumption of arthropods in June, to low if any consumption of arthropods late in the season. This trophic shift was further supported by a sharp decrease in N content of diet (consistent with reduction of high-protein animal matter in the diet) and was also consistent with seasonal reduction in $\delta^{15}N$, although the latter may be influenced by factors other than trophic position.

## Literature Cited

Ben-David M, Flaherty EA. 2012. Stable isotopes in mammalian research: a beginner's guide. Journal of Mammalogy. 93(2):312-328.

Bergstrom BJ. 2013. Would East African savanna rodents inhibit woody encroachment? Evidence from stable isotopes and microhistological analysis of feces. Journal of Mammalogy. 94(2):436-447.

Brassington JA, Chacko AE, Jang WJ, Kisser B, Passey BH, Goodwin HT. 2013. Seasonal and spatial variation in diet recorded by $\delta^{13}C$ of incisor enamel of free-ranging thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*). American Society of Mammalogists, Philadelphia, PA.

Clark BK, Kaufman DW, Kaufman GA, Gurtz SK, Bixler SH. 1992. Population ecology of thirteen-lined ground squirrels in ungrazed tallgrass prairie manipulated by fire. Proceedings of the twelfth North American Prairie Conference; 1990; Cedar Falls, Iowa. University of Northern Iowa. p. 51-54

Ehleringer JR, Sage RF, Flanagan LB, Pearcy RW. 1991. Climate change and the evolution of C4 photosynthesis. Trends in Ecology & Evolution. 6(3):95-99.

Epstein H, Lauenroth W, Burke I, Coffin D. 1997. Productivity patterns of C3 and C4 functional types in the US Great Plains. Ecology. 78(3):722-731.

Fagerstone KA, Tietjen HP, Williams O. 1981. Seasonal variation in the diet of black-tailed prairie dogs. Journal of Mammalogy. 62(4):820-824.

Fitzpatrick FL. 1923. The ecology and economic status of *Citellus tridecemlineatus tridecemlineatus* (Mitchill) in Iowa. [Iowa City]: University of Iowa.

Flake LD. 1973. Food habits of four species of rodents on a short-grass prairie in Colorado. Journal of Mammalogy. 54(3):636-647.

Hobbie EA, Shamhart J, Sheriff M, Ouimette AP, Trappe M, Schuur EA, Hobbie JE, Boonstra R, Barnes BM. 2017. Stable isotopes and radiocarbon assess variable importance of plants and fungi in diets of arctic ground squirrels. Arctic, Antarctic, and Alpine Research. 49(3):487-500.

Hwang Y, Millar J, Longstaffe F. 2007. Do $\delta^{15}$N and $\delta^{13}$C values of feces reflect the isotopic composition of diets in small mammals? Canadian Journal of Zoology. 85(3):388-396.

Jang WJ, Goodwin HT, Passey BH, Kisser B. 2012. Stable isotope analysis of incisor enamel in thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*); in Michigan Academy of Science, Arts Letters, Alma College, Alma, MI.

Khanam S, Howitt R, Mushtaq M, Russell JC. 2016. Diet analysis of small mammal pests: A comparison of molecular and microhistological methods. Integrative Zoology. 11(2):98-110.

Kincaid WB, Cameron GN. 1982. Dietary variation in three sympatric rodents on the Texas coastal prairie. Journal of Mammalogy. 63(4):668-672.

Kisser B, Goodwin HT. 2012. Hibernation and overwinter body temperatures in free-ranging thirteen-lined ground squirrels, *Ictidomys tridecemlineatus*. The American Midland Naturalist. 167(2):396-409.

Leitner BM, Leitner P. 2017. Diet of the Mohave ground squirrel (*Xerospermophilus mohavensis*) in relation to season and rainfall. Western North American Naturalist. 77(1):1-13.

McCarley H. 1966. Annual cycle, population dynamics and adaptive behavior of *Citellus tridecemlineatus*. Journal of Mammalogy. 47(2):294-316.

Minagawa M, Wada E. 1984. Stepwise enrichment of $^{15}$N along food chains: further evidence and the relation between $\delta^{15}$N and animal age. Geochimica et Cosmochimica Acta. 48(5):1135-1140.

Morton ML, Maxwell CS, Wade CE. 1974. Body size, body composition, and behavior of juvenile Belding ground squirrels. The Great Basin Naturalist.121-134.

Ode DJ, Tieszen LL, Lerman JC. 1980. The Seasonal Contribution of C3 and C4 Plant Species to Primary Production in a Mixed Prairie. Ecology. 61(6):1304-1311.

Ritchie ME. 1988. Individual variation in the ability of Columbian ground squirrels to select an optimal diet. Evolutionary Ecology. 2(3):232-252.

Schwagmeyer PL. 1984. Multiple Mating and Intersexual Selection in Thirteen-Lined Ground Squirrels. In: Murie J, Michener G, editors. The Biology of Ground-Dwelling Squirrels. Lincoln: University of Nebraska Press. p. 275-293.

Sparks DR, Malechek JC. 1968. Estimating percentage dry weight in diets using a microscopic technique. Rangeland Ecology & Management/Journal of Range Management Archives. 21(4):264-265.

Sponheimer M, Robinson T, Ayliffe L, Passey B, Roeder B, Shipley L, Lopez E, Cerling T, Dearing D, Ehleringer J. 2003. An experimental study of carbon-isotope fractionation between diet, hair, and feces of mammalian herbivores. Canadian Journal of Zoology. 81(5):871-876.

Streubel DP, Fitzgerald JP. 1978. *Spermophilus tridecemlineatus*. Mammalian Species. (103):1-5.

Van Horne B, Schooley RL, Sharpe PB. 1998. Influence of habitat, sex, age, and drought on the diet of Townsend's ground squirrels. Journal of Mammalogy. 79(2):521-537.

Vispo CR, Bakken GS. 1993. The influence of thermal conditions on the surface activity of thirteen-lined ground squirrels. Ecology. 74(2):377-389.

Voss EG, Reznicek AA. 2012. Field manual of Michigan flora. Ann Arbor: University of Michigan Press.

Whitaker JO. 1972. Food and external parasites of *Spermophilus tridecemlineatus* in Vigo County, Indiana. Journal of Mammalogy. 53(3):644-648.

Xu Q, Ding L, Zhang L, Yang D, Cai F, Lai Q, Liu J, Shi R. 2010. Stable isotopes of modern herbivore tooth enamel in the Tibetan Plateau: Implications for paleoelevation reconstructions. Chinese Science Bulletin. 55(1):45-54.

# Tables

**Table 1**. List of squirrel captures, sorted by month, including data on known recaptures and how many samples were sent to each lab for analyses.

| | June | July | August | September | October |
|---|---|---|---|---|---|
| Squirrel captures | 17 | 11 | 13 | 5 | 7 |
| Known recaptures | 2 | 0 | 4 | 1 | 1 |
| Samples for MCL | 8 | 8 | 8 | 4 | 6 |
| Samples for SIRFER | 8 | 8 | 8 | 3 | 5 |

**Table 2.** List of plants, along with their carbon fixation pathway, that were collected at the field site. Organized first by monocot/dicot, then alphabetically by family and species.

| **Family** | **Species** | **C3 or C4** | **Date of Collection** |
|---|---|---|---|
| Monocots | | | |
| Poaceae | *Cynodon dactylon* | C4 | 04 JUN 18 |
| Poaceae | *Calamagrostis canadensis* | C3 | 20 JUN 18 |
| Poaceae | *Eleusine indica* | C4 | 10 SEP 18 |
| Poaceae | *Festuca trachyphylla* | C3 | 04 JUN 18 |
| Poaceae | *Leptochloa fusca* | C4 | 10 SEP 18 |
| Poaceae | *Phalaris arundiancea* | C3 | 20 JUN 18 |
| Poaceae | *Poa pratensis* | C3 | 01 JUN 18 |
| Dicots | | | |
| Euphorbiaceae | *Euphorbia* sp. | C3 or C4 | 20 JUN 18 |
| Fabaceae | *Medicago lupulina* | C3 | 01 JUN 18 |
| Fabaceae | *Trifolium pratense* | C3 | 23 JUL 18 |
| Fabaceae | *Trifolium repens* | C3 | 01 JUN 18 |
| Oxalidaceae | *Oxalis dillenii* | C3 | 15 JUN 18 |
| Oxalidaceae | *Oxalis stricta* | C3 | 01 JUN 18 |
| Plantaginaceae | *Plantago lanceolata* | C3 | 23 JUL 18 |
| Polygonaceae | *Rumex acetosella* | C3 | 04 JUN 18 |
| Rubiaceae | *Sherardia arvensis* | C3 | 04 JUN 18 |

**Table 3**. List of squirrel food items as determined through microhistological methods by MCL and filtered by month. Plant taxa (family and species) that were collected and identified on the study plot marked with an asterisk (*). Relative abundance of food items per month determined by the average percent relative abundance of that food item (number of scanned fields with that food item/total scanned fields) across all fecal samples for that month: x=0-25%, xx=25.1-50%, xxx=>50%, and -- = not found.

| **Family** | **Species** | **C3 or C4** | **Jun** | **Jul** | **Aug** | **Sept** | **Oct** |
|---|---|---|---|---|---|---|---|
| Unknown | Arthropods | --- | xxx | xx | x | x | -- |
| <u>Monocots</u> | | | | | | | |
| Cyperaceae | Sedge | C4 | -- | -- | x | x | x |
| Poaceae | Grass seeds & glumes | C3 or C4 | -- | x | xxx | xxx | xxx |
| Poaceae | *Calamagrostis canadensis** | C3 | x | x | x | -- | -- |
| Poaceae | *Festuca** sp. | C3 | x | x | x | -- | -- |
| Poaceae | *Leptochloa* pretense* | C4 | -- | -- | -- | x | -- |
| Poaceae | Unknown grass I | --- | -- | -- | -- | x | x |
| <u>Dicots</u> | | | | | | | |
| Fabaceae | *Trifolium** | C3 | -- | -- | x | x | x |
| Fabaceae | *Trifolium pratense** | C3 | -- | -- | x | xx | x |
| Fabaceae | *Trifolium repens** | C3 | xx | xx | x | -- | -- |
| Oxalidaceae | *Oxalis** | C3 | x | x | -- | -- | -- |
| Oxalidaceae | *Oxalis dillenii** | C3 | x | -- | x | -- | -- |
| Pinaceae | *Pinus* | C3 | x | x | -- | -- | -- |
| Plantaginaceae | *Plantago** and *Plantago* seed | C3 | -- | x | -- | -- | -- |
| Unknown | Unknown forb I | --- | x | x | x | -- | -- |

# Figures

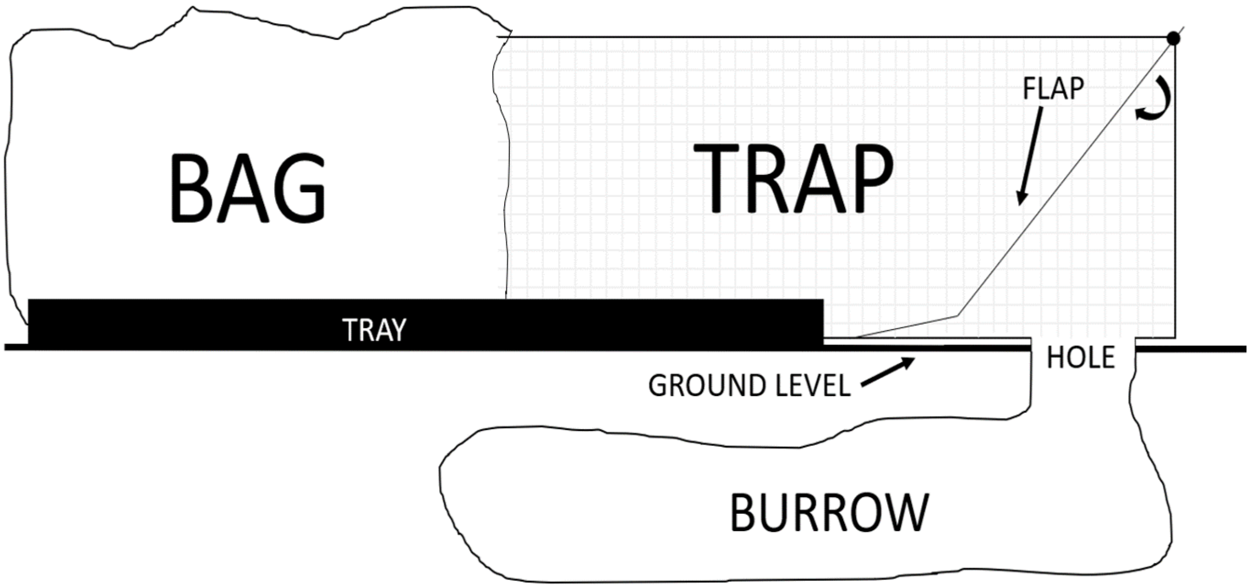

**Figure 1**. The setup used for trapping thirteen-lined ground squirrels. The flap (far right-hand side of trap) is just above the hole which let the squirrels into the trap. The wire mesh (not depicted) on the far-left side of the trap was removed to allow the squirrels access to the mesh bag. The plastic tray was slipped under this entire apparatus to collect any fecal pellets that the squirrel defecated while in the trap. Stakes, not visible, were slipped through the trap mesh not covered by the plastic tray to keep the trap stationary.

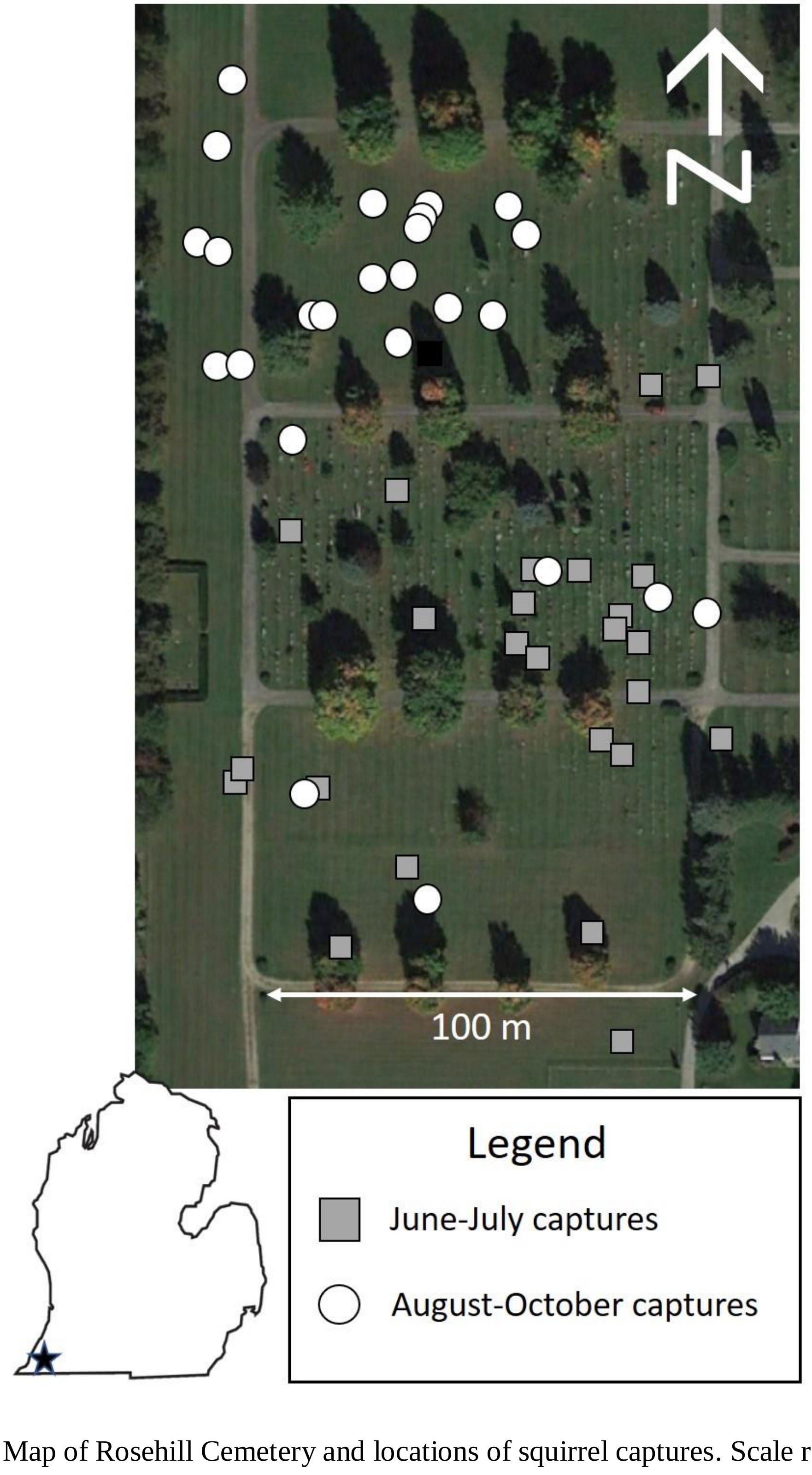

**Figure 2**. Map of Rosehill Cemetery and locations of squirrel captures. Scale represents 100 m. The star indicates location of Rosehill Cemetery in southwest Michigan.

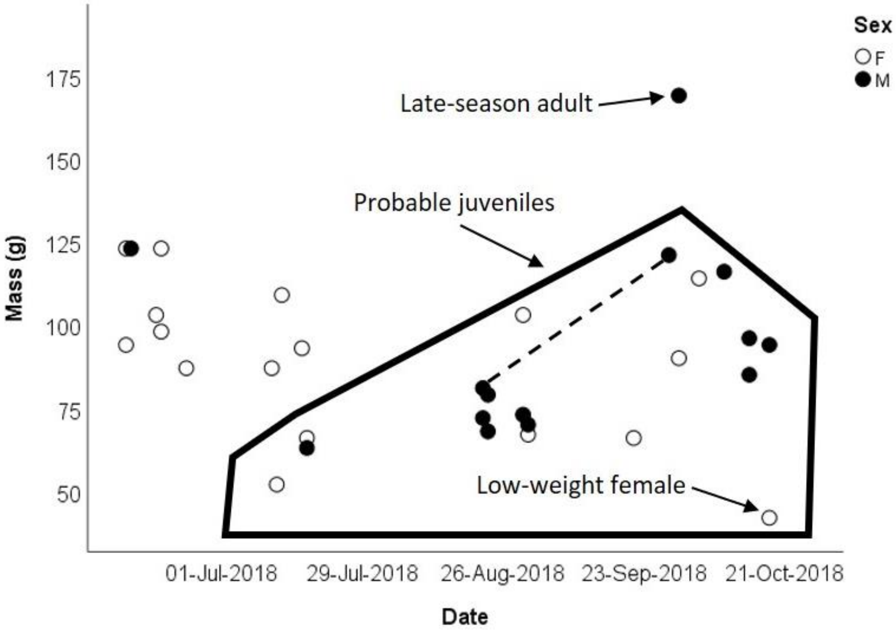

**Figure 3**. Body mass of captured squirrels plotted by date, and separated by gender. Probable juveniles (based on body mass) enclosed within polygon. A juvenile, for the purposes of this study, was described as an individual who was < 75 g when captured in July 2018, or of any weight when captured in August 2018 or later (note exception of 175-g male captured late Sept.). The dashed line connects the 22 Aug 18 and 28 Sep 18 capture weights of the same individual, and a low-weight female is also indicated.

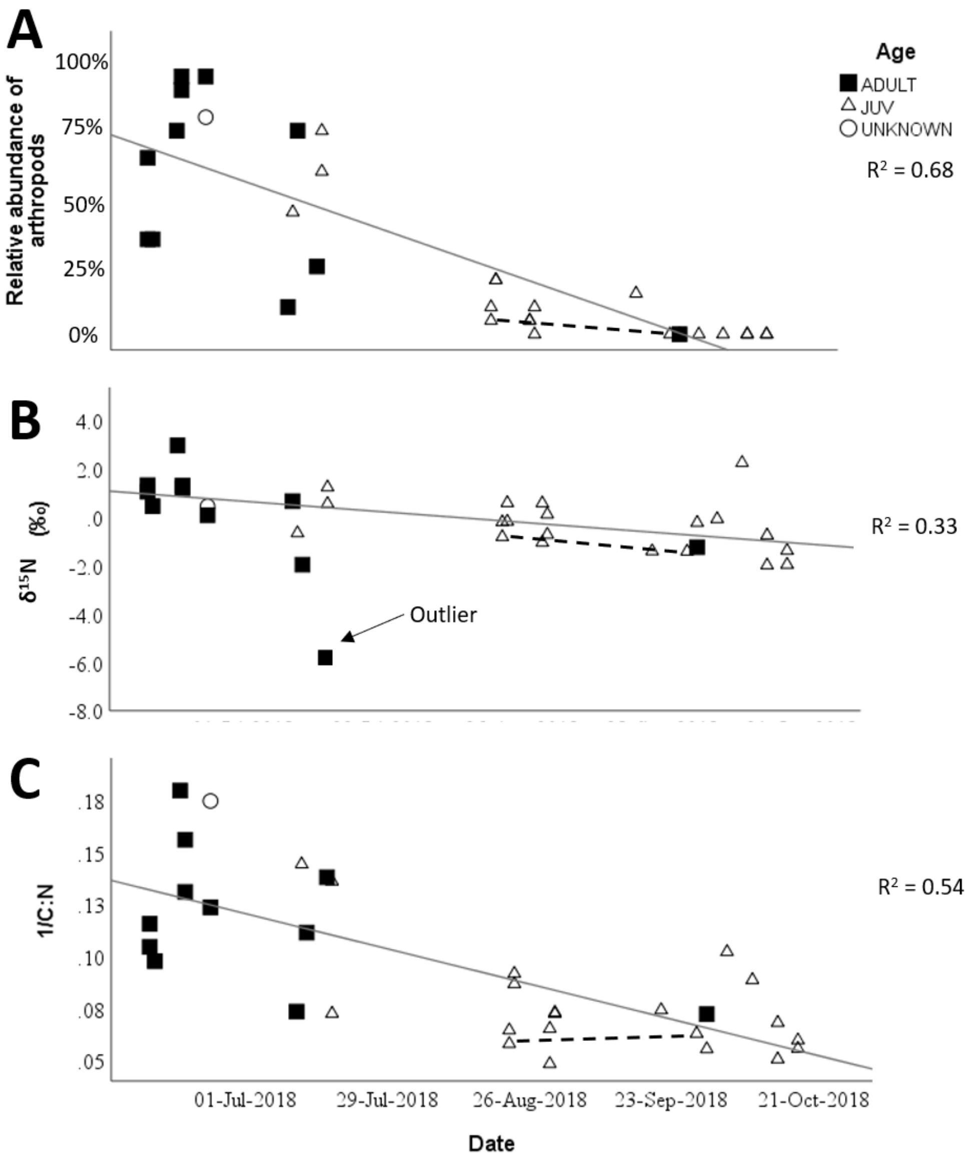

**Figure 4**. Scatterplots of microhistological and isotopic data plotted against seasonal progression. Specifically - A) arthropod consumption, B) $\delta^{15}$N values, and C) 1/C:N against date, sorted by age of individual (A: r=0.82, n=32, p<0.01; B: r=0.57, n=32, p<0.05; C: r=0.73, n=32, p<0.01). Outlier noted in B) was not included in statistical analysis. The dashed line connects the 22 Aug 18 and 28 Sep 18 values of the same individual.

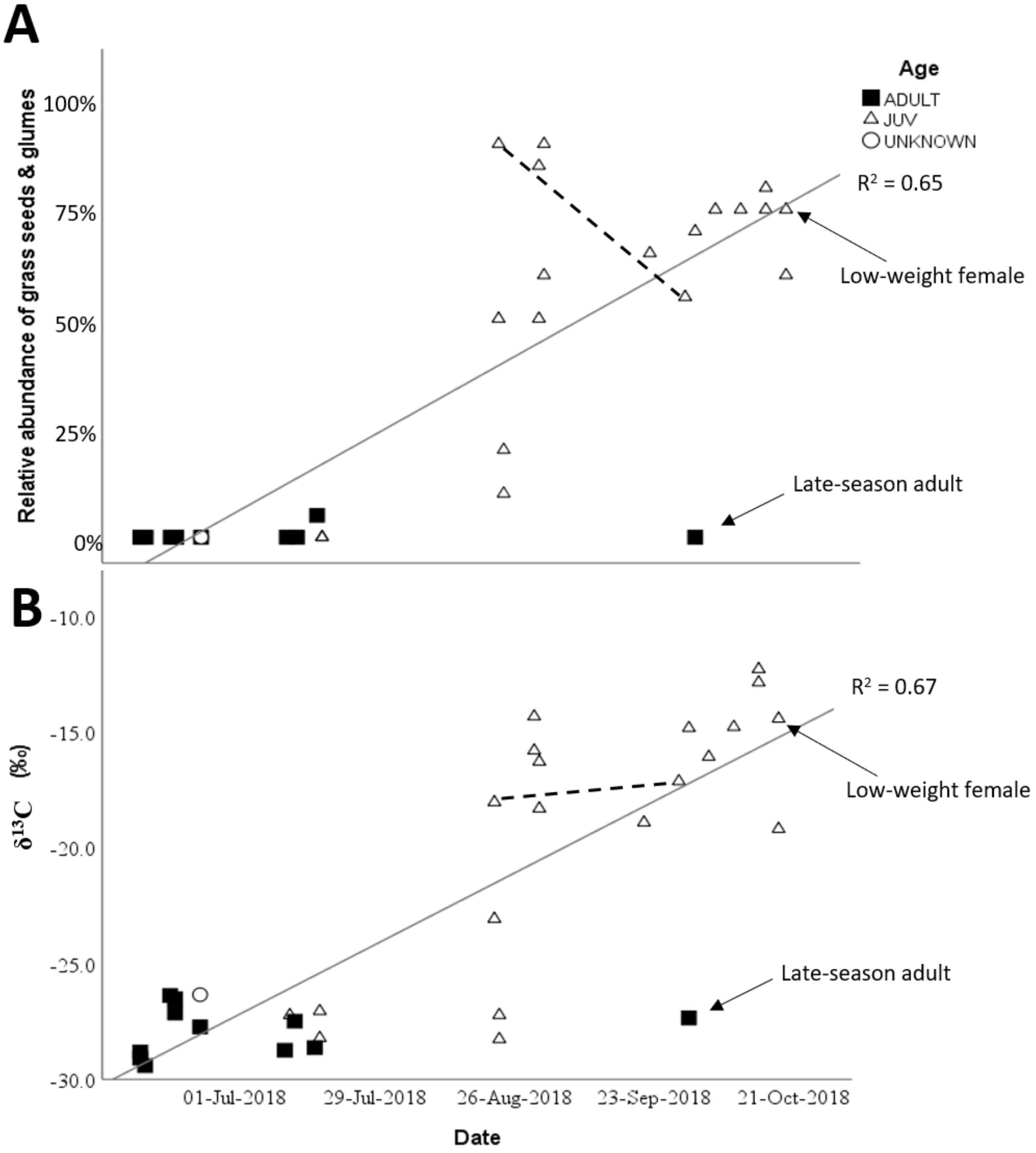

**Figure 5**. Scatterplots of microhistological and isotopic data plotted against seasonal progression. Specifically - A) grass seeds and glume consumption and B) $\delta^{13}$C values against date and sorted by age of individual (A: r=0.81, n=32, p<0.01; B r=0.82, n=32, p<0.01). The dashed line connects the 22 Aug 18 and 28 Sep 18 values of the same individual, and the low-weight female is also noted.

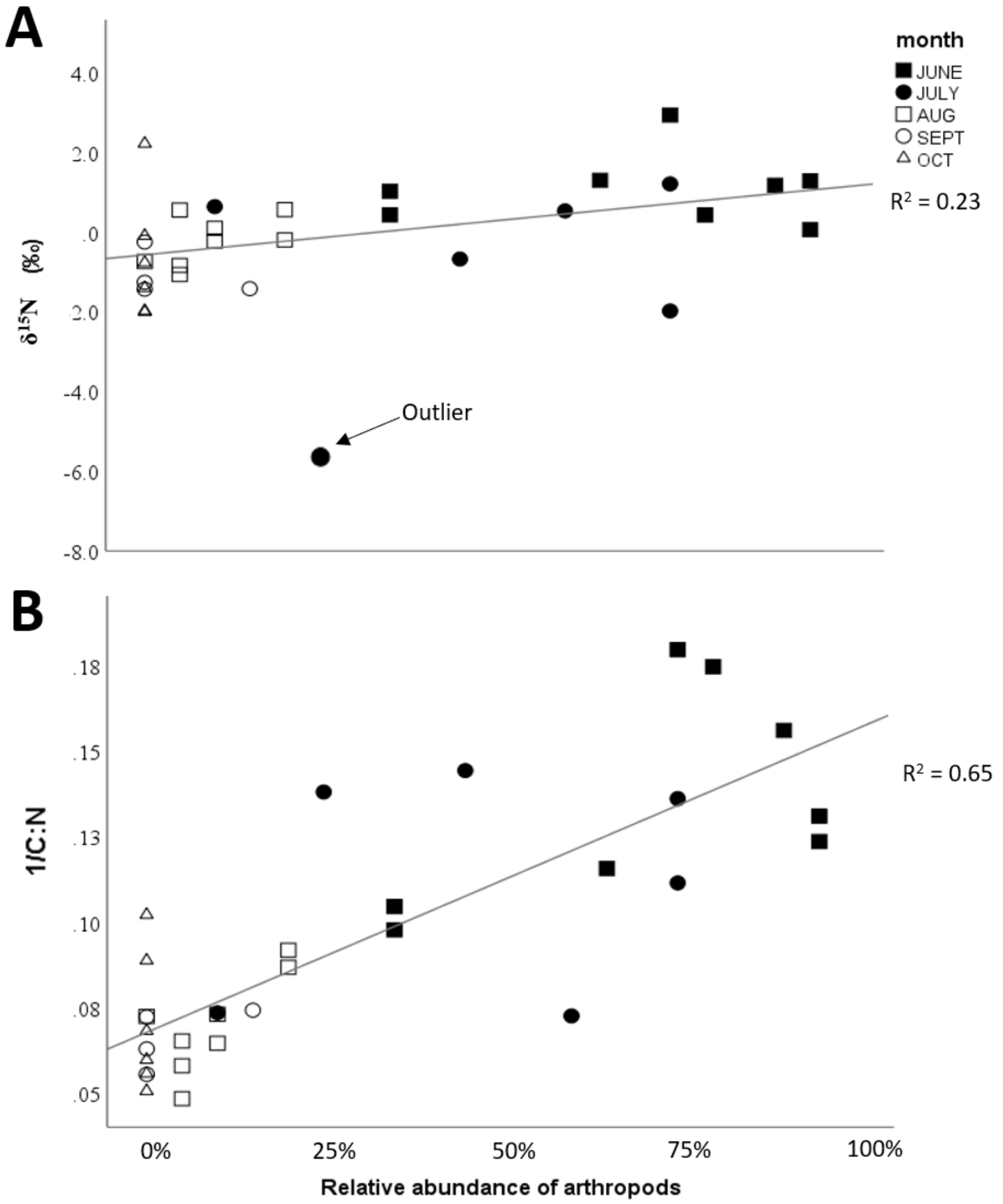

**Figure 6**. Scatterplots of isotopic data plotted against microhistological data. Specifically - A) $\delta^{15}N$ and B) 1/C:N ratio against relative dietary abundance of arthropods, and sorted by month (A: $r=0.47$, $n=32$, $p<0.05$; B: $r=0.81$, $n=32$, $p<0.01$). Statistical analysis was conducted without the outlier in Fig. 5A.

**Figure 7**. Scatterplot of isotopic data plotted against microhistological data. Specifically, $\delta^{13}$C by relative abundance of grass seeds and glumes (r=0.94, n=32, p<0.01).

**Appendix 1**. Raw data from the Micro Composition Laboratory giving frequency of each food type per fecal sample; the number is a frequency out of 20 (the number of fields scanned for each fecal sample). All data obtained from ground squirrel fecal samples of thirteen-lined ground squirrels collected at Rosehill Cemetery, Berrien Springs, MI in 2018.

| Date Collected | 6.12.18 | 6.12.18 | 6.13.18 | 6.18.18 | 6.19.18 | 6.19.18 | 6.24.18 | 6.24.18 |
|---|---|---|---|---|---|---|---|---|
| Sample ID | 26 | 28 | 29 | 33 | 34 | 57 | C | 58 |
| | | | | | | | | |
| arthropod | 7 | 13 | 7 | 15 | 19 | 18 | 19 | 16 |
| *Calamagrostis canadensis* | 1 | -- | -- | -- | -- | 1 | -- | -- |
| *Festuca* | 2 | 2 | -- | 2 | 1 | -- | -- | -- |
| grass seed & glume | -- | -- | -- | -- | -- | -- | -- | -- |
| *Leptochloa pretense* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis* | 5 | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis dilleni* | -- | -- | -- | 2 | -- | -- | -- | -- |
| *Pinus* | -- | -- | -- | -- | 1 | 1 | -- | -- |
| *Plantago* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Plantago* seed | -- | -- | -- | -- | -- | -- | -- | -- |
| sedge | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium pratense* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium repens* | 10 | 9 | 17 | 2 | 3 | 2 | 6 | 11 |
| unknown forb I | 2 | -- | 1 | -- | -- | -- | -- | -- |
| unknown grass I | -- | -- | -- | -- | -- | -- | -- | -- |

## Appendix 1 (extended).

| Date Collected | 7.11.18 | 7.12.18 | 7.13.18 | 7.17.18 | 7.18.18 | 7.18.18 | 7.24.18 | 7.24.18 |
|---|---|---|---|---|---|---|---|---|
| Sample ID | 61 | 63 | 65 | 68 | 37 | 39 | 69 | 70 |
| | | | | | | | | |
| arthropod | 2 | 9 | 15 | 5 | 15 | 12 | 10 | 12 |
| *Calamagrostis canadensis* | 1 | 11 | -- | 5 | 1 | 1 | -- | 2 |
| *Festuca* | 8 | 1 | 2 | 4 | 6 | 5 | 5 | 4 |
| grass seed & glume | -- | -- | -- | 1 | -- | -- | -- | -- |
| *Leptochloa pretense* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis* | 1 | 1 | -- | -- | 1 | -- | 3 | -- |
| *Oxalis dilleni* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Pinus* | -- | 1 | -- | -- | -- | -- | -- | -- |
| *Plantago* | -- | -- | -- | -- | -- | 1 | -- | -- |
| *Plantago* seed | 1 | -- | 1 | -- | -- | -- | -- | -- |
| sedge | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium pratense* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Trifolium repens* | 11 | -- | 3 | 8 | 1 | 4 | 7 | 7 |
| unknown forb I | -- | -- | -- | 1 | 1 | -- | 1 | -- |
| unknown grass I | -- | -- | -- | -- | -- | -- | -- | -- |

## Appendix 1 (extended).

| Date collected | 8.22.18 | 8.22.18 | 8.23.18 | 8.23.18 | 8.30.18 | 8.30.18 | 8.31.18 | 8.31.18 |
|---|---|---|---|---|---|---|---|---|
| Sample ID | 71 | 72 | 73 | 74 | 4873 | 4874 | 4850 | 4900 |
| arthropod | 2 | 1 | 4 | 4 | 1 | 1 | -- | 2 |
| *Calamagrostis canadensis* | 1 | -- | -- | -- | 2 | 5 | 1 | 3 |
| *Festuca* | -- | 1 | -- | -- | -- | -- | -- | -- |
| grass seed & glume | 10 | 18 | 2 | 4 | 17 | 10 | 18 | 12 |
| *Leptochloa pretense* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis dilleni* | 1 | -- | -- | -- | -- | -- | -- | -- |
| *Pinus* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Plantago* | -- | -- | -- | -- | -- | -- | -- | -- |
| *Plantago* seed | -- | -- | -- | -- | -- | -- | -- | -- |
| sedge | 1 | 1 | -- | -- | -- | -- | -- | -- |
| *Trifolium* | 5 | -- | 8 | 7 | 1 | 4 | -- | 1 |
| *Trifolium pratense* | 1 | -- | 4 | 5 | -- | 1 | -- | -- |
| *Trifolium repens* | 4 | 1 | 4 | 3 | -- | 1 | 1 | 3 |
| unknown forb I | -- | -- | -- | 1 | -- | -- | -- | 1 |
| unknown grass I | -- | -- | -- | -- | -- | -- | -- | -- |

## Appendix 1 (extended).

| Date collected | 9.21.18 | 9.28.18 | 9.30.18 | 9.30.18 | 10.4.18 | 10.9.18 | 10.14.18 | 10.14.18 | 10.18.18 | 10.18.18 |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample ID | S1 | 72 | S2 | 53 | 11 | 72-dark | 13 | 14 | 16 | 17 |
| | | | | | | | | | | |
| arthropod | 3 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Calamagrostis canadensis* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Festuca* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| grass seed & glume | 13 | 11 | 14 | -- | 15 | 15 | 16 | 15 | 12 | 15 |
| *Leptochloa pretense* | 2 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Oxalis dilleni* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Pinus* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Plantago* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Plantago* seed | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| sedge | -- | 3 | 2 | -- | -- | 1 | -- | -- | -- | 2 |
| *Trifolium* | 2 | 4 | 2 | 14 | 2 | 2 | 5 | 3 | 1 | 1 |
| *Trifolium pratense* | -- | 1 | -- | 4 | 4 | 5 | 1 | -- | 8 | -- |
| *Trifolium repens* | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| unknown forb I | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| unknown grass I | 2 | 1 | 3 | 2 | -- | 1 | -- | 3 | 1 | 3 |

**Appendix 2**. Raw data from the Stable Isotope Ratio Facility for Environmental Research (SIRFER) lab. All data obtained from ground squirrel fecal samples of thirteen-lined ground squirrels collected at Rosehill Cemetery, Berrien Springs, MI in 2018.

Appendix Table 2-1. List of reference materials and standard used in isotopic analysis. Provided by the SIRFER lab.

| | | | | |
|---|---|---|---|---|
| SIRFER EA-IRMS-CN | | | | |
| Job # 19-035 | | Tray | Analyst: | Suvankar Chakraborty |
| Primary reference 1 (PLRM-1): | UU-CN-1 | | Primary reference 2 (PLRM-2): | UU-CN-2 |
| Secondary reference (SLRM): | Spinach | | Wt% standard: | UU-CN-2 |
| Date analyzed: | 2/25/2019 | | | |

**Appendix 2 (continued).**

Appendix Table 2-2. Included are $\delta^{15}N_{AIR}$ values, which compare the standardized ratio of $^{15}N$ to $^{14}N$ in the samples to the same standardized ratio in air; $\delta^{13}C_{VPDB}$ that compares ratio of $^{13}C$ to $^{12}C$ in the samples to the standardized Vienna Pee Dee Belemnite standard value; %N and %C that is in each sample; and finally, C:N ratios of each sample. Nitrogen values are reported on the AIR scale where USGS40 = -4.5 per mil and USGS41 = +47.6 per mil. Carbon values are reported on the VPDB scale where USGS40 = -26.24 per mil and USGS41 = +37.76 per mil. Data provided by the SIRFER lab.

| Date | Original ID | Sample wt (mg) | $\delta^{15}N_{AIR}$ (‰) | $\delta^{13}C_{VPDB}$ (‰) | Wt% N | Wt% C | %C:%N ratio |
|---|---|---|---|---|---|---|---|
| 6/12/18 | 100 | 0.742 | 1.0 | -29.1 | 5.0 | 47.5 | 9.6 |
| 6/12/18 | 101 | 0.716 | 1.3 | -28.8 | 5.6 | 48.0 | 8.6 |
| 6/13/18 | 102 | 0.777 | 0.4 | -29.4 | 4.7 | 47.6 | 10.2 |
| 6/18/18 | 103 | 0.724 | 2.9 | -26.4 | 7.4 | 41.4 | 5.6 |
| 6/19/18 | 104 | 0.878 | 1.3 | -27.1 | 4.7 | 35.5 | 7.6 |
| 6/19/18 | 105 | 0.736 | 1.2 | -26.5 | 5.4 | 34.7 | 6.4 |
| 6/24/18 | 106 | 0.756 | 0.1 | -27.7 | 6.0 | 48.3 | 8.1 |
| 6/24/18 | 107 | 0.767 | 0.4 | -26.3 | 8.1 | 46.6 | 5.7 |
| 7/11/18 | 108 | 0.791 | 0.6 | -28.7 | 3.6 | 49.5 | 13.6 |
| 7/12/18 | 109 | 0.801 | -0.7 | -27.2 | 5.1 | 35.6 | 6.9 |
| 7/13/18 | 110 | 0.785 | -2.0 | -27.5 | 4.2 | 37.8 | 9.0 |
| 7/17/18 | 111 | 0.826 | -6.2 | -28.6 | 6.8 | 49.2 | 7.2 |
| 7/18/18 | 112 | 0.730 | 1.2 | -27.1 | 6.2 | 45.4 | 7.3 |
| 7/18/18 | 113 | 0.868 | 0.5 | -28.2 | 3.2 | 44.3 | 13.8 |

**Appendix 2 (continued).**

Appendix Table 2-2 (continued).

| Date | Original ID | Sample wt (mg) | $\delta^{15}N_{AIR}$ (‰) | $\delta^{13}C_{VPDB}$ (‰) | Wt% N | Wt% C | %C:%N ratio |
|---|---|---|---|---|---|---|---|
| 8/22/18 | 200 | 0.762 | -0.2 | -23.1 | 3.1 | 48.2 | 15.5 |
| 8/22/18 | 201 | 0.740 | -0.8 | -18.0 | 2.7 | 46.4 | 17.3 |
| 10/18/18 | 202 | 0.735 | -2.0 | -14.4 | 2.6 | 47.2 | 18.0 |
| 8/23/18 | 203 | 0.763 | 0.6 | -28.3 | 4.3 | 49.7 | 11.5 |
| 8/23/18 | 204 | 0.775 | -0.2 | -27.2 | 4.4 | 47.9 | 10.9 |
| 8/30/18 | 205 | 0.721 | -1.1 | -14.3 | 2.2 | 46.3 | 20.7 |
| 8/30/18 | 206 | 0.770 | 0.6 | -15.8 | 3.0 | 45.9 | 15.3 |
| 8/31/18 | 207 | 0.757 | -0.7 | -18.3 | 3.2 | 44.1 | 13.8 |
| 8/31/18 | 208 | 0.771 | 0.1 | -16.3 | 3.2 | 43.4 | 13.7 |
| 9/21/18 | 209 | 0.830 | -1.4 | -18.9 | 2.9 | 39.4 | 13.5 |
| 9/28/18 | 210 | 0.733 | -1.4 | -17.1 | 2.8 | 45.0 | 15.9 |
| 9/30/18 | 211 | 0.734 | -0.2 | -14.8 | 2.5 | 44.8 | 18.0 |
| 9/30/18 | 212 | 0.753 | -1.3 | -27.3 | 3.5 | 48.3 | 13.8 |
| 10/4/18 | 213 | 0.807 | -0.1 | -16.1 | 5.1 | 49.5 | 9.8 |
| 10/9/18 | 214 | 0.751 | 2.2 | -14.8 | 4.1 | 45.7 | 11.3 |
| 10/14/18 | 215 | 0.778 | -2.0 | -12.8 | 2.4 | 47.6 | 19.8 |
| 10/14/18 | 216 | 0.728 | -0.8 | -12.3 | 3.2 | 47.0 | 14.7 |
| 10/18/18 | 217 | 0.755 | -1.4 | -19.2 | 2.8 | 46.2 | 16.7 |

**Appendix 2 (continued).**

Appendix Table 2-3. A test of repeatability of analyses on samples of known isotopic composition, compared to standard reference samples. Data provided by the SIRFER lab.

| Quality Assurance | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sample ID | Sample wt (mg) | $\delta^{15}N_{AIR}$ (‰) | $\delta^{13}C_{VPDB}$ (‰) | Wt% N | Wt% C | %C:%N ratio |
| PLRM-1 | UU-CN-1 | 0.5 | 49.8 | 24.3 | 10.6 | 45.0 | 4.2 |
| $\delta^{13}C_{VPDB}$ = +23.96‰ | UU-CN-1 | 0.5 | 49.4 | 23.7 | 10.4 | 44.8 | 4.3 |
| $\delta^{15}N_{AIR}$ = +49.63‰ | UU-CN-1 | 0.5 | 49.7 | 23.9 | 10.3 | 44.6 | 4.3 |
| average | | | 49.6 | 24.0 | 10.4 | 44.8 | |
| standard uncertainty | | | 0.2 | 0.3 | 0.2 | 0.2 | |
| | Sample ID | Sample wt (mg) | $\delta^{15}N_{AIR}$ (‰) | $\delta^{13}C_{VPDB}$ (‰) | Wt% N | Wt% C | %C:%N ratio |
| PLRM-2 | UU-CN-2 | 0.5 | -4.5 | -28.4 | 9.6 | 41.0 | 4.3 |
| $\delta^{13}C_{VPDB}$ = -28.18‰ | UU-CN-2 | 0.5 | -4.5 | -28.0 | 9.3 | 40.2 | 4.3 |
| $\delta^{15}N_{AIR}$ = -4.56‰ | UU-CN-2 | 0.5 | -4.6 | -28.1 | 9.5 | 40.8 | 4.3 |
| average | | | -4.6 | -28.2 | 9.5 | 40.7 | |
| standard uncertainty | | | 0.1 | 0.2 | 0.1 | 0.4 | |

**Appendix 2 (continued).**

Appendix Table 2-4. A test of repeatability of analyses on samples of known isotopic composition, compared to biological reference samples of known composition. Data provided by the SIRFER lab.

| Quality Control | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sample ID | Sample wt (mg) | $\delta^{15}N_{AIR}$ (‰) | $\delta^{13}C_{VPDB}$ (‰) | Wt% N | Wt% C | %C:%N ratio |
| SLRM | spinach | 0.8 | -0.6 | -27.7 | 6.0 | 41.7 | 7.0 |
| $\delta^{13}C_{VPDB}$ =-27.41‰ | spinach | 0.8 | -0.3 | -27.4 | 5.9 | 41.2 | 7.0 |
| $\delta^{15}N_{AIR}$ =-0.4‰ | spinach | 0.8 | -0.3 | -27.3 | 5.9 | 41.3 | 7.0 |
| average | | | -0.4 | -27.4 | 5.9 | 41.4 | |
| standard uncertainty | | | 0.2 | 0.2 | 0.1 | 0.3 | |
| acceptable range<br>1 sigma = 0.2 | | $\delta^{13}C_{VPDB}$ | | | | | |
| acceptable range<br>1 sigma = 0.2 | | $\delta^{15}N_{AIR}$ | | | | | |